EXPERT DECLARATION

I, Grant H. Castle, Ph.D., declare and state as follows:

Credentials

a. I am a solicitor advocate of the Senior Courts of England and Wales, and have been since 1999. I am also a solicitor in Ireland. I am currently a partner at the law firm Covington & Burling LLP. Prior to qualifying as a solicitor, I obtained a Batchelor of Science degree from Imperial College London in 1991, and a Ph.D. in Organic Chemistry from the University of Cambridge in 1994. After obtaining my Ph.D., I worked as a Medicinal Chemist with Glaxo Group Research before entering the legal profession.

b. My practice focusses on life sciences regulatory law, with an emphasis on pharmaceutical and medical device regulation. I am often called upon to advise clients on a variety of issues including clinical research, adverse event and reporting obligations, manufacturing controls, product advertising and product life cycle management. In my career, I have advised clients extensively on matters relating to clinical trials, from both a UK and a pan-European perspective, and have represented their interests before both national and EU regulators. I regularly appear before the EU General Court and the Court of Justice of the EU and before the national Courts in cases involving pharmaceutical regulatory matters. On top of my practice as a solicitor, I have held several positions as a visiting lecturer, including at the University of Surrey, Cardiff University and Cranfield University, where I have delivered courses on pharmaceutical and medical device regulation.

The Dispute

c. I understand that EP2861210 owned by Ipsen Biopharm Ltd.'s ("Ipsen") was revoked by the EPO Opposition Division ("OD") because the subject matter claimed was allegedly obvious in view of a document referred to as "D15b", which is an extract from the website clinicaltrials.gov. I understand that Ipsen has appealed the decision to revoke the patent, and that a declaration from Amy McKee, M.D. ("D23") was filed in support of Ipsen's appeal. I have read document D23 and agree with all of the statements made therein.

d. I understand that the opponent/respondent (Teva Pharmaceutical Industries Ltd – "Teva") is now relying for the first time on a piece of UK statute (The Medicines for Human Use (Clinical Trials) Regulations 2004 and 2006) to argue that "*before a clinical trial can start in the UK, both ethics committee (IRB) approval and licencing authority approval is required*", and that "*the addition of Arm C would have... required both ethics committee (IRB) and licencing authority approval*"[1].

e. Based on my significant experience of UK and European regulatory law, my opinion is that Teva's arguments on this point do not accurately reflect the legal and regulatory situation. This will be explained in more detail below, with reference to enclosed D38A (*Communication from the Commission – Detailed guidance on the request to the competent authorities for authorisation*

[1] Response to appeal, paragraph 7.54.

of a clinical trial of a medicinal product for human use, the notion of substantial amendments and the declaration of the end of the trial (CT-1)".

Clinical Trial Authorisations

f. The Medicines for Human Use (Clinical Trials) Regulations 2004 and 2006 cited by Teva at paragraph 7.53 of its Response to Ipsen's appeal implement Directive 2001/20/EC (the Clinical Trials Directive, or "CTD") into UK law. I have read the Expert Declaration of Carla Schoonderbeek ("D37"), which discusses Directive 2001/20/EC insofar as it is relevant to these proceedings. I agree with all of the points made by Ms. Schoonderbeek in her Expert Declaration.

g. Article 9 of the CTD states that a clinical trial may not start until (i) the sponsor of the proposed trial has received a positive Ethics Committee opinion (Article 6(2)); and (ii) the relevant competent authority does not raise any grounds for non-acceptance:

"The sponsor may not start a clinical trial until the ***Ethics Committee has issued a favourable opinion*** *and inasmuch as the* ***competent authority[2] of the Member State concerned has not informed the sponsor of any grounds for non-acceptance.***

[...]

Before commencing any clinical trial, the sponsor shall be required to submit a valid request for authorisation to the competent authority of the Member State in which the sponsor plans to conduct the clinical trial."[3] (emphasis added)

h. Rather than a formal approval, competent authorities may only prevent the start of a clinical trial by raising "*grounds for non-acceptance*" during the sixty day period following submission of a valid request for authorisation.[4] The Recitals to the Directive clarify that:

"As a rule, authorisation should be implicit, i.e. if there has been a vote in favour by the Ethics Committee and the competent authority has not objected within a given period, it should be possible to begin the clinical trials."[5]

i. Therefore, Teva's assertion that: "*before a clinical trial can start in the UK, both ethics committee (IRB) approval* ***and licensing authority approval*** *is required*"[6] (emphasis added) is inconsistent with the language of the Directive.

j. I note that, in reaching this conclusion, Teva quoted section 12 of the UK's national implementation of the Directive (the Medicines for Human Use (Clinical Trials) Regulations 2004, the "UK Regulations")[7]. Section 12 of the UK Regulations implements Article 9 of the Directive, and requires that "*the clinical trial has* ***been authorised*** *by the licensing authority*" (emphasis added) before a CTA is valid. However, section 18(3) further clarifies that a clinical trial will be treated as "*authorised*" by way of a so-called "*tacit authorisation*" if no notice is provided by the licensing authority to the sponsor within the relevant deadline.

[2] In the Medicines for Human Use (Clinical Trials) Regulations 2004 and 2006 cited by Teva, the "competent authority" is referred to using the term "licencing authority". For the purposes of this declaration these two terms can be considered to be synonymous.
[3] Articles 9(1) and 9(2) of the CTD.
[4] Article 9(4) of the Directive.
[5] Recital 11 of the Directive.
[6] See paragraph 7.54 of Teva's "Reply to the Proprietor's Appeal" dated 27 July 2020.
[7] D36

k. This is an important consideration in establishing a competent authority's role in the clinical trial process. In particular, notwithstanding the above, certain investigational medicinal products **do** require express approval by competent authorities before the CTA becomes valid:

"Written authorisation shall be required before commencing clinical trials involving ***medicinal products for gene therapy, somatic cell therapy including xenogenic cell therapy and all medicinal products containing genetically modified organisms****."*[8] (emphasis added)

l. According to document "D15c", the Investigational Medicinal Product ("IMP") is referred to as "*nanoliposomal irinotecan*" and "*irinotecan hydrochloride*". This IMP does not fall under the categories of medicinal product listed above for which express competent authority approval is required. Thus, the NAPOLI-1 trial would only have required a "*tacit authorisation*" from the competent authority (the MHRA in the UK), as described above.

Substantial Amendments to Trial Protocols

m. During the NAPOLI-1 trial, the sponsor (Merrimack Pharmaceuticals, Inc.) added Arm C to the trial by way of a substantial amendment to the trial protocol. Contrary to Teva's assertion, such an amendment does not require an authorization by a regulatory authority. Rather, the sponsor need only obtain a positive ethics committee opinion before it can implement the change unless a competent authority objects. Article 10(a) of the CTD provides:

*"****[i]f those amendments are substantial*** *and are likely to have an impact on the safety of the trial subjects or to change the interpretation of the scientific documents in support of the conduct of the trial, or if they are otherwise significant, the* ***sponsor shall notify the competent authorities*** *of the Member State or Member States concerned of the reasons for, and content of, these amendments and shall* ***inform the ethics committee*** *or committees concerned in accordance with Articles 6 and 9.*

On the basis of the details referred to in Article 6(3) and in accordance with Article 7, the Ethics Committee shall give an opinion within a maximum of 35 days of the date of receipt of the proposed amendment in good and due form. If this opinion is unfavourable, the sponsor may not implement the amendment to the protocol.

If the opinion of the Ethics Committee is favourable and the competent authorities of the Member States have raised no grounds for non-acceptance of the abovementioned substantial amendments*, the sponsor shall proceed to conduct the clinical trial following the amended protocol."*[9] (emphasis added)

n. Section 24 of The Medicines for Human Use (Clinical Trials) Regulations 2004 cited by Teva[10] is an implementation of Article 10(a) of the CTD.

o. In summary, a substantial protocol amendment of the kind that added Arm C to the NAPOLI-1 does not require regulatory approval, but only a positive ethics opinion and the absence of an objection from the relevant regulator.

[8] Article 9(6) of the Directive and section 19 of the UK Regulations.
[9] Article 10(a) of the Directive.
[10] Paragraph 7.54 of the response.

Final comments

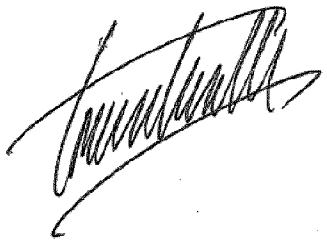
p. I note that Teva's arguments appear to suggest that, if approval from a competent authority is granted for a clinical trial, such approval means that there exists an expectation or presumption that the clinical trial will be successful. Based on my experience, I do think that such a suggestion is correct. This is reflected by the official guidance issued by from the European Commission on clinical trial authorisations, which states that the authorisation by a competent authority "*is not to be considered as scientific advice on the development programme of the investigational medicinal product (IMP) tested*" (emphasis added)[11].

q. More generally, I do not share the view expressed by Teva and the OD that the fact that a dosage regimen is being tested in a clinical trial in a particular patient population leads to an expectation or presumption of success. This is particularly true in the present circumstances, as the relevant dosage regimen had not been tested at all in the relevant patient population prior to the beginning of the NAPOLI-1 trial (see the Declaration of Bruce Belanger – "D24"). The disclosure of documents D2-D7, D12, D13, D22, and D29 do not change my conclusion on this point.

Statement of Truth

r. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true.

Declared in London, United Kingdom, on 29 June 2021.



Grant H. Castle, Ph.D.

[11] D38A, particularly paragraph 18 thereof. Issued on 30th March 2010.

II

(Information)

INFORMATION FROM EUROPEAN UNION INSTITUTIONS, BODIES, OFFICES AND AGENCIES

EUROPEAN COMMISSION

Communication from the Commission — Detailed guidance on the request to the competent authorities for authorisation of a clinical trial on a medicinal product for human use, the notification of substantial amendments and the declaration of the end of the trial (CT-1)

(2010/C 82/01)

1. INTRODUCTION

1.1. Legal basis

1. This detailed guidance is based on Article 9(8) of Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use [1] (hereinafter Directive 2001/20/EC), which establishes that:

'In consultation with Member States, the Commission shall draw up and publish detailed guidance on:

(a) the format and contents of the request referred to in paragraph 2 (i.e. submission of a valid request for authorisation to the competent authority of the Member State in which the sponsor plans to conduct the clinical trial) as well as the documentation to be submitted to support that request, on the quality and manufacture of the investigational medicinal product, any toxicological and pharmacological tests, the protocol and clinical information on the investigational medicinal product including the investigator's brochure;

(b) the presentation and content of the proposed amendment referred to in point (a) of Article 10 on substantial amendments made to the protocol;

(c) the declaration of the end of the clinical trial.'

2. This guidance does address aspects related to Ethics Committees only insofar as the provisions contained in Directive 2001/20/EC are identical with regard to both the national competent authority and the Ethics Committee. This means that the following sections in this guidance also apply to Ethics Committees:

— Procedural aspects of notification of 'substantial amendments' (Sections 3.1 to 3.3, and 3.5 to 3.8); and

— Declaration of the end of the trial (Section 4).

Regarding the other aspects, reference is made to the separate Commission guidance based on Article 8 of Directive 2001/20/EC.

3. According to Article 3(1) of Directive 2001/20/EC, all national requirements as regards clinical trials have to be consistent with the procedures and timescales set out in Directive 2001/20/EC, such as the procedures and timescales for authorisation of a clinical trial, notification of a substantial amendment, and declaration of the end of the clinical trial. This document provides guidance on these aspects.

4. EU Member States, contracting States of the European Economic Area (EEA) [2] and persons who request authorisation of a clinical trial (applicants), notify substantial amendments, and declare the end of a clinical trial in the EU should consider this guidance when applying Directive 2001/20/EC.

[1] OJ L 121, 1.5.2001, p. 34.

[2] For the purposes of this document, references to the EU, EU Member States or Member States should be understood to include the EEA or EEA contracting States, unless indicated otherwise.

1.2. **Scope**

5. This guidance addresses the requests for authorisation, amendments, and declaration of the end of clinical trials within the scope of Directive 2001/20/CE. Directive 2001/20/EC applies to all clinical trials as defined in Article 2(a) of this Directive. As regards the term 'medicinal products', this refers to medicinal products for human use as defined in Article 1(2) of Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use [1] (hereinafter Directive 2001/83/EC). This includes medicinal products where the pharmacological, immunological, or metabolic action of the product is still uncertain and being explored.

6. This includes also medicinal products which are specifically addressed in the EU law on pharmaceuticals, such as advanced therapy medicinal products [2] or medicinal products derived from human blood or human plasma as defined in Article 1(10) of Directive 2001/83/EC.

7. Directive 2001/20/EC also applies to interventional clinical trials with medicinal products for the paediatric population and interventional clinical trials with medicinal products manufactured or reconstituted in a (hospital) pharmacy and intended to be supplied directly to the clinical trials participants.

8. The exclusions contained in Article 3 of Directive 2001/83/EC are not relevant as regards the scope of Directive 2001/20/EC and of this guidance.

9. Directive 2001/20/EC does not apply to:

— medical devices, active implantable medical devices, and *in vitro* diagnostic medical devices as defined in Community legislation [3], [4], [5],

— cosmetic products as defined in Community legislation [6],

— food as defined in Community legislation [7].

10. To draw the 'borderline' between these sectoral legislations (e.g. medicinal products/food, medicinal products/cosmetic products, medicinal products/medical devices), the established criteria as set out in the case law of the European Court of Justice apply and reference is made to the relevant guidelines [8].

1.3. **Definitions**

11. The definitions contained in Directive 2001/20/EC, its implementing acts and relevant guidance documents in the current version apply also for this guidance. With regard to implementing guidelines, the following guidance documents in particular provide valuable additional definitions:

— Guidance on Investigational Medicinal Products (IMPs) and other medicinal products used in Clinical Trials (on the term 'investigational medicinal products') [9],

— Annex 13 to the Guidelines on good manufacturing practice — Manufacture of investigational medicinal products [10],

— Commission Guidelines on Pharmacovigilance for Medicinal Products for Human Use (on the term 'non-interventional trial') [11], and

— Questions and Answers Document on the Clinical Trials Directive [12].

[1] OJ L 311, 28.11.2001, p. 67.
[2] As defined in Article 2(1)(a) of Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004 (OJ L 324, 10.12.2007, p. 121) (hereinafter Regulation (EC) No 1394/2007).
[3] Council Directive 93/42/EEC of 14 June 1993 concerning medical devices (OJ L 169, 12.7.1993, p. 1).
[4] Council Directive 90/385/EEC of 20 June 1990 on the approximation of the laws of the Member States relating to active implantable medical devices (OJ L 189, 20.7.1990, p. 17).
[5] Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices (OJ L 331, 7.12.1998, p. 1).
[6] Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products (OJ L 262, 27.9.1976, p. 169).
[7] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety (OJ L 31, 1.2.2002, p. 1), as amended.
[8] cf., for example, http://ec.europa.eu/enterprise/sectors/cosmetics/cosmetic-products/borderline-products/index_en.htm
[9] http://ec.europa.eu/enterprise/sectors/pharmaceuticals/documents/eudralex/vol-10/index_en.htm
[10] http://ec.europa.eu/enterprise/sectors/pharmaceuticals/documents/eudralex/vol-10/index_en.htm
[11] Volume 9A of *The Rules Governing Medicinal Products in the European Union* (Sept. 2008), Part 1, Point 7.1. (p. 90).
[12] http://ec.europa.eu/enterprise/sectors/pharmaceuticals/documents/eudralex/vol-10/index_en.htm

12. For the purposes of this guidance, 'Member State concerned' means the Member State where the clinical trial is intended to be performed. For a given clinical trial there may be several Member States concerned (multinational clinical trials). 'ICH country' means a third country which is party to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, i.e. Japan and the US.

2. REQUEST FOR A CLINICAL TRIAL AUTHORISATION

2.1. Procedural aspects

2.1.1. *Legal basis*

13. Article 9(1), second subparagraph, and (2) of Directive 2001/20/EC reads as follows:

'The sponsor may not start a clinical trial until the Ethics Committee has issued a favourable opinion and inasmuch as the competent authority of the Member State concerned has not informed the sponsor of any grounds for non-acceptance. ...

Before commencing any clinical trial, the sponsor shall be required to submit a valid request for authorisation to the competent authority of the Member State in which the sponsor plans to conduct the clinical trial [1].

[1] cf. also recital 11 of Directive 2001/20/EC: "As a rule, authorisation should be implicit, i.e. if there has been a vote in favour by the Ethics Committee and the competent authority has not objected within a given period, it should be possible to begin the clinical trials." '

2.1.2. *Request for authorisation, applicable timelines, tacit authorisation*

14. The applicant submits a request for authorisation of a clinical trial to the national competent authority of the Member State concerned.

15. In accordance with Article 9(4) of Directive 2001/20/EC, consideration of a valid request for authorisation by the national competent authority shall be carried out as rapidly as possible and may not exceed 60 calendar days.

16. Validation of the request for authorisation is included in the period of 60 calendar days. Day 0 is the day of receipt of the request. If the request is valid, and by day 60 no ground for non-acceptance has been raised, the clinical trial is authorised by the national competent authority of the Member State concerned (tacit authorisation [1]).

[1] The term 'authorisation' will be used throughout this document.

17. However, Article 9(4), (5) and (6) of Directive 2001/20/EC sets out important exceptions to the rules on timelines and tacit authorisation as regards certain medicinal products, including medicinal products the active ingredient of which is a biological product of human or animal origin, or contains biological components of human or animal origin, or the manufacturing of which requires such components. Exceptions also apply to medicinal products for gene therapy, somatic cell therapy including xenogenic cell therapy and all medicinal products containing genetically modified organisms.

2.1.3. *Scope of authorisation*

18. The authorisation of a clinical trial by the national competent authority is valid for a clinical trial conducted in that Member State. This authorisation is not to be considered as scientific advice on the development programme of the investigational medicinal product (IMP) tested.

2.1.4. *Follow-up to request for authorisation*

2.1.4.1. Application is not valid

19. If an application is not valid, the national competent authority should inform the applicant of this within the first 10 calendar days of the period referred to in Section 2.1.2. The reasons should be given.

2.1.4.2. Changes to the submitted to documentation during the evaluation phase

20. Following the submission of a request for authorisation, the submitted documentation may change. This may happen either:

— following information by the national competent authority that the application is not valid (see Section 2.1.4.1). In this case, the time limit set out in Article 9(4) of Directive 2001/20/EC starts again when a valid request has been received;

— at the initiative of the applicant. In practice, the applicant may have an interest in changing submitted documentation. This may happen as a consequence of grounds for non-acceptance by the national competent authority of another Member State or a third country concerned if the applicant wants to ensure that the documentation submitted in all Member States/third countries concerned is identical. In this case, the time limit set out in Article 9(4) of Directive 2001/20/EC starts again; or

— following notification of grounds for non-acceptance by the competent authority of the Member State concerned: in this case Article 9(3) of Directive 2001/20/EC applies.

2.1.4.3. Withdrawals

21. Unexpected events or additional information may require the applicant to withdraw a request for authorisation before the national competent authority has reached its decision on authorisation. The applicant should inform the national competent authority of the Member State concerned as soon as he becomes aware that he intends to withdraw the application. The initial contact should be by fax or e-mail and include the EudraCT number and other trial identification. Where the initial contact is by telephone, this should be followed up, for reasons of traceability, by fax or e-mail. The initial contact should be followed as soon as possible by a formal letter of withdrawal providing a brief description of the reasons.

22. If the applicant wishes to resubmit the application, he must identify the application as a resubmission in the cover letter (resubmission letter) and in the dedicated field of the clinical trial application form. The initial EudraCT number is used with a letter after the number sequence: A for first resubmission, B for second resubmission, and so on.

2.1.5. *Interface with other authorisation requirements*

23. The applicant should make applications to fulfil other requirements that relate to clinical trials with IMPs where applicable. For example, if the IMP is a genetically modified organism (GMO) it may be necessary to obtain permission from the relevant competent authority in the Member State concerned for its contained use or deliberate release in accordance with Council Directive 90/219/EEC of 23 April 1990 on the contained use of genetically modified micro-organisms [1] or Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC [2].

2.1.6. *Other issues*

24. The application dossier should be submitted as electronic version only, i.e. via telematics system (if nationally available), e-mail, or a posted CD-ROM. If documentation is sent by paper, it should be limited to the signed cover letter only.

25. The Commission encourages national competent authorities to accept the English language in their communication with applicants and for documentation which is not destined for the public or the clinical trial participant, such as scientific documentation.

[1] OJ L 117, 8.5.1990, p. 1.
[2] OJ L 106, 17.4.2001, p. 1.

2.2. Allocation of EudraCT number

26. Before submitting an application to the national competent authority, the applicant should obtain a unique EudraCT number from the EudraCT Community Clinical Trial System [3] by the procedure described in the current version of the Detailed guidance on the European clinical trials database [4]. This number identifies the protocol for a trial, whether conducted at a single site or at multiple sites in one or more Member States. To obtain the EudraCT number automatically from the database the applicant will need to provide a few items of information [5].

2.3. Cover letter

27. The applicant should submit a signed cover letter with the application. Its subject line should contain the EudraCT number and the invariable sponsor protocol number (if available) with the title of the trial.

28. In the cover letter, the applicant should draw attention to peculiarities of the trial.

29. However, in the cover letter it is not necessary to reproduce information which is already contained in the clinical trial application form, with the following exceptions:

— specific features of the trial population, such as clinical trial participants not able to give informed consent or minors;

— whether the trial involves the first administration of a new active substance to humans;

— whether there is scientific advice related to the trial or IMP given by the European Medicines Agency (the Agency) or the national competent authority of a Member State or third country; and

— whether the trial is part or is intended to be part of a Paediatric Investigation Plan (PIP) as referred to in Title II, Chapter 3 of Regulation (EC) No 1901/2006 of the European Parliament and of the Council of 12 December 2006 on medicinal products for paediatric use [6]. If the Agency has already issued a Decision on the PIP, the cover letter should contain the link to the Decision of the Agency on its website (see also Section 2.9).

[3] https://eudract.ema.europa.eu/
[4] EudraLex, Volume 10; http://ec.europa.eu/enterprise/sectors/pharmaceuticals/documents/eudralex/vol-10/index_en.htm
[5] Note that paediatric clinical trials included in an agreed PIP and performed in a third country have to be entered into EudraCT as well (cf. point 2.2.1. of Commission Communication 2009/C28/01).
[6] OJ L 378, 27.11.2006, p. 1.

30. In the cover letter, the applicant should highlight whether the IMP or NIMP is a narcotic and psychotropic.

31. The applicant should indicate where the relevant information is contained in the application dossier.

32. The applicant should set out precisely in the cover letter where in the application dossier the reference safety information is contained for assessing whether an adverse reaction is a suspected unexpected serious adverse reaction (SUSAR).

33. In the case of a resubmission letter (see Section 2.1.4.3), the applicant should highlight the changes as compared to the previous submission.

2.4. **Clinical trial application form**

34. For clinical trials falling within the scope of the Directive 2001/20/EC, there is a unique, EU-wide clinical trial application form provided for and published in Volume 10 of EudraLex — The Rules Governing Medicinal Products in the European Union ([1]).

35. Some of the information in the form, such as information related to the applicant and the name of the investigators, will be relevant in one Member State only.

36. The applicant's signature will confirm that the sponsor is satisfied that:

— the information provided is complete,

— the attached documents contain an accurate account of the information available,

— the clinical trial will be conducted in accordance with the protocol, and

— the clinical trial will be conducted, and SUSARs and result-related information will be reported, in accordance with the applicable legislation.

37. If the form is submitted in paper form (cf. Section 2.1.6), the applicant should save the full clinical trial application form data set as an XML file using the utilities feature and submit an electronic copy of this XML file on a CD-ROM.

38. More information about the clinical trial application form, and how to fill it in, is available in the current version of these documents:

— Detailed guidance on the European clinical trials database ([2]),

— EudraCT User Manual ([3]), and

— EudraCT Frequently Asked Questions ([4]).

39. In addition, the Agency operates a help desk to support applicants who have questions related to EudraCT ([5]).

40. Certain information contained in the clinical trial application form will be made public, following its entry into EudraCT by the national competent authority of the Member State concerned. This is done by rendering certain data fields contained in EudraCT public in accordance with the applicable guidelines published by the Commission ([6]).

2.5. **Protocol**

41. According to Article 2(h), first sentence, of Directive 2001/20/EC, the protocol is 'a document that describes the objective(s), design, methodology, statistical considerations and organisation of a trial.'

42. The protocol should be identified by the title, the sponsor's protocol code number specific for all versions of it (if available), a date and number of version that will be updated when it is amended, and a short title or name assigned to it.

43. For the content and format of the protocol, reference is made to Section 6 of the Community guideline on Good Clinical Practice (CPMP/ICH/135/95) ([7]). In particular, the protocol should include:

— a clear and unambiguous definition of the end of the trial in question. In most cases this will be the date of the last visit of the last patient undergoing the trial. Any exceptions to this should be justified in the protocol; and

([1]) http://ec.europa.eu/enterprise/sectors/pharmaceuticals/documents/eudralex/vol-10/index_en.htm

([2]) http://ec.europa.eu/enterprise/sectors/pharmaceuticals/documents/eudralex/vol-10/index_en.htm

([3]) http://eudract.ema.europa.eu/document.html

([4]) http://eudract.ema.europa.eu/document.html

([5]) EudraCT Helpdesk, e-mail: eudract@ema.europa.eu; Tel. +44 2075237523; Fax +44 2074188669.

([6]) EudraLex, Volume 10, Chapter V (http://ec.europa.eu/enterprise/sectors/pharmaceuticals/documents/eudralex/vol-10/index_en.htm).

([7]) http://ec.europa.eu/enterprise/sectors/pharmaceuticals/documents/eudralex/vol-10/index_en.htm

— a description of the plan for the provision of any additional care for the trial participants once their participation in the trial has ended, where it differs from what is normally expected according to the medical condition of the clinical trial participant.

44. The protocol should clearly address sub-studies conducted at all trial sites or only at specific sites.

45. The protocol should also contain the relevant information for the assessment of the clinical trial by the Ethics Committee. To this end, the protocol should include the following information:

— a discussion of the relevance of the clinical trial and its design to allow assessment in view of Article 6(3)(a) of Directive 2001/20/EC,

— an evaluation of the anticipated benefits and risks as required in Article 3(2)(a) of Directive 2001/20/EC (cf. Article 6(3)(b) of Directive 2001/20/EC),

— a justification for including participants who are incapable of giving informed consent or other special populations, such as minors (cf. Article 6(3)(g) of Directive 2001/20/EC), and

— a detailed description of the recruitment and informed consent procedure, especially when participants are incapable of giving informed consent (cf. Article 6(3)(k) of Directive 2001/20/EC).

46. More details are provided in the separate Commission guidance based on Article 8 of Directive 2001/20/EC.

47. A sponsor may wish to conduct a clinical trial with an active substance that is available in the European Union with different trade names in a number of medicines with marketing authorisations in the Member State concerned. This may be the case, for example, in order to address local clinical practice at each clinical trial site in the Member State concerned. In this case the protocol may define the treatment in terms of the active substance or Anatomical Therapeutic Chemical (ATC) code (level 3-5) only and not specify the trade name of each product.

48. With regard to notification of adverse events, the protocol

— may identify serious adverse events which do not require immediate reporting by the investigator (cf. Article 16(1) of Directive 2001/20/EC), and

— shall identify adverse events or laboratory anomalies critical to safety evaluations to be reported to the sponsor (cf. Article 16(2) of Directive 2001/20/EC).

49. In certain cases, issues of unblinding of IMPs might need to be addressed in the protocol. For details, reference is made to the guidelines on adverse reaction reporting published in Volume 10 of EudraLex — The Rules Governing Medicinal Products in the European Union [1].

50. Regarding first-in-human clinical trials, additional guidance is provided in the Guideline on strategies to identify and mitigate risks for first-in-human clinical trials with investigational medicinal products [2].

51. The protocol should be accompanied by a synopsis of the protocol.

52. The protocol should be signed by the sponsor and:

— the overall coordinating investigator for a multi-centre (incl. multinational) trial, or

— the principal investigator in a single-site trial.

2.6. **Investigator's brochure**

53. According to Article 2(g) of Directive 2001/20/EC, the investigator's brochure (IB) is 'a compilation of the clinical and non-clinical data on the investigational medicinal product or products which are relevant to the study of the product or products in human subjects.'

54. A request for trial authorisation has to be accompanied by an IB or a document used in place of the IB (see below). Its purpose is to provide the investigators and others involved in the trial with the information to facilitate their understanding of the rationale for, and their compliance with, key features of the protocol, such as the dose, dose frequency/interval, methods of administration, and safety monitoring procedures.

[1] http://ec.europa.eu/enterprise/sectors/pharmaceuticals/documents/eudralex/vol-10/index_en.htm

[2] EMEA/CHMP/SWP/28367/07 (see http://www.ema.europa.eu/pdfs/human/swp/2836707enfin.pdf).

55. The content, format and procedures for updating the IB have to comply with Article 8(1) of Commission Directive 2005/28/EC laying down principles and detailed guidelines for good clinical practice as regards investigational medicinal products for human use, as well as the requirements for authorisation of the manufacturing or importation of such products [1] (hereinafter Directive 2005/28/EC) and with the Community guideline on Good Clinical Practice (CPMP/ICH/135/95). It should be prepared from all available information and evidence that supports the rationale for the proposed clinical trial and the safe use of the IMP in the trial and be presented in the form of summaries.

56. The approved summary of product characteristics (SmPC) may be used in place of the IB if the IMP is authorised in any Member State or ICH country and is used according to the terms of the marketing authorisation. Regarding ICH countries, the document equivalent to the SmPC is used. If the conditions of use in the clinical trial differ from those authorised, the SmPC should be supplemented with a summary of relevant non-clinical and clinical data that support the use of the IMP in the clinical trial. Where the IMP is identified in the protocol only by its active substance, the sponsor should elect one SmPC as equivalent to the IB for all medicinal products that contain that active substance and are used at any clinical trial site.

57. For a multinational trial where the medicinal product to be used in each Member State is the one authorised at national level and the SmPC varies among Member States, the sponsor should chose one SmPC to replace the IB for the whole clinical trial. This SmPC should be the one best suited to ensure patient safety.

58. The IB as last amended and approved by the national competent authority or equivalent document (e.g. SmPC for marketed products) serves as the reference safety information for the assessment of the expectedness of any adverse reaction that might occur during the clinical trial.

2.7. **IMP dossier**

59. Article 2(d) of Directive 2001/20/EC defines an IMP as follows:

'A pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical trial, including products already with a marketing authorisation but used or assembled (formulated or packaged) in a way different from the authorised form, or when used for an unauthorised indication, or when used to gain further information about the authorised form.'

60. The IMP dossier (IMPD) gives information related to the quality of any IMP (i.e. including reference product and placebo), manufacture and control of the IMP, and data from non-clinical studies and from its clinical use. However, in many cases where the IMP has a marketing authorisation, an IMPD is not required. Reference is made to Section 2.7.1 (regarding compliance with Good Manufacturing Practice, GMP) and Section 2.7.3 (regarding data).

2.7.1. *GMP compliance*

61. As regards GMP compliance, in the following cases no documentation needs to be submitted:

— the IMP has a marketing authorisation in the EU or in an ICH country, is not modified, and is manufactured in the EU, or

— the IMP is not manufactured in the EU, but has a marketing authorisation in the EU, and is not modified.

62. If the IMP does not have a marketing authorisation in the EU or an ICH country and is not manufactured in the EU, the following documentation should be submitted:

— a copy of the importation authorisation as referred to in Article 13(1) of Directive 2001/20/EC, and

— a certification by the qualified person (QP) in the EU that the manufacturing complies with GMP at least equivalent to the GMP in the EU. Regarding this certification, there are specific arrangements provided for in the Mutual Recognition Agreements between the EU and third countries [2].

63. In all other cases, to document compliance with GMP as set out in Directive 2003/94/EC and the implementing detailed guideline for IMPs [3], the applicant should submit a copy of the manufacturing/importing authorisation as referred to in Article 13(1) of Directive 2001/20/EC stating the scope of the manufacturing/importation authorisation.

2.7.2. *Data related to the IMP*

2.7.2.1. Introductory remarks

64. Regarding data, the IMPD can be replaced by other documentation which may be submitted alone or with a simplified IMPD. The details for this 'simplified IMPD' are set out in Section 2.7.3.

[1] OJ L 91, 9.4.2005, p. 13.

[2] More information is available here: http://www.ema.europa.eu/Inspections/docs/000204en.pdf

[3] Annex 13 to Volume 4 of EudraLex — The Rules Governing Medicinal Products in the European Union (http://ec.europa.eu/enterprise/sectors/pharmaceuticals/documents/eudralex/vol-10/index_en.htm).

65. The IMPD should be prefaced with a detailed table of contents and a glossary of terms.

66. The information in the IMPD should be concise. The IMPD should not be unnecessarily voluminous. It is preferable to present data in tabular form accompanied by brief narrative highlighting the main salient points.

67. Regarding various specific types of IMPs, guidance is also given by the Agency, and made available in Volume 3 of EudraLex — The Rules Governing Medicinal Products in the European Union ([1]).

2.7.2.2. Quality data

68. Quality data should be submitted in a logical structure, such as the headings of the current version of the Guideline on the requirements to the chemical and pharmaceutical quality documentation concerning investigational medicinal products in clinical trials ([2]). This document also contains guidance for quality of placebos.

69. As regards biotechnological IMPs, reference is made to the Guideline on virus safety evaluation of biotechnological investigational medicinal products, as amended ([3]).

70. In exceptional cases, where impurities are not justified by the specification or when unexpected impurities (not covered by specification) are detected, the certificate of analysis for test products should be attached. Applicants should assess the need to submit a TSE Certificate.

2.7.2.3. Non-clinical pharmacology and toxicology data

71. The applicant should also provide summaries of non-clinical pharmacology and toxicology data for any IMP used in the clinical trial. He should also provide a reference list of studies conducted and appropriate literature references. Full data from the studies and copies of the references should be made available on request. Wherever appropriate it is preferable to present data in tabular form accompanied by a brief narrative highlighting the main salient points. The summaries of the studies conducted should allow an assessment of the adequacy of the study and whether the study has been conducted according to an acceptable protocol.

72. Non-clinical pharmacology and toxicology data should be submitted in a logical structure, such as the headings of the current version of Module 4 of the Common Technical Document ([4]), or of the eCTD format.

73. Reference is made to the specific Community guidelines contained in Volume 3 of EudraLex ([5]), and especially the Note for guidance on non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals, as amended (CPMP/ICH/286/95).

74. This section should provide a critical analysis of the data, including justification for omissions of data, and an assessment of the safety of the product in the context of the proposed clinical trial rather than a mere factual summary of the studies conducted.

75. The protocols should meet the requirements of Good Laboratory Practice (GLP) guidelines where appropriate. The applicant should provide a statement of the GLP status of all studies.

76. The test material used in the toxicity studies should be representative of that proposed for clinical trial use in terms of qualitative and quantitative impurity profiles. The preparation of the test material should be subject to the controls necessary to ensure this and thus support the validity of the study.

2.7.2.4. Previous clinical trial and human experience data

77. Clinical trial and human experience data should be submitted in a logical structure, such as the headings of the current version of Module 5 of the Common Technical Document ([6]), or of the eCTD format.

78. This section should provide summaries of all available data from previous clinical trials and human experience with the proposed IMPs.

79. All studies should have been conducted in accordance with the principles of Good Clinical Practice (GCP). To this end, the applicant should submit the following:

— a statement of the GCP compliance of the clinical trials referred to,

([1]) http://ec.europa.eu/enterprise/sectors/pharmaceuticals/documents/eudralex/vol-10/index_en.htm
([2]) CHMP/QWP/185401/2004 final (http://ec.europa.eu/enterprise/sectors/pharmaceuticals/documents/eudralex/vol-10/index_en.htm).
([3]) Ref. EMEA/CHMP/BWP/398498/2005 (http://www.ema.europa.eu/pdfs/human/bwp/39849805enfin.pdf).
([4]) http://ec.europa.eu/enterprise/sectors/pharmaceuticals/files/eudralex/vol-2/b/update_200805/ctd_05-2008_en.pdf
([5]) http://ec.europa.eu/enterprise/sectors/pharmaceuticals/documents/eudralex/vol-3/index_en.htm
([6]) http://ec.europa.eu/enterprise/sectors/pharmaceuticals/files/eudralex/vol-2/b/update_200805/ctd_05-2008_en.pdf

— where a clinical trial referred to has been performed in third countries, a reference to the entry of this clinical trial in a public register, if available. Where a clinical trial is not published in a register, this should be explained and justified.

80. There are no specific requirements for data from clinical studies that must be provided before a clinical trial authorisation can be granted. Rather, this is to be assessed on a case-by-case basis. In this respect, guidance is provided in the guideline General considerations for clinical trials (CPMP/ICH/291/95) [1].

2.7.2.5. Overall risk and benefit assessment

81. This section should provide a brief integrated summary that critically analyses the non-clinical and clinical data in relation to the potential risks and benefits of the proposed trial unless this information is already provided in the protocol. In the latter case, the applicant should cross-refer to the relevant section in the protocol. The text should identify any studies that were terminated prematurely and discuss the reasons. Any evaluation of foreseeable risks and anticipated benefits for studies on minors or incapacitated adults should take account of the provisions set out in Articles 3 to 5 of Directive 2001/20/EC.

82. Where appropriate, the sponsor should discuss safety margins in terms of relative systemic exposure to the IMP, preferably based on area under the curve (AUC) data, or peak concentration (C_{max}) data, whichever is considered more relevant, rather than in terms of applied dose. The sponsor should also discuss the clinical relevance of any findings in the non-clinical and clinical studies along with any recommendations for further monitoring of effects and safety in the clinical trials.

2.7.3. *Simplified IMPD by referring to other documentation*

83. The applicant has the possibility to refer to other documentation which may be submitted alone or with a simplified IMPD to contain the information as set out in Table 1.

2.7.3.1. Possibility to refer to the IB

84. The applicant may either provide a stand-alone IMPD or cross-refer to the IB for the preclinical and clinical parts of the IMPD. In the latter case, the summaries of pre-clinical information and clinical information should include data, preferably in tables, providing sufficient detail to allow assessors to reach a decision about the potential toxicity of the IMP and the safety of its use in the proposed trial. If there is some special aspect of the preclinical data or clinical data that requires a detailed expert explanation or discussion beyond what would usually be included in the IB, the applicant should submit the preclinical and clinical information as part of the IMPD.

2.7.3.2. Possibility to refer to the SmPC or to the assessment of the IMPD in another clinical trials application

85. The applicant may submit the current version of the SmPC (or, as regards ICH countries, the documentation equivalent to the SmPC) as the IMPD if an IMP has a marketing authorisation in any Member State or in an ICH country. The exact requirements are detailed in Table 1.

86. Moreover, the IMPD may have been submitted previously by the same applicant or by another applicant and held by the national competent authority of the Member State concerned. In these cases applicants are allowed to cross-refer to the previous submission. If the submission was made by another applicant, a letter from that applicant should be submitted authorising the national competent authority to cross-refer to that data. The exact requirements are detailed in Table 1.

87. *Table 1*

Content of simplified IMPD

Types of previous assessment	Quality data	Non-clinical data	Clinical data
The IMP has an MA in any EU Member State or ICH country and is used in the trial:			
— within the conditions of the SmPC	SmPC		
— outside the conditions of the SmPC	SmPC	If appropriate	If appropriate
— after modification (e.g. blinding)	P+A	SmPC	SmPC

[1] http://www.ema.europa.eu/htms/human/ich/ichefficacy.htm

Types of previous assessment	Quality data	Non-clinical data	Clinical data
Another pharmaceutical form or strength of the IMP has an MA in any EU Member State or ICH country and the IMP is supplied by the MA holder	SmPC+P+A	Yes	Yes
The IMP has no MA in any EU Member State or ICH country but the active substance is part of a medicinal product with an MA in an EU Member State and			
— is supplied by the same manufacturer	SmPC+P+A	Yes	Yes
— is supplied by another manufacturer	SmPC+S+P+A	Yes	Yes
The IMP was subject to a previous CTA and authorised in the Member State concerned (1) and has not been modified and			
— no new data is available since last amendment to the CTA	Reference to previous submission		
— new data is available since last amendment to the CTA	New data	New data	New data
— is used under different conditions	If appropriate	If appropriate	If appropriate

(S: Data relating to the active substance; P: Data relating to the IMP; A: Appendices to the current version of the Guideline on the requirements to the chemical and pharmaceutical quality documentation concerning investigational medicinal products in clinical trials (2).)

(1) The sponsor should provide a letter of authorisation to cross-refer to the data submitted by another applicant.
(2) CHMP/QWP/185401/2004 final (http://ec.europa.eu/enterprise/sectors/pharmaceuticals/documents/eudralex/vol-10/index_en.htm).

88. If the applicant is the MA holder and he has submitted an application to vary the SmPC, which has not yet been authorised, and which is relevant for the assessment of the IMPD in terms of patient safety, the nature of the variation and the reason for it should be explained.

89. If the IMP is defined in the protocol in terms of active substance or ATC code (see above, Section 2.5), the applicant may replace the IMPD by one representative SmPC for each active substance/active substance pertaining to that ATC group. Alternatively, he may provide a collated document containing information equivalent to that in the representative SmPCs for each active substance that could be used as an IMP in the clinical trial.

2.7.4. *IMPD in cases of placebo*

90. If the IMP is a placebo, the information requirements can be reduced in line with the requirements set out in Table 2.

91. *Table 2*

IMPD in cases of placebo

IMPD in for placebo	Quality data	Non-clinical data	Clinical data
The IMP is a placebo	P+A	No	No
The IMP is a placebo and the placebo has the same composition as the tested IMP, is manufactured by the same manufacturer, and is not sterile	No	No	No

IMPD in for placebo	Quality data	Non-clinical data	Clinical data
The IMP is a placebo and has been submitted in a previous CTA in the Member State concerned	No	No	No

(S: Data relating to the active substance; P: Data relating to the IMP; A: Appendices to the current version of the Guideline on the requirements to the chemical and pharmaceutical quality documentation concerning investigational medicinal products in clinical trials [1].)

[1] CHMP/QWP/185401/2004 final (http://ec.europa.eu/enterprise/sectors/pharmaceuticals/documents/eudralex/vol-10/index_en.htm).

2.8. **Non-investigational medicinal products used in the trial**

92. Medicinal products used in the context of a clinical trial and not falling within the definition of an IMP are non-investigational medicinal products (NIMPs). The 'borderline' between IMPs and NIMPs is described in the Guidance on Investigational Medicinal Products (IMPs) and other medicinal products used in Clinical Trials [1].

93. It is strongly recommended that NIMPs with marketing authorisation in the Member State concerned are used. When this is not possible, the next choice should be NIMPs with marketing authorisation in another Member State. When this is not possible, the next choice should be NIMPs with marketing authorisation in an ICH country or a third country having a mutual recognition agreement with the EU (MRA country) [2]. When this is not possible, the next choice should be NIMPs with a marketing authorisation in another third country. Otherwise, a NIMP with no marketing authorisation may be used.

94. For the requirements of the NIMP dossier, reference is made to the applicable guideline published in EudraLex — The Rules Governing Medicinal Products in the European Union, Volume 10 [3].

2.9. **Other documents to be submitted, Overview**

95. The following additional documents should be contained in the application dossier submitted to the national competent authority of the Member State concerned:

1. A copy of the opinion of the Ethics Committee of the Member State concerned, whether the application has been submitted in parallel or in sequence, as soon as it is available, unless the Ethics Committee informs the applicant that it has copied its opinion to the national competent authority of the Member State concerned. A submission of this document subsequently to the submission of a request for authorisation is not to be considered as a change of the documentation as referred to in Section 2.1.4.2.

2. If available, a copy of the summary of scientific advice from any Member State or the Agency with regard to the clinical trial. A submission of this document subsequently to the submission of a request for authorisation is not to be considered as a change of the documentation as referred to in Section 2.1.4.2.

3. If the clinical trial is part of an agreed PIP, a copy of the Agency's Decision on the agreement on the PIP, and the opinion of the Paediatric Committee, unless these documents are fully accessible via the internet. In the latter case, the link to this documentation in the cover letter is sufficient (see Section 2.3). A submission of this document subsequently to the submission of a request for authorisation is not to be considered as a change of the documentation as referred to in Section 2.1.4.2.

4. The content of the labelling of the IMP.

5. In case of fees, proof of payment.

96. Table 3 contains the final overview of the documentation to be submitted.

Table 3

List of documentation to be provided to the national competent authority of the Member State concerned in accordance with this detailed guidance

— Cover letter with the contents set out in Section 2.3,
— Clinical trial application form,
— Protocol with the contents set out in Section 2.5,
— IB, or document replacing the IB, as set out in Section 2.6,
— IMPD/simplified IMPD, as set out in Sections 2.7 and 2.7.3,
— NIMP dossier as set out in Section 2.8,
— The additional pieces of documentation as set out in Section 2.9.

2.10. **Additional national requirements for documents**

97. The national requirements for the content of the clinical trial application dossier can be more comprehensive than the list of documentation set out in Section 2.9 in the following two cases:

[1] cf. http://ec.europa.eu/enterprise/sectors/pharmaceuticals/documents/eudralex/vol-10/index_en.htm

[2] These third countries are Australia, Canada, Japan, New Zealand and Switzerland.

[3] cf. http://ec.europa.eu/enterprise/sectors/pharmaceuticals/documents/eudralex/vol-10/index_en.htm

2.10.1. *Documents relating to information relevant for Ethics Committees but exceptionally considered by national competent authorities in accordance with Article 6(4) of Directive 2001/20/EC*

98. Documents relating to information which is, according to Article 6(2) of the Directive 2001/20/EC, only assessed by the Ethics Committee should not be submitted to the national competent authority of the Member State concerned.

99. However, if a Member State has decided, in accordance with Article 6(4) of Directive 2001/20/EC, that its national competent authority is responsible for considering:

— the provisions for indemnity or compensation,

— insurance or indemnity to cover the liability of the investigator/sponsor,

— compensation and rewards of investigators and clinical trial participants, or

— the agreement between the sponsor and the clinical trial sites.

The relevant documentation should be submitted to the national competent authority of this Member State.

100. Member States who decide to extend the scope of assessment of the national competent authority are under an obligation to notify the Commission, the other Member States, and the Agency of this. Those Member States are listed on the 'clinical trials website' of the European Commission [1].

2.10.2. *Documents relating to information on a more comprehensive protection of the clinical trial participant in accordance with Article 3(1) of Directive 2001/20/EC*

101. Some Member States may have national provisions on the protection of clinical trial subjects in place which are more comprehensive than the provisions of the Directive 2001/20/EC (cf. Article 3(1) of Directive 2001/20/EC).

102. In order for the national competent authority to assess compliance with these national provisions (hereinafter referred to as 'underlying national provisions'), Member States may require additional information in the clinical trial application dossier.

103. However, Member States may only request this additional information if the underlying national provision is compliant with Directive 2001/20/EC. This requires in particular, that the underlying national provision:

— is clearly aimed at a more comprehensive protection of the clinical trial subject than the provisions of Directive 2001/20/EC,

— is appropriate and proportionate in view of the aim pursued,

— is consistent with the procedures set out in Directive 2001/20/EC, and

— is consistent with the timescales set out in Directive 2001/20/EC.

104. The Commission is going to ensure compliance of underlying national provisions with these requirements.

3. NOTIFICATION OF AMENDMENTS AND RELATED MEASURES

3.1. Legal basis and scope

105. Article 10(a) of Directive 2001/20/EC reads as follows:

'After the commencement of the clinical trial, the sponsor may make amendments to the protocol. If those amendments are substantial and are likely to have an impact on the safety of the trial subjects or to change the interpretation of the scientific documents in support of the conduct of the trial, or if they are otherwise significant, the sponsor shall notify the competent authorities of the Member State or Member States concerned of the reasons for, and content of, these amendments and shall inform the ethics committee or committees concerned in accordance with Articles 6 (Ethics Committee) and 9 (Commencement of clinical trial).'

106. In view of the identical legal consequences of an amendment that is 'substantial and likely to have an impact on the safety of the trial subjects or to change the interpretation of the scientific documents in support of the conduct of the trial' and an amendment that is 'otherwise significant', the term 'substantial amendment' used in this guidance refers to both types of amendments.

[1] http://ec.europa.eu/enterprise/sectors/pharmaceuticals/human-use/clinical-trials/index_en.htm

107. Notification/submission of information [1] is only obligatory if the amendment is a substantial amendment. Directive 2001/20/EC does not require notification, nor immediate submission of information of non-substantial amendments. Neither national competent authorities of the Member State concerned, nor its Ethics Committee can oblige the sponsor to submit non-substantial amendments. In this regard, the rules for non-substantial amendments (cf. Section 3.6) apply.

3.2. **The notion of 'amendment'**

108. The following changes do not count as an 'amendment' as referred to in Article 10(a) of Directive 2001/20/EC:

— a change to the documentation submitted to the national competent authority during the ongoing assessment of the request for authorisation by the national competent authority (for these aspects see Section 2.1.4.2), and

— a change to the documentation submitted to the Ethics Committee during the ongoing assessment of the request for authorisation by the Ethics Committee.

109. Article 10(a) of Directive 2001/20/EC refers only to amendments to the approved protocol. This is to be understood as encompassing all documentation submitted in the context of the approved protocol.

110. The annual safety report (ASR) in accordance with Article 17(2) of Directive 2001/20/EC is not per se an amendment and thus does not have to be notified as a substantial amendment to the national competent authority of the Member State concerned. However, the sponsor has to verify whether the data presented in the ASR requires a change to the documentation submitted with the request for authorisation of a clinical trial. If this amendment is substantial, the rules for notification of substantial amendments apply to these changes.

111. A change of the contact person or in the contact details of the contact person (e.g. a change of e-mail or postal address) is not considered as an amendment, if the sponsor and legal representative remain identical. However, the sponsor should ensure that the national competent authority of the Member State concerned is aware of this change as soon as possible, in order to allow the national competent authority to exercise its supervisory function.

3.3. **The notion of 'substantial'**

112. Amendments to the trial are regarded as 'substantial' where they are likely to have a significant impact on:

— the safety or physical or mental integrity of the clinical trial participants, or

— the scientific value of the trial.

113. In all cases, an amendment is only to be regarded as 'substantial' when one or both of the above criteria are met.

114. It is up to the sponsor to assess whether an amendment is to be regarded as 'substantial'. This assessment is to be made on a case-by-case basis in view of the above criteria. While the responsibility for this assessment lies with the sponsor, in cases where the sponsor consults the national competent authority advice should be given without delay and free of charge.

115. In applying these criteria, however, care has to be taken to avoid over-reporting. In particular, not every change to the clinical trial application form is by default to be considered as a 'substantial' amendment.

116. The annual update of the IB in accordance with Article 8 of Directive 2005/28/EC is not per se a substantial amendment. However, the sponsor has to verify whether the update relates to changes which are to be considered as substantial. In that case, the rules for notification of substantial amendments apply to the change.

117. The sponsor should assess also whether the combination of substantial amendments lead to changes of the clinical trial to an extent that it has to be considered as a completely new clinical trial, which would then be subject to a new authorisation procedure.

3.4. **Examples**

118. In view of these criteria the following examples should serve as guidance for the case-by-case decision of the sponsor. These examples relate only to the aspects assessed by the national competent authority of the Member State concerned. For aspects considered by the Ethics Committee, reference is made to the Commission guidance based on Article 8 of Directive 2001/20/EC.

3.4.1. *Amendments as regards the clinical trials protocol*

119. With regard to the protocol, the following is a non-exhaustive list of amendments that are typically 'substantial':

(a) change of main objective of the clinical trial;

[1] Directive 2001/20/EC distinguishes between notification of the national competent authority and information of the Ethics Committee. For the purposes of this guidance, both submissions will be referred to as 'notification'.

(b) change of primary or secondary endpoint which is likely to have a significant impact on the safety or scientific value of the clinical trial;

(c) use of a new measurement for the primary endpoint;

(d) new toxicological or pharmacological data or new interpretation of toxicological or pharmacological data which is likely to impact on the risk/benefit assessment;

(e) a change in the definition of the end of the trial, even if the trial has in practice already ended;

(f) addition of a trial arm or placebo group;

(g) change of inclusion or exclusion criteria, such as changes to age range, if these changes are likely to have a significant impact on the safety or scientific value of the clinical trial;

(h) reducing the number of monitoring visits;

(i) change of a diagnostic or medical monitoring procedure which is likely to have a significant impact on the safety or scientific value of the clinical trial;

(j) withdrawal of an independent data monitoring board;

(k) change of IMPs;

(l) change of dosing of IMPs;

(m) change of mode of administration of IMPs;

(n) a change of study design which is likely to have a significant impact on primary or major secondary statistical analysis or the risk/benefit assessment.

120. With regard to the protocol, the following is a non-exhaustive list of amendments that are typically not 'substantial':

(a) changes to the identification of the trial (e.g. change of title, etc.);

(b) the addition/deletion of exploratory/tertiary endpoints;

(c) a minor increase in the duration of the trial (< 10 % of the overall time of the trial);

(d) an increase in duration of > 10 % of the overall time of the trial, provided that:

— the exposure to treatment with the IMP is not extended,

— the definition of the end of the trial is unchanged, and

— monitoring arrangements are unchanged;

(e) a change in the number of clinical trial participants per trial site, if the total number of participants in the Member State concerned is identical or the increase/decrease is insignificant in view of the absolute number of participants;

(f) a change in the number of clinical trial participants in the Member State concerned, if the total number of participants is identical or the increase/decrease is insignificant in view of the absolute number of participants;

(g) a change in the documentation used by the research team for recording study data (e.g. case report form or data collection form);

(h) additional safety monitoring which is not part of an urgent safety measure but is taken on a precautionary basis;

(i) minor clarifications to the protocol;

(j) correction of typographical errors.

3.4.2. *Amendments as regards the IMPD*

121. With regard to changes in the IMPD, guidance is contained in Chapter 8 of the Guideline on the requirements to the chemical and pharmaceutical quality documentation concerning investigational medicinal products in clinical trials [1].

3.4.3. *Amendments as regards the IB*

122. With regard to the IB, the following is a non-exhaustive list of amendments that are typically 'substantial':

[1] CHMP/QWP/185401/2004 final (http://ec.europa.eu/enterprise/sectors/pharmaceuticals/documents/eudralex/vol-10/index_en.htm).

(a) new toxicological or pharmacological data or new interpretation of toxicological or pharmacological data of relevance for the investigator;

(b) changes to the reference safety information for the annual safety report.

3.4.4. *Amendments as regards other initial documents supporting the request for authorisation of the clinical trial*

123. With regard to other initial documents, the following is a non-exhaustive list of amendments that are typically 'substantial':

(a) a change of sponsor or the sponsor's legal representative;

(b) the revocation or suspension of the IMP's marketing authorisation.

124. With regard to other initial documents, the following is a list of amendments that are typically *not* 'substantial':

(a) any change of persons other than the sponsor or his legal representative, for example applicant, clinical research associates (CRAs) who monitor the clinical trial for the investigator, and clinical research organisations (CROs) (note that the responsibility vis-à-vis the national competent authority for the clinical trial is always with the sponsor or his legal representative);

(b) any change in the contact details of persons referred to in the documentation (see, however, Section 3.2 as regards contact details of the contact person);

(c) changes to the internal organisation of the sponsor or of the persons to whom certain tasks have been delegated;

(d) changes in the logistical arrangements for storing/transporting samples;

(e) change of technical equipment;

(f) addition or deletion per se of another Member State or third country concerned.

3.5. **Who should be notified?**

125. Substantial amendments may relate to information relevant for assessment by the national competent authority, the Ethics Committee, or both.

126. For substantial amendments to information that is assessed only by the national competent authority of the Member State concerned, the sponsor should only notify the amendment to the national competent authority.

127. For substantial amendments to information that is assessed, according to Directive 2001/20/EC, only by the Ethics Committee of the Member State concerned, the sponsor should only notify the amendment to the Ethics Committee. This is in particular of relevance for the information relating to:

— the clinical trial site (Article 6(3)(f) of Directive 2001/20/EC),

— the written information to be given to the clinical trial participant in order to obtain informed consent (Article 6(3)(g) of Directive 2001/20/EC), and

— the investigator (Article 6(3)(d) of Directive 2001/20/EC).

128. These aspects are addressed in the separate Commission guidance based on Article 8 of Directive 2001/20/EC.

129. In the case of substantial amendments that affect information assessed by both the national competent authority and the Ethics Committee of the Member State concerned, the sponsor should submit the notifications in parallel.

130. There is no need to notify 'for information only' substantial amendments to one body (national competent authority or Ethics Committee), if this information is assessed by the respective other body.

131. In practice, it is necessary that the national competent authority and the Ethics Committee in the Member State concerned communicate with each other in order to ensure the exchange of expertise or information. This may be in particular relevant, for example, for:

— assessing scientific information requiring specific expertise,

— ensuring effective inspections of clinical trials sites, and

— updating relevant information in EudraCT.

3.6. **Non-substantial amendments**

132. The sponsor does not have to notify non-substantial amendments to the national competent authority or the Ethics Committee. However, non-substantial amendments should be recorded and contained in the documentation when it is subsequently submitted, for example in the subsequent notification of a substantial amendment. This is of particular relevance for the Clinical Trial Application Form: This form should be updated in its entirety at the occasion of a substantial amendment. Documentation of non-substantial amendments should also be available on request for inspection at the trial site or the sponsor premises as appropriate.

3.7. **Format and content of notification**

133. The notification of a substantial amendment should include the following:

(a) a signed cover letter, including:

— in its subject line the EudraCT number and the sponsor protocol number (if available) with the title of the trial and the sponsor's amendment code number allowing unique identification of the substantial amendment. Care should be taken to use the code number consistently;

— identification of the applicant;

— identification of the amendment (sponsor's substantial amendment code number [1] and date). One amendment could refer to several changes in the protocol or scientific supporting documents;

— a highlighted indication of any special issues related to the amendment and indication where the relevant information or text is in the original application dossier;

— identification of any information not contained in the Amendment Notification Form that might impact on the risk to trial participants;

— where applicable, a list of all affected clinical trials with EudraCT numbers and respective amendment code numbers (see above);

(b) the Amendment Notification Form, as amended, which is published in Volume 10 of EudraLex — The Rules Governing Medicinal Products in the European Union [2]. Only this Amendment Notification Form should be used;

(c) a description of the amendment:

— an extract from the amended documents showing previous and new wording in track changes, as well as the extract only showing the new wording;

— notwithstanding the previous point, if the changes are so widespread or far-reaching that they justify an entire new version of the document, a new version of the entire document. In this case, an additional table should list the amendments to the documents. In this list, identical changes can be grouped.

The new version should be identified with the date and an updated version number.

(d) supporting information including, where applicable:

— summaries of data,

— an updated overall risk/benefit assessment,

— possible consequences for subjects already included in the trial,

— possible consequences for the evaluation of the results;

(e) if a substantial amendment involves changes to entries on the clinical trial application form, a revised copy of the XML file incorporating amended data. If the form is not submitted via a telematics system, the fields affected by the substantial amendment should be highlighted in the revised form [3].

134. Where a substantial amendment affects more than one clinical trial of the same sponsor and the same IMP, the sponsor may make a single notification to the national competent authority/Ethics Committee of the Member State concerned. The cover letter and the notification should contain a list of all clinical trials affected with their EudraCT numbers and respective amendment code numbers. If the substantial amendment involves changes to several clinical trial application forms, all forms should be updated (see Section 3.7).

[1] The code number identifies the amendment and refers to all the documents submitted. The sponsor decides which code to be used. Section E1 of the amendment form should be completed with the date and version of the new amendment to which this form relates.

[2] http://ec.europa.eu/enterprise/sectors/pharmaceuticals/documents/eudralex/vol-10/index_en.htm

[3] Section A4 of the CTA form should contain the version and date of the protocol originally authorised and this should not be changed when the protocol is later amended. Section B4 of the amendment form should contain the version and date of the currently authorised protocol. Note that Section H of the CTA form does not need to be changed, as it concerns the status of the CTA application to the Ethics Committee at the time of the CTA submission to the CA.

3.8. **Time for response, implementation**

135. Article 10(a), second and third subparagraph, of Directive 2001/20/EC reads as follows:

> 'On the basis of the details referred to in Article 6(3) and in accordance with Article 7, the Ethics Committee shall give an opinion within a maximum of 35 days of the date of receipt of the proposed amendment in good and due form. If this opinion is unfavourable, the sponsor may not implement the amendment to the protocol.
>
> If the opinion of the Ethics Committee is favourable and the competent authorities of the Member States have raised no grounds for non-acceptance of the ... substantial amendments, the sponsor shall proceed to conduct the clinical trial following the amended protocol. Should this not be the case, the sponsor shall either take account of the grounds for non-acceptance and adapt the proposed amendment to the protocol accordingly or withdraw the proposed amendment.'

136. Accordingly, the Ethics Committee has to give within 35 calendar days an opinion on a valid submission of a proposed substantial amendment. If a submission is not considered as valid by the Ethics Committee, the Ethics Committee should inform the applicant of this within the first 10 calendar days of this 35-day period. The reasons should be given.

137. With regard to the national competent authority, no deadline is set in Directive 2001/20/EC., and in view of the approval time for requests for authorisation, the national competent authority are invited to respond within 35 calendar days of receipt of the valid notification of an amendment. Validation of the submission is included in this period. If a submission is not valid (for example, the dossier does not contain the documentation required according to this guidance), the national competent authority are invited to inform the applicant of this within the first 10 calendar days of this 35-day period. The reasons should be given. This response time may be extended if such extension is justified in view of the nature of the substantial amendment, for example if the national competent authority has to consult an expert group or committee. In such cases, the national competent authority should notify the sponsor of the duration of the extension and its reasons. If the national competent authority states that it raises no grounds for non-acceptance, the sponsor can implement the changes, even if fewer than 35 days have elapsed since the filing of the substantial amendment.

138. For amendments submitted to either the Ethics Committee alone or to the national competent authority alone, the sponsor may implement the amendment when the Ethics Committee opinion is favourable or the competent national authority has raised no grounds for non-acceptance.

139. Up until then, the trial can continue on the basis of the original documentation, unless the rules for urgent safety measures apply.

140. Applicants should be aware that these procedures are intended to ensure rapid and efficient processing of substantial amendments. Against this background, unsatisfactory documentation is likely to lead to non-acceptance of the substantial amendment. Non-acceptance does not prejudice the applicant's right to resubmission.

141. Upon approval, it is the sponsor's responsibility to ensure communication of the changes to the investigators.

3.9. **Notification of urgent safety measures**

142. Article 10(b) of Directive 2001/20/EC reads as follows:

> 'Without prejudice to point (a), in the light of the circumstances, notably the occurrence of any new event relating to the conduct of the trial or the development of the investigational medicinal product where the new event is likely to affect the safety of the subjects, the sponsor and the investigator shall take appropriate urgent safety measures to protect the subjects against any immediate hazard. The sponsor shall forthwith inform the competent authorities of those new events and the measures taken and shall ensure that the Ethics Committee is notified at the same time.'

143. Examples of urgent safety measures are if, for reasons of safety of the clinical trial participants, a trial is temporarily halted (see Section 3.10) or additional monitoring measures are set up.

144. Urgent safety measures may be taken without prior notification to the national competent authority. However, the sponsor must inform *ex post* the national competent authority and the Ethics Committee of the Member State concerned of the new events, the measures taken and the plan for further action as soon as possible. Where the initial contact is by telephone, this should be followed up, for reasons of traceability, by fax or e-mail. It should be followed by a written report.

145. The *ex post* notification of urgent safety measures is independent of the obligation to:

— notify substantial amendments (see above),

— notify early termination of the trial within 15 days in accordance with Article 10(c) of Directive 2001/20/EC (see below, Section 4.2.2), and

— notify adverse events and serious adverse reactions in accordance with Articles 16 and 17 of Directive 2001/20/EC.

3.10. **Temporary halt of a trial**

146. A temporary halt of a trial is a stoppage of the trial which is not envisaged in the approved protocol and where there is an intention to resume it.

147. A temporary halt can be:

— a substantial amendment, or

— part of an urgent safety measure as referred to in Article 10(b) of Directive 2001/20/EC. In this case, the notification of the temporary halt of a trial should be made immediately and, at the latest, in accordance with the deadline set out in Article 10(c), second sentence, of Directive 2001/20/EC, within 15 days from when the trial is temporarily halted.

148. The reasons and scope, e.g. stopping recruitment or interrupting treatment of subjects already included, should be clearly explained in the notification (in case of substantial amendment, see Section 3.7) or in the *ex post* information (in case of urgent safety measures, see Section 3.9).

149. The restart of the trial should be treated as a substantial amendment providing evidence that it is safe to restart the trial.

150. If the sponsor decides not to recommence a temporarily halted trial he should notify the national competent authority of the Member States concerned within 15 days of his decision in accordance with Article 10(c), second sentence, of Directive 2001/20/EC (see Section 4.2).

3.11. **Suspension/prohibition of a clinical trial by the national competent authority in case of doubts about safety or scientific validity**

151. Article 12(1) of Directive 2001/20/EC reads as follows:

'Where a Member State has objective grounds for considering that the conditions in the request for authorisation referred to in Article 9(2) are no longer met or has information raising doubts about the safety or scientific validity of the clinical trial, it may suspend or prohibit the clinical trial and shall notify the sponsor thereof.

Before the Member State reaches its decision it shall, except where there is imminent risk, ask the sponsor and/or the investigator for their opinion, to be delivered within one week.

In this case, the competent authority concerned shall forthwith inform the other competent authorities, the Ethics Committee concerned, the Agency and the Commission of its decision to suspend or prohibit the trial and of the reasons for the decision.'

152. If the trial is terminated following a suspension, the rules on end of trial notification apply (see below, Section 4.2).

3.12. **Non-compliance with the applicable rules on clinical trials**

153. Article 12(2) of Directive 2001/20/EC reads as follows:

'Where a competent authority has objective grounds for considering that the sponsor or the investigator or any other person involved in the conduct of the trial no longer meets the obligations laid down, it shall forthwith inform him thereof, indicating the course of action which he must take to remedy this state of affairs. The competent authority concerned shall forthwith inform the Ethics Committee, the other competent authorities and the Commission of this course of action.'

154. The 'course of action' of the national competent authority should have a timetable for its implementation and a date when the sponsor should report back to the national competent authority on the progress and completion of its implementation.

155. The sponsor should ensure that the 'course of action' set by the national competent authority is immediately implemented and report to the national competent authority of the Member State concerned on the progress in and completion of its implementation in accordance with the timetable set.

156. The national competent authority must inform the other national competent authorities, the Ethics Committee of the Member State concerned and the Commission of the 'course of action'.

4. DECLARATION OF THE END OF A CLINICAL TRIAL

4.1. Legal basis and scope

157. Article 10(c) of Directive 2001/20/EC reads as follows:

'Within 90 days of the end of a clinical trial the sponsor shall notify the competent authorities of the Member State or Member States concerned and the Ethics Committee that the clinical trial has ended. If the trial has to be terminated early, this period shall be reduced to 15 days and the reasons clearly explained.'

158. 'End of the trial' is not defined in Directive 2001/20/EC. The definition of the end of the trial should be provided in the protocol (for guidance, see Section 2.5). For changes to the definition see under Section 3.4.1.

4.2. Procedure for declaring the end of the trial

4.2.1. *General rules*

159. The sponsor has to make an end of trial declaration when the complete trial has ended in all Member States/third countries concerned. The end of the clinical trial is defined in the protocol (see Section 4.1).

160. This declaration has to be made to the national competent authority and the Ethics Committee of all Member States concerned within 90 days of the end of the clinical trial. To this end, the form published in Volume 10 of EudraLex — The Rules Governing Medicinal Products in the European Union [1] should be used.

161. The notified Member States are responsible for entering this information into the EudraCT database.

4.2.2. *Shortened deadline for early termination*

162. An earlier end of the clinical trial which is not based on grounds of safety, but on other grounds, such as faster recruitment than anticipated, is not considered as 'early termination'.

163. In the case of early termination, the sponsor must notify the end of the trial to the national competent authority and the Ethics Committee of the Member State concerned immediately and at the latest within 15 days after the trial is halted, clearly explain the reasons, and describe follow-up measures, if any, taken for safety reasons.

4.3. Clinical trial summary report

164. The clinical trial summary report is part of the end of trial notification, albeit usually submitted only subsequently to the end of trial notification. The sponsor should provide this summary report within one year of the end of the complete trial for non-paediatric clinical trials. For paediatric clinical trials, the timelines are set out in the Commission Communication 2009/C28/01. Regarding the arrangements for submitting the clinical trial summary report, its format, content, and its accessibility for the public, reference is made to the Commission Communications 2009/C28/01 and 2008/C168/02 and their implementing technical guidance documents [2].

[1] http://ec.europa.eu/enterprise/sectors/pharmaceuticals/documents/eudralex/vol-10/index_en.htm

[2] http://ec.europa.eu/enterprise/sectors/pharmaceuticals/documents/eudralex/vol-10/index_en.htm

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Appeal number: **T2963/19-3.3.07**

Communication of the Board of Appeal pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal

The Rapporteur M. Steendijk
The Registrar B. Atienza Vivancos
Tel.: 089 / 2399 - 3371

Registered letter

This document: 21 page(s) including this page

Annex(es):

Communication text

I. This communication, which is sent pursuant to Article 15(1) RPBA 2020 (OJ EPO 2019, A63), serves to prepare the oral proceedings and presents the Board's provisional and non-binding opinion. The communication

is not intended to provide an exhaustive representation of all the issues that may be dealt with at the oral proceedings. Its purpose is to express the concerns which the Board presently has in relation to the arguments or requests presented.

Document numbering

II. The Board adheres to the numbering of documents D1-D22 in the decision under appeal.

First-instance proceedings

III. The grant of European patent 2 861 210 (hereinafter: the patent) was opposed on the grounds that its subject-matter lacked inventive step and that the claimed invention was not sufficiently disclosed. The appeal filed by the patent proprietor (hereinafter: appellant) lies against the decision of the opposition division posted on 28 August 2019 to revoke the patent.

The decision was based on

- the main request, originally filed on 28 June 2019 as auxiliary request 2,

- auxiliary request 1 as filed on 28 June 2019,

- auxiliary request 2, originally filed on 24 August 2018 as main request, and

- auxiliary request 3 as filed on 28 June 2019.

IV. According to the decision under appeal:

(a) Claim 1 of the main request related to a particular dosage regimen involving administration of the triple combination of defined amounts of liposomal

irinotecan in the form of irinotecan sucrose octasulfate salt liposome injection with 5-fluorouracil (5-FU) and leucovorin (l-form) in at least one cycle of two weeks for treatment of pancreatic cancer in a human patient who has failed prior treatment with or become resistant to gemcitabine. The amendments according to the main request met the requirements of Rule 80, 123(2) and 123(3) EPC.

(b) The patent sufficiently disclosed the claimed invention as defined in accordance with the main request. The suitability of the defined treatment regimen for the defined indication was plausible in view of examples 6 and 7 of the patent and having regard to the known effectiveness of similar combination treatment involving non-liposomal irinotecan, the so-called FOLFIRI regimen, as mentioned in the the patent and reported in documents D2, D3, D5 and D6.

(c) The priority document of 13 June 2012 (hereinafter: "PD1") described the administration of leucovorin at a dose of 200 mg/m2 without specifying whether the leucovorin was in the l-form or in the racemic form. The subject-matter as defined in accordance with the main request was therefore not entitled to this priority. Accordingly, documents D4, D8, D10 and D15b represented relevant prior art.

(d) Document D15b represented the closest prior art as it related to the same purpose as the patent and required the least modifications to arrive at the claimed subject-matter. Document D15b described a protocol for a Phase 3 clinical study involving liposomal irinotecan (MM-398), alone (Arm A) or in combination with 5-FU and leucovorin (Arm C) for use in treating metastatic pancreatic cancer in

patients with failed gemcitabine based therapy. The dosage regimen disclosed in document D15b for Arm C of the study only differed from the treatment defined in claim 1 of the main request in that the order of administration was not specified.

The problem to be solved was seen in the provision of effective and safe treatment. In view of the triple treatment described in the prior art advantages of triple treatment over monotherapy could not be taken into account. Moreover, improvement over monotherapy was not plausibly derivable from the application as filed.

Whilst document D15b did not report any results of the described treatment, the mere fact that according to this document the dosage regimen of Arm C was being tested in a clinical study for the treatment of gemcitabine-resistant pancreatic cancer (as disclosed in document D15b) provided a reasonable expectation of success. Clinical studies were based on data obtained by preclinical testing both in vitro and in animals and required authority approval involving ethical considerations. The skilled person would therefore have expected the treatment in study arm C to be safe and effective unless he was dissuaded from this by the prior art. While the outcome of a clinical trial could be success or failure, no particular reason was known which would have discouraged the person skilled in the art from carrying out the therapeutic protocol according to Arm C of document D15b to simply confirm the usefulness of the dosage regimen. Finding out in a straightforward manner that the disclosed dosage regimen provided both efficacy and safety of treatment according to the purpose of the Phase 3 clinical trial could not be regarded as inventive.

No particular technical effect from the order of administration specified in claim 1 of the main request was recognized. The defined sequence corresponded to the order of administration of irinotecan, leucovorin and 5-FU as described in documents D2-D6 for the FOLFIRI treatment and was therefore obvious as available option.

Although document D15b did not indicate the meaning of MM-398, at the time of document D15b it was known from document D13 that MM-398 was nanoliposomal irinotecan, also known as PEP02. Even it were assumed that the definition of the liposoamal irinotecan as irinotecan sucrose octasulfate salt liposome injection represented a further difference with the prior art, no technical effect associated with this difference was recognized. As the original application acknowledged that irinotecan sucrose octasulfate salt liposome injection was known from US8147867, this product would represent an obvious alternative liposomal irinotecan which was available to the skilled person at the time of application

Claim 1 of the main request did therefore not meet the requirement of inventive step.

(e) The additional feature defined in accordance with auxiliary requests 1 and 3, that the patient achieves a response which is at least stable disease, merely excluded treatment failure and therefore did not represent any further distinction. Claim 1 of auxiliary request 2 only differed from the claim 1 of the main request that it did not specify the liposomal irinotecan as irinotecan sucrose octasulfate salt liposome injection.

The claims of auxiliary requests 1-3 lacked an inventive step for the same reasons as the main request.

New items of evidence

V. The following additional documents were cited during the appeal proceedings:

D23 : Declaration of Amy McKee, M.D.
D23A: Hoos et al., J Clin Oncol 31:3432-3438
D23B: Clinical Development Success Rates 2006-2015, published by Biotechnology Innovation Organization (BIO)
D24 : Declaration of Bruce Belanger, Ph.D.

D15c: EU clinical trial database for NAPOLI-1 study from 12 October 2012
D25: Pin-Yuan Chen et al, Neuro-Oncology 15(2):189-197 (December 2012)
D26: Drummond DC et al, Cancer Res 2006; 66: 3271-3277 (2006)
D27: Roy AC et al, Annals of Oncology 24(6): 1567-1573 (February 2013)
D28: Svenson S, Current Opinion in Solid State and Materials Science, 16(6) pp 287-294 (October 2012)
D29: Makrilia N et al, JOP. Journal of the Pancreas, 12(2) pp 110-113 (2011)
D30: Chen LT et al, Journal of Clinical Oncology, 30(4 Suppl) pp 613-613 (February 2012)
D31: Cunningham D et al, Journal of Clinical Oncology 29 (4 Suppl): 6-6 (2011)
D32: Gerber DE, Journal of Thoracic Oncology 7(12) Supplement 5_ S387-S389 (December 2012)
D33: Noble et al, Cancer Res 2006; 66: (5). March 1, 2006
D34: Krauze MT et al, Neuro-Oncology 9(4): 393-403 (2007)

D35: Mullard A, Nature Reviews Drug Discovery, vol. 17, page 777 (2018)
D36: The Medicines for Human Use (Clinical Trials) Regulations (MHCTR) 2004.

D37: Expert declaration of Carla Schoonderbeek
D37A:Directive 2001/20/EC
D38: Expert declaration of Grant H. Castle, Ph.D.
D38A:Communication from the Commission 2010/C 82/01

Documents D23-D24 and D37-D38A were filed by the appellant with its statement of grounds of appeal and its further submission of 30 June 2021, respectively.

Documents D15c and D25-D36 were filed by the opponent-respondent with its reply of 27 July 2020.

Parties and requests

VI. The appellant, Ipsen Biopharm Ltd., requests that the decision under appeal be set aside and the patent be maintained on the basis of the main request or any of auxiliary requests 1-3, all filed with the statement of grounds of appeal and corresponding to the requests on which the decision under appeal was based.

The appellant further requests that documents D25-D34 not be admitted into the appeal proceedings and that documents D37, D37A, D38 and D38A be admitted into the proceedings in case the Board intends to admit documents D15c, D35 and D36.

VII. The respondent, Teva Pharmaceutical Industries Ltd, requests that the appeal be dismissed.

The respondent further requests that new documents D23, D23A, D23B and D24 not be admitted into the appeal proceedings.

The following points inter alia appear to need consideration at the oral proceedings:

1. Admission additionally cited documents

1.1 Documents D23, D23A, D23B and D24 concern expert declarations with annexes relied upon by the appellant to argue that contrary to the finding in the decision under appeal (see section 5.7.3) the report of the Phase 3 clinical trial in document D15b did not provide the skilled person with a reasonable expectation of success. In view of the appellant's explanations in the statement of grounds of appeal (see section 5.86) and the letter of 30 June 2021 (see section 4) the Board is inclined to admit documents D23, D23A, D23B and D24 into the appeal proceedings as a legitimate response to the decision under appeal.

1.2 Documents D25-D34 were filed by the respondent with its reply to the appeal to support the argument that the product name "MM-398" was at the time of publication of document D15b well known to relate to liposomal irinotecan, in particular irinotecan sucrose octasulfate salt liposome injection (see reply sections 7.72-7.90). The Board is inclined to admit documents D25-D34 as legitimate response to the appellant's argument (see statement of grounds of appeal sections 5.36 and 5.48) that no documents on file identified MM-398 as irinotecan sucrose octasulfate salt liposome injection.

1.3 The appellant did not object to the admission of documents D15c, D35 and D36. These documents were filed by the respondent to argue that contrary to opinion expressed in document D23 the international Phase 3 clinical trial to which document D15b related required at least in the UK approval from the ethics committee

and the licensing authority and should thus be considered to provide a reasonable expectation of success (see reply sections 7.50-7.55). The Board therefore intends to admit documents D15c, D35 and D36 as a legitimate response to the statement of grounds of appeal.

1.4 Documents D37, D37A and D38 were filed by the appellant in support of the argument that reports of the Phase 3 clinical trial as in documents D15b and D15c did not provide the skilled person with a reasonable expectation of success (see reply of 30 June 2021 section 3). The Board intents to admit documents D15c, D35 and D36 as a legitimate response to the reply to the appeal.

2. Main request

2.1 Claim 1 of the main request defines:

"Liposomal irinotecan for use in a method of treating pancreatic cancer in a human patient, wherein the patient exhibits evidence of recurrent or persistent pancreatic cancer following primary chemotherapy and wherein the patient has failed prior treatment with gemcitabine or become resistant to gemcitabine, the method comprising co-administration of an effective amount each of liposomal irinotecan, 5-fluorouracil (5-FU) and leucovorin to the patient in at least one cycle wherein the cycle is a period of 2 weeks and, for each cycle:
(a) liposomal irinotecan is administered to patients not homozygous for the UGT1A 1 *28 allele on day 1 of each cycle at a dose of 80 mg/m2 and to patients homozygous for the UGT1A1 *28 allele on day 1 of cycle 1 at a dose of 60 mg/m2 and on day 1 of each subsequent cycle at a dose of 60 mg/m2 or 80 mg/m2;
(b) 5-FU is administered at a dose of 2400 mg/m2; and

(c) leucovorin is administered at a dose of 200 mg/m2 (1 form);
and wherein in each cycle, the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU;
and wherein the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection."

2.2 Sufficiency of disclosure

The respondent contests that the patent plausibly disclosed effectiveness of the claimed therapeutic regimen. The patent provided no experimental results of treatment involving the defined dosage regimen and essentially relied on verbal statements. The patent thereby failed to sufficiently disclose the suitability of the defined therapeutic regimen for the defined therapeutic indication (see reply section 5.21).

In line with the decision under appeal (see section 3) the Board presently takes the view that the main request complies with the requirement of Article 83 EPC.

In particular, the Board does presently not recognize that the teaching in the patent, that the defined triple therapy regimen is suitable for treatment of gemcitabine resistant pancreatic cancer as can be verified in accordance with the design for a Phase 3 trial as disclosed in example 7 of the patent (see paragraphs [0083]-[0177], is compromised by any serious doubt based on verifiable facts.

In the Board's preliminary view this teaching does further not lack plausibility taking account of:

- the results from the Phase 1 trial of example 6 of the patent (see paragraphs [0081]-[0082] involving

a triple combination of MM-398, 5-FU and leucovorin in treatment of patients with prostatic cancer and

- the known efficacy of the triple combination of non-liposomal irinotecan with 5-FU and leucovorin in treatment of patients with gemcitabine-refractory pancreatic carcinoma in the so-called FOLFIRI regimen as reported in documents D2, D3, D5 and D6 and referred to in the patent (see paragraph [0003], see also the application as filed page 1).

During the oral proceedings the parties will have the opportunity to further discuss the issue of sufficiency of disclosure.

2.3 Priority

2.3.1 It seems not in dispute that PD1 describes the same triple therapy dosage regimen as claim 1 of the main request (see PD1 claims 1, 4 and 11) except that PD1 does not explicitly refer to the l-form of the leucovorin to be administered at a dose of 200mg/m^2.

The appellant argues that PD1 implicitly disclosed the l-form of leucovorin. PD1 described "leucovorin" to act as a biochemical cofactor for 1-carbon transfer reactions (see PD1 page 11 lines 9-11). As indicated by for instance document D1 (see page 5 section 11) it was common knowledge that only the l-form of leucovorin is pharmaceutically active. The skilled person would therefore understand that in PD1 the term "leucovorin" referred to the l-form of leucovorin. Alternatively, if the term "leucovorin" in PD1 was considered to relate to "l-leucovorin or racemic leucovorin", the specification of the l-form in claim 1 of the main request represented merely a selection from two disclosed alternatives which did not affect the priority entitlement.

The Board presently considers that the term "leucovorin" as used in DP1 cannot be unambiguously considered to relate to the l-form of leucovorin. As explained by the respondent (see reply sections 6.7 and 6.16) document D1b, representing the FDA product label for leucovorin, states that "Leucovorin is a mixture of the diastereoisomers of the 5-formyl derivative of tetrahydrofolic acid (THF)", *i.e.* the racemic mixture (see D1b page 1 left column under CLINICAL PHARMACOLOGY"). Whilst document D1b recognizes that the l-form is the biologically active compound, the document nevertheless refers to leucovorin as not requiring reduction in order to participate in reactions. From the mentioned reference to the activity of leucovorin as biochemical cofactor in the patent it seems therefore not unambiguously derivable that the leucovorin defined for use in a dose of 200mg/m^2 was actually l-leucovorin and not the racemate.

The disclosure in PD1 prescribes a particular dose of 200mg/m^2 for the intended leucovorin. As explained by the respondent (see reply sections 6.24-6.25) the skilled person would be aware that the racemate and the l-form require different dosing. The appellant's alternative argument based on the interpretation that the disclosure with respect to leucovorin in PD1 implied an option between the l-form or the racemate, seems therefore presently not convincing.

2.3.2 The respondent further argues that claim 1 of the main request combines two embodiments concerning the patient populations that were only separately disclosed in PD1 (pages 11-12), namely:

- the embodiment wherein the patient exhibits evidence of recurrent or persistent pancreatic cancer following primary chemotherapy, and

- the embodiment wherein the patient has failed prior treatment with gemcitabine or become resistant to gemcitabine.

In line with the appellant's letter of 30 June 2021 (see sections 6.50-6.53) the Board is of the preliminary opinion that the mentioned combination does *per se* not present the skilled person with subject-matter that is not directly and unambiguously derivable from PD1.

2.3.3 Accordingly, the Board presently considers that the subject-matter of claim 1 of the main request is not entitled to the priority of PD1 and that documents D4,D8, D10 and D15b thus represent prior art.

During the oral proceedings the parties will have the opportunity to further discuss the question of the validity of the first priority.

2.4 Inventive step

2.4.1 The appellant has contested the finding in the decision under appeal that document D15b represents the closest prior art, because document D15b failed to disclose actual effective treatment of pancreatic cancer. In contrast, document D13 reported actual results of treatment of pancreatic cancer in patients with failed first-line gemcitabine therapy involving liposomal irinotecan and should therefore be considered as closest prior art (see statement of grounds of appeal sections 5.41-5.51).

According to the appellant monotherapy with liposomal irinotecan represented within document D13 the most promising starting point in view of the reported favourable results over triple therapy in a Phase 1

trial and in view of the disclosed subsequent Phase 2 trial, which involved monotherapy only (see statement of grounds of appeal sections 5.7-5.11).

The differences of the claimed subject-matter with this prior art involved the definition of the two weeks cycle, the lower dose of the liposomal irinotecan in the form of irinotecan sucrose octasulfate salt liposome injection and the additional administration of the defined doses of 5-FU and l-leucovorin in the defined order. Document D19 reported the results of a Phase 3 trial carried out in accordance with the design in example 7 of the patent (the NAPOLI-1 trial), which indicated advantageous efficacy with fewer adverse events for the triple therapy with respect to monotherapy. The problem solved was therefore the provision of a safe and effective treatment which is improved with respect to monotherapy (see statement of grounds of appeal sections 5.19-5.21). No prior art lead the skilled person towards the claimed subject-matter as solution to this problem (see statement of grounds of appeal section 5.64).

The differences between the claimed subject-matter and Arm C of the trial protocol described in document D15b concerned the actual effective and safe treatment of the patients, the definition of liposomal irinotecan as irinotecan sucrose octasulfate liposome salt injection instead of the product "MM-398", which remained undefined in document D15b, the order in which the drugs are administered and the distinction in the starting dose of the liposomal irinotecan depending on the patient's status concerning the UGT1A1*28 allele. In case document D15b was considered as starting point, these differences provided for safe and effective treatment which is improved relative to the monotherapy of document D15b (see statement of grounds of appeal sections 5.70-5.72). According to the appellant the

prior art provided the skilled person with no reasonable expectation that the claimed subject-matter would successfully solve such problem. In particular, contrary to the finding in the decision under appeal (see section 5.7.3), in view of documents D23, D24, D37 and D38 the mere announcement in document D15b that the mentioned Arm C was tested in a Phase 3 trial provided no reasonable expectation of success (see statement of grounds of appeal section 5.86 and reply of 30 June 2021 section 6.14). Moreover, document D15b only referred to the product MM-398 and it was not evident that this product was "irinotecan sucrose octasulfate salt liposome injection" as defined in claim 1 of the main request.

Document D5, which was additionally cited as suitable starting point in the prior art in the respondent's reply, described a clinical trial in which patients with pancreatic cancer after failed treatment with gemcitabine were administered non-liposomal irinotecan, leucovirin and 5-FU. The differences between the treatment defined in claim 1 of the main request and the treatment of document D5 concerned the use of liposomal irinotecan and its defined dose and the dose of 5-FU. According to the appellant the prior art provided no reasonable expectation that replacement of the two weekly total dose of 140mg/m^2 of non-liposomal irinotecan a 80mg/m^2 dose of liposomal irinotecan and the 20% increase of the 5-FU dose would allow for safe and effective treatment, let alone treatment resulting in improved therapy as demonstrated by the overall and progression free survival reported in document D19 (see letter of 30 June 2021 sections 6.44 and 6.49).

2.4.2 The Board recalls that the problem solution approach implies that in case an inventive step can be recognized starting from a particular item of prior art which is convincingly identified as most promising

starting point and thus represents the closest prior art, attempts to argue a lack of inventive step starting from less promising starting points are bound to fail. However, in case an inventive step is apparently convincingly denied starting from a promising particular item of prior art, the mere argument that the claimed subject-matter nevertheless involves an inventive step in view of an allegedly closer prior art, may not be persuasive, because in such case the allegedly closest prior art is likely to represent a starting point that is no more promising.

In the decision under appeal the subject-matter of claim 1 of the main request is denied an inventive step starting from document D15b as closest prior art (see sections 5.3 and 5.7.9). As pointed out by the respondent (see reply section 7.26) the patent itself does not present experimental evidence specifically demonstrating the therapeutic effect of the claimed dosage regimen. The circumstance that document D15b relates to a protocol for a Phase 3 trial without report of actual effective treatment does therefore in the Boards preliminary opinion not disqualify this document as suitable alternative starting point in the prior art. Furthermore, as explained in the decision under appeal by reference to document D13 (see section 5.3 page 12 penultimate paragraph) and as sustained by the respondent by reference to *inter alia* document D25 (see reply section 7.84) the product MM-398 mentioned in document D15b apparently concerned a known product at the time of publication of document D15b, which was identifiable as a liposomal formulation containing nano-sized irinotecan crystals complexed with sucrose octasulfate corresponding to the liposomal irinotecan defined in claim 1 of the main request (see D25 page 190 right column under "Investigational Agent"). Having regard to the similarities between the treatment regimen of claim 1 of the main request and the protocol

for treatment in document D15b the Board therefore presently considers that document D15b may not be excluded as suitable starting point in the prior art.

In view of the respondents arguments (see reply items 7.122-7.126) the Board does further not exclude that document D5 may represent a suitable starting point in the prior art, as this document describes effective treatment of pancreatic cancer after failed gemcitabine therapy involving combination treatment with non-liposomal irinotecan, 5-FU and leucovorin.

2.4.3 The differences between the claimed subject-matter and Arm C of the trial protocol described in document D15b seem to concern the actual effective and safe treatment of the patients, the order in which the drugs are administered and possibly the distinction in the starting dose of the liposomal irinotecan depending on the patient's status concerning the UGT1A1*28 allele.

As argued by the respondent (see reply section 7.24) it was already known from documents D10 and D11 that the patient's UGT1A1*28 allele-status was relevant for the irinotecan starting dose (see D10 pages 7-8 section 2.3; see D11 page 1290 right column lines 2-7). Moreover, as further explained in the decision under appeal (see section 5.7.7) and sustained by the respondent (see reply sections 7.30 and 7.31) no particular effect of the order of administration as defined in claim 1 of the main request seems to have been demonstrated, whilst the defined sequence corresponds to the order of administration of irinotecan, leucovorin and 5-FU in known combination treatment as described in documents D2, D4, D6 and D10. The appellant observes that document D15b does not mention the order of administration and the UGT1A1*28 allele-status, but does not seem to specifically contest the respondents arguments in this respect. The

Board therefore doubts whether these differences contribute to any inventive merit.

In view of the difference that document D15b only describes a protocol for treatment without reporting any results the problem to be solved could be formulated as the provision of actually effective and safe treatment of the defined patients suffering from pancreatic cancer.

In this context the Board is presently not convinced that the mere fact that a dosage regimen is reported to be tested in a Phase 3 clinical trial already by itself generally provides the skilled person with a reasonable expectation of success of that dosage regimen. As indicated in the statement of grounds of appeal (see section 5.77) the considerations in T 239/16 (see section 6.5) seem closely linked to the further circumstances of the case decided therein. Similar appears to apply with respect to the considerations in T 2506/12 (see section 3.15).

However, in the present case the patent fails to disclose any results of actual treatment in accordance with claim 1 of the main request and presents in example 7 only a design for a Phase 3 trial. Moreover, whilst the patent refers to promising efficacy and safety of triple combination treatment in the Phase 1 trial of example 6 (see paragraph [0083]), this example 6 does not seem to mention the dose of the administered 5-FU and leucovorin, the two weekly dosing cycle of the defined drugs nor the treatment status of the patients (see the respondent's reply sections 4.8, 5.2, 5.11). At the same time, as pointed out by the respondent (see reply sections 7.100-7.104 and 7.113-7.118), documents D12 and D13 already seem to have reported positive results from administration of MM-398, 5-FU and

leucovorin in treatment of gemcitabine refractory pancreatic cancer in a Phase 1 clinical trial setting.

In line with the introductory arguments of the respondent (see reply sections 5.1-5.4) the Board observes in this context that in as far as the disclosure in the patent itself essentially relies on considerations based on common knowledge and information already available from the prior art for proposing the claimed solution, similar considerations may equally apply in the assessment whether the claimed solution would be obvious to the skilled person..

According to the decision under appeal (see section 5.7.2) any evidence in documents D17-D19 of improved treatment from the defined triple dosage regimen over monotherapy lacked relevance, because the triple treatment in document D15b already represented the closest prior art. The Board recalls in this context that document D15b only describes a protocol for a trial of monotherapy and a triple dosage regimen and does not report actually effective treatment. However, the Board tends to agree with the further finding in the decision under appeal (see also section 5.7.2) that the more ambitiously formulated problem of providing an improvement over monotherapy may not be taken into account, since it seems not evident how such problem and its solution could be plausibly derived from the application as filed. In this context the Boards observes that the technical contribution actually disclosed in the patent is an aspect to be considered in the assessment of inventive step. In the present case there appears to be no disclosure of the technical contribution resulting from the distinguishing features.

2.4.4 The Board observes that the respondent acknowledges that its arguments starting from document D13 are

similar to its arguments based on document D12 (see reply section 7.113). As mentioned in section 2.4.3 above these documents seem to report positive results from administration of MM-398, 5-FU and leucovorin in treatment of gemcitabine refractory pancreatic cancer in a Phase 1 clinical trial setting. In line with the respondents arguments (see sections 7.101-7.103 and 7.118) the Board presently takes the view that the reported triple treatment seems more pertinent than the monotherapy also described in these documents.

As explained in section 2.4.1 the Board further considers that the triple treatment involving non-liposomal irinotecan as described in document D5 may not be excluded as possible starting point in the prior art.

The problem to be solved in view of documents D12/D13 or D5 could be formulated as the provision of further effective and safe treatment of the defined patients suffering from pancreatic cancer.

Faced with this problem the skilled person would likely take note of the trial protocol of document D15b. In line with the respondent's arguments (see reply sections 7.109, 7.118 and 7.127) similar considerations regarding the problem to be solved and its solution as set out in section 2.4.3 above may apply.

2.4.5 During the oral proceedings the parties will have the opportunity to further discuss the issue of inventive step.

3. Auxiliary requests 1-3

Auxiliary requests 1-3 filed with the grounds of appeal correspond to auxiliary requests 1-3 on which the decision under appeal was based.

The Board presently agrees with the decision under appeal (see sections 9.4 and 11.2 and 13.2) that the differences between auxiliary requests 1-3 and the main request do not seem of influence in the assessment of inventive step.

4. Final observations

The attention of the parties is drawn to Article 114(2) EPC and Articles 12 and 13 of the Rules of Procedure of the Boards of Appeal. The revised Rules of Procedure (RPBA 2020) entered into force on 1 January 2020 (Article 24(1) RPBA 2020). For the present case the transitional provisions pursuant to Article 25(2) RPBA apply.

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Electronically submitted

Your Ref T2963/19 - 3.3.07
Our Ref O008029EP:ECO/SJD/FJT
Date 21st December 2021

Dear Sirs,

Re: European Patent No. 2861210 deriving from EP13731230.2
Appellant: IPSEN BIOPHARM LTD.
Respondent : TEVA PHARMACEUTICAL INDUSTRIES LTD

On behalf of the appellant, I enclose a written submission, which is filed in response to the Board's communication pursuant to Art. 15(1) RPBA.

I confirm that the appellant will be represented at the oral proceedings scheduled for 18th March 2022. At the oral proceedings, we will use the English language, and we request simultaneous translation into English should any other language be used.

In view of the uncertainty caused by the ongoing COVID-19 pandemic, the appellant requests that the oral proceedings take place via videoconference. It should be noted that this request is conditional on all parties to these proceedings (i.e. the appellant, the respondent, and the Board) participating in the oral proceedings via videoconference. Should the respondent intend to attend the oral proceedings in person (i.e. on the premises of the EPO), the appellant will also attend the oral proceedings in person.

Should the Board have any queries in relation to this matter, it is requested to contact the undersigned representative by telephone or email.

A copy of this submission will be sent today by email to the respondent's representative.

Yours faithfully,

// ELECTRONICALLY SIGNED AND SUBMITTED //

OATES, Edward Christopher
Carpmaels & Ransford LLP Professional Association No. 182

Encl. Response to the communciation pursuant to Art. 15(1) RPBA

EUROPEAN PATENT 2 861 210 B1

T2963/19 - 3.3.07 | DERIVING FROM 13731230.2 | O008029EP
Appellant / proprietor: IPSEN BIOPHARM LTD.
Respondent / opponent: TEVA PHARMACEUTICAL INDUSTRIES LTD

1 INTRODUCTION AND REQUESTS

1.1 In its communication pursuant to Article 15(1) RPBA, the Board gave its preliminary and non-binding opinion on this case. This submission is filed in response to the comments made by the Board in that communication. This submission is being filed almost three months before the oral proceedings scheduled for 18th March 2022, so it is believed that there should be ample time for both the Board and the respondent to consider it and prepare for the oral proceedings accordingly.

1.2 In its communication, the Board correctly stated that D15b would not have provided the skilled person with a reasonable expectation that any of the dosage regimens mentioned therein would have been successful. The Board also correctly stated that T239/16 and T2506/12 are not relevant to the present case. In this submission, it will be further explained why the MR complies with Article 56 EPC. In particular, it will be explained that:

- "*irinotecan sucrose octasulfate salt liposome injection*" must be acknowledged as a feature which distinguishes claim 1 of the MR from D15b;
- the patent does demonstrate to the skilled person that the claimed subject matter is able to treat pancreatic cancer as required by claim 1 of the MR; and
- the prior art would have led the skilled person away from the claimed solution.

Some comments on admissibility, priority, and sufficiency will also be made later on in the submission.

1.3 For the avoidance of doubt, all of the appellant's previously-made requests are maintained.

2 INVENTIVE STEP (ART. 56 EPC)

2.1 As the appellant has previously stated, D13 should be taken as the closest prior art. However, in its communication the Board alludes to the possibility of any of D15b, D12, D13, and D5 being the closest prior art. Each of these possible starting points will be dealt with in turn below.

D15b as closest prior art

Distinguishing features

2.2 If D15b, specifically Arm C therein, is taken as the closest prior art (which the appellant believes is not the correct approach), the Board states in its communication that claim 1 of the MR differs from D15b because:

- claim 1 requires the safe and effective treatment of gemcitabine-resistant pancreatic cancer; and

- claim 1 requires a specific order of drug administration.

The communication also states that the requirement in claim 1 that patients homozygous for the UGT1A1*28 allele receive a lower starting dose of liposomal irinotecan could "*possibly*" represent a distinguishing feature[1].

2.3 The Board does not however acknowledge that the use of "*liposomal irinotecan*", specifically the use of "*irinotecan sucrose octasulfate salt liposome injection*", represents a distinguishing feature. The appellant disagrees and submits that the requirement in claim 1 that "*liposomal irinotecan*", specifically "*irinotecan sucrose octasulfate salt liposome injection*", is used in the dosage regimen **must** be seen a further feature which distinguishes the claim from D15b. This point was discussed at length in the appellant's submission of 30th June 2021 (paragraphs 6.16 – 6.30), and these arguments will not be repeated in full here purely for the sake of brevity. However, the appellant wishes to take this opportunity to comment on the specific points raised by the Board in its communication.

2.4 As a starting point, it should be emphasised that D15b merely mentions a clinical trial involving the administration of something called "*MM-398*". No information is given in D15b as to what "*MM-398*" is. That is, there no disclosure in D15b of "*MM-398*" being a type of liposomal irinotecan (or indeed any kind of irinotecan), and there is certainly no explicit or implicit disclosure of "*MM-398*" being "*irinotecan sucrose octasulfate salt liposome injection*". For this reason alone, the requirement in claim 1 that "*irinotecan sucrose octasulfate salt liposome injection*" is administered as part of the dosage regimen must be acknowledged as a distinguishing feature.

2.5 The Board appears to justify its finding that "*irinotecan sucrose octasulfate salt liposome injection*" cannot represent a distinguishing feature by stating with reference to D25 that "*MM-398 mentioned in document D15b apparently concerned a known product*"[2]. With respect, the appellant submits that the Board's reliance on D25 (should it be admitted) is misplaced. In particular, the Board's reference to MM-398 allegedly being a "*known product*" appears to indicate that the Board is of the view that the composition of MM-398 would have formed part of the common general knowledge at the relevant date. However, whilst the skilled person will, of course, consider the closest prior art (i.e. D15b) in light of their common general knowledge, D25 would not have formed part of the common general knowledge at the relevant date. This is because D25 is a single journal article and not, for example, a review article or an extract from a textbook. It should also be emphasised that the respondent has not attempted to argue that D25 would have formed part of the common general knowledge. Therefore, D25 cannot be considered in combination with the closest prior art when considering which features distinguish claim 1 from the closest prior art. Even if some of the features which distinguish claim 1 from the closest prior art are disclosed in D25 (which is not conceded), this could only ever be relevant in the final stage of the problem-solution approach when one considers whether the claimed solution would have been obvious.

2.6 In suggesting that the "*irinotecan sucrose octasulfate salt liposome injection*" feature cannot distinguish claim 1 from D15b, the Board's communication also refers to D13. However, as is the case for D25, at no point has it been established that D13 is representative of the

[1] Art. 15(1) RPBA communication, section 2.4.3, first paragraph.
[2] Art. 15(1) RPBA communication, paragraph spanning pages 16 and 17.

common general knowledge. Thus, D13 is of no relevance when considering how claim 1 is distinguished from D15b. In any case, whilst D13 does state that MM-398 is a type of liposomal irinotecan, it still does not disclose explicitly or implicitly that MM-398 is "*irinotecan sucrose octasulfate salt liposome injection*". If anything, it suggests that MM-398 is something other than "*irinotecan sucrose octasulfate salt liposome injection*". This is because the disclosure of D13 equates "*Liposomal Irinotecan*" with "*Nanoliposomal CPT-11*" and "*MM-398*"[3]. D13 subsequently defines "*CPT-11*" as irinotecan hydrochloride[4]. So, if anything, the skilled person would conclude from D13 that MM-398 is some kind of liposomal formulation of irinotecan hydrochloride – i.e. something which is different and distinct from the "*irinotecan sucrose octasulfate salt liposome injection*" required by claim 1. It should be noted that the disclosure of D13 is consistent with that of D27 in this regard[5].

2.7 Therefore, contrary to the Board's suggestion, "*irinotecan sucrose octasulfate salt liposome injection*" must be seen as a feature which distinguishes claim 1 of the MR from D15b.

Objective technical problem and non-obviousness of the claimed solution

2.8 In its communication, the Board formulates the problem as "*the provision of actually effective and safe treatment of the* [gemcitabine-resistant] *patients suffering from pancreatic cancer*"[6]. Whilst the appellant submits that one can also formulate the objective technical problem more ambitiously[7], and disagrees with the Board's statement to the contrary[8], the remainder of this section will deal with the solution to the problem formulated in the Board's communication.

2.9 The appellant acknowledges the Board's statement that the disclosure of, for example, D15b would not have provided the skilled person with a reasonable expectation that any of the dosage regimens mentioned therein would have been successful, and that decisions T239/16 and T2506/12 are not relevant to the present case[9].

2.10 However, the appellant disagrees with some of the Board's other comments on the obviousness of the claimed solution. In particular, we note that, according to the Board, "*the patent fails to disclose any results of actual treatment in accordance with claim 1*", and that "*the patent does not present experimental evidence specifically demonstrating the therapeutic effect of the claimed dosage regimen*". The Board goes on to suggest, in essence, that the disclosure of the patent does not go beyond what is disclosed in, for example, D12 and D13[10]. The appellant disagrees because the application as filed and the patent demonstrate to the skilled person for the first time that **irinotecan sucrose octasulfate salt liposome injection** administered at a dose of **80 mg/m² once every two weeks** in a combination dosage regimen according to claim 1 is able to treat pancreatic cancer in the patient population defined in claim 1. This will now be explained.

[3] D13, page 188, RH column, heading entitled "*Liposomal Irinotecan (Nanoliposomal CPT-11), PEP02, MM-398*".
[4] D13, page 188, RH column, first sentence under heading.
[5] 30th June 2021 submission, paragraphs 6.23 – 6.24.
[6] Art. 15(1) RPBA communication, page 18, first complete paragraph.
[7] Statement of grounds of appeal, paragraphs 5.55, 5.64, 5.72, and 5.101 – 5.102; 30th June 2021 submission, paragraphs 6.48 – 6.49.
[8] Art. 15(1) RPBA communication, page 19, second complete paragraph.
[9] Art. 15(1) RPBA communication, page 18, second complete paragraph.
[10] Art. 15(1) RPBA communication, paragraph spanning pages 18 and 19, and first paragraph on page 19.

The patent *does* demonstrate to the skilled person that the claimed subject matter is able to treat pancreatic cancer as required by claim 1

2.11 Example 6 of the patent describes the administration of a triple combination of irinotecan sucrose octasulfate salt liposome injection with 5-FU and leucovorin in a clinical trial to 15 patients with solid tumors. Five of these patients had pancreatic cancer. Table 2 of the patent shows that three pancreatic cancer patients received an irinotecan sucrose octasulfate salt liposome injection dose of 80 mg/m^2. Paragraph 0081 of the patent states that this 80 mg/m^2 dose was the maximum tolerated dose (MTD) and that, of the six patients who received this dose, one patient showed partial response (PR), four patients showed stable disease (SD), and one showed progressive disease (PD). PR and SD are indicative of a treatment effect, whereas PD is not. The one patient who showed PR had gastric cancer (i.e. not pancreatic cancer). This leaves five patients who received the MTD of 80 mg/m^2. Of these five, three of the five had pancreatic cancer, one of the five showed PD, and four of the five showed SD. Even if it is assumed that the one patient who showed PD had pancreatic cancer (i.e. even if, *arguendo,* one makes a negative assumption about the efficacy of the regimen in pancreatic cancer), it remains the case that two out of three pancreatic cancer patients (i.e. 67%) showed stable disease. In other words, Example 6 teaches that activity was seen in at least 67% of the pancreatic cancer patients administered the combination regimen using an 80 mg/m^2 dose of irinotecan sucrose octasulfate salt liposome injection.

2.12 The Board's communication seems to cast doubt on the reliability of Example 6 by stating that Example 6 *"does not seem to mention the dose of the administered 5-FU and leucovorin, the two weekly dosing cycle of the defined drugs nor the treatment status of the patients"*[11]. However, even though it may be the case that certain features of the dosage regimen are not *explicitly* recited in Example 6, it remains the case that the skilled person would conclude from Example 6, considered in light of the patent's teaching as a whole, that the dosage regimen defined in claim 1 is able to treat the patient population recited in claim 1.

2.13 For example, the skilled person considering the patent as a whole would note that there are two embodiments discussed therein – a monotherapy embodiment which administers liposomal irinotecan as a single active agent (see, e.g., paragraph 0057 and "*Arm A*" of Example 7), and triple combination therapy embodiment (see, e.g., paragraph 0014). Both of these embodiments are described throughout the patent in a consistent manner. For example, for the triple combination therapy embodiment, the skilled person would note that, every time specific doses and administration frequencies of 5-FU and leucovorin are mentioned, these doses and administration frequencies are those which appear in claim 1. This can be seen from, for example, paragraphs 0014 and 0058, "*Arm C*" of Example 7, and Figure 7. Thus, the skilled person reading Example 6 in light of the patent as a whole would appreciate that these doses and administration frequencies were used in Example 6. Any conclusion to the contrary would necessitate a reading of the patent which is completely artificial and one which relies on "*a mind desirous of misunderstanding*"[12]. Therefore, there is no need for the doses and administration frequencies to be recited *verbatim* in Example 6.

[11] *Ibid.* 10.
[12] Case Law of the Boards of Appeal, 9th Ed., II.E.2.3.3.

2.14 The same can be said for the order of administration of the three drugs in the triple combination therapy embodiment. Similar to the situation above, every time a specific order of administration for these three drugs is given in the patent, this order is that which appears in claim 1. This can be seen from, for example, paragraphs 0014 and 0015 (second sentence), Example 7 (row labelled "*Arm C*" of table spanning pages 15 and 16), and Figure 7. Thus, the skilled person reading Example 6 in light of the patent as a whole would conclude that this order of administration was used in Example 6. Again, any conclusion to the contrary could only be the result of an incorrect and artificial interpretation of the patent's disclosure. So, once again, it is not necessary for this feature to be recited *verbatim* in Example 6.

2.15 The Board's communication also points out that the "*treatment status*" of the patients is not explicitly mentioned in Example 6. However, the skilled person considering the disclosure of the patent as a whole would note that, after first-line therapy is mentioned in the "*Background*" section[13], it is not mentioned again. Instead, the patent focusses on the treatment of the patient population recited in claim 1 – see, for example, paragraphs 0014, 0045, 0047. In addition, the skilled person would have been aware that there was no cross-resistance between gemcitabine and irinotecan[14], and so the use of an irinotecan-based therapy following failure of gemcitabine would have been consistent with the common general knowledge at the relevant date. Therefore, once again, the skilled person considering Example 6 in light of the patent as a whole would appreciate that the pancreatic cancer patients in Example 6 had the same "*treatment status*" (to use the Board's wording) as the patients of claim 1.

2.16 Therefore, Example 6 of the patent does disclose to the skilled person that a treatment effect is associated with the subject matter of claim 1, contrary to the statement given by the Board in its communication. Any argument to the contrary assumes that the skilled person would ignore the consistent disclosure of the patent as a whole when interpreting Example 6, and would instead consider Example 6 in isolation from the remainder of the patent with a mind desirous of misunderstanding, which cannot be the correct approach.

The prior art teaches away from the claimed subject matter

2.17 As explained above, the patent describes for the first time that **irinotecan sucrose octasulfate salt liposome injection** administered at a dose of **80 mg/m² once every two weeks** in a combination dosage regimen according to claim 1 is able to treat pancreatic cancer in the patient population defined in claim 1. The Board has already acknowledged that the disclosure of D15b would not, in and of itself, have provided the skilled person with a reasonable expectation that any of the dosage regimens mentioned therein would have been successful. When this is considered in combination with the fact that the prior art would actually have dissuaded the skilled person from believing that such a dosing regimen was safe and efficacious, it becomes clearer than the claimed subject matter is nonobvious. In particular, all of the prior art which reports on the successful treatment of gemcitabine-resistant pancreatic cancer with liposomal irinotecan uses a dosage of 120 mg/m², and there is nothing in the art to suggest that a dose of 80 mg/m² once every two weeks alone or in combination would be safe and effective.

[13] Patent, paragraphs 0005, 0006, 0010, and 0011.
[14] See, for example, OD's decision, paragraph 3.3.5.

2.18 For example, D7 uses liposomal irinotecan monotherapy, and reports a partial response in a patient with pancreatic cancer. D7 reports that dosages of 60 mg/m^2, 120 mg/m^2, and 180 mg/m^2 were administered, but it is not stated which dose delivered the partial response in pancreatic cancer. However, as the maximum tolerated dose (MTD) and recommended Phase II dose was found to be 120 mg/m^2, the skilled person would have assumed that this dose was responsible for the partial response. Therefore, the teaching of D7 would have dissuaded the skilled person from reasonably expecting that a dose of 80 mg/m^2 would be efficacious, as this dose is well below the MTD, and was not even tested in D7.

2.19 D12 also tests a 120 mg/m^2 dose administered as a monotherapy, and reports that this dosage appeared to have "*both efficacy and tolerable side effects*". Again, there is nothing to suggest that 80 mg/m^2 alone or in combination will be effective.

2.20 D13 reports on liposomal irinotecan monotherapy and combination therapy regimens, although the only dosage of liposomal irinotecan it mentions is 120 mg/m^2 as a monotherapy. Very little information on the combination therapy is given, and it appears from D13 that the combination therapy was abandoned after Phase I in favour of the monotherapy. The only detail given for the combination therapy is the frequency of the administration of 5-FU/LV, which is administered weekly, in contrast to claim 1 which requires that 5-FU/LV be administered every two weeks. Thus, the skilled person consulting D13 would have had doubts about the efficacy of the combination therapy, which was seemingly abandoned after Phase I. In addition, there is still no pointer or motivation towards the use of a dose of 80 mg/m^2 liposomal irinotecan, and the skilled person is dissuaded from administration of 5-FU/LV once every two weeks.

2.21 D22 also dissuades the skilled person from expecting that a dose of 80 mg/m^2 once every two weeks would solve the objective technical problem. D22 teaches that the maximum tolerated dose of liposomal irinotecan when administered in combination with 5-FU/LV is 80 mg/m^2 once every **three** weeks. Thus, on the basis of D22, the skilled person would seriously doubt whether a dosage of 80 mg/m^2 once every **two** weeks would be safe, because this dose and administration frequency is more intense than that tolerated in D22. Thus, D22 would have dissuaded the skilled person from believing that an 80 mg/m^2 dose once every two weeks was safe and effective. D22 also requires weekly administration of 5-FU/LV rather than every two weeks, which would further complicate matters and further reduce any expectation of success for a regimen requiring administration of 5-FU/LV every two weeks. Thus, the skilled person considering D15b in combination with D22 would not have been led to the claimed subject matter with a reasonable expectation of success. In fact, this combination of documents would have dissuaded the skilled person from a dosage regimen which requires the administration of irinotecan sucrose octasulfate salt liposome injection at an 80 mg/m^2 dose with 5-FU/LV once every two weeks.

2.22 The Board has already acknowledged that the existence of document D15b would not, in and of itself, have instilled the skilled person with a reasonable expectation that the dosage regimens mentioned therein would have been successful. Moreover, it has been explained above that the other prior art documents would actually have dissuaded the skilled person from drawing such a conclusion about the dosage regimen mentioned in Arm C of D15b (which mentions the administration of an 80 mg/m^2 dose of something called "*MM-398*" with 5-FU/LV every two weeks). In particular, there is a fundamental conflict between documents

such as D7 which imply that a dose of 80 mg/m^2 might not be high enough to be effective and documents such as D22 which imply that 80 mg/m^2 every two weeks may be unsafe because that dose could only be tolerated if dosed every three weeks. The two conflicting teachings suggest, if anything, that 80 mg/m^2 once every two weeks might be both unsafe and not efficacious. This fundamental conflict confirms that the skilled person would not have been led to the claimed subject matter with a reasonable expectation of solving the objective technical problem.

The prior art would not have led the skilled person towards "*irinotecan sucrose octasulfate salt liposome injection*"

2.23 It has been explained in paragraphs 2.3 to 2.6 above that "*irinotecan sucrose octasulfate salt liposome injection*" is a feature which distinguishes claim 1 from D15b. The skilled person seeking to solve the objective technical problem formulated above would not have been led to a solution which requires the use of "*irinotecan sucrose octasulfate salt liposome injection*".

2.24 At best for the respondent, D25 demonstrates that the skilled person could have been led to irinotecan sucrose octasulfate salt liposome injection. However, the respondent has failed to establish why the skilled person would have accepted the statements in D25 at face value when other documents existed which, at the very least, suggested that MM-398 is something other than "*irinotecan sucrose octasulfate salt liposome injection*" (see, e.g., D13 discussed above at paragraph 2.6 and D27[15]). Therefore, the respondent's arguments based on D25 fail to establish that the claimed solution would have been obvious.

2.25 In any case, even if the skilled person had, for some reason, combined D15b and D25, they still would not have been led to the claimed subject matter with a reasonable expectation of success. As the Board has already acknowledged, the existence of D15b would not, in and of itself, have instilled the skilled person with a reasonable expectation of success. Moreover, the skilled person would actually have been dissuaded from the claimed subject matter had they considered the other prior art documents (see paragraphs 2.17 - 2.22 above). Therefore, the claimed subject matter involves an inventive step if D15b is taken as the closest prior art.

D12/D13 as the closest prior art

2.26 The appellant has already explained why claim 1 of the MR is inventive if either of D12 or D13 is taken as the closest prior art[16], and these arguments will not be repeated in full here purely for the sake of brevity. However, the appellant wishes to point out that it disagrees with the statement made by the Board in its communication that "*the reported triple treatment* [in D12 and D13] *seems more pertinent than the monotherapy also described in these documents*"[17].

2.27 In particular, it cannot be the case that the combination regimen described in D12 and D13 is "*more pertinent*" than the monotherapy. It is true that both D12 and D13 begin by discussing a monotherapy regimen and a triple combination regimen. Only vague details are given about the triple combination regimen, and neither document makes any explicit link between the

[15] As was stated in the 30th June 2021 submission at paragraphs 6.23 – 6.34, D27 explicitly states that "*PEP02, also known as MM-398 (Merrimack Pharmaceuticals, Inc.), is a highly stable liposomal nanocarrier formulation of irinotecan hydrochloride (CPT-11)*" [emphasis added].
[16] See, for example, statement of grounds of appeal, paragraphs 5.41 – 5.64; 30th June 2021 submission, paragraphs 7.14 – 7.25.
[17] Art. 15(1) RPBA communication, paragraph spanning pages 19 and 20.

triple combination regimen and the efficacious treatment of pancreatic cancer. This is in stark contrast with the fact that, in both documents, the monotherapy regimen is reported to have "*both activity and tolerable side effects*"[18], be a "*promising option*"[19] for the relevant patient population, and be deserving of "*further exploration*"[20]. Thus, there can be no justification whatsoever for suggesting that the combination regimen is "*more pertinent*" than the monotherapy regimen in D12 and D13.

2.28 In any case, even if one does focus on the combination regimen, neither document provides any information as to the doses of any of the drugs which should be used. In addition, neither document mentions a two-week dosing cycle, as required by claim 1. In fact, D13 teaches away from such a dosing cycle because, in the combination regimen of D13, it is stated that 5-FU/LV should be administered "*weekly*"[21]. Finally, neither document discloses that "*irinotecan sucrose octasulfate salt liposome injection*" should be used in the dosage regimen. In fact, as mentioned above at paragraph 2.6, D13 at least suggests that the MM-398 referred to therein is something other than "*irinotecan sucrose octasulfate salt liposome injection*" and thus, if anything, would have led the skilled person away from the subject matter of claim 1.

2.29 Therefore, claim 1 of the MR involves an inventive step if D12 or D13 is taken as the closest prior art.

D5 as the closest prior art

2.30 In the appellant's submission of 30th June 2021, it was explained why claim 1 of the MR involves an inventive step if D5 is taken as the closest prior art[22]. These arguments will not be repeated in full here purely for the sake of brevity.

2.31 In its communication, the Board states that, had the skilled person started from D5, they "*would likely take note of the trial protocol of document D15b*"[23]. The appellant disagrees because D5 refers to a dosage regimen which uses (non-liposomal) irinotecan, whereas D15b refers only to "*MM-398*" without any indication of what "*MM-398*" is. Thus, the skilled person consulting D15b in light of D5 would have been at a loss as to what "*MM-398*" is, and so D15b would not have assisted the skilled person seeking to solve the objective technical problem.

2.32 So, even if the skilled person had combined D5 with D15b, they would still have needed to look to a third document to find out what "*MM-398*" is. To get anywhere near the claimed solution, the skilled person would then have to focus on the disclosure of, e.g., D25 and ignore the contradictory information in, e.g., D13 and D27 in spite of the fact that there is no justification for doing so. Even then, after combining at least three documents, the skilled person would still not have had a reasonable expectation of success in view of D15b (as the Board correctly acknowledged in its communication). In fact, the skilled person would have been led away from the claimed subject matter in view of the teaching of the other prior art documents (see paragraphs 2.17 - 2.22 above). Therefore, the skilled person starting from D5

[18] D12, "*Conclusion*".
[19] *Ibid.* 18.
[20] D13, page 191, LH column above heading "*Cationic Liposome Encapsulated Paclitaxel (EndoTAG™-1)*".
[21] D13, page 189, RH column, second paragraph.
[22] 30th June 2021 submission, paragraphs 6.37 – 6.49.
[23] Art. 15(1) RPBA communication, page 20, third complete paragraph.

could not have arrived at the claimed subject matter without using inventive skill, and thus claim 1 of the MR involves an inventive step if D5 is taken as the closest prior art.

3 ADMISSIBILITY OF CITED DOCUMENTS

3.1 The appellant is grateful to the Board for stating in its communication that D23, D23A, D23B, D24, D37, D37A, and D38 are admissible[24]. However, the appellant respectfully disagrees with the Board's statements concerning the admissibility of D25-D34. In particular, the feature in claim 1 of the MR which requires that the liposomal irinotecan is "*irinotecan sucrose octasulfate salt liposome injection*" was taken from granted dependent claim 4 and was not, for example, taken solely from the description. Thus, it cannot be correct to argue, as the respondent has done, that the response to the appeal represented the first opportunity to file evidence alleging that the features of granted claim 4 were known in the art. Had the respondent wished to file evidence demonstrating that the features of this claim were allegedly known in the art, this evidence could and should have been filed in proceedings before the OD. No real justification for the late filing of these documents has been provided by the respondent. Therefore, D25-D34, filed for the first time during appeal proceedings, should not be admitted into the proceedings.

4 PRIORITY (ARTICLE 87 EPC)

4.1 The appellant is grateful to the Board for stating in its communication that claim 1 of the MR does not combine two distinct embodiments in an unallowable fashion[25]. However, for the reasons expressed in the statement of grounds of appeal and the submission of 30th June 2021, the appellant respectfully disagrees with the Board's statement that the reference to leucovorin being administered at a dose of "*200 mg/m^2 (l-form)*" in claim 1 results in the first priority claim being invalid.

5 SUFFICIENCY (ART. 83 EPC)

5.1 The appellant is grateful to the Board for acknowledging that the MR complies with Art. 83 EPC. We note that, in its communication, the Board correctly stated that there are no serious doubts substantiated by verifiable facts which compromise the teaching of the patent. However, and for the avoidance of doubt, even if the Board is minded to impose a stricter standard when assessing compliance with Art. 83 EPC (e.g. if the Board is inclined to follow the respondent's argument that the patent must "*positively demonstrate suitability*", which the appellant believes would not be the correct approach), it remains the case that Art. 83 EPC is complied with. In particular, Example 6, considered in light of the entirety of the patent's disclosure, positively demonstrates that the dosage regimen of claim 1 is able to treat

[24] Art. 15(1) RPBA communication, sections 1.1 and 1.4. With regard to section 1.4, we note that the final sentence of this section states that "*The Board intends to admit documents D15c, D35 and D36*". The appellant assumes based on the context that this was a typographical error, and the sentence was intended to state "*The Board intends to admit documents D37, D37A and D38*".
[25] Art. 15(1) RPBA communication, section 2.3.2.

pancreatic cancer in the patient population recited in claim 1. This was explained in the 30th June 2021 submission[26], and so this explanation will not be repeated here purely for the sake of brevity. In addition, the arguments made above at paragraphs 2.11 - 2.16 in the context of Article 56 EPC apply here, *mutatis mutandis*.

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[26] 30th June 2021 submission, paragraphs 7.39 – 7.44.

Europäisches Patentamt
European Patent Office
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Notice of opposition to a European patent

I. Patent opposed

Patent No.	EP3266456
Application No.	EP17169098.5
Date of mention of the grant in the European Patent Bulletin (Art. 97(3), Art. 99(1) EPC)	05 May 2021
Title of the invention	Combinations of liposomal irinotecan, 5-FU and leucovorin for the treatment of pancreatic cancer

II. Proprietor of the patent

first named in the patent specification	Ipsen Biopharm Ltd.
Opponent's or representative's reference	ESP00534SAN

III. Opponent

Name	Sandoz AG
Address:	Lichtstraße 35 4056 Basel Switzerland
State of residence or of principal place of business	Switzerland
Multiple opponents (see additional sheet)	☐

IV. Authorisation

1. Representative

	Lederer & Keller Patentanwälte Partnerschaft mbB	
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Telephone/Fax	+49 89 21 23 99 0	+49 89 21 23 99 22
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Additional representative(s) on additional sheet/see authorisation ☐

Authorisation(s)

is/are enclosed ☐

has/have been registered under No. ☐

V. Opposition is filed against

the patent as a whole ☒

claim(s) No(s).

VI. Grounds for opposition:

Opposition is based on the following grounds:

(a) the subject-matter of the European patent opposed is not patentable (Art. 100(a) EPC) because:

- it is not new (Art. 52(1); Art. 54 EPC) ☐
- it does not involve an inventive step (Art. 52(1); Art. 56 EPC) ☒
- patentability is excluded on other grounds, namely articles ☐

(b) the patent opposed does not disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art (Art. 100(b) EPC; see Art. 83 EPC). ☐

(c) the subject-matter of the patent opposed extends beyond the content of the application/of the earlier application as filed (Art. 100(c) EPC, see Art. 123(2) EPC). ☒

VII. Facts (Rule 76(2)(c) EPC)

presented in support of the opposition are submitted herewith on an attached document ☒

VIII. Other requests:

- Oral proceedings are hereby requested auxiliarily.

IX. Evidence presented

D1	Other evidence	Chen, L. et al., J. Clin. Oncol. 2010, 28(12S), abstract e13024 original file name: D1 Chen J Clin Oncol 2010 28(12S) abstract e13024.pdf attached as: Other-evidence-1.pdf
D10	Other evidence	Yoo, C. et al., Br. J. Cancer 2009, 101(10), 1658-1663 original file name: D10 Yoo Br. J. Cancer 2009, 101(10), 1658-1663.pdf attached as: Other-evidence-11.pdf
D11	Other evidence	Drummond, D.C. et al., Cancer Res. 2006, 66(6), 3271-3277 original file name: D11 Drummond Cancer Res 2006 66(6) 3271-3277.pdf attached as: Other-evidence-12.pdf
D12	Other evidence	Baker, J.H.E. et al., Clin. Cancer Res. 2008, 14(22), 7260-7271 original file name: D12 Baker Clin Cancer Res 2008 14(22) 7260-7271.pdf attached as: Other-evidence-13.pdf
D13	Other evidence	Venditto, V.J. et al., Mol. Pharmaceutics 2010, 7, 2, 307-349 original file name: D13 Venditto Mol. Pharmaceutics 2010, 7, 2, 307-349.pdf attached as: Other-evidence-14.pdf
D14	Other evidence	Tardi, P.G. et al., Biochim. Biophys. Acta 1768 2007, 678-687 original file name: D14 Tardi Biochim Biophys Acta 1768 2007 678-687.pdf attached as: Other-evidence-15.pdf
D15	Other evidence	Decision of the Opposition Division of 28th August 2019 revoking EP 2 861 210 B1 original file name: D15 Decision revoking EP 2 861 210 B1.pdf attached as: Other-evidence-16.pdf
D16	Other evidence	Preliminary opinion of 9th August 2021 of Board of Appeal 3.3.07 against decision of Opposition Division revoking EP 2 861 210B1 original file name: D16 Preliminary opinion of the Board of Appeal.pdf attached as: Other-evidence-17.pdf
D2	Other evidence	Chen, L. et al., J. Clin. Oncol. 2012, 30(4S), abstract 613 original file name: D2 Chen J Clin Oncol 2012 (30(4S) abstract 613.pdf attached as: Other-evidence-2.pdf
D3	Other evidence	Ko, A.H. et al., J. Clin. Oncol. 2011, 29(4S), abstract 237 original file name: D3 Ko J Clin Oncol 2011 29(4S) abstract 237.pdf attached as: Other-evidence-3.pdf
D4	Other evidence	Chen, L. et al., J. Clin. Oncol. 2008, 26(15), abstract 2565 original file name: D4 Chen J Clin Oncol 2008 26(15) abstract 2565.pdf attached as: Other-evidence-4.pdf
D5	Other evidence	Protocol of study NCT01494506, entitled "Study of MM-398 Versus 5-Fluorouracil ...", version 9 of 29th May 2012 original file name: D5 NCT01494506 v 9 29 May 2012 (NAPOLI-1 with 2 arms).pdf attached as: Other-evidence-5.pdf
D5a	Other evidence	Protocol of study NCT01494506, entitled "Study of MM-398 Versus 5-Fluorouacil ...", version 13 of 8th August 2012 original file name: D5a NCT01494506 v 13 8 August 2012 (NAPOLI-1 with 3 arms).pdf

		attached as: Other-evidence-6.pdf
D6	Other evidence	Protocol of study NCT01375816, entitled "Liposome-encapsulated Irinotecan Hydrochloride PEP02 ...", version 1, 16th June 2011 original file name: D6 NCT01375816 v 1 16 June 2011.pdf attached as: Other-evidence-7.pdf
D7	Other evidence	Tsai, C. et al., J. Gastrointest. Oncol. 2011, 185-194 original file name: D7 Tsai J. Gastrointest. Oncol. 2011, 185-194.pdf attached as: Other-evidence-8.pdf
D8	Other evidence	FDA label of Camptosar (irinotecan), 2009 original file name: D8 FDA irinotecan label 2009.pdf attached as: Other-evidence-9.pdf
D9	Other evidence	FDA label of leucovorin, 2008 original file name: D9 FDA leucovorin label 2008.pdf attached as: Other-evidence-10.pdf

X. Payment

Method of payment: Debit from deposit account

The European Patent Office is hereby authorised, to debit from the deposit account with the EPO any fees and costs indicated in the fees section below.

Currency: EUR

Deposit account number: 28000381

Account holder: Lederer & Keller

Refunds

Any refunds should be made to EPO deposit account: 28000381

Account holder: Lederer & Keller

Fees	Factor applied	Fee schedule	Amount to be paid
010 Opposition fee	1	815.00	815.00
Total:		EUR	815.00

	A Forms	Details:	System file name:
A-1	Form for notice of opposition		ep-oppo.pdf

	B Attached document files	Original file name:	System file name:
B-1	1. Facts and arguments	opposition.pdf	OPPO.pdf

	C Attached evidence files	Original file name:	System file name:
C-1	1. Other evidence	D1 Chen J Clin Oncol 2010 28(12S) abstract e13024.pdf	Other-evidence-1.pdf
C-2	2. Other evidence	D2 Chen J Clin Oncol 2012 (30(4S) abstract 613.pdf	Other-evidence-2.pdf
C-3	3. Other evidence	D3 Ko J Clin Oncol 2011 29(4S) abstract 237.pdf	Other-evidence-3.pdf
C-4	4. Other evidence	D4 Chen J Clin Oncol 2008 26(15) abstract 2565.pdf	Other-evidence-4.pdf
C-5	5. Other evidence	D5 NCT01494506 v 9 29 May 2012 (NAPOLI-1 with 2 arms).pdf	Other-evidence-5.pdf

C-6	6. Other evidence	D5a NCT01494506 v 13 8 August 2012 (NAPOLI-1 with 3 arms).pdf	Other-evidence-6.pdf
C-7	7. Other evidence	D6 NCT01375816 v 1 16 June 2011.pdf	Other-evidence-7.pdf
C-8	8. Other evidence	D7 Tsai J. Gastrointest. Oncol. 2011, 185-194.pdf	Other-evidence-8.pdf
C-9	9. Other evidence	D8 FDA irinotecan label 2009.pdf	Other-evidence-9.pdf
C-10	10. Other evidence	D9 FDA leucovorin label 2008.pdf	Other-evidence-10.pdf
C-11	11. Other evidence	D10 Yoo Br. J. Cancer 2009, 101(10), 1658-1663.pdf	Other-evidence-11.pdf
C-12	12. Other evidence	D11 Drummond Cancer Res 2006 66(6) 3271-3277.pdf	Other-evidence-12.pdf
C-13	13. Other evidence	D12 Baker Clin Cancer Res 2008 14(22) 7260-7271.pdf	Other-evidence-13.pdf
C-14	14. Other evidence	D13 Venditto Mol. Pharmaceutics 2010, 7, 2, 307-349.pdf	Other-evidence-14.pdf
C-15	15. Other evidence	D14 Tardi Biochim Biophys Acta 1768 2007 678-687.pdf	Other-evidence-15.pdf
C-16	16. Other evidence	D15 Decision revoking EP 2 861 210 B1.pdf	Other-evidence-16.pdf
C-17	17. Other evidence	D16 Preliminary opinion of the Board of Appeal.pdf	Other-evidence-17.pdf

Signature of opponent or representative

Place	**München**
Date:	**01 February 2022**
Signed by:	**Marco Fachini 46014**
Association:	**Lederer & Keller Patentanwälte Partnerschaft mbB**
Representative name:	**Marco Fachini**
Capacity:	**(Representative)**

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Your reference	Our reference	01 February 2021
EP 3 266 456	ESP00534SAN	MF

Re.: European Patent No. 3 266 456
European Patent Application No. 17169098.5
Patentee: Ipsen Biopharm Ltd.
Opponent: Lederer & Keller Patentanwälte Partnerschaft mbB

On behalf of

Sandoz AG
Lichtstrasse 35
4056 Basel
Switzerland

OPPOSITION

is lodged according to Article 99 EPC against the above-referenced patent of Ipsen Biopharm Ltd. entitled

"Combinations of liposomal irinotecan, 5-FU and leucovorin for the treatment of pancreatic cancer".

The opposition fee amounting to EUR 815.-- is paid via online fee payment.

The opponent has appointed us as their representatives, and it is respectfully requested to send all notifications to our address.

The European patent EP 3 266 456 (hereinafter referred to as the "contested patent") is opposed in its full extent (claims 1 to 6).

The opposition is based on the grounds of Articles 100(a) and 100(c) EPC. In particular, it is submitted that the subject matter of the contested patent extends beyond the content of the application as filed and does not involve an inventive step.

It is requested to revoke the European patent in its entirety. Oral proceedings in accordance with Article 116 EPC are requested in the event that the Opposition Division does not reach the decision to revoke the patent on the basis of the written submissions of the opponent.

Detailed statement of the grounds for opposition

1. Bibliographic information

1.1 The contested patent is based on European application no. 17169098.5, which is a divisional of application 13731230.2, now patent EP 2 861 210 B1. The parent application is the European phase of international application PCT/US2013/045495, published as WO 2013/188586 A1. Said application was filed on 12th June 2013, claiming priorities of 13th June 2012 and of 14th March 2013.

1.2 EP 2 861 210 B1 was revoked by the decision of the Opposition Division of 28th August 2019 for lack of inventive step (see document D15). The patentee appealed the decision. In their preliminary opinion of 9th August 2021, Board of Appeal 3.3.07 concurred with the appealed decision (see document D16). Oral proceedings are scheduled to be held on 18th March 2022.

2. Cited documents

2.1 Reference will be made to the following documents:

D1: Chen, L. *et al.*, *J. Clin. Oncol.* **2010**, 28(12S), abstract e13024;
D2: Chen, L. *et al.*, *J. Clin. Oncol.* **2012**, 30(4S), abstract 613;
D3: Ko, A.H. *et al.*, *J. Clin. Oncol.* **2011**, 29(4S), abstract 237;

D4: Chen, L. *et al.*, *J. Clin. Oncol.* **2008**, 26(15), abstract 2565;

D5: Protocol of study NCT01494506, entitled *"Study of MM-398 Versus 5-Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer (NAPOLI 1)"*, version 9 of 29th May **2012**;

D5a: Protocol of study NCT01494506, entitled *"Study of MM-398 Versus 5-Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer (NAPOLI 1)"*, version 13 of 8th August **2012**;

D6: Protocol of study NCT01375816, entitled *"Liposome-encapsulated Irinotecan Hydrochloride PEP02 or Irinotecan Hydrochloride, Leucovorin Calcium, and Fluorouracil as Second-Line Therapy in Treating Patients With Metastatic Colorectal Cancer"*, version 1, 16th June **2011**;

D7: Tsai, C. *et al.*, *J. Gastrointest. Oncol.* **2011**, 185-194;

D8: FDA label of Camptosar® (irinotecan), **2009**;

D9: FDA label of leucovorin, **2008**;

D10: Yoo, C. *et al.*, *Br. J. Cancer* **2009**, 101(10), 1658-1663;

D11: Drummond, D.C. *et al.*, *Cancer Res.* **2006**, 66(6), 3271-3277;

D12: Baker, J.H.E. *et al.*, *Clin. Cancer Res.* **2008**, 14(22), 7260-7271;

D13: Venditto, V.J. *et al.*, *Mol. Pharmaceutics* **2010**, 7, 2, 307–349;

D14: Tardi, P.G. *et al.*, *Biochim. Biophys. Acta 1768* **2007**, 678–687;

D15: Decision of the Opposition Division of 28th August **2019** revoking EP 2 861 210 B1;

D16: Preliminary opinion of 9th August **2021** of Board of Appeal 3.3.07 in the appeal against the decision of the Opposition Division revoking EP 2 861 210 B1.

2.2 Documents D1 to D5 and D6 to D14 were published prior to the earliest priority date of the contested patent and are thus full prior art for all claims. Document D5a was published between the priority date and the filing date of the contested patent.

2.3 Documents D7 and D13 are review articles. These documents are thus, by definition, an account of the common general knowledge and the state of the art prior to their own publication date (see e.g. T 777/08, r. 5.2, or T 1641/11). Documents D8 and D9 are the prescribing information of two authorised drugs and they too represent the general knowledge of the skilled person (T 734/12, r. 21).

2.4 In the course of these opposition proceedings we may also refer to the documents D1 to D16 listed in item IX of D15 and to documents D25 to D36 listed in item V of D16, which were cited in the opposition against the parent patent. Should the Opposition Division request copies of these documents, we can provide them at short notice.

3. The subject matter of the contested patent

3.1 Technical field and background

3.1.1 The contested patent explains that pancreatic cancer has an extremely poor prognosis and is almost always fatal, because of a lack of effective therapy combined with a generally late diagnosis, the absence of screening tools and a limited understanding of risk factors (contested patent, paragraph [0003]).

3.1.2 According to paragraphs [0004] and [0006] of the background art section, chemotherapy with one or more of 5-fluorouracil (5-FU) and gemcitabine has been shown to prolong survival. Single-agent gemcitabine was the standard of care at the relevant date in the first-line treatment of advanced and metastatic pancreatic adenocarcinoma. Moreover, various combination therapies and regimens had been developed, the acronym in parenthesis being the name with which they were known in the art:

- folinic acid (leucovorin or levoleucovorin), 5-fluorouracil, and irinotecan (FOLFIRI);
- folinic acid, 5-fluorouracil, irinotecan and oxaliplatin (FOLFIRINOX);
- folinic acid, 5-fluorouracil, and oxaliplatin (FOLFOX).

3.1.3 Irinotecan is a member of the topoisomerase I inhibitor class of drugs, which arrests uncontrolled cell growth by inhibiting the unwinding of DNA and thereby preventing DNA replication. Also known as CPT-11, irinotecan was marketed at the relevant date as Camptosar®, formulated as an aqueous solution for injection of the hydrochloride salt (contested patent, paragraph [0004]; see also prescribing information D8).

3.1.4 The contested patent further explains in the background art section that irinotecan is a prodrug that is converted into a 100-1000 fold more active metabolite, SN-38. SN-38 is not recognized by P-glycoprotein, a drug transporter that plays an important role in acquired drug resistance by pumping certain drugs out of cells, so irinotecan is likely to be active in tumours resistant to other standard chemotherapies (contested patent, paragraph [0005]).

3.1.5 The object of the contested patent appears to be the provision of new treatment options for pancreatic cancer (contested patent, paragraph [0007]).

3.2 The common general knowledge

3.2.1 Document D7 is a review article on nanovector-based therapies in advanced pancreatic cancer (D7, title). D7 explains that *"Nanovectors can provide passive drug delivery through abnormal tumor neo-vasculature microanatomy or active targeting via binding to receptors or macromolecules associated with the tumor. In such a manner, nanovector-based therapy may not only modulate the pharmacokinetics and therapeutic index of chemotherapeutic agents but also provide new treatment options in patients with advanced pancreatic cancer"* (D7, abstract).

3.2.2 A type of said nanovectors are liposomes encapsulating drugs. A liposome is often a spherical vesicle with a bilayer membrane whose size typically ranges from ~40 nanometres to several microns (D7, page 186, right-hand column, last paragraph). D7 further teaches that *"Besides its characteristic slow-release pharmacokinetic property, liposome encapsulated drugs can potentially provide improved tumor localization via the "enhanced permeability and retention" (EPR) effect. Such agents can therefore, (i) lower drug elimination to increase systemic circulation time, (ii) lower maximum plasma concentration (C_{max}) to reduce drug side effects, (iii) enhance tumor tissue uptake and exposure to the anti-cancer drug; these principles can in turn yield an improved therapeutic index for cancer therapy"*[1] (D7, page 187, left-hand column, second paragraph).

3.2.3 After a brief explanation of the mechanism of action and applications of conventional irinotecan (called also with its lab code CPT-11) (D7, page 188, second paragraph), D7 explains that *"Although the original CPT-11 drug is now of interest in pancreatic cancer management, potentially superior versions incorporating drug delivery technologies offer a next generation approach"* (D7, paragraph bridging pages 188-189). *"To realize the potential advantages of nanoparticle delivery, a novel liposome-based construct termed "nanoliposomal CPT-11 (nLs-CPT-11)" was developed, which encapsulates CPT-11 with unprecedented efficiency and stability (27). PK studies showed long circulation times for the carrier and undetectable drug release in plasma"* (D7, page 188, right-hand column, second paragraph). Liposomal irinotecan is also referred to with its lab codes PEP02 and MM-398. Also review D13 teaches that the liposomal sucrose octasulfate formulation of irinotecan shows improved

[1] The therapeutic index is a quantitative measurement of the relative safety of a drug. It is a comparison of the amount of a therapeutic agent that causes the therapeutic effect to the amount that causes toxicity. A higher therapeutic index is preferable to a lower one: a patient would have to take a much higher dose of such a drug to reach the toxic threshold than the dose taken to elicit the therapeutic effect.

overall pharmacokinetics compared to free irinotecan (D13, page 331, left-hand column, second paragraph).

3.2.4 Further, it belonged to the common general knowledge that *"In a series of preclinical studies, nanoliposomal CPT-11 demonstrated significantly superior efficacy when compared to free CPT-11 at the same or higher dose, including frequent cures in some models. The superiority of nanoliposomal CPT-11 over free CPT-11 has been observed in different tumor models including colorectal, gastric, breast, cervical, glioma, pancreatic and lung cancer models"* (D7, page 189, left-hand column, third paragraph, emphasis added).

3.2.5 After reviewing the known clinical studies with liposomal irinotecan, D7 concludes

> *"The results highlight the feasibility and activity of nanoliposomal CPT-11 in previously heavily treated patients with gemcitabine-refractory advanced pancreatic cancer, which deserves further exploration."*
> (D7, page 189, left-hand column, third paragraph, emphasis added)

3.2.6 In conclusion, the skilled person knew at the priority date of the contested patent that liposomal irinotecan had significantly superior efficacy and improved pharmacokinetics compared to conventional irinotecan. It was therefore a very promising investigational drug for the treatment of pancreatic cancer.

3.3 The claims of the contested patent

3.3.1 The contested patent comprises 6 claims. Claim 1 represents an independent claim and claims 2 to 6 refer directly or indirectly back to claim 1.

3.3.2 Independent claim 1 relates to

1. *Irinotecan sucrose octasulfate salt liposome injection*
   for use in a method of treating pancreatic cancer
   in a human patient who has failed prior treatment with gemcitabine or become resistant to gemcitabine,
   the method comprising co-administration of an effective amount each of
   irinotecan sucrose octasulfate salt liposome injection,
   5-fluorouracil (5-FU) and
   leucovorin to the patient,

wherein, in each cycle, the irinotecan sucrose octasulfate salt liposome injection is administered prior to the leucovorin,
and the leucovorin is administered prior to the 5-FU and,
in the method:

(a) the 5-FU is administered intravenously over 46 hours;
(b) the leucovorin is administered intravenously over 30 minutes, and
(c) the irinotecan sucrose octasulfate salt liposome injection is administered intravenously over 90 minutes.

3.3.3 Claim 2 specifies that, prior to each administration of irinotecan sucrose octasulfate salt liposome injection, the patient is pre-medicated with dexamethasone and/or a 5-HT^3 antagonist or another anti-emetic.

3.3.4 Claim 3 specifies that the pancreatic cancer is an exocrine pancreatic cancer selected from the group consisting of acinar cell carcinoma, adenocarcinoma, adenosquamous carcinoma, giant cell tumour, intraductal papillary-mucinous neoplasm (IPMN), mucinous cystadenocarcinoma, pancreatoblastoma, serous cystadenocarcinoma, and solid and pseudopapillary tumours. Claim 4 specifies that the cancer is advanced pancreatic cancer, which is a pancreatic tumour that exhibits distant metastasis and/or peripancreatic extension of the tumour.

3.3.6 Claim 5 specifies that each cycle is a period of 2 weeks.

3.3.7 Claim 6 specifies that (a) the irinotecan sucrose octasulfate salt liposome injection is administered intravenously at a dose of: 80 mg/m^2 every 2 weeks to patients who are not homozygous for the UGTA1*28 allele or; 60 mg/m^2 every 2 weeks to patients who are homozygous for the UGTA1*28 allele, and wherein the dose is increased to 80 mg/m^2 if the patient does not experience any drug related toxicity; (b) the 5-FU is administered intravenously at a dose of 2400 mg/m^2 over 46 hours, and the leucovorin is administered intravenously at a dose of 200 mg/m^2 over 30 minutes, every 2 weeks; wherein the patient has been premedicated with dexamethasone and a 5-HT^3 antagonist; and wherein the patient has metastatic pancreatic cancer that has progressed on gemcitabine based therapy.

3.4 The examples of the contested patent

The contested patent contains 7 examples.

3.4.1 Example 1 and figure 1 disclose the activity of MM-398 (liposomal irinotecan) in an orthotopic pancreas tumour model expressing luciferase, as compared to a control (HBS) and free irinotecan (CPT11). The results of this study belonged to the common general knowledge at the relevant date, as evidenced by review article D7, page 189, paragraph bridging the two columns.

3.4.2 Example 2 and figure 2 disclose the accumulation of the active metabolite SN-38 in human colon cancer xenografts following treatment with free Irinotecan or liposomal irinotecan (MM-398).

Example 3 and figure 3 disclose the effect of MM-398 on carbonic anhydrase IX staining in a human colon cancer xenograft model. MM-398 reduced markers of hypoxia, a hallmark of resistant and aggressive disease.

Example 4 and figure 4 disclose that MM-398 increases perfusion of Hoechst stain human colon cancer xenograft model. The increased vasculature can make the tumour more susceptible to agents such as 5-FU/LV.

The results of these studies had already been disclosed in prior art document D12 and references cited therein.

3.4.3 Example 5 and figure 5 and 6 disclose the pharmacokinetic (PK) profile of MM-398 as single agent, which were investigated in a phase I clinical study (denominated PEP0201) in patients with solid tumours at 60, 120 or 180 mg/m^2 dose levels and in a phase II clinical trial (denominated PEP0206) in gastric cancer patients at 120 mg/m^2. The results of the study on solid tumours had been published in prior art document D4 and even belonged to the common general knowledge (see D7, page 189, right-hand column, last paragraph). The results of the study in gastric cancer had been published in prior art document D31 listed in item V of D16.

3.4.4 Example 6 discloses a phase 1 dose escalation study. A regimen combining fluorouracil, leucovorin, and MM-398 was studied in a phase 1 trial (denominated PEP0203) of solid tumours in 16 subjects, of whom 5 were patients with pancreatic cancer. The overall disease control rate was 73.3%. The maximum tolerated (MTD) dose of MM-398 was

determined to be 80 mg/m^2. These results had been disclosed in prior art document D1 and even belonged to the common general knowledge (see D7, page 189, right-hand column, last paragraph).

3.4.5 Example 7 and figure 7 disclose the protocol of a phase 3 trial comparing the overall survival following treatment with MM-398, with or without 5-fluorouracil plus leucovorin, versus 5-fluorouracil and leucovorin in patients with metastatic pancreatic cancer that have progressed on gemcitabine based therapy (contested patent paragraph [0095]). The study has 3 treatment arms (A, B and C). In arm C, MM-398, 5-fluorouracil and leucovorin appear to have been administered according to the claims. No results are reported.

This study was indexed in the ClinicalTrial.gov database under number NCT01494506. Version 9 of the study protocol was disclosed in prior art document D5. The addition of arm C to the protocol is reflected in version 13 published in the priority year (see document D5a).

3.4.6 In conclusion, the examples report the known activity of liposomal irinotecan in pancreatic cancer models, its known pharmacokinetics and the known results of the phase 1 studies. The only contribution to the state of the art at the priority date can be seen as the disclosure of arm C of the protocol of study NCT01494506, without however disclosing any results.

4. Introduction of new subject matter

4.1 Claim 1

4.1.1 Reference will be made in the following to the text of the international application WO 2013/188586 A1, which is the published application underlying the parent application of the contested patent.[2]

4.1.2 According to the applicant, granted claim 1 finds basis on page 3, lines 5-9 (administration of the 3 active ingredients), on page 4, lines 2-9 (order and duration of administration), and on page 12, lines 23-24 (patient group) of the international application (letter of the applicant of 30th October 2018). This is incorrect.

[2] The text of application EP 3266456 A1 appears to be substantially identical to that of WO 2013/188586 A1, whose claims have been appended as embodiments at the end of the description.

4.1.3 If we look at the original claims of the international application, claim 3 is the one which discloses a subject matter closest to that of granted claim 1. The same disclosure can be found in the paragraph bridging pages 3 and 4 of the international application. However, granted claim 1 differs from original claim 3 in that the length of the treatment cycle (2 weeks) and the dosages of the 3 drugs have been deleted in an attempt to save the entitlement to the first priority date. To "compensate" said deletion, several features were added from the original dependent claims 4 (administration order), 6-9 (administration times), 11 (irinotecan sucrose octasulfate salt liposome injection) and from original page 12, lines 23-24 (patient group).

4.1.4 The question to be answered in relation to Article 76(1) EPC is whether or not the dosages of the 3 drugs and length of the treatment cycle were disclosed in the parent application as a whole as being optional or if they instead were inextricably linked to the disclosure of the combination of the three drugs. In the former case, said optional features can be omitted without changing the teaching of the parent application. In the latter case, claim 1 as granted provides a new technical teaching that cannot be directly and unambiguously derived from the parent application.

4.1.5 When reading the parent application, the skilled person understands that the dosages of the medicaments and their administration frequency (the length of the treatment cycle) are essential for the effective treatment of pancreatic cancer. Dosages that are too low and that are administered with insufficient frequency do not provide an effective treatment, while dosages that are too high or are administered too frequently lead to unacceptable toxicities.

4.1.6 This is evidenced also by the fact that different dosages are disclosed depending on the fact that liposomal irinotecan is administered alone or in combination with 5-fluorouracil and leucovorin. According to the international application a dosage of 120 mg/m^2 of liposomal irinotecan should be administered to a patient suffering of pancreatic cancer, if liposomal irinotecan is administered alone (claim 1). If instead liposomal irinotecan is administered together with 5-fluorouracil and leucovorin, the dosage should be reduced to 80 mg/m^2, to account for the combined toxicities of the 3 medicaments. This is in accordance to the common general knowledge that foresees a maximum tolerated dose of 120 mg/m^2 of liposomal irinotecan if it is administered alone (D7, page 189, right-hand-column, second paragraph). Said dose must be instead reduced to 80 mg/m^2 if also 5-fluorouracil and leucovorin are added to liposomal irinotecan. In said case, the maximum tolerated dose of irinotecan is only 80 mg/m^2 (see D1, *"Conclusions"*).

4.1.7 The skilled person also recognises that the frequency of the treatment is important, because the patient needs to recover from the previous chemotherapy (e.g., their white cell count needs to be high enough) before receiving the next cycle. As a matter of fact: the parent application makes clear that a frequency of 3 weeks is appropriate if liposomal irinotecan is administered alone (claim 1 of the parent application), while 2 weeks are appropriate if it is administered at a reduced dosage together with 5-fluorouracil and leucovorin.

4.1.8 Moreover, the parent application teaches that also the genetic profile of the patient must be considered. Indeed, it highlights unnumerable times the importance of a dose reduction for patients homozygous for the UGT1A1*28 allele (international application, pages 3-5, 9-11, 14, 25, 31, 38-41, claims 1-3, 5, 12-13 and figure 7). This mutation leads to increased plasma concentration of the active metabolite of irinotecan SN-38 and consequently to possible toxicities (international application, paragraph bridging page 9 and 10).

4.1.9 It is therefore evident from the parent application as a whole that the dosages of the three medicaments and their administration frequency have a fundamental impact both on the efficacy and the safety of the claimed treatment. Their omission in granted claim 1 provides a new technical teaching that is not directly and unambiguously derivable from the parent application.

4.1.10 Claim 1 thus adds matter and contravenes Article 76(1) EPC. The same reasoning applies to claims 2 to 5, which depend on claim 1.

4.2 Claim 6

4.2.1 Claim 6 depends on claim 1 and specifies the missing dosages and administration frequency. According to the letter of the applicant of 20th October 2018, claim 6 finds basis (only) in arm C of figure 7. We disagree.

4.2.2 The disclosure of figure 7 is more specific than the subject matter of claim 6, because said figure refers to MM-398 and not to the *"irinotecan sucrose octasulfate salt liposome injection"* of granted claim 1. These two wordings are not interchangeable.

4.2.3 As a matter of fact, the lab code MM-398 designates a very specific liposomal formulation of irinotecan that was being developed by the present patentee at the relevant date. Such a formulation contains a specific amount of irinotecan free base encapsulated with

the excipient sucrose octasulfate (a drug entrapment agent), in a unilamellar bilayer vesicle of a certain size and with a very specific composition (see D11).

4.2.4 On the other hand, several other liposomal formulations of irinotecan were under development at the priority date of the contested patent, as it is evidenced, e.g., by review article D13, page 330, right-hand column to page 331, left-hand column. Indeed, the whole document D12 deals with a different liposomal irinotecan formulation denominated "Irinophore C".

4.2.5 Moreover, claim 1 does not exclude that an additional drug is encapsulated in the liposomes. Document D14 discloses that a liposomal formulation of irinotecan and floxuridine had already been developed some years before the relevant date (D14, title). On the other hand, no further active ingredients in addition to irinotecan are encapsulated in the specific liposomes of MM-398.

4.2.6 The skilled person would have considered that the dosages reported in figure 7 of the parent application referred to the specific liposomal formulation of irinotecan denominated MM-398. These dosages could not be simply translated to any injectable liposomal irinotecan formulation with the only limitation that it contains the excipient sucrose octasulfate. The skilled person is well-aware that the choices of liposome type (unilamellar, bilamellar, etc.), membrane composition, liposome size, content of active ingredient, as well as the presence of further active ingredients and excipients are all critical to the stability of the liposomes and thus to their ability of delivering the active ingredient to the disease site.

4.2.7 Therefore, the skilled person would have understood that the teaching of the dose to be administered could not be generalised to other liposomal formulation of irinotecan, which have different stability and different ability of delivering the active ingredient to the disease site, thus necessarily requiring higher or lower effective doses.

4.2.8 Moreover, figure 7 is the only passage of the whole parent application to specify a dosage of leucovorin of 200 mg/m^2, half the dosage of 400 mg/m^2 disclosed in the rest of the application (see, e.g., claim 3).

4.2.9 As a matter of fact, the term *"leucovorin"* refers to the racemic drug (D,L-leucovorin), as it is evidenced by its FDA leaflet, which reflects the general knowledge of the skilled person. Instead, the term *"levoleucovorin"* is used to indicate only the l-enantiomer (see D9, page 1, left-hand column, first paragraph).

4.2.10 The names leucovorin and levoleucovorin follow the established practice in the pharmaceutical field of adding the prefix levo- and dextro-/dex- to the international proprietary names of established drugs to indicate the pure L- or D-enantiomer, respectively. Other well-known examples are levetiracetam and etiracetam, levofloxacin and ofloxacin, levocetirizine and cetirizine, levobetaxolol and betaxolol, levonorgestrel and norgestrel, levalbuterol and albuterol, dextroamphetamine and amphetamine, dexmedetomidine and medetomidine, etc....

4.2.11 Therefore the reference to a dosage of leucovorin of 200 mg/m^2, in figure 7 is understood by the skilled person to refer to the racemic mixture, i.e., to a dosage of 100 mg/m^2 of levoleucovorin and 100 mg/m^2 of dextroleucovorin. To the same preliminary opinion came also the Board of Appeal entrusted with the parent case (D16, item 2.3.1).

4.2.12 However, figure 7 is the only place of the whole international application in which a dose of leucovorin of 200 mg/m^2 is specified. The application has a whole discloses instead a dosage of either 400 mg/m^2 of leucovorin or 200 mg/m^2 of levoleucovorin. Therefore, the skilled person clearly understands that an error was made in figure 7 and that either 400 mg/m^2 leucovorin or 200 mg levoleucovorin were meant instead.

4.2.13 This is indeed what happened. The applicant modified the dosage of 200 mg/m^2 of leucovorin disclosed in the priority application to a dosage of either 400 mg/m^2 of leucovorin or 200 mg/m^2 of levoleucovorin. Due to an oversight, the amendment was not carried out in figure 7. This oversight is obvious to the eyes of the skilled person.

4.2.14 A single obviously erroneous statement cannot certainly override the overall teaching of the original application and cannot be used as basis for amendments. A dosage of 200 mg/m^2 of leucovorin in the claimed dosage regimen cannot be directly and unambiguously derived from the original application as a whole.

4.2.15 In conclusion, the disclosure of figure 7 is narrower than the subject matter of claim 1 (as far as the liposomal irinotecan formulation is concerned) and is at odds with the disclosure of the parent application as a whole (as far as the dosage of leucovorin is concerned). It cannot thus be used as basis for the subject matter of claim 6. Hence, claim 6 of the contested patent adds matter and thus does not comply with the requirements of Article 76(1) EPC.

5. Lack of inventive step

5.1 The closest prior art

5.1.1 Review article D7 provides an overview of nanovector-based therapies in advanced pancreatic cancer (D7, title), and in particular of liposome-encapsulation nanoparticles (see section 3.2 above). It is thus directed to the same purpose of the contested patent, the treatment of pancreatic cancer.

5.1.2 D7 summarises from page 188 to page 191 the pre-clinical and clinical data available on liposomal irinotecan. In particular, D7 discloses on the paragraph bridging pages 189 and 191 the results of the following 3 clinical trials:

1. first-in-human phase I trial of liposomal irinotecan in solid tumours, which corresponds to example 5 of the contested patent and is also disclosed in prior art document D4;
2. phase I trial of liposomal irinotecan in combination with 5-fluorouracil and leucovorin, which corresponds to example 6 of the contested patent and is also disclosed in prior art document D1;
3. phase II trial of liposomal irinotecan in patients with advanced pancreatic cancer after gemcitabine-based chemotherapy failure, which is disclosed in prior art document D3.

5.1.3 D7 further discloses in the same passage that the two phase I trials included a total of 7 patients with pancreatic cancer who failed gemcitabine therapy. Therefore, all three clinical trials were concerned with the treatment of pancreatic cancer in a human patient who had failed prior treatment with gemcitabine, as recited in claim 1 of the contested patent.

5.1.4 While liposomal irinotecan was administered alone in trials 1 and 3 mentioned above, the trial listed at item 2 involved the combination of all the three drugs required by claim 1: liposomal irinotecan, 5-fluorouracil and leucovorin. This embodiment of D7 has thus the most features in common with claim 1 of the contested patent and represents therefore the most promising springboard for the alleged invention.

5.1.5 We note that in the opposition against parent EP 2 861 210, the patentee considered the review of Tsai (D13 in said opposition, D7 in the present opposition) as the closest prior art (D16, item 2.4.1, first paragraph). However, they tried to argue that the monotherapy with liposomal irinotecan should be considered as the embodiment from which to apply the problem

solution approach, although it has less features in common with the claimed subject matter (D16, item 2.4.1, second paragraph).

5.1.6 This approach did not convince the Board of Appeal, which instead expressed their preliminary opinion that the disclosure of the combination treatment with liposomal irinotecan, 5-fluorouracil and leucovorin is a more promising springboard (D16, item 2.4.2). The Board also considered document D5 (D10 in the present opposition) involving the treatment of pancreatic cancer with non-liposomal irinotecan, 5- fluorouracil and leucovorin as a suitable alternative starting point (D16, item 2.4.2, last paragraph).

5.1.7 In the following we will first apply the problem solution approach starting from the embodiment of D7 involving the administration of liposomal irinotecan, 5-fluorouracil and leucovorin. We will then repeat the exercise starting from D10, which discloses the treatment of pancreatic cancer with non-liposomal irinotecan, 5-fluorouracil and leucovorin.

5.2 The objective technical problem

5.2.1 As just explained, D7 discloses the safe and effective treatment of pancreatic cancer in patients who have failed prior treatment with gemcitabine comprising co-administration of an effective amount each of injectable liposomal irinotecan, 5-fluorouracil and leucovorin.

5.2.2 While D7 does not explicitly disclose that the liposomal irinotecan formulation comprised sucrose octasulfate, the use of the specific lab codes MM-398 and PEP02 and the reference 27 to the article of Drummond (D11 in this opposition) implicitly disclose the presence of this excipient.[3] To the same conclusion came both the Opposition Division and the Board of Appeal entrusted with the parent case (D15, item 5.3, second-last paragraph; D16, item 2.4.2, second paragraph).

5.2.3 Moreover, (liposomal) irinotecan, leucovorin and 5-fluorouracil are drugs administered intravenously to cancer patients (see, e.g., D8 and D9). Therefore, although this is not explicitly stated in D7, the skilled person understands that all three drugs are administered intravenously in D7.

[3] Also review article D13, which, like D7, represents the common general knowledge, refers to the article of Drummond and the coencapsulation of irinotecan with sucrose octasulfate (D13, page 331, left-hand column, second paragraph).

5.2.4 D7 does not disclose that liposomal irinotecan is administered prior to leucovorin and leucovorin prior to 5-fluorouracil. Furthermore, D7 does not disclose that 5-fluorouracil is administered over 46 hours, leucovorin over 30 minutes, and irinotecan over 90 minutes.

5.2.5 The technical effect of these difference is not apparent from the contested patent, which does not compare the claimed regimen with alternative sequences of administration or with longer or shorter administration times. Moreover, the contested patent does not disclose that these distinguishing features are connected to any technical effect whatsoever, let alone a surprising one.

5.2.6 Therefore, in view of D7, the objective technical problem underlying claim 1 can be considered as the provision of an alternative treatment of pancreatic cancer in patients who have failed prior treatment with gemcitabine.

5.3 Obviousness in the light of the common general knowledge

5.3.1 The solution to such a problem is obvious taking into consideration the general knowledge of the skilled person, who knows well that irinotecan, 5-fluorouracil and leucovorin are invariably administered starting with irinotecan and ending with 5-fluorouracil. This is indicated, e.g., in the FDA label of standard irinotecan D8, which reflects the common general knowledge (see T 734/12, r. 21). D8 also indicates that irinotecan should be administered in 90 minutes, as recited in claim 1:

> *"CAMPTOSAR should be administered as an intravenous infusion over 90 minutes (see Preparation of Infusion Solution). For all regimens, the dose of LV should be administered immediately after CAMPTOSAR, with the administration of 5-FU to occur immediately after receipt of LV."*
> (D8, page 32 *"Dosage and administration"*)

5.3.2 This has been correctly recognised by the Opposition Division and by the Board of Appeal in charge of the parent case (D15, item 5.7.7; D16, item 2.4.3). Regarding the administration times of leucovorin and fluorouracil, it belongs to standard practice in the art to adapt them as deemed fit by the investigator. Moreover, the choice of 30 minutes and 46 hours is not connected to any technical effect and is thus arbitrary.

5.3.3 The skilled person would have had no doubt that the claimed dosage regimen is effective in the treatment of pancreatic cancer based on the known efficacy already disclosed

in the closest prior art D7. Indeed, the very patentee explained that there is no reason to doubt that this is the case based on the known efficacy of the combination of non-liposomal irinotecan, leucovorin and 5-fluorouracil:

> *"There is no reason to doubt that the claimed dosage regimen is effective, not least because "Combination therapies including ... folinic acid [a synonym for leucovorin] ... , 5-fluorouracil, and [non-liposomal] irinotecan (FOLRIRI) ... are used to treat some pancreatic cancers" (page 1 of the description, lines 2-6 of final paragraph)."*
> (letter of the applicant of 10th September 2020, page 4, third paragraph)

This must even more be the case based on the known efficacy of liposomal irinotecan, leucovorin and 5-fluorouracil in the claimed patient population disclosed in the closest prior art document D7.

5.3.4 In conclusion, the skilled person would have tested the claimed dosage regimen in the same population already disclosed in D7 (pancreatic cancer patients who have failed prior treatment with gemcitabine) with a reasonable expectation of achieving an alternative treatment of their disease.

5.3.5 Therefore, claim 1 of the contested patent does not involve an inventive step over document D7 in the light of the common general knowledge and thus does not fulfil the requirements of Article 56 EPC.

5.4 Obviousness in combination with D6 or D2

5.4.1 The skilled person would have also consulted the database clinicaltrials.gov for the currently ongoing trials in the claimed setting. The skilled person would thus have considered the clinical trial ongoing in France disclosed in D6 particularly relevant (D6, page 5, *"Contacts/Locations"*). The purpose of the trial of D6 is the following one:

> *"This randomized phase II trial is studying liposome-encapsulated irinotecan hydrochloride PEP02 given together with leucovorin calcium and fluorouracil to see how well it works compared with giving irinotecan hydrochloride together with leucovorin calcium and fluorouracil as second-line therapy in treating patients with metastatic colorectal cancer."*
> (D6, page 2, *"Study description"*, emphasis added)

5.4.2 The skilled person would have thus learned that the triple combination of liposomal irinotecan, leucovorin and 5-fluorouracil disclosed in the closest prior art document D7 for treating pancreatic cancer had already progressed to phase II in a closely related indication, colorectal cancer. Indeed, 3 out of 4 pre-clinical examples of the contested patent (see examples 2-4) are performed with colorectal cancer H-29 cells, thus confirming the close relationship between these two indications.

5.4.3 Document D6 discloses the claimed sequence of administration, as well as the administration times of 90 minutes and 46 hours of the liposomal irinotecan and 5-fluorouracil infusions, respectively:

> *"Arm II (FUPEP): Patients receive liposome-encapsulated irinotecan hydrochloride PEP02 IV over 60-90 minutes and leucovorin calcium IV over 2 hours on day 1 and fluorouracil IV over 46 hours beginning on day 1. Courses repeat every 14 days in the absence of disease progression or unacceptable toxicity."*
> (D6, middle of page 3, *"Study description"*)

5.4.4 The fact that D6 discloses an administration time of 2 hours instead of the claimed 30 minutes for leucovorin is irrelevant, because this feature is arbitrary.

5.4.5 The skilled person would have thus considered the triple therapy with liposomal irinotecan, leucovorin and 5-fluorouracil disclosed in the closest prior art document D7 particularly promising and worth being pursued in further clinical trials in pancreatic cancer. The patient population treated in D6 had already received the standard of care for their disease, as it is evidence by the title of D6 explaining that this was a *"second-line therapy"*. This follows the established practice of testing investigational therapies first in patients that have already received the standard of care and for which little or no further treatment options are available.

5.4.6 The skilled person would have thus tested the dosage regimen of D6 and/or its obvious minor modifications also in the population of the closest prior art D7, pancreatic cancer patients who have failed prior treatment with gemcitabine, thus arriving at an alternative treatment of their disease.

5.4.7 Alternatively, the skilled person would have resorted to document D2 disclosing the efficacy of biweekly liposome irinotecan (PEP02, MM-398) in second-line metastatic colorectal cancer. D2 concludes with a reference to the phase II study of D6 ongoing in France, thus highlighting the importance of further studies on this promising triple combination:

> *"A randomized Phase II study evaluating PEP02 plus 5-FU/LV (FUPEP regimen) vs. FOLFIRI is currently ongoing in France."*
> (D2, *"Conclusions"*)

The same reasoning set forth above in relation to D6 applies to D2.

5.4.8 Therefore, claim 1 of the contested patent does not involve an inventive step over document D7 in combination with any of documents D6 or D2 and thus does not fulfil the requirements of Article 56 EPC.

5.5 D10 as the closest prior art

5.5.1 Alternatively, the modified FOLFIRI.3 dosage regimen disclosed in document D10 would be a suitable springboard for the alleged invention. Mr Steendijk, the rapporteur of Board of Appeal 3.3.07 in the parent case, shares the same opinion (D16, item 2.4.2, last paragraph).

5.5.2 D10 discloses the efficacy and safety of the modified FOLFIRI.3 dosage regimen studied in a phase II clinical trial as second-line therapy in patients with gemcitabine-refractory advanced pancreatic cancer (D10, title and abstract, *"Conclusion"*). D10 thus discloses the same indication and patient population and is thus directed to the very same purpose of claim 1 of the contested patent.

5.5.3 The tested regimen consists of irinotecan (70 mg/m^2 over 1 hour on days 1 and 3), leucovorin (400 mg/m^2 over 2 hours on day 1), and 5-fluorouracil (2000 mg/m^2 over 46 hours on days 1 and 2) every 2 weeks (D10, page 1659, right-hand column, third paragraph). Therefore, D10 discloses the claimed administration sequence, as well the claimed administration time of 5-fluorouracil. The fact that a second dose of irinotecan is administered on day 3 is irrelevant in the view of the open language of claim 1 (*"comprising"*).

5.5.4 D10 does not disclose that liposomal irinotecan is administered to the patients. Standard irinotecan is administered instead. Moreover, the administration times of irinotecan and leucovorin differ slightly in D10. As already seen above, the claimed administration times

are arbitrary and thus provide no contribution to the inventive step reasoning. In the following, we will therefore focus on the fact that standard irinotecan is administered in D10, while liposomal irinotecan is claimed instead.

5.5.5 The effect of this difference is not apparent from the contested patent, which does not compare the claimed dosage regimen with that of D10. As a matter of fact, the patent contains no results whatsoever of the clinical study whose protocol is disclosed in example 7. Therefore, the objective technical problem to be solved starting from D10 is the provision of an alternative treatment of pancreatic cancer.

5.5.6 The solution to this problem is obvious in the light of the known efficacy and safety of liposomal irinotecan, also in combination with leucovorin and 5-fluorouracil, in the claimed setting, which belonged to the common general knowledge at the relevant date (see paragraphs 5.1.2-3 above with reference to review article D7).

5.5.7 Should the patentee be able to show a technical effect of the claimed regimen over the one disclosed in D10, we note that it also belonged to the common general knowledge that liposomal irinotecan was superior to standard irinotecan:

> *"In a series of preclinical studies, nanoliposomal CPT-11 demonstrated significantly superior efficacy when compared to free CPT-11 at the same or higher dose, including frequent cures in some models. The superiority of nanoliposomal CPT-11 over free CPT-11 has been observed in different tumor models including colorectal, gastric, breast, cervical, glioma, pancreatic and lung cancer models. In addition to superior efficacy, nanoliposomal CPT-11 has shown a more favorable pharmacologic profile and reduced toxicity in multiple preclinical models."*
> (D7, page 189, left-hand column, third paragraph, emphasis added)

5.5.8 Therefore, even the solution of a more ambitious problem, the provision of a somehow improved treatment of pancreatic cancer, would have been obvious in the light of the common general knowledge.

5.5.9 As explained in the problem solution approach starting from D7, the skilled person would also have consulted any of documents D2 and D6. They would have learned that the claimed combination had already progressed to phase II in the closely related indication colorectal cancer, thus reinforcing their expectation that the claimed regimen would have

provided an alternative (or even an improved) treatment of pancreatic cancer in patients who have failed prior treatment with gemcitabine.

5.5.10 Hence, claim 1 of the contested patent does not involve an inventive step over document D10 in the light of the common general knowledge or in combination with any of documents D6 or D2 and thus does not fulfil the requirements of Article 56 EPC.

5.6 Lack of inventive step of the remaining claims

5.6.1 Claim 2 specifies that, prior to each administration of irinotecan sucrose octasulfate salt liposome injection, the patient is pre-medicated with dexamethasone and/or a 5-HT^3 antagonist or another anti-emetic. However, it belonged to the common general knowledge that irinotecan is emetogenic and that patients should receive a premedication with antiemetic agents. It was thus standard practice to administer a premedication with antiemetic agents, such as dexamethasone given in conjunction with another type of antiemetic agent, such as a 5-HT^3 blocker (D8, page 19, third paragraph). Hence, claim 2 is obvious for the same reasons as claim 1.

5.6.2 Claim 3 specifies that the pancreatic cancer can be any type of exocrine pancreatic cancer. Claim 4 specifies that the cancer is advanced pancreatic cancer. All the cited prior art deals with advanced, i.e., metastatic, pancreatic cancer and specifically with adenocarcinoma of the pancreas, the most common type of pancreatic cancer (see, e.g., D5, page 5, *"Eligibility"*). Hence, the problem solution approach illustrated for claim 1 can be applied to claim 3 and 4 without modifications. Claims 3 and 4 are thus obvious.

5.6.3 Claim 5 specifies that each cycle is a period of 2 weeks. The length of the treatment cycle is already specified on page 3 of D6, *"Study Description" Arm II (FUPEP*). Therefore, claim 5 does not involve an inventive step for the same reasons of claim 1.

5.6.4 Claim 6 specifies that (a) the irinotecan sucrose octasulfate salt liposome injection is administered intravenously at a dose of: 80 mg/m^2 every 2 weeks to patients who are not homozygous for the UGTA1*28 allele or; 60 mg/m^2 every 2 weeks to patients who are homozygous for the UGTA1*28 allele, and wherein the dose is increased to 80 mg/m^2 if the patient does not experience any drug related toxicity; (b) the 5-FU is administered intravenously at a dose of 2400 mg/m^2 over 46 hours, and the leucovorin is administered intravenously at a dose of 200 mg/m^2 over 30 minutes, every 2 weeks; wherein the patient has

been premedicated with dexamethasone and a 5-HT^3 antagonist; and wherein the patient has metastatic pancreatic cancer that has progressed on gemcitabine based therapy.

5.6.5 It belonged to the common general knowledge that the dosage of irinotecan had to be reduced in patients who are homozygous for the UGTA1*28 allele. This has been correctly recognised by the Board of Appeal in charge of the parent case (D16, item 2.4.3).

5.6.6 Moreover, the Boards of Appeal have stated several times that finding the optimum dosage is a matter of routine experimentation, which does not require inventive skill (T 1760/08, T 1409/06).

5.6.7 In case T 1409/06, the data in the patent showed that the best antiemetic effects were obtained by i.v. administration of 1 mg and 3 mg granisetron as compared to 0.1 mg disclosed in the prior art, where the effect was less accentuated. The Board of Appeal revoked the opposed European patent for the following reasons:

> *"The board is of the opinion that the mere determination of the dosage which yields the best effect does not involve an inventive step when, as in the present case, the effect as such is already known or obvious. The person skilled in the art is aware that the intensity of a pharmacological effect depends inter alia on the concentration of the active agent. Finding the optimum dosage is a matter of routine experimentation, which does not require inventive skill."*
> (T 1409/06, r. 3.2.1)

5.6.8 The same applies to the present case: D7 already teaches that the combination of liposomal irinotecan, leucovorin and 5-fluorouracil is safe and effective in the treatment of second-line pancreatic cancer. No technical effect is achieved by the claimed dose that was not already known or obvious based on the prior art. Hence, claim 6 is not inventive.

6. Conclusion

It has been shown above that the contested patent is not in accordance with the requirements of Articles 56 and 76(1) EPC. Therefore, the request to revoke EP 3 266 456 in its entirety is fully justified.

Marco Fachini

Enc.
Documents D1-D16

Europäisches Patentamt
European Patent Office
Office européen des brevets

Notice of opposition to a European patent

I. Patent opposed

Patent No.	EP3266456
Application No.	EP17169098.5
Date of mention of the grant in the European Patent Bulletin (Art. 97(3), Art. 99(1) EPC)	05 May 2021
Title of the invention	COMBINATIONS OF LIPOSOMAL IRINOTECAN, 5-FU AND LEUCOVORIN FOR THE TREATMENT OF PANCREATIC CANCER

II. Proprietor of the patent

first named in the patent specification	Ipsen Biopharm Ltd.
Opponent's or representative's reference	X112963EPA KJG

III. Opponent

Name	Teva Pharmaceutical Industries Ltd
Address:	124 Dvora HaNevi'a St. 6944020 Tel Aviv Israel
State of residence or of principal place of business	Israel
Multiple opponents (see additional sheet)	☐

IV. Authorisation

1. Representative

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Association No.:	672	
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Additional representative(s) on additional sheet/see authorisation	☐	

Authorisation(s)

is/are enclosed ☐

has/have been registered under No. ☐

V. Opposition is filed against

the patent as a whole ☒

claim(s) No(s).

VI. Grounds for opposition:

Opposition is based on the following grounds:

(a) the subject-matter of the European patent opposed is not patentable (Art. 100(a) EPC) because:

- it is not new (Art. 52(1); Art. 54 EPC) ☒
- it does not involve an inventive step (Art. 52(1); Art. 56 EPC) ☒
- patentability is excluded on other grounds, namely articles ☐

(b) the patent opposed does not disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art (Art. 100(b) EPC; see Art. 83 EPC). ☒

(c) the subject-matter of the patent opposed extends beyond the content of the application/of the earlier application as filed (Art. 100(c) EPC, see Art. 123(2) EPC). ☒

VII. Facts (Rule 76(2)(c) EPC)

presented in support of the opposition are submitted herewith on an attached document ☒

VIII. Other requests:

- Oral proceedings are hereby requested auxiliarily.

IX. Evidence presented

D1	Other evidence	http://clinicaltrials.gov/archive/ NCT01494506/2011_12_16 original file name: D1 X112963EPA.pdf attached as: Other-evidence-1.pdf
D10	Other evidence	EC Decision granting MA for Onivyde-irinotecan original file name: D10 X112963EPA.pdf attached as: Other-evidence-8.pdf
D11	Other evidence	Ko A H et al, J Clin Oncol 29 (2011) abstract 237 original file name: D11 X112963EPA.pdf attached as: Other-evidence-9.pdf
D12	Other evidence	Wang-Gillam A et al, The Lancet published online on 22 November 2015 original file name: D12 X112963EPA.pdf attached as: Other-evidence-10.pdf
D13	Other evidence	Press release dated 22 October 2015 original file name: D13 X112963EPA.pdf attached as: Other-evidence-11.pdf
D14	Other evidence	Public Assessment Report - fluorouracil original file name: D14 X112963EPA.pdf attached as: Other-evidence-12.pdf
D15	Other evidence	Gebbia V et al., Am J Clin Oncol (2008) 33:461-464 original file name: D15 X112963EPA.pdf attached as: Other-evidence-13.pdf
D16	Other evidence	Chen Let al., J Clin Oncol (2008) 26:2565 original file name: D16 X112963EPA.pdf attached as: Other-evidence-14.pdf
D17	Other evidence	L. Chen, et. al., Journal of Clinical Oncology 2010 28:15_suppl, e13024 original file name: D17 X112963EPA.pdf attached as: Other-evidence-15.pdf
D18	Other evidence	Pin-Yuan Chen et al, Neuro-Oncology 15(2):189-197 (December 2012) original file name: D18 X112963EPA.pdf attached as: Other-evidence-16.pdf
D19	Other evidence	Drummond DC et al, Cancer Res 2006; 66: 3271-3277 (2006) original file name: D19 X112963EPA.pdf attached as: Other-evidence-17.pdf
D2	Other evidence	Tsai C-S et al., J Gastrointest Oncol (2011) 2(3):185-194 original file name: D2 X112963EPA.pdf attached as: Other-evidence-2.pdf
D3	Other evidence	Hoskins J M et al., J Natl Cancer Inst (2007) 99:1290-5 original file name: D3 X112963EPA.pdf attached as: Other-evidence-3.pdf
D4	Other evidence	Brixi-Benmansour H et al, Digestive and Liver Disease, 43 (2011) 912-916 original file name: D4 X112963EPA.pdf

		attached as: Other-evidence-4.pdf
D5	Other evidence	Infante et al., Cancer Chemother Pharmacol (2012) 70(5), 699 original file name: D5 X112963EPA.pdf attached as: Other-evidence-5.pdf
D6	Other evidence	http://clinicaltrials.gov/archive/ NCT01494506/2012_08_09 original file name: D6 X112963EPA.pdf attached as: Other-evidence-6.pdf
D7	Patent document	WO2013138371 (A1) original file name: D7 X112963EPA.pdf attached as: Published-Evidence-1.pdf
D8	Patent document	WO2012146610 (A1) original file name: D8 X112963EPA.pdf attached as: Published-Evidence-2.pdf
D9	Other evidence	Yoo et al., Br J Cancer (2009) 101:1658-1663 original file name: D9 X112963EPA.pdf attached as: Other-evidence-7.pdf

X. Payment

Method of payment: Debit from deposit account

The European Patent Office is hereby authorised, to debit from the deposit account with the EPO any fees and costs indicated in the fees section below.

Currency: EUR

Deposit account number: 28050042

Account holder: D Young & Co LLP

Refunds

Any refunds should be made to EPO deposit account: 28050042

Account holder: D Young & Co LLP

Fees	Factor applied	Fee schedule	Amount to be paid
010 Opposition fee	1	815.00	815.00
Total:		EUR	815.00

	A Forms	Details:	System file name:
A-1	Form for notice of opposition		ep-oppo.pdf

	B Attached document files	Original file name:	System file name:
B-1	1. Facts and arguments	Opposition Statement X112963EPA.pdf	OPPO.pdf

	C Attached evidence files	Original file name:	System file name:
C-1	1. Patent document	D7 X112963EPA.pdf	Published-Evidence-1.pdf
C-2	2. Patent document	D8 X112963EPA.pdf	Published-Evidence-2.pdf
C-3	1. Other evidence	D1 X112963EPA.pdf	Other-evidence-1.pdf
C-4	2. Other evidence	D3 X112963EPA.pdf	Other-evidence-3.pdf
C-5	3. Other evidence	D4 X112963EPA.pdf	Other-evidence-4.pdf
C-6	4. Other evidence	D9 X112963EPA.pdf	Other-evidence-7.pdf
C-7	5. Other evidence	D10 X112963EPA.pdf	Other-evidence-8.pdf

C-8	6. Other evidence	D11 X112963EPA.pdf	Other-evidence-9.pdf
C-9	7. Other evidence	D12 X112963EPA.pdf	Other-evidence-10.pdf
C-10	8. Other evidence	D13 X112963EPA.pdf	Other-evidence-11.pdf
C-11	9. Other evidence	D14 X112963EPA.pdf	Other-evidence-12.pdf
C-12	10. Other evidence	D15 X112963EPA.pdf	Other-evidence-13.pdf
C-13	11. Other evidence	D16 X112963EPA.pdf	Other-evidence-14.pdf
C-14	12. Other evidence	D17 X112963EPA.pdf	Other-evidence-15.pdf
C-15	13. Other evidence	D19 X112963EPA.pdf	Other-evidence-17.pdf
C-16	14. Other evidence	D2 X112963EPA.pdf	Other-evidence-2.pdf
C-17	15. Other evidence	D5 X112963EPA.pdf	Other-evidence-5.pdf
C-18	16. Other evidence	D6 X112963EPA.pdf	Other-evidence-6.pdf
C-19	17. Other evidence	D18 X112963EPA.pdf	Other-evidence-16.pdf

Signature of opponent or representative

Place	**London, UK**
Date:	**02 February 2022**
Signed by:	**Laura Jennings 61687**
Association:	**D Young & Co LLP**
Representative name:	**Kirk Gallagher**
Capacity:	**(Representative)**

OPPOSITION AGAINST EP3266456B

IPSEN BIOPHARM LTD

OPPOSED BY TEVA PHARMACEUTICAL INDUSTRIES LIMITED

FACTS AND ARGUMENTS (Rule 76 EPC)

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1. REQUESTS

1.1. We request revocation of EP3266456B in its entirety under Article 100(a), (b) and (c) EPC. In the event that this request is not granted on the basis of the written submissions alone, we request Oral Proceedings.

2. THE OPPOSED PATENT

2.1. EP3266456B ("the Patent") was granted on 5 May 2021, based on application no. 17169098.5 having a filing date of 12 June 2013 and claiming priority from earlier applications dated 13 June 2012 (US201261659211P) and 14 March 2013 (US201361784382P). The Patent is a divisional of EP2861210B.

2.2. The Patent is concerned with a treatment regimen for liposomal irinotecan, 5-fluorouracil (5-FU) and leucovorin in treating pancreatic cancer in patients who have failed prior treatment with gemcitabine or become resistant to gemcitabine (gemcitabine-refractory PC).

2.3. In particular, independent claim 1 is directed to:

> "Irinotecan sucrose octasulfate salt liposome injection for use in a method of treating pancreatic cancer in a human patient who has failed prior treatment with gemcitabine or become resistant to gemcitabine, the method comprising co-administration of an effective amount each of irinotecan sucrose octasulfate salt liposome injection, 5-fluorouracil (5-FU) and leucovorin to the patient,
>
> wherein, in each cycle, the irinotecan sucrose octasulfate salt liposome injection is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU and, in the method:
>
> (a) the 5-FU is administered intravenously over 46 hours;
>
> (b) the leucovorin is administered intravenously over 30 minutes, and
>
> (c) the irinotecan sucrose octasulfate salt liposome injection is administered intravenously over 90 minutes."

2.4. Irinotecan sucrose octasulfate salt liposome injection is also referred to as MM-398 and PEP02.

2.5. There are 5 dependent claims of which claim 2 relates to a pre-medication step, claims 3 and 4 further define the nature of the PC, claim 5 defines the treatment cycle period as every 2 weeks, and claim 6 reads as follows:

> "The irinotecan sucrose octasulfate salt liposome injection for use according to any one of the preceding claims, wherein
>
> (a) the irinotecan sucrose octasulfate salt liposome injection is administered intravenously at a dose of:
>
> 80 mg/m^2 every 2 weeks to patients who are not homozygous for the UGTA1*28 allele or;
>
> 60 mg/m^2 every 2 weeks to patients who are homozygous for the UGTA1*28 allele, and wherein the dose is increased to 80 mg/m^2 if the patient does not experience any drug related toxicity;
>
> (b) the 5-FU is administered intravenously at a dose of 2400 mg/m^2 over 46 hours, and the leucovorin is administered intravenously at a dose of 200 mg/m^2 over 30 minutes, every 2 weeks;
>
> wherein irinotecan sucrose octasulfate salt liposome injection is administered prior to 5-FU and leucovorin, and wherein leucovorin is administered prior to 5-FU;
>
> wherein the patient has been premedicated with dexamethasone and a 5-HT$_3$ antagonist; and
>
> wherein the patient has metastatic pancreatic cancer that has progressed on gemcitabine based therapy."

2.6. The Patent contains seven examples. Examples 1-4 describe tests with MM-398 alone in animal cancer models. Example 5 describes a human pharmacokinetic study with MM-398 alone.

2.7. Example 6 describes a phase 1 dose escalation study combining MM-398 (80 mg/m^2), 5-FU and leucovorin in 16 patients with solid tumours. The study was prior published as D17. Whilst the level of detail is different, the study described in Example 6 of the Patent is the same study reported in D17 - same number of patients enrolled (16), same number of evaluable patients (15), same number of patients receiving 80 mg/m^2 of MM-398 (6), same number of responses (PR-2 (in gastric cancer and breast cancer), SD-9 and PD-4).

2.8. Example 7 is prophetic and describes a phase 3 human study which includes a treatment arm comprising MM-398 in combination with 5-FU and leucovorin. There are no results associated with Example 7.

3. EVIDENCE

3.1. The opponent relies on all the documents listed in Annex A. D1 to D14 were cited during examination proceedings. Although, D6 filed with the opposition is dated 8 August 2012; whereas, the European search report refers to a version dated 9 August 2012.

3.2. D1 to D4, D9, D11, D14 to D17 and D19 were published before the earliest claimed priority date (13 June 2012); whereas, D5, D6, D8 and D18 were published after the earliest priority date but before the filing date of the second priority document (14 March 2013) and are therefore prior art if the claim to the earliest priority date is not valid. D7, D10, D12 and D13 are not prior art.

4. ADDED SUBJECT MATTER

4.1. The application as filed appears identical to the original PCT application and therefore the following issues arise under both Article 76(1) and 123(2) EPC. However, for convenience all references are to the published PCT application WO2013/188586.

4.2. Support for the granted claims cannot be found in the published claims since independent claims 1, 3, 12, 23 and 24 all specify the drug dosages which are absent from granted claim 1.

4.3. The subject matter of the application is outlined in the Summary section on pages 2 to 6 of the published specification.

4.4. This Summary section commences with an introductory passage describing that the application concerns a particular clinical dosage regimen for liposomal irinotecan (MM-398) either alone or in combination with 5-FU and leucovorin in treating PC.

4.5. The next paragraph sets out a first 'aspect' of the invention (monotherapy) followed by an 'embodiment' relating to that aspect of the invention. This paragraph corresponds with claims 1 and 2 of the application.

4.6. Paragraph 3 of the Summary section, discloses a second aspect of the invention (combination therapy). This paragraph corresponds with independent claim 3 of the application.

4.7. The paragraphs that follow on page 4 of the specification describe a number of embodiments:

> "In one embodiment, in each cycle, the liposomal irinotecan is administered prior to the leucovorin and the leucovorin is administered prior to the 5-FU.
>
> In another embodiment, the liposomal irinotecan is administered intravenously over 90 minutes.
>
> In another embodiment, the 5-FU is administered intravenously over 46 hours.
>
> In another embodiment, leucovorin is administered intravenously over 30 minutes."

4.8. These embodiments correspond to published claims 4, 6, 7 and 8 all of which depend from independent claim 3.

4.9. An objective reading of these embodiments would clearly link them with the dosage regimen described in the second aspect of the invention and in independent claim 3.

4.10. Granted claim 1 was arrived at by combining each of these embodiments (corresponding to dependent claims 4 and 6-8) with the introductory paragraph of the Summary section and by ignoring the core elements of the invention, i.e. the clinical dosages, which are set out in the aspects of the invention and in the independent claims.[1]

4.11. There is no direct and unambiguous basis in the application as filed for the subject matter of granted claim 1. In arriving at this subject matter the proprietor has removed the heart of its original disclosure which relates to the clinical dosages used in treating PC. This is clear from the fact that these dosages are the subject matter of each of the 'aspects' of the invention and all the independent claims. It is even clear from the introductory paragraph of the Summary section which refers to a particular clinical dosage regimen, yet the claims do not now include those clinical dosages.

4.12. Put another way, a skilled person reading the original application would have anticipated that any claims granted on this application would have included the dosage of at least irinotecan as disclosed in all the aspects of the invention, in all the clinical studies described in the application (Examples 5, 6 and 7) and in all the independent claims. Therefore, the granted claims which are without any such limitation present the skilled person with new information which is not directly and unambiguously derivable from the original application thereby contravening the gold standard set down in G2/10 and providing the proprietor with an unwarranted advantage in violation of the principles described in G1/93.

4.13. Overall, therefore, the Patent contravenes Article 76(1) and 123(2) EPC

5. SUFFICIENCY OF DISCLOSURE

5.1. The claimed subject matter cannot meet the requirements of both Article 83 EPC and Article 56 EPC.

5.2. In the present case, the claims relate to a combination of MM-398, 5-FU and leucovorin for use in a method of treating gemcitabine-refractory PC where the drug combination is administered according to a particular regimen.

5.3. The established case law of the Boards of Appeal requires that in the case of a medical use the patent / application must disclose the suitability of the product for use in the claimed therapeutic application. The relevant disclosure need not be in the form of clinical studies but it is clear that a simple verbal statement is not enough (T0609/02, reasons 9).

5.4. Moreover, in this case the prior art, e.g. D11 and D2, already describes the suitability of a combination of MM-398, 5-FU and leucovorin for use in a method of treating PC. Therefore, the alleged technical contribution relates not to the therapeutic application *per se* but the treatment regimen. Therefore, according to the case law (e.g. T1592/12) the Patent must demonstrate the suitability of the claimed regimen for treating the form of PC mentioned in the claims. Only if this suitability hurdle has been overcome may post-

[1] Applicant's letter dated 10 September 2020 filed during examination proceedings.

published evidence be taken into account to back-up the findings in the Patent in relation to the claimed medical use.

5.5. Example 6 of the Patent appears to describe the suitability of MM-398 in combination with 5-FU and leucovorin for treating PC generally, although as noted above this information was already known from the prior art. However, the Patent fails to describe the suitability of the other features of the claimed treatment regimen including that the patients have failed prior treatment with or become resistant to gemcitabine, the administration period of any of the drugs, or the order of administration of the drugs.

5.6. Therefore, in order to meet the requirements of Article 83 EPC, it is necessary to rely on the common general knowledge to teach the suitability of the claimed regimen for treating the recited form of PC. Thus, either the Patent is insufficiently disclosed because the claimed treatment regimen is not fully supported by the information content of the Patent together with the common general knowledge, or the missing features of the claimed treatment regimen are to be found in the common general knowledge, which has implications for the assessment of inventive step.

6. PRIORITY

6.1. The Patent is not entitled to benefit from the filling date (13 June 2012) of the earlier application (US201261659211P) as the Patent and the earlier application do not relate to the same invention.

6.2. The substantive reasons are similar to those set out above under the heading of Added Subject Matter. In essence, the invention presented in US201261659211P relates to a clinical dosage regimen which specifies the actual dosages of each of the component drugs, i.e. MM-398, 5-FU and leucovorin. This can be seen from the two aspects of the invention presented on page 3 of the earlier application, from all the clinical examples of the earlier application (Examples 5-7) and all the independent claims of the earlier application.

6.3. As claim 1 of the Patent does not describe the dosages of any of MM-398, 5-FU or leucovorin it relates to a different invention from that disclosed in the earlier application and therefore the requirements of Article 87(1) EPC are not fulfilled.

7. INVENTIVE STEP

Background

7.1. It was well known before the relevant date that MM-398 (formerly designated PEP02) is a liposomal formulation of irinotecan having superior properties to free irinotecan.

7.2. Thus, D2 describes that "*To realize the potential advantages of nanoparticle delivery, a novel liposome-based construct termed "nanoliposomal CPT-11 (nLs-CPT-11)" was developed, which encapsulates CPT-11 with unprecedented efficiency and stability (27)*" (page 189, first complete paragraph). Reference 27, in the above quoted passage (herein

D19) describes the preparation of nanoliposomal irinotecan formulations using sucrose octasulfate as the intraliposomal trapping agent.

7.3. D2 also describes that MM-398 has superior efficacy, a more favourable pharmacologic profile and reduced toxicity compared to free irinotecan, see page 188, col. 2; through to page 191, col. 1, especially page 189 final complete paragraph:

> "In a series of preclinical studies, nanoliposomal CPT-11 demonstrated significantly superior efficacy when compared to free CPT-11 at the same or higher dose, including frequent cures in some models. The superiority of nanoliposomal CPT-11 over free CPT-11 has been observed in different tumor models including colorectal, gastric, breast, cervical, glioma, pancreatic and lung cancer models. In addition to superior efficacy, nanoliposomal CPT-11 has shown a more favorable pharmacologic profile and reduced toxicity in multiple preclinical models."

7.4. Other disclosure confirming that MM-398 (PEP02) is a liposomal formulation of irinotecan are D16, D11 and D17.

7.5. It was also known that MM-398 is a liposomal irinotecan sucrose octasulfate salt formulation, as described in granted claim 1.

7.6. Thus, D18 describes that "*Nanoliposomal irinotecan (MM-398) is a highly stabilized liposomal formulation containing nano-sized irinotecan crystals complexed with sucrose octasulfate in the liposome interior*[15]" (page 198, col. 2). Reference 15, in this passage is document D19 mentioned above.

Selection of the closest prior art

7.7. In a proper application of the problem-solution approach the inventive step analysis is run from all documents which represent a feasible (or suitable) starting point. This is necessary to ensure that the claimed subject matter is not obvious over the state of the art as required by Article 56 EPC.

7.8. This is reflected in the established case law of the Boards of Appeal which require that the inventive step analysis is carried out starting from all feasible closest prior art documents and that to deny the existence of an inventive step, it is only necessary to demonstrate that one of the feasible starting points leads, in an obvious manner, to the claimed invention, see, e.g., T 967/97, reasons 3.2; T 1514/05, reasons 3.1.6; T 21/08, reasons 1.2.3; T 561/11, reasons 1.2.2; T 1289/09, reasons 4.5.4; T 1921/12, reasons 7.2; T 1742/1, reasons 6.6; and T 591/04, reasons 4.1 etc.

7.9. In the present case all of D11, D2, D9 and D6 are suitable starting points for the inventive step analysis.

D11 as Closest Prior Art

7.10. D11 discloses that in phase I clinical studies PEP02 (MM-398) either alone or in combination with 5-FU and leucovorin (LV) demonstrated prolonged disease control in 5 of 7 (71%) patients with gemcitabine-refractory advanced PC.

7.11. Clearly, therefore, D11 is concerned with similar issues as the Patent and is thus a suitable closest prior art document.

7.12. D11 discloses both monotherapy and combination therapy with MM-398; however, the logic of the problem-solution approach requires that the starting point of the inventive step analysis should be that embodiment requiring the minimum of structural and functional modifications. In other words, the combination therapy embodiment of D11 is directed to the same purpose as the Patent, i.e. the treatment of gemcitabine-refractory PC, and has the most features in common with the claimed subject matter, i.e. it relates to the same three drugs specified in the Patent.

7.13. Moreover, whilst the phase I results for MM-398 monotherapy and combination therapy are pooled, it is clear from the language used in D11 that both therapies were effective. Therefore, whilst it is true that D11 focuses more on the phase II study concerning MM-398 monotherapy this does not detract from the positive results reported with the combination therapy. There is no reason why the skilled person would not consider these positive results in human phase I studies as significant.

7.14. In particular, as noted by the Board in T2506/12, reasons 3.10, human clinical studies are usually only commenced when there is already favourable existing scientific data. Therefore, in the case of D11, the skilled person would reasonably expect that there is underlying scientific data which formed the basis of the decision to commence human clinical studies with MM-398, leucovorin and 5-FU in the first place. This is in addition to the positive results in phase I clinical studies disclosed in D11 itself. Such human clinical data would not be ignored by the skilled person. To the contrary, it is amongst the best type of evidence that is available in medical research. In other words, it is not a question of which one of the disclosures in D11 the skilled person would consider, the treatment with MM-398 monotherapy or the combination treatment with MM-398, leucovorin and 5-FU, there is no reason why the skilled person would not consider both of these relevant disclosures.

7.15. Starting from the disclosure in D11 of MM-398 combination therapy, the distinguishing features are the administration times for each of the drugs and the order of administration for the drugs. However, there is no evidence of any advantageous effects associated with the claimed treatment regimen.

7.16. Although D11 discloses that MM-398 is a nanoparticle liposome formulation of irinotecan it does not disclose that MM-398 is irinotecan sucrose octasulfate salt liposome injection. However, as outlined above, at the relevant date it was already known from D18 that MM-398 is a liposomal formulation containing nano-sized irinotecan crystals complexed with sucrose octasulfate in the liposome interior (page 190, col. 2).

7.17. In any event, there is no evidence that irinotecan sucrose octasulfate salt liposome is advantageous in the treatment of gemcitabine-refractory PC compared to liposomal formulations of irinotecan more generally.

7.18. Overall, therefore, in view of the absence of any advantageous effects associated with the distinguishing features, the objective technical problem may be formulated as the provision of a further treatment for gemcitabine-refractory PC.

7.19. The solution to this objective technical problem would be arrived at without inventive skill.

7.20. As noted above under the heading Sufficiency of Disclosure, the suitability of the claimed regimen for treating gemcitabine-refractory PC is not described in the Patent and therefore if the requirements of Article 83 EPC are fulfilled it must be on the basis that these distinguishing features are well-known in the prior art. For instance, the order of administration (MM-398 before leucovorin before 5-FU) appears to follow the same order used in the established FOLFIRI regimen (D15, D4 and Patent paragraph [0004]). As regards the drug administration times, D11 itself describes administering PEP02 (MM-398) by infusion over 90 minutes. Moreover, liposomal formulations of irinotecan using sucrose octasulfate as the trapping agent were known from e.g. D19.

7.21. As such, claim 1 lacks an inventive step over D11.

Dependent claims

7.22. None of the dependent claims provide any additional features that render the claimed subject matter inventive over D11. In particular, the additional subject matter is either obvious based on the analysis given above for claim 1 or has no unexpected technical effects associated with it and would be arrived at in light of the above discussed prior art or common general knowledge.

D2 as Closest Prior Art

7.23. The claimed subject matter also lacks an inventive step over D2 for reasons similar to those given above in relation to D11. In particular, the skilled person would note the positive phase I clinical data for the combination regimen. This type of data in human subjects is always significant.

7.24. As noted above, selecting the closest prior art document or selecting the closest embodiment in a prior art document as the first step in the problem-solution approach is not an exclusionary choice. The skilled person would have considered the positive results with both MM-398 alone and MM-398 combined with 5-FU and leucovorin as worthy of further study, i.e. as feasible / suitable closest prior art embodiments.

7.25. In other words, the disclosure in D2 that the combination of MM-398, leucovorin and 5-FU is efficacious in treating gemcitabine-refractory PC represents a feasible starting point for the inventive step analysis. It certainly fulfils the criteria of being "*that [disclosure] which corresponds to a similar use and requires the minimum of structural and functional modifications to arrive at the claimed invention (see T 606/89)*", GL (G-VII, 5.1).

7.26. Starting from this disclosure in D2 the problem solution analysis is essentially the same as that described above for D11.

Dependent claims

7.27. None of the dependent claims provide any additional features that render the claimed subject matter inventive over D2. In particular, the additional subject matter is either obvious based on the analysis given above for claim 1 or has no unexpected technical effects associated with it and would be arrived at in light of the above discussed prior art or common general knowledge.

D9 as Closest Prior Art

7.28. D9 describes a clinical study in patients with PC who have failed first line gemcitabine based chemotherapy (page 1659, col. 2). The patients were treated according to a modified FOLFIRI.3 (mFOLFIRI.3) regimen which consists of (non-liposomal) irinotecan, leucovorin 400 mg/m^2 over 2 hours and 5-FU and 2000 mg/m^2 over 46 hours every two weeks (page 1659, col. 2). The results describe the regimen as safe and effective.

7.29. Thus, D9 describes treatment of the same patient group as the Patent with the same active ingredients, albeit irinotecan is formulated differently, according to a similar treatment regimen.

7.30. The differences between the claimed subject matter and the disclosure of D9 are (i) the use of MM-398 instead of (non-liposomal) irinotecan, (ii) the administration time of MM-398, and (iii) the administration time of leucovorin. There is no comparison between the mFOLFIRI.3 regimen of D9 and the regimen claimed in the Patent and therefore the

7.31. technical problem should be formulated as providing an alternative treatment regimen for gemcitabine-refractory PC. However, even if a more ambitious problem is formulated the claims still lack an inventive step.

7.32. As regards point (i), at the relevant date it was already known that MM-398 had activity against gemcitabine-refractory PC (e.g. D11 and D2) and that MM-398 was superior to the free form of irinotecan in terms of improved pharmacokinetics and tumour distribution, see D16, D11, D2 and D17. As such, it would have been obvious to replace the (non-liposomal) irinotecan in D9 with MM-398 in the reasonable expectation of solving even a more ambitious technical problem.

7.33. As regards point (ii), D16, D11, D2 and D17 all describe administration of MM-398 over 90 minutes and therefore this claim feature appears routine.

7.34. As regards point (iii), it has not been shown that this difference (30 mins vs 120 mins) has any technical effect on the treatment outcome and therefore appears arbitrary.

7.35. Accordingly, claim 1 lacks an inventive step starting from D9 as closest prior art.

Dependent claims

7.36. None of the dependent claims provide any additional features that render the claimed subject matter inventive over D9. In particular, the additional subject matter is either obvious based on the analysis given above for claim 1 or has no unexpected technical

effects associated with it and would be arrived at in light of the above discussed prior art or common general knowledge.

D6 as closest prior art

7.37. D6 published on 8 August 2012 and is therefore citable under Article 54(2) EPC for subject matter not entitled to claim the filling date of the earliest priority application.

7.38. D6 is clearly directed to the same purpose as the Patent, i.e. the treatment of gemcitabine-refractory PC and also requires a small number of structural and functional modifications to arrive at the claimed subject matter, i.e. Arm C of D6 teaches a similar treatment regimen to that claimed.

7.39. Whilst D6 itself does not describe the nature of MM-398, by this time it was already known that MM-398 was irinotecan sucrose octasulfate salt liposome (e.g. D18).

7.40. The differences between the claimed subject matter and the disclosure of D6 are (i) the lack of any therapeutic results for the described study, (ii) the order of administration of the drugs in the treatment regimen, and (iii) the administration time for any of the drugs.

7.41. For the reasons discussed previously in relation to sufficiency of disclosure, there is no evidence in the Patent that the claimed treatment regimen is a safe and effective treatment for gemcitabine-refractory PC. As there is no plausible disclosure in the Patent, post-published evidence cannot be used as the sole basis for evidencing that the technical problem has been solved (CLBA-I.D.4.6). Thus, the technical problem should be formulated along the lines of providing an arbitrary treatment and the claimed subject matter therefore lacks an inventive step.

7.42. However, in the event that a more ambitious technical problem is considered appropriate, the claimed subject matter still lacks an inventive step. In particular, the skilled person would have had a reasonable expectation that the dosing regimen described in Arm C of D6 would provide a safe and effective treatment for gemcitabine-refractory PC.

7.43. With regard to difference (i), the Boards of Appeal have consistently decided that the disclosure of a detailed clinical trial protocol provides the skilled person with a reasonable expectation of the success for the therapy under investigation, unless there was evidence to the contrary (T0239/16, T2506/12 and T0096/20).

7.44. With regard to differences (ii) and (iii), there is no evidence of any particular technical effect associated with these features. Moreover, as discussed previously, the suitability of the claimed regimen for treating gemcitabine-refractory PC is not described in the Patent and therefore if the requirements of Article 83 EPC are fulfilled it must be on the basis that these distinguishing features are well-known in the prior art. For instance, the order of administration (MM-398 before leucovorin before 5-FU) appears to follow the same order used in the established FOLFIRI regimen (D15, D4 and Patent paragraph [0004]). As regards the drug administration times, D11 and D2 describe administering PEP02 (MM-398) by infusion over 90 minutes.

7.45. Thus, overall, we believe the claimed subject matter lacks an inventive step over D6.

Dependent claims

7.46. None of the dependent claims provide any additional features that render the claimed subject matter inventive over D6. In particular, the additional subject matter is either obvious based on the analysis given above for claim 1 or has no unexpected technical effects associated with it and would be arrived at in light of the above discussed prior art or common general knowledge.

Annex A

D1	http://clinicaltrials.gov/archive/ NCT01494506/2011_12_16
D2	Tsai C-S et al., J Gastrointest Oncol (2011) 2(3):185-194
D3	Hoskins J M et al., J Natl Cancer Inst (2007) 99:1290-5
D4	Brixi-Benmansour H et al, Digestive and Liver Disease, 43 (2011) 912-916
D5	Infante et al., Cancer Chemother Pharmacol (2012) 70(5), 699
D6	http://clinicaltrials.gov/archive/ NCT01494506/2012_08_09
D7	WO2013138371
D8	WO2012146610
D9	Yoo et al., Br J Cancer (2009) 101:1658-1663
D10	EC Decision granting MA for Onivyde-irinotecan
D11	Ko A H et al, J Clin Oncol 29 (2011) abstract 237
D12	Wang-Gillam A et al, The Lancet published online on 22 November 2015
D13	Press release dated 22 October 2015
D14	Public Assessment Report - fluorouracil
D15	Gebbia V et al., Am J Clin Oncol (2010) 33:461-464
D16	Chen Let al., J Clin Oncol (2008) 26:2565
D17	L. Chen, et. al., Journal of Clinical Oncology 2010 28:15_suppl, e13024
D18	Pin-Yuan Chen et al, Neuro-Oncology 15(2):189–197 (December 2012)
D19	Drummond DC et al, Cancer Res 2006; 66: 3271–3277 (2006)

Europäisches Patentamt
European Patent Office
Office européen des brevets

Notice of opposition to a European patent

I. Patent opposed

Patent No.	EP3266456
Application No.	EP17169098.5
Date of mention of the grant in the European Patent Bulletin (Art. 97(3), Art. 99(1) EPC)	05 May 2021
Title of the invention	COMBINATIONS OF LIPOSOMAL IRINOTECAN, 5-FU AND LEUCOVORIN FOR THE TREATMENT OF PANCREATIC CANCER

II. Proprietor of the patent

first named in the patent specification	Ipsen Biopharm Ltd.
Opponent's or representative's reference	RSC/R81206OP

III. Opponent

Name	Generics [UK] Limited
Address:	Building 4 Trident Place Mosquito Way Hatfield Hertfordshire AL10 9UL United Kingdom
State of residence or of principal place of business	United Kingdom
Multiple opponents (see additional sheet)	☐

IV. Authorisation

1. Representative

	Elkington and Fife LLP
Association No.:	922
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Additional representative(s) on additional sheet/see authorisation ☐

Authorisation(s)

is/are enclosed ☐

has/have been registered under No. ☐

V. Opposition is filed against

the patent as a whole ☒

claim(s) No(s).

VI. Grounds for opposition:

Opposition is based on the following grounds:

(a) the subject-matter of the European patent opposed is not patentable (Art. 100(a) EPC) because:

- it is not new (Art. 52(1); Art. 54 EPC) ☐
- it does not involve an inventive step (Art. 52(1); Art. 56 EPC) ☒
- patentability is excluded on other grounds, namely articles ☐

(b) the patent opposed does not disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art (Art. 100(b) EPC; see Art. 83 EPC). ☐

(c) the subject-matter of the patent opposed extends beyond the content of the application/of the earlier application as filed (Art. 100(c) EPC, see Art. 123(2) EPC). ☒

VII. Facts (Rule 76(2)(c) EPC)

presented in support of the opposition are submitted herewith on an attached document ☒

VIII. Other requests:

- Oral proceedings are hereby requested auxiliarily.

IX. Evidence presented

D1	Other evidence	Clinical trials register NCT01494506 16/12/2011 original file name: D1.pdf attached as: Other-evidence-1.pdf
D10	Other evidence	Drummond, D.C. et al, Development of a highly active nanoliposomal irinotecan using a novel intraliposomal stabilisation original file name: D10.pdf attached as: Other-evidence-8.pdf
D11	Other evidence	Ko, A. H. et al A multinational phase II study of liposome irinotecan (PEP02) for patients original file name: D11.pdf attached as: Other-evidence-9.pdf
D12	Other evidence	Phase I study of liposome irinotecan (PEP02) in combination with weekly infusion of 5-FU/LV in advanced solid tumors original file name: D12.pdf attached as: Other-evidence-10.pdf
D13	Other evidence	Camptosar® patient information leaflet original file name: D13.pdf attached as: Other-evidence-11.pdf
D2	Other evidence	J. Gastrointest. Oncol. 2011, 185 original file name: D2.pdf attached as: Other-evidence-2.pdf
D3	Other evidence	J. Nat Cancer. Inst. 99, 1290 original file name: D3.pdf attached as: Other-evidence-3.pdf
D4	Other evidence	Digest. Liver. Dis. 2011, 43, 912 original file name: D4.pdf attached as: Other-evidence-4.pdf
D5	Other evidence	Cancer. Chemother. Pharmacol. 2012, 70, 699 original file name: D5.pdf attached as: Other-evidence-5.pdf
D6	Other evidence	http://clinicaltrials.gov/archive/NCT01494506/2012_08_09 original file name: D6.pdf attached as: Other-evidence-6.pdf
D7	Patent document	WO 2013/138371 (A1) , 19.09.2013 original file name: D7.pdf attached as: Published-Evidence-1.pdf
D8	Patent document	WO 2012/146610 (A1) , 01.11.2012 original file name: D8.pdf attached as: Published-Evidence-2.pdf
D9	Other evidence	Br. J. Cancer 2009, 101, 1658 original file name: D9.pdf attached as: Other-evidence-7.pdf

X. Payment

Method of payment	Debit from deposit account

The European Patent Office is hereby authorised, to debit from the deposit account with the EPO any fees and costs indicated in the fees section below.

Currency:	EUR
Deposit account number:	28050051
Account holder:	Elkington and Fife LLP

Refunds

Any refunds should be made to EPO deposit account:	28050051
Account holder:	Elkington and Fife LLP

Fees	Factor applied	Fee schedule	Amount to be paid
010 Opposition fee	1	815.00	815.00
Total:		EUR	815.00

	A Forms	Details:	System file name:
A-1	Form for notice of opposition		ep-oppo.pdf

	B Attached document files	Original file name:	System file name:
B-1	1. Facts and arguments	2022-02-02-EP 3 266 456 Grounds of Opposition_FINAL.pdf	OPPO.pdf
B-2	1. Any annexes (other than citation) to an opposition letter - Covering Letter	2022-02-04-R81206OP-EPO Opposition Letter.pdf	OTHER-1.pdf

	C Attached evidence files	Original file name:	System file name:
C-1	1. Patent document	D7.pdf	Published-Evidence-1.pdf
C-2	2. Patent document	D8.pdf	Published-Evidence-2.pdf
C-3	1. Other evidence	D3.pdf	Other-evidence-3.pdf
C-4	2. Other evidence	D4.pdf	Other-evidence-4.pdf
C-5	3. Other evidence	D5.pdf	Other-evidence-5.pdf

C-6	4. Other evidence	D6.pdf	Other-evidence-6.pdf
C-7	5. Other evidence	D1.pdf	Other-evidence-1.pdf
C-8	6. Other evidence	D9.pdf	Other-evidence-7.pdf
C-9	7. Other evidence	D10.pdf	Other-evidence-8.pdf
C-10	8. Other evidence	D11.pdf	Other-evidence-9.pdf
C-11	9. Other evidence	D12.pdf	Other-evidence-10.pdf
C-12	10. Other evidence	D13.pdf	Other-evidence-11.pdf
C-13	11. Other evidence	D2.pdf	Other-evidence-2.pdf

Signature of opponent or representative

Place	**London**
Date:	**04 February 2022**
Signed by:	**Sally Kennedy 57777**
Association:	**Elkington and Fife LLP**
Representative name:	**Sally Kennedy**
Capacity:	**(Representative)**

European patent no. 3 266 456
(application no. 17169098.5)
Ipsen Biopharm Ltd.
Opponent: Generics [UK] Limited

Notice of opposition

1. Introductory comments

(01) We request revocation of the patent in its entirety. We also request oral proceedings in the event that the opposition division intend to reach any other decision.

2. Documents

(02) We refer to D1 to D9 cited during prosecution and new documents D9 to D13. A copy of each document is enclosed.

3. Background

(03) The patent relates to the treatment of advanced pancreatic cancer that is refractory to treatment with gemcitabine.

(04) The patent claims a triple combination comprising irinotecan sucrose octasulfate liposomal injection, leucovorin (LV) and 5-fluoruoracil (5-FU), given in a particular administration schedule, for the treatment of this pancreatic cancer.

4. Added subject-matter (Articles 76(1), 123(2) and 100(c) EPC)

(05) This patent has been granted from a divisional application. The divisional application was filed without claims. The disclosure of divisional application and parent application appears to be identical, because the claims of the PCT application are included as numbered embodiments in the description of the divisional application.

(06) Therefore, the basis in the PCT application under A. 76(1) EPC and the divisional application under A. 123(2) EPC can be considered together by reference to the PCT application as filed.

(07) Claim 1 claims the following:

The drug:	irinotecan sucrose octasulfate salt liposome injection; 5-fluoruoracil (5-FU); and leucovorin (LV)

The medical use:	the treatment of pancreatic cancer
The patient group:	a human patient who has failed prior treatment with gemcitabine or become resistant to gemcitabine
The dosage regimen:	the irinotecan sucrose octasulfate is administered before 5-FU and LV, the LV is administered before 5-FU and: (a) the 5-FU is administered intravensouly over 46 hours (b) the leucovorin is administered intravenously over 30 minutes, and (c) the irinotecan sucrose octasulfate salt liposome injection is administered intravenously over 90 minutes

(08) Claim 1 does not find basis in the PCT application as filed because at least two selections are required to reach the claimed subject-matter.

4.1 First selection: the combination therapy

(09) The application as filed discloses liposomal irinotecan in two methods of treating pancreatic cancer. The first employs liposomal irinotecan alone. The second employs the triple combination of liposomal irinotecan and 5-FU/LV. There is no indication that combination therapy is preferred in either the description, the examples or the claims of the PCT application as filed.

The description

(10) In the description, each disclosure of the combination therapy of liposomal irinotecan, 5-fluorouracil (5-FU) and leucovorin (LV) has a corresponding disclosure of liposomal irinotecan monotherapy at an equal level of preference:

Page 3:

> Provided are methods for treating pancreatic cancer in a patient (*i.e.*, a human patient) comprising administering to the patient liposomal irinotecan (e.g., irinotecan sucrose octasulfate salt liposome injection, also referred to as MM-398) alone or in combination with 5-fluorouracil (5-FU) and leucovorin (together, 5-FU/LV), according to a particular clinical dosage regimen. Compositions adapted for use in such methods are also provided.

Page 12:

> IV. Administration
>
> Liposomal irinotecan is administered intravenously, either alone or in combination with 5-fluorouracil (5-FU) and/or leucovorin. In one embodiment,

The examples

(11) Likewise, monotherapy and combination therapy are disclosed at an equal level of preference in the examples, which relate to both liposomal irinotecan monotherapy and combination therapy with 5-FU/LV:

- Example 1 relates to liposomal irinotecan monotherapy in an *in vitro* orthotropic pancreatic cancer model;
- Example 2 investigates the accumulation of the active species SN-38 following administration of free irinotecan monotherapy and liposomal irinotecan monotherapy in an *in vivo* colon cancer model;
- Example 3 investigates the impact of liposomal irinotecan monotherapy on hypoxia markers in an *in vitro* human colon cancer cell model;
- Example 4 investigates the effect of liposomal irinotecan monotherapy on tumour vascularisation *in vivo*;
- Example 5 is a phase I clinical study investigating the pharmacokinetics of liposomal irinotecan monotherapy;
- Example 6 is a dose escalation study relating to liposomal irinotecan and 5-FU/LV combination therapy;
- Example 7 is a prophetic trial that compares the overall survival following liposomal irinotecan monotherapy (Arm A) or liposomal irinotecan, 5-FU/LV triple combination therapy (Arm C) against the overall survival of patients treated with 5-FU/LV. Both Arm A and Arm C are stated to be 'experimental', as opposed to 'control' (Table under B. Study Design, pages 25-26). Example 7 therefore relates to both liposomal irinotecan monotherapy and the triple combination.

(12) Therefore, the examples do not indicate that there is any preference for combination therapy, either.

The claims

(13) Finally, the liposomal irinotecan monotherapy is disclosed at an equal level of preference to the combination of liposomal irinotecan and 5-FU/LV in the claims, too.

(14) There are two independent method of treatment claims in the PCT application as filed. Claim 1 claims a method of treating a pancreatic cancer using liposomal irinotecan monotherapy, and claim 3 claims a method of treating a pancreatic cancer using liposomal irinotecan in combination with 5-FU/LV.

(15) Therefore, if a higher level of preference were to be assigned to one of these treatments, it would in fact be the monotherapy, which is disclosed at claim 1.

(16) The treatment of pancreatic cancer using liposomal irinotecan monotherapy alone and in combination with 5-FU/LV are, at best, disclosed at an equal level of preference. A selection must be made to select the claimed triple combination therapy.

4.2 Second selection: the patient group

(17) A second selection is required to reach the claimed patient group. During examination, the then-applicant pointed to page 12, lines 22-23 to provide basis for this patient group. The claimed patient group is one of several options disclosed at an equal level of preference at this passage:

- patients that exhibit a recurrent or persistent pancreatic cancer following primary chemotherapy;
- patients that have failed on at least one prior platinum based chemotherapy regimen for management of primary or recurrent disease;
- patients that have failed on gemcitabine therapy;
- patients that exhibit advanced pancreatic cancer; and
- patients that exhibit a pancreatic tumour that is refractory or resistant to other anti-cancer treatments.

(18) Therefore, in order to reach the subject-matter of claim 1 from the application as filed, the skilled person must first select the combination therapy and then select the patient group.

4.3 Summary

(19) Two selections are required to reach the subject matter of claim 1 from the PCT application as filed. The claims do not fulfil Articles 76(1) or 123(2) EPC.

5. Lack of inventive step over FOLFIRI (Articles 56 and 100(a) EPC)

5.1 The closest prior art and the distinguishing feature

(20) The patent is directed towards the provision of a treatment for pancreatic cancer in patients who have failed on prior treatment with gemcitabine.

(21) D9 (A randomised phase II study of modified FOLFIRI.3 *vs* modified FOLFOX as second-line therapy in patients with gemcitabine-refractory advanced pancreatic cancer) is a phase II study investigating the use of FOLFIRI (irinotecan, 5-FU and leucovorin) and FOLFOX (oxiplatin, leucovorin and 5-FU) combination therapies on GEM-refractory pancreatic cancer.

(22) Therefore, D9 is directed towards the same purpose as the patent and is a suitable starting point for inventive step analysis.

(23) The FOLFIRI treatment schedule of D9 included the administration of irinotecan over 1 hour, leucovorin over 2 hours, 5-FU over 46 hours and an additional irinotecan dose over 1 hour (page 1659):

> **Treatment dose and schedule**
>
> The mFOlFIRI.3 regimen consisted of irinotecan 70 mg m^{-2} (over 1 h) on day 1, leucovorin 400 mg m^{-2} (over 2 h) on day 1, 5-FU 2000 mg m^{-2} (over 46 h) from day 1, and irinotecan 70 mg m^{-2} (over 1 h) at the end of the 5-FU infusion every 2 weeks. The

(24) The trial showed promising results in this difficult to treat patient group (page 180):

> The results of this trial show that both combination regimens showed favourable efficacy and toxicity profiles in gemcitabine-pretreated patients with advanced pancreatic cancer. The 6-month survival rates were 27 and 30% and disease control rates were 23% and 17%, in patients treated with mFOLFIRI.3 and mFOLFOX, respectively. Of the 12 patients whose disease was controlled by these regimens, disease stabilisation was previously achieved in nine patients in gemcitabine-based regimens. The median PFS and median OS were 8.3 weeks and 16.6 weeks in the mFOLFIRI.3 arm, and 6.0 weeks and 14.9 weeks in the mFOLFOX arm, respectively. These were in line with the survival data of several previous studies (Tsavaris *et al*, 2005; Gebbia *et al*, 2007; Novarino *et al*, 2009).

(25) And the authors recommend these therapies for administration to patients with advanced pancreatic cancer who have previously been treated with gemcitabine (page 1662):

> In conclusion, our trial not only showed that both mFOLFIRI.3 and mFOLFOX regimens could be safely used but also showed modest anti-cancer activities in gemcitabine-pretreated patients. Although further clinical trials are necessary for comparison with other regimens, these protocols may be reasonable therapeutic options in a second-line setting for patients with advanced pancreatic cancer, who were previously treated with gemcitabine-based chemotherapy.

(26) Therefore, D9 discloses the claimed combination for the claimed medical use in the claimed patient group.

(27) Claim 1 is distinguished from D9 by the use of the irinotecan sucrose octasulfate liposomal injection instead of free irinotecan, the duration of the irinotecan infusion, the duration of the LV infusion and the order of administration of the combination partners.

5.2 The objective technical problem

(28) There is no comparison in the patent of the claimed treatment against that of the prior art. No effect is shown.

(29) The administration schedule disclosed in D9 and the schedule claimed compare as follows:

D9	Claim 1
1. Irinotecan over 1 hour	1. Liposomal irinotecan over 90 minutes
2. LV over 2 hours	2. LV over 30 minutes
3. 5-FU over 46 hours	3. 5-FU over 46 hours
4. Irinotecan over 1 hour, after 5-FU	-

(30) None of the examples of the patent compares these two administration schedules. Therefore, there is no technical effect arising from the claimed administration schedule.

(31) Examples 1 to 4 are preclinical studies that cannot demonstrate any technical effect arising from the claimed administration schedule. Examples 5, 6 and 7 of the patent are clinical trials.

(32) Example 5 investigates the pharmacokinetics of MM-398 as a single agent and therefore does not provide any data regarding the administration of the triple combination claimed.

(33) Example 6 is a dose escalation study that employs the claimed triple combination. However, there is no information provided regarding the order that each drug is administered or the duration of each infusion.

(34) Example 7 is the protocol of a phase 3 clinical trial. Arm C of the trial employs the claimed triple combination and the claimed mode of administration (Table under B. Study Design, page 16). However, as no comparison is made to any other administration schedule of the triple combination, and no data are provided, Example 7 does not show any technical effect arising from the claimed administration schedule, either.

(35) The objective technical problem is the provision of an alternative treatment for gemcitabine resistant pancreatic cancer.

(36) For completeness, during examination the then-applicant argued that that the claimed treatment was unexpectedly improved over the liposomal irinotecan monotherapy or therapy with 5-FU/LV (see the then-applicant's letter of 10 September 2020). However, these two comparative treatments are not the closest prior art.

(37) When a patent and the prior art relate to particular combinations, data comparing the claimed combination to individual active ingredient is irrelevant (T 512/02; r 3.3):

> "3.3 Alleged synergistic effect
> The appellant, making reference to the tests discussed in paragraph 2.5 above, argued that two non-obvious effects were shown for the compositions as claimed: surprisingly low plasma glucose levels and a reduction of the body weight increase caused by long-term application of pioglitazone.
> The board does not contest that these effects were indeed shown by said tests. It is also correct that these effects are not mentioned in (1). However, the effects in

question cannot establish an inventive step for the following reasons: it has been established case law at the EPO that, if comparative tests are chosen to demonstrate an inventive step on the basis of an improved effect, the nature of the comparison with the closest state of the art must be such that the said effect is convincingly shown to have its origin in the distinguishing feature of the invention (T 0197/86, OJ 1989, 371). This is clearly not the case here, as the comparison was made with individual active agents rather than with combinations of an insulin sensitivity enhancer plus an alpha-reductase inhibitor encompassed by (1) but outside the scope of present claim 1." (Emphasis added.)

(38) Therefore, any arguments that the combination of liposomal irinotecan and 5-FU/LV is improved relative to liposomal irinotecan or 5-FU/LV alone are irrelevant. The closest prior art is also a combination.

5.3 Obviousness of the solution

Irinotecan sucrose octasulfate liposomes

(39) The skilled person looking to solve the objective technical problem of providing an improved treatment for pancreatic cancer would look towards D10 (Development of a highly active nanoliposomal irinotecan using a novel intraliposomal stabilisation strategy), which is directed towards the provision of a highly active form of irinotecan.

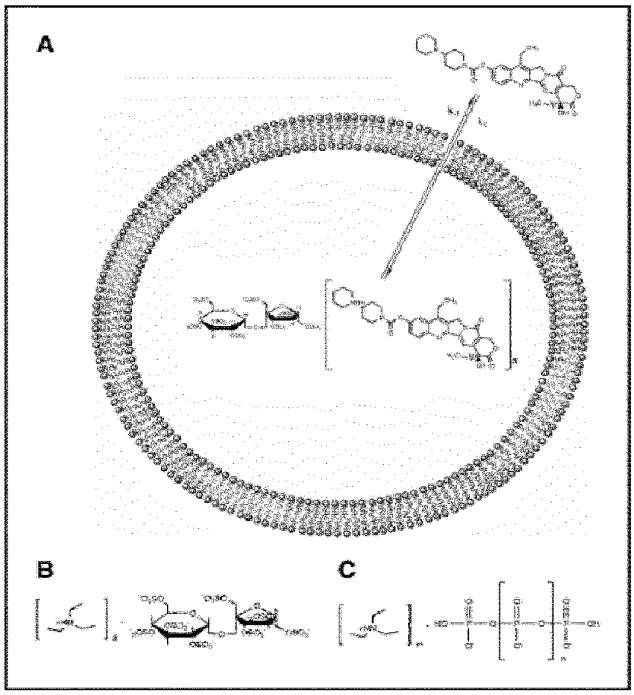
(40) D10 discloses two irinotecan liposomes, and compares each to the free irinotecan form. D10 investigates the use of the polymeric trapping agent poly(phosphate (TEA-Pn) and the non-polymeric trapping agent sucrose octasulfate (TEA-SOS) (page 3272, right hand column):

Results

Preparation of nanoliposomal CPT-11. A proposed novel process using a polyalkylammonium salt of a polymeric (poly-phosphate) or nonpolymeric (sucrose octasulfate) highly charged multivalent anion as intraliposomal trapping agents resulted in improvement of both the encapsulation efficiency and the *in vivo* stability of the liposome-encapsulated weakly basic, amphipathic drug CPT-11. The process may involve the formation of an

(41) The irinotecan forms a nanoscale complex with the trapping agent, and is thus stabilised within the liposome (page 3273):

Figure 1. *A*, schematic depicting the intraliposomal stabilization strategy for CPT-11 using polyanionic trapping agents. The basic molecule CPT-11 forms a nanoscale complex with either poly(phosphate) or sucrose octasulfate in the liposome interior, thus stabilizing the liposomal formulation to increase drug retention while in circulation. Chemical structures of the triethylammonium salts of the polyanionic liposome trapping agents, poly(phosphate) (*B*) and sucrose octasulfate (*C*).



(42) The liposomal formulations both exhibit much longer circulation times than free irinotecan. This was particularly the case for the TEA-SOS formulation (page 3273):

> **Pharmacokinetics of nanoliposomal CPT-11.** The pharmacokinetics of nanoliposomal CPT-11 formulated using either TEA-SOS or TEA-Pn were determined in normal female rats. Free CPT-11 was rapidly cleared from the circulation with $t_{1/2}$ = 0.27 hours (Fig. 3*A*). Liposome encapsulation was associated with significantly longer circulation times than free drug (Fig. 3*A* and *B*). This was especially true for liposomes loaded with TEA-SOS gradients, with blood half-lives for lipid and CPT-11 of 12.0 and 10.7 hours, respectively (Table 1).

(43) And it was shown that both liposomal forms produce a much more significant anti-tumour effect than free irinotecan. The TEA-SOS formulation was the most effective anti-tumour agent (page 3274):

> **Efficacy of liposomal CPT-11 in the HT29 colon cancer model.** In the HT29 colon tumor xenograft model, free CPT-11 again showed efficacy, albeit modest (Fig. 5*B*). However, both nanoliposomal CPT-11 formulations showed pronounced antitumor effects, including tumor regression during treatment followed by prolonged absence of tumor regrowth. Indeed, at 42 days postimplantation, all nanoliposomal CPT-11 treatments seemed to be equivalent and maximally efficacious.
>
> With continued observation, tumor regrowth was observed beginning on day 47 postimplantation. At this point, all control and free CPT-11-treated mice had been sacrificed due to excessive tumor growth. Based on regrowth rates, treatment with TEA-SOS liposomes was more efficacious than TEA-Pn liposomes administered at the same CPT-11 dose. Furthermore,

(44) D10 discusses that liposomal formulations are more effective than free drug formulations due to the enhanced permeability and retention (EPR) effect (pages 3275 and 3276):

Discussion

Liposome delivery has been shown to improve the pharmacokinetic profile and widen the therapeutic index of certain anticancer drugs, especially the anthracycline class (1, 2). Improved efficacy is in part a result of passive targeting to tumor sites based on the enhanced permeability and retention (EPR) effect (23). To

The concept of nanoparticle delivery of camptothecins is very attractive based on potential advantages, including overcoming the solubility limitations of this class, protecting drug in the active lactone configuration, rerouting of drug from sites of toxicity such as the gastrointestinal tract, prolonging circulation time, increasing tumor accumulation via the EPR effect, and providing sustained release for a so-called metronomic effect.

(45) Therefore, the skilled person reading D10 is taught that liposomal forms of irinotecan have a longer half-life, better accumulation in the tumour and ultimately a more effective anti-tumour action. The skilled person is also taught that the irinotecan sucrose octasulfate is the most advantageous liposomal form.

(46) The skilled person looking for an alternative treatment for gemcitabine resistant pancreatic cancer would consider the use of irinotecan sucrose octasulfate liposomal formulation an obvious choice. The use of the claimed form of irinotecan is obvious over D9 in view of D10.

(47) Moreover, the skilled person is also taught by D11 (A multinational phase II study of liposome irinotecan (PEP02) for patients with gemcitabine refractory metastatic pancreatic cancer) that nanoparticle liposome formulation of irinotecan, PEP02, in combination with 5-FU/LV has been shown to be effective in the treatment of GEM-refractory advanced pancreatic cancer:

Background: PEP02 is a novel nanoparticle liposome formulation of irinotecan (CPT-11) that has improved pharmacokinetics and tumor biodistribution of both CPT-11 and its active metabolite-SN38 compared to the free form drug. PEP02 has showed encouraging safety and efficacy in various tumor types, including significant antitumor activity in a human pancreatic cancer L3.6pl orthotopic nude mouse xenograft model. In previous phase I studies, PEP02 either alone or in combination with 5-FU/LV demonstrated prolonged disease control in 5 of 7 (71%) patients (pts) with gemcitabine (GEM)-refractory advanced pancreatic cancer (PC). This phase II study aims to evaluate PEP02 monotherapy as 2nd-line treatment in pts with metastatic, GEM-refractory PC.

(48) These results were known in the common general knowledge, as shown by the review article D2 (page 189, right hand column):

> one and pancreatic cancer in one (29). The observation was further extended in a phase I trial for nanoliposomal CPT-11in combination with weekly 24-hour infusion of high-dose 5-FU/LV (HDFL). In the two phase I trials, 7 pancreatic cancer patients who failed gemcitabine/HDFL +/- platinum had received PEP02 with or without HDFL. The best response was partial response in one, stable disease in 4 and progressive disease in 2, which indicated a potential activity of PEP02 in treating gemcitabine-refractory advanced pancreatic cancer. Based on these

(49) Therefore, the skilled person is also taught by D11 and the common general knowledge shown by D2 that the combination of liposomal irinotecan and 5-FU/LV is effective in the treatment of gemcitabine refractory advanced pancreatic cancer.

The administration schedule

(50) As discussed above, no technical effect is shown to arise from any of the differences between the administration schedule disclosed in D9 and that claimed. Therefore, each difference is arbitrary and obvious.

(51) Moreover, the review article D2 teaches towards the administration of liposomal irinotecan as a single 90 minute infusion for the successful treatment of pancreatic cancer (page 189):

> In the first-in-human phase I trial, patients with standard therapy-failure solid tumor were enrolled to determine the maximum tolerated dose, safety profile and pharmacokinetics of nanoliposomal CPT-11 (formerly PEP02, PharmaEngine, Inc., Taiwan, and now under the designation of MM-398, Merrimack Pharmaceuticals, Inc, USA). The drug was delivered intravenously for 90 minutes, once every 3 weeks, with starting dose of 60 mg/m^2. The maximum tolerated dose was 120 mg/m^2. Two patients achieved partial response including cervical cancer in one and pancreatic cancer in one (29). The observation

(52) And the claimed triple combination had previously been used for the successful treatment of advanced solid tumours using a single dose of irinotecan over the same infusion time (D12, Phase I study of liposome irinotecan (PEP02) in combination with weekly infusion of 5-FU/LV in advanced solid tumors):

Methods: Pts who had failed to standard chemotherapy, ECOG PS 0-1 and adequate organ functions, no prior CPT-11, were eligible. PEP02 was given as 90 mins i.v. infusion on D1 in combination with 24-hr infusion of 5FU (2,000 mg/m^2)/ LV (200 mg/m^2) on D1 and D8, every 3 weeks. Cohorts of 3-6 pts were treated at 60, 80, 100, and 120 mg/m^2. PK and PGx samples were collected.

(53) D12 teaches the skilled person that liposomal irinotecan has been successfully administered using a single 90 minute infusion on Day 1.

(54) Therefore, although no motivation is required because no effect is achieved, D2 and D12 provide motivation to administer liposomal irinotecan as a single 90 minute infusion, instead of two 60 minute infusions as in D9.

(55) The duration of infusion of LV and the order of administration are both arbitrary. There is no effect shown by reducing the LV infusion time from two hours to 30 minutes, and no effect shown to arise from the claimed order of administration.

(56) The claimed administration schedule is obvious.

7. Lack of inventive step of the dependent claims (Articles 56 and 100(a) EPC)

(57) Claim 2 is limited to a patient that is pre-medicated with dexamethasone and/or a 5-HT3 antagonist or other anti-emetic.

(58) It is acknowledged in the patent that it is standard practice to pre-medicate patients with an anti-emetic before irinotecan therapy (paragraph [0120]). This is also shown in the package insert for irinotecan hydrochloride injection, Captosar (D13, page 18, 'Precautions'). Claim 2 is obvious.

(59) Claim 3 limits the pancreatic cancer to particular types of exocrine pancreatic cancer. One of the claimed cancers is adenocarcinoma. The patients enrolled in the study of D9 exhibited histologically confirmed metastatic pancreatic adenocarcinoma (D9, page 1659, left hand column, beneath "Patients").

(60) Claim 4 requires that the cancer to be treated exhibits a distant metastasis and/or a peri-pancreatic extension of the tumour. The patients treated in D9 exhibit metastatic pancreatic cancer.

(61) Claim 5 requires that the treatment cycle is repeated every two weeks. This is the same treatment cycle employed in D9 (page 1659, right hand column, beneath "Treatment dose and schedule").

(62) Claim 6 limits the dose of the active agents. The dose required by claim 6 (80 mg/m^2 or 60 mg/m^2 irinotecan, depending on UGTA1*28 allele status; 2400 mg/m^2 5-FU and 200 mg/m^2

LV, all administered every two weeks) are very similar to the doses disclosed in D9 (70 mg/m^2 irinotecan; 2000 mg/m^2 5-FU and 400 mg/m^2 LV, all administered every two weeks, page 1659, under "Treatment dose and schedule"). No technical effect is shown to arise from the using the claimed dose, instead of the dose in the prior art. The dose is arbitrary and obvious.

(63) Claim 6 also requires that the patient is pre-medicated with dexamethasone and a 5-HT3 antagonist and that the patient has metastatic pancreatic cancer that has progressed on gemcitabine therapy. We have explained that each of these features is obvious.

Dr Richard Cooke
European Patent Attorney
Elkington and Fife LLP (ref R81206OP)
04 February 2022

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D13 Camptosar® patient information leaflet

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DEVELOPMENTAL THERAPEUTICS-CLINICAL PHARMACOLOGY AND IMMUNOTHERAPY

Phase I study of liposome irinotecan (PEP02) in combination with weekly infusion of 5-FU/LV in advanced solid tumors.

L. Chen , H. Shiah , T. Chao , R. K. Hsieh , G. Chen , J. Chang...

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Abstract

e13024

Background: PEP02 is a novel nanoparticle liposome formulation of irinotecan (CPT-11) that has improved PK and tumor biodistribution of CPT-11 and its active metabolite-SN38 with encouraging safety and tumor response in preclinical studies and a single-agent phase I study. The study is to define the DLT, MTD, and PK of PEP02 when in combination with high-dose fluorouracil/leucovorin (HDFL) in patients (pt) with advanced solid tumors.

Methods: Pts who had failed to standard chemotherapy, ECOG PS 0-1 and adequate organ functions, no prior CPT-11, were eligible. PEP02 was given as 90 mins i.v. infusion on D1 in combination with 24-hr infusion of 5FU (2,000 mg/m^2)/ LV (200 mg/m^2) on D1 and D8, every 3 weeks. Cohorts of 3-6 pts were treated at 60, 80, 100, and 120 mg/m^2. PK and PGx samples were collected.

Results: A total of 16 pts were enrolled, with 3, 6, 5, and 2 at 60, 80, 100, and 120 mg/m^2. DLTs were observed in 4 pts, including 2 each at 100 and 120 mg/m^2 dose levels. DLTs were mainly G3 diarrhea and G4 hematologic toxicities. MTD was determined as 80 mg/m^2. Grade 3 or above adverse events at the MTD dose and all dose levels were 10.6% and 18.4%, respectively. The PK of total CPT-11 after PEP02 (at 80 mg/m^2) in combination with HDFL was characterized by low clearance (mean = 116.4 mL/m^2/hr) and small volume of distribution

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Journal of Clinical Oncology 28,
no. 15_suppl

Published online May 20, 2010.

WE RECOMMEND

A phase I/IIA pharmacokinetic (PK) and serial skin and tumor pharmacodynamic (PD) study of the EGFR irreversible tyrosine kinase inhibitor EKB-569 in combination with 5-fluorouracil (5FU), leucovorin (LV) and irinotecan (CPT-11) (FOLFIRI regimen) in patients (pts) with advanced colorectal cancer (ACC)
E. Casado et al., J Clin Oncol, 2004

Phase I study of pemetrexed plus paclitaxel in patients with solid tumor
T. Graefe et al., J Clin Oncol, 2016

Phase I-II study of irinotecan hydrochloride combined with cisplatin in patients with advanced gastric cancer.
K Shirao et al., J Clin Oncol, 2016

Phase I and Pharmacokinetic Study of Two Different Schedules of Oxaliplatin, Irinotecan, Fluorouracil, and Leucovorin in Patients With Solid Tumors
Matthew P. Goetz et al., J Clin Oncol, 2016

(mean = 2.93 L/m^2, similar to plasma volume) as did of PEP02 monotherapy study. Compared to the PK of SN-38 after 250 mg/m^2 of CPT-11 (in combination with capecitabine, Ann Oncol 2005; 16: 1123-32), the C_{max} after 80 mg/m^2 of PEP02 was lower (7.98 ± 4.39 *vs* 62.0 ± 37.4 ng/mL), but the AUC_{0-t} was similar (354.77 ± 145.35 *vs* 396 ± 247 ng×h/mL). The correlation of UGT1A family with PK and toxicity was not observed. However, the only subject with the coexistence of two variants of *UGT1A1*6* and **28* had higher dose-normalized $AUC_{SN-38}$and experienced DLT. The best response of 15 evaluable pts was PR in 2 (gastric cancer and breast cancer) and SD in 9.

Conclusions: The MTD of PEP02 in combination with HDFL given every-3-week is 80 mg/m^2. The observation of tumor response in two heavily pre-treated patients suggests the combination deserves further exploration in advanced solid tumor patients who are refractory to standard therapy.

Author Disclosure

Employment or Leadership Position	Consultant or Advisory Role	Stock Ownership	Honoraria	Reser Fund
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CANCERS OF THE COLON AND RECTUM

Phase I study of biweekly liposome irinotecan (PEP02, MM-398) in metastatic colorectal cancer failed on first-line oxaliplatin-based chemotherapy.

Li-Tzong Chen , Her-Shyong Shiah , Peng-Chan Lin , Jeng-Chang Lee , Wu-Chou Su , Yi-Wen Wang...

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Abstract

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Background: PEP02 (MM-398) is a nanoliposomal formulation of irinotecan (CPT-11) that has improved pharmacokinetics (PK) and tumor distribution of CPT-11 and its active metabolite, SN-38. PEP02 single agent q3w has shown preliminary efficacy and safety in Phase II pancreatic and gastric cancer studies. Since irinotecan is approved for metastatic colorectal cancer (mCRC) and biweekly regimens are widely used, the aims of this study are to determine the maximum tolerated dose (MTD), characterize the PK and pharmacogenetics (PGx), and explore the efficacy of PEP02 q2w in mCRC.

Methods: Patients (pts) with disease progression after 1st-line oxaliplatin-based chemotherapy, ECOG PS 0-1, and without prior exposure to irinotecan were eligible. PEP02 was given on day 1 and 15 of each 28 day treatment cycle. The starting dose was 80 mg/m^2 and escalated by 10 mg/m^2 to the target dose of 100 mg/m^2. PK was evaluated during the 1st cycle and the tumor response was assessed by RECIST.

Results: A total of 18 pts (M/F 9/9; median age 57.5) were enrolled, with 6 at each dose level. Dose-limiting toxicity manifested as G3 diarrhea was observed in one pt per dose level. The target dose of 100 mg/m^2 was determined to be the MTD. Nine pts had dose delayed (4, 3, 2 at 80, 90, 100 mg/m^2),

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mostly because of neutropenia. The PK and PGx are being analysed. As of August 2011, there are 3 pts still on study treatment and 17 pts evaluable for tumor response. Four pts (2 at 80 mg/m^2, 1 each at 90 and 100 mg/m^2) showed partial response (3 after 2 cycles and 1 after 8 cycles) and 8 pts (3 each at 80 and 90 mg/m^2, 2 at 100 mg/m^2) maintained stable disease for at least 2 cycles, which resulted in a response rate (RR) of 23.5% and a disease control rate (DCR) of 70.6%. Current median progression-free survival (PFS) is 4 months and 8 pts (47%) had PFS ≥ 6 months.

Conclusions: The MTD of biweekly PEP02 is 100 mg/m^2. As a 2nd-line monotherapy after oxaliplatin-based chemotherapy, the efficacy results indicate the potential benefit of PEP02 for mCRC (FOLFIRI-1 achieved only 4% RR, 34% DCR, and 2.5 months PFS in FOLFOX pretreated pts). A randomized Phase II study evaluating PEP02 plus 5-FU/LV (FUPEP regimen) vs. FOLFIRI is currently ongoing in France.

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CANCERS OF THE PANCREAS, SMALL BOWEL, AND HEPATOBILIARY TRACT

A multinational phase II study of liposome irinotecan (PEP02) for patients with gemcitabine-refractory metastatic pancreatic cancer.

A. H. Ko, M. A. Tempero, Y. Shan, W. Su, Y. Lin, E. Dito...
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Abstract

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Background: PEP02 is a novel nanoparticle liposome formulation of irinotecan (CPT-11) that has improved pharmacokinetics and tumor biodistribution of both CPT-11 and its active metabolite-SN38 compared to the free form drug. PEP02 has showed encouraging safety and efficacy in various tumor types, including significant antitumor activity in a human pancreatic cancer L3.6pl orthotopic nude mouse xenograft model. In previous phase I studies, PEP02 either alone or in combination with 5-FU/LV demonstrated prolonged disease control in 5 of 7 (71%) patients (pts) with gemcitabine (GEM)-refractory advanced pancreatic cancer (PC). This phase II study aims to evaluate PEP02 monotherapy as 2nd-line treatment in pts with metastatic, GEM-refractory PC.

Methods: Pts were eligible if they had metastatic pancreatic adenocarcinoma, KPS ≥ 70, and progressed following one line of GEM-based therapy. Treatment consisted of PEP02 120 mg/m^2 administered as a 90-minute infusion every 3 weeks. A Simon's 2-stage design was used with 16 pts in the first stage and 39 pts in total; primary objective was 3-month survival rate ($OS_{3\text{-month}}$).

Results: Between March 2009 and August 2010, 37 pts were enrolled at 3 centers in the U.S. and Taiwan. Characteristics for the first 31 evaluable pts: 13 M/18 F; age 39-82 yrs; 19 Asian/12

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Caucasian, KPS 100/90/80/70: 5/14/4/8. Mean number of treatment cycles is 5 (range, 1-22). Disease control rate (minor response + stable disease >2 cycles) is 52%. 8 of 24 pts (33%) with elevated baseline CA19-9 have had >50% biomarker decline. To date, 23/31 pts (74%) have survived > 3 months, with 4 pts still alive after 1 year. Reasons for study discontinuation: 74% progressive disease, 9% drug-related toxicity, 17% other. Preliminary safety data is available for the first stage. Most common G3/4 adverse events included: fatigue (31%), neutropenia (25%), nausea/vomiting (19%), and diarrhea (13%).

Conclusions: This study has already met its primary endpoint (predicted $OS_{3\text{-month}}$ >65%). PEP02 appears to have both activity and tolerable side effects for pts with metastatic, GEM-refractory PC, and represents a promising option for this pt population with few standard options.

Author Disclosure

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DEVELOPMENTAL THERAPEUTICS: CYTOTOXIC CHEMOTHERAPY

Phase I study of liposome encapsulated irinotecan (PEP02) in advanced solid tumor patients

L. Chen , T. Chang , A. Cheng , C. Yang , H. Shiah, M.D. , J. Chang...
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Abstract

2565

Background: PEP02 is a novel nanoparticle liposome formulation of irinotecan aiming to enhance tumor localization and improve pharmacokinetic properties of irinotecan and its active metabolite-SN38. The aims of the study are to define the dose-limiting toxicity (DLT), maximum tolerated dose (MTD) and pharmacokinetics (PK) of PEP02 in patients with advanced refractory solid tumors. **Methods:** Pts with advanced refractory solid tumors, ECOG PS 0–1, and adequate hematological, hepatic and renal functions were eligible. PEP02 was given as 90mins i.v. infusion, repeated every 3 weeks. The doses would have been escalated from 60, 120, 180 to 240 mg/m^2 in a single-patient cohort accelerated titration design. PK samples were collected on days 1, 2, 3, 8 and 21. **Results:** A total of 11 pts (M/F 1/10; median age 47, range 41–67) were enrolled onto three dose levels, with 1, 6 and 4 pts at dose level I (60 mg/m^2), II (120 mg/m^2) and III (180 mg/m^2), respectively. DLT was observed in 3 pts, including 1 at dose level II (grade 3 catheter-related infection) and 2 at dose level III (grade 3 diarrhea and febrile neutropenia in 1 and treatment-related mortality secondary to grade 4 diarrhea and neutropenia in 1). MTD was determined as 120 mg/m^2. The PK of total irinotecan after PEP02 dosing were characterized by, i.e. after 120 mg/m^2, low clearance (mean = 0.0591 L/m^2/hr), small volume of distribution (mean = 1.8 L/m^2, similar to plasma volume), and prolonged terminal half-life (mean=29.5 hr). The plasma concentration-time profiles of encapsulated irinotecan (PEP02)

ARTICLE CITATION

DOI:
10.1200/jco.2008.26.15_suppl.2565
Journal of Clinical Oncology 26, no. 15_suppl (May 20, 2008) 2565-2565.

Published online May 20, 2008.

Cmax of SN-38 after 120 mg/m2 of PEP02 was lower (9.2±3.5 vs 26.3±11.9 ng/mL), the terminal t1/2 of SN-38 was longer (75.4±43.8 vs 10.4±3.1 hrs) and the AUC of SN-38 was larger (710±395 vs 229±108 ng.h/mL). The best response of 10 evaluable pts was PR in 2 (cervical and pancreatic cancer) and SD in 3. Conclusions: The MTD of PEP02 monotherapy at 3-week interval is 120 mg/m^2, which will be the recommended dose for future phase II studies. Preliminary data suggest that PEP02 exhibits encouraging pharmacokinetic, safety and efficacy profiles.

No significant financial relationships to disclose.

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Conquer Cancer Foundation
TAPUR Study

History of Changes for Study: NCT01494506

Study of MM-398 Versus 5-Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer (NAPOLI 1)

Latest version (submitted June 16, 2016) on ClinicalTrials.gov

- A study version is represented by a row in the table.
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Study Record Versions

Version	A	B	Submitted Date	Changes
1	○	○	December 15, 2011	None (earliest Version on record)
2	○	○	December 19, 2011	Arms and Interventions, Conditions and Study Status
3	○	○	March 7, 2012	Study Status and Contacts/Locations
4	○	○	March 9, 2012	Contacts/Locations and Study Status
5	○	○	April 18, 2012	Contacts/Locations and Study Status
6	○	○	April 20, 2012	Contacts/Locations and Study Status
7	○	○	April 25, 2012	Contacts/Locations and Study Status
8	○	○	May 18, 2012	Study Status and Contacts/Locations
9	◉	◉	May 29, 2012	Contacts/Locations and Study Status
10	○	○	June 25, 2012	Study Status and Contacts/Locations
11	○	○	June 26, 2012	Contacts/Locations and Study Status
12	○	○	July 19, 2012	Study Status and Contacts/Locations
13	○	○	August 8, 2012	Arms and Interventions, Contacts/Locations, Study Status, Study Identification, Eligibility, Study Design, Study Description and Oversight
14	○	○	August 17, 2012	Contacts/Locations and Study Status
15	○	○	October 19, 2012	Contacts/Locations and Study Status
16	○	○	November 12, 2012	Contacts/Locations and Study Status
17	○	○	December 19, 2012	Study Status and Contacts/Locations
18	○	○	January 25, 2013	Study Status, Contacts/Locations and Arms and Interventions
19	○	○	March 5, 2013	Study Status and Contacts/Locations
20	○	○	March 15, 2013	Contacts/Locations and Study Status
21	○	○	April 30, 2013	Contacts/Locations and Study Status
22	○	○	May 29, 2013	Contacts/Locations and Study Status
23	○	○	June 6, 2013	Study Status and Contacts/Locations

Version	A	B	Submitted Date	Changes
24	○	○	July 3, 2013	Study Status
25	○	○	July 10, 2013	Contacts/Locations and Study Status
26	○	○	July 22, 2013	Contacts/Locations and Study Status
27	○	○	July 23, 2013	Contacts/Locations and Study Status
28	○	○	August 1, 2013	Study Status and Contacts/Locations
29	○	○	August 20, 2013	Contacts/Locations and Study Status
30	○	○	September 4, 2013	Recruitment Status, Study Status and Contacts/Locations
31	○	○	October 10, 2014	Study Status and Contacts/Locations
32	○	○	December 23, 2014	Study Status
33	○	○	October 15, 2015	Recruitment Status and Study Status
34	○	○	February 11, 2016	Outcome Measures, Study Status, More Information, Study Design, Study Identification, Adverse Events, Baseline Characteristics, Participant Flow and Arms and Interventions
35	○	○	June 16, 2016	Study Status, Outcome Measures

Compare Comparison Format: ◉ Merged ○ Side-by-Side

Scroll up to access the controls

Study NCT01494506
Submitted Date: May 29, 2012 (v9)

Study Identification

Unique Protocol ID: MM-398-07-03-01

Brief Title: Study of MM-398 Versus 5-Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer (NAPOLI 1)

Official Title: A Randomized, Open Label Phase 3 Study of MM-398 Versus 5-Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer

Secondary IDs:

Study Status

Record Verification: May 2012

Overall Status: Recruiting

Study Start: November 2011

Primary Completion: December 2013 [Anticipated]

Study Completion: June 2014 [Anticipated]

First Submitted: December 14, 2011

First Submitted that Met QC Criteria: December 15, 2011

First Posted: December 19, 2011 [Estimate]

Last Update Submitted that Met QC Criteria: May 29, 2012

Last Update Posted: May 31, 2012 [Estimate]

Sponsor/Collaborators

Sponsor: Merrimack Pharmaceuticals

Responsible Party: Sponsor

Collaborators:

Oversight

U.S. FDA-regulated Drug:

U.S. FDA-regulated Device:

Data Monitoring: No

Study Description

Brief Summary: The study is an open label, randomized phase 3 study of MM-398 versus 5-fluorouracil (5-FU) and leucovorin (also known as folinic acid) in metastatic pancreatic cancer patients who have progressed on prior gemcitabine based therapy.

Detailed Description:

Conditions

Conditions: Metastatic Pancreatic Cancer

Keywords: Pancreatic cancer
MM-398
PEP02
Metastatic pancreatic cancer
Gemcitabine refractory pancreatic cancer
Second line pancreatic cancer treatment
Pancreatic cancer post gemcitabine therapy

Study Design

Study Type: Interventional

Primary Purpose: Treatment

Study Phase: Phase 3

Interventional Study Model: Parallel Assignment

Number of Arms: 2

Masking: None (Open Label)

Allocation: Randomized

Enrollment: 270 [Anticipated]

Arms and Interventions

Arms	Assigned Interventions
Experimental: MM-398 MM-398 Q3W IV	Drug: MM-398 MM-398 120 mg/m2 IV Q3W Other Names: • PEP02
Active Comparator: 5 Fluorouracil and Leucovorin IV 5 Fluorouracil and Leucovorin IV	Drug: 5 Fluorouracil 5 Fluorouracil 2000 mg/m2 IV for 4 weeks followed by 2 weeks of rest every 6 weeks Other Names: • 5-FU Drug: Leucovorin Leucovorin 200 mg/m2 IV for 4 weeks followed by 2 weeks of rest every 6 weeks Other Names: • Folinic Acid

Outcome Measures

Primary Outcome Measures:

1. Overall Survival
   [Time Frame: 24 months]

Secondary Outcome Measures:

1. Progression Free Survival
   [Time Frame: 24 months]
2. Time to treatment failure
   [Time Frame: 24 months]
3. Objective response rate
   [Time Frame: 24 months]

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Histologically or cytologically confirmed adenocarcinoma of the exocrine pancreas
- Metastatic disease
- Documented disease progression after prior gemcitabine based therapy
- KPS >/= 70
- Adequate bone marrow function
- Adequate hepatic function
- Adequate renal function

Exclusion Criteria:

- Prior irinotecan treatment
- Active CNS metastasis
- Clinically significant GI disorders
- Major surgery or radiotherapy within 4 weeks of enrollment
- Severe arterial thromboembolic events less than 6 months before inclusion
- NYHA Class III or IV congestive heart failure, ventricular arrhythmias or uncontrolled blood pressure
- Active infection or uncontrolled fever
- Pregnant or breast feeding patients

Contacts/Locations

Central Contact Person: Eliel Bayever, MD
Telephone: 617-441-1000
Email: ebayever@merrimackpharma.com

Locations: **United States, Arizona**

[Recruiting]
Gilbert, Arizona, United States

[Recruiting]
Scottsdale, Arizona, United States

United States, Georgia

[Recruiting]
Atlanta, Georgia, United States

United States, Missouri

[Recruiting]
St Louis, Missouri, United States

United States, Ohio

[Recruiting]
Newark, Ohio, United States

United States, Pennsylvania

[Recruiting]
Bethlehem, Pennsylvania, United States

United States, South Carolina

[Recruiting]
Greenville, South Carolina, United States

Australia, New South Wales

[Recruiting]
Westmead, New South Wales, Australia

Australia, South Australia

[Recruiting]
Kurralta Park, South Australia, Australia

Australia, Victoria

[Recruiting]
Frankston, Victoria, Australia

[Recruiting]
Heidelberg, Victoria, Australia

[Recruiting]
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History of Changes for Study: NCT01494506

Study of MM-398 With or Without 5-Fluorouracil and Leucovorin, Versus 5-Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer (NAPOLI 1)

Latest version (submitted June 16, 2016) on ClinicalTrials.gov

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5	○	○	April 18, 2012	Contacts/Locations and Study Status
6	○	○	April 20, 2012	Contacts/Locations and Study Status
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8	○	○	May 18, 2012	Study Status and Contacts/Locations
9	○	○	May 29, 2012	Contacts/Locations and Study Status
10	○	○	June 25, 2012	Study Status and Contacts/Locations
11	○	○	June 26, 2012	Contacts/Locations and Study Status
12	○	○	July 19, 2012	Study Status and Contacts/Locations
13	◉	◉	August 8, 2012	Arms and Interventions, Contacts/Locations, Study Status, Study Identification, Eligibility, Study Design, Study Description and Oversight
14	○	○	August 17, 2012	Contacts/Locations and Study Status
15	○	○	October 19, 2012	Contacts/Locations and Study Status
16	○	○	November 12, 2012	Contacts/Locations and Study Status
17	○	○	December 19, 2012	Study Status and Contacts/Locations
18	○	○	January 25, 2013	Study Status, Contacts/Locations and Arms and Interventions
19	○	○	March 5, 2013	Study Status and Contacts/Locations
20	○	○	March 15, 2013	Contacts/Locations and Study Status
21	○	○	April 30, 2013	Contacts/Locations and Study Status
22	○	○	May 29, 2013	Contacts/Locations and Study Status

Version	A	B	Submitted Date	Changes
23	○	○	June 6, 2013	Study Status and Contacts/Locations
24	○	○	July 3, 2013	Study Status
25	○	○	July 10, 2013	Contacts/Locations and Study Status
26	○	○	July 22, 2013	Contacts/Locations and Study Status
27	○	○	July 23, 2013	Contacts/Locations and Study Status
28	○	○	August 1, 2013	Study Status and Contacts/Locations
29	○	○	August 20, 2013	Contacts/Locations and Study Status
30	○	○	September 4, 2013	Recruitment Status, Study Status and Contacts/Locations
31	○	○	October 10, 2014	Study Status and Contacts/Locations
32	○	○	December 23, 2014	Study Status
33	○	○	October 15, 2015	Recruitment Status and Study Status
34	○	○	February 11, 2016	Outcome Measures, Study Status, More Information, Study Design, Study Identification, Adverse Events, Baseline Characteristics, Participant Flow and Arms and Interventions
35	○	○	June 16, 2016	Study Status, Outcome Measures

Compare Comparison Format: ◉ Merged ○ Side-by-Side

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Study NCT01494506
Submitted Date: August 8, 2012 (v13)

Study Identification

Unique Protocol ID: MM-398-07-03-01

Brief Title: Study of MM-398 With or Without 5-Fluorouracil and Leucovorin, Versus 5-Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer (NAPOLI 1)

Official Title: A Randomized, Open Label Phase 3 Study of MM-398, With or Without 5-Fluorouracil and Leucovorin, Versus 5 Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer Who Have Failed Prior Gemcitabine-based Therapy

Secondary IDs:

Study Status

Record Verification: August 2012

Overall Status: Recruiting

Study Start: November 2011

Primary Completion: December 2013 [Anticipated]

Study Completion: June 2014 [Anticipated]

First Submitted: December 14, 2011

First Submitted that Met QC Criteria: December 15, 2011

First Posted: December 19, 2011 [Estimate]

Last Update Submitted that Met QC Criteria: August 8, 2012

Last Update Posted: August 10, 2012 [Estimate]

Sponsor/Collaborators

Sponsor: Merrimack Pharmaceuticals

Responsible Party: Sponsor

Collaborators:

Oversight

U.S. FDA-regulated Drug:

U.S. FDA-regulated Device:

Data Monitoring: Yes

Study Description

Brief Summary: The study is an open label, randomized phase 3 study of MM-398 with or without 5-Fluorouracil (5-FU) and Leucovorin (also known as folinic acid), versus 5-FU and leucovorin in metastatic pancreatic cancer patients who have progressed on prior gemcitabine based therapy.

Detailed Description:

Conditions

Conditions: Metastatic Pancreatic Cancer

Keywords: Pancreatic cancer
MM-398
PEP02
Metastatic pancreatic cancer
Gemcitabine refractory pancreatic cancer
Second line pancreatic cancer treatment
Pancreatic cancer post gemcitabine therapy

Study Design

Study Type: Interventional

Primary Purpose: Treatment

Study Phase: Phase 3

Interventional Study Model: Parallel Assignment

Number of Arms: 3

Masking: None (Open Label)

Allocation: Randomized

Enrollment: 405 [Anticipated]

Arms and Interventions

Arms	Assigned Interventions
Experimental: MM-398 MM-398 Q3W IV	Drug: MM-398 Arm A: MM-398 120 mg/m2 IV Q3W Arm C: MM-398 80mg/m2 IV Q2W Other Names: • PEP02
Active Comparator: 5 Fluorouracil and Leucovorin IV 5 Fluorouracil and Leucovorin IV	Drug: 5 Fluorouracil Arm B: 5 Fluorouracil 2000 mg/m2 IV for 4 weeks followed by 2 weeks of rest every 6 weeks Arm C: 5 Fluorouracil 2400 mg/m2 IV every 2 weeks Other Names: • 5-FU Drug: Leucovorin Arm B: Leucovorin 200 mg/m2 IV for 4 weeks followed by 2 weeks of rest every 6 weeks Arm C: Leucovorin 200 mg/m2 IV every 2 weeks Other Names: • Folinic Acid
Experimental: MM-398, 5-FU and Leucovorin MM-398, 5-FU and Leucovorin Q2W IV	Drug: MM-398 Arm A: MM-398 120 mg/m2 IV Q3W Arm C: MM-398 80mg/m2 IV Q2W Other Names: • PEP02 Drug: 5 Fluorouracil Arm B: 5 Fluorouracil 2000 mg/m2 IV for 4 weeks followed by 2 weeks of rest every 6 weeks Arm C: 5 Fluorouracil 2400 mg/m2 IV every 2 weeks Other Names: • 5-FU Drug: Leucovorin Arm B: Leucovorin 200 mg/m2 IV for 4 weeks followed by 2 weeks of rest every 6 weeks Arm C: Leucovorin 200 mg/m2 IV every 2 weeks Other Names: • Folinic Acid

Outcome Measures

Primary Outcome Measures:

1. Overall Survival
   [Time Frame: 24 months]

Secondary Outcome Measures:

1. Progression Free Survival
   [Time Frame: 24 months]
2. Time to treatment failure
   [Time Frame: 24 months]
3. Objective response rate
   [Time Frame: 24 months]

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Histologically or cytologically confirmed adenocarcinoma of the exocrine pancreas
- Metastatic disease
- Documented disease progression after prior gemcitabine based therapy
- KPS >/= 70
- Adequate bone marrow function
- Adequate hepatic function
- Adequate renal function

Exclusion Criteria:

- Active CNS metastasis
- Clinically significant GI disorders
- Severe arterial thromboembolic events less than 6 months before inclusion
- NYHA Class III or IV congestive heart failure, ventricular arrhythmias or uncontrolled blood pressure
- Active infection or uncontrolled fever
- Pregnant or breast feeding patients

Contacts/Locations

Central Contact Person: Eliel Bayever, MD
Telephone: 617-441-1000
Email: ebayever@merrimackpharma.com

Locations: **United States, Arizona**

[Recruiting]
Gilbert, Arizona, United States

[Recruiting]
Scottsdale, Arizona, United States

United States, Georgia

[Recruiting]
Atlanta, Georgia, United States

United States, Louisiana

[Not yet recruiting]
Baton Rouge, Louisiana, United States

United States, Missouri

[Recruiting]
Kansas City, Missouri, United States

[Recruiting]
St Louis, Missouri, United States

United States, New Mexico

[Recruiting]
Albuquerque, New Mexico, United States

United States, Ohio

[Recruiting]
Newark, Ohio, United States

United States, Pennsylvania

[Recruiting]
Bethlehem, Pennsylvania, United States

United States, South Carolina

[Recruiting]
Greenville, South Carolina, United States

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[Recruiting]
Buenos Aires, Argentina

[Recruiting]
Santa Fe, Argentina

Australia, New South Wales

[Recruiting]
Westmead, New South Wales, Australia

Australia, South Australia

[Recruiting]
Kurralta Park, South Australia, Australia

Australia, Victoria

[Recruiting]
Frankston, Victoria, Australia

[Recruiting]
Heidelberg, Victoria, Australia

[Recruiting]
Melbourne, Victoria, Australia

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[Recruiting]
Pecs, Hungary

[Recruiting]
Szeged, Hungary

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Barcelona, Spain

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Santander, Spain

[Recruiting]
Valencia, Spain

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[Recruiting]
Tainan, Taiwan

[Recruiting]
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Oxford, United Kingdom

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History of Changes for Study: NCT01375816

Liposome-encapsulated Irinotecan Hydrochloride PEP02 or Irinotecan Hydrochloride, Leucovorin Calcium, and Fluorouracil as Second-Line Therapy in Treating Patients With Metastatic Colorectal Cancer

Latest version (submitted June 3, 2015) on ClinicalTrials.gov

- A study version is represented by a row in the table.
- Select two study versions to compare. One each from columns A and B.
- Choose either the "Merged" or "Side-by-Side" comparison format to specify how the two study versions are to be displayed. The Side-by-Side format only applies to the Protocol section of the study.
- Click "Compare" to do the comparison and show the differences.
- Select a version's Submitted Date link to see a rendering of the study for that version.
- The yellow A/B choices in the table indicate the study versions currently compared below. A yellow table row indicates the study version currently being viewed.
- Hover over the "Recruitment Status" to see how the study's recruitment status changed.
- Study edits or deletions are displayed in ~~red~~.
- Study additions are displayed in green.

Study Record Versions

Version	A	B	Submitted Date	Changes
1	◉	◉	June 16, 2011	None (earliest Version on record)
2	○	○	November 14, 2013	Recruitment Status, Study Status, Outcome Measures, Arms and Interventions, Study Identification, Study Design, Contacts/Locations, Eligibility, Study Description, Oversight and Sponsor/Collaborators
3	○	○	June 3, 2015	Recruitment Status and Study Status

Compare Comparison Format: ◉ Merged ○ Side-by-Side

Scroll up to access the controls

Study NCT01375816
Submitted Date: June 16, 2011 (v1)

Study Identification

Unique Protocol ID: CDR0000701454

Brief Title: Liposome-encapsulated Irinotecan Hydrochloride PEP02 or Irinotecan Hydrochloride, Leucovorin Calcium, and Fluorouracil as Second-Line Therapy in Treating Patients With Metastatic Colorectal Cancer

Official Title: A Randomized Phase II Study of PEP02 or Irinotecan in Combination With Leucovorin and 5-Flourouracil in Second Line Therapy of Metastatic Colorectal Cancer

Secondary IDs: FRE-GERCOR-PEPCOL-C10-1
EU-21115
EUDRACT-2010-020468-39
PHARMAENGINE-FRE-GERCOR-PEPCOL

Study Status

Record Verification:	June 2011
Overall Status:	Recruiting
Study Start:	May 2011
Primary Completion:	December 2012 [Anticipated]
Study Completion:	
First Submitted:	June 16, 2011
First Submitted that Met QC Criteria:	June 16, 2011
First Posted:	June 17, 2011 [Estimate]
Last Update Submitted that Met QC Criteria:	June 16, 2011
Last Update Posted:	June 17, 2011 [Estimate]

Sponsor/Collaborators

Sponsor:	GERCOR - Multidisciplinary Oncology Cooperative Group
Responsible Party:	
Collaborators:	

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	

Study Description

Brief Summary: RATIONALE: Drugs used in chemotherapy, such as liposome-encapsulated irinotecan hydrochloride PEP02, irinotecan hydrochloride, leucovorin calcium, and fluorouracil, work in different ways to stop the growth of tumor cells, either by killing the cells or by stopping them from dividing. It is not yet known whether giving liposome-encapsulated irinotecan hydrochloride PEP02 together with leucovorin calcium and fluorouracil is more effective than giving irinotecan hydrochloride together with leucovorin calcium and fluorouracil as second-line therapy in treating patients with metastatic colorectal cancer.

PURPOSE: This randomized phase II trial is studying liposome-encapsulated irinotecan hydrochloride PEP02 given together with leucovorin calcium and fluorouracil to see how well it works compared with giving irinotecan hydrochloride together with leucovorin calcium and fluorouracil as second-line therapy in treating patients with metastatic colorectal cancer.

Detailed Description: OBJECTIVES:

Primary

- To evaluate the objective response rates (complete response and partial response) in patients with metastatic colorectal cancer treated with liposome-encapsulated irinotecan hydrochloride PEP02, leucovorin calcium, and fluorouracil (FUPEP) Versus irinotecan hydrochloride, leucovorin calcium, and fluorouracil (FOLFIRI 1) or leucovorin calcium, fluorouracil, and irinotecan hydrochloride-modified (FOLFIRI 3-modified).

Secondary

- To determine the safety of these regimens in these patients.
- To determine progression-free survival of these patients.
- To determine overall survival of these patients.
- To assess the quality of life of these patients.
- To assess the correlation of UGT1A family polymorphism and the toxicity of liposome-encapsulated irinotecan hydrochloride PEP02 or irinotecan hydrochloride.

OUTLINE: This is a multicenter study. Patients are stratified, in terms of prognosis, according to treatment center, prognostic score (ECOG performance status [PS] 0 and normal LDH value vs ECOG PS > 1 and/or LDH > 1 times upper limit of normal), and time to progression after first-line therapy (≥ 9 months vs < 9 months). Patients are randomized to 1 of 2 treatment arms.

- Arm I: Patients are assigned to either the FOLFIRI 1 or Modified FOLFIRI 3 treatment groups according to the investigator's discretion.
  - FOLFIRI 1: Patients receive irinotecan hydrochloride over 1 hour and leucovorin calcium IV over 2 hours on day 1 and a bolus of fluorouracil followed by fluorouracil IV over 46 hours beginning on day 1. Courses repeat every 14 days in the absence of disease progression or unacceptable toxicity
  - Modified FOLFIRI 3: Patients receive irinotecan hydrochloride, leucovorin calcium, and fluorouracil as in FOLFIRI 1. Patients also receive irinotecan hydrochloride IV over 1 hour on day 3. Courses repeat every 14 days in the absence of disease progression or unacceptable toxicity.
- Arm II (FUPEP): Patients receive liposome-encapsulated irinotecan hydrochloride PEP02 IV over 60-90 minutes and leucovorin calcium IV over 2 hours on day 1 and fluorouracil IV over 46 hours beginning on day 1. Courses repeat every 14 days in the absence of disease progression or unacceptable toxicity.

Blood samples are collected periodically for pharmacogenetic analysis of UGT1A family polymorphisms. Quality of life is assessed by using a generic scale EQ-5D and the QLQ-C30 questionnaire at baseline and after courses 4 and 8.

After completion of study treatment, patients are followed up at day 30 and then every 2-3 months thereafter.

Conditions

Conditions: Colorectal Cancer

Keywords: adenocarcinoma of the colon
recurrent colon cancer
stage IV colon cancer
adenocarcinoma of the rectum
recurrent rectal cancer
stage IV rectal cancer

Study Design

Study Type: Interventional

Primary Purpose: Treatment

Study Phase: Phase 2

Interventional Study Model:

Number of Arms:

Masking: None (Open Label)

Allocation: Randomized

Enrollment: 88 [Anticipated]

Arms and Interventions

Intervention Details:

Drug: FOLFIRI regimen

Drug: fluorouracil

Drug: irinotecan hydrochloride

Drug: leucovorin calcium

Drug: liposome-encapsulated irinotecan hydrochloride PEP02

Genetic: polymorphism analysis

pharmacogenomic studies

Procedure: quality-of-life assessment

Outcome Measures

Primary Outcome Measures:

1. Tumor response, in terms of objective response rates (complete response and partial response)

Secondary Outcome Measures:

1. Safety
2. Progression-free survival
3. Overall survival
4. Quality of life
5. Correlation of UGT1A family polymorphism and the toxicity of liposome-encapsulated irinotecan hydrochloride PEP02 or irinotecan hydrochloride

Eligibility

Minimum Age: 18 Years

Maximum Age: 75 Years

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: DISEASE CHARACTERISTICS:

- Histologically proven adenocarcinoma of colon or rectum
  - Metastatic disease, exclusive of bone metastasis
  - Not suitable for complete carcinological surgical resection
- Any KRAS status allowed (wild type or mutated)
- Measurable lesion (≥ 1 cm) as assessed by CT scan or MRI according to RECIST criteria (version 1.1)
- Must have received prior oxaliplatin-based chemotherapy for metastatic disease
- No symptomatic ascites or pleural effusion not evacuated prior to study entry
- No history or evidence of CNS metastasis

PATIENT CHARACTERISTICS:

- WHO or ECOG performance status 0-2
- Absolute neutrophil count ≥ 1,500/mm^3
- Platelet count ≥ 100,000/µL
- Hemoglobin ≥ 9 g/dL (may be transfused to maintain or exceed this level)
- Serum creatinine < 150 µmol/L
- Calculated creatinine clearance > 30 mL/min
- Total bilirubin < 1.5 times upper limit of normal
- Negative serum pregnancy test
- Not pregnant or nursing
- Fertile patients must use effective contraception
- No severe arterial thromboembolic events within the past 6 months, including myocardial infarction and stroke
- No baseline diarrhea > grade 1
- No total or partial bowel obstruction
- No uncontrolled hypercalcemia
- No other prior or concurrent malignancy, except adequately treated in situ carcinoma of the uterine cervix, basal cell or squamous cell carcinoma of the skin, or cancer in complete remission for ≥ 5 years
- No other serious and uncontrolled non-malignant disease
- No known allergy to any excipients of study drugs
- Must be registered in a national health care system (CMU included)

PRIOR CONCURRENT THERAPY:

- See Disease Characteristics
- Prior anti-EGFR therapy allowed
- No prior irinotecan hydrochloride
- No concurrent agents known to have anticancer activity
- No concurrent radiotherapy
- No participation in another clinical trial with any investigational drug or treatments concurrently or within the past 30 days

Contacts/Locations

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Nanovector-based therapies in advanced pancreatic cancer

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ABSTRACT Systemic therapy for advanced pancreatic cancer has been largely disappointing owing to the unfavorable pharmacokinetic profile and poor penetration of current chemotherapeutic agents ,as well as the fragile patient population with compromised tolerance to toxic chemotherapies. Nanovectors can provide passive drug delivery through abnormal tumor neo-vasculature microanatomy or active targeting via binding to receptors or macromolecules associated with the tumor. In such a manner, nanovector-based therapy may not only modulate the pharmacokinetics and therapeutic index of chemotherapeutic agents but also provide new treatment options in patients with advanced pancreatic cancer. In this article, we present the rationale and currently available clinical results of nanovector-based therapies to highlight the potential use of this class of agent in patients with advanced pancreatic cancer.

KEY WORDS nanovector; pancreatic cancer; liposome; PEP02; nab-paclitaxel; EndoTAG-1; nanoplatin; platinum; CPT-11

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Introduction

Pancreatic cancer is one of the most detrimental malignancies and the fourth most common cause of cancer-related death in the United Stated. There were 43,140 newly diagnosed cases and 36,800 deaths in 2010 (1). Early detection is uncommon with no more than 15–20% of the patients being amenable for curative intent surgery at the time of diagnosis. Gemcitabine either alone or in combination with erlotinib are the only approved treatments for patients with advanced pancreatic cancer, of whom the overall survival time is generally around 6 months (2-5). Recently, Conroy et al showed that a gemcitabine-free triplet chemotherapy, FOLFIRINOX regimen consisting of oxaliplatin, irinotecan and infusional 5-FU/leucovorin, could achieve significantly better tumor response rate, progression-free survival and overall survival than gemcitabine monotherapy in patients with metastatic pancreatic cancer in a randomization phase III trial (6,7). However, the application of either doublet of triplet combination chemotherapy in patients with advanced pancreatic cancer is often hindered by their toxicity and the performance status of the patients.

New treatment strategies are mandatory to improve the therapeutic outcomes of patients with advanced pancreatic cancer. Recently, two major potential new approaches are emerging that may have the chance to change our practice in treating advanced pancreatic cancer. The first one is molecular targeted agent targeting on dysregulated signaling pathway and the second is the use of nanovector drug delivery system to provide 'passive" or "active" targeting drug delivery thus to modulate the pharmacokinetics and therapeutic index of chemotherapeutic agents in pancreatic cancer (8).

This review will focus on the selective nanovector treatments in pancreatic cancer, especially those with available clinical data, including albumin-bound nanoparticles, liposome-encapsulation nanoparticle, cationic liposomal nanoparticle, polymeric micellar agents, and a non-replicating, retroviral vector delivered gene therapy construct.

Albumin-bound Nanoparticle Paclitaxel (Nab-

No potential conflict of interest.

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paclitaxel)

Albumin is a particular vehicle for drug delivery in oncology because it is a natural carrier of hydrophobic molecules with reversible, noncovalent binding characteristics and able to enhance the delivery of drug into the extravascular space through a process of receptor-mediated endothelial transcytosis. Such process is initiated by the binding of albumin to an endothelium surface, 60-kDa glycoprotein (gp60) receptor (albondin), which will then bind with an intracellular protein (caveolin-1) to result in the invagination of the endothelium membrane to form transcytotic vesicles, the caveolae (9). The caveolae will subsequently move across the cytoplasm and release the albumin and its conjugated compound into the extracellular space (the peritumoral microenvironment) where the albumin will bind to SPARC (secreted protein acid and rich in cysteine), an extracellular matrix albumin-binding glycoprotein that is structurally and functionally closely related to gp60, and overexpressed in a variety of cancers, including breast cancer, gastric cancer and pancreatic cancer.

Nab-paclitaxel (Abraxane®) is a cremophor (CrEL)-free, albumin-bound, nanoparticle formulation of paclitaxel. Its CrEL-free formulation permits nab-paclitaxel to be administered within a shorter infusion period of time (30 minutes) and without the requirement of routine pre-medications for preventing the hypersensitivity reactions in association with the administration of cremophor solvent-based paclitaxel (10). In preclinical study, the transport of radiolabeled paclitaxel across the endothelial cell monolayer *in vitro,* and intratumor paclitaxel accumulation after equal doses of paclitaxel *in vivo* were both significantly enhanced by 4.2-folds ($P < 0.0001$) and 33% ($P < 0.0001$), respectively, for *nab*-paclitaxel as compared with CrEL-paclitaxel with an increase 4.2 folds. In addition, endothelial transcytosis was completely inhibited by inhibitor of gp60/caveolar transport, methyl ß-cyclodextrin (11). These observations supported that gp60-mediated transcytosis and SPARC-aided sequestration may be an important biological pathway to target tumor cells by novel albumin-bound therapeutics.

In a phase I trial, the maximum tolerated dose (MTD) of intravenous injection nab-paclitaxel monotherapy, every 3 weeks in 19 patients with standard therapy-failure solid tumors was 300 mg/m^2. No acute hypersensitivity reactions were observed. The most frequent toxicities were myelo-suppression, sensory neuropathy, nausea/vomiting, arthralgia and alopecia (12). The drug has subsequently approved for the treatment of metastatic breast cancer after failure of combination chemotherapy or relapse within 6 months of adjuvant chemotherapy. The commonly used dose/schedule was 260 mg/m^2, 30-min intravenous injection, every 3 weeks.

Because SPARC is frequently overexpressed and associated with poor clinical outcomes in pancreatic cancer, Von Hoff et al conducted a phase I/II study to evaluate the MTD of weekly nab-paclitaxel (100 – 150 mg/m^2/week) in combination with gemcitabine (1000 mg/m^2/week), and the therapeutic efficacies of the regimen. Both agents were given on day 1, 8, and 15 every 28 days (13). A total of 67 patients were treated. Despite MTD of nab-paclitaxel was determined as 125 mg/m^2/week, dose reduction was required in 30% (6/20), 18% (8/44) and 33% (1/3) of patients receiving 100 mg/m^2, 125 mg/m^2 and 150 mg/m^2, respectively. The most common grade 3-4 toxicity at the MTD dose were fatigue 23%, neutropenia 59% (grade 4 in 23%), thrombocytopenia 20% (grade 4 in 9%) and sensory neuropathy in 9%. Of the 58 patients whose CT image were revaluated with RECIST criteria by independent reviewer, the best tumor response was partial response in 40% and stable disease in 37%, with an overall disease control rate of 78%. The median progression-free and overall survival of the intent-to-treat (N=67) patients were 6.9 months and 10.3 months, respectively; while the survival parameters for the 44 patients receiving MTD dose were 7.9 months and not yet reached, respectively. Of 54 patients with available CA19.9 level, 42 (77.8%) patients had a more than 50% reduction of CA19.9 level after the treatment (14). The therapeutic efficacy of nab-paclitaxel in combination with vandetanib, a potent inhibitor of VEGF2, RET and EGFR, has also been evaluated in a phase I trial with expansion cohort of patients with pancreatic cancer (15). The MTD of vandetanib in combination with two different schedule of nab-paclitaxel, either 100 mg/m^2 weekly or 260 mg/m^2 every 3 weeks, was 300 mg daily. Of the 29 enrolled gemcitabine-refractory pancreatic cancer patients, the best tumor was partial response in 6 (20.7%) and stable disease in 10 (34.5%), and the median progression-free survival and overall survival were 5.3 (95% CI: 3.7 to 7.3) months and 8.2 (95% CI: 6.2 to 11.5) months, respectively. No statistical significant correlation between SNP (rs1059829 and rs3210714) of SPARC and clinical outcomes was observed.

Liposome-based Drugs

A liposome is often a spherical vesicle with a bilayer membrane whose size typically ranges from ~40 nanometers to several microns. Because the micro- or nanoparticles can form spontaneously and are generally easier to prepare compared to viral-mediated systems, this nontoxic phospholipid-based drug carrier has become a favorable drug delivery system for various purposes since the 1970s.

However, so-called conventional liposomes are easily bound with insoluble circulating plasma protein, i.e. opsonins and lipoproteins, and the complex will be subsequently eliminated from the circulation by reticuloendothelial cells system. Stealth liposome technology, with incorporationof high molecular weight polymers (i.e., polyethylene-glycol (PEG)) to the liposome surface, can effectively protect the liposome from circulating protein binding and subsequently phagocytosis by RER system, and thus improving its plasma clearance, prolonging the circulation time, and enhancing drug delivery efficacy.

Besides its characteristic slow-release pharmacokinetic property, liposome encapsulated drugs can potentially provide improved tumor localization via the "enhanced permeability and retention" (EPR) effect. Such agents can therefore, (i) lower drug elimination to increase systemic circulation time, (ii) lower maximum plasma concentration (C_{max}) to reduce drug side effects, (iii) enhance tumor tissue uptake and exposure to the anti-cancer drug; these principles can in turn yield an improved therapeutic index for cancer therapy.

Several liposomal formulated cancer drugs have been evaluated in various cancers, but only a limited number have been applied to pancreatic cancer.

Liposomal Doxorubicin

The first liposomal anti-cancer drug approved by the Food and Drug Administration (FDA) was pegylated liposomal doxorubicin (Caelyx®/Doxil®) in 1995 for Karposi's sarcoma (16-18). It has been subsequently approved for the treatment of multiple myeloma and recurrent epithelial ovarian cancer as well. It also has been evaluated for the treatment of pancreatic cancer in animal xenograft model and in clinical trials. In a preclinical study, Vagge et al showed that pegylated liposomal doxorubicin was significantly more effective in inhibiting the growth of human pancreatic cancer xenograft in nude mice as compared to free form doxorubicin (19). Using confocal laser scanning microscopy and microfluorimetry to quantitate the uptake of intravenously injected doxorubicin in tumor tissue, the authors found that the content of doxorubicin in tumor site of animal receiving liposomal formulated drug was 6 folds or higher compared to free doxorubicin. Based on the results, Halford et al conducted a phase II trial to evaluate the therapeutic efficacy of Caelyx® in 22 chemo-naïve patients with unresectable pancreatic carcinoma. The dose was escalated from 30 mg/m^2 (in the first two patients) to 50 mg/m^2 intravenous injection every 3 weeks (20). Of the 20 patients received the treatment, the most common grade 3 toxicity were stomatitis (20%) and nausea (10%), the best tumor response was stable diseases in 6 (30%), and the median overall survival was 3.2 months with one year survival rate of 10%. These finding excluded the use of Caelyx® monotherapy in the treatment of advanced pancreatic cancer.

The combination of Caelyx® with infusional 5-FU/leucovorin and mitomycin-C has been evaluated in a phase I trial in patients with upper gastrointestinal cancer. In that study, escalating dose of Caelyx® (15 – 35 mg/m^2) day 1 and 29 in combination with weekly 24-hour infusion of 5-FU and leucovorin (2,000 and 500 mg/m^2, respectively) for 6 weeks, and mitomycin-C 7 mg/m^2 day 8 and 36, every 8 weeks as one cycle. The most common grade 3-4 toxicities were nausea/vomiting (29%), diarrhea (18%) and leucopenia (12%). Of the 14 accruals with pre-treated pancreatic cancer, the best tumor response was partial response in one and minor response in 2, and the overall survival after the study treatment was 6.5 months (21).

Liposomal Platinum

Platinum is one of the most active and wildly used anti-cancer agents in the world, including in combination with gemcitabine to treat non-small cell lung cancer and pancreatic cancer. Although each single trial had failed to demonstrate the superiority of gemcitabine/platinum combination over gemcitabine single agent in the prolongation of the survival in patients with advanced pancreatic cancer, however, the survival benefit of gemcitabine/platinum doublets was demonstrated in a pooled, meta-analysis survival with a hazard ratio of 0.81, p = 0.031 (22).

It is also well known that the use of cisplatin is frequently limited by its nephrotoxicity, peripheral sensory neuropathy, ototoxicity and the aggravation of hematological toxicity while in combination with other cytotoxic agents. Therefore, several liposomal formulations of cisplatin have been developed aiming to reduce its toxicity profile and hopefully to enhance it activity. Based on previous experience of gemcitabine/cisplatin combination and the result of meta-analysis, several liposomal formulated cisplatin have been evaluated in patients with pancreatic cancer.

Lipoplatin is one of the pegylated liposome cisplatin, whose nanoparticulate liposomes are reverse-miscelles, composed of dipalmitoyl phosphatidyl glycerol (DPPG), soy phosphatidyl choline (SPC-3), cholesterol and methoxy- polyethylene glycol-distearoyl phosphatidylethanolamine (mPEG2000-DSPE). Lipoplatin exhibits the fundamental pharmacologic characteristics of pegylated liposomal agents, for example, protecting from the engulfment of reticuloendotheralial system

to prolong circulating time, and extravasating from the fenestrate between endothelial cells of tumor vasculature to preferentially localize in per-tumor interstitial tissue and uptake by tumor cells. The anionic, fusogenic nature of the DPPG lipids enables lipoplatin to cross cell membranes more easily than native cisplatin. In addition, with intraperitoneal injection of a "sheath" liposomes wrapped reporter β-galactosidase gene, which had same structure like lipoplatin, into human tumor bearing nude mice, Boulikas et al were able to demonstrate the preferential expression of the reporter gene in the tumor and the tumor neo-vasculature. The findings indicate the potential antiangiogenic activity of the lipoplatin (23).

In phase I trial of lipoplatin monotherapy, the drug was diluted in 5% glucose water and administered as 8 hour intravenous infusion every 14 days. The dose was escalated from 25 mg/m^2 to 125 mg/m^2. Even at the targeted dose of 125 mg/m^2, only grade 1-2 gastrointestinal and hematological toxicities were observed, but neither nephrotoxicity nor neuropathy. Higher doses, 200, 250 and 300 mg/m^2, were also tested in one each patient, respectively. The half-life of lipoplatin was estimated ranging from 60 – 117 hours. Of the 27 accruals (19 with pretreated, advanced pancreatic cancer) in this phase I trial, the objective tumor response rate and disease control rate were 11.1% and 63.0%, respectively. Based on the exciting results, the drug has been further tested in combination with gemcitabine in non-small cell lung cancer and pancreatic cancer patient cohorts (24).

In a phase I/II study, Stathopoulos GP et al evaluated the maximum tolerated dose of lipoplatin in combination with gemcitabine in patients with previously treated advanced pancreatic cancer (25). Lipoplatin was given as an 8-hour infusion followed by 60 minutes infusion of 1,000 mg/m^2 of gemcitabine at day 1 and 15 every 28 days. The dose of lipoplatin was stepwise escalated from 25 mg/m^2 to 125 mg/m^2. Of the 24 enrolled patients, two of four patients at 125 mg/m^2 experienced grade 3-4 neutropenia. Therefore, the MTD of lipoplatin in this combination was determined to be 100 mg/m^2. In this dose escalating study, there were two (8.3%) partial responders and 14 (58.3%) disease stabilizers, and the median overall survival was 4 month. Further randomized phase II/III trial against gemcitabine monotherapy is under evaluation.

Liposome-entrapped *cis*-bisneodecanoato-*trans*-R,*R*-1,2-diaminocyclohexane (DACH) platinum(II) (L-NDDP, Aroplatin™) is a lipophilic cisplatin analog that has been formulated in relatively large-size multi-lamellar liposomes measuring from 1 to 3 μm in diameter. L-NDDP has been demonstrated to be non-cross-resistant with cisplatin in cisplatin-resistant Lovo DDP 3.0 (human colon cancer cells) and L1210/PPD (human leukemia cells) both *in vitro* and *in vivo* models. In a phase I study, L-NDDP was given intravenously once every 4 weeks, ranging from 7.5 mg/m^2 to 390 mg/m^2 (26). The infusion rate was set at 4 mg NDDP per minute for all cases. In this particular study, intra-patient dose escalation was allowed. Grade 1-2 nausea/vomiting, diarrhea and fever were frequently observed in patients receiving 100 mg/m^2 or higher dose of L-NDDP. Six out of the 10 patients who had 390 mg/m^2 experienced grade 4 hematological toxicities manifesting as thrombocytopenia, granulocytopenia or both. The MTD of intravenous L-NDDP every 4 weeks was determined as 300 mg/m^2. In 2004, Aronex Pharmaceuticals had registered a phase I/II study of L-NDDP and gemcitabine combination in patients with advanced pancreatic cancer resistant to standard therapy in a public clinical trial registration website, the clinicaltrials.gov, with an indentifier of NCT00081549. Unfortunately, the latest trial information was updated in June 2005, and no further publication on this trial can be found.

Liposomal Irinotecan (Nanoliposomal CPT-11, PEP02, MM-398)

Irinotecan hydrochloride (CPT-11) is a water-soluble semi-synthetic derivative of camptothecin targeting topoisomerase I, and has been an approved agent for the treatment of metastatic colorectal cancer worldwide, and also for gastric cancer (Japan and Korea), non-small cell lung cancer, small cell lung cancer, cervical cancer, and non-Hodgkin's lymphoma in Japan. In pancreatic cancer, earlier trial showed that combination of gemcitabine and CPT-11 did not provide any survival benefit over gemcitabine monotherapy in patients with advanced pancreatic cancer, and thus CPT-11 has not been considered to be a clinically useful drug in this disease. However, in the recent PRODIGE 4/ACCORD 11 trial, Conroy et al demonstrated that a gemcitabine-free, CPT-11-containing regimen, FOLFIRINOX (CPT-11, oxaliplatin plus intermittent infusion of 5-FU/leucovorin), provided significantly better objective tumor response rate, progression-free survival and overall survival versus gemcitabine monotherapy in patients with metastatic pancreatic cancer. Notable and not unexpectedly, this triplet regimen is associated with significant hematologic toxicity including higher rates of grade-3/4 febrile neutropenia. The results of the PRODIGE/ACCORD 11 trial have revived interest in CPT-11-based therapy in advanced pancreatic cancer (6,7).

Although the original CPT-11 drug is now of interest in pancreatic cancer management, potentially superior versions incorporating drug delivery technologies offer a

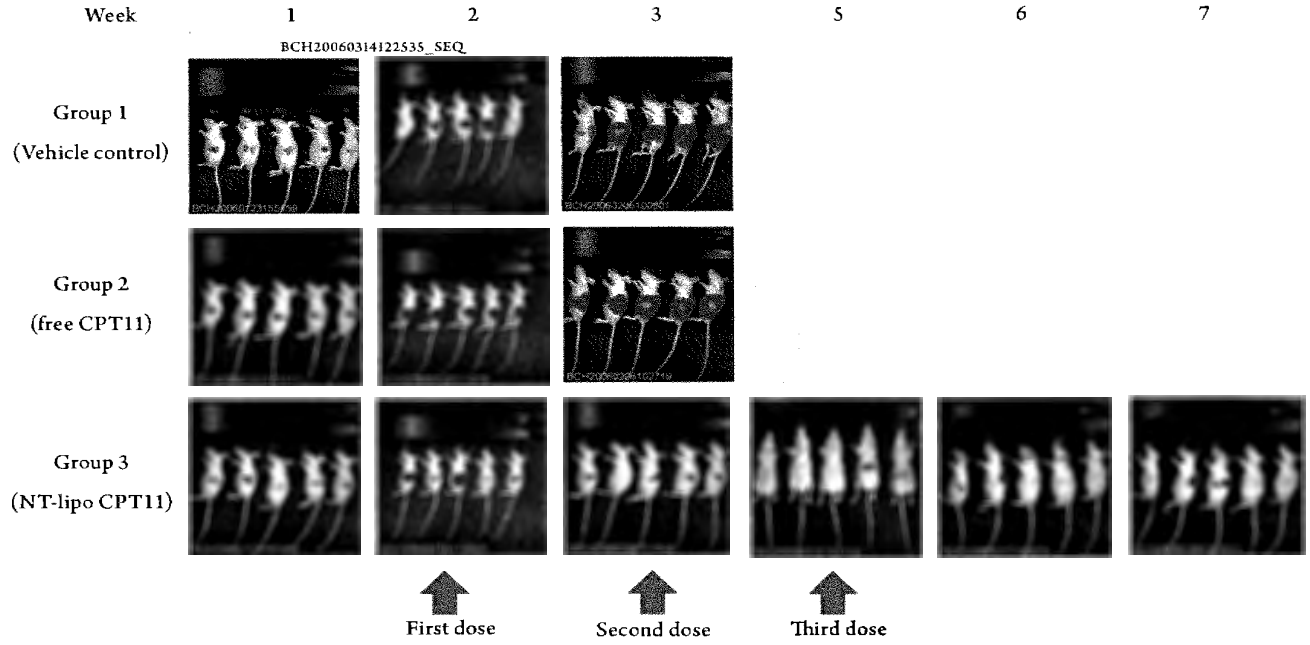
next generation approach. CPT-11 exhibits well-known pharmacologic liabilities and significant associated toxicities, which in turn make it an obvious candidate for drug delivery strategies The camptothecins exist in a pH-dependent equilibrium between an inactive carboxylate form (predominant at neutral-to-basic pH) and an active lactone form (predominant under acidic conditions); hence, intravenous injection of free CPT-11 results in rapid inactivation as well as clearance. Furthermore, CPT-11 is largely a prodrug which is converted into the much more potent metabolite SN-38. Hepatic activation and hepatobiliary excretion of SN-38 result in substantial risk of GI injury, especially in individuals having impaired SN-38 glucuronidation. These metabolic conversions contribute to notable heterogeneities in both efficacy and toxicity, and ultimately to a rather narrow therapeutic index. The concept of nanoparticle delivery of CPT-11 is thus very attractive based on potential advantages including: overcoming solubility limitations of the camptothecins; protecting drug in the active lactone configuration; chaperoning drug away from sites of toxicity such as the GI tract; prolonging circulation time and increasing tumor accumulation via the enhanced permeability and retention (EPR) effect; and providing sustained release and prolonged tumor exposure.

To realize the potential advantages of nanoparticle delivery, a novel liposome-based construct termed "nanoliposomal CPT-11 (nLs-CPT-11)" was developed, which encapsulates CPT-11 with unprecedented efficiency and stability (27). PK studies showed long circulation times for the carrier and undetectable drug release in plasma. Furthermore, nanoliposomal CPT-11 provides protection of drug in its active lactone form within the liposome aqueous interior, preventing its hydrolysis as well as premature conversion to the potent and toxigenic metabolite, SN-38. This contrasts markedly with free CPT-11, which is rapidly cleared from circulation, is subject to immediate hydrolysis of the lactone ring, and is also converted to SN-38 contributing to its dose-limiting GI toxicity.

In a series of preclinical studies, nanoliposomal CPT-11 demonstrated significantly superior efficacy when compared to free CPT-11 at the same or higher dose, including frequent cures in some models. The superiority of nanoliposomal CPT-11 over free CPT-11 has been observed in different tumor models including colorectal, gastric, breast, cervical, glioma, pancreatic and lung cancer models. In addition to superior efficacy, nanoliposomal CPT-11 has shown a more favorable pharmacologic profile and reduced toxicity in multiple preclinical models.

In order to evaluate this novel agent as a potential therapy for pancreatic cancer, a bioluminescence-based orthotopic xenograft model of pancreas cancer was developed (28). COLO357, a human pancreatic cell line, was passaged multiple times in vivo to generate the subline L3.6pl. This cell line was then modified by lentiviral transduction (L3.6pl-T) to express firefly luciferase. L3.6pl-T cells were implanted during open surgery directly into the pancreas of a nude mouse to form an orthotopic tumor xenograft. Therapeutic studies in this model compared nanoliposomal CPT-11 versus free drug at the equivalent dose, along with vehicle control (Figure 1). All treatments were administered intravenously by tail vein beginning at 7 days post-tumor implantation and continued weekly for a total of 3 planned treatments. At 20 mg/kg, free CPT-11 showed some tumor growth inhibition, but all mice required euthanization after 2 doses due to massive tumor progression. In contrast, nanoliposomal CPT-11 at the equivalent 20 mg/kg dose showed potent antitumor activity, including complete tumor inhibition during the entire post-treatment period. Systemic toxicity was not observed with any treatment. These studies indicated that nanoparticle- mediated delivery via nanoliposomal CPT-11 greatly enhances antitumor efficacy in the COLO357/L3.6pl-T orthotopic pancreatic xenograft model.

In the first-in-human phase I trial, patients with standard therapy-failure solid tumor were enrolled to determine the maximum tolerated dose, safety profile and pharmacokinetics of nanoliposomal CPT-11 (formerly PEP02, PharmaEngine, Inc., Taiwan, and now under the designation of MM-398, Merrimack Pharmaceuticals, Inc, USA). The drug was delivered intravenously for 90 minutes, once every 3 weeks, with starting dose of 60 mg/m^2. The maximum tolerated dose was 120 mg/m^2. Two patients achieved partial response including cervical cancer in one and pancreatic cancer in one (29). The observation was further extended in a phase I trial for nanoliposomal CPT-11in combination with weekly 24-hour infusion of high-dose 5-FU/LV (HDFL). In the two phase I trials, 7 pancreatic cancer patients who failed gemcitabine/HDFL +/- platinum had received PEP02 with or without HDFL. The best response was partial response in one, stable disease in 4 and progressive disease in 2, which indicated a potential activity of PEP02 in treating gemcitabine-refractory advanced pancreatic cancer. Based on these clinical observations and preclinical results, clinical testing of nanoliposomal CPT-11 was pursued in patients with gemcitabine-based chemotherapy failure advanced pancreatic cancer in an international phase II trial with the target of the primary end-point of 3-month overall survival rate ($OS_{3\text{-month}}$) = 65%. The results have been presented at the 2011 ASCO meeting (30). Of the 40 treated patients, more than three fourths had failed to first-line gemcitabine-based doublet or triplet chemotherapy. Mean cycle of treatment



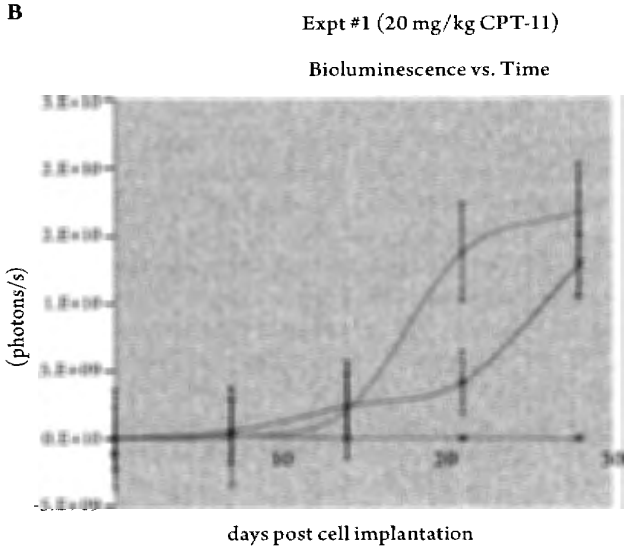


Figure 1 Nude mice were orthotopically implanted with COLO357/L3.6pI-T xenografts into the pancreas. Following ip administration of luciferin, animals were immediately imaged using a Xenogen IVIS 100 bioluminescence system, and subsequently imaged at weekly intervals. The signal was quantified by defining regions of interest (ROIs) and measuring photons/sec/str. Quantitative BLI values at post implantation day 7 were used to assign mice to treatment groups of five mice per group. Treatments included nanoliposomal CPT- 11 at 20 mg/kg, free CPT-11 at 20 mg/kg or vehicle control. All treatments were administered i.v. by tail vein injection beginning at 7 days post- tumor implantation and continued weekly for a total of 3 planned treatments. (A) Bioluminescence images of nude mice on weeks 1-7. (B) BLI values over time. Free CPT-11 treatment (diamonds) produced partial inhibition of tumor growth at initial time points, followed by rapid growth approaching that of the vehicle control group (+). Nanoliposomal CPT-11 treatment (circles) produced complete inhibition of tumor growth at all time points.

was 5.4 (range, 1 – 26) cycles. The most common G3/4 toxicities were: neutropenia (30%), leucopenia (22.5%), anemia (15%), diarrhea (7.5%), and fatigue (7.5%). Dose modification due to adverse events was required in 10 (25%) patients. The best tumor response rate was partial response in 7.5% and stable disease in 40% (overall disease control rate of 47.5%). The overall survival was 5.2 months with a 3-month and 6-month survival rate of 75% and 42.5%, respectively. The results highlight the feasibility and activity of nanoliposomal CPT-11 in previously heavily treated patients with gemcitabine-refractory advanced pancreatic cancer, which deserves further exploration.

Cationic Liposome Encapsulated Paclitaxel (EndoTAG™-1)

Tumor angiogenesis, the formation of neovasculature from pre-existed peri-tumor vessels, is a crucial process in supporting the development and growth of tumor mass, and the dissemination of tumor metastases. Tumor angiogenesis is mainly triggered by growth factors that are secreted by tumor cells per se and/or by miscellaneous types of cell within the microenvironment, for example, tumor associated macrophages or fibroblasts. Tumor vessels are often dilated and torturous, and characterized by large inter-endothelial cell gap (up to 100 – 600 nm *versus* < 6 nm in normal vessels), aberrant pericytes and basement membrane coverage, overexpression of specific surface receptor or antigen, and the presence of negative charged macro-molecules for example, anionic phospholipids and glycoprotein. Based on these characters, several strategies have been used to develop neo-vascular targeting liposomal drugs, which include conjugating with specific antibody again surface antigen or receptor and modified, non-functional receptor binding ligand, or incorporating positive (cationic) charged molecules in the surface of liposome. Of them, cationic liposome is a unique and interesting approach (31). In a preclinical study, Kalra and Campbell showed 5-FU and doxorubicin-loaded cationic liposome could preferentially bind with human endothelial (HMEC-1 and HUVEC) rather than pancreatic cancer cells. (HPAF-II and Capan-1)(32). Subsequently, Eichhorn et al showed that both cationic lipid complexed paclitaxel (EndoTAG™-1) and camptothecin (EndoTAG™-2) could preferentially bind at endothelial cells of neo-vasculature in solid tumor preclinical model (33-35). The selectively targeting of both agents on tumor microvasculature was confirmed by quantitative fluorescence microscopy. Further study suggested the anti-vascular effect of cationic liposome encapsulated paclitaxel (EndoTAG™-1) is schedule-dependent with metronomic schedule better than the maximum tolerated dose schedule. In addition, the combination of EndoTAG™-1 and gemcitabine could significantly inhibit the incidence of metastatsis in L3.6pl orthotopic pancreatic cancer mice model.

Based on these data, EndoTAG™-1, a cationic liposome (prepared from 1,2 dioleoyl-3-trimethyl- ammonium-propane (DOTAP) and 1,2 dioleoyl-sn-glycero-3-phosphocholine (DOPC)) encapsulated paclitaxel, has been used in combination with gemcitabine to treat chemo-naïve pancreatic cancer patients. The latest follow-up data of the four-arm randomized, phase II trial comparing weekly gemcitabine 1,000 mg/m^2 alone *versus* gemcitabine plus twice weekly EndoTAG™-1 at three different doses, 11, 22 and 44 mg/m^2) was presented in the 2009 ASCO Annual Meeting (36). Of the 200 chemo-naïve advanced pancreatic cancer patients who participated the study, 80% had metastatic diseases and 20% had locally advanced diseases. Disease-control rates in the gemcitabine monotherapy arm and the three gemcitabine plus EndoTAG-1 arms was 43% and ranging from 53% to 69%, respectively. The median progression-free survival time in corresponding group of patients were 2.7 months versus 4.1 to 4.6 months, respectively. The median overall survival time of patients receiving gemcitabine plus either high-dose (44 mg/m^2) or intermediate-dose of EndoTAG-1 were 9.4 months and 8.7 months, respectively, as compared with the 7.2 months in the gemcitabine monotherapy arm. The adjusted hazard ratio for overall survival for either arm was 0.72 (95% CI, 0.46 to 1.13) and 0.67 (95% CI, 0.43 to 1.07), respectively. The data is exciting but large-scale study to validate the data is mandatory.

Polymeric Micelles

Polymeric micelles-based anticancer drug, consisting of the incorporation of chemotherapeutic agent into polymeric micelles in size of 20–100 nm, was originally developed by Professor Kataoka(37). The polymeric micelle has two major components, a polyethylene glycol (PEG) constituted hydrophilic outer shell and a cytotoxic chemotherapeutic agent incorporated hydrophobic inner core. The main action mechanism of the polymeric micelles is similar to lipomosal agents and through the passive targeting based on the enhanced permeability of tumor neo-vasculature and the impeding clearance of macromolecules from lymphatic-deficient tumor interstitial tissue. Several cytotoxic chemotherapy-incorporating polymeric micellar nanoparticles have been in clinical trials, including paclitaxel-incorporating PEG-polyaspartate (NK105), cisplatin-incorporating PEG-polyglutamate/cisplatin complex (NC-6004) and SN-38-incorporating PEG-

ployglutamate/SN-38 (NK012). Of them, NC-6004 is currently evaluated in a phase Ib/II trial for patients with advanced pancreatic cancer, and will be discussed (38-41).

Cisplatin-incorporating Polymeric Micelles, NC-6004

In animal study, NC-6004 showed characteristic delayed total body clearance and higher area-under curve as compared with free cisplatin with a ratio of 1/19 and 65 folds, respectively (42). In addition, both histopathological and biochemical studies suggested NC-6004 significantly reduced cisplatin-associated nephrotoxicity. In phase I trial for patients with refractory advanced solid tumor, escalating dose of NC-6004 was administered intravenously every 3 weeks. Despite the implantation of pre-medication and post-therapy hydration, nephrotoxicity and allergic reaction were observed in patients receiving 120 mg/m^2 and further dose escalation was withheld. The MTD and the recommended dose were determined as 120 mg/m^2 and 90 mg/m^2, respectively. Pharmacokinetic study showed the maximum plasma concentration and area under curve of ultra-filterable platinum after 120 mg/m^2 of NC-6004 were 1/34 and 8.5 folds of those with free cisplatin (43). Seven out of 17 accruals achieved stable diseases, including two of two pancreatic cancer patients who had NC-6004 at dose level of 90 mg/m^2. Perhaps owing to earlier meta-analysis showed he combination of gemcitabine and platinum could significantly improved the overall survival of advanced pancreatic cancer patients as compared to gemcitabine monotherapy, NC-6004 is currently proceeded into a phase Ib/II trial to evaluate the maximum tolerated dose of NC-6004 in combination with gemcitabine and the therapeutic efficacy of the combination in patients with chemo-naïve advanced pancreatic cancer, clinicaltrials.gov identifier NCT00910741.

Rexin-G

Rexin-G is a highly engineered, nonreplicating retroviral vector displaying a von Willebrand factor–derived collagen-binding motif at its amphotropic envelope, and expressing a dominant negative cyclin G1 gene (44-46). This Willebrand factor-derived collagen-binding motif on the retrovector' s surface enables the nanoparticle drug to seek and be selectively delivered to primary and secondary tumor sites where angiogenesis and collagen matrix exposure characteristically occur. The encoded dominant negative cyclin G1 gene will thus to disrupt tumor cell cyclin G1 activity to lead to the destruction and/or growth inhibition of tumor.

There were two dose escalating phase I trials evaluating different dose/schedule of Rexin-G in patients with gemcitabine-failed advanced pancreatic cancer. The first trial evaluating 3 dose levels of Rexin-G administered intravenously, level I, 7.5 x 10^9 colony forming units (CFU) per day, days 1-7 and 15-21 every 28 days; level II, 1.1 x 10^{10} CFU per day, days 1-7 and 15-21 every 28 days; and level III, 3 x 10^{10} CFU per day, 5 days per week x 4 weeks/cycle with 6 weeks rest between two cycles. A total of 12 patients were enrolled, only one patient with dose-limiting toxicity manifesting as grade 3 transaminitis was observed at dose level II. However, the best tumor response was stable disease in one (8.3%) and the median time to tumor progression and overall survival of intent-to-treat population were 32 days and 3.5 months, respectively (47). In the second trial, the dose of Rexin-G was increased to 1 x 10^{11} CFU per day, twice or thrice per week for 4 weeks as one cycle (dose levels 0 and I), and 2 x 10^{11} CFU per day, thrice per week for 4 weeks as one cycle (dose levels II). A total of 13 patients were enrolled, 6 in dose level 0-I and 7 in dose level II. There was no DLT observed. On intent-to –treat analysis, the tumor control rate was 50% (3/6) and 85.7% (6/7 with one partial responder) of patients at dose level 0-I and II, respectively. The median overall survival in corresponding group of patients was 2.6 months and 9.3 months, respectively (48). Based on the results, the US FDA has granted Rexin-G fast-track designation as second-line treatment for pancreatic cancer in June 2009. Currently, a phase II/III pivotal two-arm randomized study aiming to validate the survival benefit of Rexin-G monotherapy *versus* physician's choice in gemcitabine-refractory pancreatic cancer is under discussion.

Conclusion

Systemic therapy for advanced pancreatic cancer has been largely disappointed owing to the unfavorable pharmacokinetic profile and poor penetration of current chemotherapeutic agents and the fragile patient population hard to tolerate toxic combination chemotherapy. Nanovector can provide passive or active targeting drug delivery to reduce the system exposure and enhance local drug retention in tumor tissue. In this review, we provide pre-clinical and clinical evidence to support the potential use of nanovector-based therapy in patients with advanced pancreatic cancer. Unfortunately, most of trials reported here are relatively small and without control group. Prospective, large-scale randomization trials are warranted to confirm their efficacy in this difficult tumor. In addition, the combination of the relatively low toxic nanoparticle drug with conventional cytotoxic agent and/or

Table 1 Nanovectors in pancreatic cancer treatment

Name	Compound	Nanocarrier	Size	Status
Abraxane™	Paclitaxel	Nanoparticle-albumin	130 nm	Phase I/II
Caelyx™	Doxorubicin	Liposome	100 nm	Phase I/II
Lipoplatin™	Cisplatin	Liposome	110nm	Phase I/II
Aroplatin™	Platunum	Liposome	1-3 μm	Phase I/II
MM-398	Irinotecan	Liposome	110±30 nm	Phase II
Endotag-1™	Paclitaxel	Liposome	180-200 nm	Phase II
Nanoplatin™	Cisplatin	Polymer Micelle	30 nm	Phase I/II
Rexin-G™	Cyclin G1 gene	Viral vector	110 nm	Phase I/II

recently emergent molecular targeted agent should also be investigated to improve the clinical outcomes of patients with advanced pancreatic cancer.

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Camptosar®
irinotecan hydrochloride injection

For Intravenous Use Only

WARNINGS

CAMPTOSAR Injection should be administered only under the supervision of a physician who is experienced in the use of cancer chemotherapeutic agents. Appropriate management of complications is possible only when adequate diagnostic and treatment facilities are readily available. CAMPTOSAR can induce both early and late forms of diarrhea that appear to be mediated by different mechanisms. Both forms of diarrhea may be severe. Early diarrhea (occurring during or shortly after infusion of CAMPTOSAR) may be accompanied by cholinergic symptoms of rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, and intestinal hyperperistalsis that can cause abdominal cramping. Early diarrhea and other cholinergic symptoms may be prevented or ameliorated by atropine (see PRECAUTIONS, General). Late diarrhea (generally occurring more than 24 hours after administration of CAMPTOSAR) can be life threatening since it may be prolonged and may lead to dehydration, electrolyte imbalance, or sepsis. Late diarrhea should be treated promptly with loperamide. Patients with diarrhea should be carefully monitored and given fluid and electrolyte replacement if they become dehydrated or antibiotic therapy if they develop ileus, fever, or severe neutropenia (see WARNINGS). Administration of CAMPTOSAR should be interrupted and subsequent doses reduced if severe diarrhea occurs (see DOSAGE AND ADMINISTRATION).

Severe myelosuppression may occur (see WARNINGS).

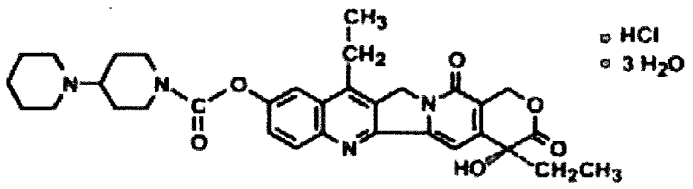
DESCRIPTION

CAMPTOSAR Injection (irinotecan hydrochloride injection) is an antineoplastic agent of the topoisomerase I inhibitor class. Irinotecan hydrochloride was clinically investigated as CPT-11.

CAMPTOSAR is supplied as a sterile, pale yellow, clear, aqueous solution. It is available in two single-dose sizes: 2 mL-fill vials contain 40 mg irinotecan hydrochloride and 5 mL-fill vials contain 100 mg irinotecan hydrochloride. Each milliliter of solution contains 20 mg of irinotecan hydrochloride (on the basis of the trihydrate salt), 45 mg of sorbitol NF powder, and 0.9 mg of lactic acid, USP. The pH of the solution has been adjusted to 3.5 (range, 3.0 to 3.8) with sodium hydroxide or hydrochloric acid. CAMPTOSAR is intended for dilution with 5% Dextrose Injection, USP (D5W), or 0.9% Sodium Chloride Injection, USP, prior to intravenous infusion. The preferred diluent is 5% Dextrose Injection, USP.

Irinotecan hydrochloride is a semisynthetic derivative of camptothecin, an alkaloid extract from plants such as *Camptotheca acuminata* or is chemically synthesized.

The chemical name is *(S)*-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo1*H*-pyrano[3',4':6,7]-indolizino[1,2-b]quinolin-9-yl-[1,4'bipiperidine]-1'-carboxylate, monohydrochloride, trihydrate. Its structural formula is as follows:



Irinotecan Hydrochloride

Irinotecan hydrochloride is a pale yellow to yellow crystalline powder, with the empirical formula $C_{33}H_{38}N_4O_6 \cdot HCl \cdot 3H_2O$ and a molecular weight of 677.19. It is slightly soluble in water and organic solvents.

CLINICAL PHARMACOLOGY

Irinotecan is a derivative of camptothecin. Camptothecins interact specifically with the enzyme topoisomerase I which relieves torsional strain in DNA by inducing reversible single-strand breaks. Irinotecan and its active metabolite SN-38 bind to the topoisomerase I-DNA complex and prevent religation of these single-strand breaks. Current research suggests that the cytotoxicity of irinotecan is due to double-strand DNA damage produced during DNA synthesis when replication enzymes interact with the ternary complex formed by topoisomerase I, DNA, and either irinotecan or SN-38. Mammalian cells cannot efficiently repair these double-strand breaks.

Irinotecan serves as a water-soluble precursor of the lipophilic metabolite SN-38. SN-38 is formed from irinotecan by carboxylesterase-mediated cleavage of the carbamate bond between the camptothecin moiety and the dipiperidino side chain. SN-38 is approximately 1000 times as potent as irinotecan as an inhibitor of topoisomerase I purified from human and rodent tumor cell lines. In vitro cytotoxicity assays show that the potency of SN-38 relative to irinotecan varies from 2- to 2000-fold. However, the plasma area under the concentration versus time curve (AUC) values for SN-38 are 2% to 8% of irinotecan and SN-38 is 95% bound to plasma proteins compared to approximately 50% bound to plasma proteins for irinotecan (see Pharmacokinetics). The precise contribution of SN-38 to the activity of CAMPTOSAR is thus unknown. Both irinotecan and SN-38 exist in an active lactone form and an inactive hydroxy acid anion form. A pH-dependent equilibrium exists between the two forms such that an acid pH promotes the formation of the lactone, while a more basic pH favors the hydroxy acid anion form.

Administration of irinotecan has resulted in antitumor activity in mice bearing cancers of rodent origin and in human carcinoma xenografts of various histological types.

Pharmacokinetics

After intravenous infusion of irinotecan in humans, irinotecan plasma concentrations decline in a multiexponential manner, with a mean terminal elimination half-life of about 6 to 12 hours. The mean terminal elimination half-life of the active metabolite SN-38 is about 10 to 20 hours. The half-lives of the lactone (active) forms of irinotecan and SN-38 are similar to those of total irinotecan and SN-38, as the lactone and hydroxy acid forms are in equilibrium.

Over the recommended dose range of 50 to 350 mg/m^2, the AUC of irinotecan

increases linearly with dose; the AUC of SN-38 increases less than proportionally with dose. Maximum concentrations of the active metabolite SN-38 are generally seen within 1 hour following the end of a 90-minute infusion of irinotecan. Pharmacokinetic parameters for irinotecan and SN-38 following a 90-minute infusion of irinotecan at dose levels of 125 and 340 mg/m^2 determined in two clinical studies in patients with solid tumors are summarized in Table 1:

Table 1.Summary of Mean (±Standard Deviation) Irinotecan and SN-38 Pharmacokinetic Parameters in Patients with Solid Tumors

Dose (mg/m^2)	Irinotecan					SN-38		
	C_{max} (ng/mL)	AUC_{0-24} (ng·h/mL)	$t_{1/2}$ (h)	V_z (L/m^2)	CL (L/h/m^2)	C_{max} (ng/mL)	AUC_{0-24} (ng·h/mL)	$t_{1/2}$ (h)
125 (N=64)	1,660 ±797	10,200 ±3,270	5.8[a] ±0.7	110 ±48.5	13.3 ±6.01	26.3 ±11.9	229 ±108	10.4[a] ±3.1
340 (N=6)	3,392 ±874	20,604 ±6,027	11.7[b] ±1.0	234 ±69.6	13.9 ±4.0	56.0 ±28.2	474 ±245	21.0[b] ±4.3

C_{max} - Maximum plasma concentration
AUC_{0-24} - Area under the plasma concentration-time curve from time 0 to 24 hours after the end of the 90-minute infusion
$t_{1/2}$ - Terminal elimination half-life
V_z - Volume of distribution of terminal elimination phase
CL - Total systemic clearance
[a] Plasma specimens collected for 24 hours following the end of the 90-minute infusion.
[b] Plasma specimens collected for 48 hours following the end of the 90-minute infusion. Because of the longer collection period, these values provide a more accurate reflection of the terminal elimination half-lives of irinotecan and SN-38.

Irinotecan exhibits moderate plasma protein binding (30% to 68% bound). SN-38 is highly bound to human plasma proteins (approximately 95% bound). The plasma protein to which irinotecan and SN-38 predominantly binds is albumin.

Metabolism and Excretion: The metabolic conversion of irinotecan to the active metabolite SN-38 is mediated by carboxylesterase enzymes and primarily occurs in the liver. *In vitro* studies indicate that irinotecan, SN-38 and another metabolite aminopentane carboxylic acid (APC), do not inhibit cytochrome P-450 isozymes. SN-38 is subsequently conjugated predominantly by the enzyme UDP-glucuronosyl transferase 1A1 (UGT1A1) to form a glucuronide metabolite. UGT1A1 activity is reduced in individuals with genetic polymorphisms that lead to reduced enzyme activity such as the UGT1A1*28 polymorphism. Approximately 10% of the North American population is homozygous for the UGT1A1*28 allele (also referred to as UGT1A1 7/7 genotype). In a prospective study, in which irinotecan was administered as a single-agent (350 mg/m^2) on a once-every-3-week schedule, patients with the UGT1A1 7/7 genotype had a higher exposure to SN-38 than patients with the wild-type UGT1A1 allele (UGT1A1 6/6 genotype) (See WARNINGS and DOSAGE AND ADMINISTRATION). SN-38 glucuronide had 1/50 to 1/100 the activity of SN-38 in cytotoxicity assays using two cell lines in vitro. The disposition of irinotecan has not been fully elucidated in humans. The urinary excretion of irinotecan is 11% to 20%; SN-38, <1%; and SN-38 glucuronide, 3%. The cumulative biliary and urinary excretion of irinotecan and its metabolites (SN-38 and SN-38 glucuronide) over a period of 48 hours following administration of irinotecan in

two patients ranged from approximately 25% (100 mg/m^2) to 50% (300 mg/m^2).

Pharmacokinetics in Special Populations

Geriatric: The pharmacokinetics of irinotecan administered using the weekly schedule was evaluated in a study of 183 patients that was prospectively designed to investigate the effect of age on irinotecan toxicity. Results from this trial indicate that there are no differences in the pharmacokinetics of irinotecan, SN-38, and SN-38 glucuronide in patients <65 years of age compared with patients ≥65 years of age. In a study of 162 patients that was not prospectively designed to investigate the effect of age, small (less than 18%) but statistically significant differences in dose-normalized irinotecan pharmacokinetic parameters in patients <65 years of age compared to patients ≥65 years of age were observed. Although dose-normalized AUC_{0-24} for SN-38 in patients ≥65 years of age was 11% higher than in patients <65 years of age, this difference was not statistically significant. No change in the starting dose is recommended for geriatric patients receiving the weekly dosage schedule of irinotecan. (see DOSAGE AND ADMINISTRATION).

Pediatric: See **Pediatric Use** under **PRECAUTIONS.**

Gender: The pharmacokinetics of irinotecan do not appear to be influenced by gender.

Race: The influence of race on the pharmacokinetics of irinotecan has not been evaluated.

Hepatic Insufficiency: Irinotecan clearance is diminished in patients with hepatic dysfunction while exposure to the active metabolite SN-38 is increased relative to that in patients with normal hepatic function. The magnitude of these effects is proportional to the degree of liver impairment as measured by elevations in total bilirubin and transaminase concentrations. However, the tolerability of irinotecan in patients with hepatic dysfunction (bilirubin greater than 2 mg/dl) has not been assessed sufficiently, and no recommendations for dosing can be made (see DOSAGE AND ADMINISTRATION and PRECAUTIONS: Patients at Particular Risk Sections).

Renal Insufficiency: The influence of renal insufficiency on the pharmacokinetics of irinotecan has not been evaluated. Therefore, caution should be undertaken in patients with impaired renal function. Irinotecan is not recommended for use in patients on dialysis.

Drug-Drug Interactions

5-fluorouracil (5-FU) and leucovorin (LV): In a phase 1 clinical study involving irinotecan, 5-fluorouracil (5-FU), and leucovorin (LV) in 26 patients with solid tumors, the disposition of irinotecan was not substantially altered when the drugs were co-administered. Although the C_{max} and AUC_{0-24} of SN-38, the active metabolite, were reduced (by 14% and 8%, respectively) when irinotecan was followed by 5-FU and LV administration compared with when irinotecan was given alone, this sequence of administration was used in the combination trials and is recommended (see DOSAGE AND ADMINISTRATION). Formal in vivo or in vitro drug interaction studies to evaluate the influence of irinotecan on the disposition of 5-FU and LV have not been conducted.

Anticonvulsants: Exposure to irinotecan and its active metabolite SN-38 is substantially reduced in adult and pediatric patients concomitantly receiving the CYP3A4 enzyme-inducing anticonvulsants phenytoin, phenobarbital or

carbamazepine. The appropriate starting dose for patients taking these anticonvulsants has not been formally defined. The following drugs are also CYP3A4 inducers: rifampin, rifabutin. For patients requiring anticonvulsant treatment, consideration should be given to substituting non-enzyme inducing anticonvulsants at least 2 weeks prior to initiation of irinotecan therapy. Dexamethasone does not appear to alter the pharmacokinetics of irinotecan.

St. John's Wort: St. John's Wort is an inducer of CYP3A4 enzymes. Exposure to the active metabolite SN-38 is reduced in patients receiving concomitant St. John's Wort. St. John's Wort should be discontinued at least 2 weeks prior to the first cycle of irinotecan, and St. John's Wort is contraindicated during irinotecan therapy.

Ketoconazole: Ketoconazole is a strong inhibitor of CYP3A4 enzymes. Patients receiving concomitant ketoconazole have increased exposure to irinotecan and its active metabolite SN-38. Patients should discontinue ketoconazole at least 1 week prior to starting irinotecan therapy and ketoconazole is contraindicated during irinotecan therapy.

Neuromuscular blocking agents. Interaction between irinotecan and neuromuscular blocking agents cannot be ruled out. Irinotecan has anticholinesterase activity, which may prolong the neuromuscular blocking effects of suxamethonium and the neuromuscular blockade of non-depolarizing drugs may be antagonized.

Atazanavir sulfate: Coadministration of atazanavir sulfate, a CYP3A4 and UGT1A1 inhibitor has the potential to increase systemic exposure to SN-38, the active metabolite of irinotecan. Physicians should take this into consideration when co-administering these drugs.

CLINICAL STUDIES

Irinotecan has been studied in clinical trials in combination with 5-fluorouracil (5-FU) and leucovorin (LV) and as a single agent (see DOSAGE AND ADMINISTRATION). When given as a component of combination-agent treatment, irinotecan was either given with a weekly schedule of bolus 5-FU/LV or with an every-2-week schedule of infusional 5-FU/LV. Weekly and a once-every-3-week dosage schedules were used for the single-agent irinotecan studies. Clinical studies of combination and single-agent use are described below.

First-Line Therapy in Combination with 5-FU/LV for the Treatment of Metastatic Colorectal Cancer

Two phase 3, randomized, controlled, multinational clinical trials support the use of CAMPTOSAR Injection as first-line treatment of patients with metastatic carcinoma of the colon or rectum. In each study, combinations of irinotecan with 5-FU and LV were compared with 5-FU and LV alone. Study 1 compared combination irinotecan/bolus 5-FU/LV therapy given weekly with a standard bolus regimen of 5-FU/LV alone given daily for 5 days every 4 weeks; an irinotecan-alone treatment arm given on a weekly schedule was also included. Study 2 evaluated two different methods of administering infusional 5-FU/LV, with or without irinotecan. In both studies, concomitant medications such as antiemetics, atropine, and loperamide were given to patients for prophylaxis and/or management of symptoms from treatment. In Study 2, a 7-day course of fluoroquinolone antibiotic prophylaxis was given in patients whose diarrhea persisted for

greater than 24 hours despite loperamide or if they developed a fever in addition to diarrhea. Treatment with oral fluoroquinolone was also initiated in patients who developed an absolute neutrophil count (ANC) <500/mm^3, even in the absence of fever or diarrhea. Patients in both studies also received treatment with intravenous antibiotics if they had persistent diarrhea or fever or if ileus developed.

In both studies, the combination of irinotecan/5-FU/LV therapy resulted in significant improvements in objective tumor response rates, time to tumor progression, and survival when compared with 5-FU/LV alone. These differences in survival were observed in spite of second-line therapy in a majority of patients on both arms, including crossover to irinotecan-containing regimens in the control arm. Patient characteristics and major efficacy results are shown in Table 2.

Table 2. Combination Dosage Schedule: Study Results

	Study 1			Study 2	
	Irinotecan + Bolus 5-FU/LV weekly x 4 q 6 weeks	**Bolus 5-FU/LV daily x 5 q 4 weeks**	**Irinotecan weekly x 4 q 6 weeks**	**Irinotecan + Infusional 5-FU/LV**	**Infusional 5-FU/LV**
Number of Patients	231	226	226	198	187
Demographics and Treatment Administration					
Female/Male (%)	34/65	45/54	35/64	33/67	47/53
Median Age in years (range)	62 (25-85)	61 (19-85)	61 (30-87)	62 (27-75)	59 (24-75)
Performance Status (%)					
0	39	41	46	51	51
1	46	45	46	42	41
2	15	13	8	7	8
Primary Tumor (%)					
Colon	81	85	84	55	65
Rectum	17	14	15	45	35
Median Time from Diagnosis to Randomization (months, range)	1.9 (0-161)	1.7 (0-203)	1.8 (0.1-185)	4.5 (0-88)	2.7 (0-104)
Prior Adjuvant 5-FU Therapy (%)					
No	89	92	90	74	76
Yes	11	8	10	26	24
Median Duration of Study Treatment[a] (months)	5.5	4.1	3.9	5.6	4.5
Median Relative Dose Intensity (%)[a]					
Irinotecan	72	—	75	87	—
5-FU	71	86	—	86	93
Efficacy Results					
Confirmed Objective Tumor Response Rate[b] (%)	39 (p<0.0001)[c]	21	18	35 (p<0.005)[c]	22
Median Time to Tumor Progression[d] (months)	7.0 (p=0.004)[d]	4.3	4.2	6.7 (p<0.001)[d]	4.4
Median Survival (months)	14.8 (p<0.05)[d]	12.6	12.0	17.4 (p<0.05)[d]	14.1

[a] Study 1: N=225 (irinotecan/5-FU/LV),N=219 (5-FU/LV),N=223 (irinotecan)
Study 2: N=199 (irinotecan/5-FU/LV),N=186 (5-FU/LV)
[b] Confirmed ≥ 4 to 6 weeks after first evidence of objective response
[c] Chi-square test
[d] Log-rank test

Improvement was noted with irinotecan-based combination therapy relative to 5-FU/LV when response rates and time to tumor progression were examined across the following demographic and disease-related subgroups (age, gender, ethnic origin, performance status, extent of organ involvement with cancer, time from diagnosis of cancer, prior adjuvant therapy, and baseline laboratory abnormalities). Figures 1 and 2 illustrate the

Kaplan-Meier survival curves for the comparison of irinotecan/5-FU/LV versus 5-FU/LV in Studies 1 and 2, respectively.

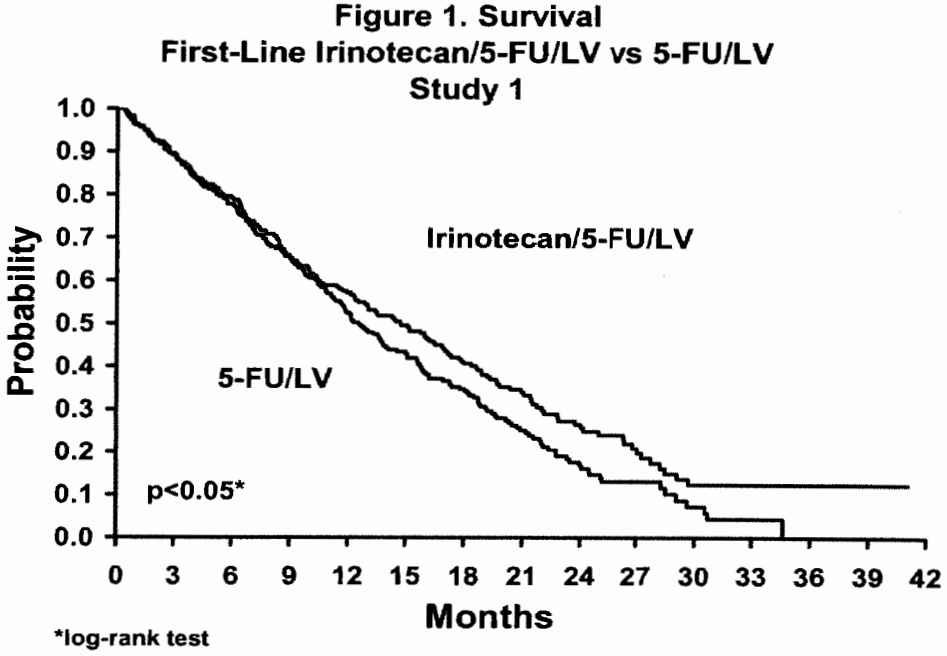
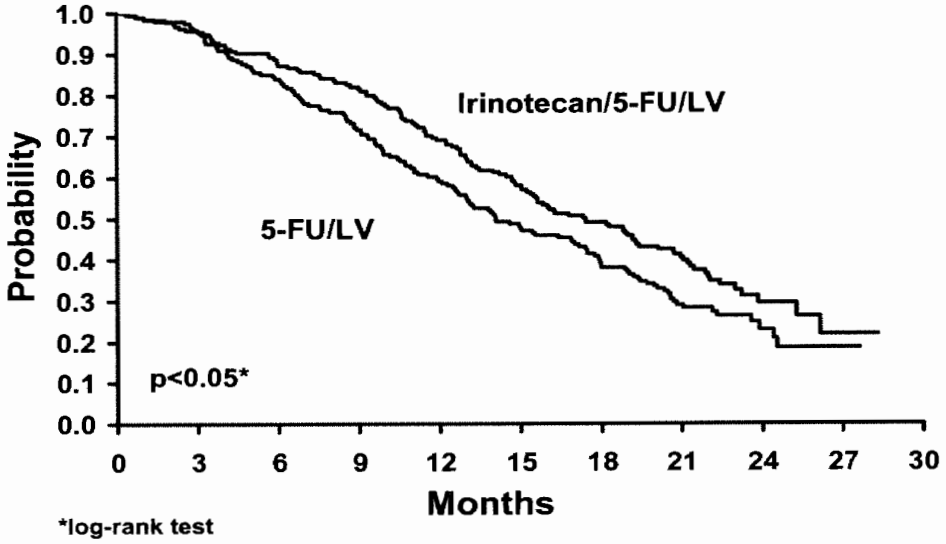


Figure 2. Survival
First-Line Irinotecan/5-FU/LV vs 5-FU/LV
Study 2



*log-rank test

After 5-FU-Based Treatment

Weekly Dosage Schedule

Data from three open-label, single-agent, clinical studies, involving a total of 304 patients in 59 centers, support the use of CAMPTOSAR in the treatment of patients with metastatic cancer of the colon or rectum that has recurred or progressed following treatment with 5-FU-based therapy. These studies were designed to evaluate tumor response rate and do not provide information on actual clinical benefit, such as effects on survival and disease-related symptoms. In each study, CAMPTOSAR was administered in repeated 6-week cycles consisting of a 90-minute intravenous infusion once weekly for 4 weeks, followed by a 2-week rest period. Starting doses of CAMPTOSAR in these trials were 100, 125, or 150 mg/m^2, but the 150-mg/m^2 dose was poorly tolerated (due to unacceptably high rates of grade 4 late diarrhea and febrile neutropenia). Study 1 enrolled 48 patients and was conducted by a single investigator at several regional hospitals. Study 2 was a multicenter study conducted by the North Central Cancer Treatment Group. All 90 patients enrolled in Study 2 received a starting dose of 125 mg/m^2. Study 3 was a multicenter study that enrolled 166 patients from 30 institutions. The initial dose in Study 3 was 125 mg/m^2 but was reduced to 100 mg/m^2 because the toxicity seen at the 125-mg/m^2 dose was perceived to be greater than that seen in previous studies. All patients in these studies had metastatic colorectal cancer, and the majority had disease that recurred or progressed following a 5-FU-based regimen administered for metastatic disease. The results of the individual studies are shown in Table 3.

Table 3. Weekly Dosage Schedule: Study Results

	Study			
	1	2	3	
Number of Patients	48	90	64	102
Starting Dose (mg/m^2/wk x 4)	125[a]	125	125	100
Demographics and Treatment Administration				
Female/Male (%)	46/54	36/64	50/50	51/49
Median Age in years (range)	63 (29-78)	63 (32-81)	61 (42-84)	64 (25-84)
Ethnic Origin (%)				
White	79	96	81	91
African American	12	4	11	5
Hispanic	8	0	8	2
Oriental/Asian	0	0	0	2
Performance Status (%)				
0	60	38	59	44
1	38	48	33	51
2	2	14	8	5
Primary Tumor (%)				
Colon	100	71	89	87
Rectum	0	29	11	8
Unknown	0	0	0	5
Prior 5-FU Therapy (%)				
For Metastatic Disease	81	66	73	68
≤ 6 months after Adjuvant	15	7	27	28
> 6 months after Adjuvant	2	16	0	2
Classification Unknown	2	12	0	3
Prior Pelvic/Abdominal Irradiation (%)				
Yes	3	29	0	0
Other	0	9	2	4
None	97	62	98	96
Duration of Treatment with CAMPTOSAR (median, months)	5	4	4	3
Relative Dose Intensity[b] (median %)	74	67	73	81
Efficacy				
Confirmed Objective Response Rate (%)[c]	21	13	14	9
(95% CI)	(9.3 - 32.3)	(6.3 - 20.4)	(5.5 - 22.6)	(3.3 - 14.3)
Time to Response (median, months)	2.6	1.5	2.8	2.8
Response Duration (median, months)	6.4	5.9	5.6	6.4
Survival (median, months)	10.4	8.1	10.7	9.3
1-Year Survival (%)	46	31	45	43

[a] Nine patients received 150 mg/m^2 as a starting dose; two (22.2%) responded to CAMPTOSAR.

[b] Relative dose intensity for CAMPTOSAR based on planned dose intensity of 100, 83.3, and 66.7 mg/m^2/wk corresponding with 150, 125, and 100 mg/m^2 starting doses, respectively.

[c] Confirmed ≥ 4 to 6 weeks after first evidence of objective response.

In the intent-to-treat analysis of the pooled data across all three studies, 193 of the 304 patients began therapy at the recommended starting dose of 125 mg/m^2. Among these 193 patients, 2 complete and 27 partial responses were observed, for an overall response rate of 15.0% (95% Confidence Interval [CI], 10.0% to 20.1%) at this starting dose. A considerably lower response rate was seen with a starting dose of 100 mg/m^2. The majority of responses were observed within the first two cycles of therapy, but responses did occur in later cycles of treatment (one response was observed after the eighth cycle). The median response duration for patients beginning therapy at 125 mg/m^2 was 5.8 months (range, 2.6 to 15.1 months). Of the 304 patients treated in the three studies, response rates to CAMPTOSAR were similar in males and females and among patients older and younger than 65 years. Rates were also similar in patients with cancer of the colon or cancer of the rectum and in patients with single and multiple metastatic sites. The response rate was 18.5% in patients with a performance status of 0 and 8.2% in patients with a performance status of 1 or 2. Patients with a performance status of 3 or 4 have not been studied. Over half of the patients responding to CAMPTOSAR had not responded to prior 5-FU. Patients who had received previous irradiation to the pelvis responded to CAMPTOSAR at approximately the same rate as those who had not previously received irradiation.

Once-Every-3-Week Dosage Schedule

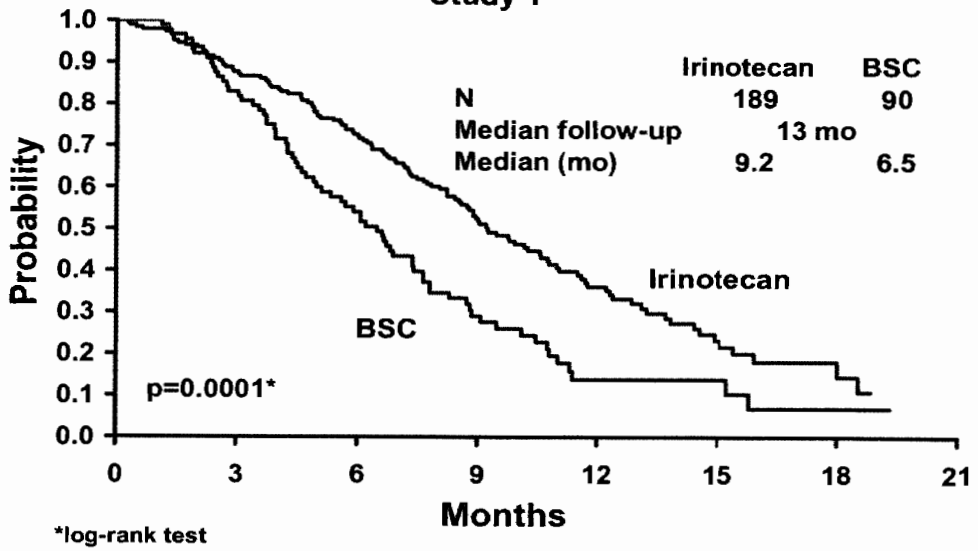
Single-Arm Studies: Data from an open-label, single-agent, single-arm, multicenter, clinical study involving a total of 132 patients support a once every-3-week dosage schedule of irinotecan in the treatment of patients with metastatic cancer of the colon or rectum that recurred or progressed following treatment with 5-FU. Patients received a starting dose of 350 mg/m^2 given by 30-minute intravenous infusion once every 3 weeks. Among the 132 previously treated patients in this trial, the intent-to-treat response rate was 12.1% (95% CI, 7.0% to 18.1%).

Randomized Trials: Two multicenter, randomized, clinical studies further support the use of irinotecan given by the once-every-3-week dosage schedule in patients with metastatic colorectal cancer whose disease has recurred or progressed following prior 5-FU therapy. In the first study, second-line irinotecan therapy plus best supportive care was compared with best supportive care alone. In the second study, second-line irinotecan therapy was compared with infusional 5-FU-based therapy. In both studies, irinotecan was administered intravenously at a starting dose of 350 mg/m^2 over 90 minutes once every 3 weeks. The starting dose was 300 mg/m^2 for patients who were 70 years and older or who had a performance status of 2. The highest total dose permitted was 700 mg. Dose reductions and/or administration delays were permitted in the event of severe hematologic and/or nonhematologic toxicities while on treatment. Best supportive care was provided to patients in both arms of Study 1 and included antibiotics, analgesics, corticosteroids, transfusions, psychotherapy, or any other symptomatic therapy as clinically indicated. In both studies, concomitant medications such as antiemetics, atropine, and loperamide were given to patients for prophylaxis and/or management of symptoms from treatment. If late diarrhea persisted for greater than 24 hours despite loperamide, a 7-day course of fluoroquinolone antibiotic prophylaxis was given. Patients in the control arm of the second study received one of the following 5-FU regimens: (1)

LV, 200 mg/m^2 IV over 2 hours; followed by 5-FU, 400 mg/m^2 IV bolus; followed by 5-FU, 600 mg/m^2 continuous IV infusion over 22 hours on days 1 and 2 every 2 weeks; (2) 5-FU, 250 to 300 mg/m^2/day protracted continuous IV infusion until toxicity; (3) 5-FU, 2.6 to 3 g/m^2 IV over 24 hours every week for 6 weeks with or without LV, 20 to 500 mg/m^2/day every week IV for 6 weeks with 2-week rest between cycles. Patients were to be followed every 3 to 6 weeks for 1 year.

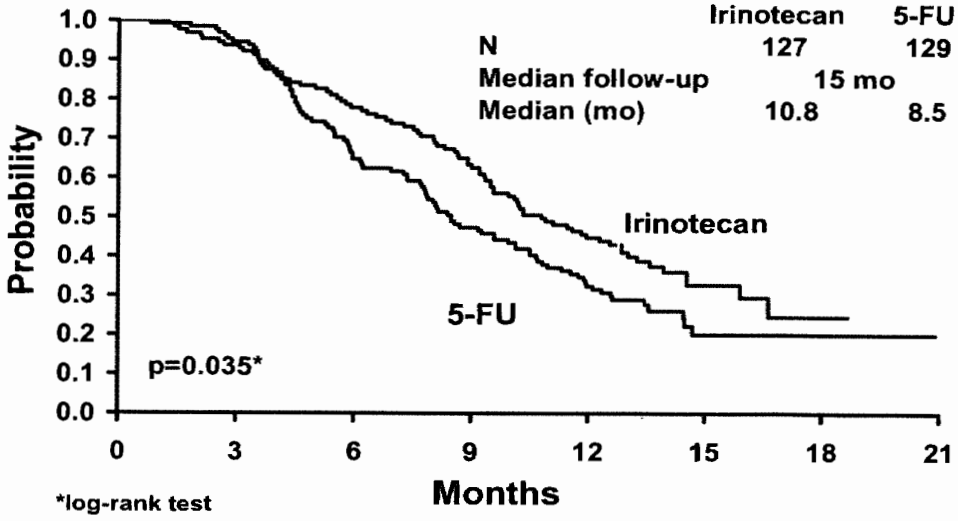
A total of 535 patients were randomized in the two studies at 94 centers. The primary endpoint in both studies was survival. The studies demonstrated a significant overall survival advantage for irinotecan compared with best supportive care (p=0.0001) and infusional 5-FU-based therapy (p=0.035) as shown in Figures 3 and 4. In Study 1, median survival for patients treated with irinotecan was 9.2 months compared with 6.5 months for patients receiving best supportive care. In Study 2, median survival for patients treated with irinotecan was 10.8 months compared with 8.5 months for patients receiving infusional 5-FU-based therapy. Multiple regression analyses determined that patients' baseline characteristics also had a significant effect on survival. When adjusted for performance status and other baseline prognostic factors, survival among patients treated with irinotecan remained significantly longer than in the control populations (p=0.001 for Study 1 and p=0.017 for Study 2). Measurements of pain, performance status, and weight loss were collected prospectively in the two studies; however, the plan for the analysis of these data was defined retrospectively. When comparing irinotecan with best supportive care in Study 1, this analysis showed a statistically significant advantage for irinotecan, with longer time to development of pain (6.9 months versus 2.0 months), time to performance status deterioration (5.7 months versus 3.3 months), and time to > 5% weight loss (6.4 months versus 4.2 months). Additionally, 33.3% (33/99) of patients with a baseline performance status of 1 or 2 showed an improvement in performance status when treated with irinotecan versus 11.3% (7/62) of patients receiving best supportive care (p=0.002). Because of the inclusion of patients with non-measurable disease, intent-to-treat response rates could not be assessed.

Figure 3. Survival
Second-Line Irinotecan vs Best Supportive Care (BSC)
Study 1



*log-rank test

Figure 4. Survival
Second-Line Irinotecan vs Infusional 5-FU
Study 2



*log-rank test

In the two randomized studies, the EORTC QLQ-C30 instrument was utilized. At the start of each cycle of therapy, patients completed a questionnaire consisting of 30 questions, such as "Did pain interfere with daily activities?" (1 = Not at All, to 4 = Very Much) and "Do you have any trouble taking a long walk?" (Yes or No). The answers from the 30 questions were converted into 15 subscales, that were scored from 0 to 100, and the global health status subscale that was derived from two questions about the patient's sense of general well being in the past week. In addition to the global health status subscale, there were five functional (i.e., cognitive, emotional, social, physical, role) and nine symptom (i.e., fatigue, appetite loss, pain assessment, insomnia, constipation, dyspnea, nausea/vomiting, financial impact, diarrhea) subscales. The results as summarized in Table 5 are based on patients' worst post-baseline scores. In Study 1, a multivariate analysis and univariate analyses of the individual subscales were performed and corrected for multivariate testing. Patients receiving irinotecan reported significantly better results for the global health status, on two of five functional subscales, and on four of nine symptom subscales. As expected, patients receiving irinotecan noted significantly more diarrhea than those receiving best supportive care. In Study 2, the multivariate analysis on all 15 subscales did not indicate a statistically significant difference between irinotecan and infusional 5-FU.

Table 4. Once-Every-3-Week Dosage Schedule: Study Results

	Study 1		Study 2	
	Irinotecan	BSC[a]	Irinotecan	5-FU
Number of Patients	189	90	127	129
Demographics and Treatment Administration				
Female/Male (%)	32/68	42/58	43/57	35/65
Median Age in years (range)	59 (22-75)	62 (34-75)	58 (30-75)	58 (25-75)
Performance Status (%)				
0	47	31	58	54
1	39	46	35	43
2	14	23	8	3
Primary Tumor (%)				
Colon	55	52	57	62
Rectum	45	48	43	38
Prior 5-FU Therapy (%)				
For Metastatic Disease	70	63	58	68
As Adjuvant Treatment	30	37	42	32
Prior Irradiation (%)	26	27	18	20
Duration of Study Treatment (median, months) (Log-rank test)	4.1	--	4.2 (p=0.02)	2.8
Relative Dose Intensity (median %)[b]	94	--	95	81-99
Survival				
Survival (median, months) (Log-rank test)	9.2 (p=0.0001)	6.5	10.8 (p=0.035)	8.5

[a] BSC = best supportive care

[b] Relative dose intensity for irinotecan based on planned dose intensity of 116.7 and 100 mg/m^2/wk corresponding with 350 and 300 mg/m^2 starting doses, respectively.

Table 5. EORTC QLQ-C30: Mean Worst Post-Baseline Score[a]

QLQ-C30 Subscale	Study 1			Study 2		
	Irinotecan	BSC	p-value	Irinotecan	5-FU	p-value
Global Health Status	47	37	0.03	53	52	0.9
Functional Scales						
Cognitive	77	68	0.07	79	83	0.9
Emotional	68	64	0.4	64	68	0.9
Social	58	47	0.06	65	67	0.9
Physical	60	40	0.0003	66	66	0.9
Role	53	35	0.02	54	57	0.9
Symptom Scales						
Fatigue	51	63	0.03	47	46	0.9
Appetite Loss	37	57	0.0007	35	38	0.9
Pain Assessment	41	56	0.009	38	34	0.9
Insomnia	39	47	0.3	39	33	0.9
Constipation	28	41	0.03	25	19	0.9
Dyspnea	31	40	0.2	25	24	0.9
Nausea/Vomiting	27	29	0.5	25	16	0.09
Financial Impact	22	26	0.5	24	15	0.3
Diarrhea	32	19	0.01	32	22	0.2

[a] For the five functional subscales and global health status subscale, higher scores imply better functioning, whereas, on the nine symptom subscales, higher scores imply more severe symptoms. The subscale scores of each patient were collected at each visit until the patient dropped out of the study.

INDICATIONS AND USAGE

CAMPTOSAR Injection is indicated as a component of first-line therapy in combination with 5-fluorouracil and leucovorin for patients with metastatic carcinoma of the colon or rectum. CAMPTOSAR is also indicated for patients with metastatic carcinoma of the colon or rectum whose disease has recurred or progressed following initial fluorouracil-based therapy.

CONTRAINDICATIONS

CAMPTOSAR Injection is contraindicated in patients with a known hypersensitivity to the drug or its excipients.

WARNINGS

General

Outside of a well-designed clinical study, CAMPTOSAR Injection should not be used in combination with the "Mayo Clinic" regimen of 5-FU/LV (administration for 4-5 consecutive days every 4 weeks) because of reports of increased toxicity, including toxic deaths. CAMPTOSAR should be used as recommended (see DOSAGE AND ADMINISTRATION, Table 11).

In patients receiving either irinotecan/5-FU/LV or 5-FU/LV in the clinical trials, higher rates of hospitalization, neutropenic fever, thromboembolism, first-cycle treatment discontinuation, and early deaths were observed in patients with a baseline performance status of 2 than in patients with a baseline performance status of 0 or 1.

Diarrhea

CAMPTOSAR can induce both early and late forms of diarrhea that appear to be mediated by different mechanisms. Early diarrhea (occurring during or shortly after infusion of CAMPTOSAR) is cholinergic in nature. It is usually transient and only infrequently is severe. It may be accompanied by symptoms of rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, and intestinal hyperperistalsis that

can cause abdominal cramping. Early diarrhea and other cholinergic symptoms may be prevented or ameliorated by administration of atropine (see PRECAUTIONS, General, for dosing recommendations for atropine).

Late diarrhea (generally occurring more than 24 hours after administration of CAMPTOSAR) can be life threatening since it may be prolonged and may lead to dehydration, electrolyte imbalance, or sepsis. Late diarrhea should be treated promptly with loperamide (see PRECAUTIONS, Information for Patients, for dosing recommendations for loperamide). Patients with diarrhea should be carefully monitored, should be given fluid and electrolyte replacement if they become dehydrated, and should be given antibiotic support if they develop ileus, fever, or severe neutropenia. After the first treatment, subsequent weekly chemotherapy treatments should be delayed in patients until return of pretreatment bowel function for at least 24 hours without need for anti-diarrhea medication. If grade 2, 3, or 4 late diarrhea occurs subsequent doses of CAMPTOSAR should be decreased within the current cycle (see DOSAGE AND ADMINISTRATION).

Neutropenia

Deaths due to sepsis following severe neutropenia have been reported in patients treated with CAMPTOSAR. Neutropenic complications should be managed promptly with antibiotic support (see PRECAUTIONS). Therapy with CAMPTOSAR should be temporarily omitted during a cycle of therapy if neutropenic fever occurs or if the absolute neutrophil count drops $<1000/mm^3$. After the patient recovers to an absolute neutrophil count $\geq 1000/mm^3$, subsequent doses of CAMPTOSAR should be reduced depending upon the level of neutropenia observed (see DOSAGE AND ADMINISTRATION).

Routine administration of a colony-stimulating factor (CSF) is not necessary, but physicians may wish to consider CSF use in individual patients experiencing significant neutropenia.

Patients with Reduced UGT1A1 Activity

Individuals who are homozygous for the UGT1A1*28 allele (UGT1A1 7/7 genotype) are at increased risk for neutropenia following initiation of CAMPTOSAR treatment.

In a study of 66 patients who received single-agent CAMPTOSAR (350 mg/m^2 once-every-3-weeks), the incidence of grade 4 neutropenia in patients homozygous for the UGT1A1*28 allele was 50%, and in patients heterozygous for this allele (UGT1A1 6/7 genotype) the incidence was 12.5%. No grade 4 neutropenia was observed in patients homozygous for the wild-type allele (UGT1A1 6/6 genotype).

In a prospective study (n=250) to investigate the role of UGT1A1*28 polymorphism in the development of toxicity in patients treated with CAMPTOSAR (180 mg/m^2) in combination with infusional 5-FU/LV, the incidence of grade 4 neutropenia in patients homozygous for the UGT1A1*28 allele was 4.5%, and in patients heterozygous for this allele the incidence was 5.3%. Grade 4 neutropenia was observed in 1.8% of patients homozygous for the wild-type allele.

In another study in which 109 patients were treated with CAMPTOSAR (100-125 mg/m^2) in combination with bolus 5-FU/LV, the incidence of grade 4 neutropenia in patients homozygous for the UGT1A1*28 allele was 18.2%, and in patients heterozygous for this allele the incidence was 11.1%. Grade 4 neutropenia was observed in 6.8% of patients homozygous for the wild-type allele.

When administered in combination with other agents, or as a single-agent, a reduction in the starting dose by at least one level of CAMPTOSAR should be considered for patients known to be homozygous for the UGT1A1*28 allele. However, the precise dose reduction in this patient population is not known and subsequent dose modifications should be considered based on individual patient tolerance to treatment (see DOSAGE AND ADMINISTRATION and PRECAUTIONS, Laboratory Tests).

Hypersensitivity

Hypersensitivity reactions including severe anaphylactic or anaphylactoid reactions have been observed.

Colitis/Ileus

Cases of colitis complicated by ulceration, bleeding, ileus, and infection have been observed. Patients experiencing ileus should receive prompt antibiotic support (see PRECAUTIONS).

Renal Impairment/Renal Failure

Rare cases of renal impairment and acute renal failure have been identified, usually in patients who became volume depleted from severe vomiting and/or diarrhea.

Thromboembolism

Thromboembolic events have been observed in patients receiving irinotecan-containing regimens; the specific cause of these events has not been determined.

Pulmonary Toxicity

Interstitial Pulmonary Disease (IPD)-like events, including fatalities, have been reported in patients receiving irinotecan (in combination and as monotherapy) for treatment of colorectal cancer and other advanced solid tumors. In the event of an acute onset of new or progressive, unexplained pulmonary symptoms such as dyspnea, cough, and fever, irinotecan and other co-prescribed chemotherapeutic agents should be interrupted pending diagnostic evaluation. If IPD is diagnosed, irinotecan and other chemotherapy should be discontinued and appropriate treatment instituted as needed (see ADVERSE REACTIONS: Overview of Adverse Events: *Respiratory*).

Pregnancy

CAMPTOSAR may cause fetal harm when administered to a pregnant woman. Radioactivity related to ^{14}C-irinotecan crosses the placenta of rats following intravenous administration of 10 mg/kg (which in separate studies produced an irinotecan C_{max} and AUC about 3 and 0.5 times, respectively, the corresponding values in patients administered 125 mg/m^2). Administration of 6 mg/kg/day intravenous irinotecan to rats (which in separate studies produced an irinotecan C_{max} and AUC about 2 and 0.2 times, respectively, the corresponding values in patients administered 125 mg/m^2) and rabbits (about one-half the recommended human weekly starting dose on a mg/m^2 basis) during the period of organogenesis, is embryotoxic as characterized by increased post-implantation loss and decreased numbers of live fetuses. Irinotecan was teratogenic in rats at doses greater than 1.2 mg/kg/day (which in separate studies produced an irinotecan C_{max} and AUC about 2/3 and 1/40th, respectively, of the corresponding values in patients administered 125 mg/m^2) and in rabbits at 6.0 mg/kg/day (about one-half the recommended human weekly starting dose on a mg/m^2 basis). Teratogenic effects included a variety of external, visceral, and skeletal abnormalities. Irinotecan

administered to rat dams for the period following organogenesis through weaning at doses of 6 mg/kg/day caused decreased learning ability and decreased female body weights in the offspring. There are no adequate and well-controlled studies of irinotecan in pregnant women. If the drug is used during pregnancy, or if the patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant while receiving treatment with CAMPTOSAR.

PRECAUTIONS

General

Care of Intravenous Site: CAMPTOSAR Injection is administered by intravenous infusion. Care should be taken to avoid extravasation, and the infusion site should be monitored for signs of inflammation. Should extravasation occur, flushing the site with sterile water and applications of ice are recommended.

Premedication with Antiemetics: Irinotecan is emetigenic. It is recommended that patients receive premedication with antiemetic agents. In clinical studies of the weekly dosage schedule, the majority of patients received 10 mg of dexamethasone given in conjunction with another type of antiemetic agent, such as a 5-HT^3 blocker (e.g., ondansetron or granisetron). Antiemetic agents should be given on the day of treatment, starting at least 30 minutes before administration of CAMPTOSAR. Physicians should also consider providing patients with an antiemetic regimen (e.g., prochlorperazine) for subsequent use as needed.

Treatment of Cholinergic Symptoms: Prophylactic or therapeutic administration of 0.25 to 1 mg of intravenous or subcutaneous atropine should be considered (unless clinically contraindicated) in patients experiencing rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, abdominal cramping, or diarrhea (occurring during or shortly after infusion of CAMPTOSAR). These symptoms are expected to occur more frequently with higher irinotecan doses.

Immunosuppressant Effects/Increased Susceptibility to Infections: Administration of live or live-attenuated vaccines in patients immunocompromised by chemotherapeutic agents including CAMPTOSAR, may result in serious or fatal infections. Avoid vaccination with a live vaccine in patients receiving irinotecan. Killed or inactivated vaccines may be administered; however, the response to such vaccines may be diminished.

Patients at Particular Risk: In patients receiving either irinotecan/5-FU/LV or 5-FU/LV in the clinical trials, higher rates of hospitalization, neutropenic fever, thromboembolism, first-cycle treatment discontinuation, and early deaths were observed in patients with a baseline performance status of 2 than in patients with a baseline performance status of 0 or 1. Patients who had previously received pelvic/abdominal radiation and elderly patients with comorbid conditions should be closely monitored.

The use of CAMPTOSAR in patients with significant hepatic dysfunction has not been established. In clinical trials of either dosing schedule, irinotecan was not administered to patients with serum bilirubin >2.0 mg/dL, or transaminase >3 times the upper limit of normal if no liver metastasis, or transaminase >5 times the upper limit of normal with liver metastasis. In clinical trials of the weekly dosage schedule, patients with modestly elevated baseline serum total bilirubin levels (1.0 to 2.0 mg/dL) had a significantly greater likelihood of experiencing first-cycle, grade 3 or 4 neutropenia than those

with bilirubin levels that were less than 1.0 mg/dL (50% [19/38] versus 18% [47/226]; $p<0.001$). (Also see CLINICAL PHARMACOLOGY: Pharmacokinetics in Special Populations: *Hepatic Insufficiency*). Patients with deficient glucuronidation of bilirubin, such as those with Gilbert's syndrome, may be at greater risk of myelosuppression when receiving therapy with CAMPTOSAR.

Ketoconazole, enzyme-inducing anticonvulsants and St. John's Wort are known to have drug-drug interactions with irinotecan therapy. (See Drug-Drug Interactions sub-section under CLINICAL PHARMACOLOGY)

Irinotecan commonly causes neutropenia, leucopenia, and anemia, any of which may be severe and therefore should not be used in patients with severe bone marrow failure.

Patients must not be treated with irinotecan until resolution of the bowel obstruction.

Patients with hereditary fructose intolerance should not be given CAMPTOSAR, as this product contains sorbitol.

Information for Patients

Patients and patients' caregivers should be informed of the expected toxic effects of CAMPTOSAR, particularly of its gastrointestinal complications, such as nausea, vomiting, abdominal cramping, diarrhea, and infection. Each patient should be instructed to have loperamide readily available and to begin treatment for late diarrhea (generally occurring more than 24 hours after administration of CAMPTOSAR) at the first episode of poorly formed or loose stools or the earliest onset of bowel movements more frequent than normally expected for the patient. One dosage regimen for loperamide used in clinical trials consisted of the following (Note: This dosage regimen exceeds the usual dosage recommendations for loperamide.): 4 mg at the first onset of late diarrhea and then 2 mg every 2 hours until the patient is diarrhea-free for at least 12 hours. Loperamide is not recommended to be used for more than 48 consecutive hours at these doses, because of the risk of paralytic ileus. During the night, the patient may take 4 mg of loperamide every 4 hours. Premedication with loperamide is not recommended. The use of drugs with laxative properties should be avoided because of the potential for exacerbation of diarrhea. Patients should be advised to contact their physician to discuss any laxative use.

Patients should be instructed to contact their physician or nurse if any of the following occur: diarrhea for the first time during treatment; black or bloody stools; symptoms of dehydration such as lightheadedness, dizziness, or faintness; inability to take fluids by mouth due to nausea or vomiting; inability to get diarrhea under control within 24 hours; or fever or evidence of infection.

Patients should be warned about the potential for dizziness or visual disturbances which may occur within 24 hours following the administration of CAMPTOSAR, and advised not to drive or operate machinery if these symptoms occur.

Patients should be alerted to the possibility of alopecia.

Laboratory Tests

Careful monitoring of the white blood cell count with differential, hemoglobin, and platelet count is recommended before each dose of CAMPTOSAR.

UGT1A1 Testing

A laboratory test is available to determine the UGT1A1 status of patients._Testing can detect the UGT1A1 6/6, 6/7 and 7/7 genotypes (See WARNINGS).

Drug Interactions

The adverse effects of CAMPTOSAR, such as myelosuppression and diarrhea, would be expected to be exacerbated by other antineoplastic agents having similar adverse effects.

Patients who have previously received pelvic/ abdominal irradiation are at increased risk of severe myelosuppression following the administration of CAMPTOSAR. The concurrent administration of CAMPTOSAR with irradiation has not been adequately studied and is not recommended.

Lymphocytopenia has been reported in patients receiving CAMPTOSAR, and it is possible that the administration of dexamethasone as antiemetic prophylaxis may have enhanced the likelihood of this effect. However, serious opportunistic infections have not been observed, and no complications have specifically been attributed to lymphocytopenia.

Hyperglycemia has also been reported in patients receiving CAMPTOSAR. Usually, this has been observed in patients with a history of diabetes mellitus or evidence of glucose intolerance prior to administration of CAMPTOSAR. It is probable that dexamethasone, given as antiemetic prophylaxis, contributed to hyperglycemia in some patients.

The incidence of akathisia in clinical trials of the weekly dosage schedule was greater (8.5%, 4/47 patients) when prochlorperazine was administered on the same day as CAMPTOSAR than when these drugs were given on separate days (1.3%, 1/80 patients). The 8.5% incidence of akathisia, however, is within the range reported for use of prochlorperazine when given as a premedication for other chemotherapies.

It would be expected that laxative use during therapy with CAMPTOSAR would worsen the incidence or severity of diarrhea, but this has not been studied.

In view of the potential risk of dehydration secondary to vomiting and/or diarrhea induced by CAMPTOSAR, the physician may wish to withhold diuretics during dosing with CAMPTOSAR and, certainly, during periods of active vomiting or diarrhea.

Drug-Laboratory Test Interactions

There are no known interactions between CAMPTOSAR and laboratory tests.

Carcinogenesis, Mutagenesis & Impairment of Fertility

Long-term carcinogenicity studies with irinotecan were not conducted. Rats were, however, administered intravenous doses of 2 mg/kg or 25 mg/kg irinotecan once per week for 13 weeks (in separate studies, the 25 mg/kg dose produced an irinotecan C_{max} and AUC that were about 7.0 times and 1.3 times the respective values in patients administered 125 mg/m^2 weekly) and were then allowed to recover for 91 weeks. Under these conditions, there was a significant linear trend with dose for the incidence of combined uterine horn endometrial stromal polyps and endometrial stromal sarcomas. Neither irinotecan nor SN-38 was mutagenic in the in vitro Ames assay. Irinotecan was clastogenic both in vitro (chromosome aberrations in Chinese hamster ovary cells) and in vivo (micronucleus test in mice). No significant adverse effects on fertility and general reproductive performance were observed after intravenous administration of irinotecan in doses of up to 6 mg/kg/day to rats and rabbits. However, atrophy of male reproductive organs was observed after multiple daily irinotecan doses both in rodents at 20 mg/kg (which in separate studies produced an irinotecan C_{max} and AUC about 5 and 1 times, respectively, the corresponding values in patients administered 125 mg/m^2 weekly) and

dogs at 0.4 mg/kg (which in separate studies produced an irinotecan C_{max} and AUC about one-half and 1/15th, respectively, the corresponding values in patients administered 125 mg/m^2 weekly).

Pregnancy

Pregnancy Category D—see WARNINGS.

Nursing Mothers

Radioactivity appeared in rat milk within 5 minutes of intravenous administration of radiolabeled irinotecan and was concentrated up to 65-fold at 4 hours after administration relative to plasma concentrations. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants, it is recommended that nursing be discontinued when receiving therapy with CAMPTOSAR.

Pediatric Use

The effectiveness of irinotecan in pediatric patients has not been established. Results from two open-label, single arm studies were evaluated. One hundred and seventy children with refractory solid tumors were enrolled in one phase 2 trial in which 50 mg/m^2 of irinotecan was infused for 5 consecutive days every 3 weeks. Grade 3-4 neutropenia was experienced by 54 (31.8%) patients. Neutropenia was complicated by fever in 15 (8.8%) patients. Grade 3-4 diarrhea was observed in 35 (20.6%) patients. This adverse event profile was comparable to that observed in adults. In the second phase 2 trial of 21 children with previously untreated rhabdomyosarcoma, 20 mg/m^2 of irinotecan was infused for 5 consecutive days on weeks 0, 1, 3 and 4. This single agent therapy was followed by multimodal therapy. Accrual to the single agent irinotecan phase was halted due to the high rate (28.6%) of progressive disease and the early deaths (14%). The adverse event profile was different in this study from that observed in adults; the most significant grade 3 or 4 adverse events were dehydration experienced by 6 patients (28.6%) associated with severe hypokalemia in 5 patients (23.8%) and hyponatremia in 3 patients (14.3%); in addition Grade 3-4 infection was reported in 5 patients (23.8%) (across all courses of therapy and irrespective of causal relationship).

Pharmacokinetic parameters for irinotecan and SN-38 were determined in 2 pediatric solid-tumor trials at dose levels of 50 mg/m^2 (60-min infusion, n=48) and 125 mg/m^2 (90-min infusion, n=6). Irinotecan clearance (mean ± S.D.) was 17.3 ± 6.7 L/h/m^2 for the 50mg/m^2 dose and 16.2 ± 4.6 L/h/m^2 for the 125 mg/m^2 dose, which is comparable to that in adults. Dose-normalized SN-38 AUC values were comparable between adults and children. Minimal accumulation of irinotecan and SN-38 was observed in children on daily dosing regimens [daily x 5 every 3 weeks or (daily x 5) x 2 weeks every 3 weeks].

Geriatric Use

Patients greater than 65 years of age should be closely monitored because of a greater risk of early and late diarrhea in this population (see CLINICAL PHARMACOLOGY, Pharmacokinetics in Special Populations and ADVERSE REACTIONS, Overview of Adverse Events). The starting dose of CAMPTOSAR in patients 70 years and older for the once-every-3-week-dosage schedule should be 300 mg/m^2 (see DOSAGE AND ADMINISTRATION).

ADVERSE REACTIONS

First-Line Combination Therapy

A total of 955 patients with metastatic colorectal cancer received the recommended regimens of irinotecan in combination with 5-FU/LV, 5-FU/LV alone, or irinotecan alone. In the two phase 3 studies, 370 patients received irinotecan in combination with 5-FU/LV, 362 patients received 5-FU/LV alone, and 223 patients received irinotecan alone. (See Table 11 in DOSAGE AND ADMINISTRATION for recommended combination-agent regimens.)

In Study 1, 49 (7.3%) patients died within 30 days of last study treatment: 21 (9.3%) received irinotecan in combination with 5-FU/LV, 15 (6.8%) received 5-FU/LV alone, and 13 (5.8%) received irinotecan alone. Deaths potentially related to treatment occurred in 2 (0.9%) patients who received irinotecan in combination with 5-FU/LV (2 neutropenic fever/sepsis), 3 (1.4%) patients who received 5-FU/LV alone (1 neutropenic fever/sepsis, 1 CNS bleeding during thrombocytopenia, 1 unknown) and 2 (0.9%) patients who received irinotecan alone (2 neutropenic fever). Deaths from any cause within 60 days of first study treatment were reported for 15 (6.7%) patients who received irinotecan in combination with 5-FU/LV, 16 (7.3%) patients who received 5-FU/LV alone, and 15 (6.7%) patients who received irinotecan alone. Discontinuations due to adverse events were reported for 17 (7.6%) patients who received irinotecan in combination with 5FU/LV, 14 (6.4%) patients who received 5-FU/LV alone, and 26 (11.7%) patients who received irinotecan alone.

In Study 2, 10 (3.5%) patients died within 30 days of last study treatment: 6 (4.1%) received irinotecan in combination with 5-FU/LV and 4 (2.8%) received 5-FU/LV alone. There was one potentially treatment-related death, which occurred in a patient who received irinotecan in combination with 5-FU/LV (0.7%, neutropenic sepsis). Deaths from any cause within 60 days of first study treatment were reported for 3 (2.1%) patients who received irinotecan in combination with 5-FU/LV and 2 (1.4%) patients who received 5-FU/LV alone. Discontinuations due to adverse events were reported for 9 (6.2%) patients who received irinotecan in combination with 5FU/LV and 1 (0.7%) patient who received 5-FU/LV alone.

The most clinically significant adverse events for patients receiving irinotecan-based therapy were diarrhea, nausea, vomiting, neutropenia, and alopecia. The most clinically significant adverse events for patients receiving 5-FU/LV therapy were diarrhea, neutropenia, neutropenic fever, and mucositis. In Study 1, grade 4 neutropenia, neutropenic fever (defined as grade 2 fever and grade 4 neutropenia), and mucositis were observed less often with weekly irinotecan/5-FU/LV than with monthly administration of 5-FU/LV.

Tables 6 and 7 list the clinically relevant adverse events reported in Studies 1 and 2, respectively.

Table 6. Study 1: Percent (%) of Patients Experiencing Clinically Relevant Adverse Events in Combination Therapies[a]

Adverse Event	Study 1					
	Irinotecan + Bolus 5-FU/LV weekly x 4 q 6 weeks N=225		Bolus 5-FU/LV daily x 5 q 4 weeks N=219		Irinotecan weekly x 4 q 6 weeks N=223	
	Grade 1-4	Grade 3&4	Grade 1-4	Grade 3&4	Grade 1-4	Grade 3&4
TOTAL Adverse Events	100	53.3	100	45.7	99.6	45.7
GASTROINTESTINAL						
Diarrhea						
late	84.9	22.7	69.4	13.2	83.0	31.0
grade 3	--	15.1	--	5.9	--	18.4
grade 4	--	7.6	--	7.3	--	12.6
early	45.8	4.9	31.5	1.4	43.0	6.7
Nausea	79.1	15.6	67.6	8.2	81.6	16.1
Abdominal pain	63.1	14.6	50.2	11.5	67.7	13.0
Vomiting	60.4	9.7	46.1	4.1	62.8	12.1
Anorexia	34.2	5.8	42.0	3.7	43.9	7.2
Constipation	41.3	3.1	31.5	1.8	32.3	0.4
Mucositis	32.4	2.2	76.3	16.9	29.6	2.2
HEMATOLOGIC						
Neutropenia	96.9	53.8	98.6	66.7	96.4	31.4
grade 3	--	29.8	--	23.7	--	19.3
grade 4	--	24.0	--	42.5	--	12.1
Leukopenia	96.9	37.8	98.6	23.3	96.4	21.5
Anemia	96.9	8.4	98.6	5.5	96.9	4.5
Neutropenic fever	--	7.1	--	14.6	--	5.8
Thrombocytopenia	96.0	2.6	98.6	2.7	96.0	1.7
Neutropenic infection	--	1.8	--	0	--	2.2
BODY AS A WHOLE						
Asthenia	70.2	19.5	64.4	11.9	69.1	13.9
Pain	30.7	3.1	26.9	3.6	22.9	2.2
Fever	42.2	1.7	32.4	3.6	43.5	0.4
Infection	22.2	0	16.0	1.4	13.9	0.4
METABOLIC & NUTRITIONAL						
↑ Bilirubin	87.6	7.1	92.2	8.2	83.9	7.2
DERMATOLOGIC						
Exfoliative dermatitis	0.9	0	3.2	0.5	0	0
Rash	19.1	0	26.5	0.9	14.3	0.4
Alopecia[b]	43.1	--	26.5	--	46.1	--
RESPIRATORY						
Dyspnea	27.6	6.3	16.0	0.5	22.0	2.2
Cough	26.7	1.3	18.3	0	20.2	0.4
Pneumonia	6.2	2.7	1.4	1.0	3.6	1.3
NEUROLOGIC						
Dizziness	23.1	1.3	16.4	0	21.1	1.8
Somnolence	12.4	1.8	4.6	1.8	9.4	1.3
Confusion	7.1	1.8	4.1	0	2.7	0
CARDIOVASCULAR						
Vasodilatation	9.3	0.9	5.0	0	9.0	0
Hypotension	5.8	1.3	2.3	0.5	5.8	1.7
Thromboembolic events[c]	9.3	--	11.4	--	5.4	--

[a]Severity of adverse events based on NCI CTC (version 1.0)

[b]Complete hair loss = Grade 2

[c]Includes angina pectoris, arterial thrombosis, cerebral infarct, cerebrovascular accident, deep thrombophlebitis, embolus lower extremity, heart arrest, myocardial infarct, myocardial ischemia, peripheral vascular disorder, pulmonary embolus, sudden death, thrombophlebitis, thrombosis, vascular disorder.

Table 7. Study 2: Percent (%) of Patients Experiencing Clinically Relevant Adverse Events in Combination Therapies[a]

Adverse Event	Study 2			
	Irinotecan + 5-FU/LV infusional d 1&2 q 2 weeks N= 145		5-FU/LV infusional d 1&2 q 2 weeks N=143	
	Grade 1-4	Grade 3&4	Grade 1-4	Grade 3&4
TOTAL Adverse Events	100	72.4	100	39.2
GASTROINTESTINAL				
Diarrhea				
late	72.4	14.4	44.8	6.3
grade 3	--	10.3	--	4.2
grade 4	--	4.1	--	2.1
Cholinergic syndrome[b]	28.3	1.4	0.7	0
Nausea	66.9	2.1	55.2	3.5
Abdominal pain	17.2	2.1	16.8	0.7
Vomiting	44.8	3.5	32.2	2.8
Anorexia	35.2	2.1	18.9	0.7
Constipation	30.3	0.7	25.2	1.4
Mucositis	40.0	4.1	28.7	2.8
HEMATOLOGIC				
Neutropenia	82.5	46.2	47.9	13.4
grade 3	--	36.4	--	12.7
grade 4	--	9.8	--	0.7
Leukopenia	81.3	17.4	42.0	3.5
Anemia	97.2	2.1	90.9	2.1
Neutropenic fever	--	3.4	--	0.7
Thrombocytopenia	32.6	0	32.2	0
Neutropenic infection	--	2.1	--	0
BODY AS A WHOLE				
Asthenia	57.9	9.0	48.3	4.2
Pain	64.1	9.7	61.5	8.4
Fever	22.1	0.7	25.9	0.7
Infection	35.9	7.6	33.6	3.5
METABOLIC & NUTRITIONAL				
↑ Bilirubin	19.1	3.5	35.9	10.6
DERMATOLOGIC				
Hand & foot syndrome	10.3	0.7	12.6	0.7
Cutaneous signs	17.2	0.7	20.3	0
Alopecia[c]	56.6	--	16.8	--
RESPIRATORY				
Dyspnea	9.7	1.4	4.9	0
CARDIOVASCULAR				
Hypotension	3.4	1.4	0.7	0
Thromboembolic events[d]	11.7	--	5.6	--

[a] Severity of adverse events based on NCI CTC (version 1.0)

[b] Includes rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, abdominal cramping or diarrhea (occurring during or shortly after infusion of irinotecan)

[c] Complete hair loss = Grade 2

[d] Includes angina pectoris, arterial thrombosis, cerebral infarct, cerebrovascular accident, deep thrombophlebitis, embolus lower extremity, heart arrest, myocardial infarct, myocardial ischemia, peripheral vascular disorder, pulmonary embolus, sudden death, thrombophlebitis, thrombosis, vascular disorder.

Second-Line Single-Agent Therapy

Weekly Dosage Schedule

In three clinical studies evaluating the weekly dosage schedule, 304 patients with metastatic carcinoma of the colon or rectum that had recurred or progressed following 5-FU-based therapy were treated with CAMPTOSAR. Seventeen of the patients died within 30 days of the administration of CAMPTOSAR; in five cases (1.6%, 5/304), the deaths were potentially drug-related. These five patients experienced a constellation of medical events that included known effects of CAMPTOSAR. One of these patients died of neutropenic sepsis without fever. Neutropenic fever occurred in nine (3.0%) other patients; these patients recovered with supportive care.

One hundred nineteen (39.1%) of the 304 patients were hospitalized a total of 156 times because of adverse events; 81 (26.6%) patients were hospitalized for events judged to be related to administration of CAMPTOSAR. The primary reasons for drug-related hospitalization were diarrhea, with or without nausea and/or vomiting (18.4%); neutropenia/leukopenia, with or without diarrhea and/or fever (8.2%); and nausea and/or vomiting (4.9%).

Adjustments in the dose of CAMPTOSAR were made during the cycle of treatment and for subsequent cycles based on individual patient tolerance. The first dose of at least one cycle of CAMPTOSAR was reduced for 67% of patients who began the studies at the 125-mg/m^2 starting dose. Within-cycle dose reductions were required for 32% of the cycles initiated at the 125-mg/m^2 dose level. The most common reasons for dose reduction were late diarrhea, neutropenia, and leukopenia. Thirteen (4.3%) patients discontinued treatment with CAMPTOSAR because of adverse events. The adverse events in Table 8 are based on the experience of the 304 patients enrolled in the three studies described in the CLINICAL STUDIES, Studies Evaluating the Weekly Dosage Schedule, section.

Table 8. Adverse Events Occurring in >10% of 304 Previously Treated Patients with Metastatic Carcinoma of the Colon or Rectum[a]

Body System & Event	% of Patients Reporting	
	NCI Grades 1-4	NCI Grades 3 & 4
GASTROINTESTINAL		
Diarrhea (late)[b]	88	31
7-9 stools/day (grade 3)	—	(16)
≥10 stools/day (grade 4)	—	(14)
Nausea	86	17
Vomiting	67	12
Anorexia	55	6
Diarrhea (early)[c]	51	8
Constipation	30	2
Flatulence	12	0
Stomatitis	12	1
Dyspepsia	10	0
HEMATOLOGIC		
Leukopenia	63	28
Anemia	60	7
Neutropenia	54	26
500 to <1000/mm^3 (grade 3)	—	(15)
<500/mm^3 (grade 4)	—	(12)
BODY AS A WHOLE		
Asthenia	76	12
Abdominal cramping/pain	57	16
Fever	45	1
Pain	24	2
Headache	17	1
Back pain	14	2
Chills	14	0
Minor infection[d]	14	0
Edema	10	1
Abdominal enlargement	10	0
METABOLIC & NUTRITIONAL		
↓ Body weight	30	1
Dehydration	15	4
↑ Alkaline phosphatase	13	4
↑ SGOT	10	1
DERMATOLOGIC		
Alopecia	60	NA[e]
Sweating	16	0
Rash	13	1
RESPIRATORY		
Dyspnea	22	4
↑ Coughing	17	0
Rhinitis	16	0
NEUROLOGIC		
Insomnia	19	0
Dizziness	15	0
CARDIOVASCULAR		
Vasodilation (flushing)	11	0

[a] Severity of adverse events based on NCI CTC (version 1.0)
[b] Occurring > 24 hours after administration of CAMPTOSAR
[c] Occurring ≤24 hours after administration of CAMPTOSAR
[d] Primarily upper respiratory infections
[e] Not applicable; complete hair loss = NCI grade 2

Once-Every-3-Week Dosage Schedule

A total of 535 patients with metastatic colorectal cancer whose disease had recurred or progressed following prior 5-FU therapy participated in the two phase 3 studies: 316 received irinotecan, 129 received 5-FU, and 90 received best supportive care. Eleven (3.5%) patients treated with irinotecan died within 30 days of treatment. In three cases (1%, 3/316), the deaths were potentially related to irinotecan treatment and were attributed to neutropenic infection, grade 4 diarrhea, and asthenia, respectively. One (0.8%, 1/129) patient treated with 5-FU died within 30 days of treatment; this death was attributed to grade 4 diarrhea.

Hospitalizations due to serious adverse events (whether or not related to study treatment) occurred at least once in 60% (188/316) of patients who received irinotecan, 63% (57/90) who received best supportive care, and 39% (50/129) who received 5-FU-based therapy. Eight percent of patients treated with irinotecan and 7% treated with 5-FU-based therapy discontinued treatment due to adverse events.

Of the 316 patients treated with irinotecan, the most clinically significant adverse events (all grades, 1-4) were diarrhea (84%), alopecia (72%), nausea (70%), vomiting (62%), cholinergic symptoms (47%), and neutropenia (30%). Table 9 lists the grade 3 and 4 adverse events reported in the patients enrolled to all treatment arms of the two studies described in the CLINICAL STUDIES, Studies Evaluating the Once-Every-3-Week Dosage Schedule, section.

Table 9. Percent Of Patients Experiencing Grade 3 & 4 Adverse Events In Comparative Studies Of Once-Every-3-Week Irinotecan Therapy[a]

	Study 1		Study 2	
Adverse Event	**Irinotecan N=189**	**BSC [b] N=90**	**Irinotecan N=127**	**5-FU N=129**
TOTAL Grade 3/4 Adverse Events	79	67	69	54
GASTROINTESTINAL				
Diarrhea	22	6	22	11
Vomiting	14	8	14	5
Nausea	14	3	11	4
Abdominal pain	14	16	9	8
Constipation	10	8	8	6
Anorexia	5	7	6	4
Mucositis	2	1	2	5
HEMATOLOGIC				
Leukopenia/Neutropenia	22	0	14	2
Anemia	7	6	6	3
Hemorrhage	5	3	1	3
Thrombocytopenia	1	0	4	2
Infection				
without grade 3/4 neutropenia	8	3	1	4
with grade 3/4 neutropenia	1	0	2	0
Fever				
without grade 3/4 neutropenia	2	1	2	0
with grade 3/4 neutropenia	2	0	4	2
BODY AS A WHOLE				
Pain	19	22	17	13
Asthenia	15	19	13	12
METABOLIC & NUTRITIONAL				
Hepatic [c]	9	7	9	6
DERMATOLOGIC				
Hand & foot syndrome	0	0	0	5
Cutaneous signs [d]	2	0	1	3
RESPIRATORY [e]	10	8	5	7
NEUROLOGIC [f]	12	13	9	4
CARDIOVASCULAR [g]	9	3	4	2
OTHER [h]	32	28	12	14

[a] Severity of adverse events based on NCI CTC (version 1.0)
[b] BSC = best supportive care
[c] Hepatic includes events such as ascites and jaundice
[d] Cutaneous signs include events such as rash
[e] Respiratory includes events such as dyspnea and cough
[f] Neurologic includes events such as somnolence
[g] Cardiovascular includes events such as dysrhythmias, ischemia, and mechanical cardiac dysfunction
[h] Other includes events such as accidental injury, hepatomegaly, syncope, vertigo, and weight loss

Overview of Adverse Events

Gastrointestinal: Nausea, vomiting, and diarrhea are common adverse events following treatment with CAMPTOSAR and can be severe. When observed, nausea and vomiting usually occur during or shortly after infusion of CAMPTOSAR. An increased incidence of late diarrhea was observed in two studies, one using a 3-week schedule and the other using a weekly schedule. In the clinical studies testing the every 3-week-dosage schedule, the median time to the onset of late diarrhea was 5 days after irinotecan infusion. In the clinical studies evaluating the weekly dosage schedule, the median time to onset of late diarrhea was 11 days following administration of CAMPTOSAR. For patients

starting treatment at the 125-mg/m^2 weekly dose, the median duration of any grade of late diarrhea was 3 days. Among those patients treated at the 125-mg/m^2 weekly dose who experienced grade 3 or 4 late diarrhea, the median duration of the entire episode of diarrhea was 7 days. The frequency of grade 3 or 4 late diarrhea was somewhat greater in patients starting treatment at 125 mg/m^2 than in patients given a 100-mg/m^2 weekly starting dose (34% [65/193] versus 23% [24/102]; p=0.08). The frequency of grade 3 and 4 late diarrhea by age was significantly greater in patients ≥65 years than in patients <65 years (40% [53/133] versus 23% [40/171]; p=0.002). In another study of 183 patients treated on the weekly schedule, the frequency of grade 3 or 4 late diarrhea in patients ≥65 years of age was 28.6% [26/91] and in patients <65 years of age was 23.9% [22/92].

In one study of the weekly dosage treatment, the frequency of grade 3 and 4 late diarrhea was significantly greater in male than in female patients (43% [25/58] versus 16% [5/32]; p=0.01), but there were no gender differences in the frequency of grade 3 and 4 late diarrhea in the other two studies of the weekly dosage treatment schedule. Colonic ulceration, sometimes with gastrointestinal bleeding, has been observed in association with administration of CAMPTOSAR.

Hematology: CAMPTOSAR commonly causes neutropenia, leukopenia (including lymphocytopenia), and anemia. Serious thrombocytopenia is uncommon. When evaluated in the trials of weekly administration, the frequency of grade 3 and 4 neutropenia was significantly higher in patients who received previous pelvic/abdominal irradiation than in those who had not received such irradiation (48% [13/27] versus 24% [67/277]; p=0.04). In these same studies, patients with baseline serum total bilirubin levels of 1.0 mg/dL or more also had a significantly greater likelihood of experiencing first-cycle grade 3 or 4 neutropenia than those with bilirubin levels that were less than 1.0 mg/dL (50% [19/38] versus 18% [47/266]; p<0.001). There were no significant differences in the frequency of grade 3 and 4 neutropenia by age or gender. In the clinical studies evaluating the weekly dosage schedule, neutropenic fever (concurrent NCI grade 4 neutropenia and fever of grade 2 or greater) occurred in 3% of the patients; 6% of patients received G-CSF for the treatment of neutropenia. NCI grade 3 or 4 anemia was noted in 7% of the patients receiving weekly treatment; blood transfusions were given to 10% of the patients in these trials.

Body as a Whole: Asthenia, fever, and abdominal pain are generally the most common events of this type.

Cholinergic Symptoms: Patients may have cholinergic symptoms of rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, and intestinal hyperperistalsis that can cause abdominal cramping and early diarrhea. If these symptoms occur, they manifest during or shortly after drug infusion. They are thought to be related to the anticholinesterase activity of the irinotecan parent compound and are expected to occur more frequently with higher irinotecan doses.

Hepatic: In the clinical studies evaluating the weekly dosage schedule, NCI grade 3 or 4 liver enzyme abnormalities were observed in fewer than 10% of patients. These events typically occur in patients with known hepatic metastases.

Dermatologic: Alopecia has been reported during treatment with CAMPTOSAR.

Rashes have also been reported but did not result in discontinuation of treatment.
Respiratory: Severe pulmonary events are infrequent. In the clinical studies evaluating the weekly dosage schedule, NCI grade 3 or 4 dyspnea was reported in 4% of patients. Over half the patients with dyspnea had lung metastases; the extent to which malignant pulmonary involvement or other preexisting lung disease may have contributed to dyspnea in these patients is unknown.

Interstitial pulmonary disease presenting as pulmonary infiltrates is uncommon during irinotecan therapy. Interstitial pulmonary disease can be fatal. Risk factors possibly associated with the development of interstitial pulmonary disease include pre-existing lung disease, use of pneumotoxic drugs, radiation therapy, and colony stimulating factors. Patients with risk factors should be closely monitored for respiratory symptoms before and during irinotecan therapy (see WARNINGS).

Neurologic: Insomnia and dizziness can occur, but are not usually considered to be directly related to the administration of CAMPTOSAR. Dizziness may sometimes represent symptomatic evidence of orthostatic hypotension in patients with dehydration.

Cardiovascular: Vasodilation (flushing) may occur during administration of CAMPTOSAR. Bradycardia may also occur, but has not required intervention. These effects have been attributed to the cholinergic syndrome sometimes observed during or shortly after infusion of CAMPTOSAR. Thromboembolic events have been observed in patients receiving CAMPTOSAR; the specific cause of these events has not been determined.

Other Non-U.S. Clinical Trials

Irinotecan has been studied in over 1100 patients in Japan. Patients in these studies had a variety of tumor types, including cancer of the colon or rectum, and were treated with several different doses and schedules. In general, the types of toxicities observed were similar to those seen in U.S. trials with CAMPTOSAR. There is some information from Japanese trials that patients with considerable ascites or pleural effusions were at increased risk for neutropenia or diarrhea. A potentially life-threatening pulmonary syndrome, consisting of dyspnea, fever, and a reticulonodular pattern on chest x-ray, was observed in a small percentage of patients in early Japanese studies. The contribution of irinotecan to these preliminary events was difficult to assess because these patients also had lung tumors and some had preexisting nonmalignant pulmonary disease. As a result of these observations, however, clinical studies in the United States have enrolled few patients with compromised pulmonary function, significant ascites, or pleural effusions.

Post-Marketing Experience

The following events have been identified during postmarketing use of CAMPTOSAR in clinical practice. Myocardial ischemic events have been observed following irinotecan therapy (See also Table 7, thromboembolic events) Infrequent cases of ulcerative and ischemic colitis have been observed. This can be complicated by ulceration, bleeding, ileus, obstruction, and infection, including typhlitis. Patients experiencing ileus should receive prompt antibiotic support (see PRECAUTIONS). Cases of megacolon, intestinal perforation, symptomatic pancreatitis, and asymptomatic pancreatic enzyme elevation have been reported.

Hypersensitivity reactions including severe anaphylactic or anaphylactoid reactions have also been observed (see WARNINGS).

Cases of hyponatremia mostly related with diarrhea and vomiting have been reported. Increases in serum levels of transaminases (i.e., AST and ALT) in the absence of progressive liver metastasis; transient increase of amylase and occasionally transient increase of lipase have been reported.

Infrequent cases of renal insufficiency including acute renal failure, hypotension or circulatory failure have been observed in patients who experienced episodes of dehydration associated with diarrhea and/or vomiting, or sepsis (see WARNINGS).

Early effects such as muscular contraction or cramps and paresthesia have been reported.

Hiccups have been reported.

Transient dysarthria has been reported in patients treated with CAMPTOSAR; in some cases, the event was attributed to the cholinergic syndrome observed during or shortly after infusion of irinotecan.

OVERDOSAGE

In U.S. phase 1 trials, single doses of up to 345 mg/m^2 of irinotecan were administered to patients with various cancers. Single doses of up to 750 mg/m^2 of irinotecan have been given in non-U.S. trials. The adverse events in these patients were similar to those reported with the recommended dosage and regimen. There have been reports of overdosage at doses up to approximately twice the recommended therapeutic dose, which may be fatal. The most significant adverse reactions reported were severe neutropenia and severe diarrhea. There is no known antidote for overdosage of CAMPTOSAR. Maximum supportive care should be instituted to prevent dehydration due to diarrhea and to treat any infectious complications.

DOSAGE AND ADMINISTRATION

Combination-Agent Dosage

Dosage Regimens

CAMPTOSAR Injection in Combination with 5-Fluorouracil (5-FU) and Leucovorin (LV)

CAMPTOSAR should be administered as an intravenous infusion over 90 minutes (see Preparation of Infusion Solution). For all regimens, the dose of LV should be administered immediately after CAMPTOSAR, with the administration of 5-FU to occur immediately after receipt of LV. CAMPTOSAR should be used as recommended; the currently recommended regimens are shown in Table 10.

Table 10. Combination-Agent Dosage Regimens & Dose Modifications[a]

Regimen 1 6-wk cycle with bolus 5-FU/LV (next cycle begins on day 43)	CAMPTOSAR LV 5-FU	125 mg/m^2 IV over 90 min, d 1,8,15,22 20 mg/m^2 IV bolus, d 1,8,15,22 500 mg/m^2 IV bolus, d 1,8,15.22		
		Starting Dose & Modified Dose Levels (mg/m^2)		
		Starting Dose	Dose Level -1	Dose Level -2
	CAMPTOSAR	125	100	75

	LV		20	20	20
	5-FU		500	400	300
Regimen 2 6-wk cycle with infusional 5-FU/LV (next cycle begins on day 43)	CAMPTOSAR LV 5-FU Bolus 5-FU Infusion[b]		180 mg/m^2 IV over 90 min, d 1,15,29 200 mg/m^2 IV over 2 h, d 1,2,15,16,29,30 400 mg/m^2 IV bolus, d 1,2,15,16,29,30 600 mg/m^2 IV over 22 h, d 1,2,15,16,29,30		
			Starting Dose & Modified Dose Levels (mg/m^2)		
			Starting Dose	Dose Level -1	Dose Level -2
	CAMPTOSAR		180	150	120
	LV		200	200	200
	5-FU	Bolus	400	320	240
	5-FU	Infusion[b]	600	480	360

[a]Dose reductions beyond dose level –2 by decrements of ≈20% may be warranted for patients continuing to experience toxicity. Provided intolerable toxicity does not develop, treatment with additional cycles may be continued indefinitely as long as patients continue to experience clinical benefit.
[b]Infusion follows bolus administration.

Dosing for patients with bilirubin >2 mg/dL cannot be recommended because there is insufficient information to recommend a dose in these patients. It is recommended that patients receive premedication with antiemetic agents. Prophylactic or therapeutic administration of atropine should be considered in patients experiencing cholinergic symptoms. See PRECAUTIONS, General.

Dose Modifications

Patients should be carefully monitored for toxicity and assessed prior to each treatment. Doses of CAMPTOSAR and 5-FU should be modified as necessary to accommodate individual patient tolerance to treatment. Based on the recommended dose-levels described in Table 10, Combination-Agent Dosage Regimens & Dose Modifications, subsequent doses should be adjusted as suggested in Table 11, Recommended Dose Modifications for Combination Schedules. All dose modifications should be based on the worst preceding toxicity. After the first treatment, patients with active diarrhea should return to pre-treatment bowel function without requiring anti-diarrhea medications for at least 24 hours before the next chemotherapy administration.

A new cycle of therapy should not begin until the toxicity has recovered to NCI grade 1 or less. Treatment maybe delayed 1 to 2 weeks to allow for recovery from treatment-related toxicity. If the patient has not recovered, consideration should be given to discontinuing therapy. Provided intolerable toxicity does not develop, treatment with additional cycles of CAMPTOSAR/5-FU/LV may be continued indefinitely as long as patients continue to experience clinical benefit.

Table 11. Recommended Dose Modifications for CAMPTOSAR/5-Fluorouracil (5-FU)/Leucovorin (LV) Combination Schedules

Patients should return to pre-treatment bowel function without requiring antidiarrhea medications for at least 24 hours before the next chemotherapy administration. A new cycle of therapy should not begin until the granulocyte count has recovered to ≥1500/mm^3, and the platelet count has recovered to ≥100,000/mm^3, and treatment-related diarrhea is fully resolved. Treatment should be delayed 1 to 2 weeks to allow for recovery from treatment-related toxicities. If the patient has not recovered after a 2-week delay, consideration should be given to discontinuing therapy

Toxicity NCI CTC Grade[a] (Value)	During a Cycle of Therapy	At the Start of Subsequent Cycles of Therapy[b]
No toxicity	Maintain dose level	Maintain dose level

Neutropenia		
1 (1500 to 1999/mm^3)	Maintain dose level	Maintain dose level
2 (1000 to 1499/mm^3)	↓ 1 dose level	Maintain dose level
3 (500 to 999/mm^3)	Omit dose until resolved to ≤ grade 2, then ↓ 1 dose level	↓ 1 dose level
4 (<500/mm^3)	Omit dose until resolved to ≤ grade 2, then ↓ 2 dose levels	↓ 2 dose levels
Neutropenic fever	Omit dose until resolved, then ↓ 2 dose levels	
Other hematologic toxicities	Dose modifications for leukopenia or thrombocytopenia during a cycle of therapy and at the start of subsequent cycles of therapy are also based on NCI toxicity criteria and are the same as recommended for neutropenia above.	
Diarrhea		
1 (2-3 stools/day > pretx[c])	Delay dose until resolved to baseline, then give same dose	Maintain dose level
2 (4-6 stools/day > pretx)	Omit dose until resolved to baseline, then ↓ 1 dose level	Maintain dose level
3 (7-9 stools/day > pretx)	Omit dose until resolved to baseline, then ↓ 1 dose level	↓ 1 dose level
4 (≥10 stools/day > pretx)	Omit dose until resolved to baseline, then ↓ 2 dose levels	↓ 2 dose levels
Other nonhematologic toxicities[d]		
1	Maintain dose level	Maintain dose level
2	Omit dose until resolved to ≤ grade 1, then ↓ 1 dose level	Maintain dose level
3	Omit dose until resolved to ≤ grade 2, then ↓ 1 dose level	↓ 1 dose level
4	Omit dose until resolved to ≤ grade 2, then ↓ 2 dose levels	↓ 2 dose levels
	For mucositis/stomatitis decrease only 5-FU, not CAMPTOSAR	*For mucositis/stomatitis decrease only 5-FU, not CAMPTOSAR.*

[a] National Cancer Institute Common Toxicity Criteria (version 1.0)
[b] Relative to the starting dose used in the previous cycle
[c] Pretreatment
[d] Excludes alopecia, anorexia, asthenia

Single-Agent Dosage Schedules

Dosage Regimens

CAMPTOSAR should be administered as an intravenous infusion over 90 minutes for both the weekly and once-every-3-week dosage schedules (see Preparation of Infusion Solution). Single-agent dosage regimens are shown in Table 12.

Table 12. Single-Agent Regimens of CAMPTOSAR and Dose Modifications

Weekly Regimen[a]	125 mg/m^2 IV over 90 min, d 1,8,15,22 then 2-wk rest		
	Starting Dose & Modified Dose Levels[c] (mg/m^2)		
	Starting Dose	Dose Level -1	Dose Level -2
	125	100	75
Once-Every-3-Week Regimen[b]	350 mg/m^2 IV over 90 min, once every 3 wks[c]		
	Starting Dose & Modified Dose Levels (mg/m^2)		
	Starting Dose	Dose Level -1	Dose Level -2
	350	300	250

[a]Subsequent doses may be adjusted as high as 150 mg/m^2 or to as low as 50 mg/m^2 in 25 to 50 mg/m^2 decrements depending upon individual patient tolerance.
[b]Subsequent doses may be adjusted as low as 200 mg/m^2 in 50 mg/m^2 decrements depending upon individual patient tolerance.
[c]Provided intolerable toxicity does not develop, treatment with additional cycles may be continued indefinitely as long as patients continue to experience clinical benefit.

A reduction in the starting dose by one dose level of CAMPTOSAR may be considered for patients with any of the following conditions: prior pelvic/abdominal radiotherapy, performance status of 2, or increased bilirubin levels. Dosing for patients with bilirubin >2 mg/dL cannot be recommended because there is insufficient information to recommend a dose in these patients.

It is recommended that patients receive premedication with antiemetic agents.

Prophylactic or therapeutic administration of atropine should be considered in patients experiencing cholinergic symptoms. See PRECAUTIONS, General.

Dose Modifications

Patients should be carefully monitored for toxicity and doses of CAMPTOSAR should be modified as necessary to accommodate individual patient tolerance to treatment. Based on recommended dose-levels described in Table 12, Single-Agent Regimens of CAMPTOSAR and Dose Modifications, subsequent doses should be adjusted as suggested in Table 13, Recommended Dose Modifications for Single-Agent Schedules. All dose modifications should be based on the worst preceding toxicity.

A new cycle of therapy should not begin until the toxicity has recovered to NCI grade 1 or less. Treatment may be delayed 1 to 2 weeks to allow for recovery from treatment-related toxicity. If the patient has not recovered, consideration should be given to discontinuing this combination therapy. Provided intolerable toxicity does not develop, treatment with additional cycles of CAMPTOSAR may be continued indefinitely as long as patients continue to experience clinical benefit.

Table 13. Recommended Dose Modifications For Single-Agent Schedules[a]

A new cycle of therapy should not begin until the granulocyte count has recovered to ≥1500/mm^3, and the platelet count has recovered to ≥100,000/mm^3, and treatment-related diarrhea is fully resolved. Treatment should be delayed 1 to 2 weeks to allow for recovery from treatment-related toxicities. If the patient has not recovered after a 2-week delay, consideration should be given to discontinuing CAMPTOSAR.

Worst Toxicity NCI Grade[b] (Value)	During a Cycle of Therapy	At the Start of the Next Cycles of Therapy (After Adequate Recovery), Compared with the Starting Dose in the Previous Cycle[a]	
	Weekly	Weekly	Once Every 3 Weeks
No toxicity	Maintain dose level	↑ 25 mg/m^2 up to a maximum dose of 150 mg/m^2	Maintain dose level
Neutropenia			
1 (1500 to 1999/mm^3)	Maintain dose level	Maintain dose level	Maintain dose level
2 (1000 to 1499/mm^3)	↓ 25 mg/m^2	Maintain dose level	Maintain dose level
3 (500 to 999/mm^3)	Omit dose until resolved to ≤ grade 2, then ↓ 25 mg/m^2	↓ 25 mg/m^2	↓ 50 mg/m^2
4 (<500/mm^3)	Omit dose until resolved to ≤ grade 2, then ↓ 50 mg/m^2	↓ 50 mg/m^2	↓ 50 mg/m^2
Neutropenic fever	Omit dose until resolved, then ↓ 50 mg/m^2 when resolved	↓ 50 mg/m^2	↓ 50 mg/m^2
Other hematologic toxicities	Dose modifications for leukopenia, thrombocytopenia, and anemia during a cycle of therapy and at the start of subsequent cycles of therapy are also based on NCI toxicity criteria and are the same as recommended for neutropenia above.		
Diarrhea			
1 (2-3 stools/day > pretx[c])	Maintain dose level	Maintain dose level	Maintain dose level
2 (4-6 stools/day > pretx)	↓ 25 mg/m^2	Maintain dose level	Maintain dose level
3 (7-9 stools/day > pretx)	Omit dose until resolved to ≤ grade 2, then ↓ 25 mg/m^2	↓ 25 mg/m^2	↓ 50 mg/m^2
4 (≥10 stools/day > pretx)	Omit dose until resolved to ≤ grade 2 then ↓ 50 mg/m^2	↓ 50 mg/m^2	↓ 50 mg/m^2
Other nonhematologic[d] toxicities			
1	Maintain dose level	Maintain dose level	Maintain dose level
2	↓ 25 mg/m^2	↓ 25 mg/m^2	↓ 50 mg/m^2
3	Omit dose until resolved to ≤ grade 2, then ↓ 25 mg/m^2	↓ 25 mg/m^2	↓ 50 mg/m^2
4	Omit dose until resolved to ≤ grade 2, then ↓ 50 mg/m^2	↓ 50 mg/m^2	↓ 50 mg/m^2

[a] All dose modifications should be based on the worst preceding toxicity
[b] National Cancer Institute Common Toxicity Criteria (version 1.0)
[c] Pretreatment
[d] Excludes alopecia, anorexia, asthenia

Dosage in Patients with Reduced UGT1A1 Activity

When administered in combination with other agents, or as a single-agent, a reduction in the starting dose by at least one level of CAMPTOSAR should be considered for patients known to be homozygous for the UGT1A1*28 allele (see CLINICAL PHARMACOLOGY and WARNINGS). However, the precise dose reduction in this

patient population is not known and subsequent dose modifications should be considered based on individual patient tolerance to treatment (see Tables 10-13).

Preparation & Administration Precautions

As with other potentially toxic anticancer agents, care should be exercised in the handling and preparation of infusion solutions prepared from CAMPTOSAR Injection. The use of gloves is recommended. If a solution of CAMPTOSAR contacts the skin, wash the skin immediately and thoroughly with soap and water. If CAMPTOSAR contacts the mucous membranes, flush thoroughly with water.

Several published guidelines for handling and disposal of anticancer agents are available.[1-7]

Preparation of Infusion Solution

Inspect vial contents for particulate matter and repeat inspection when drug product is withdrawn from vial into syringe.

CAMPTOSAR Injection must be diluted prior to infusion. CAMPTOSAR should be diluted in 5% Dextrose Injection, USP, (preferred) or 0.9% Sodium Chloride Injection, USP, to a final concentration range of 0.12 to 2.8 mg/mL. In most clinical trials, CAMPTOSAR was administered in 250 mL to 500 mL of 5% Dextrose Injection, USP.

The solution is physically and chemically stable for up to 24 hours at room temperature (approximately 25°C) and in ambient fluorescent lighting. Solutions diluted in 5% Dextrose Injection, USP, and stored at refrigerated temperatures (approximately 2° to 8°C), and protected from light are physically and chemically stable for 48 hours. Refrigeration of admixtures using 0.9% Sodium Chloride Injection, USP, is not recommended due to a low and sporadic incidence of visible particulates. Freezing CAMPTOSAR and admixtures of CAMPTOSAR may result in precipitation of the drug and should be avoided. Because of possible microbial contamination during dilution, it is advisable to use the admixture prepared with 5%Dextrose Injection, USP, within 24 hours if refrigerated (2° to 8°C, 36° to 46°F). In the case of admixtures prepared with 5% Dextrose Injection, USP, or Sodium Chloride Injection, USP, the solutions should be used within 6 hours if kept at room temperature (15° to 30°C, 59° to 86°F).

Other drugs should not be added to the infusion solution. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

HOW SUPPLIED

Each mL of CAMPTOSAR Injection contains 20 mg irinotecan (on the basis of the trihydrate salt); 45 mg sorbitol; and 0.9 mg lactic acid. When necessary, pH has been adjusted to 3.5 (range, 3.0 to 3.8) with sodium hydroxide or hydrochloric acid.

CAMPTOSAR Injection is available in single-dose amber glass vials in the following package sizes:

2 mL	NDC 0009-7529-02
5 mL	NDC 0009-7529-01

The vial should be inspected for damage and visible signs of leaks before removing from the carton. If damaged, incinerate the unopened package.

Store at controlled room temperature 15° to 30°C (59° to 86°F). Protect from light. It is recommended that the vial should remain in the carton until the time of use.

Rx only

REFERENCES

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Camptosar brand of irinotecan hydrochloride injection

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HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use Fusilev safely and effectively. See full prescribing information for Fusilev.

Fusilev (levoleucovorin) INJECTION, POWDER, LYOPHILIZED, FOR SOLUTION for INTRAVENOUS use

Initial U.S. Approval: 1952 (d,l-leucovorin), 2008 (levoleucovorin)

----------INDICATIONS AND USAGE----------

Fusilev is a folate analog. (1)

Fusilev rescue is indicated after high-dose methotrexate therapy in osteosarcoma. (1)

Fusilev is also indicated to diminish the toxicity and counteract the effects of impaired methotrexate elimination and of inadvertent overdosage of folic acid antagonists. (1)

Limitations of Use

Fusilev is not approved for pernicious anemia and megaloblastic anemias. Improper use may cause a hematologic remission while neurologic manifestations continue to progress. (1.1)

----------DOSAGE AND ADMINISTRATION----------

Fusilev Rescue After High-Dose Methotrexate Therapy

Do not administer intrathecally. (2.1)

Fusilev is dosed at one-half the usual dose of the racemic form. (2.1)

Fusilev rescue recommendations are based on a methotrexate dose of 12 grams/m^2 administered by intravenous infusion over 4 hours. Fusilev rescue at a dose of 7.5 mg (approximately 5 mg/m^2) every 6 hours for 10 doses starts 24 hours after the beginning of the methotrexate infusion. Determine serum creatinine and methotrexate levels at least once daily. Continue Fusilev administration, hydration, and urinary alkalinization (pH of 7.0 or greater) until the methotrexate level is below 5 x 10^{-8} M (0.05 micromolar). The Fusilev dose may need to be adjusted. (2.3)

----------DOSAGE FORMS AND STRENGTHS----------

Each 50 mg single-use vial of Fusilev contains a sterile lyophilized powder consisting of 64 mg levoleucovorin calcium pentahydrate (equivalent to 50 mg levoleucovorin) and 50 mg mannitol. (16) It is intended for intravenous administration after reconstitution with 5.3 mL of sterile 0.9% Sodium Chloride for Injection, USP. (2.5, 11)

----------CONTRAINDICATIONS----------

Fusilev is contraindicated for patients who have had previous allergic reactions attributed to folic acid or folinic acid. (4)

----------WARNINGS AND PRECAUTIONS----------

Due to Ca^{++} content, no more than 16 mL (160 mg) of levoleucovorin solution should be injected intravenously per minute. (5.1)

Fusilev enhances the toxicity of fluorouracil. (5.2,7)

Concomitant use of *d,l*-leucovorin with trimethoprim-sulfamethoxazole for Pneumocystis carinii pneumonia in HIV patients was associated with increased rates of treatment failure in a placebo-controlled study. (5.3)

----------ADVERSE REACTIONS----------

Allergic reactions were reported in patients receiving Fusilev. (6.2)

Vomiting (38%), stomatitis (38%) and nausea (19%) were reported in patients receiving Fusilev as rescue after high dose methotrexate therapy. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Spectrum Pharmaceuticals, Inc. at 1-877-387-4538 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch

----------DRUG INTERACTIONS----------

Fusilev may counteract the anticpileptic effect of phenobarbital, phenytoin and primidone, and increase the frequency of seizures in susceptible patients. (7)

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

- Fusilev™ is a folate analog.
- Fusilev rescue is indicated after high-dose methotrexate therapy in osteosarcoma.
- Fusilev is also indicated to diminish the toxicity and counteract the effects of impaired methotrexate elimination and of inadvertent overdosage of folic acid antagonists.

1.1 Limitations of Use

- Fusilev is not approved for pernicious anemia and megaloblastic anemias secondary to the lack of vitamin B_{12}. Improper use may cause a hematologic remission while neurologic manifestations continue to progress.

2 DOSAGE AND ADMINISTRATION

2.1 Administration Guidelines

Fusilev is dosed at **one-half** the usual dose of the racemic form.

Fusilev is indicated for intravenous administration only. **Do not administer intrathecally.**

2.2 Co-administration of Fusilev with other agents

Due to the risk of precipitation, do not co-administer Fusilev with other agents in the same admixture.

2.3 Fusilev Rescue After High-Dose Methotrexate Therapy

The recommendations for Fusilev rescue are based on a methotrexate dose of 12 grams/m^2 administered by intravenous infusion over 4 hours (see methotrexate package insert for full prescribing information). Fusilev rescue at a dose of 7.5 mg (approximately 5 mg/m^2) every 6 hours for 10 doses starts 24 hours after the beginning of the methotrexate infusion.

Serum creatinine and methotrexate levels should be determined at least once daily. Fusilev administration, hydration, and urinary alkalinization (pH of 7.0 or greater) should be continued until the methotrexate level is below 5×10^{-8} M (0.05 micromolar). The Fusilev dose should be adjusted or rescue extended based on the following guidelines.

Table 1 Guidelines for Fusilev Dosage and Administration

Clinical Situation	Laboratory Findings	Fusilev Dosage and Duration
Normal Methotrexate Elimination	Serum methotrexate level approximately 10 micromolar at 24 hours after administration, 1 micromolar at 48 hours, and less than 0.2 micromolar at 72 hours	7.5 mg IV q 6 hours for 60 hours (10 doses starting at 24 hours after start of methotrexate infusion).
Delayed Late Methotrexate Elimination	Serum methotrexate level remaining above 0.2 micromolar at 72 hours, and more than 0.05 micromolar at 96 hours after administration.	Continue 7.5 mg IV q 6 hours, until methotrexate level is less than 0.05 micromolar.
Delayed Early Methotrexate Elimination and/or Evidence of Acute Renal Injury	Serum methotrexate level of 50 micromolar or more at 24 hours, or 5 micromolar or more at 48 hours after administration, OR; a 100% or greater increase in serum creatinine level at 24 hours after methotrexate administration (e.g., an increase from 0.5 mg/dL to a level of 1 mg/dL or more).	75 mg IV q 3 hours until methotrexate level is less than 1 micromolar; then 7.5 mg IV q 3 hours until methotrexate level is less than 0.05 micromolar.

Patients who experience delayed early methotrexate elimination are likely to develop reversible renal failure. In addition to appropriate Fusilev therapy, these patients require continuing hydration and urinary alkalinization, and close monitoring of fluid and electrolyte status, until the serum methotrexate level has fallen to below 0.05 micromolar and the renal failure has resolved.

Some patients will have abnormalities in methotrexate elimination or renal function following methotrexate administration, which are significant but less severe than the abnormalities described in the table above. These abnormalities may or may not be associated with significant clinical toxicity. If significant clinical toxicity is observed, Fusilev rescue should be extended for an additional 24 hours (total of 14 doses over 84 hours) in subsequent courses of therapy. The possibility that the patient is taking other medications which interact with methotrexate (e.g., medications

which may interfere with methotrexate elimination or binding to serum albumin) should always be reconsidered when laboratory abnormalities or clinical toxicities are observed.

Delayed methotrexate excretion may be caused by accumulation in a third space fluid collection (i.e., ascites, pleural effusion), renal insufficiency, or inadequate hydration. Under such circumstances, higher doses of Fusilev or prolonged administration may be indicated.

Although Fusilev may ameliorate the hematologic toxicity associated with high dose methotrexate, Fusilev has no effect on other established toxicities of methotrexate such as the nephrotoxicity resulting from drug and/or metabolite precipitation in the kidney.

2.4 Dosing Recommendations for Inadvertent Methotrexate Overdosage

Fusilev rescue should begin as soon as possible after an inadvertent overdosage and within 24 hours of methotrexate administration when there is delayed excretion. As the time interval between antifolate administration [e.g., methotrexate] and Fusilev rescue increases, Fusilev's effectiveness in counteracting toxicity may decrease. Fusilev 7.5 mg (approximately 5 mg/m^2) should be administered IV every 6 hours until the serum methotrexate level is less than 10^{-8} M.

Serum creatinine and methotrexate levels should be determined at 24 hour intervals. If the 24 hour serum creatinine has increased 50% over baseline or if the 24 hour methotrexate level is greater than 5 x 10^{-6} M or the 48 hour level is greater than 9 x 10^{-7} M, the dose of Fusilev should be increased to 50 mg/m^2 IV every 3 hours until the methotrexate level is less than 10^{-8} M. Hydration (3 L/day) and urinary alkalinization with $NaHCO_3$ should be employed concomitantly. The bicarbonate dose should be adjusted to maintain the urine pH at 7.0 or greater.

2.5 Reconstitution and Infusion Instructions

- Prior to intravenous injection, the 50 mg vial of Fusilev for Injection is reconstituted with 5.3 mL of 0.9% Sodium Chloride Injection, USP to yield a levoleucovorin concentration of 10 mg per mL. Reconstitution with Sodium Chloride solutions with preservatives (e.g. benzyl alcohol) has not been studied. The use of solutions other than 0.9% Sodium Chloride Injection, USP is not recommended.
- The reconstituted 10 mg per mL levoleucovorin contains no preservative. Observe strict aseptic technique during reconstitution of the drug product.
- Saline reconstituted levoleucovorin solutions may be further diluted, immediately, to concentrations of 0.5 mg/mL to 5 mg/mL in 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP. Initial reconstitution or further dilution using 0.9% Sodium Chloride Injection, USP may be held at room temperature for not more than a total of 12 hours. Dilutions in 5% Dextrose Injection, USP may be held at room temperature for not more than 4 hours.
- Visually inspect the reconstituted solution for particulate matter and discoloration, prior to administration. CAUTION: Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use if cloudiness or precipitate is observed.
- No more than 16 mL of reconstituted solutions (160 mg of levoleucovorin) should be injected intravenously per minute, because of the calcium content of the levoleucovorin solution.

3 DOSAGE FORMS AND STRENGTHS

Fusilev is supplied in sterile, single-use vials containing 64 mg levoleucovorin calcium pentahydrate (equivalent to 50 mg levoleucovorin) and 50 mg mannitol.

4 CONTRAINDICATIONS

Fusilev is contraindicated for patients who have had previous allergic reactions attributed to folic acid or folinic acid.

5 WARNINGS AND PRECAUTIONS

5.1 Rate of Administration

Because of the Ca^{++} content of the levoleucovorin solution, no more than 16 mL (160 mg of levoleucovorin) should be injected intravenously per minute.

5.2 Potential for Enhanced Toxicity with 5-Fluorouracil

Fusilev enhances the toxicity of 5-fluorouracil. Deaths from severe enterocolitis, diarrhea, and dehydration have been reported in elderly patients receiving weekly *d,l*-leucovorin and 5-fluorouracil.

5.3 Potential for interaction with trimethoprim-sulfamethoxazole

The concomitant use of *d,l*-leucovorin with trimethoprim-sulfamethoxazole for the acute treatment of Pneumocystis carinii pneumonia in patients with HIV infection was associated with increased rates of treatment failure and morbidity in a placebo-controlled study.

6 ADVERSE REACTIONS

6.1 Clinical Studies Experience

Since clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. The following table presents the frequency of adverse reactions which occurred during the administration of 58 courses of high dose methotrexate 12 grams/m^2 followed by Fusilev rescue for osteosarcoma in 16 patients age 6-21. Most patients received Fusilev 7.5 mg every 6 hours for 60 hours or longer beginning 24 hours after completion of methotrexate.

Table 2 Adverse Reactions

Body System/Adverse Reactions	Number (%) of Patients with Adverse Reactions (N =16)		Number (%) of Courses with Adverse Reactions (N = 58)	
	All	Grade 3+	All	Grade 3+
Gastrointestinal				
Stomatitis	6 (37.5)	1 (6.3)	10 (17.2)	1 (1.7)
Vomiting	6 (37.5)	0	14 (24.1)	0
Nausea	3 (18.8)	0	3 (5.2)	0
Diarrhea	1 (6.3)	0	1 (1.7)	0
Dyspepsia	1 (6.3)	0	1 (1.7)	0
Typhlitis	1 (6.3)	1 (6.3)	1 (1.7)	1 (1.7)
Respiratory				
Dyspnea	1 (6.3)	0	1 (1.7)	0
Skin and Appendages				
Dermatitis	1 (6.3)	0	1 (1.7)	0
Other				
Confusion	1 (6.3)	0	1 (1.7)	0
Neuropathy	1 (6.3)	0	1 (1.7)	0
Renal function abnormal	1 (6.3)	0	3 (5.2)	0
Taste perversion	1 (6.3)	0	1 (1.7)	0
Total number of patients	9 (56.3)		2 (12.5)	
Total number of courses	25 (43.1)		2 (3.4)	

The incidence of adverse reactions may be underestimated because not all patients were fully evaluable for toxicity for all cycles in the clinical trials. Leukopenia and thrombocytopenia were observed, but could not be attributed to high dose methotrexate with Fusilev rescue because patients were receiving other myelosuppressive chemotherapy.

6.2 Postmarketing Experience

Since adverse reactions from spontaneous reports are provided voluntarily from a population of uncertain size, it is not always possible to estimate reliably their frequency or establish a causal relationship to drug exposure. Spontaneously reported adverse reactions collected by the WHO Collaborating Center for International Drug Monitoring in Uppsala Sweden have yielded seven cases where levoleucovorin was administered with a regimen of methotrexate. The events were dyspnea, pruritus, rash, temperature change and rigors. For 217 adverse reactions (108 reports) where levoleucovorin was a suspected or interacting medication, there were 40 occurrences of "possible allergic reaction."

7 DRUG INTERACTIONS

Folic acid in large amounts may counteract the antiepileptic effect of phenobarbital, phenytoin and primidone, and increase the frequency of seizures in susceptible children. It is not known whether folinic acid has the same effects. However, both folic and folinic acids share some common metabolic pathways. Caution should be taken when taking folinic acid in combination with anticonvulsant drugs.

Preliminary human studies have shown that small quantities of systemically administered leucovorin enter the CSF, primarily as its major metabolite, 5-methyltetrahydrofolate (5-MTHFA). In humans, the CSF levels of 5-MTHFA remain 1-3 orders of magnitude lower than the usual methotrexate concentrations following intrathecal administration.

Fusilev increases the toxicity of 5-fluorouracil *[see Warnings and Precautions (5.2)]*.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C. It is not known whether Fusilev can cause fetal harm when administered to a pregnant woman or if it can affect reproduction capacity. Animal reproduction studies have not been conducted with Fusilev. Fusilev should be given to a pregnant woman only if clearly needed.

8.3 Nursing Mothers

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Fusilev is administered to a nursing mother.

8.4 Pediatric Use

[See Clinical Studies (14)]

8.5 Geriatric Use

Clinical studies of Fusilev in the treatment of osteosarcoma did not include subjects aged 65 and over to determine whether they respond differently from younger subjects.

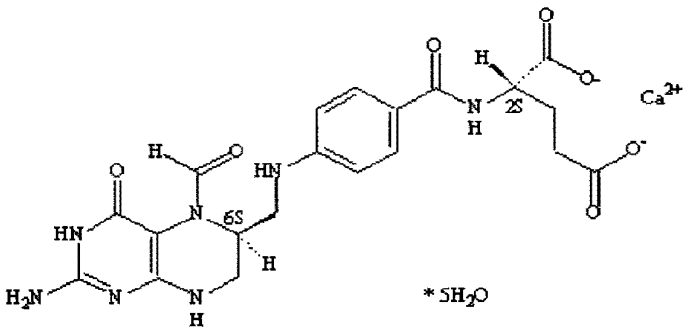
10 OVERDOSAGE

No data are available for overdosage with levoleucovorin.

11 DESCRIPTION

Levoleucovorin is the levo isomeric form of racemic *d,l*-leucovorin, present as the calcium salt. Levoleucovorin is the pharmacologically active isomer of leucovorin [(6-S)-leucovorin].

Fusilev for injection contains levoleucovorin calcium, which is one of several active, chemically reduced derivatives of folic acid. It is useful as antidote to the inhibition of dihydrofolate reductase by methotrexate. This compound has the chemical designation calcium (6S)-N-{4-[[(2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridinyl)methyl] amino]benzoyl}-L-glutamate pentahydrate. The molecular weight is 601.6 and the structural formula is:



Its molecular formula is: $C_{20}H_{21}CaN_7O_7 \cdot 5\ H_2O$.

Fusilev for injection is supplied as a sterile lyophilized powder consisting of 64 mg levoleucovorin calcium pentahydrate (equivalent to 50 mg levoleucovorin) and 50 mg mannitol per 50 mg vial.

Sodium hydroxide and/or hydrocholoric acid are used to adjust the pH during manufacture. It is intended for intravenous administration after reconstitution with 5.3 mL of sterile 0.9% Sodium Chloride Injection, USP [*See Dosage and Administration (2.5)*]

12 CLINICAL PHARMACOLOGY

12.1 Mechanism Of Action

Levoleucovorin is the pharmacologically active isomer of 5-formyl tetrahydrofolic acid. Levoleucovorin does not require reduction by the enzyme dihydrofolate reductase in order to participate in reactions utilizing folates as a source of "one-carbon" moieties. Administration of levoleucovorin can counteract the therapeutic and toxic effects of folic acid antagonists such as methotrexate, which act by inhibiting dihydrofolate reductase.

12.2 Pharmacodynamics

Levoleucovorin is actively and passively transported across cell membranes. In vivo, levoleucovorin is converted to 5-methyltetrahydrofolic acid (5-methyl-THF), the primary circulating form of active reduced folate. Levoleucovorin and 5-methyl-THF are polyglutamated intracellularly by the enzyme folylpolyglutamate synthetase. Folylpolyglutamates are active and participate in biochemical pathways that require reduced folate.

12.3 Pharmacokinetics

The pharmacokinetics of levoleucovorin after intravenous administration of a 15 mg dose was studied in healthy male volunteers. After rapid intravenous administration, serum total tetrahydrofolate (total-THF) concentrations reached a mean peak of 1722 ng/mL. Serum (6S)-5-methyl-5,6,7,8-tetrahydrofolate concentrations reached a mean peak of 275 ng/mL and the mean time to peak was 0.9 hours. The mean terminal half-life for total-THF and (6S)-5-methyl-5,6,7,8-tetrahydrofolate was 5.1 and 6.8 hours, respectively.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment Of Fertility

No studies have been conducted to evaluate the potential of levoleucovorin for carcinogenesis, mutagenesis and impairment of fertility.

13.2 Animal Toxicology And/Or Pharmacology

The acute intravenous LD_{50} values in adult mice and rats were 575 mg/kg (1725 mg/m^2) and 378 mg/kg (2268 mg/m^2), respectively. Signs of sedation, tremors, reduced motor activity, prostration, labored breathing, and/or convulsion were

observed in these studies. Anticipated human dose for each administration is approximately 5 mg/m^2, which represents a 3-log safety margin.

14 CLINICAL STUDIES

The safety and efficacy of Fusilev rescue following high-dose methotrexate were evaluated in 16 patients age 6-21 who received 58 courses of therapy for osteogenic sarcoma. High-dose methotrexate was one component of several different combination chemotherapy regimens evaluated across several trials. Methotrexate 12 g/m^2 IV over 4 hours was administered to 13 patients, who received Fusilev 7.5 mg every 6 hours for 60 hours or longer beginning 24 hours after completion of methotrexate. Three patients received methotrexate 12.5 g/m^2 IV over 6 hours, followed by Fusilev 7.5 mg every 3 hours for 18 doses beginning 12 hours after completion of methotrexate. The mean number of Fusilev doses per course was 18.2 and the mean total dose per course was 350 mg. The efficacy of Fusilev rescue following high-dose methotrexate was based on the adverse reaction profile. *[See Adverse Reactions (6)]*

16 HOW SUPPLIED/STORAGE AND HANDLING

Each 50 mg single-use vial of Fusilev for Injection contains a sterile lyophilized powder consisting of 64 mg levoleucovorin calcium pentahydrate (equivalent to 50 mg levoleucovorin) and 50 mg mannitol.

50 mg vial of freeze-dried powder – NDC 68152-101-00.

Store at 25° C (77 °F) in carton until contents are used. Excursions permitted from 15-30° C (59-86 °F). [See USP Controlled Room Temperature]. Protect from light.

SPECTRUM
PHARMACEUTICALS

Manufactured for Spectrum Pharmaceuticals, Inc.
Irvine, CA 92618
Manufactured by Chesapeake Biological Laboratories, Inc.
Baltimore, MD 21230

Spectrum Pharmaceuticals, Inc.

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www.bjcancer.com

Clinical Studies

A randomised phase II study of modified FOLFIRI.3 *vs* modified FOLFOX as second-line therapy in patients with gemcitabine-refractory advanced pancreatic cancer

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BACKGROUND: Only a few clinical trials have been conducted in patients with advanced pancreatic cancer after failure of first-line gemcitabine-based chemotherapy. Therefore, there is no current consensus on the treatment of these patients. We conducted a randomised phase II study of the modified FOLFIRI.3 (mFOLFIRI.3; a regimen combining 5-fluorouracil (5-FU), folinic acid, and irinotecan) and modified FOLFOX (mFOLFOX; a regimen combining folinic acid, 5-FU, and oxaliplatin) regimens as second-line treatments in patients with gemcitabine-refractory pancreatic cancer.

METHODS: The primary end point was the 6-month overall survival rate. The mFOLFIRI.3 regimen consisted of irinotecan (70 mg m^{-2}; days 1 and 3), leucovorin (400 mg m^{-2}; day 1), and 5-FU (2000 mg m^{-2}; days 1 and 2) every 2 weeks. The mFOLFOX regimen was composed of oxaliplatin (85 mg m^{-2}; day 1), leucovorin (400 mg m^{-2}; day 1), and 5-FU (2000 mg m^{-2}; days 1 and 2) every 2 weeks.

RESULTS: Sixty-one patients were randomised to mFOLFIRI.3 ($n = 31$) or mFOLFOX ($n = 30$) regimen. The six-month survival rates were 27% (95% confidence interval (CI) = 13–46%) and 30% (95% CI = 15–49%), respectively. The median overall survival periods were 16.6 and 14.9 weeks, respectively. Disease control was achieved in 23% (95% CI = 10–42%) and 17% patients (95% CI = 6–35%), respectively. The number of patients with at least one grade 3/4 toxicity was identical (11 patients, 38%) in both groups: neutropenia (7 patients under mFOLFIRI.3 regimen *vs* 6 patients under mFOLFOX regimen), asthaenia (1 *vs* 4), vomiting (3 in both), diarrhoea (2 *vs* 0), and mucositis (1 *vs* 2).

CONCLUSION: Both mFOLFIRI.3 and mFOLFOX regimens were tolerated with manageable toxicity, offering modest activities as second-line treatments for patients with advanced pancreatic cancer, previously treated with gemcitabine.

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Keywords: second-line chemotherapy; pancreatic cancer; irinotecan; oxaliplatin; gemcitabine

Pancreatic cancer accounts for 3% of all cancers, but is the fifth leading cause of cancer death in Western countries (Yeo *et al*, 2005). At the time of diagnosis, approximately half of the patients have metastases, and the median survival time barely exceeds 6 months, whereas approximately one-third of patients diagnosed with locally advanced disease have median survival times ranging between 6 and 9 months. Thus, a small proportion of patients are eligible for surgery, the only curative treatment option, at diagnosis (Bilimoria *et al*, 2007). Even with surgery, prognosis remains poor; the 5-year overall survival was only 23.4% for patients undergoing pancreatectomy (Sener *et al*, 1999).

Although 5-fluorouracil (5-FU)-based chemotherapy has been reported to be superior to best supportive care alone (Palmer *et al*, 1994; Glimelius *et al*, 1996), and a pivotal phase III trial showed that gemcitabine offers a survival advantage over a weekly bolus infusion of 5-FU, accompanied by an improved clinical benefit (Burris *et al*, 1997), the overall therapeutic results are still disappointing; the response rate was 5.4% with a clinical benefit response rate of 23.8% and a 1-year survival rate of 18% in patients treated with gemcitabine.

Therefore, a number of clinical studies have been undertaken to enhance the effectiveness of front-line chemotherapy. Despite promising results in early-phase clinical studies, the majority of newer approaches have failed to show clinically meaningful therapeutic advantages over the standard infusion of gemcitabine alone. Although regimens consisting of gemcitabine in combination with erlotinib or capecitabine have shown statistically significant increases in survival duration, the small amount of survival benefit and accompanying toxicities result in difficulties related to their translation into clinically meaningful improvements (Cunningham *et al*, 2005; Moore *et al*, 2007).

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Considering the poor response rate (20% or less) of gemcitabine-based doublet treatment in the first-line setting, the short progression-free survival (PFS) (<4 months), and the increased use of gemcitabine as adjuvant treatment (Oettle *et al*, 2007), an additional problem in the therapeutic management of this common malignant disease, is the need for effective treatment alternatives in patients failing to respond to gemcitabine-based chemotherapy. To date, few studies have assessed second-line chemotherapy, primarily because of poor prognosis (Nakachi *et al*, 2007) and because of the limited life expectancy of those with advanced pancreatic cancer after failure of first-line chemotherapy (Kozuch *et al*, 2001; Tsavaris *et al*, 2005; Kulke *et al*, 2007; Xiong *et al*, 2008; Novarino *et al*, 2009). There is, therefore, a growing unmet need for a second-line chemotherapy regimen to treat patients with gemcitabine-refractory pancreatic cancer (Boeck and Heinemann, 2008; Kang and Saif, 2008).

The clinical benefit and safety of the FOLFIRI and FOLFOX regimens have been well established in a study of gastrointestinal cancer patients (Tournigand *et al*, 2004). In several phase II trials, irinotecan-based and oxaliplatin-based regimens have shown modest activity against advanced pancreatic cancer. A French group has reported that the FOLFIRI.3 regimen, composed of a split irinotecan infusion on days 1 and 3, with 5-FU for 2 days, showed promising activity in chemotherapy-naive and pre-treated patients with advanced pancreatic cancer. The confirmed response rate was 37.5%, with a median PFS of 5.6 months (Taieb *et al*, 2007). The study also suggested that there was no cross-resistance between gemcitabine and FOLFIRI.3 regimen. Furthermore, an oxaliplatin and 5-FU combination, at various doses and schedules, has been evaluated as second-line chemotherapy in pancreatic cancer patients after gemcitabine failure (Tsavaris *et al*, 2005; Gebbia *et al*, 2007; Novarino *et al*, 2009). Recently, a German group has reported that the 5FU/folinic acid (FA) plus oxaliplatin (OFF) regimen could prolong survival and improve the quality of life of advanced pancreatic cancer patients after gemcitabine failure compared with best supportive care alone with or without 5FU/FA (FF) (Oettle *et al*, 2005; Pelzer *et al*, 2008).

On the basis of these results, we conducted a randomised phase II study of the modified FOLFIRI.3 (mFOLFIRI.3) and modified FOLFOX (mFOLFOX) regimens as second-line treatments in patients with gemcitabine-refractory pancreatic cancer. The aim of this study was to select a better regimen, which should be investigated in future studies.

MATERIALS AND METHODS

Patients

Patients at least 18 years of age with histologically confirmed, locally advanced, or metastatic pancreatic adenocarcinoma, who were previously treated with gemcitabine-based first-line chemotherapy were eligible for this study if they met the following inclusion criteria: Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0–2; measurable disease based on Response Evaluation Criteria in Solid Tumors (RECIST) criteria; no previous second-line chemotherapy; adequate bone marrow function, defined as a condition with leukocyte count >4000 per μl, absolute neutrophil count >1500 per μl, haemoglobin >9.0 g per 100 ml, platelets >100 000 per μl; adequate renal and hepatic function, defined as a condition with serum creatinine <1.5 mg per 100 ml, bilirubin <1.5 mg per 100 ml (<2.5 mg per 100 ml in patients with obstructive jaundice and adequately decompressed bile duct obstruction), and serum transaminase <three-fold the upper normal limit (<five-fold the upper normal limit for patients with liver metastasis); adequate nutritional status, defined as a condition with albumin >3.0 g per 100 ml; and the giving of written informed consent. Patients were excluded if they had histology indicating a condition other than adenocarcinoma, brain metastasis, significant gastrointestinal bleeding or obstruction, any serious co-morbidity, axial skeletal radiotherapy within 6 months before study commencement, or peripheral neuropathy of grade 2 or worse. This study was initially approved by the Institutional Review Board of the Asan Medical Center. The study was conducted according to the tenets of the Declaration of Helsinki and guidelines on good clinical practice. The clinical trial registration number was NCT00786006.

Study design and randomisation

This was an open-label, single-centre, randomised phase II trial using the two treatment arms of mFOLFIRI.3 and mFOLFOX. Random assignment was performed at a 1:1 ratio and patients were stratified by age (≤65 years *vs* >65 years), ECOG PS (0–1 *vs* 2), and an earlier best overall response to gemcitabine (non-disease progression *vs* disease progression).

Treatment dose and schedule

The mFOlFIRI.3 regimen consisted of irinotecan 70 mg m^{-2} (over 1 h) on day 1, leucovorin 400 mg m^{-2} (over 2 h) on day 1, 5-FU 2000 mg m^{-2} (over 46 h) from day 1, and irinotecan 70 mg m^{-2} (over 1 h) at the end of the 5-FU infusion every 2 weeks. The mFOLFOX regimen was composed of oxaliplatin 85 mg m^{-2} (over 2 h) on day 1, leucovorin 400 mg m^{-2} (over 2 h) on day 1, and 5-FU 2,000 mg m^{-2} (over 46 h) every 2 weeks. When haematologic or non-haematologic toxicities of grade ≥2 occurred, chemotherapy was delayed until recovery to grade ≤1. The doses of subsequent schedules were reduced by 25% in patients with grade ≥3 haematologic and non-haematologic toxicities, and if toxicity was considered to be attributable, by the attending physician, to only one drug; the doses of other drugs were not modified. Treatment was continued until the occurrence of disease progression, unacceptable toxicity, or patient's refusal to continue. If disease progression was observed and patient performance was good, crossover to the alternate treatment arm was permitted.

Pre- and on-treatment evaluation

Within 2 weeks before study enrolment, patients gave a complete medical history; underwent a full physical examination including ECOG PS; were sampled for a complete blood count, serum chemistry with electrolyte levels, a coagulation battery, and carbohydrate antigen 19–9 (CA 19–9) level; underwent urinalysis; underwent a chest X-ray; were assessed by electrocardiography; and were evaluated by computed tomography of the abdomen and pelvis (chest or any other region, if metastasis was suspected or previously detected). Before the administration of each cycle of chemotherapy, each patient was examined and reviewed for complete and differential blood counts and serum chemistry. More frequent review and monitoring were performed if clinically indicated. Tumour response was assessed every three cycles according to the RECIST criteria (Therasse *et al*, 2000). For each of these assessments, similar imaging techniques as used at baseline were used. The National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0, was used to assess toxicity.

Statistical analysis

The primary end point was the 6-month survival rate. The randomised two-arm phase II design was used to select the more promising regimen of the two in terms of this criterion (Simon *et al*, 1985). Using this design, the regimen with the better survival rate is selected, irrespective of the difference between protocols. To permit at least a 90% probability of selecting a truly better regimen when the absolute difference in the 6-month survival rate was 15%

or greater, 29 evaluable patients were needed in each arm. Survival time was calculated from the date of randomisation to the date of death from any cause. The secondary end points were overall response rate, PFS, overall survival (OS), and toxicity. Overall response rate was analysed on an intention-to-treat basis. PFS was defined as the time from randomisation to disease progression or death from any cause. PFS was censored at the date of the last visit for those patients who were alive without documented disease progression. OS and PFS were estimated by the Kaplan–Meier method. Patients were considered assessable if they had received at least two cycles of chemotherapy (over 4 weeks) and had at least one follow-up imaging study. However, patients were also considered assessable if they received less than two cycles because of rapid tumour progression. Survival curves were compared by the log-rank test. In multivariate analysis, Cox's proportional hazards model was used to identify independent prognostic factors for PFS and OS. All tests were two-sided and a P-value <0.05 was considered to be statistically significant. SPSS version 14.0 (SPSS, Chicago, IL, USA) was used for statistical analysis.

RESULTS

Patient characteristics

From January 2007 to December 2008, 61 pancreatic cancer patients were enrolled at the Asan Medical Center, Seoul, Korea; 31 were randomly assigned to the mFOLFIRI.3 arm and 30 to the mFOLFOX arm. One patient in the mFOLFIRI.3 arm withdrew consent after the first cycle of chemotherapy and was lost to follow-up. Baseline characteristics were well balanced between the two treatment arms (Table 1). The median patient age was 55 years (range 35–73 years) and all but one patient was of ECOG PS 0 or 1. Twenty-one patients (34%) had undergone previous surgery and two (3%) had received palliative radiotherapy. Of the 16 patients who were prescribed adjuvant chemotherapy, gemcitabine was administered to three patients. Gemcitabine plus capecitabine was given to most patients (75%). After disease progression to a stage at which a salvage regimen was required, a crossover to the alternate protocol was undertaken by 12 patients (39%) in the mFOLFIRI.3 arm and by 7 (23%) in the mFOLFOX arm. The median time to crossover to the alternate treatment was 8.3 weeks (range 3.3–18.1 weeks) in the mFOLFIRI.3 arm, and 15 weeks (range 7.0–32.6 weeks) in the mFOLFOX arm.

Primary end points

A total of 98 cycles of the mFOLFIRI.3 and 93 cycles of the mFOLFOX regimens were delivered with a median of 3 cycles (range 1–12 and 1–10 cycles, respectively) in both arms. With a median follow-up period of 24.4 weeks (range 0.8–40.8 weeks), 50 of 61 patients (82%) died. The 6-month survival rate was 27% in the mFOLFIRI.3 arm (95% confidence interval (CI) = 13–46%) patients and 30% for those in the mFOLFOX arm (95% CI = 15–49%). Except for two patients who died because of treatment-related complications, all deaths were attributable to disease progression *per se*.

Secondary end points

The overall response rate values are listed in Table 2. Response evaluation was possible in 28 patients in the mFOLFIRI.3 arm and in 26 patients in the mFOLFOX arm. In the mFOLFIRI.3 arm, two patients could not be evaluated because of early death, and were lost to follow-up before the first response evaluation. In the mFOLFOX arm, response evaluation could not be achieved in four patients because of early death (two patients), loss to follow-up (one patient), and patient's refusal to continue with the trial (one patient). The overall response rate in the intention-to-treat population was 7% in the mFOLFOX arm (95% CI = 1–22%). Overall response could not be ascertained in the mFOLFIRI.3 arm. The disease control rate (PR and stable disease) was 23% in the mFOLFIRI.3 arm (95% CI = 11–40%) and 17% in the mFOLFOX arm (95% CI = 7–34%).

Table 1 Patient characteristics

Characteristic	mFOLFIRI.3 ($n=31$) No. of patients (%)	mFOLFOX ($n=30$) No. of patients (%)
Age, median (range)	55 (37–73)	55 (35–69)
<60 years	19 (61)	18 (60)
⩾60 years	12 (39)	12 (40)
Gender		
Male	24 (77)	20 (67)
Female	7 (23)	10 (33)
ECOG PS		
0	5 (16)	5 (17)
1	26 (84)	24 (80)
2	0 (0)	1 (3)
Metastatic site		
Liver	19 (61)	21 (70)
Peritoneum	19 (61)	11 (37)
Lung	6 (19)	5 (17)
Lymph nodes	15 (48)	14 (47)
Others	9 (29)	5 (17)
Prior treatment		
Surgery	10 (32)	11 (37)
Palliative radiotherapy	1 (3)	1 (3)
Adjuvant chemotherapy	7 (23)	9 (30)
Neoadjuvant chemoradiotherapy	0 (0)	1 (3)
Prior gemcitabine-based regimen		
Gemcitabine	4 (13)	2 (7)
Gemcitabine/capecitabine	20 (64)	26 (86)
Gemcitabine/erlotinib	4 (13)	2 (7)
Gemcitabine/cisplatin	3 (10)	0 (0)
Previous response to gemcitabine-based regimen		
CR	0 (0)	1 (3)
PR	10 (32)	9 (30)
SD	11 (35)	13 (43)
PD	10 (32)	7 (23)
Survival at analysis		
Alive	6 (20)	5 (17)
Dead	25 (81)	25 (83)
Crossover to alternative regimen	12 (39)	7 (23)

Abbreviations: ECOG PS = Eastern Cooperative Oncology Group performance status; CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease.

Table 2 Overall response rate

Overall Response	mFOLFIRI.3 No. of patients (%, 95% CI)	mFOLFOX No. of patients (%, 95% CI)
PR	0 (0, 0–10)	2 (7, 1–22)
SD	7 (23, 11–40)	3 (10, 3–26)
PD	21 (68, 49–83)	21 (70, 52–84)
Not evaluable	3 (10, 3–26)	4 (13, 5–30)
Disease control	7 (23, 11–40)	5 (17, 7–34)

Abbreviations: PR = partial response; SD = stable disease; PD = progressive disease.

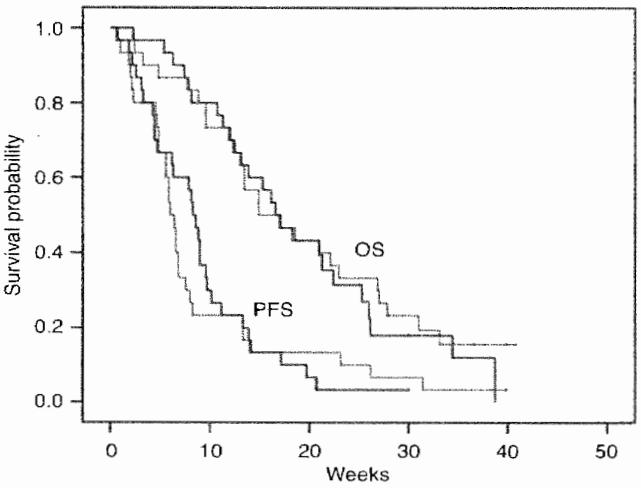

Figure 1 Survival curves for progression-free survival (PFS) and overall survival (OS). Modified FOLFIRI.3 (a regimen combining 5-fluorouracil, folinic acid, and irinotecan) is depicted as solid lines and modified FOLFOX (a regimen combining folinic acid, 5-FU, and oxaliplatin) as dotted lines.

The median PFS was 8.3 weeks for patients treated with mFOLFIRI.3 (95% CI = 6.9–9.6 weeks) and 6.0 weeks for those given mFOLFOX (95% CI = 5.1–6.9 weeks) (Figure 1). The median OS was 16.6 weeks for patients treated with mFOLFIRI.3 (95% CI = 12.5–20.6 weeks) and 14.9 weeks for those given mFOLFOX (95% CI = 8.0–21.8 weeks) (Figure 1). Turning to survival outcomes from the commencement of first-line chemotherapy, the median PFS was 34.9 weeks (95% CI = 30.8–38.9 weeks) and 37.0 weeks (95% CI = 32.0–42.0 weeks) for mFOLFIRI.3 and mFOLFOX, respectively. The median OS was identical at 47.1 weeks (95% CI = 39.0–55.2 weeks and 36.0–58.3 weeks, respectively).

Toxicity

The numbers of patients experiencing adverse events are presented in Table 3. In each treatment arm, 29 patients were available for toxicity assessment, and only two patients in the mFOLFOX arm were free from adverse events. The prevalence of severe toxicities was the same between the two regimens (38%); however, grade 3/4 asthaenia (3% *vs* 14%) developed more frequently in patients receiving mFOLFOX, whereas grade 3/4 diarrhoea (7% *vs* 0%) was more common in patients prescribed mFOLFIRI.3. Treatment-related mortality occurred in one patient in each group. One patient in the mFOLFIRI.3 arm died of septic shock complicated by febrile neutropaenia after 2 weeks of the first cycle. In one patient in the mFOLFOX arm, early death after the first cycle of chemotherapy was caused by severe pneumonia.

Prognostic factors

In a univariate analysis of survival outcomes according to the clinical variables of all 60 patients (gender, age, ECOG PS, hypoalbuminaemia, anaemia, resectability at initial diagnosis, liver metastasis, and PFS under gemcitabine), hypoalbuminaemia ($\leqslant 3.5$ mg 100 ml^{-1}) and ECOG PS $\geqslant 1$ were significant prognostic factors for poor PFS and OS. In multivariate analysis, however, only hypoalbuminaemia predicted poor PFS ($P = 0.02$, hazard ratio = 1.97, 95% CI = 1.14–3.39), but not OS.

DISCUSSION

Pancreatic cancer is well known to be refractive to chemotherapy and to show rapid progression. Until recently, patients with pancreatic cancer after gemcitabine-based chemotherapy failure have had little opportunity to receive second-line chemotherapy because of rapid performance deterioration (Nakachi *et al*, 2007; Kang and Saif, 2008). Therefore, few studies have focused on patients with advanced pancreatic cancer in a second-line setting. Moreover, as gemcitabine is known to be effective when used as adjuvant therapy, many patients who underwent curative resection received gemcitabine in this setting. This means that oncologists urgently require data on other chemotherapeutic options for gemcitabine-pretreated patients.

Gemcitabine plus oxaliplatin (GEMOX), oxaliplatin plus capecitabine (XELOX), capecitabine plus erlotinib, docetaxel plus gefitinib, and FOLFOX have been tested in gemcitabine-refractory pancreatic cancer patients and showed disease control rates of 19–53% and a median OS range of 2.9–6.7 months (Tsavaris *et al*, 2005; Demols *et al*, 2006; Kulke *et al*, 2007; Xiong *et al*, 2008; Brell *et al*, 2009; Novarino *et al*, 2009). Recently, another oxaliplatin-based regimen, 5-FU/FA plus oxaliplatin (OFF), was shown to offer significantly improved survival compared with 5-FU/FA (FF) in a phase III trial (CONKO 003) (Pelzer *et al*, 2008). In this randomised trial, including 160 gemcitabine-pretreated patients with advanced pancreatic cancer, patients receiving OFF achieved a median PFS of 13 weeks ($P = 0.012$) and a median OS of 26 weeks ($P = 0.014$), compared with 9 and 13 weeks, respectively, for FF-treated patients. However, there is no current consensus on optimal second-line therapy for gemcitabine-refractory advanced pancreatic cancer (Boeck and Heinemann, 2008; Kang and Saif, 2008). Both FOLFIRI.3 and FOLFOX have shown modest activity as first-line and second-line chemotherapy regimens (Tsavaris *et al*, 2005; Gebbia *et al*, 2007; Taieb *et al*, 2007; Novarino *et al*, 2009). We were also of the view that neither regimen showed significant cross-resistance to gemcitabine-based protocols (Gebbia *et al*, 2007; Taieb *et al*, 2007).

The results of this trial show that both combination regimens showed favourable efficacy and toxicity profiles in gemcitabine-pretreated patients with advanced pancreatic cancer. The 6-month survival rates were 27 and 30% and disease control rates were 23% and 17%, in patients treated with mFOLFIRI.3 and mFOLFOX, respectively. Of the 12 patients whose disease was controlled by these regimens, disease stabilisation was previously achieved in nine patients in gemcitabine-based regimens. The median PFS and median OS were 8.3 weeks and 16.6 weeks in the mFOLFIRI.3 arm, and 6.0 weeks and 14.9 weeks in the mFOLFOX arm, respectively. These were in line with the survival data of several previous studies (Tsavaris *et al*, 2005; Gebbia *et al*, 2007; Novarino *et al*, 2009).

Toxicities related to both regimens were quite expectable and generally manageable. Patients with toxicities of grade 3 or worse constituted 38% of each treatment arm. Common toxicities of both regimens included anaemia, neutropenia, asthaenia, nausea, vomiting, and mucositis. In accordance with the known toxicities of both regimens, diarrhoea developed more frequently in mFOLFIRI.3 arm patients and neuropathy was more common in those in the mFOLFOX arm. Although half the patients treated with mFOLFOX experienced peripheral neuropathy, this was mostly of grade 1. This may be related to a lower cumulative dose of oxaliplatin because of the early dropout caused by rapid disease progression. However, treatment-related mortality occurred in patients prescribed either regimen, and hence physicians need to guard against infectious complications in patients treated with these protocols.

Turning to prognostic factors affecting PFS and OS, hypoalbuminaemia, implying poor nutritional status, was a poor prognostic factor for PFS in this study. In contrast to a previous study (Herrmann *et al*, 2008), we could not find an association between the time to progression under first-line chemotherapy ($\leqslant 6$ months) and PFS under second-line therapy, or residual survival. However, it is hard to draw conclusions with regard to this, because this study had small sample sizes, which might result in insufficient statistical power detecting significant prognostic factors.

Table 3 Treatment-related toxicities

	mFOLFIRI.3 no. of patients (%)			mFOLFOX no. of patients (%)		
Toxicity	**G 1-2**	**G 3-4**	**All G**	**G 1-2**	**G 3-4**	**All G**
Anaemia	14 (48)	1 (3)	15 (52)	15 (50)	1 (3)	16 (55)
Neutropenia	6 (20)	7 (24)	13 (45)	8 (27)	6 (20)	14 (48)
Thrombocytopenia	3 (10)	1 (3)	4 (14)	9 (31)	1 (3)	10 (34)
Febrile neutropenia		1 (3)	1 (3)		0 (0)	0 (0)
Alopecia	3 (10)	0 (0)	3 (10)	0 (0)	0 (0)	0 (0)
Asthaenia	17 (58)	1 (3)	18 (62)	22 (76)	4 (14)	26 (90)
Diarrhoea	10 (34)	2 (7)	12 (41)	5 (17)	0 (0)	5 (17)
Anorexia	5 (17)	1 (3)	6 (21)	6 (21)	2 (7)	8 (28)
Nausea	12 (41)	1 (3)	13 (45)	13 (45)	1 (3)	14 (48)
Vomiting	6 (20)	3 (10)	9 (31)	11 (38)	3 (10)	14 (48)
Mucositis	8 (27)	1 (3)	9 (31)	8 (28)	2 (7)	10 (34)
Neurotoxicity	1 (3)	0 (0)	1 (3)	13 (44)	0 (0)	13 (45)
Maximum/patients*	18 (62)	11 (38)		16 (57)	11 (38)	

Abbreviation: G = grade. *Maximum/patients, maximal toxicity in an individual patient. The numbers of patients experiencing adverse events are listed.

Clinical Studies

Although this trial used adequate primary and secondary outcomes to represent the characteristics of the two regimens, the lack of assessment of clinical benefit or quality of life is a limitation of our study.

In conclusion, our trial not only showed that both mFOLFIRI.3 and mFOLFOX regimens could be safely used but also showed modest anti-cancer activities in gemcitabine-pretreated patients. Although further clinical trials are necessary for comparison with other regimens, these protocols may be reasonable therapeutic options in a second-line setting for patients with advanced pancreatic cancer, who were previously treated with gemcitabine-based chemotherapy.

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Development of a Highly Active Nanoliposomal Irinotecan Using a Novel Intraliposomal Stabilization Strategy

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Abstract

Liposome formulations of camptothecins have been actively pursued because of the potential for significant pharmacologic advantages from successful drug delivery of this important class of anticancer drugs. We describe nanoliposomal CPT-11, a novel nanoparticle/liposome construct containing CPT-11 (irinotecan) with unprecedented drug loading efficiency and *in vivo* drug retention. Using a modified gradient loading method featuring a sterically hindered amine with highly charged, multivalent anionic trapping agents, either polymeric (polyphosphate) or nonpolymeric (sucrose octasulfate), liposomes were capable of entrapping CPT-11 at extremely high drug-to-lipid ratios (>800 g CPT-11/mol phospholipid) and retaining encapsulated drug *in vivo* with a half-life of drug release in the circulation of 56.8 hours. CPT-11 was also protected from hydrolysis to the inactive carboxylate form and from metabolic conversion to SN-38 while circulating. The maximum tolerated dose in normal mice was determined to be 80 mg/kg for free CPT-11 and >320 mg/kg for nanoliposomal CPT-11. Nanoliposomal CPT-11 showed markedly superior efficacy when compared with free CPT-11 in human breast (BT474) and colon (HT29) cancer xenograft models. This study shows that intraliposomal stabilization of CPT-11 using a polymeric or highly charged, nonpolymeric polyanionic trapping agent results in a markedly active antitumor agent with low toxicity. (Cancer Res 2006; 66(6): 3271-7)

Introduction

Liposome-based systems have been used to enhance efficacy and/or ameliorate toxicity of certain drugs (1, 2). Thus far, the most successful approach has involved constructs engineered for long circulation times, combined with stable encapsulation of the active compound within the liposome; this allows liposomes to accumulate at sites of cancer, followed by intratumoral drug release. An example is PEGylated liposomal doxorubicin (3), which has received Food and Drug Administration approval for cancer treatment. However, the successful case of liposomal anthracyclines has not yet been matched by liposome constructs containing other anticancer drug classes, although recent progress has been made with vincristine (4–6) and certain camptothecin analogues (7–9). One of the key reasons for this has been the technical facility with which anthracyclines can be stably encapsulated in the liposome interior using remote-loading methodologies (10, 11), giving rise to stable liposome formulations that have been difficult to replicate with other classes of drugs.

CPT-11 {irinotecan; 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycampothecin} is a water-soluble camptothecin derivative currently used in cancer chemotherapy. The pharmacology of CPT-11 is complex, with extensive metabolic conversions involved in the activation, inactivation, and elimination of the drug (12, 13). CPT-11 is a prodrug that is converted by nonspecific carboxylesterases into a 100- to 1,000-fold more active metabolite, SN-38 (14). SN-38 is cleared via glucuronidation, for which major pharmacogenetic differences have been shown (15), and biliary excretion. In addition, CPT-11 and other camptothecins exist in a pH- and serum protein-dependent equilibrium between an active lactone form of the drug (predominant under acidic conditions) and an inactive carboxylate form (predominant at neutral or basic pH; ref. 16). These drug properties contribute to the marked heterogeneities in efficacy and toxicity observed clinically with CPT-11 (12, 17). Hence, drug carrier technologies represent a rational strategy to improve the pharmacokinetics and biodistribution of CPT-11 while protecting it from premature metabolism.

In this report, we describe a novel intraliposomal drug stabilization technology for encapsulation of CPT-11 into long-circulating liposome-based nanoparticles with high drug load and high *in vivo* stability, matching or surpassing previous liposomal drugs. This was achieved using polymeric or nonpolymeric highly charged anions, polyphosphate or sucrose octasulfate, as intraliposomal trapping agents in conjunction with a high-pK_a polyalkylamine gradient. The approach also allowed for preservation of the drug in its active lactone form within the liposome interior, protecting it from hydrolysis as well as premature conversion to SN-38. Here we use the term "nanoliposomal drug" to describe a nanoparticle consisting of a lipid bilayer scaffold encapsulating a nanoscale drug complex or aggregate that facilitates *in vivo* drug retention.

Materials and Methods

Liposome Preparation and Drug Loading

Solutions of triethylammonium salts of a linear poly(phosphate) (TEA-Pn, 13-18 phosphate units; Sigma Corp., St. Louis, MO) and sucrose octasulfate (TEA_8SOS) were prepared from commercially obtained sodium salts (Toronto Research Chemicals, Inc., North York, Ontario, Canada) by ion-exchange chromatography on the Dowex 50Wx8-200 resin in the H^+ form, immediately followed by titration with neat triethylamine. Residual sodium in either solution, as determined by potentiometry using a Na^+-selective electrode, was <1% of the cation content. Phosphate content was determined by inorganic phosphate assay following acid hydrolysis and was adjusted to 0.55 mol/L for TEA-Pn (osmolality, 430-480 mmol/kg). The TEA concentration was calculated from the amount of added TEA and was

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adjusted to 0.65 mol/L for TEA_8SOS solution (osmolality, 480-530 mmol/kg). The final pH for both solutions was 5.5 to 6.0.

Distearoylphosphatidylcholine (3 mol. parts), methoxypoly(ethylene)glycol (PEG2000)-derivatized distearoylphosphatidylethanolamine (0.015 mol. parts; Avanti Polar Lipids, Alabaster, AL), and cholesterol (2 mol. parts; Calbiochem, La Jolla, CA) were combined in ~50% (w/v) ethanolic solution and mixed with 10 volumes of the solution of TEA-Pn or TEA_8SOS at 60°C to 65°C. For pharmacokinetic studies, a nonexchangeable lipid label, [^{3}H] cholesteryl hexadecyl ether (Perkin-Elmer, Boston, MA), was added to the lipids in the amount of 0.5 mCi/mmol phospholipid. The lipid suspension was extruded 15 times through two stacked polycarbonate membranes (Nucleopore, Corning-Costar, Acton, MA) with 0.08-μm pore size using argon pressure at 60°C to 65°C. The extruded liposomes were 88 to 95 nm in diameter by dynamic light scattering.

Unentrapped triethylammonium polyanions were removed by chromatography on a Sepharose CL-4B size exclusion column eluted with HEPES-buffered dextrose (5 mmol/L HEPES, 5% dextrose, pH 6.5). CPT-11·HCl (kindly provided by TTY Biopharmaceuticals, Taipei, Taiwan) was added to the liposomes at a ratio of 500 g CPT-11/mol phospholipid and the pH adjusted to 6.5. The resulting solution was heated to 60°C for 30 minutes and then quenched on ice for 15 minutes. Unencapsulated CPT-11 was subsequently removed using a Sephadex G-75 column eluted with HEPES-buffered saline (5 mmol/L HEPES, 145 mmol/L NaCl, pH 6.5). The loading efficiency was determined in all preparations by quantitating both drug and phospholipid and comparing the resulting drug/phospholipid ratio to its input value. CPT-11 was determined spectrophotometrically at 372 nm in acid/methanol (20 volume % 0.5 mol/L phosphoric acid/80 volume % methanol). Phospholipid was quantitated using a standard phosphate assay (18).

Pharmacokinetic Studies

Female Sprague-Dawley rats (190-210 g) with indwelling central venous catheters were injected with a 0.2 to 0.3 mL bolus of ^{3}H-CHE-labeled CPT-11 liposomes (10 mg/kg). Blood samples (0.2-0.3 mL) were drawn at various times postinjection using a heparin-treated syringe. The withdrawn blood volume was replaced using PBS. Blood samples were diluted with 0.3 mL of ice-cold PBS containing 0.04% EDTA, weighed, and centrifuged. Plasma was assayed for CPT-11 [by fluorometry or high-performance liquid chromatography (HPLC)] and for liposome label (scintillation radioactivity counting). The percent of drug remaining in the liposomes was calculated by dividing the drug/lipid ratio in the blood samples by that of the injected liposomes (taken as 100%). Because free CPT-11 is cleared at a much faster rate than liposomes (Fig. 3*A*), a change in the CPT-11-to-liposomal lipid ratio was indicative of drug leakage from the carrier. Noncompartmental pharmacokinetics data analysis was done using PK Solutions 2.0 software (Summit Research Services, Montrose, CO).

Drug Stability and Metabolism Studies

Liposomal and free CPT-11 were administered i.v. at a dose of 25 mg/kg in female albino rats (180-220 g) as above, and blood samples were withdrawn at intervals up to 48 hours. The blood samples were mixed with ice-cold PBS containing 0.04% EDTA and quickly centrifuged. The plasma was assayed for CPT-11, SN-38, and their carboxylate forms by HPLC using a modification of the method of Warner and Burke (19). Briefly, samples were extracted with 400 μL of ice-cold methanol by vortexing and centrifugation at 14,100 × *g* for 5 minutes. The mobile phase consisted of 3% triethylammonium acetate pH 5.5 (solution A) and acetonitrile (solution B) delivered at 1.0 mL/min in a linear gradient of 20 volume % A to 50 volume % B in 14 minutes. The eluted products were detected by fluorescence with an excitation at 375 nm and emission at 500 nm. The retention times were 5.3 minutes (CPT-11 carboxylate), 6.8 minutes (SN-38 carhoxylate), 9.3 minutes (CPT-11), and 11.0 minutes (SN-38).

Conversion of CPT-11 to SN-38 was assayed in macrophages isolated from the peritoneum of female NCR *nu/nu* mice and plated at a density of 150,000 cells per well in a 12-well plate. After 24 hours, nanoliposomal CPT-11 was added to macrophages at a concentration of 10 μg CPT-11/mL and incubated for 24 hours in RPMI 1640 with 10% FCS. At indicated times, the medium was removed and the cells washed twice with Hanks buffered saline. The cells were treated with 0.2 mL of 1% Triton X-100 at room temperature for 5 minutes and solubilized in 0.8 mL of 80 volume % methanol/20 volume % 0.1 mol/L H_3PO_4 with shaking for an additional 5 minutes. The cell debris was removed by centrifugation at 13,000 rpm for 10 minutes and the supernatant was assayed by HPLC as described above.

Acute Toxicity Studies

The maximum tolerated dose following single i.v. administration was evaluated in healthy female Swiss Webster mice following a protocol adapted from the protocol communicated by the National Cancer Institute (NCI) Developmental Therapeutics Program. Briefly, in the first range-seeking step, the drug was administered via the tail vein in groups of two mice, beginning with the dose of 60 mg/kg CPT-11 and continuing with the dose escalation factor of 1.8 until acute mortality or terminal morbidity (within 1 day postinjection) was observed in any animal. The second range-seeking step was similarly done using a dose escalation factor of 1.15 and starting with the highest dose at which no mortality or terminal morbidity was observed (the highest tolerated dose) in the first step. Finally, in a validation step, a group of five mice were injected at the highest tolerated dose achieved in the second step and followed for up to 11 days for signs of general health daily and body weight twice a week. If during the observation period there was no mortality, irreversible (terminal) morbidity, or weight loss in excess of 15% of the preinjection body weight, the administered dose was considered the acute single injection maximum tolerated dose.

Antitumor Efficacy Studies

BT474 tumor model. NCR *nu/nu* athymic female mice (4-6 weeks old; Taconic Farms, Germantown, NY) were s.c. implanted at the base of tail with 60-day sustained release 0.72-mg 17β-estradiol pellets (Innovative Research of America, Inc., Sarasota, FL). Two days later, 2×10^7 BT474 human breast cancer cells were implanted s.c. in the upper back area as a 0.1-mL suspension. Tumor growth was measured by caliper along the largest (length) and smallest (width) axes twice a week. Tumor volumes were calculated using the following formula (20): tumor volume = [(length) × (width)^2] / 2. At day 13 posttumor implantation (mean tumor volume, 200 mm^3), animals were randomized to three treatment groups of 13 to 15 animals per group and treated via i.v. (tail vein) injection as described in the text. The study was continued until day 60, which also represented the duration of estrogen supplementation. Animals were weighed twice weekly. If tumors reached 20% of the mouse body weight, the animals were euthanized.

HT29 tumor model. NCR *nu/nu* athymic male mice (6-week-old, weight >16 g; Charles River, Wilmington, MA) were injected s.c. in the right flank with 0.1-mL suspensions containing 5×10^6 HT-29 human colon cancer cells. Eleven days later (mean tumor volume, 150-350 mm^3), mice were randomized to six treatment groups of 11 animals per group. Starting on day 13, the animals received four tail vein injections at intervals of 4 days of various treatments as described in the text.

Results

Preparation of nanoliposomal CPT-11. A proposed novel process using a polyalkylammonium salt of a polymeric (polyphosphate) or nonpolymeric (sucrose octasulfate) highly charged multivalent anion as intraliposomal trapping agents resulted in improvement of both the encapsulation efficiency and the *in vivo* stability of the liposome-encapsulated weakly basic, amphipathic drug CPT-11. The process may involve the formation of an intraliposomal drug-polyanion complex (Fig. 1). Sucrose octasulfate is a high-charge density molecule with one strongly acidic, negatively charged sulfate group per 1.5 carbon atoms. The triethylammonium component of the salt assists drug loading as well, ensuring the charge neutrality of the liposome interior by allowing the efflux of cations accompanying the influx of the drug and possibly by formation of a self-perpetuating pH gradient to provide a driving force for progressive drug accumulation (10).

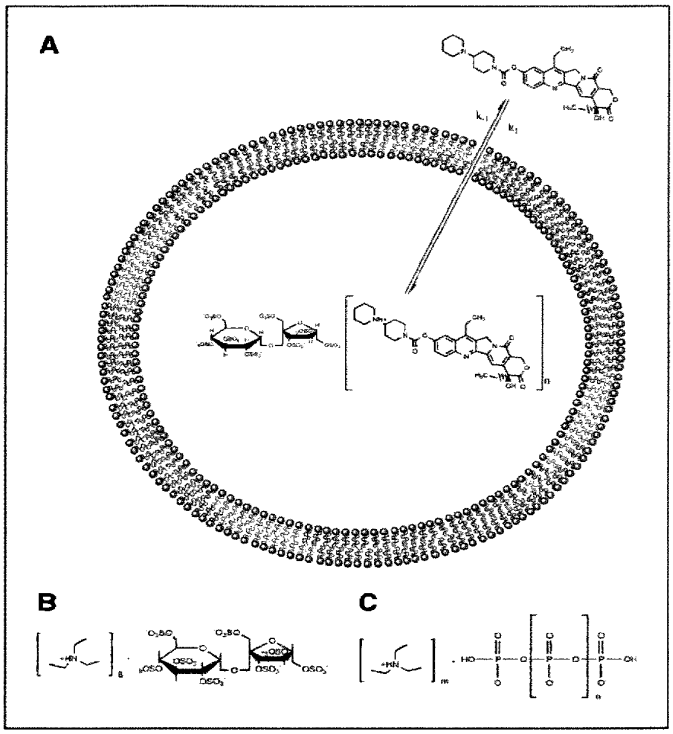

Figure 1. *A*, schematic depicting the intraliposomal stabilization strategy for CPT-11 using polyanionic trapping agents. The basic molecule CPT-11 forms a nanoscale complex with either poly(phosphate) or sucrose octasulfate in the liposome interior, thus stabilizing the liposomal formulation to increase drug retention while in circulation. Chemical structures of the triethylammonium salts of the polyanionic liposome trapping agents, poly(phosphate) (*B*) and sucrose octasulfate (*C*).

To minimize the treatment-associated lipid burden, encapsulation of CPT-11 was attempted up to drug-to-lipid ratios far exceeding the usual ratios achievable by traditional transmembrane-gradient drug loading techniques (Fig. 2). Remarkably, we found that CPT-11 encapsulation in liposomes was quantitative up to 800 g CPT-11/mol phospholipid. The final molar ratio of drug-to-phospholipid corresponds to 1.36:1 for liposomes loaded at 800 g CPT-11/mol phospholipid or 109,000 drug molecules per particle. This represents a 10- to 20-fold improvement over other liposomal formulations, including anthracyclines (3) or camptothecins lurtotecan (8) and SN-38 (21). We hypothesize that the high loading capacity of triethylammonium sucrose octasulfate liposomes is due to the formation of a stable complex between the drug and polyanion whereas the displaced triethylammonium ion dissociates and traverses the lipid bilayer as triethylamine, ensuring that the loading process continues until all added drug is encapsulated or the charge stoichiometry is achieved between the added drug and the liposomally encapsulated anion (Fig. 1).

Pharmacokinetics of nanoliposomal CPT-11. The pharmacokinetics of nanoliposomal CPT-11 formulated using either TEA-SOS or TEA-Pn were determined in normal female rats. Free CPT-11 was rapidly cleared from the circulation with $t_{1/2}$ = 0.27 hours (Fig. 3*A*). Liposome encapsulation was associated with significantly longer circulation times than free drug (Fig. 3*A* and *B*). This was especially true for liposomes loaded with TEA-SOS gradients, with blood half-lives for lipid and CPT-11 of 12.0 and 10.7 hours, respectively (Table 1).

Whereas both liposome constructs displayed long circulation for the lipid component, drug associated with TEA-SOS liposomes unexpectedly showed less rapid clearance from the blood than with TEA-Pn liposomes (Fig. 3*A* and *B*). This likely reflects that the $t_{1/2}$ of CPT-11 release from TEA-Pn liposomes was 14 hours, significantly shorter than that for TEA-SOS liposomes with a $t_{1/2}$ of CPT-11 release of 56.8 hours.

Drug stability of free and nanoliposomal CPT-11. *In vivo*, CPT-11 undergoes transformation to its more active metabolite, SN-38, and both molecules are also subject to inactivation by hydrolysis of the lactone forms to the respective carboxylate forms (Fig. 4*A* and *B*). Liposome encapsulation and delivery markedly altered these bioconversions in rats. Free CPT-11 was rapidly cleared from circulation, with only 2% of the injected dose remaining at 30 minutes and 35% of this present in the carboxylate form (Fig. 4*C*). In contrast, nanoliposomal CPT-11 showed both prolonged circulation, with 23.2% of injected dose still remaining at 24 hours, and drug protection, with no detectable conversion of CPT-11 to either SN-38 or the carboxylate form of CPT-11 (Fig. 4*D*). Thus, the high-charge density polyanionic nanoliposomal matrix provided a chaperone for the stably entrapped prodrug CPT-11, improving its pharmacokinetics and preventing its inactivation or premature conversion to the toxic metabolite SN-38.

Once deposited in tumors, liposomes are known to be taken up avidly by tumor-resident macrophages (22). To determine if macrophages could metabolically activate drug from nanoliposomal CPT-11, an *ex vivo* assay using macrophages isolated from the peritoneum of nude mice was done. Incubation of nanoliposomal CPT-11 with macrophages showed no detectable conversion to SN-38 at 24 hours but 100% conversion to SN-38 by 72 hours. This time course suggested that at least 24 hours was required for macrophage-mediated disruption of the liposome, drug release, and conversion to SN-38.

Acute toxicity of nanoliposomal CPT-11. The acute toxicity of free and nanoliposomal CPT-11 was determined in normal Swiss Webster mice using an NCI-based protocol. The maximum tolerated dose of free CPT-11 was 80 mg/kg whereas the maximum

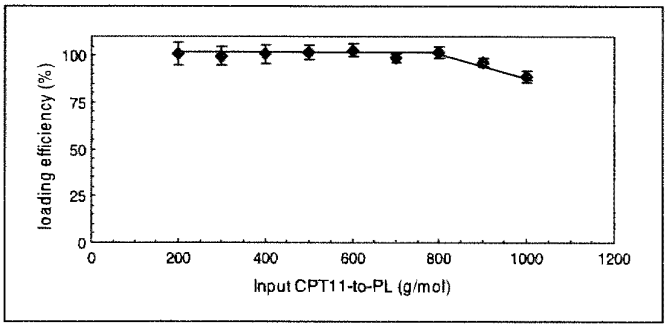

Figure 2. Liposomal loading efficiency as a function of input CPT-11-to-phospholipid (*PL*) ratio. Distearoylphosphatidylcholine/cholesterol/methoxypoly(ethylene)glycol (PEG2000)-derivatized distearoylphosphatidylethanolamine (3:2:0.015) liposomes were loaded with CPT-11 as described in Materials and Methods. The resulting CPT-11-to-phospholipid ratio following loading was determined by quantitating both CPT-11 and phospholipid in the resulting purified liposomal CPT-11 formulation, and the loading efficiency by comparing this ratio to the input ratio.

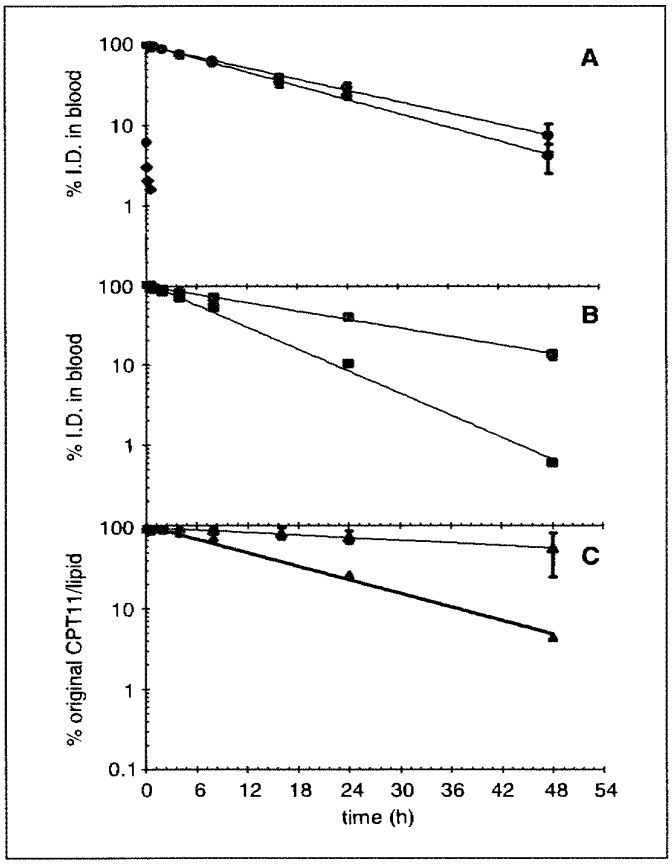

Figure 3. Pharmacokinetics of nanoliposomal CPT-11 in rats. Nanoliposomal CPT-11 prepared using either triethylammonium sucrose octasulfate (*A*) or poly(phosphate) (*B*) was administered i.v. in 9-week-old female Sprague-Dawley rats (body weight ~ 200 g) with indwelling central venous catheters at a dose of 10 mg CPT-11/kg (17.6 μmol phospholipid/kg). Free CPT-11 was administered i.v. as a bolus injection at 25 mg/kg (*A*, ♦). Plasma was sampled at the indicated times and analyzed for drug and liposomal lipid content. *Points*, % of injected dose (*% I.D.*) of lipid (○, □) or drug (●, ■, ♦). *C*, drug retention was calculated as percent of original drug associated with liposomal lipid at each time point for the poly(phosphate) (▲) and sucrose octasulfate (△) formulations.

tolerated dose of nanoliposomal CPT-11 formulated using a TEA-SOS gradient was not achieved even at the highest administered dose of 324 mg CPT-11/kg. A dose of >324 mg CPT-11/kg was impossible to administer because of concentration and injection volume limitations. Therefore, nanoliposomal CPT-11 delivery reduced drug toxicity in the mouse by at least 4-fold.

Efficacy of nanoliposomal CPT-11 in the BT474 breast cancer model. Treatment using nanoliposomal CPT-11, formulated using the TEA-Pn loading strategy, was evaluated in the BT474 breast tumor xenograft model (Fig. 5*A*). Free CPT-11 was clearly efficacious in this model with noticeable inhibition of tumor growth. However, treatment with nanoliposomal CPT-11 provided further advantage with dramatic regressions in tumor volumes and 100% cures of mice (defined as no residual tumor at study end).

Treatment-related toxicities were not observed. There was a slight decrease in mean body weight by 3.3% on the final treatment day in the animals receiving liposomal CPT-11; this decrease was not statistically significant compared with pretreatment weight (P = 0.274, Student's t test). All other weight measurements were within the expected range.

Efficacy of liposomal CPT-11 in the HT29 colon cancer model. In the HT29 colon tumor xenograft model, free CPT-11 again showed efficacy, albeit modest (Fig. 5*B*). However, both nanoliposomal CPT-11 formulations showed pronounced antitumor effects, including tumor regression during treatment followed by prolonged absence of tumor regrowth. Indeed, at 42 days postimplantation, all nanoliposomal CPT-11 treatments seemed to be equivalent and maximally efficacious.

With continued observation, tumor regrowth was observed beginning on day 47 postimplantation. At this point, all control and free CPT-11-treated mice had been sacrificed due to excessive tumor growth. Based on regrowth rates, treatment with TEA-SOS liposomes was more efficacious than TEA-Pn liposomes administered at the same CPT-11 dose. Furthermore, treatment with either liposome type at 50 mg/kg dose was more efficacious than at 25 mg/kg. In an analysis of cure rates, no mice receiving control or free CPT-11 were cured. Mice receiving TEA-Pn liposomal drug at 50 mg/kg per injection, despite initial tumor regressions, showed eventual regrowth. In the two groups receiving 25 mg/kg of either liposome formulation, one animal (9.1%) from each group was tumor-free at study end. In the group receiving 50 mg/kg of the TEA-SOS liposome formulation, 4 animals (36.4%) showed no regrowth and remained tumor-free.

Animals receiving free CPT-11, but not any of the nanoliposomal CPT-11 preparations, showed morbidity (loss of alertness, humped posture, ruffled fur, decreased mobility) for 1 hour after drug injection. Animals receiving free CPT-11 also lost 6% of weight

Table 1. Pharmacokinetic variables for free and nanoliposomal CPT-11 in rats

Formulation	$t_{1/2}$ (h)	AUC_∞ (μg h/mL)	CL (mL/h)	V_d (mL)	MRT (h)	$t_{1/2}$ CPT-11 release (h)
Free CPT-11	0.27	6.2	1,609	616.4	0.4	—
Ls-CPT-11 [TEA-Pn]	6.80	1,407.8	7.10	69.7	9.8	14.0
Ls-CPT-11 [TEA-SOS]	10.7	2,134.4	4.69	72.3	15.4	56.8

NOTE: The data used to calculate the pharmacokinetic variables for CPT-11 when formulated either in the free form or liposomal form refer to the actual drug concentrations measured in the blood that were then used to calculate the %ID values found in the corresponding curves for Fig. 3*B*.
Abbreviations: AUC_∞, area under the concentration versus time curve in plasma based on the sum of exponential terms; MRT, mean residence time calculated from exponential terms; CL, clearance calculated from exponential terms; V_d, volume of distribution.

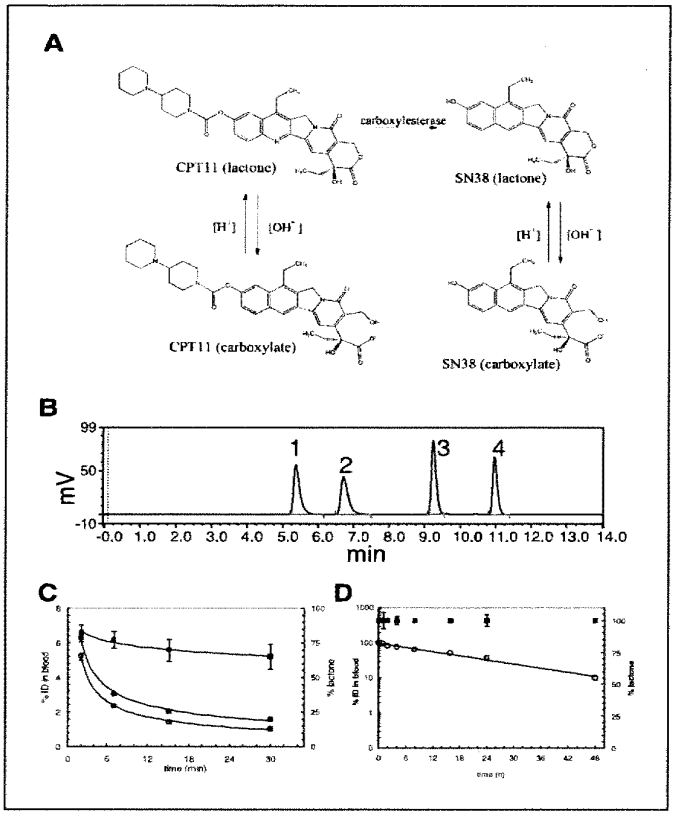

Figure 4. Drug stability of free and nanoliposomal CPT-11. *A*, CPT-11 and SN-38 exist in a pH-dependent equilibrium between closed lactone and open carboxylate configurations. CPT-11 is converted to its more active metabolite, SN-38, by carboxylesterases. *B*, HPLC chromatogram showing the separation of these species: CPT-11 carboxylate (*peak 1*), SN-38 carboxylate (*peak 2*), CPT-11 lactone (*peak 3*), and SN-38 lactone (*peak 4*). The *in vivo* drug stability of free (*C*) and nanoliposomal (*D*) CPT-11 was evaluated following single i.v. bolus administration at a dose of 25 mg CPT-11/kg in 9-week-old female Sprague-Dawley rats (body weight ~200 g). Levels of total CPT-11 (●) and CPT-11 lactone (○) in the blood were determined by HPLC analysis and expressed as percent of initial CPT-11 dose. *Right y axis*, percentage of CPT-11 in the lactone form is plotted as a function of time (■).

during treatment and did not recover, probably because of the effects of the growing tumor. Animals receiving nanoliposomal CPT-11 formulations experienced transient weight loss of 5% (at 25 mg/kg) or 9% (at 50 mg/kg) between the second and third injections as compared with pretreatment values; however, weights recovered following completion of treatment.

Discussion

Liposome delivery has been shown to improve the pharmacokinetic profile and widen the therapeutic index of certain anticancer drugs, especially the anthracycline class (1, 2). Improved efficacy is in part a result of passive targeting to tumor sites based on the enhanced permeability and retention (EPR) effect (23). To fully exploit this process, drug carriers must be engineered to retain drug while circulating, thereby preventing premature drug release before accumulating in the tumor but still allowing for release of drug once in the vicinity of the tumor. Antibody-targeted nanoparticles, such as immunoliposomes against HER2 (24) or epidermal growth factor receptor (25), represent another strategy for more efficient drug delivery to tumor cells.

Gradient-based drug loading technologies, in which electrochemical gradients drive the accumulation of drugs in the liposome interior, represent a key advance in liposome research (11, 26). This approach was further refined when transmembrane gradients of ammonium ion were proposed to form a self-sustaining pH-gradient that can load drugs inside liposomes (10). However, weakly basic anthracyclines represented the only drug class that afforded slow *in vivo* release rates when loaded using gradients involving common anionic counterions, such as sulfate or citrate. With other drug classes, gradient-based loading has been achieved with variable efficiency. To stabilize other cationic drugs against premature escape from liposomes, the use of pre-entrapped polyanionic polymers was proposed (9, 27).

In the present study, we used a drug loading transmembrane gradient system with two components, a substituted ammonium and a poly(anionic) trapping agent of either polymeric (polyphosphate) or nonpolymeric (sucrose octasulfate) nature. The use of polymeric polyanions such as heparin or dextran sulfate to improve liposomal drug retention has been reported (9, 27). Polyphosphate was effective in stabilizing intraliposomal CPT-11

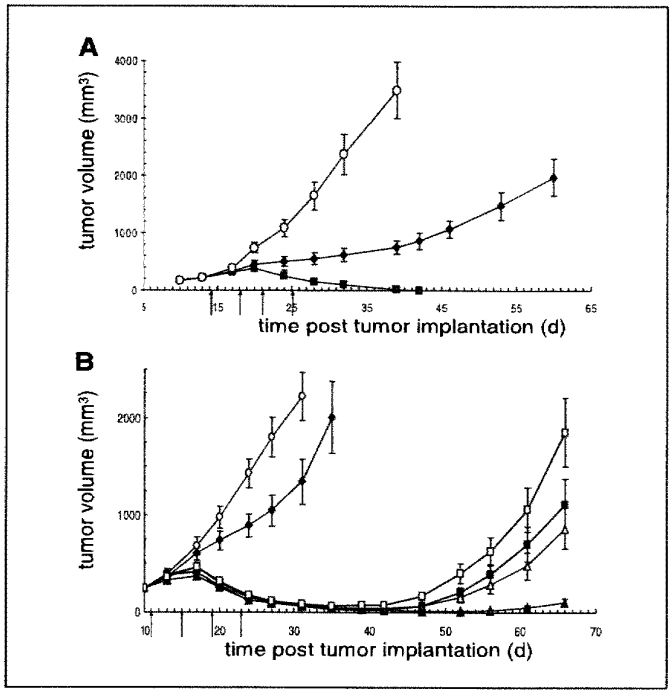

Figure 5. Antitumor efficacy of nanoliposomal CPT-11 in tumor xenograft models. *A*, BT474 breast cancer cells were implanted s.c. in nude mice along with estrogen pellets. When tumors were well established and had reached mean volumes of 200 mm^3, the following treatments were initiated: control (○), drug- and liposome-free vehicle only; free CPT-11 (◆); or nanoliposomal CPT-11 stabilized with TEA-Pn (■). Free and nanoliposomal CPT-11 were injected at 50 mg CPT-11/kg/dose i.v. twice per week for four doses (*arrows*). *B*, HT-29 colon cancer cells were implanted s.c. in nude mice. When tumors were well established and had reached mean volumes of 150 to 350 mm^3, the following treatments were administered: control (saline; ○); free CPT-11, 50 mg/kg/dose (◆); nanoliposomal CPT-11 using TEA-Pn, 25 mg/kg/dose (□); nanoliposomal CPT-11 using TEA-Pn, 50 mg/kg/dose (■); nanoliposomal CPT-11 using TEA-SOS, 25 mg/kg/dose (△); and nanoliposomal CPT-11 using TEA-SOS, 50 mg/kg/dose (▲).

against *in vivo* release, having the added advantage of being more readily biodegradable than dextran sulfate. However, polyanionic polymers such as heparin and dextran sulfate have notable anticoagulant activity and, in the case of dextran sulfate, toxic to Kupffer cells (28). The undefined chemical nature of many functionalized polymers may also contribute to variability in *in vivo* properties. Unexpectedly, we observed that a highly charged, nonpolymeric anion, such as sucrose octasulfate, provided even better drug retention than a polyanionic polymer, resulting in outstanding *in vivo* drug encapsulation stability. Sucrose octasulfate is a product of exhaustive esterification of sucrose, using chlorosulfonic acid or sulfur trioxide in pyridine or methylpyridine, and is a known pharmaceutical ingredient, the basic aluminum salt (Sucralfate) of which is widely used to treat gastric hyperacidity (29). Compared with dextran sulfate, sucrose octasulfate is chemically well defined; it does not have known anticoagulant or antimacrophage activity (29) and its salts can be produced in pure crystalline form ensuring less interlot variability.

The concept of nanoparticle delivery of camptothecins is very attractive based on potential advantages, including overcoming the solubility limitations of this class, protecting drug in the active lactone configuration, rerouting of drug from sites of toxicity such as the gastrointestinal tract, prolonging circulation time, increasing tumor accumulation via the EPR effect, and providing sustained release for a so-called metronomic effect. Using a novel intraliposomal stabilization technology, we have developed a nanoliposomal CPT-11 featuring drug loading efficiency and drug payload ($>10^5$ per particle) in far excess of that previously reported for this type of encapsulation; this agent showed marked *in vivo* retention of CPT-11 during long circulation times while simultaneously protecting the drug from lactone hydrolysis or premature activation. Compared with free CPT-11, this liposome-based nanoparticle reduced host toxicity in rats by >4-fold and greatly increased antitumor efficacy in animal models. In a separate study, we showed similar improvements in efficacy and host toxicity when nanoliposomal CPT-11 was administered locally to brain tumors using convection-enhanced delivery (30).

Previously reported liposomal camptothecin preparations have shown increased efficacy but not necessarily improved toxicity when compared with free drug (8, 9, 31). Other examples have shown prolonged circulation (32, 33), but not to the extent observed for the TEA-SOS-stabilized liposomes described here. In addition, a liposomal version of SN-38 is cleared even more rapidly with an AUC_∞ that seems to be at least 2 orders of magnitude less than that observed for nanoliposomal CPT-11 (34).

Another aspect of nanoliposomal CPT-11 is that it delivers a prodrug. Cytotoxic drugs encapsulated in liposomes are normally unable to act on their therapeutic targets or cause toxicity until they can be released from the confines of the carrier, and thus liposomal drug delivery can itself be regarded as a prodrug strategy. Hence, in this dual prodrug strategy, liposome delivery of CPT-11 chaperones the camptothecin until it reaches tumor sites where the prodrug can then be activated locally. Although local activation of CPT-11 to SN-38 has yet to be shown, carboxylesterases have a widespread distribution in different tumor types (35–37) and are active in macrophages, the principal scavenger of liposomes. Indeed, we observed that nanoliposomal CPT-11 was completely converted to SN-38 by macrophages after 72-hour incubation. We hypothesize that nanoliposomal CPT-11 may be acted on by tumor-resident macrophages, which convert drug to SN-38 with subsequent diffusion to nearby tumor cells. Alternatively, CPT-11 may be activated directly by tumor cells following release from its liposome carrier.

We conclude that nanoliposomal CPT-11 generated by novel intraliposomal drug stabilization resulted in advantageous pharmacologic properties with increased efficacy and reduced host toxicity *in vivo*. The drug-loading and stabilization technologies used for CPT-11 may also be broadly applicable to other weakly basic anticancer drugs as we have recently shown using a novel histone deacetylase inhibitor, LAQ824 (38). Nanoliposomal CPT-11 may provide a robust and useful nanoparticle-based treatment for cancer.

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Development of a Highly Active Nanoliposomal Irinotecan Using a Novel Intraliposomal Stabilization Strategy

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Irinophore C, a Novel Nanoformulation of Irinotecan, Alters Tumor Vascular Function and Enhances the Distribution of 5-Fluorouracil and Doxorubicin

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Abstract

Purpose: To examine the antitumor effects of Irinophore C, a nanopharmaceutical formulation of irinotecan, on the tissue morphology and function of tumor vasculature in HT-29 human colorectal tumors.

Experimental Design: Fluorescence microscopy was used to map and quantify changes in tissue density, tumor vasculature, hypoxia, and the distribution of Hoechst 33342, a perfusion marker, and the anticancer drug, doxorubicin. Noninvasive magnetic resonance imaging was used to quantify K_{trans}, the volume transfer constant of a solute between the blood vessels and extracellular tissue compartment of the tumor, as a measure of vascular function. Following treatment with Irinophore C, ^{19}F magnetic resonance spectroscopy was used to monitor the delivery of 5-fluorouracil (5-FU) to the tumor tissue, whereas scintigraphy was used to quantify the presence of bound [^{14}C]5-FU.

Results: Irinophore C decreased cell density ($P = 8.42 \times 10^{-5}$), the overall number of endothelial cells in the entire section ($P = 0.014$), tumor hypoxia ($P = 5.32 \times 10^{-9}$), and K_{trans} ($P = 0.050$). However, treatment increased the ratio of endothelial cells to cell density ($P = 0.00024$) and the accumulation of Hoechst 33342 ($P = 0.022$), doxorubicin ($P = 0.243 \times 10^{-5}$), and 5-FU ($P = 0.0002$) in the tumor. Vascular endothelial growth factor and interleukin-8, two proangiogenic factors, were down-regulated, whereas the antiangiogenic factor TIMP-1 was up-regulated in Irinophore C–treated tumors.

Conclusions: Irinophore C treatment improves the vascular function of the tumor, thereby reducing tumor hypoxia and increasing the delivery and accumulation of a second drug. Reducing hypoxia would enhance radiotherapy, whereas improving delivery of a second drug to the tumor should result in higher cell kill.

The clinical management of metastatic disease originating from colon/colorectal cancer remains challenging. The liver is the most common site of distant metastases for colorectal cancer, with 70% of patients presenting with liver metastases followed by the lungs, bone, and brain (1, 2). At present, the only cure is complete surgical removal of the primary tumor if diagnosed early; however, up to 45% of these patients still relapse with metastatic disease. Standard of care for first-line therapy in patients is a combination of 5-fluorouracil (5-FU; plus leucovorin) with either irinotecan (FOLFIRI) or oxaliplatin (FOLFOX; ref. 3). The treatments are associated with prolonged median survivals of 18 to 21 months. Capecitabine, an oral fluoropyrimidine carbamate, has also been used in combination with 5-FU, and clinical data suggest that this combination is comparable with the FOLFIRI and FOLFOX regimens (4, 5). In practice, however, combination therapy with capecitabine is limited because of severe toxicities such as hand-foot syndrome, diarrhea, nausea, vomiting, and bone marrow suppression (4, 5). More recently, monoclonal antibodies targeting the epidermal growth factor receptor, such as cetuximab and panitumumab, have been used in combination with standard chemotherapy with promising results (6). The safety and efficacy of bevacizumab, the monoclonal antibody that targets vascular endothelial growth factor (VEGF; ref. 7), in combination with FOLFIRI or FOLFOX, was also evaluated recently (8, 9). Although both studies were carried out with small

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Translational Relevance

Median survival times of 18 to 21 months are associated with irinotecan-based treatments for metastatic colorectal cancer. We report here a novel formulation of irinotecan, Irinophore C, which improves oxygenation levels and the delivery and accumulation of a second drug in a tumor model for colorectal cancer. Increasing oxygen levels would potentiate radiotherapy, and improving delivery of a second drug should increase cell kill. If human tumors respond similarly to Irinophore C, replacing irinotecan with Irinophore C in current combination therapies could improve the delivery of the partner agents (oxaliplatin, 5-FU, or leucovorin). Imaging techniques (magnetic resonance imaging and positron emission tomography) could be used to monitor changes in the tumor and help delineate the use of sequential therapies to target specific treatment induced changes in the tumor microenvironment (e.g., radiotherapy when oxygen levels improve). Integrating Irinophore C into clinical use should be relatively easy because irinotecan is clinically approved for colorectal cancer.

patient numbers, Kang et al. concluded that the combination treatment had modest activity and was relatively tolerable in metastatic colorectal cancer that had failed FOLFIRI or FOLFOX. A retrospective study where bevacizumab and FOLFOX were used in first-line treatment of metastatic colorectal cancer (8) further indicated that the treatment improved time to disease progression and overall survival. The authors concluded that this combination should be explored further. Finally, there is interest in using radiotherapy concurrently with chemotherapy in an adjuvant setting (10, 11).

Of particular interest to our group, the positive results with the antiangiogenic activity of bevacizumab indicate that the vasculature of colorectal tumors is an important therapeutic target in metastatic colorectal cancer. The clinical utility of combination therapies underlines the fact that novel approaches targeting more than one of the hallmarks of aggressive cancer should be explored more fully. More specifically, there is interest in targeting tumor vasculature and cancer cells in combination. Thus, for example, vascular disrupting agents have been used as single agents to shut down blood vessels in tumors and also in combination with chemotherapy (12, 13). The utility of antiangiogenic agents in clinical trials as single agents and in combination with chemoradiation is also being examined (14, 15).

Although newer treatments have improved the outcome of metastatic colorectal cancer from 6 months without treatment to 2 years with treatment, we believe that further improvements in survival time are achievable using innovative therapeutics and carefully designed treatment scheduling. An attractive proposition is to devise a sequence of therapies designed to take advantage of treatment-induced changes in the tumor microenvironment that can be readily measured using noninvasive imaging methods. We recently reported on the improved efficacy of a novel lipid-based formulation of irinotecan (Irinophore C) where treatment outcomes in 5 xenograft models, including the HT-29 and LS-180 models for colorectal cancer, were significantly improved (16, 17). In brief, the difference in pharmacokinetic profiles between free Irinotecan (Camptosar) and Irinophore C is best emphasized by the 1,000-fold increase in the plasma area-under-the-curve of the lactone form of irinotecan, the active form of the drug, seen with Irinophore C compared with that of Camptosar. Irinotecan is also metabolized by carboxylesterase to yield SN-38, which is 100- to 1,000-fold more potent *in vitro* than the parent drug. Like irinotecan, SN-38 also exists in equilibrium as the lactone and carboxylate forms, and we have also shown that both forms of SN-38 were detected in the plasma 5 min after injection of Camptosar. In contrast, only the lactone form was detected in the plasma following Irinophore C administration. The plasma levels of SN-38 lactone peaked at 2 μg/mL within 1 h, decreased to 1 μg/mL after 4 h, and remained constant thereafter over 24 h; however, the plasma concentrations of SN-38 at 24 h were still greater than those observed for Camptosar 10 min after administration.

To gain a better understanding of the mechanism behind the antitumor activity of Irinophore C, we examined the treatment-induced effects on the tumor microenvironment in the HT-29 colorectal cancer model. Our interest in assessing the tumor microenvironment was driven in part by publications suggesting that irinotecan inhibits angiogenesis (18) and also by the potential for systemically administered nanopharmaceuticals to achieve antivascular effects comparable with those achieved with metronomic dosing (19). The studies described here used multimodality imaging methods to assess tumor-associated vascular structure and function. These include noninvasive magnetic resonance imaging/spectroscopy and tumor mapping of biological markers in tumor sections. Fluorescence microscopy and tumor mapping techniques were used to quantify the observed decreases in tumor blood vessel content, hypoxia, and viable cell density. Treatment with Irinophore C increased the accumulation of a perfusion marker (Hoechst 33342). Magnetic resonance imaging indicated that vascular function improved following treatment with Irinophore C. The vascular changes were further associated with enhanced delivery of doxorubicin and 5-FU determined with fluorescence microscopy and ^{19}F magnetic resonance spectroscopy (MRS), respectively.

Materials and Methods

Tumor model. Animal studies were approved by the University of British Columbia Animal Care Committee and conducted in accordance with guidelines from the Canadian Council for Animal Care. The HT-29 tumor model for colon cancer was used in these studies; 5×10^6 cells (50 μL medium) were injected subcutaneously into the lower backs of female Rag2M mice. Tumors appeared within 2 weeks following cell inoculation and mice were randomly separated into 7 groups at this time (6 mice per group, unless indicated otherwise). Treatments were initiated when tumors reached an average volume of ~150 mm^3 (~0.5 cm in diameter). Tumor volume was calculated as volume = 0.5 length (cm) × width $(cm)^2$. Dimensions were measured using calipers by the same technician throughout the duration of the studies.

Treatment groups and drug/marker administration. Procedures for encapsulating irinotecan in liposomes have been described previously (16). In brief, irinotecan was encapsulated in 1,2-distearoyl-*sn*-glycerophosphocholine and cholesterol distearoyl phosphatidylcholine/cholestrol at a molar ratio of 55:45. The distearoyl phosphatidylcholine/

cholestrol/cholesterol lipid films formed were hydrated at 65°C in a solution of 300 mmol/L copper sulfate solution and subjected to 5 cycles of freeze-and-thaw. The multilamellar vesicle suspensions were extruded 10 times through polycarbonate filters with defined pore size to obtain unilamellar vesicles with a mean size distribution of 100 to 140 nm. The external buffer of the liposomes was then exchanged using Sephadex G-50 size exclusion chromatography, with HEPES-buffered solution containing EDTA (pH 7.5). The liposomes were then preincubated in the presence of the divalent metal ionophore A23187 at 60°C for 30 min. Subsequently, irinotecan hydrochloride trihydrate was added to liposomes at 50°C in a drug-to-lipid ratio of 0.2:1 (mol/mol); under these conditions, >98% of the added drug associates with the liposomes. These drug-loaded liposomes are then exchanged into a non-EDTA-containing buffer and adjusted to an irinotecan concentration suitable for administration to mice. It should be noted that a scaled version of this formulation process has been developed for batch sizes of 1 L; stability studies to date suggest that the drug-loaded preparation is stable for >6 months at 4°C. The formulation was administered (25 mg/kg, active drug ingredient) to one group once a week for 3 weeks and the tumors allowed to regrow after treatment was stopped. The remaining 6 groups of mice received either saline or Irinophore C once a week for 6 weeks with a 10-day break after week 3 of the treatment regimen. The tumors in one group (and saline control group) were used to assess tumor growth delay, K_{trans} values, and levels of hypoxia. The hypoxia marker EF5 (30 mg/kg; ref. 20) and the fluorescent dye Hoechst 33342 (16 mg/kg; Sigma) were injected intravenously into mice 180 and 20 min before sacrifice, respectively. Tumors were harvested and portions were cryopreserved in tissue preservative (OCT) or flash-frozen in liquid nitrogen for subsequent molecular analysis. The remaining two groups (and controls) were used to assess the tumor accumulation of 5-FU and doxorubicin (Sigma-Aldrich). A single bolus injection of 5-FU [200 mg/kg, labeled with [^{14}C]5-FU (0.6 μCi/mL)] was administered intravenously via a catheter placed into the animal's lateral tail vein for ^{19}F MRS scans. One hour after scans were completed, the mice were euthanized and the tumors were harvested for scintillation counting. Doxorubicin (30 mg/kg) was injected intravenously 40 min before harvesting tumors that were cryopreserved (OCT) in liquid N_2 vapor. A portion of tumors from the group used to examine the distribution of 5-FU with MRS was also fixed in formalin and embedded in paraffin blocks.

Immunohistochemistry. Tumor cryosections (10 or 20 μm, as indicated) were cut using a Cryostar HM560 (Microm International), air-dried, and imaged for exogenous marker native fluorescence (Hoechst 33342 and doxorubicin, visualized at 365 and 546 nm, respectively). Sections were fixed in 50% (v/v) acetone/methanol for 10 min at room temperature and endothelial cells were stained using an antibody to PECAM/CD31 (BD Pharmingen) and fluorescent Alexa 647 secondary antibody (Invitrogen). Reduced EF5 adducts in viable hypoxic cells were stained using the monoclonal antibody specific for EF5 adducts, ELK3-51 (21), followed by a fluorescent Alexa 488 secondary. Evaluation of cell density was carried out by imaging sections following their immersion in Hoechst 33342 (8 μg/mL at 37°C) for 30 min.

Analysis of hypoxia using flow cytometry. The methods used for analyzing the hypoxic fraction of cells present in solid tumors were reported previously (22, 23). Harvested tumors were rinsed in HBSS (Stem Cell Technologies) containing 0.4% bovine serum albumin. A portion of the tumor (~25-30%) was chopped with scalpels in ice-cold HBSS-0.4% bovine serum albumin and transferred to 14 mL tubes with disaggregation mixture [HBSS-1% bovine serum albumin fraction V (Calbiochem) collagenase type 2 and 4, final concentration 250 units/mL (Worthington Biochemical)] and rotated at 37°C for 2 h. The resulting suspensions were placed in 50 μm Medicones and run (3×, 1 min each) through a Medimachine (BD Biosciences) with extensive PBS-0.1% bovine serum albumin (PBSB) washes after each run. Collected cells were washed once with PBSB. Pellets were resuspended in 2.5 mmol/L EDTA and incubated at 37°C for 5 min to reduce cell clumping. Lastly, cells were washed, resuspended in PBSB, and stored on ice for subsequent processing. Tumor cell suspensions were diluted in 0.1% trypan blue in PBS and cells were counted using a hemocytometer. Fragments of cells, erythrocytes, and cells with 2× erythrocyte volume or smaller were excluded. All flow cytometric analyses were done using the FACSCalibur (Becton Dickinson). Hypoxia was measured by detecting EF5 adducts using the ELK3-51-Cy5 antibody developed and generously provided by Dr. C.J. Koch et al. (University of Pennsylvania). Tumor cells were fixed with 2% FA, permeabilized with 1% Tween 20, and blocked overnight to reduce nonspecific binding. ELK3-51-Cy5 antibody was titrated, and the concentration with the best signal-to-noise ratio was chosen for staining. Fixed cells (5×10^6) in 300 μL were incubated with ELK3-51-Cy5 antibody (room temperature for 3 h on a rotator) and washed three times with PBSB-0.5% Tween 20, with the third wash rotating for 1 h. Finally, cells were washed and resuspended in PBSB with 1 μg/mL Sytox Green (DNA dye; Molecular Probes) and analyzed the same day on a flow cytometer. Controls for this assay consisted of cells cultured *in vitro* for 3 h with or without 200 μmol/L EF5 in normoxic (air) or hypoxic (0.005% O_2 + 99.995% N_2) conditions. Mouse cells were differentiated from tumor cells based on DNA content compared.

Image acquisition and analysis. The imaging system consists of a robotic fluorescence microscope (Zeiss Imager Z1), a cooled, monochrome CCD camera (Retiga 4000R, QImaging), a motorized slide loader and x-y stage (Ludl Electronic Products), and customized NIH-ImageJ software.[7] The system allows adjacent microscope fields of view to be photographed and automatically tiled to produce a montage of the entire tumor cryosections at a resolution of 0.75 μm/pixel for qualitative and quantitative analysis. All variables stained on the same section were imaged separately using the monochrome camera and subsequently overlaid and aligned to generate false-color images using Adobe Photoshop (CS) or for quantitative analysis examining the spatial relationships between two and three factors of interest. NIH software applications and user-supplied algorithms were used to quantify the degree of staining on images by measuring the percentage of pixels above a threshold, determined to be a minimum of SDs above background, for the markers CD31 (>7 SDs above background) and EF5 (>7 SDs above background), and intravenously administered perfusion marker Hoechst 33342 (>18 SDs above background). Accumulation of doxorubicin was quantified by determining the average intensity of doxorubicin native fluorescence for pixels located within the tumor margins, and the data were normalized to the average intensity of flooded Hoechst 33342 staining as a control for cell density. Background autofluorescence was determined similarly using tumors untreated with doxorubicin, and this average value was subtracted from doxorubicin-treated tumors. A Leica DLM-100 microscope with a RGB filter was used to image formalin-fixed, H&E-stained sections.

Magnetic resonance imaging and spectroscopy. All magnetic resonance experiments were carried out using a 7.0 Tesla MR scanner (Bruker). Signal transmission and reception was achieved with a three-turn solenoidal radiofrequency coil (1.7 cm inner diameter) with the tumor situated in its interior. This coil was tuned to the hydrogen proton frequency (300.3 MHz) for the K_{trans} measurements and to the ^{19}F frequency (282.58 MHz) for the 5-FU measurements. The K_{trans} values were obtained from serial images acquired to monitor changes in the concentration of a MR-visible contrast agent (Gd-DTPA) within each pixel during the initial uptake and subsequent washout of the agent in the tumor. The magnetic resonance imaging scans follow the protocol reported by Lyng et al. (24); briefly, mice were anesthetized with isofluorane (5% induction, 2% maintenance), a catheter was inserted into the lateral tail vein, and the tumor was placed in the solenoid coil. A proton-density weighted scan was first acquired to

[7] Public domain program developed at the NIH (http://rsb.info.nih.gov/nih-image) running on a G5 Macintosh computer (Apple).

serve as a baseline for conversion of pixel intensity to absolute concentration values of the contrast agent. A volume equivalent to 10 μL/g body weight of the contrast agent (0.3 mmol/kg Gd-DTPA in saline) was injected via the tail vein catheter for 15 to 25 s. Starting at the time of injection, a series of 41 consecutive T_1-weighted scans was acquired with each scan lasting 64 s (spin echo MSME, TR/TE = 11.9/500 ms, field of view = 4 cm, matrix = 128 × 128, slice thickness = 1.5 mm, number of slices = 12). The concentration-time curve for each pixel was fit to a two-compartment Kety model (25), which describes the pharmacokinetics of the contrast agent using two variables: v_e (volume of extracellular extravascular space) and K_{trans} (volume transfer constant between the vasculature and tissue compartment). To assess the relative amounts of 5-FU present within treated and untreated tumors in a second group of mice, 0.05 mL of 0.12 mol/L trifluoroacetic acid in a small glass sphere, placed at the bottom of the solenoid, was used as a fluorine reference. Animals were immobilized with ketamine and acepromazine (175 and 6 mg/kg, respectively). 5-FU (200 mg/kg) was injected intravenously 1 min before the start of spectroscopy measurements. Nonlocalized fluorine spectra were acquired with a bandwidth of 50 kHz, 8192 digitizer points, repetition time of 1 s, and 300 averages, leading to a time resolution of 5 min. The evolving 5-FU peak was observed at 94.1 ppm down-frequency from the trifluoroacetic acid peak. The ratio of integrals between the 5-FU peak and the trifluoroacetic acid peak was calculated to estimate the relative amount of 5-FU present in the tumor at a particular time. Scans were repeated up to a maximum of 2 h. One hour after ^{19}F MRS scans, animals were sacrificed and the tumors were harvested. The tissue was weighed and digested in 500 μL Solvable at 50°C overnight (Sigma) before the addition of EDTA (200 mmol/L, 50 μL), H_2O_2 (30%, 200 μL), and HCl (10 N, 25 μL). The digested tissue was then added to 5 mL scintillation fluid and placed in a scintillation counter (Packard Tri-carb LS 1900 TR) to evaluate the presence of bound [^{14}C]5-FU present per gram tumor tissue.

Expression levels of VEGF-A, VEGF-C, TIMP-1, and interleukin-8. Tumors were homogenized in lysis buffer (150 mmol/L NaCl, 1% NP-40, 0.5% sodium deoxycholate, 2.5 mmol/L EDTA, 0.1% SDS, Mini protease inhibitor cocktail tablets from Roche Diagnostics) using a Polytron homogenizer (Kinematica) and stored at -80°C. Protein determination was done in triplicate using a Micro BCA protein assay (Pierce). Portions of the tumor lysates were pooled and used with the TransSignal Angiogenesis Antibody Array (Panomics) to assay relative changes in proangiogenic and antiangiogenic factors according to the manufacturer's instructions. For Western blots, ~60 μg total protein was loaded and weight separated on a NuPAGE 4% to 15% Bis-Tris gel (Invitrogen). Protein was transferred to 0.45 μm nitrocellulose membranes (Invitrogen) and blocked with 5% skim milk powder in TBST [150 mmol/L NaCl, 50 mmol/L Tris, 0.1% Tween 20 (pH 7.5)] for 2 h. The membranes were then probed for VEGF-A (Santa Cruz Biotechnology), VEGF-C (Zymed), and TIMP-1 (Chemicon) expression using rabbit IgG antibodies diluted in TBST with 5% bovine serum albumin (Sigma-Aldrich) at concentrations of 1:5,000, 1:5,000, and 1:1,000, respectively. Membranes were incubated with primary antibodies overnight at 4°C with gentle shaking. Following incubation, the membrane was washed in TBST (3 × 10 min). The corresponding anti-rabbit horseradish peroxidase-conjugated secondary antibody (Promega) was applied at a 1:5,000 dilution in TBST and 3% skim milk for 1 h. The membrane was washed again in TBST (3 × 10 min) and covered with enhanced chemiluminescent solution (Amersham Biosciences). After 1 min, excess enhanced chemiluminescent solution was poured off, and the membrane was sandwiched in a transparent sheet protector. Bands were then visualized using autoradiography film (Biomax MR Film; Kodak) in a safe-light darkroom. Tumor lysates were analyzed for levels of interleukin-8 (IL-8) using a human CXCL8/IL-8 ELISA (Quantikine; R&D Systems). All samples consisted of 160 μg total protein and were done in duplicate according to the manufacturer's protocol. The standard curve was generated using a four-variable logistic curve fit.

Statistics. Statistical analyses were done with Statistica software. One-way ANOVA was used to calculate P values. Differences were considered significant at $P \leq 0.05$.

Results

Irinophore C treatment inhibits growth and reduces the cell density of HT-29 tumors. All data presented were derived from tumors harvested from mice treated for 6 weeks. The inhibitory effect of Irinophore C on subcutaneous HT-29 tumors is shown in Fig. 1A. As outlined in Materials and Methods, mice were randomly assigned to 7 different groups when tumors were ~150 to 200 mm^3. Mice in the control group were treated with saline (□), and mice in the remaining groups were treated with Irinophore C for 3 weeks (●) or for 6 weeks with a 12-day break after week 3 (▣). Treatment with Irinophore C inhibited tumor growth but did not cause tumor regression at this dose. When treatment was suspended, the tumors started to grow again within 5 to 8 days (●). If treatment was started again (▣), further stabilization of the tumor was observed. Mice in the 6-week treatment group were sacrificed following imaging procedures to determine K_{trans}, and the tumors were collected for analysis as specified in Materials and Methods.

Tumor cell density was assessed by staining cell nuclei with Hoechst 33342. Image analysis of these sections indicated that the average intensity of Hoechst 33342 staining, summarized in Fig. 1B, was significantly lower for those tumors treated with 6 weeks of Irinophore C (▣) (~35% lower; $P = 8.42 \times 10^{-5}$) compared with untreated tumors (□), reflecting a lower density of nuclei or cell density. H&E-stained tumor sections obtained from animals treated with saline or Irinophore C are shown in Fig. 1C. The tissue structure in untreated tumors is composed of densely packed cancer cells whose nuclei are stained dark purple and are permeated with river-like bands of stroma (pink). However, after treatment with Irinophore C, the density of the cancer cells is much lower and the tumor cells appear to be grouped in small islands amidst what appears to be fatty tissue, as determined by an experienced pathologist. Tumor cell nuclei from treated tumors are larger and lack prominent nucleoli, which are easily detected in tumors from saline-treated animals. Necrosis was not widespread or confluent and confined to single cells.

Irinophore C treatment is associated with a decrease in K_{trans}. Noninvasive magnetic resonance imaging was used to assess K_{trans}, the volume transfer constant of a solute between the blood vessels and extracellular tissue compartment of the tumor. The median viable values of K_{trans} for the tumors within the control and treated groups are graphed individually in Fig. 2A and show the relative spread of K_{trans} values between the control group (□) and the Irinophore C-treated group (▣). The average values for K_{trans} in untreated tumors was ~1.5 times greater than in treated tumors (0.0375 and 0.025 mL/g/min for untreated and treated tumors, respectively; $P = 0.050$). The values for K_{trans} in untreated tumors were more variable compared with the treated group (SD = ±0.01 and ±0.001, respectively).

Irinophore C treatment is associated with increases in Hoechst dye perfusion and in the CD31$^+$ cells to tumor cell ratio. Hoechst 33342 dye was injected intravenously into the lateral tail vein

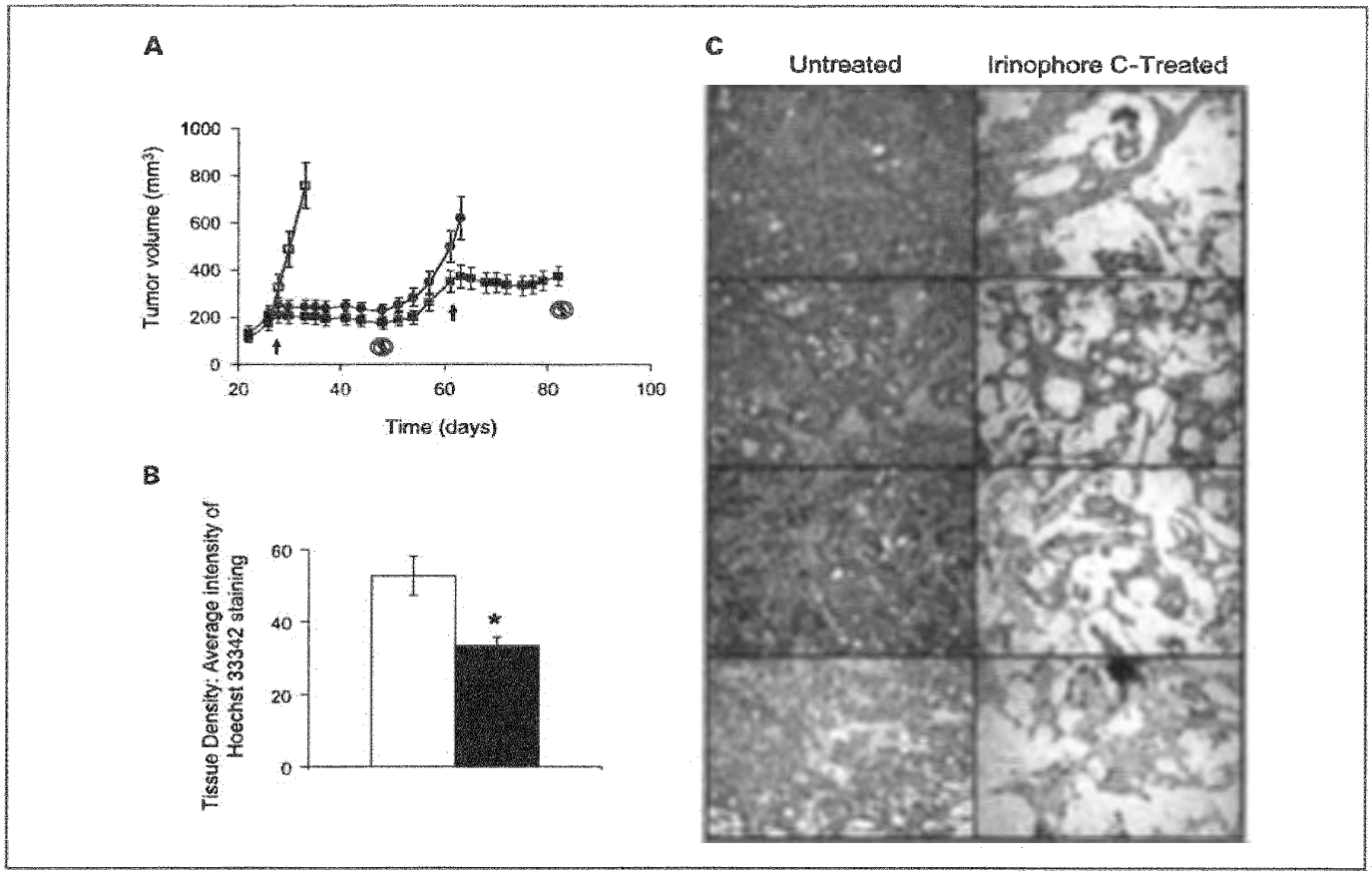

Fig. 1. *A* to *C*, Irinophore C treatment has a cytostatic effect on HT-29 tumors and reduces the cell density of the tumor. *A*, inhibitory effect of Irinophore C on HT-29 tumors grown subcutaneously in Rag2M mice. *Arrows*, start and end of treatment. Tumors in mice treated with saline reached predetermined endpoints within 2 wk and were harvested immediately (□). Tumor growth in mice treated with Irinophore C for 3 wk (●) or 6 wk with a 10- to 12-day break after week 3 (■) was inhibited, but the tumor was not eradicated. When treatment was suspended, the tumors started growing again (●); however, when treatment recommenced, growth was inhibited again (■). *B*, cell density of tumors treated with 6 wk of Irinophore C (■) was significantly reduced compared with untreated tumors (□). Cryosections of the tumors were flooded with Hoechst 33342 to stain cell nuclei as a measure of cell density. The average intensity of Hoechst 33342 staining shows that the cell density in treated tumors is reduced by ~35% ($P = 8.42 \times 10^{-5}$). *C*, images of H&E-stained tumor sections (magnification, ×20) from 4 sets of control and treated tumors show representative fields of view, clearly illustrating that the effect of Irinophore C is consistent in dramatically changing the tissue morphology and reducing the cell density.

of animals 20 min before tumor harvest to assess perfusion within the tumor. Significantly higher amounts of the fluorescent DNA-binding dye were found in tumors treated with Irinophore C. Figure 2B shows representative false-color composite images of cryosections from HT-29 tumors; Irinophore C-treated tumors contain greater quantities of dye labeling (blue) compared with controls. The proportion of pixels per unit area with staining intensity greater than threshold for Hoechst 33342 were quantified, and the data show that there is ~2.8-fold more Hoechst 33342 labeling in tumors from Irinophore C-treated animals (Fig. 2C; $P = 0.022$). The cryosections from HT-29 tumors shown in Fig. 2B were also stained for $CD31^+$ cells (green), and the results indicate that treatment with Irinophore C significantly decreased the number of $CD31^+$ pixels present in tumor sections. More specifically, tumor mapping analysis of entire sections showed that the overall percentage of $CD31^+$ pixels above threshold, as summarized in Fig. 2D, was significantly reduced ($P = 0.014$) in tumors from Irinophore C-treated animals (■) compared with untreated controls (□). Furthermore, when the percentage of $CD31^+$ pixels was normalized to the cell density, the results show a 1.8-fold increase in $CD31^+$ pixels relative to the number of tumor cells in the treated tumors compared with controls ($P = 0.00024$; Fig. 2E).

Irinophore C reduces tumor hypoxia. The level of tissue oxygenation in HT-29 tumors from control and Irinophore C-treated animals was examined with the hypoxic marker EF5. Because control, untreated tumors were substantially larger than treated tumors, the levels of hypoxia in the HT-29 model were first examined as a function of size. Untreated HT-29 tumors ranging from 200 to 600 mm^3 in size were exposed to EF5 as described in Materials and Methods. Subsequently, these tumors were disaggregated and analyzed with flow cytometry for the presence of viable hypoxic cells as reported previously (23). The results, plotted as a function of volume, are summarized in Fig. 3A and indicate that the percentage of viable hypoxia tumor cells is not correlated to size in HT-29 tumors ($R^2 = 0.0766$) within the range of volumes examined.

These results seemingly contradict previously published data where HT-29 tumors <1 mm in diameter were shown to be intensely hypoxic (and avascular) compared with tumors 1 to 4 mm in diameter (26); however, the size of tumors used in these studies were considerably larger (>0.5 cm^3). As shown in Fig. 3B, untreated tumors analyzed with flow cytometry were found on average to have a population of viable hypoxic levels of ~18% (consistent with the results shown in Fig. 3A), whereas treatment with Irinophore C for 6 weeks significantly reduced the population of viable hypoxic cells by 3-fold (~5% viable hypoxic cells; $P = 5.32 \times 10^{-9}$). Representative HT-29 sections for tumors from untreated and treated animals are shown in Fig. 3C and D, where a false-color image depicting cells stained positive for EF5 (red) is overlaid with an image for $CD31^+$ endothelial cells (green) against a hematoxylin background (gray). The images show that tumors from saline-treated animals have larger areas of hypoxic cells compared with tumors obtained from Irinophore C-treated animals.

Irinophore C treatment increases the accumulation of a second drug. Increased accumulation of Hoechst 33342 (Fig. 2C) and improvements in oxygenation levels (Fig. 3B-D) led us to investigate the effects of Irinophore C treatment on the delivery of 5-FU. The appearance of 5-FU in HT-29 tumors from animals treated with Irinophore C or saline was monitored with ^{19}F spectroscopy noninvasively over time in live animals. The doses used in this study were not meant to reflect therapeutically relevant doses but chosen to ensure a good signal-to-noise ratio for ^{19}F MRS. The amplitudes of the peak corresponding to the single fluorine atom in 5-FU, relative to an external standard (trifluoroacetic acid) and normalized to the tumor size, in individual control (□) and treated (■) mice are shown in Fig. 4A. The data suggest that the appearance of 5-FU is more variable in Irinophore C-treated tumors but that the tumors are exposed to up to 10 times more drug compared with untreated controls over the same period. The levels of bound ^{14}C-labeled 5-FU, added at tracer levels to the injected drug, in tumors from saline-treated (□) or Irinophore C-treated (■) animals were also measured using scintigraphy. The results show that ~1.5 times more 5-FU was present per gram of tumor tissue in tumors from Irinophore C-treated animals compared with

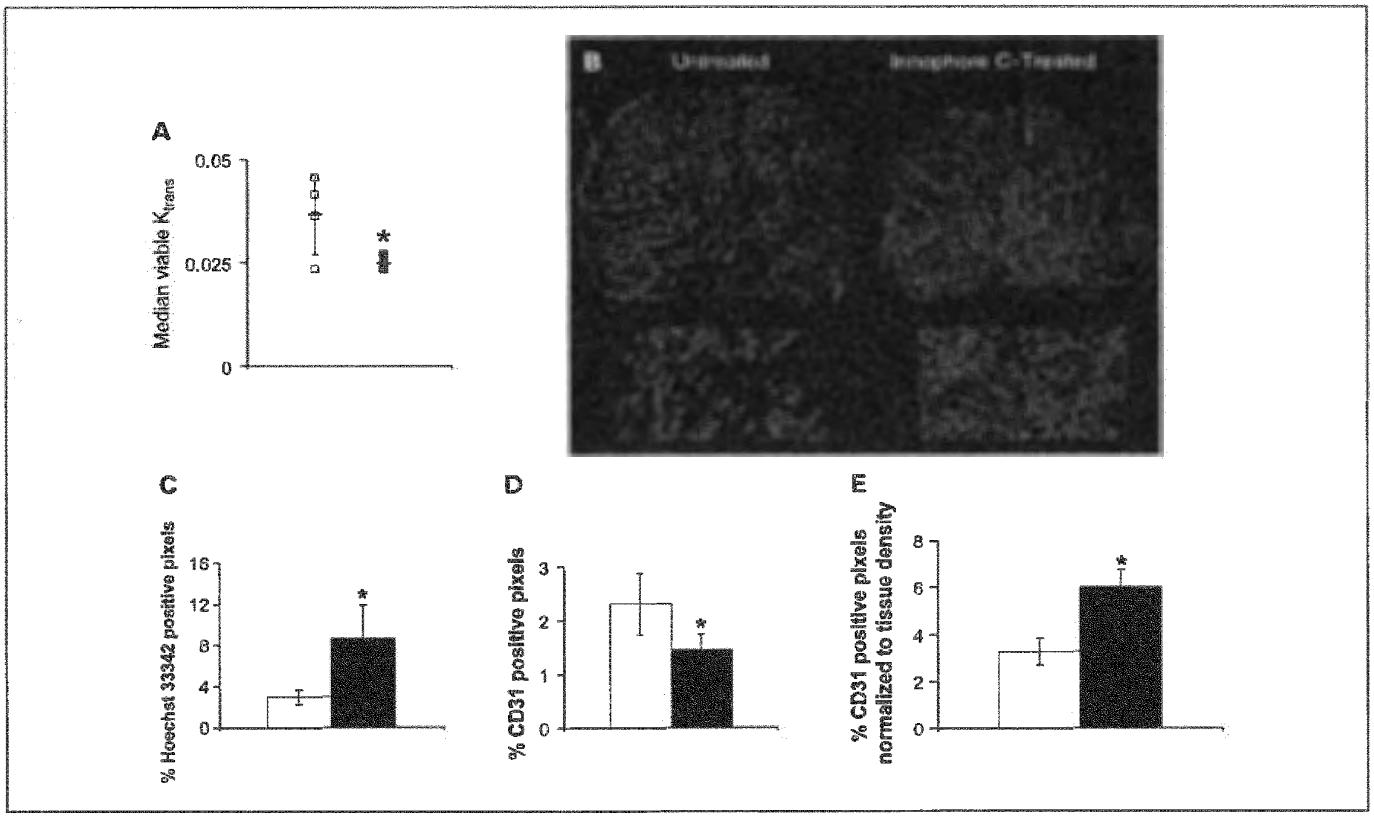

Fig. 2. *A* to *E*, vascular function in HT-29 tumors is improved with Irinophore C treatment. *A*, median viable values for K_{trans} in individual HT-29 tumors show the relative spread of K_{trans} values between the control group (□; 4 animals) and Irinophore C-treated group (■; 5 animals). The average value for K_{trans} (-) was significantly lower in the treated group ($P = 0.050$). The values for K_{trans} were more variable for the untreated group compared with the treated group of mice (SD = ±0.01 and ±0.001, respectively). *B*, significantly higher amounts of the fluorescent DNA-binding dye Hoechst 33342 injected intravenously are delivered to HT-29 tumors following treatment with Irinophore C relative to untreated controls. Representative images of sections from tumors harvested 20 min after intravenous injection of Hoechst 33342 (*blue*) show that more dye is present and that the overall amount of CD31 staining (*green*) is decreased in treated tumors. *C*, percentage of Hoechst 33342-positive pixels in sections from control (□) and treated tumors (■) was quantified, and the data show that ~2.8-fold more Hoechst 33342 is present in the Irinophore C-treated tumors ($P = 0.022$). *D*, tumor mapping analysis of sections stained specifically for endothelial cells indicates that the percentage of $CD31^+$ pixels per section in Irinophore C-treated tumors (■) is significantly reduced ($P = 0.014$) compared with untreated controls (□). Additional CD31 images can be seen in Figs. 3C and 4C. *E*, when the percentage of $CD31^+$ pixels is normalized to cell density (Hoechst 33342 flooding), a net 1.8-fold increase is seen ($P = 0.00024$) following treatment with Irinophore C.

controls (Fig. 4B; $P = 0.0002$). The scintigraphy data confirm, at a single time point, the ^{19}F spectroscopy data.

The accumulation of another commonly used anticancer drug, doxorubicin, was also evaluated in HT-29 tumor-bearing animals that were treated with saline or Irinophore C. Animals were injected with a single dose of doxorubicin (30 mg/kg) in animals treated with saline or with 6 weeks of Irinophore C. Size-matched tumors were subsequently harvested and the native fluorescence of bound doxorubicin present in the sections was visualized with fluorescence microscopy before subsequent staining and reimaging for endothelial cells (CD31) and nuclear density (Hoechst 33342 flooding). Representative false-color images of tumor sections from mice injected with doxorubicin are shown in Fig. 4C, where the native fluorescence of doxorubicin in tumors from mice treated with Irinophore C had a greater distribution compared with untreated control tumors. It should be noted that the cell density is not discernible in these images, and gaps in doxorubicin fluorescence within the tumors from Irinophore C-treated animals were associated with low tumor cell density (see Fig. 1B). Consequently, the visual assessment was corroborated by normalizing the average intensity of doxoru-

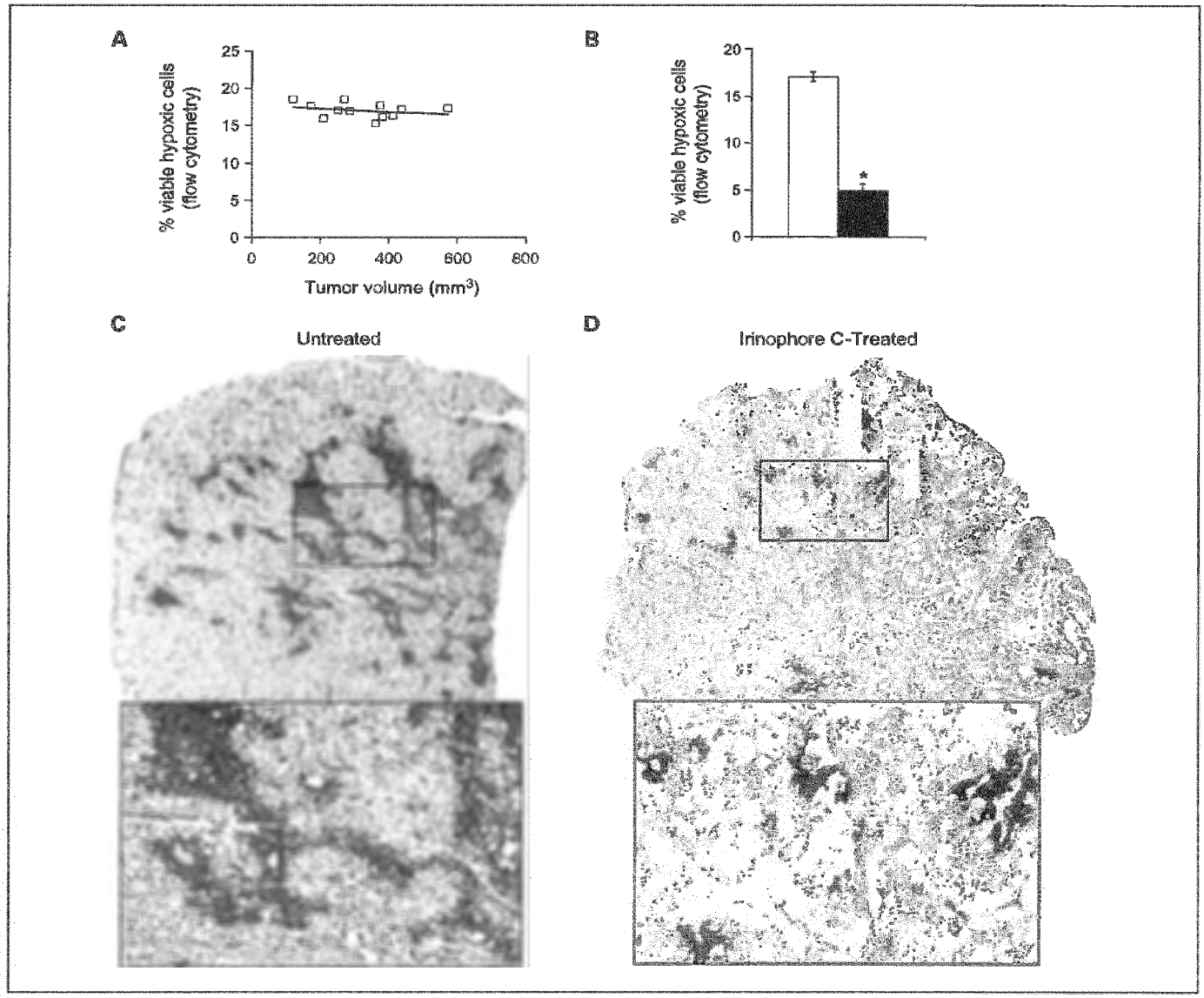

Fig. 3. *A* to *D*, Irinophore C treatment reduces levels of hypoxia in HT-29 tumors. *A*, percentage of viable hypoxic cells in untreated tumors ranging in size from 200 to 600 mm^3 was determined with EF5 staining and flow cytometry. No correlation was found between levels of hypoxia and the size of the tumor ($R^2 = 0.0766$). *B*, ~18% of viable cells in untreated tumors were found to be hypoxic; in contrast, Irinophore C-treated tumors were better oxygenated containing a significantly smaller population of viable hypoxic cells (~5%; $P = 5.32 \times 10^{-9}$). *C* and *D*, images of representative HT-29 sections with hypoxic and endothelial cells colored red and green, respectively, against a hematoxylin background show that untreated tumors have larger areas of hypoxic cells compared with the treated tumors. *Squares*, magnified images immediately below each section.

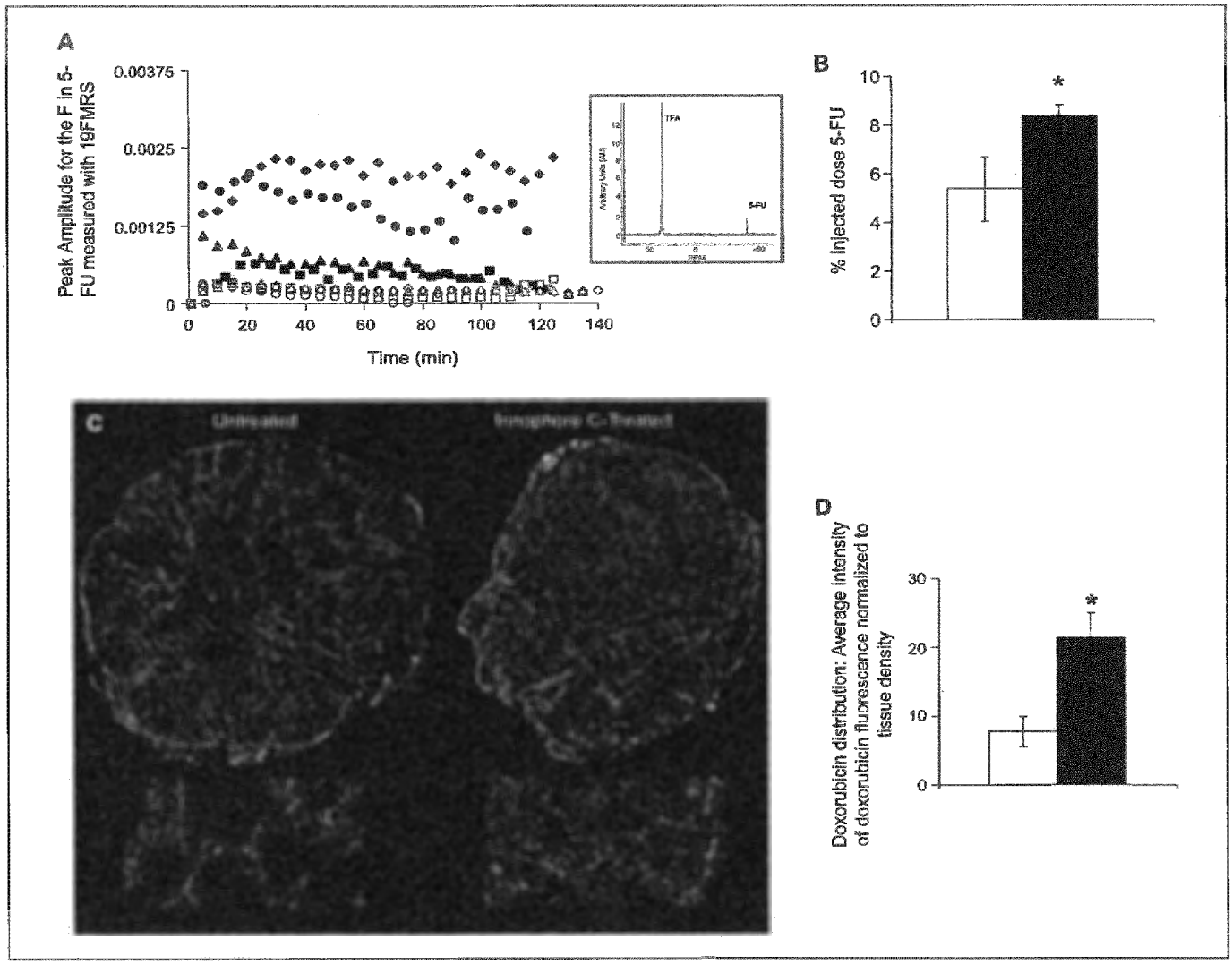

Fig. 4. *A* to *D*, treatment with Irinophore C increases the accumulation of a second drug in HT-29 tumors. *A*, ^{19}F spectroscopy was used to evaluate the delivery of 5-FU noninvasively in HT-29 tumors over 2 h in living animals. The relative amplitudes of the peak corresponding to the single fluorine atom in the 5-FU, normalized to an external standard (trifluoroacetic acid), in individual animals from untreated groups (□) and treated groups (■). *Inset*, representative spectrum for 5-FU measured *in vivo*. Data indicate that exposure of the tumor to 5-FU is more variable in Irinophore C-treated tumors but that higher concentrations of drug are present compared with untreated controls (up to 10-fold more drug in the same period). *B*, levels of [^{14}C]5-FU, added at tracer levels to the injected drug dose for ^{19}F MRS, bound to untreated tumors (□) and Irinophore C-treated tumors (■) were measured using scintigraphy. The percent injected dose of 5-FU present in the tumor tissue was ~1.5 times greater in Irinophore C-treated tumors compared with controls ($P = 0.0002$). *C*, similar results were obtained with the anticancer drug doxorubicin; control and Irinophore C-treated animals were injected with a single dose of doxorubicin, and the tumors were subsequently harvested and analyzed with fluorescence microscopy. The images of the tumor sections show that more doxorubicin is present in the Irinophore C-treated tumor tissue compared with untreated controls. *D*, average intensity of doxorubicin-positive pixels normalized to the cell density (Hoechst 33342 flooding) shows that Irinophore C-treated tumors (■) have significantly more doxorubicin present (~2.7-fold increase; $P = 0.2.43 \times 10^{-5}$) compared with control tumors (□).

bicin native fluorescence to the cell density as measured by the average intensity of flooded Hoechst 33342 staining. As summarized in Fig. 4D, in tumors from Irinophore C-treated animals (■), the amount of doxorubicin is ~2.7-fold greater than in control tumors (□; $P = 0.2.43 \times 10^{-5}$).

Antiangiogenic and antivascular effects of Irinophore C. To better understand the observed effects of Irinophore C on tumor vasculature (see Fig. 2B and D), a survey of promoters and inhibitors of angiogenesis was completed. The results obtained from an ELISA screen of proangiogenic and antiangiogenic activators are summarized in Table 1. Two proangiogenic factors, VEGF and IL-8, were down-regulated and an inhibitor of angiogenesis, TIMP-1, was up-regulated in tumors from Irinophore C-treated animals compared with saline-treated controls. Other markers in the array were not detected by this assay, so no conclusions can be drawn regarding these activators and inhibitors. To confirm results obtained in the ELISA screen, Western blot analysis for VEGF

and TIMP-1 in tumor lysates from individual animals treated with saline or Irinophore C was completed. The results, shown in Fig. 5, corroborate the results of the initial screen for two forms of VEGF and for TIMP-1. An ELISA kit for human CXCL8/IL-8 (Quantikine; R&D Systems) was used to assess the levels of IL-8 in the treated and control tumors (154 and 265 pg/mL, respectively); a strong trend toward lower levels of IL-8 in treated tumors (~1.7-fold less) was observed, although the results did not achieve significance ($P = 0.07$).

Discussion

Lipid-based nanopharmaceuticals are reasonably well-established (27–29), and in the context of anticancer drugs, most investigators would suggest that the benefits of these drug carriers include prolonged systemic drug exposure, enhanced delivery of the associated drug to tumors, and/or protection of the associated drug from premature metabolism in the plasma. Drug carrier formulations of camptothecins have been aggressively pursued in part because the formulations improve the availability of the active lactone form of the drug (30). The liposomal formulation of irinotecan developed by our group (Irinophore C) maintains the drug in its active form within the liposome, extends its plasma half-life, and improves accumulation of drug in the tumor (16). Prolonged systemic exposure to the active form of irinotecan as well as its more active metabolite SN-38, along with other data suggesting that these drugs can have antivascular or antiangiogenic effects, prompted us to study the effects of Irinophore C on tumor morphology and vasculature. We recently reported that Irinophore C was significantly more active than irinotecan in 5 different xenograft models (17), so here we focus on one of those tumor models (HT-29) and completed a multimodality imaging analysis of tumor-associated vascular structure and function. The results of this study clearly show that Irinophore C treatment has a striking effect on the morphology and vasculature of the tumor. The changes in vascular structure and function have important implications for using this drug in a combination setting and, as discussed below, allow us to speculate on the potential use of this nanopharmaceutical to increase the penetration and accumulation of a second chemotherapeutic agent.

Table 1. Irinophore C treatment of HT-29 tumors inhibits angiogenesis

Activators			Inhibitors
ANG	IL-1α	FGF-α	IFN-γ
G-CSF	IL-1β	FGF-β	IL-12
HGF	IL6	TNF-α	IP-10
Leptin	*IL-8*	TGF-β	**TIMP-1**
VEGF	PIGF	*Negative controls*	TIMP-2
		Positive controls	

NOTE: Pooled tumor lysates from untreated and treated tumors were assayed with an ELISA-based screen of proangiogenic and antiangiogenic activators. The proangiogenic factors VEGF and IL8 were down-regulated in Irinophore C-treated tumors relative to untreated controls (italicized), whereas the inhibitor of angiogenesis TIMP-1 was up-regulated (bold). The remaining factors in the assay were not detected in either group of tumors.

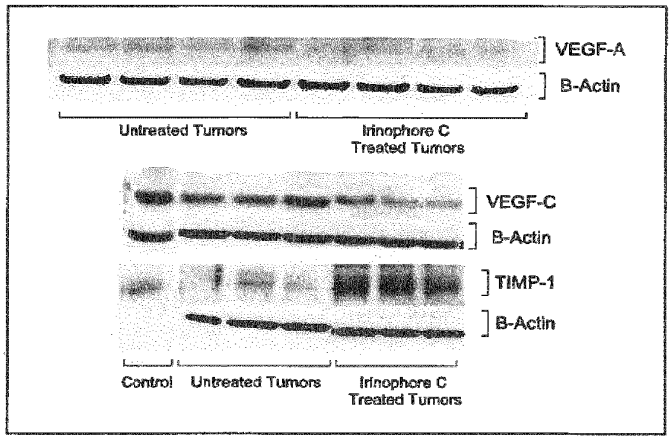

Fig. 5. Irinophore C treatment of HT-29 reduced expression levels of VEGF A and C but up-regulated expression levels of TIMP-1. Western blot analysis of the treated tumor lysates confirm that VEGF-A and VEGF-C were down-regulated (~2- and ~3.5-fold, respectively), whereas TIMP-1 was up-regulated (~4-fold) compared with untreated tumors.

It is notable that Irinophore C treatment stabilized the disease and did not cause tumor regression at these doses (Fig. 1A). This is similar to data that we have generated with a lipid-based formulation of vincristine, which when used to treat a human squamous cell carcinoma resulted in stable disease over an extended time course (31). Treatment effects that are associated with the appearance of stable disease are consistent with the observed effects obtained with metronomic chemotherapy (32, 33), where low doses of anticancer drugs are administered on a frequent or continuous schedule without extended breaks (33). The mechanistic basis of metronomic chemotherapy is believed to be primarily antiangiogenic, from either direct kill of endothelial cells in the growing tumor vasculature (34, 35) and/or destruction of bone marrow-derived endothelial progenitor cells (36, 37). The effects on tumor-associated endothelial cells is not necessarily surprising because many anticancer drugs, including irinotecan, have preferential activity against endothelial cells *in vitro* (38). In fact, the free drug was examined as an antiangiogenic agent in the late 1990s (39, 40) and was shown to inhibit neovascularization in a cornea model of angiogenesis (40). More recently, irinotecan was used to examine angiogenesis in a green fluorescent protein transgenic nude mouse model of human colon cancer (18). The use of irinotecan in a metronomic dosing regimen in a preclinical model of colorectal cancer (HT-29) was recently described (41); the authors indicate that metronomic dosing with irinotecan alone significantly inhibited tumor growth and also decreased microvessel density. The same group has now examined the pharmacokinetic and pharmacodynamic effects of metronomic irinotecan in metastatic colorectal patients, although the study did not include an examination of antivascular effects in the tumor tissue (42). Irinophore C, which behaves as a circulating drug depot that maintains low doses of active drug in the circulation for extended periods, may in fact be fundamentally akin to metronomic chemotherapy. We therefore speculate that the prolonged exposure to irinotecan and its active metabolite SN-38 (17) following intravenous use of Irinophore C may

elicit effects consistent with those achieved with metronomic dosing (42). Liposomal formulations of doxorubicin have also been shown to produce antivascular activity in models of glioma (43), which further corroborates our postulate that the liposomal formulation of Irinophore C indeed does have antiangiogenic effects.

Treatments with inhibitory effects on tumor vasculature have also been postulated to transiently and partially "normalize" the typically torturous, redundant, and inefficient vasculature found in tumors (44, 45). The concept of "vascular normalization" was originally conceived in 1972 (46) and more recently described in terms of a rebalancing of angiogenic processes gone awry in tumor growth (45). Whatever the precise mechanism behind this process, normalization of vasculature in window dorsal chamber tumor models treated with agents against VEGRF-2 improves blood flow and oxygen delivery to the cancer cells. These changes are associated with subsequent increases in drug penetration (47) and the sensitivity of the cells to radiation (48). Efficient delivery and distribution of anticancer agents is crucial in the treatment of solid tumors where every cancer cell, regardless of its local microenvironment, must be exposed to toxic levels of drug (49). Many small-molecule cancer therapeutics have shown evidence of poor tumor tissue penetration in both *in vitro* and *in vivo* model systems, including doxorubicin (50), gemcitabine (51), docetaxel, and paclitaxel (52). Thus, the concept of modifying the tumor vasculature, even temporarily, to improve radiotherapy by decreasing hypoxia, or increasing delivery of systemically administered chemotherapeutics, is highly compelling from the clinical perspective. The present studies indicate that Irinophore C treatment significantly decreases the number of endothelial cells and reduces K_{trans} while increasing the accumulation of the perfusion marker Hoechst 33342 in HT-29 tumors. The decrease in CD31 staining in treated tumors is consistent with an antiangiogenic effect (Fig. 2D). However, when CD31 staining is expressed relative to tumor cell density (see Fig. 2E), the ratio of endothelial cells to the number of cancer cells present in the section is actually ~1.8-fold higher in treated tumors. Thus, relative to the size of the tumor, the overall vasculature is decreased; however, in terms of tumor cell density, the ratio of vasculature to tumor cells is increased presumably improving the coverage of the vascular network.

These data suggest that improved delivery of a second compound, as seen here with Hoechst 33342, 5-FU, and doxorubicin, would be expected based on the drug-induced changes in vascular structure and function. We also believe that decreases in K_{trans} values in tumors following treatment with Irinophore C are consistent with improved vascular function. If the vasculature is normalized in treated tumors, and vessels become less chaotic and leaky, then vessel permeability becomes the rate-limiting step for determining K_{trans}, and the values would be expected to drop, as was the case. Thus, the large variability in K_{trans} values associated with untreated tumors (see Fig. 2A) likely reflects the random nature of chaotic and leaky blood vessels in tumors (45). Leaky tumor vasculature allows the MR-visible contrast agent to enter the extracellular matrix easily, so blood flow is the rate-limiting step in the process and K_{trans} values in untreated tumors are more likely to approximate blood flow rates. The reduction of overall vasculature in the treated tumors may seem to be at odds with the levels of Hoechst 33342 delivered and one might expect that fewer blood vessels would be associated with reduced dye delivery to the tumor. However, the possibility that Irinophore C treatment may normalize tumor vasculature, rendering the vessels more functional and thus able to deliver more of the dye, is a reasonable interpretation of the results. Improved vascular function, as evidenced by the K_{trans} data, in combination with the dramatic changes to tissue morphology and density, may explain significantly enhanced accumulation of secondary agents in tumors from animals previously treated with Irinophore C.

The antiangiogenic effect of Irinophore C is corroborated by data showing that two promoters of angiogenesis, VEGF (53) and IL-8 (54), are down-regulated, whereas an inhibitor of angiogenesis, TIMP-1 (55), is activated in the treated tumors (see Table 1). VEGF-A is known to promote endothelial cell proliferation, sprouting, and tube formation; VEGF-C also contributes to angiogenesis by activating VEGF receptors (53). VEGF levels have also been correlated with vessel permeability (56); lower VEGF levels in treated tumors would be expected to decrease blood vessel permeability, further supporting the interpretation of the observed K_{trans} values. Evidence also exists for IL-8 being a stimulus for endothelial cell proliferation, tube formation, and endothelial cell survival (54). In contrast, TIMP-1 is a known inhibitor of matrix metalloproteinases, which are necessary for breaking down the extracellular matrix to permit endothelial cell invasion (55); an increase in expression levels of TIMP-1 would thus have an antiangiogenic effect by preventing this process. However, angiogenesis is a complex process and the precise molecular mechanisms behind the effects of Irinophore C on tumor vascular function is a focus of ongoing studies.

Because treatment with Irinophore C appeared to improve vascular function and accumulation of Hoechst 33342, the effect of treatment on hypoxia as well as the delivery and accumulation of a second drug in these tumors was examined. Tumor hypoxia in tumors from saline-treated and Irinophore C-treated tumors was evaluated with the EF5/ELK3-51 system, which is specific for viable hypoxic cells (20, 21). The data indicate that Irinophore C treatment significantly reduces the proportion of viable, hypoxic tumor cells and this may be equated with improved delivery of oxygen. A decrease in hypoxia would have important implications for scheduling radiation treatments in colon cancer as hypoxia adversely affects radiation treatment (57, 58). Enhanced accumulation and improved distribution of doxorubicin and 5-FU in HT-29 tumors from animals treated for 6 weeks with Irinophore C were significantly higher compared with untreated controls. Exposure of cancer cells to higher levels of either drug would presumably result in better cell kill, and it will be important to now study in preclinical models the influence of Irinophore C treatment in combination with second agents, either given simultaneously or sequentially. More specifically, the results reported here raise the intriguing possibility of using subsequent or concurrent therapies to take advantage of changes in the tumor microenvironment engendered by Irinophore C. Because 5-FU and irinotecan are used in combination (FOLFIRI) to treat colon cancer (3), restoring or improving vascular function in the tumor by replacing irinotecan with Irinophore C could be beneficial if it

improved the therapeutic efficacy of 5-FU by increasing the delivery and subsequent accumulation of the drug in the tumor. Likewise, the efficacy of ionizing radiation could be increased if tumor hypoxia is decreased by treatment with Irinophore C in an adjuvant setting. Our group thus believes that the potential for Irinophore C to change the tumor microenvironment and render cancer cells more vulnerable to sequential chemotherapy or radiotherapy is a novel observation with immediate implications for the treatment of advanced colon cancer. Although the results reported here are relevant to colon cancer, the basic principles should be applicable to all solid tumors because angiogenesis and the expansion of a vascular network is a requirement for tumor proliferation and metastasis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Clinical Cancer Research

Irinophore C, a Novel Nanoformulation of Irinotecan, Alters Tumor Vascular Function and Enhances the Distribution of 5-Fluorouracil and Doxorubicin

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molecular pharmaceutics

reviews

Cancer Therapies Utilizing the Camptothecins: A Review of the *in Vivo* Literature

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Abstract: This review summarizes the *in vivo* assessment—preliminary, preclinical, and clinical—of chemotherapeutics derived from camptothecin or a derivative. Camptothecin is a naturally occurring, pentacyclic quinoline alkaloid that possesses high cytotoxic activity in a variety of cell lines. Major limitations of the drug, including poor solubility and hydrolysis under physiological conditions, prevent full clinical utilization. Camptothecin remains at equilibrium in an active lactone form and inactive hydrolyzed carboxylate form. The active lactone binds to DNA topoisomerase I cleavage complex, believed to be the single site of activity. Binding inhibits DNA religation, resulting in apoptosis. A series of small molecule camptothecin derivatives have been developed that increase solubility, lactone stability and bioavailability to varying levels of success. A number of macromolecular agents have also been described wherein camptothecin(s) are covalently appended or noncovalently associated with the goal of improving solubility and lactone stability, while taking advantage of the tumor physiology to deliver larger doses of drug to the tumor with lower systemic toxicity. With the increasing interest in drug delivery and polymer therapeutics, additional constructs are anticipated. The goal of this review is to summarize the relevant literature for others interested in the field of camptothecin-based therapeutics, specifically in the context of biodistribution, dosing regimens, and pharmacokinetics with the desire of providing a useful source of comparative data. To this end, only constructs where *in vivo* data is available are reported. The review includes published reports in English through mid-2009.

Keywords: Camptothecins; topoisomerase I inhibitors; polymer therapeutics; *in vivo*; cancer therapy

1. Introduction

Wall and Wani isolated 20-(*S*)-camptothecin (CPT) in 1966 from the bark of *Camptotheca acuminata*, and quickly observed that CPT suffers from many limitations including poor stability and solubility.[1] A year after the discovery of CPT, Wall and Wani discovered paclitaxel, another anticancer drug, which also showed great promise.[2] While both drugs showed powerful anticancer activity,[3] CPT's poor solubility and unpredictable adverse drug interactions favored the development of paclitaxel as a broad spectrum chemotherapeutic.[4] However, the CPTs gained much interest in the late 1980s when the molecular target was identified: DNA topoisomerase I (TOP I) is believed to be the single point of biological activity.[5−10] Crystal structures later confirmed the binding pocket for CPT as well as for a series of other

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compounds.[11−13] TOP I is an essential enzyme that relaxes supercoiled DNA prior to transcription through the formation of a single strand break and religation. Upon binding to TOP I, CPT prevents religation and causes apoptosis. Pommier has reviewed the literature focusing on mechanisms of molecular determinants for response of TOP I inhibitors.[14]

To overcome the solubility and stability issues associated with camptothecin, various derivatives have been developed. Although a number of small and large molecule compounds are currently in clinical trials, only two CPT derivatives, irinotecan and topotecan, are approved for clinical use. Irinotecan is currently used for metastatic colorectal cancer. Topotecan has been approved for ovarian cancer, cervical cancer and small-cell lung cancer. These derivatives employ tertiary amine cations to improve solubility and subsequently improve lactone stability. Currently, the CPTs—notably topotecan,[15−21] irinotecan,[22−27] 9-aminocamptothecin,[28,29] 9-nitrocamptothecin[30,31] and belotecan[32]—are being investigated for use as a late-stage therapy either alone or in combination therapies.

Polymer therapeutics for cancer therapy is a burgeoning field[33−36] that combines the therapeutic capacity of small

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molecule drugs with polymers designed to exploit tumor physiology[37,38] to achieve improved efficacy. These constructs complement the arsenal of self-assembling systems including micelles and liposomes. Both covalent and noncovalent strategies have been applied, and accordingly, macromolecular and supramolecular drug constructs present new possibilities to treat a variety of diseases. Incorporation of these small molecules into any construct is pursued with the intent of surmounting limitations that preclude broad clinical application including poor solubility, rapid clearance, high systemic toxicity and/or poor selectivity toward cancer cells.[39]

We have organized this review by small molecule and macromolecular agents. Small molecule derivatives are classified according to their site of modification (quinoline ring, lactone or 20-hydroxyl), with a specific section for each drug. Macromolecular agents are subdivided into noncovalent assemblies and covalent constructs with sections dedicated to each architecture and the small molecule agent investigated. As these molecules progressed from benchtop to bedside, new names have been introduced that represent the chemical name, the company name, the generic name and the brand name. The situation is further exacerbated when pharmaceutical companies merge or sell their products to other companies. Accordingly, the section heading refers to the most common name with alternate names indicated in parentheses. To simplify our discussion of pharmacokinetics, we focus on the half-life and area under the curve (AUC) values. Available pharmacokinetic parameters such as clearance rates and mean residence times are not included here because it muddies our efforts to providing a concise summary of the field as it applies to future therapeutics.

Furthermore, interpatient variability has been seen in many studies using the CPTs. The inability to accurately determine an optimal dose for all patients has limited the utility of these drugs until better methods for patient specific therapies are developed. For example, one study using irinotecan suggests that body weight and surface area do not accurately determine dosing.[40] That is, while many drugs are delivered as a mg/m^2 dose in human patients, a flat dose across all patients reported in milligrams, regardless of patient size, is suggested to be a more appropriate. The current convention for reporting drug doses in animals is mg/kg while human studies use mg/m^2. For easiest comparison, we report the published values and convert everything to mg/kg using body weight/surface area conversions found in the *Toxicologist's Pocket Handbook*.[41] Doses are reported as the dose administered by day rather than the total dose administered over the duration of the experiment. Doses of macromolecular constructs are reported with respect to the amount of CPT rather than total molecular weight of the construct. Toxicity data is included using maximum tolerated doses (MTDs) that are generally classified as the highest dose that does not cause death to the organism or specific organs, does not cause toxic manifestations reducing the life of the animal and does not appreciably decrease the body weight of the animal.[42] In most cases described here, the MTD caused mild neutropenia, leucopenia and thrombocytopenia along with other nonhematologic toxicities including fatigue, nausea and diarrhea, but the effects were generally reversible after treatment. Additional details about the pharmacokinetics, pharmacodynamics and toxicity may be found in the appropriate references.

Many of the compounds described in this review were developed in academic laboratories providing ample reports of the synthesis, characterization *in vitro* and *in vivo* evaluation. Naturally, some new drugs developed and being investigated in the pharmaceutical industry lack publications. One such drug is NKTR-102 from Nektar, which is currently in phase II trials for second-line colorectal cancer, metastatic breast cancer, platinum-resistant breast cancer and metastatic cervical cancer. We make efforts to mention promising drugs even when publications do not exist, but a lack of information precludes some candidates.

In an attempt to deliver a single, clear evaluation and comparison of the *in vivo* data, we have summarized all of the data collected into tabular format at the end of each

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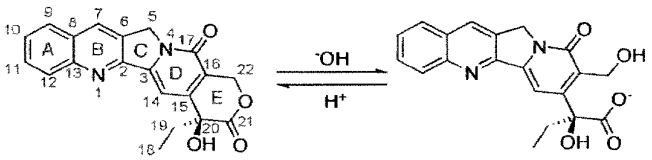
section. The data derives from peer-reviewed journals written in English as of September 2009. Other supporting articles leading to the reports of pharmacokinetic data and efficacy are also included. Furthermore, some small molecule derivatives with preliminary efficacy data are included to show the direction of current efforts with small molecules. Macromolecular architectures are only included when pharmacokinetic data of the small molecule drug was available to make the appropriate comparisons.

2. The Camptothecins

2.1. Camptothecin (NSC100880). The camptothecins are cytotoxic, quinoline alkaloids characterized by the planar pentacyclic ring system (Scheme 1).[1,43] While the A−D rings

Scheme 1. Camptothecin in the Lactone Form and Open Carboxylate Form



of CPT are necessary to maintain activity, modifications are permissible.[44] The E-ring lactone, however, is necessary for activity by binding to TOP I.[45] Hydrolysis or removal of the lactone leads to loss of all activity. The equilibrium between the closed, active lactone and the open, inactive carboxylate form is influenced by both the affinity of the carboxylate for human serum albumin and the local pH *in vivo*.[46] Originally, CPT was delivered as the sodium salt of the carboxylate to help overcome solubility issues, however, the poor efficacy created a need for new alternatives.[47]

Even given the hydrolytic sensitivity, the drug remains highly active as an anticancer agent. When delivered in an intralipid formulation through im administration, CPT showed nearly 100% growth inhibition and regression in colon, lung, breast, stomach, ovary and malignant melanoma xenografts.[48]

Pharmacokinetic studies of CPT in the lactone and carboxylate forms have been performed in rats.[49] In various buffers at 37 °C, the carboxylate is the predominant form. In PBS at pH 7.2, 7.4 and 8.0, the half-lives for the lactone are 33 min, 22 min and 5.3 min, respectively. Furthermore, equilibrium was achieved between both forms 90 min after injection of either 1 mg/kg lactone or carboxylate in rats. The carboxylate was cleared at a much faster rate through the urine and bile as compared to the lactone form. Clearance was also shown to be pH dependent. Decreasing pH of the urine may also reduce bladder toxicity caused by the carboxylate form.[50] Additional studies in dogs, monkeys, rats and mice showed toxic effects including emesis, diarrhea, dehydration, coma and death. Intravenous administration of 80 mg/kg or five doses of 0.625 mg/kg/day in dogs showed cumulative toxicity that was entirely reversible in survivors.[51] In human subjects, unpredictable toxicity associated with CPT halted clinical trials and opened the door for new antitumor agents.[52−56] The preparation and assessment of derivatives through classical structure activity relationships led to increased efficacy and better understanding of the basis for such activity.

Detailed structure activity relationships (SARs) have led to new CPTs with potent antitumor activity.[10,44,47,57−63] Many efforts focused on stabilizing the lactone without compromising cytotoxicity. To summarize the SAR studies, the A and B rings are the most tolerant to modification with

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substitutions at positions 7, 9, 10, and 11 improving or retaining activity. Altering the C and D rings or substituting positions 12 and 14, however, inactivates the molecules. Interestingly, von Hoff has provided evidence that substitutions that increase hydrogen bonding at the 7-position improve binding to TOP I, thus increasing activity over CPT.[64] The E ring, where binding to TOP I occurs, tolerates only minor modifications without dramatic, negative effects. For example, enlargement of the ring to form the beta-hydroxy lactone improves stability and drug activity. Additionally, modification of the C20 hydroxyl group through alkylation or acylation has been shown to stabilize the lactone. Acylation is the favored method for linking CPT covalently to macromolecules.

3. Camptothecin Modifications

Camptothecin modifications have attracted a great deal of research in an effort to increase the therapeutic index of the parent drug. Shortly after the discovery and initial investigations with CPT, new semisynthetic derivatives were developed that addressed the solubility and stability issues associated with CPT. Herein we focus only on derivatives with reported *in vivo* evaluation, but certainly, many other derivatives than those described below have been developed including Low's peptide folate conjugate,[65] Chen's 20-O-linked esters[66] and Battaglia's polyamine conjugate.[67]

3.1. Quinoline Modifications. The quinoline ring of CPT is the most commonly modified region. These derivatives show increased solubility, lactone stability and antitumor activity. Derivatives include the FDA approved drugs irinotecan and topotecan among many others. All of the quinoline modified CPTs are shown in Figure 1.

3.1.1. 10-Hydroxycamptothecin. Animal Models. Like CPT, 10-hydroxycamptothecin is naturally occurring.[68] Pharmacokinetic studies in rats after iv bolus with 10-hydroxy-CPT showed a short distribution half-life and long elimination half-life, with a majority of drug excreted in the urine within the first 6 h while fecal excretion occurred later, dependent on dose.[69] Very little systemic toxicity was observed at doses of 1, 3, and 10 mg/kg, including no gastrointestinal toxicity common in many CPTs. Furthermore, it was found that, after iv administration, the carboxylate form predominated in all organs except in the bone marrow, where the lactone was favored.

3.1.2. Topotecan (Hycamtin, NSC 609669, SK&F 104864). Animal Models. To improve solubility over CPT and 10-hydroxyCPT, solubilizing groups have been added to the quinoline ring, yielding the approved therapeutics topotecan[70] and irinotecan.[63] Topotecan owes its increased solubility to a tertiary amine at the 9-position, while irinotecan presents solubilizing groups through the 10-hydroxyl moiety. Topotecan was the first topoisomerase I inhibitor approved for clinical trials by the US Food and Drug Administration following CPT. Initial studies using a murine L1210 model showed an MTD in mice of 30 μmol/kg (13.7 mg/kg) as compared to 22 μmol/kg (10.1 mg/kg) for CPT, but the dosing strategy is unclear.[70] In a subsequent study, topotecan was administered subcutaneously over 72 h

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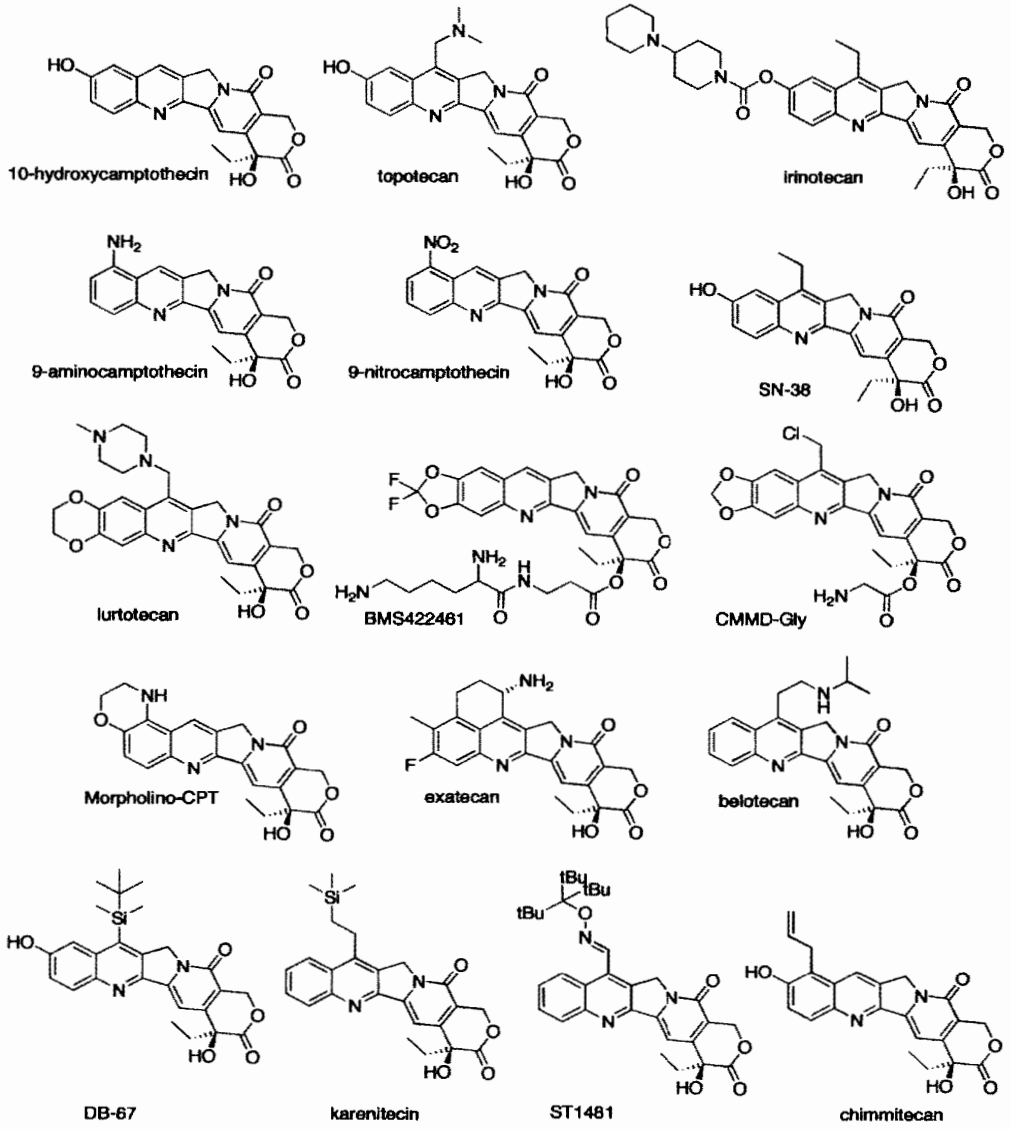

Figure 1. Quinoline modified camptothecin derivatives.

to SCID mice with B-lineage acute lymphoblastic leukemia at higher doses of 15.3 mg/m^2 (~5.1 mg/kg) as compared to an iv dosage of 1.75 mg/m^2 (~0.58 mg/kg). The survival rate for treated mice was 57% at 175 days whereas the control mice died at 40 days.[71] To further improve the efficacy of topotecan, a mitochondrial inhibitor was given to animals with RIF-1 tumors to decrease the local pH of the tumor to between 6.8 and 6.4 under the hypothesis that the equilibrium of CPT in the open and closed lactone forms could be influenced. However, the improvement in efficacy seen *in vitro* did not translate to *in vivo* studies.[46]

Topotecan was also investigated for activity against both subcutaneous (sc) and intracranial (ic) xenografts in mice with 1.9 mg/kg (LD_{10}) administered on days 1−5 and 8−12 by ip injection.[72] Tumor regression was observed in both sc and ic xenografts, with 39% increase in survival of mice bearing intracranial glioblastoma multiforme xenografts.[72] When delivered intraperitoneally (ip) to mice bearing solid human tumor xenografts once every four days for four total doses, an MTD of 12.5 mg/kg was observed, with modest reduction in tumor volume.[73] When 2 mg/kg topotecan was delivered through oral gavage, a maximum plasma concentration was achieved at 0.25 h, with alpha and beta half-

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lives of 0.55 and 2.8 h, respectively. These times are significantly longer than those observed for human subjects.[73] Similar half-lives were obtained when 1.75 mg/kg topotecan was delivered intraperitoneally, however, AUC values were three times higher than those obtained through oral gavage (0.09 and 0.29 μg·h/mL). An improved response was observed in rhabdomyosarcoma xenografts when delivered at daily doses of up to 2.0 mg/kg for five consecutive days, every three weeks, for up to 20 weeks, with tumor regression and minor toxicity.[73] This study was followed with a more thorough investigation of sustained topotecan exposure in Daoy and Rh30 xenografts. The results suggest that sustained exposure is more effective than delivering single, high doses of drug.[74]

In addition to traditional dosing strategies, oral dosing has been investigated. One study with topotecan compared the modes of delivery for the drug in mice: oral, intravenous, intraperitoneal and subcutaneous.[75] When topotecan was delivered as a single dose orally or ip, the matching MTD values of 10 mg/kg suggested that oral formulations may have clinical relevance. Subcutaneous administration with an MTD of 20 mg/kg suggested lower bioavailability. Correlations between dosing and the route of tumor inoculation were also examined. When mice were inoculated intravenously with L1210 lymphocytic leukemia, oral administration showed 216% increased life span (ILS) as compared to 183% ILS with ip administration. Mice with iv-inoculated Lewis lung carcinoma showed an ILS of 86−110% for oral administration as compared to complete regression observed when the drug was delivered subcutaneously. Lewis lung tumors implanted subcutaneously showed similar results for oral and iv administration, with 90% tumor growth inhibition when topotecan was delivered three times every four days. Mice bearing B16 melanoma through iv inoculation experienced increased median survival time through the oral route as compared to ip (56% ILS to 49% ILS). Finally, mice with ip M5067 reticulum cell sarcoma showed significantly diminished activity for oral administration as compared to ip or sc administration. These results suggest a potential for oral administration of topotecan.

In a later study, topotecan was orally administered to mice with sc NCI-H460 lung tumor xenografts in four doses of 15 mg/kg every four days. No toxicity was observed and 98% tumor growth inhibition was seen for orally administered drug. Upon iv administration, 93% inhibition at the same dose was achieved, but lethal toxicity was observed in one of four mice.[76] Similar results were also obtained in JCA-1 prostate cancer xenografts. When oral and iv routes were compared on the same schedule in POVD small-cell lung tumor, U87 glioblastoma tumor, COCF colon tumor, SKOV-3 ovarian tumor and A549 non-small-cell lung tumor xenografts, improved tumor growth inhibition was observed in each case regardless of route of administration. Increased weight loss, however, was observed through oral administration on this schedule. An increased half-life was also observed when delivered orally as compared to iv (2.77 h vs 1.95 h), however, AUC values were nearly 5 times lower for oral delivery (0.6 μg·h/mL vs 2.5 μg·h/mL), suggesting that drug persistence in the plasma may be more important than concentration.

An ideal therapeutic range was later determined to be between 0.2 and 0.7 μM drug for periods greater than 10 h.[77] This window was determined after mice with OVCAR-3 xenografts were treated with a total dose of 12.5 mg/kg topotecan at 1, 5, 10, 20, 40, or 80 daily ip injections. Maximal toxicity was observed when the total dose was delivered in the first 5−10 days of treatment, and the maximum efficacy was observed when delivered using 20 daily injections of 0.625 mg/kg without any major toxicity.

Human Patients. Preliminary phase I pharmacokinetic studies in which topotecan (MTD of 2.0 mg/m^2 (~0.05 mg/kg)) was administered for 30 min over five consecutive days every three weeks in patients with advanced cancer found AUC values of 4.09 μg·h/mL and alpha and beta half-lives of 0.06 and 3.5 h, respectively.[78] In phase I clinical trials, a dose of 1.5 mg/m^2 (~0.041 mg/kg) is the most common MTD, which may be achieved in a variety of ways, including a weekly 24 h iv infusion[79,80] or 0.5 h iv infusion for five

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consecutive days every three weeks.[81,82] Promising preliminary results with this dosing regime were observed in phase I trials while a pharmacokinetic profile showed biexponential elimination from the body, with mean alpha and beta half-lives of 0.1 and 3 h and 39% of the drug excreted in the urine within the first 24 h.[81] Similar results were obtained in a later study.[82] Other MTDs and associated dosing strategies for topotecan have been obtained such as 5.5 mg/m^2 (~0.18 mg/kg; 24 h iv infusion every 21 days)[83] and 22.5 mg/m^2 (~0.61 mg/kg; 0.5 h iv infusion every 21 days).[84] Interestingly, however, MTDs of 0.68 mg/m^2 (~0.018 mg/kg) in patients with solid tumors,[85] and 10.0 mg/m^2 (~0.27 mg/kg) in patients with acute leukemia[86] were obtained using a 120 h iv infusion every 21 days. Kantarjian attributes the higher MTD to the difference in cancer type.[86] Two studies suggest that using higher doses can achieve efficacy with subsequent successful treatment of the toxicities.[87,88] Additionally, each of the clinical trials discussed here was performed to investigate a variety of variables from pediatric patients with solid malignant tumors[83,88] and adult patients with solid tumors[79–82,84,85,89] to refractory or relapsed acute leukemia.[86,87]

In phase II trials, 30 min iv infusions of topotecan at 1.5 mg/m^2 (~0.041 mg/kg) administered for five consecutive days every three weeks to patients with advanced pancreatic cancer yielded a 122 day median survival time with no significant antitumor response.[90] The lack of tumor volume reduction led to recommendation against topotecan for pancreatic cancer treatment. A higher dose of 3.5 mg/m^2 given for 30 min five consecutive days every three weeks was also investigated in patients with colorectal cancer. This study relied on coadministration with granulocyte-colony stimulating factor (GCSF) to counteract toxic effects of the higher dose.[91] At this dose, a mean AUC of 0.34 μg·h/mL was obtained with alpha and beta half-lives of 0.2 and 4 h, respectively. While pharmacokinetic parameters remained similar to those for lower doses reported in the absence of GCSF, an insufficient increase in efficacy did not justify further study.

3.1.3. Irinotecan (CPT-11, Camptosar). Animal Models. Although topotecan was the first topoisomerase inhibitor approved for clinical trials since CPT, irinotecan entered the clinic only slightly later. Irinotecan (CPT-11) has an ethyl substituent at position 7 and a dipiperidyl carbamate at position 10, which is metabolized to SN-38, a 7-ethyl-10-hydroxy derivative that is 100 to 1000 times more cytotoxic than the prodrug.[92] Bioactivation of the prodrug has been shown to occur through human carboxylesterase 2 (hCE-2)[93] and human hepatic microsomes in the liver, with evidence of participation of the enzyme P-450 3A through an oxidized form.[94,95] Rabbit carboxylesterase, however, has been shown to activate CPT-11 more efficiently than the

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human enzymes.[96] This route of activation suggests the opportunity for targeted therapies in cancer cells transfected to overexpress the carboxylesterase proteins. Analysis of human plasma collected from patients receiving CPT-11 infusions showed that the lactone is more stable in the metabolized SN-38 (64% lactone) than in parent CPT-11 (37% lactone).[97] The area under the curve (AUC) pharmacokinetics of CPT-11 delivered intravenously to mice with L1210 tumors was determined to be 3 μg·h/mL and 23.5 μg·h/mL, corresponding to an 8-fold increase in residence time with only a 4-fold increase in dose from 10 to 40 mg/kg.[98] Additionally, SN-38 was found to have AUC values between 0.41 and 1.08 μg·h/mL after CPT-11 administration, whereas the AUC value rose to 1.35 μg·h/mL when 10 mg/kg of SN-38 was delivered directly. However, the concentration of SN-38 remained above the ED_{95} value for 5 h when delivered as CPT-11 as compared to 1 h when given as SN-38.[98] This data supports the use of CPT-11 over SN-38 due to beneficial solubility and the steady-state kinetics obtained from the prodrug form. However, gastrointestinal toxicity remains a major side effect of CPT-11 therapy.[99,100] Irinotecan was also investigated for oral delivery by administering it as a powder-filled capsule daily for five days every three weeks showing AUC values of 0.65 μg·h/mL and 0.76 μg·h/mL on days 1 and 5, respectively. An MTD of 50 mg/m^2 (~1.35 mg/kg) was found in patients with advanced solid tumors.[101] Half-lives for irinotecan were determined to be 7 h on day one and 12 h on day five using this strategy. This dosing schedule and pharmacokinetics suggest the potential for further study with orally available irinotecan, however, the gastrointestinal toxicity and myelosuppression remain a drawback.

The antitumor effect of irinotecan was demonstrated *in vitro* and *in vivo* in vincristine and adriamycin resistant P388 xenografts.[102] Tumor suppression was measured by the percent increase in life span (ILS) compared to control mice after intravenous administration on days 1, 5, and 9 after tumor inoculation. In vincristine resistant tumors, a 130% ILS was observed at total dose of 200 mg/kg of CPT-11 and a 20% ILS was observed with a 4 mg/kg dose of vincristine. Similar results were observed with adriamycin resistant cells.

Human Patients. While irinotecan was able to treat tumors resistant to other therapies, a study with irinotecan as a first line therapy proved interesting. In a phase II trial of 90 patients including those previously treated with 5-fluorouracil and 31 untreated patients, the percentage of partial responses increased from 13.3% to 25.8%, respectively.[103] Irinotecan was also investigated in untreated patients with metastatic colorectal cancer with 90 min infusions of 125 mg/m^2 (~3.38 mg/kg) weekly for four consecutive weeks every six weeks.[104] This study is one of the few studies involving previously untreated patients, thus providing a clear investigation of irinotecan as a first-line therapy. Thirteen patients showed partial response with median survival of 12.1 months. The authors compare this to the commonly used fluorouracil plus leucovorin combination therapy, which offers an 11.5-month median survival time for patients. Side effects of this therapy were observed to be neutropenia in 22% of the patients and diarrhea in 29% of the patients, which was counteracted with diphenhydramine. Similar toxicities and responses were observed at the same dosing schedule in patients with squamous cell carcinoma of the cervix.[105]

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To counteract the gastrointestinal toxicity and resulting diarrhea observed in patients receiving irinotecan, alternative schedules were investigated. With side effects resulting from treatments given weekly, a study of 90 min infusions of irinotecan every three weeks found an MTD of 240 mg/m^2 (~6.5 mg/kg) with an AUC value of 11.5 μg·h/mL and a mean elimination half-life of 6.7 h.[106] At this dose, only 3 out of 72 patients experienced diarrhea who received initial doses higher than the MTD. A later study in patients with metastatic cancer previously treated with surgery, chemotherapy or radiation therapy found a higher MTD of 290 mg/m^2 (~7.8 mg/kg) using the same dosing schedule with an AUC value of 18.1 μg·h/mL and a half-life of 13 h.[107] This study also showed promising tumor growth inhibition, with four patients experiencing partial response and one with complete response. Furthermore, another study found an MTD of 350 mg/m^2 (~9.5 mg/kg) when using the same dosing schedule.[108] Due to the success seen with doses up to 350 mg/m^2, it is unclear what parameters led to lower MTDs in each study. The effectiveness and length of previous treatments as well as alternative complications associated with the cancer may all contribute to the disparity of reports. To better understand these differences, hepatic function of patients was investigated. The recommended doses between 200 mg/m^2 (~5.4 mg/kg) and 350 mg/m^2 (~9.5 mg/kg) depend on bilirubin levels, which is a marker for disease states of the liver.[109,110] From the data presented here it is clear that many factors must go into devising a treatment regimen with irinotecan.

While much of the research with irinotecan has focused on solid malignancies and metastases in the lung, liver, pancreas and digestive tract, targeting the brain has been investigated. One phase II trial, built from positive data obtained in preclinical and phase I trials,[111−116] tested the efficacy of irinotecan in patients with malignant gliomas.[117] This study, however, gave less than desired results. Eighteen patients were treated with 90 min iv infusions once a week for four weeks with two weeks off after treatment to complete a six week cycle. The patients received up to 10 cycles of treatment before stopping the study, with a median of 2 cycles. One patient had complete response, five patients experienced disease stability, five patients progressed, six patients were removed from the study due to toxicity and another refused further therapy. Most recently, a case study reporting the use of irinotecan in combination therapy with 5-fluorouracil and leucovorin acid to treat an ovarian tumor during pregnancy found no adverse side effects observed in the baby up to 6 months after birth.[118]

3.1.4. 9-Aminocamptothecin (9-AC). Animal Models. Shortly after the development of irinotecan, a series of CPTs were developed with substitutions on the A ring.[59] One derivative, 9-aminocamptothecin, showed the highest activity in cell culture, and later showed antitumor activity *in*

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vitro[10,47] and *in vivo*.[119] A pharmacokinetic comparison of 9-aminocamptothecin with CPT in the lactone and sodium carboxylate forms was conducted after iv administration in mice.[120] Although at different doses, the elimination of 9-AC occurs more rapidly than CPT with elimination half-lives of 1.4 h for 9-AC (5 mg/kg) and 24.6 h for CPT (10 mg/kg). The initial plasma lactone concentration was higher after iv injection of 9-AC compared CPT at the same doses. Due to fast clearance of 9-AC, the plasma concentration fell below 10 nM at 8 h compared to 48 h for CPT. The promising cytotoxic effects and lactone stability of 9-AC led to the suggestion that continuous iv infusion was the ideal method to obtain steady state pharmacokinetics and efficacy.

Human Patients. A pharmacokinetic study of 9-AC in human patients showed dose-dependent half-lives ranging from 4.5 to 21 h with doses of 0.208 mg/m^2 to 1.5 mg/m^2 (~0.006−0.041 mg/kg).[121] This work was followed by phase I clinical trials of 9-AC administered in a colloidal dispersion as a 72 h continuous iv infusion. While there was no evidence of tumor regression, tumor growth did not progress during therapy.[122] Various other studies have shown that 9-AC has broad activity in human xenografts including melanoma, breast, colon and brain tumors,[123−126] however, when delivered through continuous intravenous infusion to patients with non-small-cell lung cancer, a response rate of only 9% was observed.[127]

Similarly, in phase II trials, as second-line therapy for ovarian carcinoma, 18 of 28 patients saw no response to 9-AC, while only 1 had complete response, 2 had partial response and 6 had stable disease.[128] While poor efficacy of the drug prevents full scale clinical use, 9-AC has shown potential as a method for sensitizing cells prior to radiation therapy.[28]

3.1.5. 9-Nitrocamptothecin (9-NC, Rubitecan, Orathecin). Animal Models. After investigations of 9-AC showed little promise, an intermediate in its synthesis, 9-NC, was tested for cytotoxic properties and was found to be converted to 9-AC *in vivo*.[129] Compared with 9-AC, half-life for 9-NC increased from 1.2 to 10 h in mice given 4.1 mg/kg through iv injection in cottonseed oil. Similarly, AUC increased from 63 μg·h/mL to 441 μg·h/mL. However, upon oral delivery of 0.1 mg/kg gelatin capsule, a comparison to 9-AC showed that 9-NC had a higher AUC (2.6 μg·h/mL vs 0.3 μg·h/mL) but lower half-lives (2.5 h vs 7.1 h) and lower maximum plasma concentrations (3.4 h vs 10.3 h). Although the drug suffered from poor solubility, oral availability prompted further investigation toward the use of 9-NC clinically.

A pharmacokinetic study between iv and oral administration of 9-NC to rats concluded that oral administration of 9-NC may be more effective clincially.[130] When 9-NC was delivered through iv administration at doses 1.5 mg/kg, 3 mg/kg or 6 mg/kg, half-lives of 0.5 h were obtained for lactone, carboxylate and total drug, regardless of dose. AUC values for the lactone, carboxylate and total drug were 0.25, 0.75, and 1.2 μg·h/mL for each increasing dose. Oral administration of 6 mg/kg of 9-NC provided a slightly longer half-life of 0.8 h with a lower AUC value of 0.25 μg·h/mL for the lactone and carboxylate forms.

Human Patients. Phase I trials with oral 9-NC in patients with metastatic cancer found an MTD of 1.5 mg/m^2 (~0.04

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mg/kg) on a weekly schedule.[131] At this dose in phase II trials, hematological and gastrointestinal toxicities similar to those of irinotecan were observed with modest efficacy in patients with ovarian, tubal or peritoneal cancers.[132] Pharmacokinetics in these patients showed great variability with AUC values ranging from 0.6 μg·h/mL to 2.8 μg·h/mL and a mean half-life of 11 h. While the results with 9-NC showed moderate promise and the lactone stability improved over CPT, the insolubility and equilibrium of the drug favoring the inactive carboxylate prevented further exploration with this drug without further modification. Similar to 9-AC, however, 9-NC has been investigated as a sensitizer for radiation therapy.[133]

3.1.6. Lurtotecan (GI147211, GG211). Animal Models. Lurtotecan is water-soluble by virtue of a methylpiperazino group at position 7 and an ethylenedioxy ring bridging positions 10 and 11.[134] Initial comparisons to topotecan found that lurtotecan was to be both more soluble (5.8 mg/mL vs 3.1 mg/mL) and more cytotoxic *in vitro*.[135] Lurtotecan was evaluated in mice with HT-29 and SW48 colon tumor xenografts dosing twice a week for five weeks using a ratio of tumor volume after treatment to tumor volume before treatment (T/B). Success was defined by a T/B ratio <1, meaning that the tumor regressed in size. Lurtotecan provided T/B values of 0.8 and 0.4 at doses of 9 and 12 mg/kg, respectively, in HT-29 xenografts and 0.9 and 0.6 at the same doses in SW48 xenografts. However, body weight loss was observed in both tumor models with two out of six animals dying at the higher dose. Topotecan on the other hand showed T/B values of 4.3 and 2.9 at 9 and 11 mg/kg, respectively, in HT-29 cells and 3.1 and 2 in SW48 cells at the same doses with significant body weight loss at all doses.

Human Patients. In phase I clinical trials with doses ranging from 0.3 to 1.75 mg/m^2 (~0.008 to 0.047 mg/kg) for five consecutive days every three weeks, an MTD was determined to be 1.5 mg/m^2 (0.041 mg/kg).[136,137] Lurtotecan was determined to have concentration pharmacokinetic profiles following a three compartment model with total drug alpha, beta and gamma half-lives of 0.095 h, 0.91 h and 7.1 h on day one and 0.062 h, 1.2 h and 15 h on day four of treatment. One and four day AUC values of 0.057 μg·h/mL and 0.064 μg·h/mL were obtained for total drug with 25% corresponding to lactone.

Two different phase I trials investigated the potential of delivering lurtotecan through continuous infusion for 3 days[138] or 7, 14, and 21 day continuous infusions.[139] When delivered as a 3 day continuous infusion every four weeks to heavily pretreated patients, an MTD of 1.2 mg/m^2 (~0.03 mg/kg) was determined, while a slightly higher MTD of 2.0 mg/m^2 (~0.05 mg/kg) was found for minimally pretreated patients.[138] Over the dose range studied, a mean half-life of 7.5 h was observed for the lactone, with total drug blood concentration four times higher than lactone concentration. Of the 44 patients in this study, only three patients experienced partial responses, while two others observed decreases in hepatic lesions. In the subsequent study, where lurtotecan was administered in 0.3 to 0.5 mg/m^2 (~0.008 to 0.013 mg/kg) as a 7, 14, or 21 day continuous infusion, AUC values increased from 0.031 μg·h/mL to 0.18 μg·h/mL when delivered at 0.3 mg/m^2 everyday for 7 days and 21 days, respectively. Additionally, only a slight increase to 0.19 μg·h/mL was observed at 0.5 mg/m^2 for 21 days. However, these studies and one later study showed significant patient variation between correlation of AUC values and dose,

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suggesting further investigation into the cause and potential clinical solutions.[140]

Lurtotecan moved swiftly to phase II trials despite unpredictable pharmacokinetics due to the mild side effects associated with the drug, but only modest antitumor activity was observed.[141] Patients with breast cancer (23 patients), colorectal cancer (19 patients) and non-small-cell lung cancer (22 patients) were treated with 30 min iv injections of 1.2 mg/m^2 (0.03 mg/kg) for five consecutive days every three weeks. No complete responses were obtained in any of the patients, with 13% of breast cancer patients and 9.1% of non-small-cell lung cancer patients experiencing partial responses. In breast cancer patients, 39.1% had stable disease with disease progression in 48%. Colorectal cancer patients showed 37% stable disease and 63% cancer progression. In lung cancer patients, 22.7% had stable disease and 68.2% had progressive cancer. With such modest results, lurtotecan was also investigated as a second-line treatment for small-cell lung cancer, but only 11 out of 66 patients experienced partial response.[142]

3.1.7. 10,11-Methylenedioxy Camptothecins. Animal Models. To overcome the gastrointestinal toxicity of CPT-11,[99,100] a series of fluorinated derivatives were developed. Two fluorinated derivatives, a free hydroxyl and a 20-O-linked ester (BMS422461), showed great promise.[143] The compounds showed positive gross log cell kill ability (at MTD) in A2780 (0.06 mg/kg), HT29 (0.13 mg/kg) and HCT116 (0.06 mg/kg) tumors in athymic nude mice when administered iv every two days for ten days. Furthermore, BMS422461 showed similar lactone stability as compared to CPT-11 in mouse and human plasma as well as in the presence of mouse or human albumin (between 20 and 34% lactone). The parent compound also possessed a 4-fold increase in AUC pharmacokinetics as compared to the prodrug and an 8-fold increase as compared to the β-alanyl intermediate upon intraarterial administration. While the data questions the necessity of the prodrug strategy, the improved solubility of the prodrug over the parent molecule provides clear explanation. A semiquantitative, histopathological assessment of GI injury after subcutaneous injections of the prodrug was performed with the parent drug and irinotecan dosed every day for five days at the MTD. A relative injury scale of 0 (no injury) to 4 (mucosal atrophy and ulceration) was employed, which provided evidence of diminished toxicity over irinotecan in the new fluorinated molecules with injury values of 0.5, 1.5 and 2.8, respectively.

Wadkins and co-workers explored esters of 10,11-methylenedioxy camptothecins.[144] The parent compound, 10,11-methylenedioxy camptothecin showed a 3-fold decrease in half-life and a 5-fold decrease in plasma AUC as compared to CPT after a 10 mg/kg iv injection in tumor free mice.[120] The poor results prevented further studies until Wadkins and co-workers investigated ester derivatives six years later. All of the compounds were tested in a series of breast cancer cell lines showing nanomolar IC_{50} values in ZR-75, MDA-231 and BT-20 cells. Two of the derivatives contained an electrophilic chloromethyl group at the 7-position poised for covalent attachment to DNA .[144] During *in vivo* studies with MX-1 and MDA-231 human breast tumor xenografts, the chloromethyl groups did not show cytotoxic enhancement. Furthermore, while the glycinate ester derivatives were more water-soluble than CPT-11, there was no enhanced toxicity observed in either cell line. Success could be achieved using smaller doses over a longer period of time. For example, dosing 0.50 mg/kg every day for five days resulted in eight out of eight complete responses, while 5.0 mg/kg dosed once gave seven out of eight complete responses.[144] Additional studies in monolayer cell culture as well as in histocultures provided evidence that the acidic conditions (pH 6.8) of tumor cells increases potency of CPTs including this chloromethyl derivative.[145,146]

3.1.8. Morpholino Camptothecins. Animal Models. Kim and co-workers synthesized a library with a variety of A-ring substituents to investigate the effects on the stability of the

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lactone.[147] Initial screening of the compounds found a subgroup that maintained the cytotoxicity of CPT. One compound with a morpholine ring bridging positions 9 and 10 showed retention of TOP I inhibition and increased lactone stability in human serum compared to CPT (but not SN-38). However, the additional solubility prompted further *in vivo* investigations in WiDr xenografts in nude mice. The molecule showed efficacy that was comparable to SN-38 at 1/10 the dose delivered ip every four days for eight total doses. That is, at an MTD of 10 μmol/kg (4.05 mg/kg), the morpholino compound showed tumor growth inhibition at 98.6% while SN-38 dosed at 100 μmol/kg (39.2 mg/kg) gave a 98.2% inhibitory rate.

3.1.9. Exatecan (DX-8951). Animal Models. While many of the previously described CPTs have suffered from poor lactone stability, the exatecan equilibrium favors the closed, active lactone form. Exatecan owes its stability and solubility to a six-membered ring containing an exocyclic amine connecting carbons 7 and 9, as well as a methyl at position 10 and fluorine at position 11. Each of the modifications have been shown to increase lactone stability, solubility and *in vitro* activity over CPT and irinotecan without the need for metabolic activation.[148] Activity has been noted in pancreatic tumors *in vitro*[149–153] and in subcutaneous xenografts *in vivo*.[154] With such promising activity, Hoffman aimed to investigate the activity of exatecan through the treatment of surgical orthotopic implantation to determine the activity of the drug in normal tissue with metastatic capability as compared to gemcitabine.[148] Single doses of the drug delivered to mice with early stage MIA-PaCa-2-GFP tumors provided 93% inhibition at 25 mg/kg and 79% inhibition at 15 mg/kg compared to the control. Gemcitabine gave only 67% inhibition for the high dose (300 mg/kg) and 43% for the low dose (150 mg/kg). Similar results were obtained for the early stage BxPC-3 orthotopic human pancreatic model. Furthermore, when using the same dosing strategy, exatecan was effective in inhibiting lymphatic metastasis and completely eliminating lung metastases in late stage BxPC-3 orthotopic tumors. Little to no effect was observed for gemcitabine, with only 45% and 25% tumor growth inhibition for each tumor, respectively.

When exatecan was investigated for efficacy in mice with SC-6 gastric cancer xenografts, a dosing schedule of four total doses given once every fourth day proved more efficacious than three total doses given once every fourth day or three total doses given once every seventh day.[154] Using four doses, between 6.25 mg/kg and 18.75 mg/kg was delivered to mice with greater than 94% tumor growth inhibition and no significant toxicity. Similar potency was observed in a number of cell lines. At 18.75 mg/kg, toxicity manifests in significant loss in body weight and death. Although cell dependent toxicity was observed, exatecan proved to be more potent than irinotecan without the need for metabolic activation while retaining the solubility and improving the lactone stability.

Human Patients. Exatecan was eventually taken into clinical trials, with antitumor activity shown in non-small-cell lung cancer, ovarian cancer, tubal cancer, peritoneal cancer, endometrial cancer, colon cancer, hepatoma, thyoma and small-cell carcinoma of the bladder, as well as patients with platinum, topotecan and taxane resistance.[155–158] In patients with advanced solid malignancies on a schedule of 30 min iv infusions five days a week every three weeks, MTDs of 0.3 and 0.5 mg/m^2 (~0.008 and 0.013 mg/kg) were recommended for heavily pretreated patients and mildly pretreated patients, respectively.[155] An average half-life of

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8.75 h was determined, with severe myelosuppression experienced at doses above the MTD. Patients with advanced leukemia, however, were treated for 30 min on five consecutive days for three weeks through iv infusion, resulting in a recommended dose of 0.9 mg/m^2 (~0.024 mg/kg).[159] This dose appears to be double the MTD for solid tumors.[155]

When the dosing strategy was changed from every five days to a single 30 min iv infusion every three weeks, the MTD increases to 5.33 mg/m^2 (~0.14 mg/kg).[160] At this dose, the mean half-life is 7.5 h. While promising pharmacokinetic data was obtained, only six of 11 patients had stable disease, while five showed progressive disease. A subsequent study afforded similar results, with pharmacokinetic analysis showing a lactone AUC value and half-life of 0.663 μg·h/mL and 8 h, respectively, and total drug values of 2.09 μg·h/mL and 10 h.[161] However, by increasing the dosing to consecutive weeks on a schedule of 30 min infusions for three out of four weeks, a recommended dose of 2.1 mg/m^2 (~0.057 mg/kg) in heavily pretreated patients and 2.75 mg/m^2 (~0.074 mg/kg) in minimally pretreated patients was obtained.[162] At the higher dose, an AUC value of 1.095 μg·h/mL and half-life of 8 h was determined, suggesting slight advantage for every three weeks at a higher dose.

While pharmacokinetic data proved promising, poor efficacy prompted investigation of an extended dosing regimen in phase I trials. Exetecan mesylate showed mild toxicity and a mean plasma elimination half-life of 7 h after 24 h iv infusions for three consecutive weeks in patients with solid tumors.[163] The authors of this study expressed their desire to abandon this route before moving to phase II trials due to the inconvenience associated with the dosing regimen. However, the recommended doses were 0.8 mg/m^2 (~0.022 mg/kg) for minimally pretreated patients and 0.53 mg/m^2 (~0.014 mg/kg) for heavily pretreated patients. An alternative extended dosing strategy involved a 21 day continuous iv infusion at a dose of 0.15 mg/m^2 (~0.004 mg/kg) for the first 5 days and incremental increases from days 5 to 21 to reach a steady state plasma concentration.[157] Increasing the dose to 0.3 mg/m^2 led to AUC values that were significantly higher, 465.8 μg·h/mL, than those obtained when drug is administered over short periods of time. While this dosing schedule was even more cumbersome than a 24 h infusion, the greatly improved pharmacokinetics sets a benchmark for future studies, namely, macromolecular delivery of CPTs.

Phase II evaluation of exatecan mesylate on a schedule of 30 min iv infusion for five days every three weeks provided 8 h half-lives in patients with metastatic breast cancer. The infusion dose given to minimally pretreated patients was 0.5 mg/m^2 (~0.014 mg/kg) while that given to heavily pretreated patients was 0.3 mg/m^2 (~0.008 mg/kg).[164] Out of 39 patients, no patients experienced a complete response, while three experienced partial and four had minor responses. Sixteen and 14 patients, however, experienced stable and progressive disease, respectively. The authors suggest that although mild toxicity was observed from this dosing strategy, poor efficacy suggests that an alternate schedule be used or that the drug was not effective in treating this tumor. A subsequent study in patients with ovarian, tubal or peritoneal cancer showed slightly higher efficacy at the same dosing schedule, with 7 of 16 patients experiencing stable disease.[158] Poor efficacy using this dosing strategy, however, was also observed in patients with non-small-cell lung cancer[165] and metastatic colorectal adenocarcinoma.[166] Poor efficacy was also observed in patients with platinum and taxane resistant ovarian cancer.[167] Slight improvement of efficacy was observed when administered at a dose of 0.3 mg/m^2 (~0.008 mg/kg) for five consecutive days every three weeks as compared to a dose of 2.1 mg/m^2 (~0.057 mg/kg) every week for three consecutive weeks out of four. The modest improvement, however, did not warrant further investigation. Patients with metastatic gastric cancer also experienced poor efficacy, with only 2 out of 41 patients experiencing partial response, 18 exhibiting stable disease and 18 showing progressive disease.[168] The median survival time in this cohort of patients was determined to be 197 days with 59% survival at 6 months. Biliary tract cancers treated

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with this dosing regimen provided similar results and modest survival.[169] Modest success was also reported for patients with soft tissue sarcoma, a disease typified by poor survival rates and lack of therapeutic options.[170] Phase III studies using exatecan and gemcitabine were also performed and compared to gemcitabine therapy alone showing no extended survival time with cotherapy.[171] While initial investigations with exatecan mesylate proved to be promising, results from phase II and phase III clinical trials in a variety of cancers suggest further investigation must be completed to identify the role for exatecan mesylate in cancer therapy.

3.1.10. Belotecan (CKD-602, Camtobell). Animal Models. More recently a water-soluble CPT derivative with an (isopropylamino)ethyl moiety at position 7, known as belotecan, has been developed. Initial studies in nude mice with human tumor xenografts (CX-1, HT-29, WIDR, LX-1, MX-1, SKOV-3 tumors) showed broad antitumor activity. Potency was three times that of topotecan and slightly higher than that of CPT.[172] The schedule dependence of belotecan was also investigated with doses being administered intraperitoneally to mice bearing L1210 leukemia xenografts on the following dosing schedules: a single dose, 5-daily doses, four total doses every fourth day, two total doses every fourth day and two total doses every seventh day. The antitumor effect and increased life span (ILS) were apparent when four doses were delivered every four days, with very little body weight loss occurring at a dose of 25 mg/kg and 213% ILS.

Significant efforts have focused on the acute toxicity of belotecan in a variety of tumor free animals. Studies have been performed in embryonic and adult rats,[173–175] dogs,[176] pregnant does and rabbits[177] and human subjects with small-cell lung cancer.[178] In general, daily doses of 0.01 mg/kg were well tolerated in both maternal and embryonic subjects depending on the length of administration. Furthermore, the maximum tolerated dose was found to be 0.5 mg/kg when delivered to rats for five consecutive days through iv injection.[179] At this dose, no deaths were observed, but minor toxicities were found to affect the spleen and thymus. Acute toxicity, with adverse effects on the gastrointestinal, hematopoietic and reproductive systems, occurred at single iv doses of 40 mg/kg in male rats and 50 mg/kg in female rats.[180] While acute toxicity has been demonstrated, little evidence of pharmacokinetic analysis or

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efficacy in the literature precludes additional discussion here, but macromolecular constructs containing belotecan will be discussed later.

3.1.11. Silatecans. Animal Models. While much work with the CPTs has focused on improving solubility, a series of molecules with increased lipophilicity have been prepared in an attempt to increase cellular uptake and oral availability. One such library of CPT derivatives, known as "silatecans", employ silyl substituents at the 7-position to increase lipophilicity in an attempt to promote drug trafficking across the blood-brain barrier. Curran and co-workers developed a library of silyl-modified CPTs with such properties.[181] The lactone stability of the silyl modified derivatives exceeded that of CPT and other 10,11-methylenedioxyCPTs, with sustained or increased TOP I activity in various cell lines including a U87 glioma cell line. This activity was highest with 7-*tert*-butyl-dimethylsilyl-10-hydroxycamptothecin (TBDMS-10-hydroxyCPT), which provided promising results in subcutaneous U87 human glioma tumor xenografts. Furthermore, intracranial U87 tumor xenografts were employed to investigate blood−brain barrier trafficking of this silatecan. Median survival time in the control group was 58 days, with all animals dead by day 70, whereas all animals treated with subcutaneous injections of the silatecan were alive at 120 days. The pharmacokinetics of DB-67 were later measured in SCID mice and found a 1.4 h plasma half-life of the lactone with a 17 μg·h/mL plasma AUC value.[182] Liver showed the highest AUC value of 57 μg·h/mL with the kidney (30 μg·h/mL) and lung (20 μg·h/mL) providing lower AUC values.

A slightly different silatecan, called karenitecin, was developed by Van Hattum and co-workers, which employed a trimethylsilane attached through an ethyl linkage at position 7.[183] The maximum tolerated dose was determined to be 1.0 mg/kg for five consecutive days when administered through ip injection, whereas a dose of 1.5 mg/kg administered orally on the same schedule gave equitoxic results. While the *in vivo* efficacy of karenitecin was the same as or slightly better than other CPTs in four colon cancer xenografts, oral bioavailability was a major advantage: 67% of karenitecin is bioavailable compared to 30% for topotecan[184] and 49% for 9-AC capsules.[185]

This study was expanded into human ovarian cell lines and showed promising effects *in vivo*.[186] When delivered to mice with human tumor xenografts through ip administration daily for five days, topotecan showed >75% growth inhibition in only one cell line at doses of 1.5 or 2.0 mg/kg. Conversely, karenitecin showed >80% growth inhibition in all three cell lines when given at a dose of 1.0 mg/kg. Additional studies showed potent activity against lung, prostate, breast, melanoma, head and neck cancers, medulloblastoma, neuroblastoma and rhabdomyosarcoma.[187−189] During pharmacokinetic studies in non-human primates, which best represent a model for cerebrospinal fluid (CSF) uptake, it was determined that the lactone in karenitecin was present at greater than 90% of the measurable drug.[190] Furthermore, only 5% of karenitecin was observed in the CSF, with a whole body mean distribution half-life of 0.96 h and an elimination half-life of 7.6 h. Peak CSF distribution was observed between 12 and 25 min after a 0.1 mg/kg iv infusion.

Human Patients. In phase II clinical trials in 41 patients with malignant melanoma, karenitecin was delivered on five consecutive days every three weeks.[191] Only one patient showed complete response, while three showed minor response, ten showed stabilized disease during treatment and 27 saw no effect. Clinical trials with karenitecin are still underway.

3.1.12. ST1481. Animal Models. Other lipophilic derivatives of CPT, 7-oxyiminomethyl derivatives, were investi-

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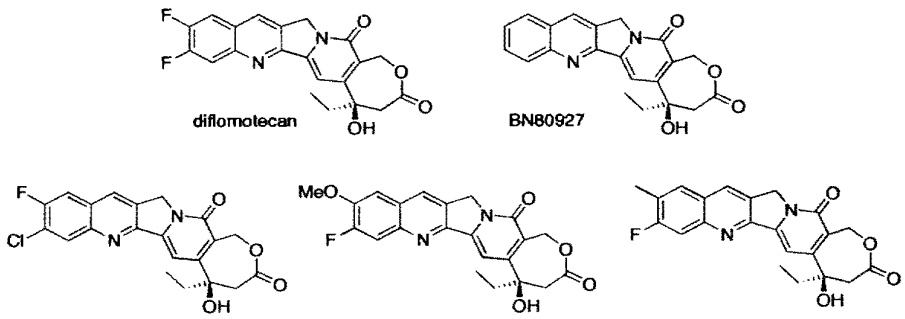

Figure 2. Diflomotecan and related modified camptothecins.

gated by Zunino and co-workers.[192] From the 37 derivatives synthesized by this group, 27 showed increased activity in cell culture as compared to topotecan and 12 were more active than SN-38. Correlations between drug activity and steric or electronic substituents on the oxime were identified with a tri(*tert*-butyl) compound, ST1481, proving to be the most potent derivative *in vitro*. In athymic nude mice with NCI-H460 and LX-1 lung tumor xenografts, the MTD of ST1481 was determined to be 3 mg/kg as compared to 15 mg/kg for topotecan when administered on a schedule of every four days for four total doses. A 100% tumor volume inhibition (TVI) was observed when using ST1481 at the MTD, with 100% complete responses (CR) in LX-1 tumors as compared to 99% TVI and 50% CR observed with topotecan 10 days after treatment. Furthermore, when topotecan was administered at 2 mg/kg five times a week for 10 weeks, 4 out of 10 tumors had regressed by 30 days but all tumors were present at 100 days. However, at 0.5 mg/kg five times a week for 5 weeks, the ST1481 group had no detectable tumors on day 30. At 100 days, 5 of 8 tumors were not detectable.[193] From the data, the oxime derivative proved to be about five times more potent than topotecan, with a 5-fold increase in AUC (0.55 μg·h/mL; 2.43 μg·h/mL) and half-life (2.77 h; 11.8 h) when delivered orally at 15 mg/kg for topotecan and 5 mg/kg for the oxime derivative.

3.1.13. Chimmitecan. Animal Models. Ding and co-workers developed a series of 9-alkyl derivatives that inhibited TOP I effectively *in vitro*. Initial studies concluded that chimmitecan, with an allyl group at position 9 and a hydroxyl at position 10, showed the most promise for *in vivo* investigation.[194] Chimmitecan was delivered every three weeks through iv injection at 15 mg/kg in three of four human xenograft nude mouse models with different experimental end points (A549 lung cancer, 15 weeks; MDA-MB-435 breast cancer, 12 weeks; BEL-7402 hepatocellular cancer, 12 weeks). HCT-116 colon cancer was treated by dosing every two weeks for 6 total weeks. Antitumor efficacies were reported as percent tumor inhibtion/control for each of the cell lines. Against the four tumor xenograft models, chimmitecan showed efficacies of 23.0%, 24.2%, 28.2% and 17.6%, respectively, while CPT-11 showed efficacies of 34%, 42%, 15% and 21% for each cell line, respectively. When compared to CPT-11 at equivalent doses, chimmitecan was significantly more potent in BEL-7402 and A549 models. When delivered orally to treat A549 tumors every two days for seven total doses, tumor inhibition was observed at low doses of 4.5 mg/kg and antitumor activity was observed at 9.0 mg/kg with 22.2% efficacy.

3.2. E-Ring Modifications. As previously described, modifications to the A and B rings of CPT improve solubility and lactone stability while often retaining, if not improving, efficacy. The C and D rings are the least common sites for modifications due to complete inactivation of the molecule. Very few modifications to the E ring have been reported, due to the poor efficacy upon manipulation of the lactone. However, the homocamptothecins shown in Figure 2 have offered promise with E-ring stabilization and antitumor activity.

3.2.1. Diflomotecan (BN80915). Animal Models. Bigg and co-workers developed a series of enlarged E rings, called homocamptothecins (hCPT), which are characterized by a β-hydroxylactone instead of the natural α-hydroxylactone.[195] The addition of a methylene group within the E ring stabilizes

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the lactone, leaving 87% in the intact lactone form at pH 7.4 after 24 h compared to only 20% at 1 h for CPT, while retaining TOP I activity comparable to CPT. The *in vitro* investigation of this series of compounds led to the identification of four lead compounds, which possessed subnanomolar IC_{50} values in one or more cell lines (A427, PC-3, K562adr and MCF7mdr). Interestingly, each of the compounds contains a fluorine substituent at position 10, 11 or both. Each compound was tested in subcutaneous HT-29 tumor xenograft models in nu/nu female athymic nude mice. When administered through ip injection 12 times over three weeks using a four days on and three days off schedule, an MTD of 0.32 mg/kg was determined. At this MTD, using the methoxy, methyl or difluoro compounds, a tumor growth delay of 12 days, 7 days and 25 days was observed for the derivatives, respectively. The chlorinated compound, however, showed a tumor growth delay of 7 days at 1.25 mg/kg as compared to a 4 day delay at 0.625 mg/kg for CPT. This study was expanded to additional tumor xenografts and compared to topotecan, CPT, and SN-38 with similar results showing higher stability of cleavage complexes and subnanomolar IC_{50} values.[196]

Human Patients. In phase I trials, the MTD was determined to be 0.27 mg/day when administered five times orally every three weeks to adults with solid tumors.[197] Pharmacokinetics were measured on the fifth consecutive day of treatment showing AUC values of 0.014 $\mu g \cdot h/mL$ and a half-life of 3.7 h. Furthermore, several patients in this study that had been heavily treated prior to this study still showed signs of extended periods of stable disease. When administered through the iv route, the MTD of diflomotecan was determined to be 0.15 mg/m^2 (~0.0041 mg/kg) as a 20 min iv infusion for five days every three weeks with a recommended dose of 0.125 mg/m^2 (~0.0034 mg/kg).[198] The treatment also showed very few toxic side effects and either stabilized patients or produced a partial response although this was outside the scope of the study.

A recent phase I study utilizing the flat dosing strategy finds that toxic doses range from 2 mg to 4 mg, due to interpatient variability.[199] Upon administration of 2 mg of diflomotecan through 20 min iv infusion every three weeks, an AUC value of 0.11 $\mu g \cdot h/mL$ was found with a half-life of 4 h. When using 3 mg and 4 mg doses, the AUC values increased slightly to 0.12 $\mu g \cdot h/mL$ and 0.16 $\mu g \cdot h/mL$, with half-lives of 3.3 and 4.6 h, respectively. From the pharmacokinetic data and the toxicities observed, it was determined that the toxic variability was due to drug exposure and not specific dose. With such variability, the authors suggest that further investigation using this strategy for delivery of diflomotecan is not warranted. Interpatient variability with diflomotecan complicates the future utilization of this drug, but future studies at different doses using different schedules may prove advantageous.

3.2.2. Homocamptothecin and BN80927. Animal Models. Although the fluorinated hCPTs showed promising results *in vitro* and *in vivo*, Bigg and co-workers investigated a hCPT without a quinoline substituent,[200] and a hCPT with a 4-methyl-piperazinomethyl group at position 7, a methyl at position 10 and a chloride at position 11.[201] The study of unsubsitituted hCPT provided results similar to the fluorinated compound, showing increased lactone stability and increased TOP I inhibition. While the lactone undergoes slow hydrolysis to the carboxylate form, the seven-membered ring does not spontaneously recyclize. During *in vivo* studies in athymic mice with HT-29 tumor xenografts, hCPT was administered using a schedule of four days on and three days off for a total of twelve injections at a dose of 1.25 mg/kg as compared to 0.625 mg/kg CPT. Results from this study showed that unsubstituted hCPT inhibited tumor growth as compared to CPT, with tumor volumes of 900, 750, and 400 mm^3 for the control, CPT and hCPT, respectively.

The trisubsitituted hCPT showed greater than 90% closed lactone after 3 h in human plasma, with 50% of the lactone form still present at 24 h. This new hCPT also showed broad antitumor efficacy *in vitro* in breast, colon, prostate, ovarian, bladder, leukemia and lung cancers. *In vivo* efficacy was demonstrated through oral administration to mice with either PC3 or DU145 prostate cancer xenografts. In both models, the preferred schedule was twice a day for 14 days, giving 125% and 175% increase in survival for each cell line, respectively. Only minor toxicity resulted in each model, which rebounded after treatment.

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3.3. 20-Hydroxy-Linked Modifications. Lactone stability has been shown to increase upon esterification or alkylation of the 20-hydroxyl group. A hypothesis proposed in 1992 implicates the hydroxyl group as a mediator of lactone hydrolysis by activation of water through a hydrogen bond interaction.[202] While various ester derivatives have been prepared, only a small number of 20-*O*-hydroxyl modifications have been tested *in vivo*. A few examples have already been discussed in the context of A-ring modifications. We address the remaining examples here and discuss utilization of this hydroxyl to covalently append CPT and its derivatives to macromolecular architectures later.

3.3.1. Hydrophobic Esters of CPT. Animal Models. Cao and co-workers reported the synthesis of a series of esters of CPT and 9-nitrocamptothecin (9-NC).[203] In human plasma, the lactone of propanoate of CPT diminished to 56% over 6 h as compared to only 0.5% at 2 h for CPT. Comparatively, the propanoate of 9-NC exhibited higher stability in human plasma, with 64.4% lactone present at 6 h and 5.8% present at 51 h, compared to only 7% at one hour for 9-NC. The propanoate of CPT was investigated in CLO-breast tumor and SPA lung tumor xenografts in nude mice, while the propanoate of 9-NC was investigated in SQU colon cancer cells. The breast tumor sizes were measured at 56 days, with average tumor sizes of mice treated with propanoate ranging from ~500 to 100 mm^3 at doses of 5–8 mg/kg, as compared to tumor sizes of ~4500 mm^3 in the control animals. Similar results were observed in SPA and SQU tumor xenografts. In a subsequent study with 9-NC, the propanoate ester and butyrate esters showed the greatest toxicity in HL-60 cells and U-937 cells *in vitro*.[204] *In vivo* data using Doyle lung carcinoma, BRO-melanoma, SPA lung carcinoma and BRE stomach tumor xenografts also suggested promising antitumor activity, however, the poor solubility of the constructs prompted the investigators to dissolve the drugs in cottonseed oil and inject the solutions into the stomach cavity through the anterior wall of the abdomen every day for five consecutive days each week for the duration of the experiment. While the compounds showed promising lactone stability and antitumor activity, the route of administration was not ideal for prolonged therapy.

3.3.2. Amino Acid Esters of CPT. Animal Models. Lerchen and co-workers at Bayer AG developed a series of 20-hydroxyl linked glycoconjugates of CPT with preferential cellular uptake in cancer cells.[152] CPT was acylated with a series of dipeptides, which were then linked to the carbohydrate targeting moiety (*p*-aminophenyl 3-*O*-methyl-β-L-fucopyranoside) through a thiourea linkage. Interestingly, when the amino acid adjacent to the CPT was glycine, stability in cell culture was diminished, while valine improved stability. The conjugate with the greatest stability in culture medium and lowest IC_{50} value in HT29 cells proved to be Sug-HisVal-CPT (Figure 3). When delivered intravenously at the MTD of 32 mg/kg for three consecutive days to mice bearing breast cancer MX-1 xenografts, treated tumor growth/control group growth (T/C %) values were determined to be 1.8%, as compared to 12.7% for topotecan at an MTD dosage of 2.5 mg/kg on the same schedule. Fluorescence experiments revealed that cellular uptake in HT29 cells occurs through active transport into the lysosomes. While this study indicates a significant improvement in the design of novel CPTs, further investigation of this conjugate must be completed to determine the pharmacokinetics as compared to the parent molecule.

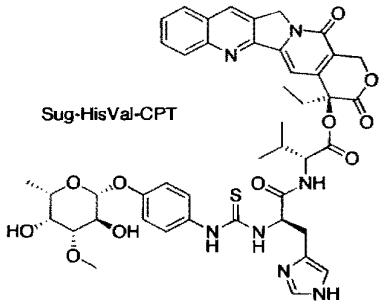

Figure 3. Amino acid linked 20-hydroxy ester of camptothecin.

4. Macromolecular Architectures for Passive Drug Delivery

Although a significant library of CPTs has been developed, macromolecular delivery agents have focused on CPT or SN-38. Here, we describe the noncovalent and covalent approaches toward increasing *in vivo* efficacy using macromolecular constructs. While a large number of architectures have also been developed, many will not be discussed here due to the absence of *in vivo* data. The compounds described in the literature, that have not yet been investigated *in vivo*, include noncovalent dendrimer constructs from Ghandehari,[205] Grinstaff[206] and Simanek[207] and covalent dendrimer constructs from Shabat[208–212] and Simanek,[213] "clicked"

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polymers from Emrick,[214] micelles from Torchilin[215] and Kataoka,[216] and PEGylated nanoscale graphene oxide from Dai.[217]

4.1. Noncovalent Drug Delivery Systems. Various noncovalent drug delivery systems have been developed to improve solubility and lactone stability of CPTs, including micelles, liposomes, dendrimers, nanoparticle drug formulations and hydrogels. Each noncovalent drug delivery vehicle is summarized herein, with comparisons made between the pharmacokinetics and efficacy of the complex to that of the free drug and further summarized in Table 2.

4.1.1. Micelles. Micelles are macromolecular constructs formed from an aggregation of amphiphilic molecules, which display charged or charge-neutral hydrophilic head groups at the water interface and hydrophobic chains toward the center of the vesicle, commonly forming a spherical structure. The hydrophobic interior of the structure enables efficient encapsulation of hydrophobic molecules, such as the CPTs, for drug delivery.

Camptothecin Micelles. Animal Models. Poly(ethylene glycol) is a polymer commonly used to increase solubility and bioavailability of otherwise insoluble drugs. Through the development of poly(ethylene glycol)-poly(aspartic acid) block copolymers and subsequent partial esterification with benzyl alcohol, the formation of micellar structures containing a benzyl rich core capable of encapsulating CPT and a water-soluble PEG corona has been realized.[218] Micelles formed from block copolymers containing 5 kDa PEG chains and a poly(aspartic acid) block 25 monomer units long esterified with benzyl groups to 70% were used to encapsulate CPT for treatment of mice with C26 colon tumor xenografts.[219] The micelles were found to have an average diameter of 190 nm and 63% incorporation efficiency. Incorporation efficiency reflects the amount of drug encapsulated in the vesicle after removal of unencapsulated drug. In this example, 2 mg of free CPT was mixed with 5 mg of PEG-P(AspBz) resulting in vesicles with approximately 20 wt % CPT. When the micelles were delivered through iv injection to tumor bearing mice at doses of 15 mg/kg and 30 mg/kg, 72.5% and 81.5% tumor growth inhibition at 8 days was observed as compared to 51.4% for the solution of free CPT at a dose of 1.5 mg/kg. The micelles released nearly 50% of CPT at 24 h, however, blood plasma levels were 150 times higher at 24 h as compared to free CPT. Furthermore, tumor levels showed an 8-fold increase in CPT when using the micelle as compared to the free drug.

10-Hydroxycamptothecin Micelles. Animal Models. Micelles of poly(ethylene glycol)-poly(γ-benzyl-L-glutamate) were also developed to encapsulate hydroxycamptothecin at 57% efficiency.[220] The micelles had an average diameter of 200 nm, with 7.5 wt % drug loading capacity. After ip administration of 3 mg/kg for five consecutive days, the micelles showed a slow release of hydroxycamptothecin, with maximum blood concentrations at 1 h as compared to the carboxylate form of the free drug with lower concentrations at 0.25 h. The beta half-lives of the carboxylate and micellar 10-hydroxycamptothecin forms were determined to be 5.8 and 10.2 h, respectively, with AUC values of 431 μg·h/mL and 1034 μg·h/mL, respectively. Antitumor effects in golden hamsters with cheek pouch carcinomas showed a 66% decrease in tumor volume when treated with the micellar formulation and only 50% decrease in tumor volume when treated with the free hydroxycamptothecin. This delivery method, however, suffers from poor loading efficiency and cellular inflammation due to the toxicity of the micelles. Pharmacokinetic data and efficacy data show moderate success, but minimal investigation using noncovalent

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Table 1. Pharmacokinetic Data of Small Molecule Camptothecin Derivatives[a]

drug	subject (sex)	tumor type	route (time)	dosing schedule[b]	dose (mg/kg)	MTD (mg/kg)	$t_{1/2}$ (h)	plasma AUC (μg·h/mL)	ref
CPT	SD rats (M)	none	iv	q1d × 1	1.0	NR	NR	0.21	49
CPT	Balb/c mice	none	iv	q1d × 1	10	NR	α: 0.3 β: 4	1.80	120
Na-CPT	SD rats (M)	none	iv	q1d × 1	1.0	NR	NR	0.37	49
Na-CPT	Balb/c mice	none	iv	q1d × 1	10	NR	α: 0.1 β: 2	1.47	120
TPT	SCID mice	none	sc	q1d × 1	5.3	NR	2	0.14	71
TPT	mice	none	po (gavage)	q1d × 1	2.0	2	α: 0.6 β: 3	0.09	73
TPT	mice	none	ip	q1d × 1	1.75	1.75	α: 0.3 β: 3	0.29	73
TPT	athymic mice (F)	none	iv	q1d × 1	15	NR	2	2.5	76
TPT	athymic mice (F)	none	po	q1d × 1	15	NR	3	0.55	76
TPT	adult humans	solid	iv (0.5 h)	q1d × 5, q3w	0.05	0.05	α: 0.1 β: 4	4.1	78
TPT	adult humans	solid	iv (24 h)	q1d × 1, q1w	0.04	0.04	4	0.12	79
TPT	adult humans	solid	iv (24 h)	q1d × 1, q1w	0.04	0.04	3	0.06	80
TPT	adult humans	solid	iv (0.5 h)	q1d × 5, q3w	0.04	0.04	α: 0.2 β: 3	3.34	82
TPT	pediatric humans	solid/leukemia	iv (24 h)	q1d × 1, q3w	0.15	0.15	α: 0.2 β: 3	NR	83
TPT	adult humans	solid	iv (0.5 h)	q1d × 1, q3w	0.61	0.61	6	3.38	84
TPT	adult humans	solid	iv (24 h)	q1d × 3, q3w	0.03	0.03	3	NR	88
TPT	adult humans	solid	iv (24 h)	q1d × 5, q3w	0.06	0.06	NR	0.11	89
TPT	adult humans	colorectal	iv (0.5 h)	q1d × 5	0.10	0.10	4	0.34	91
CPT-11	Balb/c mice (F)	L1210 leukemia	iv	q1d × 1	10	NR	β: 1	2.96	98
CPT-11	Balb/c mice (F)	L1210 leukemia	iv	q1d × 1	20	NR	β: 1	7.65	98
CPT-11	Balb/c mice (F)	L1210 leukemia	iv	q1d × 1	40	NR	β: 1	23.45	98
CPT-11	adult humans	solid	po (PFC)	q1d × 5, q 3w	1.35	1.35	12	0.8	101
CPT-11	adult humans	colorectal	iv (1.5 h)	q1d × 1, q1w, 4/6	3.38	3.38	NR	3.08	104
CPT-11	adult humans	solid	iv (1.5 h)	q1d × 1, q3w	6.49	6.49	6	15.2	106
CPT-11	adult humans	solid	iv (1.5 h)	q1d × 1, q3w	7.84	7.84	13	22.3	107
CPT-11	adult humans	solid	iv (0.5 h)	q1d × 1, q3w	9.46	9.46	7	25.6	109
9-AC	Balb/c mice	none	iv	q1d × 1	10	NR	α: 0.2 β: 1	3.58	120
9-AC	adult humans	peritoneal	ip	q2d × 6, q4w	0.04	0.04	21	0.46	121
9-AC	adult humans	solid	iv (24 h)	q1d × 3, q3w	0.11	0.11	31	0.11	122
9-AC	mouse	none	po (gavage)	q1d × 1	4.1	NR	1	0.06	129
9-AC	dog	none	po	q1d × 1	1.0	NR	21	0.31	129
9-AC	adult human	none	po	q1d × 1	0.10	NR	7	0.31	129
9-AC	adult human	none	po	q1d × 1	1.0	NR	13	9.12	129
9-NC	mouse	none	po (gavage)	q1d × 1	4.1	NR	10	0.44	129
9-NC	dog	none	po	q1d × 1	1.0	NR	6	0.19	129
9-NC	adult human	none	po	q1d × 1	0.1	NR	3	2.60	129
9-NC	adult human	none	po	q1d × 1	1.0	NR	5	17.1	129
9-NC	SD rats (M)	none	iv	q1d × 1	1.5	NR	0.5	0.55	130
9-NC	SD rats (M)	none	iv	q1d × 1	3.0	NR	0.5	1.48	130
9-NC	SD rats (M)	none	iv	q1d × 1	6.0	NR	0.5	2.38	130
9-NC	SD rats (M)	none	po (tube)	q1d × 1	6.0	NR	0.8	0.54	130
9-NC	adult humans	ovarian, tubal, peritoneal	po	q1d × 4	0.04	NR	14	1.51	132
GG211	adult humans	solid	iv (0.5 h)	q1d × 5, q3w	0.03	0.03	4	0.02	136
GG211	adult humans	solid	iv (0.5 h)	q1d × 5, q3w	0.03	0.03	α: 0.1 β: 0.9 γ: 9.6	0.02	137
GG211	adult humans	solid	iv (24 h)	q1d × 3, q4w	0.03	0.03	6	NR	138
GG211	adult humans	solid	iv (24 h)	q1d × 21	0.01	0.01	NR	0.15	139
GG211	adult human	solid	iv (0.5 h)	q1d × 5, q3w	0.03	0.03	0.9	0.03	140
BMS-422461	SD rats (M)	none	ia	q1d × 1	1.36	NR	0.2	0.47	143
10,11-MC	Balb/c mice	none	iv	q1d × 1	10	NR	α: 0.1	0.32	120

Table 1. Continued

drug	subject (sex)	tumor type	route (time)	dosing schedule[b]	dose (mg/kg)	MTD (mg/kg)	$t_{1/2}$ (h)	plasma AUC (μg·h/mL)	ref
							β: 0.8		
DX-8951	adult humans	solid	iv (0.5 h)	q1d × 5, q3w	0.01	0.01	11	3.2	155
DX-8951	adult humans	solid	iv (24 h)	q1d × 1, q3w	0.06	0.06	NR	1.8	156
DX-8951	adult humans	solid	iv (24 h)	q1d × 21	0.004	0.004	27	0.17	157
DX-8951	adult humans	solid	iv (0.5 h)	q1d × 1, q3w	0.14	0.19	7	3.42	160
DX-8951	adult humans	solid	iv (0.5 h)	q1d × 1, q3w	0.14	0.14	10	2.1	161
DX-8951	adult humans	solid	iv (0.5 h)	q1d × 1, q1w	0.06	0.06	8	1.2	162
DX-8951	adult humans	solid	iv (24 h)	q1d × 1, q3w	0.01	0.01	7	NR	163
DX-8951	adult humans	breast	iv (0.5 h)	q1d × 5, q3w	0.01	0.01	8	NR	164
DX-8951	adult humans	NSC lung	iv (0.5 h)	q1d × 5, q3w	0.01	0.01	8	NR	165
DB-67	SCID mice	none	iv	q1d × 1	10	NR	1.4	17	182
BNP1350	Rhesus monkeys	none	iv (1 h)	q1d × 1	0.1	NR	α: 1	0.15	190
							β: 8		
ST1481	athymic mice (F)	none	po	q1d × 1	5	NR	11	2.5	193
BN80915	adult humans	solid	iv (0.3 h)	q1d × 5, q3w	0.004	0.004	4	0.01	198
BN80915	adult humans	solid	po	q1d × 5, q3w	0.004	0.004	4	0.01	197
BN80915	adult humans	solid	iv (0.3 h)	q1d × 1, q3w	0.05	NR	3	0.16	199

[a] Abbreviations: MTD, maximum tolerated dose; AUC, area under the curve; CPT, camptothecin; Na-CPT, camptothecin sodium carboxylate; TPT, topotecan; CPT-11, irinotecan; GG211, lurtotecan; DX-8951, exatecan; BNP1350, karenitecin; BN80915, diflomotecan; SD, Sprague–Dawley; SCID, severe combined immunodeficiency; M, male; F, female; NSC, non-small-cell; iv, intravenous; sc, subcutaneous; po, oral; ip, intraperitoneal; ia, intrarterial; PFC, powder filled capsule; NR, not reported. [b] Dosing abbreviations: q1d × 1, one dose; q1d × 5, q3w, five consecutive days every three weeks; q1d × 1, q1w, one dose per week; q1d × 1, q3w, one dose per week every three weeks; q1d × 5, one dose per day for five days; q1d × 1, q1w × 4/6, one dose per week every week for four out of six weeks; q2d × 6, q4w, every two days for six total doses every four weeks; q1d × 21, every day for 21 consecutive days.

Table 2. Pharmacokinetic Data of Macromolecular Constructs with Noncovalent Attachment of Camptothecin Derivatives[a]

compound (drug)	structure	wt % CPT	subject (sex)	tumor type	route (time)	dosing schedule[b]	dose (mg/kg)	MTD (mg/kg)	$t_{1/2}$ (h)	plasma AUC (μg·h/mL)	ref
PEG-P(bzAsp) (CPT)	micelle	40	ddY mice (M)	none	iv	q1d × 1	1	NR	NR	47	218
HSA-DB-L (CPT)	liposome	3	ddY mice	none	iv	q1d × 1	0.08	NR	0.8	25	225
DSPC-Chol (TPT)	liposome	12	Balb/c mice	none	iv	q1d × 1	0.6	NR	α: 2	360000	227
									β: 3		
TEA-Pn (CPT-11)	liposome	41	SD rats	none	iv	q1d × 1	4.1	>130	7	1400	229
TEA-SOS (CPT-11)	liposome	41	SD rats	none	iv	q1d × 1	4.1	>130	11	2100	229
LE-SN-38 (SN-38)	liposome	3	CD2F1 mice (M)	none	iv	q1d × 5	0.15	0.15	NR	3	232
LE-SN-38 (SN-38)	liposome	3	CD2F1 mice (F)	none	iv	q1d × 5	0.23	0.23	NR	3	232
LE-SN-38 (SN-38)	liposome	3	beagle dogs	none	iv	q1d × 1	0.04	0.04	3	0.5	232
OSI-211 (GG211)	liposome	14	SD rats (M)	none	iv	q1d × 1	1.4	NR	21	1900	233
OSI-211 (GG211)	liposome	14	athymic mice (F)	none	iv	q1d × 1	1.26	NR	2	130	234
OSI-211 (GG211)	liposome	14	adult humans	solid	iv	q1d × 1, q3w	0.01	0.01	7	12	237
OSI-211 (GG211)	liposome	14	adult humans	solid	iv (0.5 h)	q1d × 3, q3w	0.01	0.01	7	10	238
OSI-211 (GG211)	liposome	14	adult humans	solid	iv (0.5 h)	q1d × 3, q3w	0.006	0.006	5	4	239
9NC.NP (9-NC)	NP	0.6	Wistar rats (M)	none	iv	q1d × 1	2	NR	2	4	251
SMEDDS-T (9-NC)	NP	0.5	SD rats (M)	none	po (gavage)	q1d × 1	0.18	0.18	4	0.36	252
SMEDDS-C (9-NC)	NP	0.5	SD rats (M)	none	po (gavage)	q1d × 1	0.18	0.18	6	0.35	252

[a] Abbreviations: MTD, maximum tolerated dose; AUC, area under the curve; CPT, camptothecin; TPT, topotecan; CPT-11, irinotecan; GG211, lurtotecan; NP, nanoparticle; M, male; F, female; SD, Sprague–Dawley; iv, intravenous; po, oral; NR, not reported. [b] Dosing abbreviations: q1d × 1, one dose; q1d × 1, q3w, one dose per week every three weeks.

drug–micelle complexes has been completed due to success with other forms of noncovalent drug delivery. Furthermore, CPT attached covalently to micelles has proven successful as will be discussed later.

4.1.2. Liposomes. Liposomal drug delivery has received much attention for the delivery of a variety of insoluble therapeutics, including the CPTs.[221] Burke observed the need for an alternative route to deliver CPTs and investigated liposomal drug delivery with CPT, 9-AC, 9-NC, 10-hydroxyCPT and topotecan. Lactone stability increases when drugs were noncovalently complexed with liposomes.[222,223] Current *in vivo* efforts with liposomal formulations are summarized below.

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Camptothecin Liposomes. Animal Models. A series of lipids were investigated to develop liposomes with high levels of CPT loading. Results showed that cardiolipin and *N*-glutaryl phosphatidyl ethanolamine (NGPE) had 67% and 97% drug loading, respectively, while other neutral or single, negatively charged head groups provided <5% encapsulation.[224] Loading is a measure of the difference between total drug in solution and free drug. At a 12.5:1 wt/wt ratio of lipid to drug, 95% loading is observed, which corresponds to 7 wt % CPT. Antitumor activity in an ip injected P388 leukemia mouse model was evaluated using a T/C value, which represents the ratio of median survival in treated mice over control mice. CPT delivered intraperitoneally at a dose of 40 mg/kg resulted in a T/C value of 2.07, whereas toxicity was observed at this dose using liposomal CPT. Decreasing the dose of liposomal CPT to 20 mg/kg resulted in a T/C value of 1.86. An L1210 leukemia model gave T/C values between 0.85 and 0.92 for free CPT at doses between 30 and 60 mg/kg, whereas liposomal CPT afforded a T/C of 1.46 at a dose of 20 mg/kg. Biodistribution studies after iv administration of 10 mg/kg CPT found high quantities of drug in the lung at 6 h, with decreasing levels at 24 h, while liposomal CPT afforded negligible uptake in all organs.

Alternatively, liposomes developed from bis(dodecyl)benzoic acid and poly(ethylene glycol) with a coating of human serum albumin (HSA) achieved 80% CPT encapsulation efficiency.[225] Blood plasma levels increased dramatically from an AUC value of 1.1 μg·h/mL for the CPT solution to an AUC value of 24.8 μg·h/mL for the HSA-coated liposome after a 2.5 mg/kg dose with respect to CPT. When delivered to mice with C26 colon carcinomas through iv injection of 15 mg/kg, 84.6% tumor growth inhibition was observed as compared to control mice. However, when delivered at a 10 mg/kg dose on days 1 and 3, significant weight loss (>20%), a common marker for CPT toxicity, was observed. Biodistribution studies with this liposome showed nearly 10-fold increase in tumor accumulation with a 60-fold increase in blood plasma at 8 h as compared to the tumor accumulation seen when using the free drug.

Topotecan Liposomes. Animal Models. The success observed in liposomal formulations of CPT extends to more soluble topotecan. One study produced a 400-fold increase in plasma AUC when topotecan was encapsulated in sphingomyelin and cholesterol liposomes as compared to free drug.[226] Furthermore, lactone stability was enhanced with 84% lactone present at 24 h after injection of the liposome compared with only 50% lactone present at five minutes after injection of free drug. Mice bearing L1210 ascites treated with a single iv dose of 20 mg/kg free drug or three 4 mg/kg doses on days 1, 5, and 9 provided an 11 day median survival time and 15 day median survival time at three doses of 8 mg/kg. Administration of liposomal topotecan at a single dose of 20 mg/kg or the multiple dosing strategy of 4 mg/kg or 8 mg/kg afforded a median survival time of >60 days. However, liposomal topotecan led to more weight loss as compared to an equivalent dose of free drug. To overcome the toxicity, liposomal doses at half the free drug dose were utilized, resulting in efficacy that was still superior to that seen using the free drug. In a liver metastasis model, similar results were obtained, with liposomal topotecan providing >60 day survival in all animals at all doses half that of free drug. A human breast carcinoma model (MDA-435/LCC6) showed median survival times between 20 and 30 days depending on dose, however, liposomal topotecan provided median survival times between 37 and 52 days.

A subsequent study using DSPC/Chol lipids found that at 48 h greater than 70% topotecan existed in the lactone form when encapsulated in liposomes, while the free drug showed a hydrolysis half-life of 0.33 h.[227] Pharmacokinetic data of the DSPC/Chol−topotecan complex show an approximate 40-fold increase in the AUC values of free drug from 9400 μg·h/mL to 358400 μg·h/mL with an increase in alpha half-life from 0.1 to 2.1 h after a single iv injected dose of 5 mg/kg. Only a small increase, however, was observed in beta half-life from 2.6 to 2.9 h. Although liposomal topotecan was found to leak significantly in the presence of plasma, the antitumor efficacy of liposomal topotecan surpassed that of the free drug. When delivered at this dose weekly for two weeks, topotecan showed a 21% tumor growth inhibition at 32 days in mice bearing C26 tumors, while liposomal topotecan exhibited 57% tumor growth inhibition.

Irinotecan Liposomes. Animal Models. Irinotecan was also investigated using DSPC/Chol liposomes which could be loaded with up to 35 wt % drug. This formulation could provide a significant increase in plasma levels when the complex was delivered through iv injection at 50 mg/kg to SCID/Rag-2 M mice.[228] Furthermore, the lactone stability of irinotecan was increased due to liposomal protection, with

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>95% lactone at 4 h and 80% at 24 h as compared to only 40% for the free drug at 1 h. Toxicity studies showed that a 100 mg/kg dose of free irinotecan elicited toxic effects within 1 min. This toxicity was not observed with the same dosage of the liposomal formulation, but weight loss over time was observed as a toxic marker. When free irinotecan was delivered as a single 50 mg/kg dose to mice inoculated with sc LS180 tumors, tumor growth was delayed for 22 days post inoculation as compared to 19 days for control animals. This tumor growth inhibition increased to 30 days when the irinotecan−liposome complex was delivered at 50 mg/kg on days 11, 15, and 19. The liposomal formulation showed delayed growth at 34 days, and no growth at 40 days for the single and triple doses, respectively. Similar results were obtained in LS174T tumors, which exhibit relatively slow growth and mimic liver metastases secondary to colorectal cancer. The potential for the treatment of liver metastases was suggested by histology wherein accumulation was observed at the tumor's periphery, while no tumor uptake at 24 h post iv injection was observed.

To improve drug loading in liposomes, Drummond and co-workers developed a method of delivery that utilizes polyphosphate or sucrose octasulfate coencapsulation. This method achieved a loading efficacy of 1.4 mol of CPT-11/mol of phospholipids or 109,000 drug molecules per liposome, corresponding to a 10- to 20-fold increase in drug loading.[229] Blood plasma half-lives of 7 and 11 h were obtained in Sprague−Dawley rats after iv administration of the polyphosphate and sucrose octasulfate liposome complexes, respectively. The sucrose octasulfate formulation showed improved overall pharmacokinetics, with an AUC value of 2134 μg·h/mL and 57 h half-life release of CPT-11 from the liposome. Rats bearing HT29 xenografts received the sucrose encapsulated formulation resulting in four (36.4%) tumor free animals at study end (66 days) as compared to a maximum survival time of 35 days for mice receiving 50 mg/kg CPT-11 every four days for four total iv doses of CPT-11. This method of encapsulation has proven optimal for increasing drug loading, plasma half-life and efficacy as compared to the small molecule. The impact that this strategy has on *in vivo* activity given the need for metabolic activation in the liver or at tumor remains unclear.

SN-38 Liposomes (LE-SN-38). Animal Models. To further improve the antitumor activity of liposomes, SN-38, rather than CPT-11, was encapsulated in liposomes to circumvent the need for metabolic activation of CPT-11. Liposomes were formed from DOPC, cholesterol and cardiolipin with a drug to lipid ratio of 1:18.[230] Studies in P388 tumor bearing mice showed median survival of 20 days when CPT-11 was administered at 16 mg/kg over five consecutive days, whereas administration of 4 mg/kg of LE-SN-38 for five consecutive days offered 100% long-term survival (>60 days). In mice bearing HT29 xenografts a dose of 8 mg/kg gave 88% tumor growth inhibition for LE-SN-38 as compared to a 36% tumor inhibition observed using free CPT-11. Similar results were observed in mice bearing Capan1 and MX-1 xenografts. Similar tumor growth inhibition was observed when administered as a single dose of 10 mg/kg, 20 mg/kg or 40 mg/kg in the same cell lines, but increased body weight loss at the higher doses suggests an optimal delivery of multiple low doses rather than a single high dose.[231]

Detailed pharmacokinetics were also reported with this construct, showing plasma AUC values of 3.92 μg·h/mL and a half-life of 6.38 h for SN-38 after a single 10 mg/kg iv injection.[232] AUC values at 24 h were significant for liver and spleen (~400 μg·h/mL), suggesting recognition by the reticuloendothelial system (RES), which was supported with the presence of extramedullary hematopoesis in dogs. Furthermore, multiple dosing strategies gave MTDs of 5 mg/kg and 7.5 mg/kg for male and female mice, respectively. Single doses afforded an MTD of 37 mg/kg and 46 mg/kg for male and female mice, respectively. Together, these results suggest significant promise for LE-SN-38 at multiple low doses, however, accumulation in the RES warrants concern. Recognition by the RES has been overcome through the use of STEALTH liposomes coated in poly(ethylene glycol). Furthermore, the sex dependent variability of pharmacokinetics in small animals also raises interesting questions toward the treatment of cancer in human subjects.

Lurtotecan Liposomes (SPI-355, NX 211, OSI-211). Animal Models. Liposomal encapsulation of lurtotecan has progressed into phase II clinical trials. PEGylated liposomes, known as STEALTH liposomes, are formed from HSPC/PEG-DSPE with cholesterol resulting in 90% drug encapsulation efficiency in particles of 100 nm diameter.[233] Liposomes were determined to have a half-life of 21 h and an AUC of 1852 μg·h/mL after iv injection of 10 mg/kg of liposomal drug as compared to 1.58 h and 1.49 μg·h/mL after injection of 8.72 mg/kg free drug. In the first study using mice bearing HT29 colon xenografts, drug doses of 15 mg/kg and 24 mg/kg, given once weekly for three weeks, proved toxic, while 6 mg/kg showed minimal toxicity with

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complete responses in all animals >70 days. The free drug on the same dosing schedule showed less toxicity with only 3 out of 10 complete responses and 1 partial response at 24 mg/kg. In the second study using the same tumor model, lower doses of liposomal lurtotecan were investigated from 0.1 mg/kg to 5 mg/kg as compared to 20 mg/kg for free drug. The MTD for liposomal lurtotecan was determined to be between 3 and 5 mg/kg. Although deaths were observed, complete regression was experienced in 10 out of 10 mice at 5 mg/kg. At a dose of 3 mg/kg, seven out of 10 complete responses were observed with one partial response and one death not associated with the therapy, while 20 mg/kg of free drug afforded no complete responses and only 1 partial response.

A subsequent study with liposomal lurtotecan showed 99.5% tumor growth inhibition with no deaths and 19% body weight loss when 9 mg/kg was delivered through 30 min iv infusion to mice with ES-2 tumor xenografts on days 1, 8, and 15. This compares favorably to 95% TGI with 14 mg/kg lurtotecan and 57% TGI with 16 mg/kg topotecan, the respective MTDs.[234] Liposomal lurtotecan also proved successful at tumor inhibition when delivered on days 1 and 9 in a KB xenograft model, with 98% TGI at 9 mg/kg. Administration of a 4 mg/kg dose of liposomal drug proved to be as effective at tumor growth inhibition as 16 mg/kg dose of free drug. Pharmacokinetic analysis after administration of 1 mg/kg of liposomal lurtotecan provided an AUC value of 127 $\mu g \cdot h/mL$ and a 2 h half-life as compared to an AUC value of 0.069 $\mu g \cdot h/mL$ and a half-life of 0.83 h for the free drug. Biodistribution studies showed that lurtotecan accumulated in tumors 9- to 67-fold more effectively when administered in the liposome as compared to the free drug. However, significantly high splenic uptake was observed for liposomal lurtotecan with maximum concentration at 6 h and a decreased concentration in all organs after that time. Similar pharmacokinetic and biodistribution results were also reported in a later paper from this group.[235]

Promise was seen in studies comparing different dosing strategies in SCID mice bearing acute myelogenous leukemia and acute lymphocytic leukemia.[236] To investigate the effectiveness of liposomal lurtotecan when administered either as an early treatment or as a delayed treatment, mice were injected intravenously with KBM-3B cells followed by an incubation period (6−8 days for early treatment; 15−19 days for delayed treatment). After the predetermined incubation period, treatment began and continued on a schedule of five consecutive days, every two days for three total doses or once a week for two consecutive weeks. When delivered at a dose of 2 mg/kg on days 1, 3 and 5, in either early or delayed therapy, significant toxicity was observed. When the dose was decreased to 1.75 mg/kg, poor efficacy resulted in all cases. Treatment with 6 mg/kg on days 1 and 8 proved to be the most successful in both early and delayed therapy, with an average increased life span of 146% in early and 196% in delayed therapy. In early therapy the lowest and most prolonged dose of 1 mg/kg for five consecutive days provided a survival increase of 174%. Similar results were obtained in HL-60 A5 and Molt-4 A4 leukemia models.

Human Patients. In phase I trials with liposomal lurtotecan, using the same 30 min iv infusion once a week for three weeks provided a recommended dose of 3.8 mg/m^2 (~0.1 mg/kg).[237] At this dose, AUC values were determined to be between 2.21 and 28.0 $\mu g \cdot h/mL$, with a mean value of 12.0 $\mu g \cdot h/mL$, and a half-life range of 2.5−11 h with a mean of 6.8 h. To further investigate the potential to increase blood plasma concentration of liposomal lurtotecan, 30 min iv infusions were administered for three consecutive days to patients with leukemia.[238] An MTD similar to what was found previously (3.7 mg/m^2) provided an AUC value of 7.1 $\mu g \cdot h/mL$ after a single injection with a mean half-life of 7.2 h. In patients with refractory solid tumors administered liposomal lurtotecan as a 30 min iv infusion on days 1, 2, and 3 for three consectutive weeks, MTDs of 2.1 mg/m^2 (~0.057 mg/kg) and 1.8 mg/m^2 (~0.049 mg/kg) were found for minimally pretreated patients and heavily pretreated patients, respectively.[239] Pharmacokinetic data obtained from the patients receiving 2.1 mg/m^2 gave AUC values of 4.6 $\mu g \cdot h/mL$ on day one and 7.3 $\mu g \cdot h/mL$ on day three with half-lives of 6.9 and 9.3 h, respectively. Significant variability in pharmacokinetic values was once again observed, with AUC ranges from 0.6 to 24 $\mu g \cdot h/mL$ and half-lives from 3 to 20 h. Although efficacy was not determined under this set of studies, it was determined that lower doses could be

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given over extended periods of time with minimal toxicity, however, less than optimal pharmacokinetics were observed.

In phase II trials, liposomal lurtotecan was delivered to patients with topotecan resistant ovarian cancer on days 1 and 8 of a three-week cycle at doses of 2.4 mg/m^2 (~0.065 mg/kg). No complete regression was observed. Only 8 of 22 patients had stable disease suggesting further investigation with a different schedule or a different tumor is warranted.[240] In a similar study of patients with squamous cell carcinoma of the head and neck using the same dosing regimen, similar results with mild drug toxicity and poor efficacy were found.[241] A comparative study using either this dosing schedule or a 30 min infusion of 1.8 mg/m^2 (~0.049 mg/kg) on days 1, 2, and 3 every three weeks found significant advantage using the latter dosing schedule.[242] In this study, doses on three consecutive days afforded 1 complete response, 5 partial responses, 22 patients with stable disease and 8 patients with progressive disease. No complete responses and only 2 partial responses were observed with the once weekly administration. Interestingly, AUC values in the three daily doses schedule (4.8 μg·h/mL) were slightly lower than the once weekly schedule (5.8 μg·h/mL). Lurtotecan has shown great potential *in vitro* and in preliminary *in vivo* studies, surpassing topotecan efficacy. Phase I and II clinical trials, however, have given less than promising results suggesting a need for alternative routes of delivery.

Belotecan Liposomes (CKD-602 Liposomes). Animal Models. STEALTH liposomes containing CKD602 were investigated in a series of tumor xenografts in mice to determine the optimal dosing schedules and the MTDs.[243] Tumor inhibition was observed when free drug was administered through iv administration to mice bearing A375 human melanoma xenografts on a schedule of once a week for three weeks at doses between 10 mg/kg and 20 mg/kg. Higher doses of 30 mg/kg resulted in tumor regression with no observed toxicity in any case. However, when liposomal CKD-602 was administered intravenously, regression was observed at doses as low as 0.15 mg/kg and up to 1.5 mg/kg with only minimal toxicity. Larger doses between 0.3 mg/kg and 3.0 mg/kg administered every two weeks for three total doses resulted in tumor regression with toxicity occurring at 2.5 mg/kg. Similar results were also observed in ES-2 human ovarian xenografts, with a tumor growth delay of >60 days at doses of 2.25 mg/kg and 8 out of 12 mice were cured. Slightly lower efficacy, however, was observed in H82 human small-cell lung cancer and HT-29 human colon xenografts.

A pharmacokinetic analysis of liposomal CKD-602 was conducted in mice bearing A375 tumors.[244] Liposomal CKD-602 was administered at 1 mg/kg as an iv injection, providing a plasma AUC value of 202 μg·h/mL and tumor AUC value of 13 μg·h/mL. The complex showed higher efficacy as compared to a 30 mg/kg injection of free drug, which displayed a plasma AUC value of 9 μg·h/mL and a tumor AUC value of 12 μg·h/mL. Furthermore, uptake in the liver, kidney and spleen showed 2- to 6-fold increase over plasma AUC values when delivered as free drug, whereas liposomal CKD-602 delivery showed an approximate 5-fold decrease. Interestingly, the brain showed a 2.5-fold increase in AUC for liposomal CKD-602 as compared to free drug, suggesting potential utilization of the complex in the treatment of gliomas.

Human Patients. A phase I and pharmacokinetic investigation of liposomal CKD-602 demonstrated an MTD of 2.1 mg/m^2 (~0.057 mg/kg) when the complex was administered intravenously once every three weeks.[245] This dosage provided a plasma AUC value of 45 μg·h/mL. However, significant interpatient variability was observed, with AUC values ranging from 7 μg·h/mL to 86 μg·h/mL. Partial response was seen in 2 out of 5 patients with ovarian cancer. Stable disease was seen in 6 out of 45 patients with sarcoma, hepatocellular, prostate and thyroid cancer. Although the response was not significant in the series of tumors studied here, phase II studies are currently ongoing, with a focus on ovarian, gastric and small-cell lung cancers.

DB-67 Liposomes (AR-67 Liposomes). Animal Models. Liposomal encapsulation of the silatecan, DB-67, was also investigated in SCID mice to monitor plasma and tissue disposition after delivery of the liposomal derivative as compared to the free drug.[182] A dose of 10 mg/kg of liposomal DB-67 or nonliposomal DB-67 was administered

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through tail vein injection to the mice. Interestingly, the AUC values for nonliposomal and liposomal DB-67 were 17.3 μg·h/mL and 8.2 μg·h/mL, respectively. The lactone half-lives of nonliposomal and liposomal DB-67 were 1.4 and 0.9 h, respectively. This data suggests that the liposome releases DB-67 somewhat rapidly after injection. Furthermore, AUC values measured for each organ show that the lactone form of nonliposomal DB-67 has an extended lifetime in the plasma as compared to the liposomal treatment (17 μg·h/mL vs 7 μg·h/mL). Decreased splenic (14 μg·h/mL vs 29 μg·h/mL) and lung (20 μg·h/mL vs 39 μg·h/mL) AUC values were also observed for the nonliposomal treatment. Each of these data suggests that while DB-67 may be a good candidate for cancer therapy, the use of liposomes drastically change the pharmacokinetic data in a somewhat unexpected fashion.

4.1.3. Nanoparticle Formulations and Emulsions. While CPT has previously been delivered in complex formulations to improve solubility, nanoparticulate structures and emulsions utilizing polymers are described here. Alternative block copolymers including constructs by Onishi[246,247] and Jiang[248] have also been developed, however, a lack of complete pharmacological data prevents their inclusion here.

Hydrophobic Chitosan Nanoparticals with Camptothecin. Animal Models. Chitosan was modified with cholanic acid to increase the hydrophobicity of the nanoparticles for encapsulation of CPT.[249] An 80% encapsulation efficiency was obtained using this construct. While complete tumor regression was not observed, tumor growth inhibition was apparent. Mice bearing MDA-MB231 human breast cancer xenografts were administered a 30 mg/kg iv dose of free CPT, resulting in a 49% tumor growth inhibition. Chitosan nanoparticles containing 10 wt % CPT were given doses of 10 mg/kg and 30 mg/kg resulting in 68% and 77% tumor growth inhibition, respectively. While survival data showed promising results with 75% and 50% of mice alive at 40 days when utilizing 10 mg/kg and 30 mg/kg doses, there were only four mice used in each experiment. Curiously, 25% of mice receiving saline injections were alive at 40 days whereas those treated with free CPT were dead at 35 days. This data suggests that the chitosan nanoparticles slightly improve survival times in mice, however, additional studies with larger test populations are required along with pharmacokinetic investigation to determine their potential for future studies.

Proprietary Nanoparticles with SN-38. Animal Models. In addition to increasing solubility through drug encapsulation, macromolecular constructs often increase lactone stability. When SN-38 is encapsulated in "soft" nanoparticle formulations of 100 to 300 nm in diameter, lactone stability was shown to be 80% at 3 h as compared to 40% with free SN-38.[250] Blood plasma levels of 1 μg/mL in the nanoparticle at 24 h compared favorably to free drug at less than 0.01 μg/mL. Mice bearing HT-29 xenografts were treated with the formulations on days 6, 9, 13, and 16 days after implantation and evaluated for drug efficacy by measuring the time for the tumor to reach 1 g in weight. The tumors took 46, 64, 47, and 50 days to reach 1 g using four different formulations as compared to 22 days for no treatment and 35 days with free irinotecan. Although this study offers a brief glimpse into the use of soft nanoparticles for therapy, extensive investigation with these constructs is limited. Challenges of characterization and polydispersity are overshadowed by the positive results, suggesting that additional investigation into the pharmacokinetics and efficacy may be warranted.

Poly(lactic-co-glycolic acid) Nanoparticles with 9-Nitrocamptothecin. Animal Models. Nanoparticles measuring 200 nm in diameter with 9-NC at 33% drug loading were prepared from poly(lactic-*co*-glycolic acid).[251] The elimination half-life increased as compared to free drug from 0.8 h to 2.45 h at doses of 2 mg/kg through iv injection to rats. Similar results were obtained for the half-life of the lactone, suggesting extended lifetime of the active form. The AUC values for the total free drug and lactone were 0.68 μg·h/mL and 0.45 μg·h/mL, respectively, as compared to 3.7 μg·h/mL for the nanoparticle. *In vitro* cytotoxicity assessments showed that the nanoparticles containing 9-NC were 10 times more cytotoxic than the free drug, presumably due to a higher cellular uptake through endocytosis. Additional *in vivo* studies in tumor bearing mice must be completed to determine the clinical relevance of this construct.

A self-microemulsifying drug delivery system (SMEDDS) was also developed from a mixture of oil (ethyloleat), surfactant (Tween-80 or cremophor EL), cosurfactant (PEG-400) and drug (9-NC).[252] The mean particle size was determined to be between 30 and 40 nm depending on whether Tween-80 (T-form) or cremophor EL (C-form) was utilized as the surfactant, respectively. The half-lives of the

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9-NC suspension, SMEDDS C-form, SMEDDS T-form and 9-NC solution delivered orally to rats were 3.3 h, 6.3 h, 3.9 h and 3.5 h, respectively. The AUC values obtained for each delivery system were found to be 0.16 μg·h/mL, 0.35 μg·h/mL, 0.36 μg·h/mL and 0.24 μg·h/mL, respectively. Furthermore, oral bioavailability increased from 17% in the free drug suspension to 37% for both SMEDDS. Efficacy studies in nude mice bearing sc SKOV-3 ovarian tumors that were treated with 6 mg/kg 9-NC every four days led to 100% tumor growth inhibition at 24 days. This result is significantly better than the 50% growth inhibition observed for the 9-NC suspension. Untreated mice survived between 5 and 10 days.

4.1.4. Hydrogels. Hydrogels are polymers that swell in the presence of water effectively entrapping guest molecules, such as drugs, within the matrix. This technique for drug delivery is attractive due to the biocompatibility, durability, flexibility and ease of injection at the site of interest. Hydrogels have shown great promise in delivering CPTs for cancer therapy.

Camptothecin Hydrogels. Animal Models. The addition of glycerol-2-phosphate to chitosan leads to hydrogel formation at body temperatures due to a lower critical solution temperature (LCST) of 37 °C.[253−255] CPT was loaded into the hydrogel at 4.5 wt % and showed 85% release at 30 days when studied *in vitro*. When administered to mice bearing RIF-1 tumor xenografts through intratumoral injection at 24 mg/kg, tumor growth delays of 25 days were reported. This compares favorably to the delay of only 8 days when using a 6 mg/kg ip injected CPT.[256]

Alternatively, two biodegradable PLGA-PEG-PLGA copolymers with 5 kDa total polymer molecular weight were prepared that formed gels from room to body temperature.[257] These polymers degraded by 50% and 80% over 30 days *in vitro*. CPT was PEGylated prior to encapsulation due to large pore sizes within the gel that otherwise led to fast release of the unmodified drug. Although incorporation of the modified drug decreased the gel's LCST, drug release was observed for over 1 month. Release was dependent on the wt % of copolymer in solution and, to a lesser extent, drug loading. The optimal polymer and drug loading was found to be 18 wt % of a PLGA-PEG-PLGA copolymer with block weights of 1730-1500-1730 Da and 1.5 wt % drug loading. A 65% tumor inhibition rate was observed when this construct was injected subcutaneously into mice with intradermal S-180 sarcomas introduced by injection in the armpits. Higher drug loading (3 wt %) caused toxicity in nearly 50% of animals.

Topotecan Hydrogels. Animal Models. A two-phase system of the PEG hydrogel and liposomes containing topotecan was investigated to exploit the pore size of the parent hydrogel. Free drug entrapped within the liposome-free, one-phase system and free drug were used for comparison.[258] A 60-fold increase in the release of topotecan entrapped in liposomes within the hydrogel was observed *in vitro*. Both systems, however, were evaluated for drug release through subcutaneous administration of 5 mg/kg doses, with observed alpha half-lives of 0.84 h, 0.72 h and 2.70 h for iv topotecan, sc topotecan liposomes and sc hydrogels with topotecan liposomes, respectively. Beta half-lives of 6.2 h, 35.1 h and 89.3 h, respectively, were obtained, however, AUC values of 5.8 μg·h/mL, 3.6 μg·h/mL and 3.0 μg·h/mL for sc topotecan, sc topotecan liposomes and sc hydrogels with topotecan liposomes, respectively, were observed. The poor area under the curve data for all of the suspensions suggests poor bioavailability in sc tissue. The tumor growth suppression in rats with MAT B III tumors, however, gave mixed results, suggesting that free topotecan was the superior in both small and large tumor models.

4.2. Covalent Drug Delivery Systems. The covalent conjugation of CPTs to macromolecular architectures has shown great potential for improving pharmacokinetics and increasing tumor efficacy. Most commonly, CPT is attached to the polymer through an ester bond with the 20-hydroxy moiety. This linkage not only conveys solubility through conjugation with a water-soluble polymer, but also improves lactone stability. Some linkages are chosen as specific substrates for enzymatic cleavage, while others are used due to their pH sensitivity, but may also undergo hydrolysis. The advances with covalently linked CPTs to polymers are discussed here with a table of summarized pharmacokinetics (Table 3) for comparison to the small molecule derivatives in Table 1 and noncovalent pharmacokinetics in Table 2. Covalent constructs offer advantages and disadvantages over noncovalent assemblies. Of the advantages, the opportunity to execute structure−activity studies in a very narrowly defined composition space is attractive. Disadvantages include, in addition to constituting a new drug entity, the

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Table 3. Pharmacokinetic Data of Macromolecular Constructs with Covalent Attachment of Camptothecin Derivatives[a]

compound (drug)	structure	MW (kDa)	wt % CPT	subject (sex)	tumor type	route (time)	dosing schedule[b]	dose (mg/kg)	MTD (mg/kg)	$t_{1/2}$ (h)	plasma AUC (μg·h/mL)	ref
NK012 (SN-38)	micelle	19	20	Balb/c mice (F)	HT-29 (colon)	iv	q4d × 3	6	6	31	5000	259
PEG(CPT)$_2$ (CPT)	linear polymer	40	2	CD1 mice (F)	none	iv	q1d × 1	6	NR	α: 0.1 β: 4	18300	267
PEG(GlyCPT)$_2$ (CPT)	linear polymer	40	2	CD1 mice (F)	none	iv	q1d × 1	17.5	NR	α: 0.1 β: 10	53300	268
Pegamotecan (CPT)	linear polymer	40	2	adult humans	solid	iv (1 h)	q1d × 1, q3w	3.8	3.8	94	29	269
Pegamotecan (CPT)	linear polymer	40	2	adult humans	solid + lymphoma	iv (1 h)	q1d × 1, q3w	1.75	1.75	44	27	270
IT-101 (CPT)	linear polymer	85	5	SD rats	none	iv	q1d × 1	1	8.8	α: 0.4 β: 6 γ: 20	700	273
PLGA (CPT)	linear polymer	33	38	athymic mice (F)	HT29 (colon)	iv	q1d × 1	18	NR	97	240	279
PLGA (CPT)	linear polymer			adult humans	solid	iv	q1d × 1, q1w	25	25	63	27	280
HPMA (CPT)	linear polymer	28	5	Balb/c mice (M)	none	iv	q1d × 1	20	NR	27	1121	281
HPMA (CPT)	linear polymer	21	10	Balb/c mice (M)	none	iv	q1d × 1	20	NR	20	500	281
HPMA (CPT)	linear polymer	20	10	adult humans	solid	iv	q1d × 3	68	68	8	8700	283
T-0128 (SN-38)	linear polymer	130	5	Wistar rats (F)	Walker 256 (sarcoma)	iv	q1d × 1	1	100	α: 4 β: 17	101	288
T-0128 (SN-38)	linear polymer	130	5	Balb/c mice (M)	St-4 (gastric)	iv	q1d × 1	40	80	30	23900	292
DE-310 (DX-8951)	linear polymer	300	8	mice	Meth A (sarcoma)	iv	q1d × 1	5.7	5.7	NR	6300	293
DE-310 (DX-8951)	linear polymer	300	8	adult humans	solid	iv (3 h)	q1d × 1, q6w	0.016	0.016	418	1180	298
PEG-PLL (CPT)	branched polymer	40	6	Balb/c mice (F)	C26 (colon)	iv	q1d × 1	10	NR	31	1400	303
DTS-108 (SN-38)	peptide targeted	3.6	11	beagle dogs	none	iv (0.8 h)	q1d × 1	2.2	2.2	NR	4.8	310

[a] Abbreviations: PLGA, poly(L-glutamic acid); MTD, maximum tolerated dose; AUC, area under the curve; CPT, camptothecin; TPT, topotecan; CPT-11, irinotecan; GG211, lurtotecan; DX-8951, exatecan; M, male; F, female; SD, Sprague–Dawley; iv, intravenous; NR, not reported. [b] Dosing abbreviations: q4d × 3, one dose every four days for three total doses; q1d × 1, one dose; q1d × 1, q3w, once a week every three weeks; q1d × 1, q6w, once a week every six weeks.

burden of characterization. The characterization of covalent macromolecular constructs is oftentimes not trivial, and enthusiasm for biological results needs to be tempered with the critical evaluation of the claims on composition. The literature summarized here and the following section clearly contains many well-characterized systems.

4.2.1. Micelles. The individual components in micelles can provide sites for covalent attachment of drugs. Amphiphilic block copolymers with two water-soluble blocks are oftentimes used to obtain micellar structures. In this case, one block is chemically inert while the other is reactive. Covalent attachment of hydrophobic drugs provides an amphiphile that forms micelles. This strategy has seen a great deal of success, particularly for SN-38.

SN-38 Micelles (NK012). Animal Models. Block copolymers of poly(ethylene glycol)-poly(glutamic acid) were developed for covalent attachment of SN-38 to the carboxylate moieties. Esterification of the phenolate hydroxyl group of SN-38 with acid backbone produces the hydrophobic block for micelle formation.[259] Micelles were formed from copolymers 19 kDa in length with a 12 kDa PEG segment, a 7 kDa poly(Glu) segment and incorporation of about 20% SN-38 (Figure 4). In nude mice bearing HT-29 colon cancer xenografts, pharmacokinetic studies following iv administration of 30 mg/kg micelle or 66.7 mg/kg CPT-11 provided

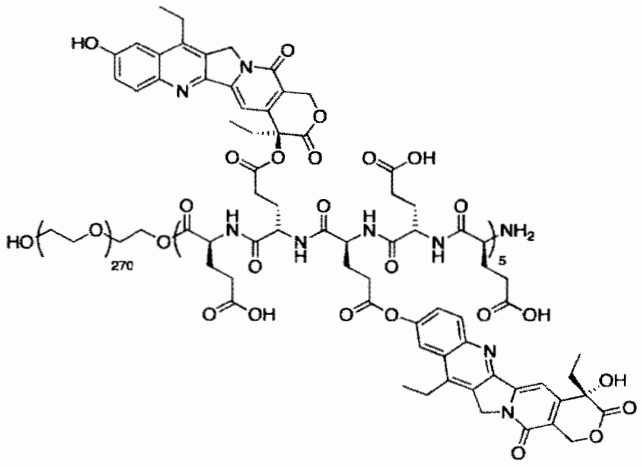

Figure 4. NK012 showing 12 kDa PEG block and 7 kDa poly(L-glutamic acid) block with 20 wt % overall SN-38.

plasma AUC values of 5,010 μg·h/mL for the micelle, 0.022 μg·h/mL for irinotecan and 0.001 μg·h/mL for irinotecan metabolized to SN-38. The half-lives for each of the agents were 31 h, 3 h and 4 h, respectively. Antitumor effects were studied *in vivo* in mice with highly vascularized SBC-3/VEGF tumors as compared to SBC-3/Neo tumors, which do

not promote angiogenesis. The micelles showed significant antitumor activity against the highly vascularized tumors with eradication of bulky masses in VEGF positive tumors.

In mice with sc Renca renal cell carcinoma xenografts, NK012 was delivered intravenously at 20 mg/kg resulting in complete tumor disappearance by day 21. In contrast, free CPT-11 administered at 30 mg/kg led to only partial response at day 15, followed by progressive disease.[260] NK012 treatment led to a 10% decrease in body weight in tumor bearing mice, suggesting that there was little toxicity associated with the complex. In lung metastasis models, significant uptake was observed and an overall decrease in metastatic nodules compared to irinotecan and no treatment. Prior to treatment, at day 0, 126 nodules were observed, which increased to 287 nodules at day 21 in untreated mice, as compared to 236 nodules observed after treatment with free irinotecan and only 32 nodules observed after treatment with the NK012 micelles. Furthermore, 6 of 10 mice were alive at 90 days when treated with NK012 as compared to only one remaining mouse at 90 days following treatment with irinotecan. Untreated mice expired by day 65.

Similar results were also observed in orthotopic gastric cancer with peritoneal metastases.[261] Mice bearing 44As3Luc tumors were treated with MTDs of either NK012 (30 mg/kg) or CPT-11 (67 mg/kg) for three total doses every four days. NK012 treatment resulted in 80% survival at 150 days using the micelle. Free drug led to no survival at 80 days. Similar results were observed with the 58As tumor model. Tumor uptake of both NK012 and CPT-11 was observed, and the extended half-life of NK012 resulted in increased antitumor activity in both the gastric tumor and peritoneal nodules.

While the success observed with NK012 is believed to be due to the enhanced vasculature in solid tumors, investigation of tumor xenografts with highly vascularized (PSN1) and poorly vascularized tumors (Capan1) suggests otherwise.[262] When irinotecan was delivered intravenously to mice bearing tumor xenografts at an MTD of 66.7 mg/kg every fourth day for three total doses, a reduction in tumor size was observed from days 4 to 12 in PSN1 tumors but not in Capan1 tumors. Conversely, NK012 caused complete regression of both tumors, regardless of vascularity. Drug distribution monitored with fluorescence distribution showed peak fluorescence intensity at 1 h using CPT-11 as compared to 24 h using NK012, with detection extending past 96 h. One concludes that although differences in vascularization are observed, the extended plasma retention times associated with the increased molecular weight of constructs enable eventual accumulation in tumors, including those with poor blood flow.

Most recently, NK012 was compared to CPT-11 for treatment of malignant gliomas.[263] Subcutaneous xenografts treated at MTD (30 mg/kg) every four days for three total doses showed tumor regression beginning on day 5 and reaching complete regression on day 23 until day 80, when relapse was observed. After administration of 30 mg/kg for NK012 and 67 mg/kg CPT-11 to mice bearing orthotopic U87MG/Luc intracranial xenografts, the pharmacokinetic analysis showed 1113 ng/mL SN-38 in the plasma at 2 h decreasing to 90 ng/mL at 24 h and 6.88 ng/mL at 72 h with tumor concentrations of 67.7 ng/mL, 137 ng/mL and 24.6 ng/mL, respectively. Plasma and tumor concentrations were significantly higher using the micelle as compared to free drug, suggesting significant trafficking of SN-38 from the micelle to the tumor. It is unclear what form is transported into the brain. While the antitumor effect was not statistically significant between CPT-11 and NK012, the Kaplan–Meier plot showed all mice had died by 30 days for both the control and CPT-11 treated mice, whereas NK012-treated mice survived for 43 days. While treating gliomas with NK012 was not as successful as treating mice bearing subcutaneous xenografts, this work represents a significant step toward improving treatment of gliomas clinically. Additional studies have shown success in combination therapy with 5-fluorouracil[264] and cisplatin,[265] expanding the therapeutic potential of NK012, which will likely be the goal of future studies using this micellar construct in both monotherapy and combination therapy.

4.2.2. Linear Polymers. Poly(ethylene glycol)-Camptothecin (PEG(CPT)$_2$, PEG(GlyCPT)$_2$, Prothecan and Pegamotecan). Animal Models. Poly(ethylene glycol) (PEG) has been used extensively to increase the water solubility of hydro-

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phobic small molecule drugs and macromolecular drug delivery vehicles. PEGylation has been used to increase biocompatibility, masking agents from the reticuloendothelial system while also increasing molecular weight to improve retention in the circulatory system. PEGylation of CPT led to the discovery that CPT esters stabilize the lactone ring.[266] Various derivatives of PEGylated camptothecins have been developed, which employ different linkers to attach CPT to 40 kDa PEG. Initially, PEG(CPT)$_2$ was developed through acylation of CPT with PEG-dicarboxylic acid. Glycine was then added as a linker between PEG and CPT to form PEG(GlyCPT)$_2$. Finally, alanine was employed as the linker to form PEG-CPT and later termed Prothecan and Pegamotecan. Each of these derivatives was developed by Enzon Pharmaceuticals Inc.

The antitumor efficacy of PEG(CPT)$_2$ was evaluated in mice bearing either P388/0 leukemia or HT-29 colon xenografts.[267] After a 5.2 mg/kg iv injection of PEG(CPT)$_2$, alpha and beta half-lives were determined to be 0.07 h and 3.5 h, respectively with an AUC of 0.018 μg·h/mL. In mice bearing HT-29 colon tumor xenografts, a dose of 2.5 mg/kg CPT afforded a 20% decrease in tumor volume after a dosing schedule of five days a week for five weeks, with 62% increase in tumor volume 2 weeks after treatment. However, using a dose of 3 mg/kg on the same schedule, PEG(CPT)$_2$ afforded an 87% decrease in tumor volume after treatment and a 93% decrease two weeks later. Utilization of PEG-(GlyCPT)$_2$ improved the alpha and beta half-lives to 0.1 and 10 h, respectively, with an increased AUC value of 0.05 μg·h/mL.[268] The glycinate ester form appears to be 1.5 times less toxic than free CPT, and shows improved pharmacokinetics than the parent PEG dicarboxylate derivative while maintaining similar efficacy. This derivative also showed a significant increase in % ID/g out to 72 h in the HT-29 tumor xenografts as compared to other organs, which was not observed using free CPT.

Human Patients. A phase I and pharmacokinetic study of Pegamotecan (Figure 5) found an MTD of 122 mg/m^2 (~3.3 mg/kg) as a 1 h iv infusion every 3 weeks.[269] At this dosing schedule, a plasma AUC value of 29 μg·h/mL was observed, with a 94 h half-life and minimal toxicity seen in patients with solid tumors. While one partial response was noted out of 37 patients and only two minor responses were observed, the promising pharmacokinetics suggested further study. A subsequent study in patients with solid tumors, however, showed a lower MTD of 56 mg/m^2 (~1.5 mg/kg), with a similar AUC value of 27 μg·h/mL and a 44 h half-life.[270] Minimal toxicity was observed in a later study with two unconfirmed partial responses out of 27 patients. Enzon Pharmaceuticals Inc. halted further phase trials in 2005 due to poor efficacy in phase II trials and redirected efforts toward alternative antitumor research targets.

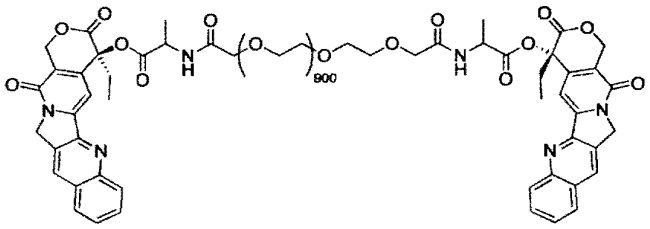

Figure 5. Pegamotecan made using 40 kDa PEG-diacid with two camptothecin moieties attached through alanine linker.

Investigation continued in outside laboratories with phase II trials of patients with gastric and gastroesophageal adenocarcinoma.[271] Using a dosing strategy of 1 h doses every three weeks, an MTD of 122 mg/m^2 (~3.3 mg/kg) was determined. Limited efficacy was also noted, with five of 35 patients experiencing a partial response. While this drug alone provided little evidence of efficacy, utilization of this drug in combination therapy may prove more successful.

Cyclodextrin-PEG Polymers (IT-101). Animal Models. Davis and co-workers synthesized a PEG-containing polymer containing disubstituted β-cyclodextrin moieties and CPT linked through a glycine ester linkage. The final construct had a molecular weight of 97 kDa, with 6.8% drug loading capacity.[272] The MTD was determined to be 9 mg/kg, with a drug release half-life of 1.7 h in human plasma. When

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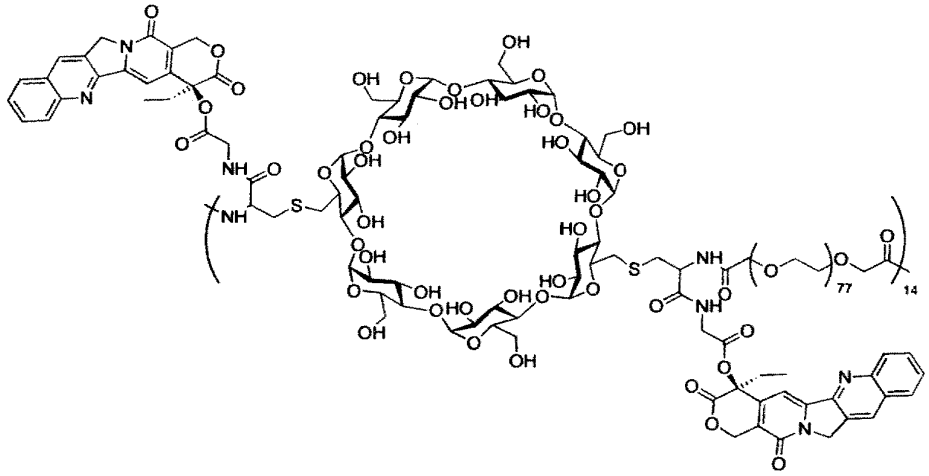

Figure 6. IT-101 with a 3.4 kDa PEG chain and overall molecular weight of 85 kDa.

delivered intravenously to nude mice bearing sc LS174T colon carcinoma tumors every four days for three total doses at MTD, a 227% tumor growth delay (TGD) was obtained as compared to only 47% TGD observed when only two doses of 9 mg/kg CPT were delivered on the same schedule. These positive results prompted additional studies toward eventual clinical use. In a later pharmacokinetic study with an 85 kDa polymer–CPT conjugate (Figure 6), a single iv injection of 8.8 mg/kg in rats found an elimination half-life of 20 h and an AUC of 693 μg·h/mL.[273] Biodistribution studies found that plasma concentrations increased with a significant increase in total CPT. Furthermore, cleavage of CPT from the polymer at the tumor site created a tumor to plasma ratio of 2.5 at 24 h increasing to 21.2 at 48 h, with concentrations of drug higher in the tumor than any other organ. To find an optimal dosing strategy, nude mice with LS174T colon cancer, HT29 colon cancer, H1299 non-small-cell lung cancer, MDA-MB-231 breast cancer, H69 small-cell lung cancer or Panc-1 pancreatic cancer xenografts were treated with either a single dose of IT-101 or multiple high and low doses.[274] Three doses every week provided increased efficacy over single dose administration, however, efficacy did not increase when delivered at five total doses every four days or five times a week for three weeks due to the extended half-life. In the majority of tumor xenografts, three doses of 16.1 mg/kg over three weeks provided the most promising results, showing the least body weight loss, highest tumor growth delay and highest number of complete regressions. IT-101 has shown significant progress in cancer therapy.

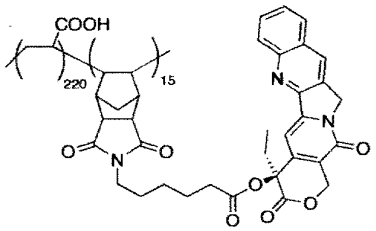

Figure 7. Phthalimide polymers with molecular weight of 25.5 kDa.

Phthalimide Polymers. Animal Models. Theodorakis and co-workers developed phthalimide-*co*-acrylic acid polymers synthesized through photopolymerization to afford a 25.5 kDa (Figure 7) construct with 21 wt % CPT in one derivative[275] or a 15.4 kDa construct with 26 wt % CPT in another.[276] *In vivo* studies with the high molecular weight polymer architectures showed an increase in activity compared to free CPT. Doses of 10 mg/kg of CPT, 21 mg/kg CPT equivalents in the polymer or 2.1 mg/kg CPT equivalents in the polymer afforded ~90 day survival time. The low molecular weight polymer architecture, however, did not provide the same antitumor efficacy at low doses.

Poly(L-glutamic acid) Polymers (CT-2106). Animal Models. Li and co-workers developed a poly(L-glutamic acid) polymer containing 7.7 wt % CPT through esterification. *In vitro* studies of this derivative showed base catalyzed hydrolysis of CPT from the polymer and cytotoxicity in a variety of cell lines.[277] Antitumor studies of sc H322 tumors in nude mice showed a 32 day tumor growth delay after four

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40 mg/kg iv injections were administered every four days. Furthermore, when H322 tumors were grown in the lung of nude mice, a dosing schedule of five doses of 10 mg/kg every four days afforded a median survival time of 157 days as compared to the survival time of only 86 days seen for untreated mice and 108 days seen for mice treated with free CPT. When this dose was increased to 40 mg/kg, a median survival of 238 days was obtained as compared to a survival time of only 59 days using one complete dose of 160 mg/kg (less than the LD_{10} of 177 mg/kg).

Klein and de Vries utilized a similar polymer *in vivo*, wherein glycine linkers were introduced to investigate the difference in MTD (40 mg/kg) and efficacy.[278] While the glycine linkers did not alter efficacy or MTD, drug loading on 50 kDa polymers increased from 15 wt % to 50 wt % when glycine was used. Solubility limited drug loading at ~37 wt %. Increased polymer molecular weights from 33 kDa to 50 kDa increased antitumor effect in C57BL/6 mice with B16 melamona. Similarly, improvement was seen by increasing CPT wt % from 15 wt % to 35 wt %, using the same 40 mg/kg dose. An idealized architecture consisting of a 49 kDa polymer with 37 wt % CPT was used in preliminary *in vivo* studies of NCI-H460 lung cancer xenografts in athymic mice. Results were successful, showing increased tumor growth delay to 50% after a 30 mg/kg dose. It was later determined that, in nude mice with HT-29 colon carcinoma tumors, this polymer had a 97 h plasma half-life and plasma AUC value of 240 $\mu g \cdot h/mL$, as well as a tumor half-life of 84 h and tumor AUC value of 696 $\mu g \cdot h/mL$.[279]

Human Patients. In phase I trials, CT-2106 was administered intravenously weekly.[280] The MTD was determined to be 25 mg/m^2 (~0.68 mg/kg) after higher doses of 30 mg/m^2 (~0.82 mg/kg) and 35 mg/m^2 (~0.95 mg/kg) showed signs of toxicity. Pharmacokinetic data at the MTD provided a 63 h half-life for conjugated CPT and a 36 h half-life for released CPT. At this dose, the plasma AUC value for the conjugate was 27 $\mu g \cdot h/mL$, while the plasma AUC for unconjugated CPT was 14 $\mu g \cdot h/mL$. While PLGA polymers with CPT offer extended plasma half-lives and slow release of CPT, only 3 of 25 patients experienced stable disease, suggesting that additional work needs to be completed to determine the clinical relevance of this construct (Figure 8).

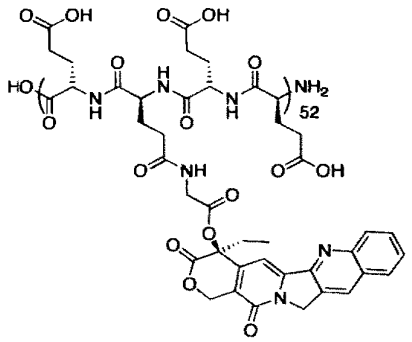

Figure 8. PLGA polymer with glycine linker to CPT containing 37 wt % drug in a 49 kDa polymer.

Poly[N-(2-hydroxypropyl) methacrylamide] Copolymers (MAG-CPT). Animal Models. Caiolfa and co-workers developed HPMA polymers of 28 kDa and 21 kDa with 5.4 wt % and 10 wt % CPT, respectively.[281] CPT was also linked through the tetrapeptide linker GlyPheLeuGly for esterlytic cleavage at the tumor. Pharmacokinetic studies showed 27 h and 20 h half-lives for the high and low molecular weight polymers, respectively, with AUC values of 1023 $\mu g \cdot h/mL$ and 480 $\mu g \cdot h/mL$, respectively. Studies in mice bearing HT-29 colon xenografts showed higher efficacy after the administration of six doses of either 25 mg/kg or 10 mg/kg every four days for the high and low MW polymers, respectively. Each polymer gave 98% tumor growth inhibition one week after the last treatment and 72 day and 62 day tumor growth delay for the high and low MW polymers, respectively. Although a 2-fold increase in AUC for the higher molecular weight polymer was observed, the low molecular weight polymer showed a 2-fold increase in potency, presumably due to an increase in polymer metabolism for the lower molecular weight polymer.

In a subsequent study, Phe-Leu of the tetrapeptide linkage was replaced by 6-aminohexanoic acid.[282] The construct, known as MAG-CPT, contains 10 wt % CPT and has a mass of 20 kDa. The polymer with the tetrapeptide linker showed a 2-fold higher potency against a variety of sc tumor xenografts in nude mice due to the increased potential for proteolytic cleavage. Although the construct with the Gly-hexanoic acid-Gly linker was less potent, decreased toxicity

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was also observed resulting in more complete responses when administered at higher doses as compared to the construct with the tetrapeptide linker. This difference in toxicity and efficacy may be attributed to the decreased rate of hydrolysis in the non-peptide linker as compared to the peptide linker.

Human Patients. MAG-CPT has been utilized in phase I studies, administered as a 30 min iv infusion for three consecutive days every four weeks.[283] The MTD of MAG-CPT was determined to 68 mg/m^2 (~1.8 mg/kg) with dose limiting cumulative bladder toxicity at higher doses. The plasma AUC value of the construct was found to be 8661 μg·h/mL with an 8 h half-life. Approximately 70% of the dose was excreted through the kidneys within 4 days. The route of excretion likely leads to the bladder toxicity observed at high doses. Changing this dosing regimen to a once weekly schedule for three weeks in a four week cycle at doses of 80 mg/m^2 (~2.2 mg/kg) and 120 mg/m^2 (~3.2 mg/kg) saw similar results.[284] At the low dose, no adverse toxicities were observed until the second cycle of treatment, whereas cumulative bladder toxicity was observed during the first cycle at the high dose. Carrier bound plasma AUC values for the low dose were 1540 μg·h/mL and 1226 μg·h/mL for the high dose. Alpha and beta plasma half-lives were about 2.5 h and 100 h, respectively, regardless of dose. Unpredictable excretion kinetics and the resulting variable toxicities suggest that this dosing strategy is not practical for clinical development. The dosing regimen was changed once again to a 30 min infusion once every four weeks at doses between 30 mg/m^2 (~0.81 mg/kg) and 240 mg/m^2 (~6.5 mg/kg).[285] An MTD of 200 mg/m^2 (~5.4 mg/kg) was determined, which afforded a 237 h half-life and a plasma AUC value of 9305 μg·h/mL. Once again highly variable urinary excretion proved problematic in determining toxicity and determining an effective dose.

In another study MAG-CPT was administered intravenously at a dose of 60 mg/m^2 (~1.6 mg/kg) over 24 h, 3 days or 7 days prior to surgery for colorectal cancer.[286] After infusion of MAG-CPT (Figure 9) patients had mean plasma concentrations of 29,378 ng/mL of polymer bound CPT and 17.3 ng/mL, which decreased to 2588 ng/mL and 12.2 ng/mL at 7 days, respectively. Furthermore, normal tissue had

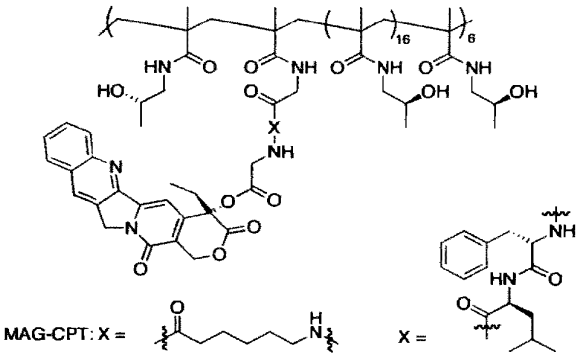

Figure 9. HPMA polymer with a GlyPheLeuGly linker or a Gly-hexanoic acid-Gly linker (MAG-CPT) to CPT with 10 wt % drug in a 21 kDa polymer.

451 ng/mL MAG-CPT at 7 days as compared to 434 ng/mL in the tumor. The high plasma concentration and poor tumor uptake relative to normal tissue suggest that this construct is perhaps too small to selectively partition into the colorectal tumors through the EPR effect. The variable pharmacokinetics and bladder toxicities associated with this construct prevented further evaluation, however, it is assumed that increasing the size of the construct to bypass glomerular filtration and increase EPR effect may prove to be efficacious.

Carboxymethyl Dextran (Delimotecan, MEN4901, T-0128). Animal Models. The carbohydrate backbone of carboxymethyl dextran displays acid groups for attaching SN-38. Here, SN-38 is modified with an aminopropyl group and triglycine. The starting polymer has approximately 0.40−0.45 carboxylate per sugar with a molecular weight of about 130 kDa and 3−6 wt % drug loading. This linker has shown 9% release of accumulated polymer in the liver at 6 h after administration of a 1 mg/kg dose as compared to 1.4% and 23% for diglycine and tetraglycine linkers, respectively.[287] Furthermore, it appears that the triglycine linker is a selective substrate for cathepsin B.

A comparison of the polymeric material containing SN-38 and the aminopropyl derivative of SN-38 showed lower potency for the polymeric material in a series of cell lines when compared to topotecan, SN-38 propylamine ether and CPT.[288] The ED_{50} and MTDs of the SN-38 derivative and topotecan were reported as 23 mg/kg (MTD 60 mg/kg) and 5.4 mg/kg (MTD 25 mg/kg), respectively. The ED_{50} and

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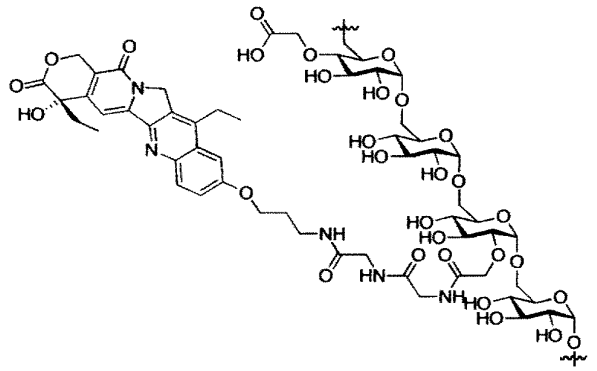

Figure 10. T-0128 polymer containing SN-38 linked with glycine and propanolamine to a 130 kDa polymer with 3 wt % drug loading and 0.5 carboxylate per sugar.

MTD of the polymer conjugate, however, were 2.3 mg/kg and 100 mg/kg, respectively. Mice bearing MX-1 tumor xenografts experienced 99.8% maximum tumor growth inhibition, with 5 out of 6 mice tumor free at 6 days, after the administration of a 6 mg/kg iv dose of T-0128. This inhibition was higher than the 67% maximum tumor growth inhibition observed using the SN-38 derivative at 80 mg/kg. Various tumors and doses were investigated, with significant efficacy enhancement observed using the polymer in all cases. Investigation of the pharmacokinetics provided alpha and beta half-lives of 4 h and 17 h for the carboxymethyl dextran polymer as compared to 0.017 h and 0.88 h for the SN-38 derivative. Plasma AUC values also showed significant enhancement for the polymer, as expected, with 101 $\mu g \cdot h/mL$ and 0.14 $\mu g \cdot h/mL$, respectively. Tumor uptake of the polymeric material was significant when compared to free drug. The liver, spleen and lymph nodes, however, also showed significant uptake, suggesting recognition by the reticuloendothelial system, which was later investigated and confirmed in studies involving macrophage-mediated activation of T-0128.[289,290]

To investigate the effects of molecular weight and degree of substitution on the pharmacokinetics of T-0128 (Figure 10), a series of carboxymethyl dextran analogues with varying degrees of substitution of FITC dye were developed.[291] Using 110 kDa polymer, the optimal degree of substitution of carboxylates of 0.4 provided an AUC value of 6361 $\mu g \cdot h/mL$ and half-life of 10 h. Varying the molecular weight at a fixed degree of substitution, 0.4 per sugar, established that AUC values increased with molecular weight with 40 kDa < 70 kDa < 250 kDa < 110 kDa. This trend is due to the increased renal excretion of low molecular weight polymers (40 kDa and 70 kDa). Significant hepatic uptake is observed for the 250 kDa polymer. Furthermore, low anionic charge, 0.2 to 0.6 carboxylate per sugar, enables decreased hepatic uptake suggesting that the 110 kDa polymer with 0.4 substitution per sugar would possess the highest tumor accumulation. Pharmacokinetic analysis of polymer bound SN-38 at the highest dose tested (25 mg/kg) gave an AUC of 4450 $\mu g \cdot h/mL$ and a half-life of 8.2 h in rats bearing Walker-256 tumor xenografts. Nontumor bearing rats, however, experienced significant increase in AUC and half-life (13675 $\mu g \cdot h/mL$; 28 h). A subsequent study in mice bearing human tumor xenografts found significant tumor inhibition at one-third the MTD of 80 mg/kg once a week for four weeks (H81 gastric, 97.5%; H-110 colon, 98.5%; Mqnu-1 lung, 99.7%; H-74 lung, 90.7%; H-204 esophageal, 78.8%; H-181 liver, 81.2%; H48 pancreatic, 98.8%).[292] Pharmacokinetic evaluation of polymer bound SN-38 in nude mice bearing St-4 xenografts found plasma AUC values of 23,900 $\mu g \cdot h/mL$ and a 30 h half-life after a 40 mg/kg iv injection.

Carboxymethyl Dextran Polyalcohol-DX8951 (DE-310). Animal Models. Exatecan showed significant promise in preliminary studies of antitumor activity with minimal toxicity as compared to other CPT derivatives. The success in preclinical studies did not translate well into human patients, and the need for a macromolecular architecture became apparent. Exatecan was incorporated into a carboxymethyl dextran polyalcohol polymer through Gly-GlyPheGly spacer with an average molecular weight of 300 kDa and 8 wt % drug loading.[293]

Mice bearing Meth A fibrosarcoma xenografts were administered a single MTD dose of 11.4 mg/kg resulting in tumor shrinkage out to three weeks.[294] This improved efficacy over the free drug was accompanied by a 30% loss in body weight. When a single dose at one-quarter of the MTD is given to mice, similar efficacy is observed without the loss in body weight. In a long-term study, mice administered four doses of 5.7 mg/kg every three days or

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every seven days experienced complete tumor regression at 85 days, with significant body weight loss when delivered every three days. A single dose of 11.4 mg/kg also resulted in complete tumor regression at 85 days, however, weight loss was also observed using this dosing strategy. It should also be noted that body weight decreased significantly in the first 17 days with multiple injections and 8 days with a single injection, but returned to normal within 10 days of reaching a minimum. DE-310 also showed similar efficacy in mice bearing HCT116 colon cancer, PC-6 and PC-12 lung cancers, CDDP liver metastasis and CPA lung metastasis models. A later study showed similar results, but also found evidence that DE-310 was taken up into tumors or macrophages and broken down to release the drugs.[295] The release of drug through the amino acid linker cleavage was also determined to be mainly due to the activity of cathepsin B in the tumor.[296] Another study shows evidence of meningocele induction in rat fetuses after their mothers received four iv doses of 0.3 mg/kg or a single dose of 1 mg/kg.[297] Administration between days 7 and 13 of gestation resulted in 100% meningocele formation and suggests significant caution when utilizing DE-310.

Human Patients. In human patients, a dramatic increase in plasma AUC values was obtained when DE-310 was administered using a 3 h iv infusion once every six weeks at an MTD of 7.5 mg/m^2 (~0.2 mg/kg).[298] The plasma AUC was determined to be 1,124 μg·h/mL with a 338 h half-life. Furthermore, a total of 27 patients received doses from 1.0 mg/m^2 (~0.03 mg/kg) every two weeks to 9.0 mg/m^2 (~0.24 mg/kg) every six weeks. Dose limiting toxicities were observed when 9.0 mg/m^2 (~0.24 mg/kg) was delivered every six weeks, but lower doses of 6.0 mg/m^2 (~0.16 mg/kg) resulted in no dose limiting toxicity. An intermediary dose of 7.5 mg/m^2 (~0.20 mg/kg) was also investigated, resulting in only one patient with reversible toxicity, suggesting this dose to be used in phase II clinical trials. Efficacy was also measured in all 27 patients receiving DE-310. One patient experienced complete remission for over 2 years after receiving two doses of 9.0 mg/m^2 (~0.24 mg/kg). Another patient had a partial response for 3 months after one dose of 9.0 mg/m^2 and two subsequent doses of 6.0 mg/m^2. A third patient had a partial response after seven cycles of 2.0 mg/m^2 with progression occurring after 8 months. Furthermore, 1 patient experienced disease stabilization for 6 weeks, another for 8 weeks and another for 10 weeks. Five other patients had disease stabilization for 12 weeks, one for 16 weeks, 2 patients each for 18 weeks and 24 weeks and one patient for 32 weeks. The significant pharmacokinetic improvement and efficacy of DE-310 over the free drug suggested further investigation was warranted. In related studies, however, tumor accumulation is not improved significantly over other organs, perhaps posing problems with systemic toxicity if the drug is capable of releasing in normal tissue.[299] It remains clear, however, that the significant increase in pharmacokinetics and the convenient dosing schedule with DE-310 will warrant further investigation and may find clinical utility in terminal patients who will benefit from the antitumor efficacy, which may outweigh the toxic side effects.

Carboxymethyl Dextran Polyalcohol-Camptothecin (XMT-1001). Animal Models. Camptothecin was also employed in a carboxymethyl dextran polyalcohol polymer. The polyalcohol polymer was functionalized with succinic acid, which was later acylated with glycine-camptothecin as shown in Figure 11.[300] The starting polymer contained 0.2 carboxylate per sugar, which resulted in a 5−7 wt % CPT after partial acylation to yield a final carboxylate construct of 70 kDa. The polymer was labeled with ^{111}In and CPT was labeled with ^{3}H for dual labeling biodistribution studies in mice. CPT uptake in HT29 colon cancer human tumor xenografts was 2.52% ID/g at 24 h with 6% ID/g of carrier in the blood and 7% ID/g of CPT in the liver. XMT-1001 was delivered intravenously to mice bearing LS174T tumors at doses of 59 mg/kg, 44 mg/kg and 22 mg/kg resulted in 223%, 196% and 207% tumor growth delay, respectively. Pharmacokinetic studies were also performed in rats and dogs through iv administration of 30−300 mg/kg and 5−50 mg/kg, respectively. The conjugated CPT half-life was found to be 3.5−4.0 h in rats and 4.5−5.2 h in dogs.

Human Patients. Little animal or human data is available in the literature for this construct, however, phase 1 studies are currently ongoing. Initial studies in patients bearing solid tumors have not reached an MTD after administration of construct between 1 mg/m^2 and 20.5 mg/m^2. Release of hydrolysis products in the plasma have also been determined with less than 1% CPT present in the urine. Furthermore, patients with advanced disease prior to treatment have experienced extended periods of stable disease. Although few

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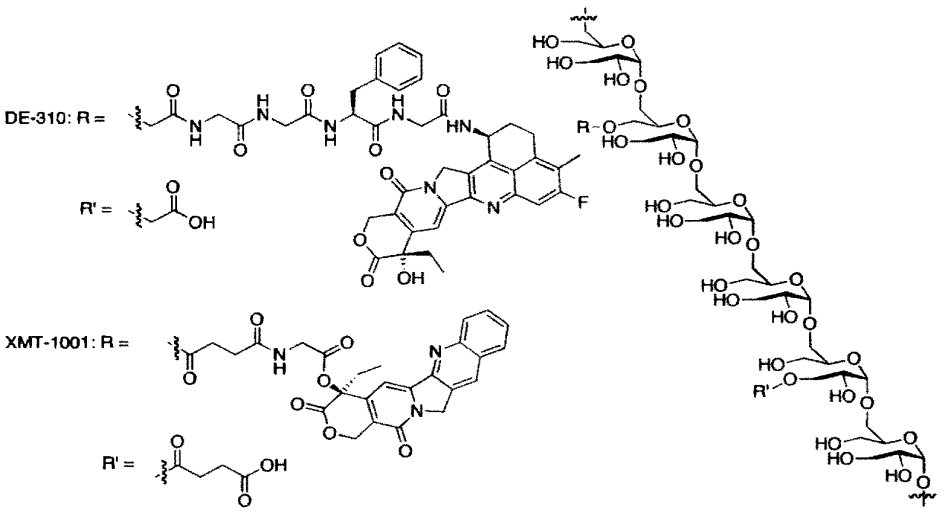

Figure 11. Carboxymethyl dextran polyalcohol polymers. DE-310 containing 5–7 wt % exatecan in a 360 kDa polymer with 0.4 carboxylate per sugar. XMT-1001 containing 5–7 wt % camptothecin in a 70 kDa polymer with 0.2 carboxylate per sugar.

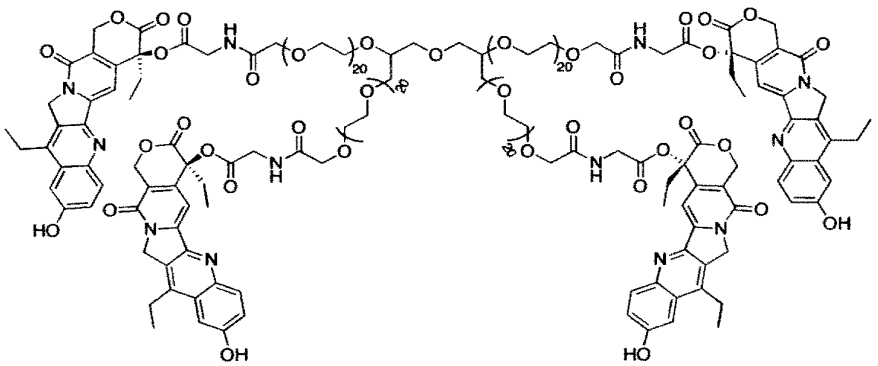

Figure 12. EZN-2208 is a 40 kDa four arm PEG with four SN-38 moieties attached.

results with XMT-1001 have been published, this construct has provided some promising data and addition efforts utilizing this construct are warranted.

4.2.3. Branched Polymers. Poly(ethylene glycol) SN-38 (EZN-2208). Animal Models. Although limited success was observed with PEG-CPT conjugates, SN-38 was incorporated into 4-arm PEG through glycine linkers, increasing the drug loading from 1.7 wt % in PEG-CPT to 3.7 wt % in PEG-SN-38.[301] In MX-1 tumors, EZN-2208 (Figure 12) was administered intravenously at an MTD of 20 mg/kg for six total doses every other day affording 100% tumor growth inhibition with cures in all animals at 16 weeks as compared to cures seen in 44% of animals using CPT-11, regardless of dose. In bulky tumors, both dosing schedules of EZN-2208 resulted in 100% tumor growth inhibition at 10 days and complete regression was observed until the end of the study at 115 days. CPT-11 was slightly more efficacious at a multiple dosing schedule with tumor regrowth occurring at 13 days using the single dosing schedule and at 45 days for the multiple dosing schedule. Similar results were obtained in MiaPaCa-2 and HT-29 cells for CPT-11 regardless of dose. Slightly less efficacy was observed in MiaPaCa-2 xenografts, with 71% tumor growth inhibition on day 69 and 100% animal survival at the termination of the study (day 125) using a single dose. Multiple doses of EZN-2208 resulted in 95% tumor growth inhibition and 66% animals cured at the termination of the study (day 147). In HT-29 xenografts, 68% and 92% tumor growth inhibition was observed using single and multiple doses, respectively. Furthermore, HT-29 xenografts were treated with EZN-2208 upon remission of tumor for up to three cycles, displaying evidence of response to repeated cycles of therapy. Additionally, EZN-2208 showed increased response from CPT-11 resistant cells, with 193% increase in tumor volume 32 days after therapy as compared to 1298% increase when using CPT-11. Pharmacokinetic studies showed that this delivery system displayed a 12 h plasma half-life for SN-38

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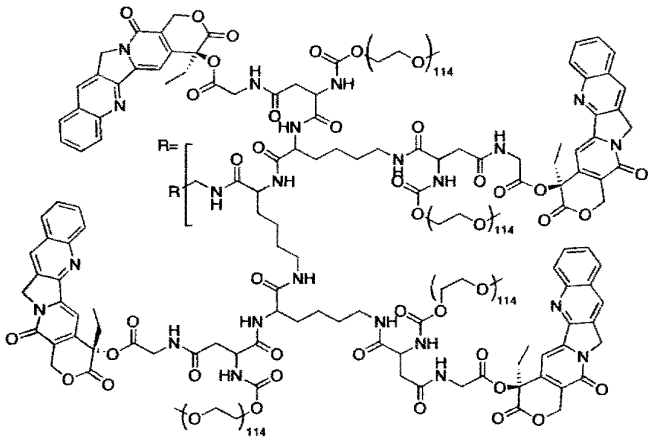

Figure 13. Poly(L-lysine) dendrimer containing eight CPT and eight PEG5000 chains.

and an elimination half-life of 26 h as compared to a plasma half-life of 1.7 h and elimination half-life of 2.1 h using CPT-11 as a free drug. Plasma AUC values of EZN-2208 and released SN-38 were 107,065 μg·h/mL and 129 μg·h/mL, respectively. When using CPT-11, AUC values of 194 μg·h/mL and 3 μg·h/mL were obtained. Tumor AUC values were also significantly higher for EZN-2208 with a value of 38825 μg·h/g versus 83 μg·h/g for CPT-11.

Structure activity relationships aimed at the linker were pursued using different amino acids. Hydrolysis half-lives in human plasma depended on amino acid: alanine, 0.21 h; methionine, 0.45 h; sarcosine, 0.32 h; glycine, 0.21 h.[302] While the MTDs of the alanine and glycine analogues were determined after a single iv injected dose to be 20 mg/kg, antitumor efficacy was investigated using a single MTD dose and six doses of 5 mg/kg. Complete regression was observed in 100% of mice with MX-1 tumor xenografts using both single or multiple dosing schedules in all derivatives except in the single dose with the glycine derivative (83%).

Poly-(L-lysine) Dendrimer. Animal Models. Dendrimers are hyperbranched polymers synthesized in a controlled fashion to afford a monomolecular entity with dense terminal functionality at the periphery. Peripheral functionalization with drug moieties through covalent attachment affords macromolecular constructs with a large number of drug molecules. Fréchet and Szoka utilized poly(L-lysine) dendrimers as an architecture for peripheral functionalization with CPT and poly(ethylene glycol) (Figure 13) to increase size and solubility of the dendrimer to effectively improve the efficacy of CPT.[303] CPT was attached to the dendrimer through both glycine and β-alanine linkage to afford ~35 kDa conjugate with 6.0 wt % and 4.5 wt % drug loading, respectively. The glycine linkage proved to be 2 orders of magnitude more toxic *in vitro* than the β-alanine linkage due to the increased hydrolysis in the former. In pharmacokinetic studies, a 31 h half-life was observed for the dendrimer construct, with an AUC value of 460% of the injected dose-h/gram tissue (~1380 μg·h/g) after a 10 mg/kg i.v injection. The biodistribution data showed 4% ID/g tumor accumulation at 24 h, compared to 0.3% for free drug. Furthermore, free CPT accumulated in the lung, liver and spleen at 24 h, while dendrimeric CPT was observed in the tumor, serum, spleen and to a lesser extent in the lungs at 48 h. Mice with C26 colorectal sc tumors were treated with a single dose of 24 mg/kg eight days after inoculation, resulting in 72% tumor growth delay as compared to no significant tumor growth delay observed when using a single 10 mg/kg dose of CPT and 18% tumor growth delay with four 50 mg/kg doses of irinotecan in one week. Lower doses over prolonged periods proved successful with 155% tumor growth delay at 12 mg/kg doses once a week for three weeks. When the same dosing strategy was used in a HT29 mouse model, a 122% tumor growth delay was obtained with all mice surviving for the length of the experiment while free CPT and irinotecan proved to be less efficacious.

4.2.4. Proteins. Human Serum Albumin. Animal Studies. Blood proteins are another attractive macromolecular architecture for delivery of anticancer drugs to tumors. Human serum albumin (HSA) is the most abundant blood protein and has been shown to accumulate in solid tumors due to the EPR effect. This has led some to link anticancer drugs to the protein for drug delivery. One method used with CPT involves a short poly(ethylene glycol) linker between a camptothecin ester and a maleimide group (Figure 14). The maleimide is then capable of reacting with a cysteine-34 in HSA.[304] The final product, HSA-PEG-CPT, is well-defined and contains a single CPT (0.51 wt % CPT). Conjugation of the drug to albumin also provides a 27-fold increase in water solubility compared to CPT alone depending on the length of the poly(ethylene glycol) linker. When delivered to mice bearing subcutaneous HT-29 human tumor xenografts at four doses of 25 mg/kg (two times the MTD of camptothecin), no adverse effects were observed and an improved T/C % of 47% was achieved as compared to 89% for CPT at 12.5 mg/kg.

Alternatively, the PEG-linker was replaced with a peptide spacer (maleimide-Arg-Arg-Ala-Leu-Ala-Leu-Ala-CPT) susceptible to cleavage by cathepsin B, which is present in lysozomes and overexpressed in various ma-

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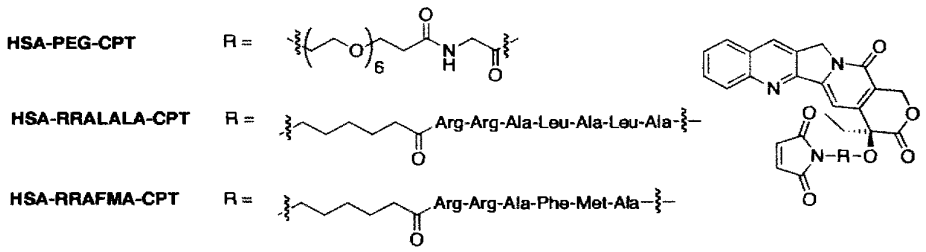

Figure 14. Compounds investigated for human serum albumin linked CPT conjugates.

lignant tumors.[305] Cleavage studies showed that mice bearing HT-29 human tumor xenografts treated three times with HSA-RRALALA-CPT at the MTD of the free drug (3 × 12.5 mg/kg) gave a T/C % of 17% compared to 40% for the free drug alone. To enhance the cleavage properties of the peptide sequence used, a peptide positional scanning library was developed to determine the optimal sequence for peptidase activity in tumor homogenates.[306] From the library of peptide sequences, two sequences were found to show optimal cleavage profiles (maleimide-Arg-Arg-Ala-Phe-Met-Ala-CPT and maleimide-Arg-Arg-Phe-Tyr-Met-Ala-CPT). The sequence containing Arg-Ala-Phe-Met was then investigated *in vivo* in nude mice with HT-29 human tumor xenografts. The optimized sequences, HSA-RRAFMA-CPT, showed a similar T/C % of 17% at a dose of 2 × 12.5 mg/kg as compared to a 40% T/C % for free drug at a dose of 3 × 12.5 mg/kg.

While the peptide positional scanning technique has shown promise at developing new cleavage sequences, the *in vivo* results suggest that there is no tumor selectivity. Although tumor homogenates were shown to be active at cleaving this optimized sequences, healthy cells also express the same proteases presenting an issue with potential systemic toxicity. Further optimization of these sequences to target tumor cells while providing higher drug loading may prove beneficial to a range of antitumor therapeutics.

5. Macromolecular Architectures for Targeted Drug Delivery

Few constructs have utilized targeting moieties to localize CPT to tumors through receptor−ligand mediated interactions. While passive tumor targeting has been shown to successfully reach solid tumors with increased vasculature quite efficiently through the EPR effect, actively targeting receptors present on tumor cells allows for tumor localization for both solid tumors and leukemias. Active targeting relies on the presence of a specific receptor overexpressed on cancer cells relative to non-cancerous cells for tumor specific drug delivery. Targeted therapy has proven to be useful in some cases, but significant barriers toward successful clinical implementation are present including the aforementioned challenges to characterization. Some targeted therapies have been reported but have not been investigated *in vivo*.[307]

5.1. Luteinizing Hormone-Releasing Hormone-PEG-Camptothecin (LHRH-PEG-CPT). *Animal Models.* Breast, ovarian and prostate cancer cells have been shown to overexpress luteinizing hormone-releasing hormone (LHRH) receptors, which are not detected in most other organs. To exploit this tumor targeting potential, LHRH was attached to a 5 kDa PEG chain with CPT attached to the other end.[308] Cysteine links CPT through an ester and PEG through a thioether. The molecular weight of the final construct is ~7 kDa, which represents a 5 wt % drug loading. In mice without tumors, tritium labeled PEG and LHRH-PEG showed limited uptake in major organs with the highest in the liver. Mice with tumors showed a significant increase in PEG and LHRH-PEG accumulation. As expected, the ovaries showed an increase in accumulation with targeted PEG in mice without tumors and with tumors. Nude mice with sc A2780 ovarian cancer xenografts were treated with 0.5 mg/kg of targeted and nontargeted constructs through ip injection. Surprisingly, tumors decreased in size 20 h after treatment. Other macromolecular nontargeted constructs, which have 100% tumor regression >60 days generally do not see statistically significant tumor response until one week after treatment. The nontargeted CPT-PEG-constructs developed by Minko afforded tumor maintenance up to 40 h and slow increase in tumor size from 40 to 100 h. Furthermore, a 28-fold increase in relative apoptosis values was observed in tumors compared to the untargeted construct. Apoptosis was measured using ELISA assays for protein expression in tumor homogenates. Additional studies to investigate the physi-

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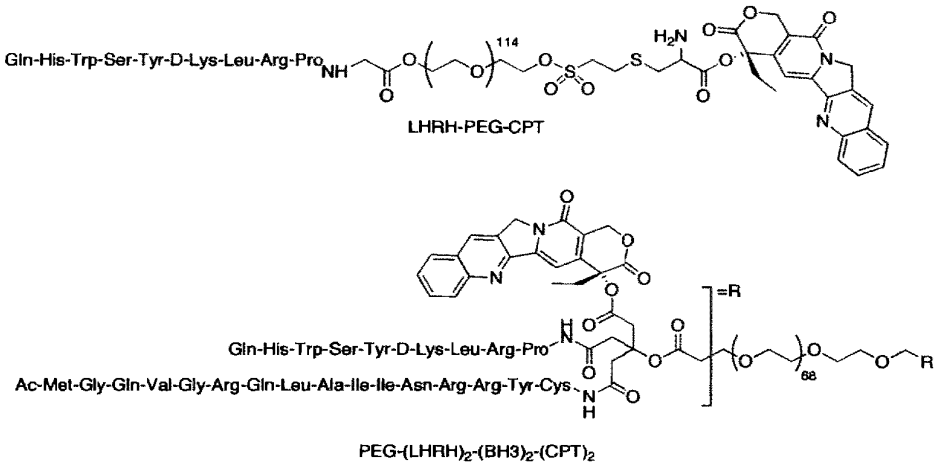

Figure 15. Ideal structures of PEG-CPT constructs containing LHRH or LHRH and BH3 antiapoptotic peptide.

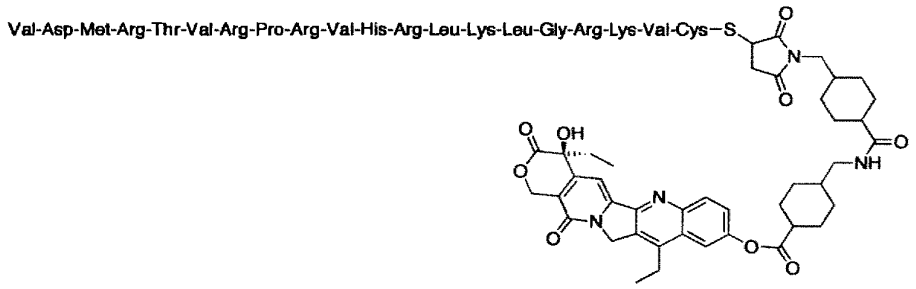

Figure 16. SN-38 linked to Vectocell cell penetrating peptide, through a maleimide linkage.

ologic effects of LHRH targeting moieties showed no change in serum levels of luteinizing hormone and no change in progeny numbers at the next generation.

More complex constructs with citric acid groups installed on the termini of the PEG chain were reported. The resulting six terminal carboxylates were used to attach CPT, LHRH and the BCL2 homology 3 domain (BH3).[309] The BH3 peptide is added to suppress the cellular antiapoptotic defense system. A series of derivatives were synthesized with the construct bearing 2 CPT, 2 LHRH and 2 BH3 moieties judged most effective construct. Apoptosis was measured in each construct in mice bearing A2780 tumor xenografts, with the saline control having a relative value of one. The drug construct containing 2 CPT, 2 LHRH and 2 BH3 moieties showed a relative apoptosis unit of 55. A 28% decrease in apoptosis was measured when one of each moiety was present with an approximate 64% decrease in apoptosis when two CPT and 2 BH3 were present without LHRH. Furthermore, the absence of BH3 caused a 55% decrease in apoptosis. Tumor size was also measured 96 h after treatment of 0.4 mg/kg (1 CPT) or 0.7 mg/kg (2 CPT) of construct, showing the most significant response when two of each moiety was present on the PEG construct (Figure 15).

5.2. Vectocell SN-38 (DTS-108). *Animal Models.* DTS-108 is a prodrug of SN-38 with a 20-amino acid peptide sequence, known as Vectocell (Figure 16), which enables increased cellular trafficking.[310] This construct has a molecular weight of 3.2 kDa and contains a highly charged sequence, allowing for delivery of the topoisomerase I inhibitor directly into the nucleus of the cell. In dogs, the MTD after iv infusion of a single dose was determined to be 20 mg/kg or 2.2 mg/kg with respect to SN-38, which is significantly lower than the MTD of irinotecan (30 mg/kg). At this dose, the prodrug AUC values decreased dramatically from 36 μg·h/mL with irinotecan to 4 μg·h/mL with DTS-108 at their respective MTDs. However, the AUC values of SN-38 increase significantly from 0.018 μg·h/mL with irinotecan to 4.8 μg·h/mL for DTS-108. The increase in active drug suggests that more SN-38 is available in the plasma. However, this observation also suggests that a portion is not entering the cell with the aid of the peptide. In nude mice bearing HCT116 tumors, a slight enhancement in antitumor activity was observed when DTS-108 was administered intravenously at a dose of 10.4 mg/kg on days 3, 7, and 11 after tumor implantation, as compared to 20

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mg/kg for irinotecan. When delivered on a more frequent schedule (3 times a week for 3 weeks), DTS-108 provides significant tumor growth inhibition, however, this data is not compared with irinotecan. In mice bearing HCT116 colorectal carcinoma, a 3% T/C was observed, with a 23% and 29% T/C in mice bearing NCI-H460 and MDA-MB-231 tumors, respectively. A 44% T/C was observed in rats with LS-174T colon tumor xenografts. Furthermore, efficacy was improved in combination therapy with 5-fluorouracil or bevacizumab suggesting further investigation with DTS-108 is warranted. It is unclear from this study whether DTS-108 has an advantage over irinotecan with respect to efficacy, but pharmacokinetics seem to improve and interpatient variability may also improve due to the ability to function without further metabolism to form SN-38. Further studies with DTS-108 are currently ongoing.

6. Conclusions

Interest in the CPTs has undergone a significant evolution from the initial discovery in the late 1960s through the investigation of small molecule derivatives to macromolecular constructs and formulations. The initial modifications of the quinoline ring provided increased solubility and cytotoxicity, which led to further structure activity relationships to determine the necessity of the E-ring lactone. The importance of the lactone was confirmed with reports of the TOP I binding site. Further modifications of the E ring and the 20-(*S*)-hydroxyl moiety have led to a series of water-soluble, highly efficacious CPTs.

While small molecule CPTs have received much attention, macromolecular architectures and supramolecular assemblies have improved pharmacokinetic parameters over the small molecule counterparts. Increasing plasma half-life and AUC values correlate to antitumor efficacy, which continues to improve in a broad series of tumor cell lines in mouse xenograft models as well as clinical trials. A compilation of the *in vivo* trials for the small molecule drugs suggests that irinotecan and exatecan are the most promising derivatives based on half-life and plasma AUC values. This conclusion may be supported by the number of clinical trials completed and ongoing using irinotecan (>250 according to clinicaltrials.gov). This comparison, however, is not as straightforward when evaluating macromolecular constructs. While each construct has specific half-life and AUC values associated, a number of variables play a role in the selection of the optimal construct. Synthetic ease, linker technology, solubility, drug loading, molecular weight, drug accessibility to esterases and other proteins and polymer degradability must all be taken into account. Furthermore, physiological variables also play a role, which is not as easy to account for.

In selecting a drug to utilize, a number of options are available. Implementation is limited by availability, cost and the need for metabolic activation. Antagonistic functional groups, such as the phenolate on 10-hydroxyCPT, and potential side reactions must also be considered. Irinotecan has been successful as a small molecule drug, however, implementation onto a polymer would contradict the benefits of macromolecular delivery to the tumor since drug trafficking to the liver would be necessary for activation. The use of camptothecin then seems to be a logical choice.

The polymers used have also provided evidence of optimal properties. In most cases a biodegradable polymer backbone is employed such as cyclodextrans, polyamides and polyacetals. The molecular weights of these constructs also provide insight. For example, MAG-CPT, an HPMA based construct with a molecular weight of 20 kDa, suffers from renal excretion and bladder toxicity. Higher molecular weight constructs tend to stay in the blood longer and potentially allow more uptake in the tumor. However, DE-310 has a molecular weight of 300 kDa and a plasma half-life of 7 days, which may be beneficial for sustained drug concentrations, but may also present chronic toxicity issues over time. DE-310, however, has had the most significant efficacy data in a phase 1 study of any other macromolecular construct with extended periods of remission in slightly more than 50% of the patients. It is unclear, however, what parameter is most indicative of these results. The tetrapeptide linker may be of more importance than the molecular weight of the polymer itself. Each of these parameters may only be corroborated after methodical modifications of each unit in the macromolecular architecture. Interestingly, XMT-1001 has changed the tetrapeptide linker for a Gly-hexanoic acid-Gly linker and decreased the polymer from 300 kDa to 70 kDa. Preliminary data with this construct also suggests disease stabilization, but further studies are needed to support this data.

Furthermore, changes in dose and schedule greatly affect the pharmacokinetics and efficacy of the constructs. These data are summarized in the tables. The doses listed in the tables generally correspond to the MTD unless a recommended dose other than the MTD is mentioned in the specific reference. Some references generated pharmacokinetic data from doses that were not the most efficacious. In general, many different dosing strategies have been evaluated for all species.

The MTD dose with small molecules is generally the dose that offers the greatest therapeutic efficacy, while macromolecular agents were generally tested at doses lower than the MTD of the construct to establish improved efficacy over the small molecules. While macromolecules improve the pharmacokinetic data observed in small molecules, comparisons between different macromolecular constructs cannot be superficially made from the reported data. Data in each report is presented in a slightly different manner. For example, half-lives are sometimes reported using a two-compartment model or a three compartment model, while others just report elimination half-life. Additionally, it is unclear in some cases whether the half-lives are reported with respect to macromolecular drug construct, total drug construct or free drug. Furthermore, such disparity in data may be due to the difference in chemical makeup of the architectures as well as genetic differences in animal and human subjects.

Interpatient variability with both macromolecular constructs and free drug continues to hamper the widespread use of CPTs. Some variability in pharmacokinetics has been shown to occur due to a mutation in ABCG2 when using diflomotecan.[311] This protein is believed to be responsible for natural detoxification and has been found to be overexpressed in the placenta, liver and intestine. Allele mutations have shown dramatically increased plasma AUC values for molecule substrates, which include 9-AC,[312] SN-38[313] and topotecan.[314] Although allele variants may provide insight into potential pharmacokinetic outcomes, it is likely that other physiological differences in tumors, such as vascularization and expression of other proteins, may also cause variability. Furthermore, a significant number of clinical trials have been completed in patients who have previously been treated with therapy, showing further interpatient variability with improved efficacy in some cases and diminished efficacy in others. One method to decrease interpatient variability and improve efficacy using the CPTs utilizes cellular transfection prior to therapy to overexpress E2F-1 and thus sensitize the tumors to CPT. Promising results have been obtained using this technique, but further assessment of transfection and camptothecin cotherapy are needed to verify the clinical relevance of such a technique.

While research aims toward the development of a "magic bullet" capable of treating all cancers in all patients with a single compound, current data suggests the need to tailor therapy for individual patients either through the choice of drugs and dosing schedules or through the use of combination therapy. This review identifies a number of interesting leads for these pursuits as well as comparative data useful for assessing the next generation of candidates.

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Coencapsulation of irinotecan and floxuridine into low cholesterol-containing liposomes that coordinate drug release in vivo

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Abstract

A liposomal delivery system that coordinates the release of irinotecan and floxuridine in vivo has been developed. The encapsulation of floxuridine was achieved through passive entrapment while irinotecan was actively loaded using a novel copper gluconate/triethanolamine based procedure. Coordinating the release rates of both drugs was achieved by altering the cholesterol content of distearoylphosphatidylcholine (DSPC)/distearoylphosphatidylglycerol (DSPG) based formulations. The liposomal retention of floxuridine in plasma after intravenous injection was dramatically improved by decreasing the cholesterol content of the formulation below 20 mol%. In the case of irinotecan, the opposite trend was observed where increasing cholesterol content enhanced drug retention. Liposomes composed of DSPC/DSPG/Chol (7:2:1, mole ratio) containing co-encapsulated irinotecan and floxuridine at a 1:1 molar ratio exhibited matched leakage rates for the two agents so that the 1:1 ratio was maintained after intravenous administration to mice. The encapsulation of irinotecan was optimal when copper gluconate/triethanolamine (pH 7.4) was used as the intraliposomal buffer. The efficiency of irinotecan loading was approximately 80% with a starting drug to lipid molar ratio of 0.1/1. Leakage of floxuridine from the liposomes during irinotecan loading at 50 °C complicated the ability to readily achieve the target 1:1 irinotecan/floxuridine ratio inside the formulation. As a result, a procedure for the simultaneous encapsulation of irinotecan and floxuridine was developed. This co-encapsulation method has the advantage over sequential loading in that extrusion can be performed in the absence of chemotherapeutic agents and the drug/drug ratios in the final formulation can be more precisely controlled.

Keywords: Liposomes; Irinotecan; Floxuridine; Coordinated release; Metal loading

1. Introduction

Liposomes have been used extensively to improve the therapeutic index of a variety of drugs by ameliorating toxicity and/or increasing the therapeutic potency of the encapsulated agent [1,2]. This is perhaps best exemplified in the delivery of anticancer drugs where it has been well documented both preclinically and clinically that small (approximately 100 nm) liposomes reduce exposure of entrapped drugs to susceptible healthy tissues while preferentially accumulating in sites of tumor growth due to enhanced permeability and retention (EPR) effects associated with solid tumors [3–6]. This in turn has often resulted in improvements of the overall therapeutic activity of the drug and has led to the regulatory approval of several liposome-based anticancer products [7,8]. Interestingly, very little work has been undertaken to deliver drug combinations in liposomes. This is likely the result of difficulties associated with the efficient and stable encapsulation of two chemotherapeutics inside a single liposome as well as challenges in controlling the release of chemically disparate drugs with one liposome composition.

Our interest in developing liposome formulations containing co-encapsulated anticancer drug combinations stems from the fact that virtually all curative cancer treatment regimens utilize drug combinations. We hypothesized that enhanced antitumor activity may be achieved by simultaneously delivering and

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exposing anticancer drug combinations to tumor cells in vivo, since tumor cells would be less able to develop compensatory resistance mechanisms compared to single or sequentially administered agents. This approach became of even greater importance when we recently observed that the antitumor activity of drug combinations can be dramatically dependent on the molecular ratio of the combined drugs [9]. Specifically, some ratios of a drug combination can be synergistic whereas other ratios of the same drugs can be additive or even antagonistic [10]. This highlighted the need to control drug ratios being exposed to tumor cells after systemic administration since the uncontrolled and dissimilar pharmacokinetics of individual drugs utilized in conventional drug "cocktails" no doubt results in exposure to sub-optimal drug ratios with a concomitant loss in therapeutic activity. Further, although an increasing number of individual liposomal anticancer drugs are being developed, the pharmacokinetics of currently available formulations vary greatly which would hinder attempts to coordinate the exposure of different drugs using existing liposome formulations [11,12]. In addition, combining multiple liposome-based anticancer drugs poses potential difficulties due to high lipid doses which have been shown in humans to lead to infusion-related adverse events [13,14].

In view of the above considerations, we undertook a series of studies aimed at developing single liposome formulations that are able to stably co-encapsulate drug combinations and coordinate the release of the two agents after intravenous (i.v.) injection. In this report we describe the co-encapsulation of irinotecan and floxuridine. This drug combination was selected based on the fact that irinotecan and fluoropyrimidine (typically 5-fluorouracil, 5-FU) combination treatment is standard of care for metastatic colorectal cancer. The focus on floxuridine rather than 5-FU was based on the superior encapsulation and retention properties of floxuridine compared to 5-FU [15] and the fact that trials conducted in the 1960s established the clinical equivalency of these two fluoropyrimidines [16,17]. We have shown in previous studies that a 1:1 molar ratio of these drugs optimizes drug synergy in a panel of gastrointestinal tumor lines [9]. We describe here the identification of liposome encapsulation techniques and lipid compositions that result in stable drug entrapment and drug release rates that maintain irinotecan and floxuridine at a 1:1 molar ratio after i.v. administration.

2. Materials and methods

2.1. Lipids, drugs and chemicals

Distearoylphosphatidylglycerol (DSPG), distearoylphosphatidylcholine (DSPC) and cholesterol were obtained from Avanti Polar Lipids (Alabaster, AL, USA). Irinotecan hydrochloride trihydrate (Camptosar) is a product of Pharmacia and Upjohn Company (Kalamazoo, MI, USA) and was obtained from the pharmacy of the British Columbia Cancer Agency. It was also obtained as a dry powder from SciinoPharm Taiwan, Ltd. (Tainan, Taiwan). Floxuridine was obtained from the Zhejiang Hisun Pharmaceutical Company (Taizhou City, China). Radiolabelled [^{3}H]floxuridine was obtained from Moravek Biochemicals (Brea, CA, USA). Cholesterol hexadecyl ether (CHE) radiolabelled with [^{3}H] or [^{14}C] was obtained from NEN Life Science Products (Oakville, ON, Canada). All other chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA).

2.2. Preparation of liposomes

Based on the appropriate molar composition, DSPC and DSPG were dissolved in chloroform/methanol/water (5/1/.1) at 50 mg/ml and combined with cholesterol dissolved in chloroform. Where appropriate, cholesteryl hexadecyl ether [^{14}C]CHE or [^{3}H]CHE was added as a non-exchangeable, non-metabolizable lipid marker [18]. The solvent was removed under a stream of nitrogen gas and the lipid films placed under high vacuum overnight. Typically, the lipid films were rehydrated at 70 °C with 100 mM copper gluconate, 220 mM triethanolamine (TEA), pH 7.4. The pH of the copper gluconate solution was adjusted by varying the TEA content and not through the addition of sodium hydroxide. In experiments involving the sequential loading of floxuridine and irinotecan, the floxuridine was passively trapped in the liposomes by adding 122 mM floxuridine plus [^{3}H]floxuridine as a tracer to the copper gluconate buffer. The newly formed multilamellar vesicles (MLVs) were passed 10 times through an extruding apparatus (Northern Lipids) containing two stacked 100 nm polycarbonate filters. The mean diameter and size distribution of each liposome preparation, analyzed by a NICOMP Model 270 Submicron particle sizer (Pacific Scientific, Santa Barbara, CA, USA) operating at 632.8 nm, was typically 110±20 nm. Following extrusion the external liposomal buffer was exchanged for 300 mM sucrose, 20 mM HEPES, 30 mM EDTA, pH 7.4 (SHE) using tangential flow chromatography.

2.3. Time course of irinotecan encapsulation

The following conditions were used to examine irinotecan encapsulation into liposomes containing various metal salts, copper gluconate at different pH and irinotecan to lipid ratios. Irinotecan (Camptosar) and liposomes were heated separately at 50 °C for 1 min and then combined at $t=0$ by adding the lipid to the drug while vortexing. The final lipid concentration was diluted to 30 mM using SHE buffer. Aliquots of 100 μl were removed at various time points and applied to 1 ml Sephadex G-50 spin columns. The columns were prepared by adding glass wool to a 1 ml syringe and Sephadex G-50 beads hydrated in HEPES buffered saline (HBS; 20 mM HEPES, 150 mM NaCl, pH 7.4). The columns were packed by spinning at 500×g for 2 min. Following addition of the sample to the column, the liposome fraction was collected in the void volume by centrifuging at 650×g for 1 min. Aliquots of the spin column eluant and the pre-column solution were taken and analyzed by liquid scintillation counting to determine the lipid concentration at each time point. The irinotecan concentration in each liposomal fraction was determined using a UV based assay. Briefly, a 100 μl aliquot of each liposomal sample (or smaller volume adjusted to 100 μl with distilled water) was solubilized in 100 μl of 10% Triton X-100 plus 800 μl of 50 mM citrate/trisodium citrate, 15 mM EDTA, pH 5.5 and heated in boiling water until the cloud point was reached. The samples were cooled to room temperature and the absorbance at 370 nm measured and compared to a standard curve. For mouse studies utilizing irinotecan containing formulations, the unencapsulated irinotecan and EDTA was removed by replacing the external buffer with SH buffer (300 mM sucrose, 20 mM HEPES, pH 7.4) using tangential flow chromatography.

2.4. Simultaneous encapsulation of irinotecan and floxuridine

A lipid film composed of DSPC/DSPG/Chol (7:2:1, mol/mol/mol) was prepared as previously described in Section 2.2 containing [^{14}C]CHE as the lipid marker. The film was hydrated with 100 mM copper gluconate, 220 mM TEA, pH 7.4 at 70 °C and the resulting MLVs were extruded at 70 °C through two stacked 100 nm filters for a total of ten passes. The liposomes were subsequently exchanged for SHE using tangential flow chromatography and concentrated to 60 mg/ml. To prepare a drug solution containing floxuridine and irinotecan, ^{3}H-floxuridine was first added to a test tube and dried under a stream of nitrogen. Subsequently, floxuridine and irinotecan were added to the same tube as a powder and hydrated with SHE buffer. The pH of the drug solution was adjusted to 7.4 with NaOH. The amount of irinotecan in the loading solution was based on a 0.12 drug to lipid molar ratio. The final floxuridine concentration was adjusted so that a concentration of 122 mM would be achieved after its addition to the liposome solution. The drug solution and the liposomes were incubated separately at 50 °C for approximately 5 min to equilibrate the temperature. The

two solutions were then combined and 100 μL aliquots were removed at various time points and applied to a Sephadex G-50 spin column as previously described. The drug to lipid ratio for floxuridine, from the eluted column sample, was determined using dual label liquid scintillation counting. The irinotecan concentration was determined by measuring the irinotecan absorbance at 370 nm as previously described.

2.5. Mice

Female Balb/c mice (6–8 weeks), 20–22 g breeders were purchased from Charles River Laboratories (St. Constant, PQ, Canada) and bred in house. Mice were housed in microisolator cages and given free access to water and food. The animals were maintained according to the procedures established at the BC Cancer Agency Joint Animal Facility. All animal studies were approved by the University of British Columbia Animal Care Committee in accordance with the guidelines established by the Canadian Council of Animal Care.

2.6. Plasma elimination of liposomes

To determine the retention of irinotecan and floxuridine in various liposomal formulations in vivo, plasma elimination studies were performed. Radiolabelled liposomes containing floxuridine and/or irinotecan were administered at the appropriate concentration intravenously into the lateral tail vein of mice. At 1, 4 and 24 h after administration, mice were asphyxiated with CO_2 and the blood collected by cardiac puncture and placed into EDTA coated microtainer tubes. The blood was centrifuged at 900×g for 10 min at 4 °C to isolate the plasma fraction. Liposomes and floxuridine concentrations were determined by liquid scintillation counting while irinotecan concentrations were determined by HPLC.

2.7. Irinotecan quantitation using HPLC

To determine the $T=0$ irinotecan concentration in the liposome sample prior to injection, the liposomes were diluted 1:100 in saline. Subsequently, a 100 μL aliquot of the diluted liposome sample was mixed with internal standard (camptothecin), followed by adding 600 μL acidified methanol (pH 2.0). For determination of irinotecan HCl in plasma, 10 μL (1-h and 4-h time points) or 20 μL (24-h time points) mouse plasma was mixed with 10 μL internal standard (camptothecin) followed by adding 600 μL acidified methanol (pH 2.0). Samples were then vortexed vigorously and stored in −70 °C freezer for 1 h. After cooling, the samples were centrifuged for 10 min at 1500×g and the supernatant was analyzed for irinotecan HCl using HPLC. The chromatographic system consisted of a Waters Alliance 2695 system and 2475 fluorescence detector. Waters Symmetry reverse phase C18 column (4.6×250 mm, 5μ) protected by a Symmetry Sentry guard column (3.9×20 mm, 5μ) was used for chromatographic separation. The analytical column was maintained at a temperature of 30 °C. The mobile phase was composed of acetonitrile −75 mM ammonium acetate containing 7.5 mM tetrabutylammonium bromide (24:76, v/v), with pH adjusted to 6.4 using glacial acetic acid. The mobile phase was delivered isocratically at a flow rate of 1.5 mL/min. Column elute was monitored fluorimetrically at excitation wavelength of 362 nm and emission wavelength of 425 nm. Detection and integration of chromatographic peaks was performed by Waters Empower software. Irinotecan HCl concentration was calculated using a calibration curve (linear range 2–100 μg/mL).

3. Results

3.1. Irinotecan encapsulation into transition metal containing liposomes

Based on the structure of irinotecan and the documented ability of transition metals to interact with drugs [19], we hypothesized that the presence of a free hydroxyl on the E-ring adjacent to the lactone group could be a candidate for the formation of a coordination complex with metal ions [20,21], thus enabling active loading of the irinotecan into preformed liposomes containing a metal salt solution. To determine if irinotecan could be encapsulated into liposomes using transition metals, DSPC/Chol (55:45 mol/mol) liposomes were prepared containing 300 mM sulfate salts of manganese, zinc, copper, nickel or cobalt. The liposomes were incubated with irinotecan at 50 °C using a drug to lipid ratio of 0.1:1 (mol/mol) for 1 h. This temperature was selected based on the fact that temperatures ≤40 °C provided very slow irinotecan encapsulation rates (data not shown). At various time points, aliquots were removed and the encapsulated drug to lipid ratio was determined (Fig. 1). Less than 10% encapsulation was observed under these conditions for all metals except zinc and copper which rapidly accumulated irinotecan. Zinc encapsulation was less efficient than copper with a maximum irinotecan to lipid ratio of 0.075:1 (mol/mol) and showed a gradual decrease in trapped irinotecan over the 60-min time course. Irinotecan encapsulation into copper containing liposomes was very effective with >95% encapsulation efficiency at all time points tested. It should be noted that although evidence of transition metal–fluoropyrimidine interactions have been reported [22], attempts to actively encapsulate floxuridine into metal containing liposomes in a manner similar to irinotecan were unsuccessful (data not shown).

Numerous copper salts (chloride, sulfate, nitrate, gluconate and tartrate) are commercially available, however, only the sulfate and gluconate forms were found to be sufficiently soluble and stable to promote efficient irinotecan encapsulation described here. When dissolved in water, both the sulfate and gluconate forms of copper are acidic with a pH of approximately 4. Since it was desirable to have an internal liposomal pH near neutrality, the copper solutions were adjusted to pH 7.4. When copper sulfate was pH adjusted with sodium hydroxide, however, a significant copper oxide

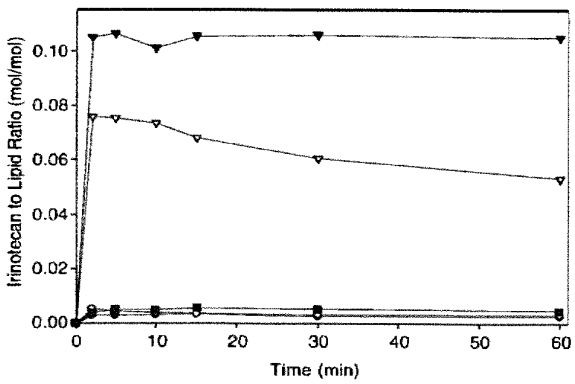

Fig. 1. Irinotecan encapsulation into DSPC/Chol (55:45, mol%) liposomes using various metal salts. Liposomes radiolabelled with [^{3}H]CHE were extruded in the presence of various unbuffered metal salt solutions and subsequently exchanged into SHE buffer (pH 7.4). The liposomes were incubated at 50 °C in the presence of irinotecan at a 0.1/1 molar drug to lipid ratio. At various time points aliquots were removed and assayed for drug encapsulation as outlined in Section 2.3. The following metal solutions, $CuSO_4$ (▼); $ZnSO_4$ (▽); $MnSO_4$ (■); $CoSO_4$ (●) and $NiSO_4$ (○) were all prepared at a concentration of 300 mM and were not pH adjusted (representative data shown).

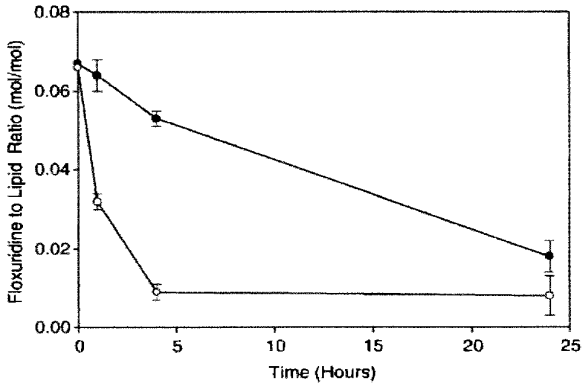

Fig. 2. The influence of liposome composition on the retention of floxuridine in vivo. Liposomes composed of either DSPC/DSPG (8:2 molar ratio, ●) or DSPC/Chol (55: 45 molar ratio, ○) were radiolabelled with [^{14}C]CHE were extruded in the presence of a 100 mM solution of [^{3}H]floxuridine dissolved in HBS (pH 7.4). The unencapsulated floxuridine was exchanged for saline using tangential flow chromatography. The liposomes were injected into the tail vein of female Balb/c mice at a floxuridine dose of 5 mg/kg. Blood was collected via cardiac puncture at 1, 4 and 24 h after injection (3 mice per time point). Lipid and floxuridine recovery was determined by liquid scintillation counting.

precipitate formed immediately after mixing. The biological buffer triethanolamine (TEA) was found to be the most compatible buffer for both copper sulfate and copper gluconate pH adjustment. When pH 7.4 solutions of copper gluconate and copper sulfate were compared for their ability to encapsulate irinotecan, the copper gluconate solution was found to be superior in total capacity, percent encapsulation and drug retention (data not shown). As a result, all subsequent formulation studies focused on copper gluconate buffered with TEA. It should be noted that stable liposome formulations containing gluconate salts of the other transition metals buffered to physiological pH could not be prepared due to instability of the salt solution which lead to significant precipitation (data not shown).

3.2. Floxuridine encapsulation and retention in liposomes

Since floxuridine could not be actively loaded into liposomes via metal complexation, the drug was passively encapsulated into liposomes by hydrating lipid films in the presence of floxuridine solutions. A previous study reported that the trapping efficiency of floxuridine can be increased when trapped in negatively charged liposomes [15]. Also, recent studies have shown that cholesterol-free liposomes can enhance the retention properties of certain drugs [23]. Taken together, this information led us to investigate the in vivo drug retention properties of cholesterol-free, negatively charged liposomes composed of DSPC/DSPG (80:20, mol/mol) compared to DSPC/Chol (55:45, mol/mol) formulations. As shown in Fig. 2, the retention of floxuridine (reflected by maintenance of the encapsulated drug to lipid ratio) inside liposomes after i.v. injection to mice was found to be superior in the cholesterol free formulation. The half life for the release of floxuridine from the liposomes increased from approximately 1 h for DSPC/Chol (55:45, mol/mol) liposomes to approximately 16 h for the DSPC/DSPG (80:20, mol/mol) formulation. Based on the superior floxuridine retention properties and circulation lifetimes, the DSPC/DSPG formulation was studied as a potential delivery system for the drug combination of irinotecan and floxuridine.

3.3. Co-formulation of irinotecan and floxuridine

Initial studies on the co-formulation of irinotecan and floxuridine focused on the hydration of lipid films with solutions of copper gluconate/TEA that contained floxuridine. However, irinotecan encapsulation was not affected by the presence or absence of floxuridine within the liposomes. As a result, the optimization of irinotecan loading was determined in the absence of entrapped floxuridine. The liposomes hydrated from lipid films were extruded and the unencapsulated copper was removed by tangential flow dialysis. The conditions that were found to impact the subsequent encapsulation of irinotecan to the greatest degree were pH and temperature. To determine the optimal pH for irinotecan loading, liposomes were prepared with matching internal and external buffer pH. The time course for irinotecan loading into liposomes at 50 °C is presented in Fig. 3. Maximum irinotecan encapsulation leading to a final drug to lipid ratio of approximately 0.8:1 (mol/mol) was achieved with buffers at pH 7.0 and 7.5. This reflected an encapsulation efficiency of 80% based on the starting irinotecan to lipid ratio of 0.1:1 (mol/mol). The rate of irinotecan accumulation inside the copper containing liposomes was most rapid at pH 7.5 where uptake levels were 85% of maximum within 5 min. Decreasing the pH to 6.5 or 6.0 resulted in decreased rates of irinotecan accumulation and liposome uptake levels after incubating for 60 min were 0.061 and 0.039 μmole drug/μmol lipid, respectively. Due to the similar

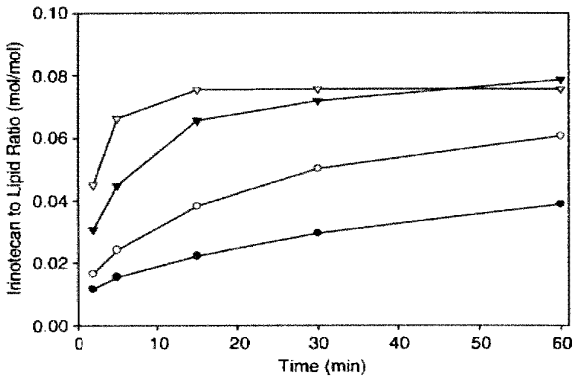

Fig. 3. The effect of pH on irinotecan encapsulation. Liposomes composed of DSPC/DSPG (80:20 molar ratio) were radiolabelled with [^{3}H]CHE and extruded in the presence of 100 mM copper gluconate that was pH adjusted with TEA to pH 7.5 (▽); pH 7 (▼); pH 6.5 (○) or pH 6 (●). The external liposomal solution was replaced with 150 mM NaCl, 10 mM HEPES and 10 mM MES which was pH adjusted to match the internal liposomal pH. Irinotecan was incubated with the liposomes at 50 °C and at various times aliquots were removed and passed through a spin column to determine the extent of irinotecan encapsulation. The irinotecan to lipid ratio was determined as described previously in Section 2.3 (representative data shown).

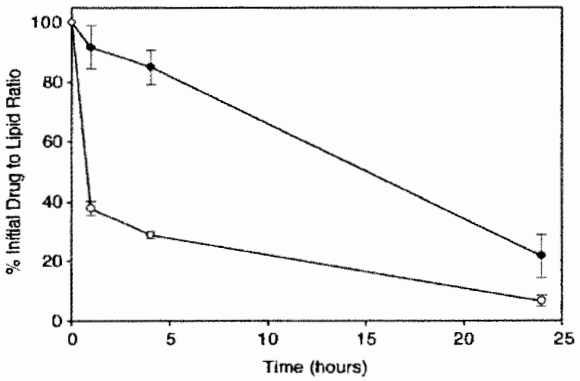

Fig. 4. The in vivo retention of floxuridine and irinotecan coformulated in DSPC/DSPG (80:20 molar ratio) liposomes. Liposomes were radiolabelled with [^{14}C]CHE and extruded in the presence of 100 mM copper gluconate/TEA pH 7.4 containing [^{3}H] labelled floxuridine. The external buffer was exchanged for SHE (pH 7.4) and loaded with irinotecan at a drug to lipid molar ratio of 0.1/1. The unencapsulated irinotecan and EDTA was removed by exchanging into SH buffer using tangential flow chromatography. Mice were injected via the tail vein at a lipid dose of 200 μmol/kg (16 μmol/kg of irinotecan and floxuridine). Blood was recovered at 1, 4 and 24 h after injection and centrifuged to isolate plasma (3 mice per time point). Lipid and floxuridine (●) levels were determined by liquid scintillation counting while irinotecan (○) concentrations were determined by HPLC analysis.

irinotecan loading properties at pH 7 and 7.5, the physiological pH of 7.4 was chosen for all subsequent studies.

Liposomes composed of DSPC/DSPG (80:20, mol/mol), containing both floxuridine and irinotecan at a molar drug ratio of 1:1 (drug to lipid molar ratios of approximately 0.08:1), were administered i.v. into mice to determine if this formulation could coordinate drug release after injection. The drug to lipid ratio for both drugs was monitored in the plasma over 24 h as an indicator of drug release from the liposomes in the circulation. As shown in Fig. 4, the retention of floxuridine in this cholesterol-free formulation was far greater than that observed for irinotecan. The floxuridine drug to lipid ratio decreased linearly over time with a half life of approximately 14 h and 22% of the original drug to lipid ratio remained after 24 h. In contrast, the retention of irinotecan was poor with greater than 60% drug leakage within the first hour after injection and the plasma irinotecan to lipid ratio at 24 h was only 5% of the starting drug to lipid ratio. This large difference in drug release rates lead to circulating irinotecan/floxuridine ratios that rapidly deviated from the desired 1:1 ratio. It should be noted that the rates of irinotecan and floxuridine release when co-formulated inside the same liposome were similar to those obtained for each respective drug encapsulated individually inside the liposomes (data not shown).

In order to better coordinate the release of irinotecan and floxuridine co-formulated inside 100 nm liposomes, an iterative variation of lipid composition was performed. Since cholesterol content was shown to have a dramatic impact on the retention of floxuridine (Fig. 2), we reasoned that it may also play a role in the retention of irinotecan. Various liposome formulations containing cholesterol at levels ranging from 0 to 15% were tested for drug retention in vivo. At 1, 4 and 24 h after injection, blood was collected and the plasma was analyzed for irinotecan, floxuridine and lipid levels. Irinotecan to lipid ratios were found to be significantly affected by cholesterol content (Fig. 5, top panel). In the formulations containing 0 or 5% cholesterol, rapid leakage of irinotecan is observed. One hour after injection of these formulations, the circulating liposomes retained only 38%

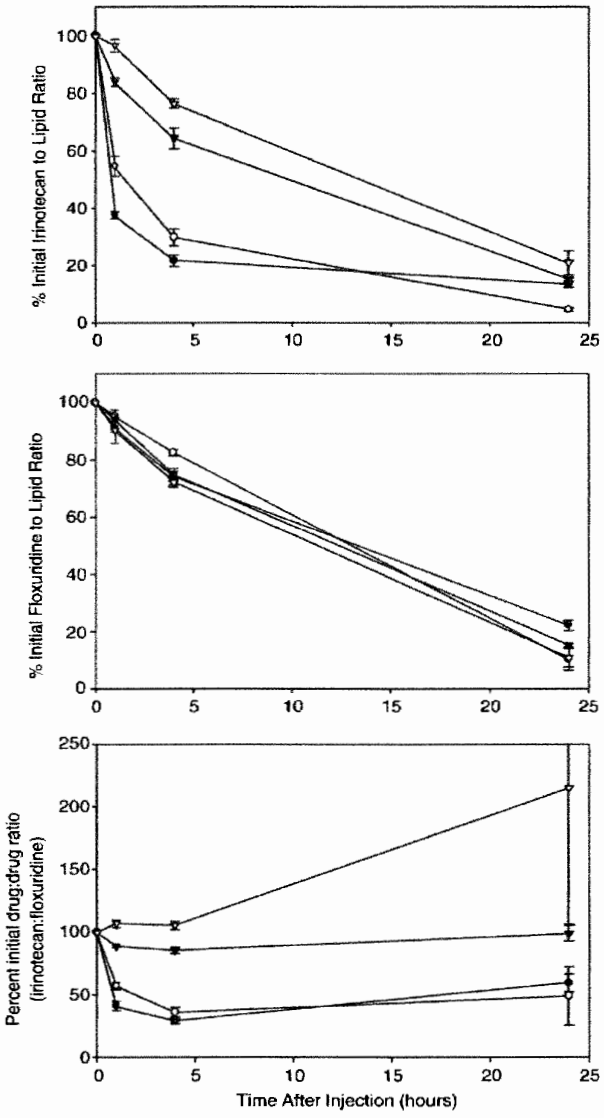

Fig. 5. The in vivo retention of floxuridine and irinotecan coformulated in various liposomal formulations. Liposomes composed of DSPC/Chol/DSPG (65:15:20 molar ratio); ▽, DSPC/Chol/DSPG (70:10:20 molar ratio); ▼, DSPC/Chol/DSPG (75:5:20 molar ratio); ○, and DSPC/DSPG (80:20 molar ratio); ● were radiolabelled with [^{14}C]CHE and coformulated with irinotecan and floxuridine as previously described. Samples were injected into the tail vein of mice at a lipid dose of 370 μmoles/kg (37 μmoles/kg irinotecan and floxuridine). At 1, 4 and 24 h after injection blood was collected and assayed for lipid, irinotecan and floxuridine levels (3 mice per time point). Changes to irinotecan to lipid levels with time are monitored in the upper panel while the middle panel plots the floxuridine to lipid levels from the same formulation. The lower panel charts changes in the drug: drug molar ratio in the various liposomal formulations over time.

and 55% of the initial encapsulated irinotecan, respectively. When the cholesterol content was increased to 10 and 15 mol%, we observed 84 and 97% of initial irinotecan to lipid ratios in the plasma after 1 h. The small increase in cholesterol content from 5 to 10 mol% increased the irinotecan half life by approximately 6.5-fold from 1.5 h to 10 h. This large dependency of irinotecan retention in vivo on cholesterol content was not observed at liposome cholesterol levels above 10 mol%. The sensitivity of irinotecan retention in the 10 mol% cholesterol range was subsequently investigated with formulations containing 7.5, 10 and 12.5 mol% cholesterol. Analysis of irinotecan release rates over 24 h found no significant differences between these three formulations (data not shown). As a result it appears that irinotecan retention is not correlated with cholesterol content in a linear fashion but requires a minimum threshold level. In addition, it should be noted that irinotecan can exist in an open ring carboxylate or closed ring lactone form. Only the lactone form of the drug is biologically active and was found to be the predominant form (>90%) in all the liposomal formulations tested.

The release of floxuridine from DSPC/DSPG-based liposomes as a function of cholesterol content was monitored and the results are presented in Fig. 5 (middle panel). Unlike irinotecan, floxuridine retention was not significantly influenced by cholesterol content between 0 and 15 mol%. The drug to lipid half life for floxuridine ranged from a high of approximately 13 h in the cholesterol free formulation to a low of approximately 11 h in the 15 mol% formulation. This was somewhat surprising since the results shown in Fig. 2 suggest that floxuridine leakage is dramatically enhanced in the presence of 45 mol% cholesterol. The fact that little difference was observed between 0 and 15 mol% cholesterol suggests that, similar to irinotecan leakage, there appears to be a threshold amount of cholesterol that is required before large changes in drug release are observed. When the encapsulated irinotecan to floxuridine molar ratio was plotted (Fig. 5, lower panel) for the 0 and 5 mol% formulations, the starting drug to drug molar ratio was observed to drop from 1:1 to 1:2 within 1 h. The initial drop in the molar drug ratio is the result of rapid leakage of irinotecan from these formulations. Following the initial drop at 1 h, the molar drug ratio is maintained out to 24 h. In the formulation containing 15 mol% cholesterol, the opposite trend was observed where the drug to drug ratio increases as a result of enhanced irinotecan retention relative to floxuridine. The synergistic 1:1 molar drug ratio was optimally maintained in the formulation containing 10 mol% cholesterol for which the release rates of both agents was matched.

3.4. Optimization of drug encapsulation

The studies described thus far have demonstrated that irinotecan and floxuridine can be stably co-encapsulated at a 1:1 molar ratio inside DSPC/DSPG/Chol (70:20:10, mol/mol) liposomes containing copper gluconate/TEA pH 7.4 and that these liposomes coordinate the release of the two drugs, thereby maintaining the irinotecan/floxuridine ratio for extended times after i.v. administration. In an attempt to increase the liposomal drug capacity, copper gluconate/TEA pH 7.4 containing liposomes were incubated at elevated starting irinotecan to lipid ratios to determine the maximum amount of drug that could be loaded into these formulations. Irinotecan encapsulation was not affected by the presence or absence of floxuridine within the liposomes, therefore irinotecan loading was determined in the absence of entrapped drug. At a starting drug to lipid ratio of 0.1:1, approximately 80% drug encapsulation is observed (Fig. 6). A similar encapsulation efficiency was observed at a starting irinotecan to lipid molar ratio of 0.2:1 where the final encapsulated drug to lipid ratio was approximately 0.15:1. However, when the starting irinotecan to lipid ratio was elevated to 0.3:1 the encapsulated irinotecan to lipid ratio increased only to 0.18:1, reflecting a decrease in encapsulation efficiency to approximately 60%. As a result, incubating the liposomes with irinotecan at a 0.2:1 ratio provided the highest drug to lipid ratio in the final formulation (0.14–0.15/1) without significantly decreasing the irinotecan loading efficiency.

Floxuridine levels were also increased in the liposome formulations in order to compensate for the increase in irinotecan to lipid ratio and maintain the 1:1 molar drug ratio inside the liposomes. This was achieved by extruding the liposomes in the presence of elevated floxuridine concentrations. However, floxuridine leakage during irinotecan encapsulation at 50 °C when the higher drug to lipid ratios were utilized caused significant difficulty in controlling final irinotecan to floxuridine ratios in the liposomes. This effect is reflected in Fig. 7 which presents the encapsulated floxuridine to lipid ratio over time for liposomes incubated at temperatures ranging from 40 °C to 60 °C. Floxuridine release from DSPC/DSPG/Chol (70:20:10, mol/mol) liposomes at 40 °C and 45 °C was <10% over 20 min, however under these conditions, irinotecan encapsulation was <50% (data not shown). In contrast, >90% floxuridine release was observed within 5 min when the liposomes were incubated at ≥55 °C. The rapid drug release at this temperature occurred as a result of the liposomal membrane

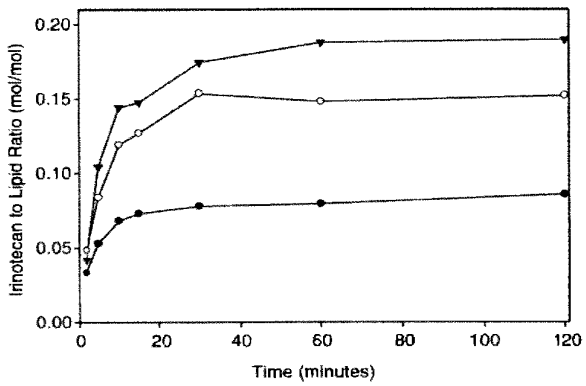

Fig. 6. The extent of irinotecan encapsulation at various starting drug to lipid ratios. Liposomes composed of DSPC/Chol/DSPG (70:10:20 molar ratio) were radiolabelled with [^{3}H]CHE and incubated with irinotecan at drug to lipid molar ratios of 0.1:1 (●), 0.2:1 (○) and 0.3 :1 (▼). The reaction was carried out at 50 °C for various times and assayed for drug encapsulation as previously described (representative data shown).

converting from a gel state to a liquid–crystalline state with a phase transition temperature (T_m) of 54.5 °C as measured using differential scanning calorimetry (data not shown). By decreasing the loading temperature to 50 °C, approximately 10% drug leakage was observed after 10 min, at which time irinotecan loading achieved maximum levels (see Fig. 3).

3.5. Simultaneous drug loading

In view of the difficulties associated with reproducibly achieving target irinotecan/floxuridine ratios inside DSPC/DSPG/Chol (70:20:10, mol/mol) liposomes with descending floxuridine entrapment due to leakage during irinotecan encapsulation, we examined alternative entrapment procedures whereby the two drugs could be simultaneously loaded subsequent to liposome formation and extrusion. We reasoned that if floxuridine could leak from liposomes when incubated at 50 °C, it should also be possible for the drug to load into preformed liposomes by following its concentration gradient and equilibrating across the liposome bilayer during irinotecan encapsulation. This approach relied on the ability of floxuridine and irinotecan to permeate the membrane under conditions where copper remained inside the liposomes. Liposomes were extruded in the presence of copper gluconate/TEA (pH 7.4) in the absence of floxuridine and then buffer exchanged to remove unencapsulated copper. Just prior to liposome loading, a single drug solution containing irinotecan and floxuridine was prepared. As shown in Fig. 8, floxuridine passively accumulated into liposomes simultaneously with irinotecan active encapsulation without compromising the integrity of irinotecan loading. The similar kinetics of liposome accumulation for floxuridine and irinotecan facilitated the ability to precisely control the drug/drug ratios in the final formulation and reliably

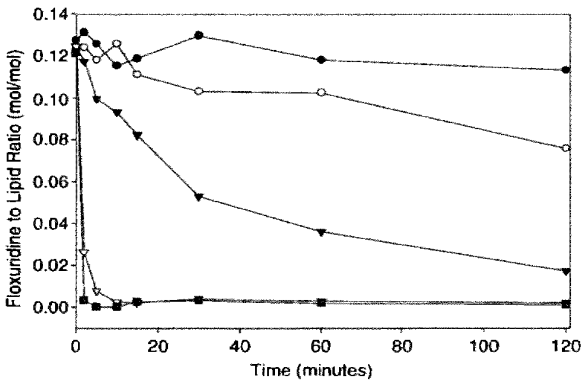

Fig. 7. The extent of floxuridine leakage from DSPC/Chol/DSPG (70:10:20 molar ratio) liposomes at various temperatures. Liposomes labelled with [^{14}C]CHE were extruded in the presence of 122 mM [^{3}H]floxuridine in HBS pH 7.4 and subsequently buffer exchanged to remove unencapsulated drug. To monitor the leakage rates of floxuridine from the formulation at a range of temperatures, the liposomes were incubated at 40 °C (●), 45 °C (○), 50 °C (▼), 55 °C (▽) and 60 °C (■) for 2 h. At various time points, aliquots were removed and passed though a Sephadex G-50 spin column and assayed for lipid and floxuridine content using liquid scintillation counting (representative data shown).

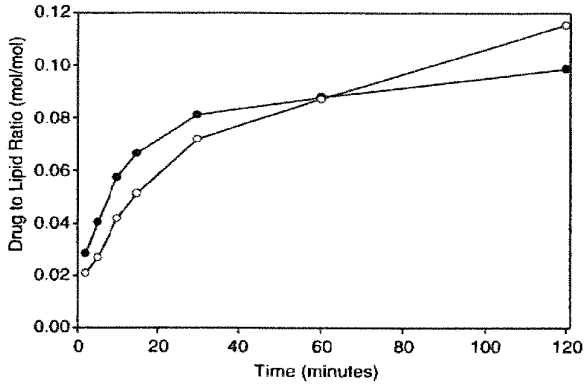

Fig. 8. The drug to lipid ratio of irinotecan and floxuridine during simultaneous drug loading. Liposomes composed of DSPC/Chol/DSPG (70:10:20 molar ratio) were radiolabelled with [^{14}C]CHE and incubated at 50 °C with [^{3}H] floxuridine and irinotecan as outlined in Section 2.4. At various time points aliquots were removed and passed through a Sephadex G-50 spin column to determine the irinotecan to lipid ratio (●) and the floxuridine to lipid ratio (○) (representative data shown).

achieve the target 1:1 molar ratio. It should be noted that the pharmacokinetics and drug release properties of this formulation did not differ from those observed with liposome formulations in which floxuridine and irinotecan were sequentially loaded (see Fig. 5).

4. Discussion

The use of drug combinations has been standard of care in the treatment of cancer for many years. Given this and the expanded use of liposomal delivery vehicles for cancer chemotherapy, it is somewhat surprising that very little research has focused on the development of liposomal drug combinations, either in separate liposomes or co-encapsulated in the same liposome. This may be related to expectations that the activity of liposomal drug combinations would simply be the sum of the individual liposomal drug components. In fact, the limited examples where liposomal drug combinations have been investigated resulted in therapeutic activity that was less than predicted for additivity [24,25]. Such adverse outcomes could have resulted from unfavorable pharmacological interactions such as restricted delivery of encapsulated drugs to tumor sites, counteractive drug activities or altered drug release rates.

We have recently identified a new and potentially important application for liposome delivery of drug combinations. This application arose from in vitro observations in cytotoxicity assays where the degree of drug synergy or antagonism has been shown to be dependent on the ratio of drugs in the combination [9]. The implications of this observation are that unless drug combinations are maintained in a synergistic range after administration in vivo, therapeutic activity will be compromised. Clearly, such control of systemic drug ratios after injection of unencapsulated drug cocktails is difficult, if not impossible, to achieve. Our approach to this problem is therefore to: (1) identify the optimum drug/drug ratio in a range of in vitro tumor cell lines, (2) design liposomes that can co-

encapsulate the desired drug/drug ratio and (3) maintain the drug/drug ratio in the synergistic range after administration so it can be exposed to tumor cells in vivo. One of the first drug combinations we applied this approach to was irinotecan and floxuridine, which are commonly used in the treatment of colorectal cancer [26–28]. This drug combination exhibited drug ratio-dependent synergy when examined in vitro where a 1:1 molar ratio was shown to be optimal over a broad range of tumor cell lines [9,29]. Consequently, we focused efforts to generate liposome formulations that could both encapsulate and maintain this drug combination at a 1:1 ratio.

In practical terms, the most challenging hurdle to achieving coordinated pharmacokinetics of drug combinations co-encapsulated in liposomes is the development of a single formulation that can simultaneously, yet independently control the release of two drugs that exhibit very differing physico-chemical properties, as is the case with irinotecan and floxuridine. Considering the significant differences in solubility properties of irinotecan and floxuridine, we contemplated establishing formulation conditions that would differentially control the entrapment and retention of these two drugs. As a very water-soluble compound (>400 mg/ml), floxuridine can be readily entrapped passively during liposome production and its release from liposomes will be predominately controlled by lipid permeability properties. In comparison, irinotecan can be actively entrapped inside liposomes with high efficiency using transmembrane pH gradients [30]. However, the use of pH gradients can be problematic due to the instability of phospholipids exposed to the acidic liposome interior which can lead to altered drug retention properties [31]. We therefore examined the use of encapsulated transition metals to actively load irinotecan without the use of pH gradients.

The rationale for utilizing encapsulated transition metals to actively load and retain irinotecan was based on the more than 40 years of evidence documenting interactions between transition metals and drugs [19]. We observed that only copper and zinc were able to promote the efficient encapsulation of irinotecan when high concentrations of liposome encapsulated unbuffered metal sulfates were utilized. The other metal sulfates tested (Ni, Mn, Co) provided little or no encapsulation under the conditions utilized even though $MnSO_4$ was previously reported to promote the encapsulation of doxorubicin into liposomes [32]. The pH of these unbuffered metal solutions ranged from a low of 3.3 for $MnSO_4$ to a high of 5.5 for $CoSO_4$. Based on this information and the loading curves, there appeared to be no correlation between the pH of the encapsulated salt solution and the extent of drug encapsulation. The lack of irinotecan uptake in the presence of $MnSO_4$ observed here contrasts the results obtained previously with another weak base camptothecin, topotecan, where liposomes containing this metal and the ionophore A23187 actively accumulated the drug [33]. This difference is related to the fact that in the presence of the ionophore, a transmembrane pH gradient (inside acidic) is generated which drives drug uptake. The fact that irinotecan does not load into the $MnSO_4$ without an ionophore indicates that the encapsulation observed here is not due to a pH gradient, but rather is related to selective interactions between irinotecan and the transition metals copper and zinc. The strength of the irinotecan interaction, correlates with the stability constants commonly observed with metal complexes [34]. Specific interactions between copper and the anthracyclines have been previously reported [35,36].

We were also able to achieve efficient loading using liposomes containing copper gluconate buffered to physiological pH with TEA, thereby alleviating potential difficulties with lipid degradation at acidic pH. When the influence of internal and external pH on the encapsulation of irinotecan was investigated, significantly lower irinotecan encapsulation was observed with decreasing pH. This may be the result of a decreased irinotecan permeability at lower pH due to a larger fraction of protonated amine, decreased interactions between irinotecan and copper gluconate/TEA or a combination of both. The mechanism responsible for this decrease has not been fully elucidated at this time. When the starting drug to lipid ratio was increased to 0.3:1, irinotecan encapsulation reached a maximum drug to lipid ratio of 0.18:1. This saturation effect suggests a stoichiometric relationship between irinotecan and copper gluconate/TEA. Further investigations are underway to fully characterize the nature of the interaction between irinotecan and copper gluconate/TEA inside liposomes. It is important to note that preliminary evidence for this buffer system indicates that TEA plays a major role in mediating the encapsulation and retention of irinotecan in contrast to the case of unbuffered copper sulfate where evidence for a direct irinotecan–copper interaction was obtained [37]. The copper gluconate/TEA irinotecan loading process described here provided an encapsulation system that was more amenable to pharmaceutical applications and was therefore utilized in an iterative optimization process to coordinate the release of irinotecan and floxuridine in vivo. This copper based formulation of irinotecan and floxuridine has been evaluated in dog toxicological studies and no evidence of copper toxicity was detected.

When floxuridine and irinotecan encapsulated in liposomes were administered i.v. to mice, surprising trends were observed in drug pharmacokinetics as a function of lipid composition. Historically, liposomes composed of saturated phospholipids and high cholesterol content (>33 mol%) have provided maximum drug retention in vivo for a wide range of drugs [38,39]. More recently, we demonstrated that for some drugs, low cholesterol-containing gel phase liposomes can reduce in vivo drug release compared to cholesterol-enriched systems [23]. In the studies here we observed a rather striking dependence of irinotecan and floxuridine leakage from DSPC-based liposomes in that the relationship between drug release and cholesterol content in the liposome was diametrically opposed for the two drugs. Whereas cholesterol-free DSPC/DSPG liposomes displayed optimal floxuridine retention, irinotecan was rapidly released from these systems. By titrating the cholesterol content in DSPC/DSPG liposomes containing floxuridine/irinotecan at a 1:1 ratio and assessing the plasma drug release properties after i.v. injection, we were able to identify that liposomes composed of DSPC/DSPG/Chol at a 70:20:10 molar ratio were able to coordinate the

pharmacokinetics of the two agents such that the circulating drug/drug ratio was maintained at approximately 1:1 for up to 24 h. The fact that the circulating drug to lipid ratios for both irinotecan and floxuridine decrease at the same rate over time indicates that both drugs are bioavailable and are being exposed systemically at the 1:1 ratio.

When liposomes were passively encapsulated with floxuridine and subsequently loaded with irinotecan, the temperature of loading had a dramatic impact on the drug content of the final formulation. In the cholesterol-free and low cholesterol formulations, irinotecan encapsulation was almost immediate at 60 °C. Unfortunately, this temperature resulted in over 90% floxuridine leakage within 2 min. This rapid leakage is likely the result of heating the liposomes above their transition temperature of 55 °C. Since these liposomes contain very little cholesterol, bilayer fluidity at these temperatures prevents adequate retention of floxuridine. Complete retention of floxuridine could be achieved at 40 °C, however, the encapsulation of irinotecan at this temperature was observed to be very poor. Consequently, a temperature of 50 °C was identified at which irinotecan could be encapsulated with high efficiency with manageable loss of floxuridine such that the target 1:1 drug ratio could be achieved. Although this was achievable in formulation batches less than 10 mL, it was not possible to achieve the desired accuracy and reproducibility in larger volumes.

In order to resolve this problem, an alternative loading scheme was developed based on the observed leakage of floxuridine at 50 °C. The high permeability of floxuridine near the phase transition temperature of the formulation allowed for the passive loading of floxuridine during irinotecan encapsulation. Analysis of the drug loading curves in Fig. 8 for the two drugs revealed very similar kinetics. This is somewhat surprising since the mechanisms driving the encapsulation of floxuridine and irinotecan are quite different and the two drugs display very different drug release dependence on cholesterol content. This method, however, has allowed us to control the encapsulated drug/drug molar ratio over a wide range of time. This unique dual loading method has the advantage over sequential loading in that the liposomes are prepared and extruded in the absence of chemotherapeutics and the final drug/drug ratio in the formulation can be tightly controlled.

This report describes one of the first attempts to co-formulate drug combinations in a manner that coordinates the pharmacokinetics of the different drugs after i.v. administration. By combining copper gluconate/TEA-based drug encapsulation and low-cholesterol containing gel phase liposomes, we were able to encapsulate and maintain irinotecan and floxuridine at a fixed, synergistic molar ratio of 1:1. We believe that maintaining this ratio will be critical to maximizing the therapeutic activity of this drug combination, based on results in tumor models [9,29]. As more cancer therapies utilize a greater number of agents, it will become increasingly important to avoid antagonistic drug interactions in order to maximize therapeutic activity. Since the pharmacokinetics of the individual drugs in conventional combination treatments cannot be controlled, formulating multiple drugs into delivery vehicles that can coordinate their pharmacokinetics is a viable approach to optimize the therapeutic activity of multiple agent combinations.

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Application No. / Patent No.	Ref.	Date
13 731 230.2 - 1109 / 2 861 210 /	P067376EP:ECO	28.08.2019
Proprietor Ipsen Biopharm Ltd.		

Decision revoking the European Patent (Art. 101(3)(b) EPC)

The Opposition Division - at the oral proceedings dated 10.07.2019 - has decided:

European Patent No. EP-B- 2 861 210 is revoked.

The reasons for the decision are enclosed.

Possibility of appeal
This decision is open to appeal. Attention is drawn to the attached text of Articles 106 to 108 and Rules 97 to 98 EPC.

Opposition Division:

Registered letter with advice of delivery
EPO Form 2331 07.19TRI

Chairman: Hoff, Philippe
2nd Examiner: Gradassi, Giulia
1st Examiner: Bazzanini, Rita

Kobylkova Fingerova
Formalities Officer
Tel. No.: +31 70 340-3260 **Branch at The Hague**

Enclosure(s): 22 page(s) reasons for the decision (Form 2916)
Wording of Articles 106 - 108 and Rules 97-98 EPC (Form 2019)
Minutes of oral proceedings

to EPO postal service: 22.08.19

Registered letter with advice of delivery
EPO Form 2331 07.19TRI

DECISION

Facts and Submissions

I. European patent 2 861 210 having the title "METHODS FOR TREATING PANCREATIC CANCER USING COMBINATION THERAPIES COMPRISING LIPOSOMAL IRINOTECAN" is based upon European patent application No. 13 731 230.2 filed on 12-06-2013. It claims priority of US 201261659211 filed on 13-06-2012 and US201361784382 filed on 14-03-2013. The mention of the grant of the patent has been published in the European Patent Bulletin of 03-05-2017.

Proprietor of the patent is:
Ipsen Biopharm Ltd.
Ash Road, Wrexham Industrial Estate, Wrexham, LL13 9UF, GB.

II. A notice of opposition has been filed by:
Teva Pharmaceutical Industries Ltd
5 Basel Street, P.O. Box 3190, 49131 Petah Tiqva, IL
on 05-02-2018.

III. The opponent (further referred to as OI) requests revocation of the patent in its entirety based on Articles 100(a) and (b) EPC because the patent lacks inventive step (Article 56 EPC) and is insufficiently disclosed (Article 83 EPC). The validity of the priority is also challenged by OI. In the notice of opposition OI made reference to documents D1-D16.

As an auxiliary request OI requested oral proceedings (Article 116 EPC).

IV. The opposition is filed in due time, in proper form and is supported by reasoned statement, and is therefore considered to be admissible in that it complies with the requirements of Articles 99(1), 100 EPC and Rules 3(1), 76 EPC.

V. In a reasoned statement received on 24-08-2018 in reply to the notice of opposition, the proprietor (further referred to as P) requested for the patent to be maintained on the basis of a new claim set entitled "Main Request". With his reply, P enclosed documents D15a, D17-D21 and relabelled document D15 cited by OI D15b.

As an auxiliary request P requested oral proceedings (Article 116 EPC).

VI. With official communication of 30.01.2019 the parties were summoned to Oral proceedings, to be held on 10.07.2019. In the annex to the summons the OD expressed a preliminary positive opinion with regard to Article 83, 87 and 56.

VII. With letter of 10.05.2019, within the limits under Rule 116(1) EPC, OI filed documents D1b and D22 and submitted further arguments in support of his objections under Articles 83, 87 and 56 EPC.

VIII. With letter of 28.06.2019, in reply to OI's submissions, P filed Auxiliary Requests 1-3.

IX. Reference will be made to the documents D1-D22 according to the following consolidated list:

D-number	Reference
D1	FDA label (Highlights of Prescribing Information) for FUSILEV (levoleucovorin) (2008)
D2	Gebbia V et al., *Am J Clin Oncol* (2008) 33:461-464
D3	Zaniboni A et al., *Cancer Chemother Pharmacol* (2012) 69:1641-1645
D4	Neuzillet C et al., *World J Gastroenterol* (September 2012) 18(33):4533-4541
D5	Yoo et al., *Br J Cancer* (2009) 101 :1658-1663
D6	Taieb J et al., *Ann Oneal* (2007) 18:498-503
D7	Chen Let al., *J Clin Oneal* (2008) 26:2565
D8	Infante et al., *Cancer Chemother Pharmacol* (2012) 70(5), 699
D9	Waterhouse et al., *Nanomedicine* (2011) 6(9), 1645-1654
D10	FDA label (Highlights of Prescribing Information) for CAMPTOSAR (irinotecan) (2012)
D11	Hoskins J M et al., *J Natl Cancer Inst* (2007) 99:1290-5
D12	Ko AH et al., *J Clin Oneal* (2011) 29(15), 4069
D13	Tsai C-S et al., *J Gastrointest Oneal* (2011) 2(3):185-194
D14	T 1409/06
D15a	"Study of MM-398 Versus 5-Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer" clinicaltrials.gov posting NCT01494506 as updated on 2011_12_16
D15b	"Study of MM-398 With or Without 5-Fluorouracil and Leucovorin, Versus 5-D15 Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer", Clinical Trials Identifier: NCT01494506 (25 January 2013)
D16	T 1592/12
D17	Commission Implementing Decision and Annexes (Summary of Product Characteristics for Onivyde®)
D18	"FDA approves new treatment for advanced pancreatic cancer" (2015), FDA News Releases
D19	"Nanoliposomal irinotecan with fluorouracil and folinic acid in metastatic pancreatic cancer after previous gemcitabine-based therapy (NAPOLI-1): a global, randomised, open-label, phase 3 trial", *Lancet*, 2016 Feb 6;387(10018):545-57

D20	MHRA Public Assessment Report for 5-FU (2006)
D21	EP 1 210 115 B1 with relevant parts highlighted
D1b	Leucovorin calcium product label, November 2011
D22	L.Chen, et al., "Phase I study of liposome irinotecan (PEP02) in combination with weekly infusion of 5-FU/LV in advanced solid tumours", Journal of Clinical Oncology, 2010, 28:15 suppl, e13024

X. During the oral proceedings, held on 10.07.2019, OI confirmed his previous requests. P requested to promote AR2 filed on 28.06.2019 to Main Request. The previous Main Request filed on 24-08-2018 was designated as AR2. After the OD came to the conclusion that the main request did not comply with Article 56 EPC, P requested to discuss AR3.

XI. Following the discussion between the parties, the OD came to the conclusion that the requirements of Rule 80, 123(2) and 123(3) EPC were met. However, none of the requests on file complied with the requirements of Article 56 EPC. Therefore, the patent was revoked under Articles 101(3)(b).

Reasons for the decision

MAIN REQUEST (MR)

Claim 1 of the MR recites as follows:

Liposomal irinotecan for use in a method of treating pancreatic cancer in a human patient, wherein the patient exhibits evidence of recurrent or persistent pancreatic cancer following primary chemotherapy and wherein the patient has failed prior treatment with gemcitabine or become resistant to gemcitabine, the method comprising co-administration of an effective amount each of liposomal irinotecan, 5-fluorouracil (5-FU) and leucovorin to the patient in at least one cycle wherein the cycle is a period of 2 weeks and, for each cycle:

(a) liposomal irinotecan is administered to patients not homozygous for the UGT1A 1 28 allele on day 1 of each cycle at a dose of 80 mg/m^2 and to patients homozygous for the UGT1A1* 28 allele on day 1 of cycle 1 at a dose of 60 mg/m^2 and on day 1 of each subsequent cycle at a dose of 60 mg/m^2 or 80 mg/m^2 ;*

(b) 5-FU is administered at a dose of 2400 mg/m^2 ; and

(c) leucovorin is administered at a dose of 200 mg/m^2 (l form); and wherein in each cycle, the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU; and wherein the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection.

1. Rule 80 EPC

1.1. Claim 1 of the MR has been modified by the deletion of the alternative "or 400 mg/m^2 (l + d racemic form)". This amendment was made in an attempt to restore the validity of the priority, challenged by OI. The validity of the priority claim in turn has an influence on the relevance of certain documents (D4, D8, D10 and D15b) cited by OI in the attack against inventive step.

1.2. Claim 1 of the MR has been further modified by incorporation of the feature "*and wherein the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection*". This amendment was made in response to the attack under Article 83 EPC raised by OI in the submission of 10.05.2019.

1.3. OI did not raise objections under Rule 80 EPC.

1.4. Thus, the OD is of the opinion that the amendments are occasioned by grounds of opposition (Article 56 and 83 EPC) and therefore are admissible under Article 80 EPC.

2. Articles 123(2) and (3) EPC

2.1. The claims of the MR are identical to the claims as granted with the deletion of the alternative "or 400 mg/m^2 (l + d racemic form)" for the dose of leucovorine, and with the addition of the limitation of liposomal irinotecan to its sucrose octasulfate salt liposome injection with basis in several parts of the original application (e.g. original claims 11 and 20 as well as in the examples) and in claim 4 of the patents as granted.

2.1. OI did not raise objections under Article 123(2) or (3) EPC.

2.2. In the opinion of the OD, the deletion of an alternative appears as well as the limitation to the specific preferred salt comply with the requirements of both Articles 123(2) and 123(3) EPC.

3. Sufficiency of disclosure (Article 83 EPC)

The OD is of the opinion that the MR complies with the requirements of Article 83 EPC.

3.1. In the notice of opposition OI submitted that the patent is devoid of any adequate information demonstrating that the claimed drug dosing regimen is suitable for treating pancreatic cancer in patients who have failed gemcitabine therapy and therefore the requirements of Article 83 EPC are not met.

3.1.1. OI referred to the decision T1592/12 (D16) relating to a herceptin dosing regimen for use in treating breast cancer. The Board held that it was not enough that herceptin itself was known to be useful for the treatment of breast cancer, to meet the requirements of Article 83 EPC it was necessary that the patent described the suitability of the claimed dosage regimen for treating breast cancer.

During OP, O1 further argued that in the framework of suitability, in accordance with T1592/12, the application must provide the required support for both the therapeutic indication, i.e. gemcitabine (GEM)-resistant pancreatic cancer (PC), and the claimed dosage regimen. However, according to OI Example 6 fails to do so since it does not indicate that GEM-resistant PC patients were treated, does not provide the doses for 5-fluorouracil (5-FU) or leucovorin (LV), does not mention the 2-week cycle, and does not indicate the order of administration of the drugs of the triple combination. Therefore, Example 6 is very different from original claim 3, which forms the basis of claim 1 of the MR.

3.1.2. With regard to the clinical trial described in Example 7, OI argued that it is only prophetic, and according to T609/02 a simple verbal statement of success is not enough to ensure sufficiency of disclosure in relation to a claim to a pharmaceutical use.

3.1.3. OI added that the original application did not provide consistent disclosure about the dosage regimen, with several variations of doses, order of administration, cycle duration and patients to be treated: e.g. page 14, lines 12-20 disclose a dose of 120 mg/m^2 with a cycle of 3 weeks; page 13, lines 5-18 indicate that simultaneous administration is also possible; page 12, line 15-34 disclose the treatment of all pancreatic cancer, including those that are refractory or resistant to other anti-cancer treatments (carboplatin, cisplatin), not only gemcitabine.

3.1.4. OI also alleged that, based on the number of patients and general outcome, the phase I clinical study described in document D22 was obviously the same study of Example 6. Thus it was possible to extract the information contained in D22 on the dosage regimen of this study to know that in the study of Example 6 the dose of 5-FU was 2000 mg/mg^2 (not 2400 mg/mg^2 according to the claims of the MR); racemic LV was used instead of its levo form; and a 3-week dosing cycle was used instead of a 2-week cycle. Thus it is not credible that study of Example 6 relates to the claimed regimen.

3.1.5. OI concluded that as the credibility test failed, P could not rely on post-published evidence.

3.2. P argued that the claims of the MR met the requirements of sufficiency.

3.2.1. P submitted that absolute proof is not required under Article 83 EPC. Nevertheless, the application provides lots of preclinical and clinical data, relative to pancreatic cancer, and using a regimen combining 5-FU, leucovorin and MM-398. In particular, the specific claimed dosage regimen is rendered plausible by the combined teaching of Example 6 and 7.

3.2.2. According to P the absence of specific indication in Example 6 that GEM-resistance PC patients have been treated does not undermine the plausibility, because at the time of the application it was known from e.g. D3, D5 and D6 that there is no cross-resistance between gemcitabine and irinotecan.

3.2.3. P also invoked decision T108/09, where although no data was provided for the claimed third line therapy, the patent was still considered sufficiently disclosed based on the results provided for the second line therapy.

3.3. The OD is of the opinion that the claims of the MR comply with the requirements of Article 83 EPC since the claimed dosage regimen is sufficiently disclosed and the claimed therapeutic application appears to be plausible at the relevant date, based on the original disclosure in combination with the common general knowledge.

3.3.1. Example 7 of the patent in suit, describes a prophetic Phase 3 clinical trial protocol for which no results are provided. The patients of the Phase 3 clinical trial of Example 7 are the same of claim 1 of the MR, i.e. patients with metastatic pancreatic cancer that have progressed on gemcitabine based therapy (see page 25, lines 15-16; page 27, lines 25-26 of the original application). The dosage regimen of the triple combination according to Arm C (see paragraph bridging page 25 and 26) is identical the the claimed dosage regimen.

Example 7 of the patent in suit, concerning a protocol for a phase 3 clinical trial which may or may not be carried out in the future, does not put into practice the invention defined in the claims as granted and can therefore, if taken alone, cannot serve as a basis for sufficient disclosure. However, the evaluation of sufficiency of disclosure takes account of the entire information to be found in the patent, including claims, description, examples and figures. The OD notes that the heading of Example 7, on page 24, below table 2, reads as follows: "*The promising efficacy and safety data from the Phase 1 trial (described above) warrant the MM-398 and 5-FU plus leucovorin combination to expand further in a phase 3 study*". This clearly indicates that Example 7 is not to be read in isolation, but in combination with the "promising" results of the previous Phase I trial, i.e. the study of Example 6.

3.3.2. Example 6 of the patent (and of the original application) shows that the triple combination of liposomal irinotecan, 5-FU and leucovorin showed promising efficacy and safety data in a Phase 1 trial (PEP0203) which included 5 patients with pancreatic cancer (see Table 2 and paragraph [0083] of the patent). Table 2 indicates that of these 5 pancreatic cancer patients, one patient received a dose of 60 mg/m^2, three patients a dose 80 mg/m^2, and one patient a dose of 120 mg/m^2 of MM-398 (i.e. liposomal irinotecan). Thus, three pancreatic cancer patients where among the 6 patients who received the dose of 80 mg/m^2 in the phase I clinical trial described in example 6 as mentioned in paragraph [0044] of the patent. This paragraph specifies that among these 6 patients there were 1 partial response (PR), 4 stable disease (SD) and 1 progressive disease (PD). By consequence, even assuming that one of the three pancreatic cancer patients showed PD, it follows that the other two exhibited either PR or SD. Consequently Example 6 appears to indicate that a positive effect was shown in at least 67% of the pancreatic cancer patients who received liposomal irinotecan at the dose of 80 mg/m^2 in combination with 5-FU and leucovorin. Although Example 6 does not indicate the doses of 5-FU and leucovorin used in the phase I clinical trial, said doses are consistently indicated in the patent and in the original applications as in the claim of the MR. Therefore, Example 6 appears to sufficiently indicate that at the time of priority date the claimed triple combination was suitable for the treatment of pancreatic cancer.

3.3.3. OI relied on document D22 in an attempt to extract information on the possible dosage regimen used in Example 6 of the application. However, the OD is the opinion that D22 cannot be used to interpret Example 6, since this isolated abstract publication cannot be considered to represent the common general knowledge at the time of the application and does not relate to the treatment of PC.

3.3.4. The OD agrees with OI that the application as filed also mentions different dosages for liposomal irinotecan and a 3-week cycle. However, these different dosages and duration consistently relate to monotherapy, not to the triple combination according to the claims. The OD also concurs with OI that the description disclose both sequential and simultaneous administration of liposomal irinotecan, 5-FU and LV. However, the OD notes that whenever more details are provided for the triple combination (e.g. on pages 13 and 14), the description consistently specifies in the same paragraph the same dosages, the same order of administration and the same cycle duration as in claim 1 of the MR, which in turn adhere to the dosage regimen according to Arm C of Example 7.

3.3.5. With regard to the type of patient, the OD considers that the treatment of this specific group of GEM-resistant pancreatic cancer patients is rendered plausible by the common knowledge that combinations comprising (non-liposomal) irinotecan, 5-FU and LV were already successfully used at the time of application in the treatment of GEM-resistant pancreatic cancer. This previous knowledge is indicated in paragraph 4 of page 1 of the application as originally filed, with the support of the convergent

disclosures of D2, D3 , D5 and D6, all relating to the so called FOLFIRI regimens mentioned in the application as filed. As the liposomal preparation of irinotecan according to the patent in suit is only directed to improve the therapeutic index (see e.g. page 9, lines 5-10), there are no reasons to doubt that the claimed combination including liposomal irinotecan would also be suitable for the treatment of GEM-resistant PC. This view is further supported by the knowledge from the same documents D3 (page 1644, left column, last sentence of 2nd paragraph), D5 (page 1659, left column, second paragraph) and D6 (abstract) that, having gemcitabine and irinotecan very different mechanism of action, there was no cross-resistance between these active compounds. Thus, the skilled person knew that the failure of GEM-therapy did not imply the failure of a therapy involving irinotecan, as confirmed by the success of the FOLFIRI treatments.

3.3.6. OI objected that according to the CLBA, II.C.3.3.1 and T2059/13, common general knowledge does not normally include patent literature and scientific articles. Accordingly D3, D5 and D6 could not be relied on for the purpose of Article 83 EPC. However, the OD considers that although D3, D5 and D6 are not monographs or textbooks, their convergent and consistent disclosure about FOLFIRI in the period spanning from 2007 to 2012, reflects the common knowledge of the skilled person at the time of the application.

3.3.7. In the opinion of the OD, the decision T1592/12 (D16) does not apply to the facts of the patent in suit. The patent at issue in T1592/12 (EP1 210 115 B1 see D21) related to a new dosage regimen for herceptin. In T1592/12, however, the reason for denying plausibility was the fact that the patent taught weekly administration of herceptin as being most preferred, whereas the claims related to subsequent doses every three weeks. In addition to that the half-life of herceptin was known to be only about 1 week (see reasons 28). Based on this technical information, the Board had reason to doubt that herceptin was suitable for treating breast cancer by administration every three weeks. Therefore, in T1592/12 there were serious doubts as to the suitability of the claimed dosing schedule for treating the claimed disease.

This appears to be different from the situation of the patent in suit, which consistently refers to a very specific combination regimen, the same of Arm C of Example 7, which is also claimed in claim 1 of the MR, and which appears to be supported and rendered plausible by the preliminary results of Example 6 in combination with the common general knowledge as indicated above. There is no inconsistency here between the results provided in Example 6, the regimen according to Example 7 and the claims of the MR, and there are no serious doubt about the efficacy of the claimed dosage regimen for the treatment of GEM-resistant PC.

3.3.8. As the plausibility hurdle appears to have been overcome by the application as originally filed, the post-published documents D17 and D19 can be taken into account as evidence that liposomal irinotecan can effectively treat GEM-resistant pancreatic cancer when administered according to the specific combination regimen of claim 1 of the MR.

4. Priority claim (Article 87 EPC).

The OD is of the opinion that the MR does not comply with the requirements of Article 87 EPC.

4.1. In the notice of opposition OI submitted that the first priority claim of the patent as granted (13.06.2012) is not valid since claim 3 of the priority document US2012/61659211P (PD1) states that "....leucovorin is administered at a dose of 200 mg/m^2" without specifying whether leucovorin is in the l form or in the l+d (racemic) form.

According to OI the term leucovorin without any further definition refers to the racemic form, and therefore, PD1 describes a different dose from that specified in claim 1 of the patent as granted, which relates to a dose of 200 mg/m^2 of leucovorin in the l form or 400 mg/m^2 of leucovorin in the l+d form. OI expressed the view that at the time of the priority date the term "leucovorin" without any further definition would have been understood to exclusively refer to the racemic form of the drug, based on the fact that D1 reported the term "levoleucovorin" to refer to the levo isomeric form.

4.2. In the MR the dose of 400 mg/m^2 has been deleted, and claim 1 has been limited to a dose of 200 mg/m^2 of leucovorin in the l form.

According to P, although PD1 refers to "leucovorin" only, this difference is not prejudicial to the validity of the priority claim to PD1, since at the time of the priority date it was well known that, being leucovorin an optically active molecule, it could exist in the l-, d- or racemic-form.

In addition, P argued that the priority document PR1 on page 11, line 19 indicates that "leucovorin acts as a biochemical co-factor" and at the priority date it was already well known (e.g. from D1) that the l-form of leucovorin was the pharmaceutically active form. Moreover, a number of documents published before the priority date, e.g. D2 (see title; page 461, right column, par. 4; page 462, left-column, par. 4) and D3 (page 1642, right column, par. 1) relating to the FOLFIRI regimen, use the generic term "leucovorin" but then clearly use the active l-form (levoleucovorin, levofolinic acid). The priority document PR1 on page 1, line 20 refers to the FOLFIRI regimen, thus based on D2 and D3, the l-form is implicitly disclosed.

According to P, the skilled person at the time of PD1 would have considered the generic term "leucovorin" to encompass "l-leucovorin" and its racemic form. Therefore, both forms appear to be implicitly, directly and unambiguously disclosed in PD1, in accordance with the approach followed in T658/91.

Consequently, the reference in claim 1 of the MR to the l-form of leucovorin is considered to find basis in PD1 as it is a limitation from two possible alternatives, i.e. l-form and racemic-form.

4.3. The OD is of the opinion that the priority claim of PD1 is not valid.

It is well established under EPO case law that the concept of disclosure is the same for both the assessment of novelty and the determination of the right to claim priority and therefore, the same tests can be applied.

According to decisions T269/87 and T1048/92, with regard to chiral compounds, the disclosure of a racemate does not anticipate the novelty of either enantiomer (see e.g. CLBA-I.C.6.2.3). Thus, the disclosure of leucovorin in PD1, is not considered a direct and unambiguous disclosure of each of the individual enantiomer of leucovorin, let alone e.g. levoleucovorin. Indeed, if the priority document PD1 were a prior art disclosure, the dose 200 mg/m^2 leucovorin in the l-form according to claim 1 of the MR would still be novel.

The decision T658/91 invoked by P does not seem to apply in present case. In fact, the prior art document at issue in T658/91 specifically taught that the described compound had an asymmetric centre and that the disclosure concerned both the single enantiomers as well as the racemic mixture. The Board considered this teaching tantamount to an individualized disclosure of the later claimed enantiomer and therefore the Board denied the novelty. In the present case, however, there is no corresponding disclosure in the priority document that leucovorin has an asymmetric centre nor is there a teaching that both enantiomers of leucovorin, as well as the racemate, are contemplated.

This approach is further confirmed by T600/95 which indicated that it is not sufficient that the compound in question belongs conceptually to a disclosed class of possible compounds, without any pointer to the individual member. It might be the case that levo-leucovorin belongs conceptually to the class covered by the term leucovorin, but this does not equate to an unambiguous disclosure.

Moreover, although it might have been obvious for the skilled person to use the active enantiomer, the concept of obviousness does not correspond to the concept of direct and unambiguous disclosure required here. Additionally, at the time of PR1 the racemic form was also commonly marketed and used, as indicated by D1, D1b as well as D5 and D6, were no specific reference is made to the l form.

Accordingly, the claimed subject-matter which describes a dose of leucovorin in the l form cannot be derived directly and unambiguously from PD1 and therefore the priority claim of PD1 is not valid.

The valid priority date is the one of PR2, i.e. 14.03.2013. Consequently, documents D4, D8, D10 and D15b cited by OI become relevant prior art because they were made available to the public before that date.

5. Inventive step

The OD is of the opinion that the MR does not comply with the requirements of Article 56 EPC.

5.1. In the notice of opposition OI raised an objection of lack of inventive step starting from D12 or D13 as the closest prior art using routine methods and/or in combination with the information available in D15b, or in alternative starting from D15b as the closest prior art in combination with the teaching of D12 or D13.

During the OP, OI indicated that D15b, D13 and D5 could all represent good starting points, however, according to OI, D15b represented the closest prior art.

5.2. Conversely, according to P, D13 is to be regarded as the closest prior art, since it contains data on the beneficial effects of a treatment regimen using a triple combination with liposomal irinotecan, 5-FU and LV on GEM resistant PC patients. P submitted that D13 contains a more comprehensive disclosure than D15b. Based on purposive considerations, in view of the reported results, and in line with T2154/14, D13 should be preferred over D15b which does not provide any result of the disclosed treatment.

Moreover, P pointed out that D13 clearly relates to nanoliposomal CPT-11 (PEP02 also known as MM-398) whereas D15b does not indicate what MM-398 stands for.

P further argued that a phase III clinical trial may imply a certain expectation of safety, but no expectations of actual efficacy. This particularly applies to D15b which aims to treat the specific patient population of GEM-refractory PC patients whose life expectations is of 6 months or less. Thus, rather than an expectations of success, there was a hope of prolonging patients survival with the regimen of D15b, according to a try and see approach.

5.3. In the opinion of the OD, D15b represents a better starting point compared to D13.

D13 (page 189, right-hand column, paragraph 2, to page 191, left-hand column, paragraph 1) discloses the PEP02 (liposomal irinotecan) monotherapy for the treatment of patients with gemcitabine refractory pancreatic cancer, and also mentions a PEP02/5-FU/LV combination treatment used in a phase I study. However, it is silent with regard

to the respective dosage of the three components of the combination treatment, and does not provide any result specifically relative to said combination treatment either. Results are only provided for a Phase II clinical trial using PEP02 as monotherapy at a dosage of 120 mg/m^2 every three weeks (see also D12, corresponding to Reference 30 in D13).

Moreover, when referring to the combination treatment of the phase I clinical trial, D13 discloses that nanoliposomal CPT-11 (irinotecan) was used in combination with "weekly" 24-hour infusion of high-dose 5-FU/leucovorin (HDFL). Thus, D13 discloses a different schedule regimen for the claimed combination, i.e. a weekly cycle as opposed to the 2-week cycle according to claim 1 of the MR.

Therefore, not only D13 does not disclose specific results for the combination treatment, but it also fails to disclose the dosage regimen according to the patent in suit.

D15b describes the protocol for a phase III clinical study of liposomal irinotecan (MM-398), alone or in combination with 5-FU and leucovorin for use in treating metastatic pancreatic cancer in patients who have failed gemcitabine based therapy. 5-FU and leucovorin without liposomal irinotecan is the active comparator. Of the two experimental Arms (A and C), Arm A represents a 3-week (Q3W) dosing cycle of MM-398 (120 mg/m^2) and Arm C represents a 2-week (Q2W) dosing cycle of MM-398 (80 mg/m^2), 5-FU (2400 mg/m^2) and leucovorin (400 mg/m^2). Therefore, Arm C is a combination treatment using the same drugs in the same amounts of claim 1 of the MR, with the only difference that the order of administration is not specified.

It is correct what stated by P that D15b does not disclose the outcome of the clinical study, however, the Boards of Appeal have on multiple occasions selected the disclosure of a clinical trial protocol with no results as the closest prior art document, e.g. T239/16 and T2506/12.

The official title of D15b, reads: "A randomized, open label phase 3 study of MM-398, with or without 5-fluorouracil and leucovorin in patients with metastatic pancreatic cancer who have failed prior gemcitabine-based therapy". Thus, based on the title, the OD considers that D15b clearly relates to the same purpose of D13 and the same purpose of the patent in suit, i.e. the treatment of GEM-resistant PC patients.

Moreover, although D15b does not indicates the meaning of MM-398, at the time of D15b it was known from D13 that the MM-398 was nanoliposomal CPT-11, also known as PEP02.

All in all, the OD considers that D15b clearly relates to the same purpose of the patent in suit, and it is the document which requires less modifications to arrive at the dosage regimen of the MR, and therefore it represents the closest prior art.

5.4. During the OP, the parties discussed inventive step using the problem and solution approach starting from D15b as the closest prior.

5.5.1. According to OI, D15 differs from claim 1 of the MR in that the order of administration of the drugs of the combination treatment is not disclosed. However, this order would be obvious in view of the same order of administration used in the FOLFIRI or CAMPTOSAR treatment, as disclosed e.g. in D2, D4, D6 and D10.

5.5.2. A further difference indicated by OI is the lack of therapeutic results. The objective technical problem could be formulated in a more ambitious or less ambitious way, i.e. as an improved or simply as an effective treatment of GEM-resistant PC, but still claim 1 of the MR would not be inventive. In fact, OI submitted that human clinical trials in general, and phase III clinical trials in particular, are only authorised once it has been shown that the treatment under investigation has a high level of safety and efficacy in earlier studies. Thus, a skilled person reading D15b knows that the regimen described therein has already demonstrated safety and efficacy in earlier human clinical studies, earlier animal studies and in other preclinical studies. Therefore, in line with established case law, such as T239/16 and T2506/12, starting from D15b the skilled person has a reasonable expectation that the technical problem would be solved.

5.6.1. P submitted that not only D15b does not disclose the order in which the drugs of the combination treatment are administered, but also does not contain any data on the outcome of the treatment, thus failing to provide actual disclosure of both safe and effective treatment. According to P, the disclosure that a particular treatment is undergoing clinical trials is merely speculative of the treatment being safe and therapeutically effective. The selection of Arm C in D15b could not be done in the expectation of a safe and effective treatment but only in "the hope" of such an effect (emphasis added) based on a "try and see" approach.

5.6.2. According to P, the objective technical problem could be formulated as a low threshold or as a more ambitious problem, and in both case the dosage regimen of claim 1 of the MR would be inventive.

The low threshold problem could be regarded as the provision of a safe and effective treatment of GEM-resistant PC. To this regard, P indicated that the post-published documents D17, D18 and D19 all confirm that the problem has been solved, providing sound data relative to both safety and efficacy.

The more ambitious problem could be regarded as the provision of an improved safe and effective treatment of GEM-resistant PC compared to the monotherapy. To this regard, P argued that liposomal irinotecan for use as recited in claim 1 of the MR was approved following a pivotal Phase III trial, referred to as NAPOLI-1 (see e.g. D17 and D19). The protocol for this trial is explained in detail in Example 7 of the patent. The results of this trial show that the claimed invention is associated with a number of

beneficial technical effects. In the NAPOLI-1 trial, and as explained in section B of Example 7, patients were separated into three arms. Patients in Arm A were administered 120 mg/m^2 of liposomal irinotecan (referred to as "MM-398" in Example 7) over 90 minutes every three weeks. Arm B was referred to as the control arm, in which patients were treated with 5-FU/LV. Patients in Arm C were administered 80 mg/m^2 of liposomal irinotecan over 90 minutes every two weeks in combination with 5-FU/LV according to claim 1 of the MR. The results from each of Arms A (monotherapy) and C (triple therapy) were individually compared with those from Arm B (5-FU/LV). The claimed dosage regimen shows clinically and statistically relevant improved efficacy with regard to all primary and secondary endpoints studied, i.e. overall survival (OS - defined in paragraph [0164] of the patent as the time from the date of patient randomisation to the date of death or the date the patient was last known alive); progression free survival (PFS - defined in paragraph [0169] of the patent as the number of months from the data of randomization to the date of death or progression, whichever occurred earlier); median time to treatment failure (TTF - defined in paragraph [0171] of the patent as the time from randomisation to either disease progression, death or study discontinuation due to toxicity); objective response rate (ORR - discussed in paragraphs [0172] and [0173] of the patent); levels of the pancreatic cancer tumour marker CA19-9 (discussed in paragraph [0174] of the patent). The efficacy data endpoints from the NAPOLI-1 trial illustrated by P are discussed in D19, and are reproduced in the table below:

	Arm A (monotherapy)	**Arm C (combination)**
Median Overall survival / months (Arm B value)	4.9 (4.2)	6.1 (4.2)
Median progression-free survival / months (Arm B value)	2.7 (1.6)	3.1 (1.5)
Median time to treatment failure / months (Arm B value)	1.7 (1.4)	2.3 (1.4)
Overall response rate / % (Arm B value)	6.0 (1)	16 (1)
Patients showing ≥50% reduction in CA19-9 levels / % (Arm B value)	24 (11)	29 (9)

Moreover, P added that in the patient reported outcome analysis there were no substantial differences in patient quality of life between the three arms, indicating that the increased efficacy of the claimed combination regimen surprisingly does not have a detrimental effect on patients' quality of life. Therefore, P submitted that these data demonstrate that the claimed combination therapy regimen is associated with therapeutic advantages, without having a detrimental effect on patients' quality of life.

Furthermore, P submitted that the improved efficacy of the claimed dosage regimen was also surprisingly associated with a lower frequency of serious treatment emergent adverse events (TEAEs) compared to liposomal irinotecan monotherapy regimen as discussed in D19 and summarized in the table below:

	Arm A (monotherapy)	Arm B (control)	Arm C (combination)
Frequency of serious TEAEs (%)	61.2	44.8	47.9
Frequency of patients experiencing severe diarrhoea (%)	21	5	13
Frequency of patients experiencing alopecia (%)	22	5	14

5.6.3. Moreover, P pointed to the fact that the skilled person reading D15b was faced with a choice between the monotherapy (Arm A) and the combination therapy (Arm C). At the time of application the skilled person would have been aware that the field of pancreatic cancer treatment was unpredictable and prone to unsuccess and, therefore, he would have adopted a conservative approach. The conservative skilled person would have been dissuaded from the combination regimen in favour of the monotherapy, in view of the fact that liposomal irinotecan monotherapy had ben already successfully tested in a phase 2 clinical trial (see D12), whereas the combination with other drugs such as 5-FU and LV went only through phase 1, and could have caused enhanced side effects and further concerns regarding safety, especially in view of the known adverse reactions associated to irinotecan, 5-FU and LV as indicated in documents D10, D20 and D1a respectively.

Additionally, P argued that none of the prior art documents suggested the efficacy and safety of liposomal irinotecan at a dose of 80 mg/m^2 against GEM-resistant PC, neither in monotherapy nor in a combination treatment with 5-FU and LV. In particular, both D7 and D12 are rather pointing towards the use of a dose of 120 mg/m^2 of liposomal irinotecan in monotherapy; D13 does not provide the doses used in the combination therapy, which in addition is administered in a 3-week cycle; D8 in table 2 discloses a dose of 80 mg/m but does not specify whether said dose was used in the pancreatic cancer patients; D22 does not specifically mention pancreatic cancer, let alone GEM-resistant, and merely discloses that the maximum tolerated dose (MTD) of PEP02 is of 80 mg/m^2 when administered in a combination treatment with 5-FU and LV in a 3-week cycle.

Based on the considerations above, P submitted that the prior art dissuaded from the use of the triple combination, as well as from the dosage of liposomal irinotecan according to the MR. Therefore, the alleged expectation of safe and effective treatment according to T239/16 was taken away by the fact that the skilled person was dissuaded from this by the prior art.

5.6.4. P further argued that the nature of compound MM-398 is not defined in D15b.

P submitted that there are no documents on file identifying MM-398 with "irinotecan sucrose octasulfate salt liposome injection" according to claim 1 of the MR, therefore, the skilled person would not have been incited to use this specific form of liposomal irinotecan.

5.7. The OD came to the following conclusions:

5.7.1. The OD does not share the view of P that the skilled person reading D15b would be faced by the choice between monotherapy and combination therapy. The teaching forming the closest prior art in D15b is not the monotherapy but the combination therapy, i.e. the dosage regimen according to Arm C.

5.7.2. The OD acknowledges that in view of the data provided in D17-D19, the dosage regimen according to the MR results in an improved treatment compared to the monotherapy. However, since the closest prior art D15b is not the monotherapy but the combination treatment according to Arm C, for the assessment of inventive step it appears irrelevant whether the combination provides an improvement over the monotherapy. Moreover, it also appears that an ambitious problem formulated as an improvement over the monotherapy cannot be taken into account, since said effect is not plausibly derivable from the application as filed (GL G-VII, 5.2; T386/89).

Therefore, the problem to be solved cannot be regarded as an improved treatment but merely as the provision of an effective (and safe) treatment.

5.7.3. As indicated by OI, human clinical trials in general and phase III clinical trials in particular are only authorised once it has been shown that the treatment under investigation has a high level of safety and efficacy in earlier preclinical and clinical studies. In line with T239/16 reasons 6.5, the OD considers that the mere fact that the dosage regimen of Arm C was being tested in a clinical study for the treatment of GEM-resistant PC (as disclosed in document D15b) leads to an expectation of success, due to the fact that clinical studies are based on data obtained by preclinical testing both in vitro and in animals and require authority approval which takes ethical considerations into account. This means in the present case that the skilled person would expect the study arm C to treat GEM-resistant PC safely and effectively, unless he was dissuaded from this by the prior art.

The OD does not share the view of P that T239/16 does not apply in the present case because the skilled person would be dissuaded from an expectation of success by the prior art. In fact, none of the documents of the prior art appear to provide a particular disincentive to the use of the triple combination, nor to the dosage 80 mg/m^2 of liposomal irinotecan according to the MR as alleged by P. In fact, it is common practice to lower the dosage of a drug when used in a combination treatment. Therefore, lowering the recommended dose of 120 mg/m^2 liposomal irinotecan monotherapy as in D7 or D12 to 80 mg/m^2 as in the combination treatment of Arm C of D15b, would not take away the expectation of successful treatment using the regimen of Arm C, especially in the presence of a synergistic effect between irinotecan and 5-FU which is clearly suggested by D4 (see e.g. page 4534, right-hand column, paragraph 2). D13 appears to support rather then provide a disincentive to the successful expectation of a combined use of liposomal irinotecan, 5FU and LV. D8 is vague with regard to the doses used on PC patients, thus it is irrelevant with regard to the reasonable expectations of the skilled person. D22 indicates an MTD of 80 mg/m^2 for PEP02 given every 3-weeks, which however is given in combination with 5-FU and LV on day 1 and day 8, i.e. in a dosage regimen different from the one according to the MR, thus this document does not cast a doubt on exceeding toxicity when using the dosage regimen of Arm C. On the other hand, the skilled person also knows from D2-D6 that triple combinations of the same drugs, i.e. irinotecan (although not liposomal), 5-FU and LV, administered in similar dosage regimens, already proved to be promising for the treatment of GEM-resistant PC.

All in all, the prior art does not undermine, but rather support, the presumption of the official authority who authorized the clinical trial of D15c that the treatment according to Arm C would work. This presumption of success is based on careful risk/benefit evaluation by the authority. As recited in reasons 6.6. of T239/16, ethical and economical considerations require that the "benefit" will arise with reasonable certainty and will not only "be hoped for". The set-up of the clinical study of D15b thus inherently creates an expectation of success.

Similar considerations are made in T2506/12 (see reasons 3.10), wherein the Board pointed out that drugs to be used in a clinical trial with human subjects are not selected based on a general "try and see" attitude, but based on existing favourable scientific data, for both ethical and economical reasons. In line with reasons 3.15 of T2506/12, the OD considers that while the outcome of a clinical trial could be success or failure, no particular reason was known which would have discouraged the person skilled in the art from carrying out the therapeutic protocol according to Arm C of D15b, to simply confirm the usefulness of the dosage regimen. Finding out in this straightforward manner that

the disclosed dosage regimen provided indeed both efficacy and safety of treatment in GEM-resistant PC patients according to the purpose of the phase 3 clinical trial, cannot be regarded as inventive.

5.7.4. The fact that the combined treatment may give cumulative side effects, in view of the known side effects associated to each drug of the combination (e.g. see D1a, D10 and D20) does not appear to represent a disincentive either. In fact, the combination of irinotecan, 5-FU and LV is already known from the FOLFIRI regimens, which are disclosed in D2-D6. In particular, D3, D5 and D6 relate to phase 2 clinical studies, for which safety and efficacy evaluations were already made. The skilled person would not expect that the use of liposomal irinotecan (instead of non-liposomal irinotecan used in FOLFIRI) would negatively impact on the safety of very similar combination dosage regimens of the same active compounds.

5.7.5. With regard to the side effects mentioned by P, i.e. nausea, vomiting and diarrhea, the OD considers that safe treatment does not equate to the absence of side effects. The mentioned side effects appear to be common side effects of many, if not all, anticancer treatments, but in a balancing act the benefits arising from the treatment weighs in favour of treatment despite said side effects. Even taking said side effects into account, the skilled person in the art would not have been deterred from, or prejudiced against, applying the dosage regimen according to Arm C of D15b.

5.7.6. The dosage regimen disclosed for Arm C of D15b not only already discloses the dosage of 80 mg/m^2 of liposomal irinotecan according to the MR, but it already discloses also its combination with a dosage of 2400 mg/m^2 of 5-FU and 400 mg/m^2 of LV in a 2-week cycle. The OD notes that D15b does not disclose 200 mg/m^2 of LV in the I form. However, rightly enough none of the parties identified this as a difference. In fact the 400 mg/m^2 of LV in D15b are equivalent to the 200 mg/m^2 of LV in the I form according to the MR. Thus, as the dosages according to the MR are already recited in D15b, the skilled person does not need to find any further motivations or pointers to use said dosages.

5.7.7. With regard to the order of administration specified in claim 1 of the MR, i.e. "the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU", it appears that no particular technical effect is associated to it. Thus, the claimed sequence of administration would be one the skilled person could chose from, among those which were available at the time of application. The OD notices that the very same order (irinotecan - LV - 5-FU) is consistently mentioned in the prior art when the triple combination of the same drugs (only where irinotecan is not in liposomal form) is used in the FOLFIRI treatment (see e.g. D2-D6). Therefore, it would be obvious for the skilled person to follow the same order.

5.7.8. With regard to the identity of compound MM-398, it is true that there are no indications on file that the compound MM-398 used in D15b was irinotecan sucrose octasulfate salt liposome injection. However, even assuming that this is a further difference, this feature does not appear to be associated with any technical effect. In page 8, lines 23-28 of the original application, it is indicated that the claimed irinotecan sucrose octasulfate salt liposome injection was known from US 8,147,867, and therefore, it would just represent an alternative liposomal irinotecan which was available to the skilled person at the time of application.

5.7.9. For these reasons the OD concludes that the subject-matter of claim 1 of the MR does not involve an inventive step and therefore, does not comply with the requirements of Article 56 EPC.

AUXILIARY REQUEST 3 (AR3)

6. AR3 is discussed first here since during OP, P did not request to discuss AR1 and AR2.

7. AR3 differs from the MR in that it requires that "*the patient achieves a response which is at least stable disease*".

8. Rule 80, Article 123 and 83 EPC

8.1. OI raised no objections under Rule 80 and Article 123 EPC with regard to AR3.

8.2. The OD concludes that AR3 complies with the requirements of Rule 80 and Articles 123(2),(3).

8.3. The OD also concludes that AR3 meets the requirements of Article 83 EPC for the same reasons provided above for the MR.

9. Inventive step

The OD is of the opinion that the AR3 does not comply with the requirements of Article 56 EPC.

9.1. D15b is the closest prior art for AR3.

9.2. P submitted that the claim 1 now required the treatment not just to be effective but to reach a particular level of effect, i.e. "at least stable disease".

P argued that, as indicated in paragraphs [0063] of the patent specification and on pages 16-17 of the application as filed, responses to therapy include four different responses, i.e. pathologic complete response (pCR), complete response (CR), partial

response (PR) and stable disease (SD), which would represent the best responses. However, also progressive disease (PD, defined in paragraph [0065]) can be considered as a somehow effective treatment, since in the absence of said treatment the disease might have been progressed at a much higher rate.

Accordingly, the new threshold introduced in claim 1 of the MR may be used to formulate a new objective technical problem, i.e. to provide a safe and effective treatment of GEM-resistant PC wherein the patient achieves a response which is at least stable disease.

P submitted that there were no expectations, neither for the skilled person nor the FDA, deriving from the prior art that the dosage regimen of D15b would have provided at least stable disease.

9.3. OI responded that "progressive disease" could not be considered part of an effective treatment, and that within the meaning of an "effective" treatment the minimum to be expected is "at least stable disease". Moreover, OI argued that the mere exclusion of the failures from claim 1 of AR3 did not allow a reformulation of the problem. OI added that even if the obtention of "at least stable disease" was to be taken into account in the formulation of the problem, this would have been obvious in view of the effects already shown for FOLFIRI, e.g. in D2 (see Table 2) and D3 (see Table 1)

9.4. The OD does not agree with P that progressive disease may be considered as an effective treatment. Clearly the patent specification refers to the Response evaluation criteria in solid tumors (RECIST) as indicated on page 27, line 23-24 of the application as originally filed. RECIST is a set of published rules that define when tumors in cancer patients improve ("respond"), stay the same ("stabilize"), or worsen ("progress") during treatment. According to the RECIST criteria, "progressive disease" is characterized by worsening and clearly cannot be considered as a "somehow" successful treatment as alleged by P. The slower progression referred to by P appears rather to pertain to the group of "partial response". As a consequence, in the framework of an effective treatment, the obtention of stable disease is considered to represent the minimum level of therapeutic effect.

The introduction of the feature "*the patient achieves a response which is at least stable disease*" in claim 1 of AR3 simply excludes the failures from the claim.

However, this new feature does not imply that all patients treated with the regimen according to claim 1 will reach stable disease. It rather excludes the patients with progressive disease from the claim. Still this does not appear to represent an actual distinction from claim 1 of the MR, since the patients treated with the claimed regimen and showing progressive disease would simply not fall under the claim.

Therefore, the new feature cannot serve to reformulate the technical problem. At most, the technical problem to be solved could be re-worded as to provide a safe and effective treatment of GEM-resistant PC, where "at least some patients" reach at least stable disease. However, the expectation to achieve stable disease, at least in some of the patients, already derived from D15b for the same reasons provided above.

Moreover, reasonable expectation to achieve at least stable disease is already provided by D2-D6 for FOLFIRI. In particular Table 2 of D2 and Table 1 of D3 clearly indicates that 28-35% of the patients reach stable disease.

The OD considers that the prior art does not contain any information which would have dissuaded the person skilled in the art from trying the protocol of D15b to confirm the expected effects.

For theses reasons and for the reasons provided in items 5.7 to 5.7.8, the person skilled in the art, starting from D15b, had reasonable expectation to achieve "safe and effective treatment" of GEM-resistant PC, this inherently implying the achievement of "stable disease" in at least some of the patients, when administering said patients the dosage regimen according to Arm C of D15b, following the order of administration generally known for the FOLFIRI combination.

Therefore, Claim 1 of AR3 does not involve an inventive step over the prior art, and does not comply with the requirements of Article 56 EPC.

AUXILIARY REQUEST 1 (AR1)

10. AR1 differs from the MR in that, like AR3, it requires that "*the patient achieves a response which is at least stable disease*". AR1 differs from both the MR and AR3 in that it does not contain the limitation that "*the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection*".

11. Rule 80, Article 123, 83 and 56 EPC

11.1. The OD concludes that AR1 complies with the requirements of Rule 80, Articles 123(2),(3), Article 83 EPC for the same reasons provided above for the MR.

11.2. However, AR1 does not comply with the requirements of Article 56 EPC for the same reasons provided above for both MR and AR3.

AUXILIARY REQUEST 2 (AR2)

12. AR2 (former MR filed on 24.08.2018) differs from the MR and AR3, in that it does not requires that "*the patient achieves a response which is at least stable disease*" nor that *"the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection*".

13. Rule 80, Article 123, 83 and 56 EPC

13.1. The OD concludes that AR1 complies with the requirements of Rule 80, Articles 123(2),(3), and Article 83 EPC for the same reasons provided above for the MR.

13.2. However, the OD considers that the conclusions on lack of inventive step provided above for both MR and AR3, apply *mutatis mutandis* to AR2.

Decision

Taking account of the amendments made by the patent proprietor during opposition proceedings (i.e. MR, AR1, AR2 and AR3), the European patent EP 2 861 210 is revoked on the ground of Article 100(a) EPC because it does not meet the requirements of Article 52(1) EPC in conjunction with Article 56 EPC (Article 101(3)(b) EPC).

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Appeal number: **T2963/19-3.3.07**

Communication of the Board of Appeal pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal

The Rapporteur M. Steendijk
The Registrar B. Atienza Vivancos
Tel.: 089 / 2399 - 3371

Registered letter

This document: 21 page(s) including this page

Annex(es):

Communication text

I. This communication, which is sent pursuant to Article 15(1) RPBA 2020 (OJ EPO 2019, A63), serves to prepare the oral proceedings and presents the Board's provisional and non-binding opinion. The communication

is not intended to provide an exhaustive representation of all the issues that may be dealt with at the oral proceedings. Its purpose is to express the concerns which the Board presently has in relation to the arguments or requests presented.

Document numbering

II. The Board adheres to the numbering of documents D1-D22 in the decision under appeal.

First-instance proceedings

III. The grant of European patent 2 861 210 (hereinafter: the patent) was opposed on the grounds that its subject-matter lacked inventive step and that the claimed invention was not sufficiently disclosed. The appeal filed by the patent proprietor (hereinafter: appellant) lies against the decision of the opposition division posted on 28 August 2019 to revoke the patent.

The decision was based on

- the main request, originally filed on 28 June 2019 as auxiliary request 2,

- auxiliary request 1 as filed on 28 June 2019,

- auxiliary request 2, originally filed on 24 August 2018 as main request, and

- auxiliary request 3 as filed on 28 June 2019.

IV. According to the decision under appeal:

(a) Claim 1 of the main request related to a particular dosage regimen involving administration of the triple combination of defined amounts of liposomal

irinotecan in the form of irinotecan sucrose octasulfate salt liposome injection with 5-fluorouracil (5-FU) and leucovorin (l-form) in at least one cycle of two weeks for treatment of pancreatic cancer in a human patient who has failed prior treatment with or become resistant to gemcitabine. The amendments according to the main request met the requirements of Rule 80, 123(2) and 123(3) EPC.

(b) The patent sufficiently disclosed the claimed invention as defined in accordance with the main request. The suitability of the defined treatment regimen for the defined indication was plausible in view of examples 6 and 7 of the patent and having regard to the known effectiveness of similar combination treatment involving non-liposomal irinotecan, the so-called FOLFIRI regimen, as mentioned in the the patent and reported in documents D2, D3, D5 and D6.

(c) The priority document of 13 June 2012 (hereinafter: "PD1") described the administration of leucovorin at a dose of 200 mg/m2 without specifying whether the leucovorin was in the l-form or in the racemic form. The subject-matter as defined in accordance with the main request was therefore not entitled to this priority. Accordingly, documents D4, D8, D10 and D15b represented relevant prior art.

(d) Document D15b represented the closest prior art as it related to the same purpose as the patent and required the least modifications to arrive at the claimed subject-matter. Document D15b described a protocol for a Phase 3 clinical study involving liposomal irinotecan (MM-398), alone (Arm A) or in combination with 5-FU and leucovorin (Arm C) for use in treating metastatic pancreatic cancer in

patients with failed gemcitabine based therapy. The dosage regimen disclosed in document D15b for Arm C of the study only differed from the treatment defined in claim 1 of the main request in that the order of administration was not specified.

The problem to be solved was seen in the provision of effective and safe treatment. In view of the triple treatment described in the prior art advantages of triple treatment over monotherapy could not be taken into account. Moreover, improvement over monotherapy was not plausibly derivable from the application as filed.

Whilst document D15b did not report any results of the described treatment, the mere fact that according to this document the dosage regimen of Arm C was being tested in a clinical study for the treatment of gemcitabine-resistant pancreatic cancer (as disclosed in document D15b) provided a reasonable expectation of success. Clinical studies were based on data obtained by preclinical testing both in vitro and in animals and required authority approval involving ethical considerations. The skilled person would therefore have expected the treatment in study arm C to be safe and effective unless he was dissuaded from this by the prior art. While the outcome of a clinical trial could be success or failure, no particular reason was known which would have discouraged the person skilled in the art from carrying out the therapeutic protocol according to Arm C of document D15b to simply confirm the usefulness of the dosage regimen. Finding out in a straightforward manner that the disclosed dosage regimen provided both efficacy and safety of treatment according to the purpose of the Phase 3 clinical trial could not be regarded as inventive.

No particular technical effect from the order of administration specified in claim 1 of the main request was recognized. The defined sequence corresponded to the order of administration of irinotecan, leucovorin and 5-FU as described in documents D2-D6 for the FOLFIRI treatment and was therefore obvious as available option.

Although document D15b did not indicate the meaning of MM-398, at the time of document D15b it was known from document D13 that MM-398 was nanoliposomal irinotecan, also known as PEP02. Even it were assumed that the definition of the liposoamal irinotecan as irinotecan sucrose octasulfate salt liposome injection represented a further difference with the prior art, no technical effect associated with this difference was recognized. As the original application acknowledged that irinotecan sucrose octasulfate salt liposome injection was known from US8147867, this product would represent an obvious alternative liposomal irinotecan which was available to the skilled person at the time of application

Claim 1 of the main request did therefore not meet the requirement of inventive step.

(e) The additional feature defined in accordance with auxiliary requests 1 and 3, that the patient achieves a response which is at least stable disease, merely excluded treatment failure and therefore did not represent any further distinction. Claim 1 of auxiliary request 2 only differed from the claim 1 of the main request that it did not specify the liposomal irinotecan as irinotecan sucrose octasulfate salt liposome injection.

The claims of auxiliary requests 1-3 lacked an inventive step for the same reasons as the main request.

New items of evidence

V. The following additional documents were cited during the appeal proceedings:

D23 : Declaration of Amy McKee, M.D.
D23A: Hoos et al., J Clin Oncol 31:3432-3438
D23B: Clinical Development Success Rates 2006-2015, published by Biotechnology Innovation Organization (BIO)
D24 : Declaration of Bruce Belanger, Ph.D.

D15c: EU clinical trial database for NAPOLI-1 study from 12 October 2012
D25: Pin-Yuan Chen et al, Neuro-Oncology 15(2):189-197 (December 2012)
D26: Drummond DC et al, Cancer Res 2006; 66: 3271-3277 (2006)
D27: Roy AC et al, Annals of Oncology 24(6): 1567-1573 (February 2013)
D28: Svenson S, Current Opinion in Solid State and Materials Science, 16(6) pp 287-294 (October 2012)
D29: Makrilia N et al, JOP. Journal of the Pancreas, 12(2) pp 110-113 (2011)
D30: Chen LT et al, Journal of Clinical Oncology, 30(4 Suppl) pp 613-613 (February 2012)
D31: Cunningham D et al, Journal of Clinical Oncology 29 (4 Suppl): 6-6 (2011)
D32: Gerber DE, Journal of Thoracic Oncology 7(12) Supplement 5_ S387-S389 (December 2012)
D33: Noble et al, Cancer Res 2006; 66: (5). March 1, 2006
D34: Krauze MT et al, Neuro-Oncology 9(4): 393-403 (2007)

D35: Mullard A, Nature Reviews Drug Discovery, vol. 17, page 777 (2018)
D36: The Medicines for Human Use (Clinical Trials) Regulations (MHCTR) 2004.

D37: Expert declaration of Carla Schoonderbeek
D37A:Directive 2001/20/EC
D38: Expert declaration of Grant H. Castle, Ph.D.
D38A:Communication from the Commission 2010/C 82/01

Documents D23-D24 and D37-D38A were filed by the appellant with its statement of grounds of appeal and its further submission of 30 June 2021, respectively.

Documents D15c and D25-D36 were filed by the opponent-respondent with its reply of 27 July 2020.

Parties and requests

VI. The appellant, Ipsen Biopharm Ltd., requests that the decision under appeal be set aside and the patent be maintained on the basis of the main request or any of auxiliary requests 1-3, all filed with the statement of grounds of appeal and corresponding to the requests on which the decision under appeal was based.

The appellant further requests that documents D25-D34 not be admitted into the appeal proceedings and that documents D37, D37A, D38 and D38A be admitted into the proceedings in case the Board intends to admit documents D15c, D35 and D36.

VII. The respondent, Teva Pharmaceutical Industries Ltd, requests that the appeal be dismissed.

The respondent further requests that new documents D23, D23A, D23B and D24 not be admitted into the appeal proceedings.

The following points inter alia appear to need consideration at the oral proceedings:

1. Admission additionally cited documents

1.1 Documents D23, D23A, D23B and D24 concern expert declarations with annexes relied upon by the appellant to argue that contrary to the finding in the decision under appeal (see section 5.7.3) the report of the Phase 3 clinical trial in document D15b did not provide the skilled person with a reasonable expectation of success. In view of the appellant's explanations in the statement of grounds of appeal (see section 5.86) and the letter of 30 June 2021 (see section 4) the Board is inclined to admit documents D23, D23A, D23B and D24 into the appeal proceedings as a legitimate response to the decision under appeal.

1.2 Documents D25-D34 were filed by the respondent with its reply to the appeal to support the argument that the product name "MM-398" was at the time of publication of document D15b well known to relate to liposomal irinotecan, in particular irinotecan sucrose octasulfate salt liposome injection (see reply sections 7.72-7.90). The Board is inclined to admit documents D25-D34 as legitimate response to the appellant's argument (see statement of grounds of appeal sections 5.36 and 5.48) that no documents on file identified MM-398 as irinotecan sucrose octasulfate salt liposome injection.

1.3 The appellant did not object to the admission of documents D15c, D35 and D36. These documents were filed by the respondent to argue that contrary to opinion expressed in document D23 the international Phase 3 clinical trial to which document D15b related required at least in the UK approval from the ethics committee

and the licensing authority and should thus be considered to provide a reasonable expectation of success (see reply sections 7.50-7.55). The Board therefore intends to admit documents D15c, D35 and D36 as a legitimate response to the statement of grounds of appeal.

1.4 Documents D37, D37A and D38 were filed by the appellant in support of the argument that reports of the Phase 3 clinical trial as in documents D15b and D15c did not provide the skilled person with a reasonable expectation of success (see reply of 30 June 2021 section 3). The Board intents to admit documents D15c, D35 and D36 as a legitimate response to the reply to the appeal.

2. Main request

2.1 Claim 1 of the main request defines:

"Liposomal irinotecan for use in a method of treating pancreatic cancer in a human patient, wherein the patient exhibits evidence of recurrent or persistent pancreatic cancer following primary chemotherapy and wherein the patient has failed prior treatment with gemcitabine or become resistant to gemcitabine, the method comprising co-administration of an effective amount each of liposomal irinotecan, 5-fluorouracil (5-FU) and leucovorin to the patient in at least one cycle wherein the cycle is a period of 2 weeks and, for each cycle:
(a) liposomal irinotecan is administered to patients not homozygous for the UGT1A 1 *28 allele on day 1 of each cycle at a dose of 80 mg/m2 and to patients homozygous for the UGT1A1 *28 allele on day 1 of cycle 1 at a dose of 60 mg/m2 and on day 1 of each subsequent cycle at a dose of 60 mg/m2 or 80 mg/m2;
(b) 5-FU is administered at a dose of 2400 mg/m2; and

(c) leucovorin is administered at a dose of 200 mg/m2 (1 form);
and wherein in each cycle, the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU;
and wherein the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection."

2.2 Sufficiency of disclosure

The respondent contests that the patent plausibly disclosed effectiveness of the claimed therapeutic regimen. The patent provided no experimental results of treatment involving the defined dosage regimen and essentially relied on verbal statements. The patent thereby failed to sufficiently disclose the suitability of the defined therapeutic regimen for the defined therapeutic indication (see reply section 5.21).

In line with the decision under appeal (see section 3) the Board presently takes the view that the main request complies with the requirement of Article 83 EPC.

In particular, the Board does presently not recognize that the teaching in the patent, that the defined triple therapy regimen is suitable for treatment of gemcitabine resistant pancreatic cancer as can be verified in accordance with the design for a Phase 3 trial as disclosed in example 7 of the patent (see paragraphs [0083]-[0177], is compromised by any serious doubt based on verifiable facts.

In the Board's preliminary view this teaching does further not lack plausibility taking account of:

- the results from the Phase 1 trial of example 6 of the patent (see paragraphs [0081]-[0082] involving

a triple combination of MM-398, 5-FU and leucovorin in treatment of patients with prostatic cancer and

- the known efficacy of the triple combination of non-liposomal irinotecan with 5-FU and leucovorin in treatment of patients with gemcitabine-refractory pancreatic carcinoma in the so-called FOLFIRI regimen as reported in documents D2, D3, D5 and D6 and referred to in the patent (see paragraph [0003], see also the application as filed page 1).

During the oral proceedings the parties will have the opportunity to further discuss the issue of sufficiency of disclosure.

2.3 Priority

2.3.1 It seems not in dispute that PD1 describes the same triple therapy dosage regimen as claim 1 of the main request (see PD1 claims 1, 4 and 11) except that PD1 does not explicitly refer to the l-form of the leucovorin to be administered at a dose of 200mg/m^2.

The appellant argues that PD1 implicitly disclosed the l-form of leucovorin. PD1 described "leucovorin" to act as a biochemical cofactor for 1-carbon transfer reactions (see PD1 page 11 lines 9-11). As indicated by for instance document D1 (see page 5 section 11) it was common knowledge that only the l-form of leucovorin is pharmaceutically active. The skilled person would therefore understand that in PD1 the term "leucovorin" referred to the l-form of leucovorin. Alternatively, if the term "leucovorin" in PD1 was considered to relate to "l-leucovorin or racemic leucovorin", the specification of the l-form in claim 1 of the main request represented merely a selection from two disclosed alternatives which did not affect the priority entitlement.

The Board presently considers that the term "leucovorin" as used in DP1 cannot be unambiguously considered to relate to the l-form of leucovorin. As explained by the respondent (see reply sections 6.7 and 6.16) document D1b, representing the FDA product label for leucovorin, states that "Leucovorin is a mixture of the diastereoisomers of the 5-formyl derivative of tetrahydrofolic acid (THF)", *i.e.* the racemic mixture (see D1b page 1 left column under CLINICAL PHARMACOLOGY"). Whilst document D1b recognizes that the l-form is the biologically active compound, the document nevertheless refers to leucovorin as not requiring reduction in order to participate in reactions. From the mentioned reference to the activity of leucovorin as biochemical cofactor in the patent it seems therefore not unambiguously derivable that the leucovorin defined for use in a dose of 200mg/m^2 was actually l-leucovorin and not the racemate.

The disclosure in PD1 prescribes a particular dose of 200mg/m^2 for the intended leucovorin. As explained by the respondent (see reply sections 6.24-6.25) the skilled person would be aware that the racemate and the l-form require different dosing. The appellant's alternative argument based on the interpretation that the disclosure with respect to leucovorin in PD1 implied an option between the l-form or the racemate, seems therefore presently not convincing.

2.3.2 The respondent further argues that claim 1 of the main request combines two embodiments concerning the patient populations that were only separately disclosed in PD1 (pages 11-12), namely:

- the embodiment wherein the patient exhibits evidence of recurrent or persistent pancreatic cancer following primary chemotherapy, and

- the embodiment wherein the patient has failed prior treatment with gemcitabine or become resistant to gemcitabine.

In line with the appellant's letter of 30 June 2021 (see sections 6.50-6.53) the Board is of the preliminary opinion that the mentioned combination does *per se* not present the skilled person with subject-matter that is not directly and unambiguously derivable from PD1.

2.3.3 Accordingly, the Board presently considers that the subject-matter of claim 1 of the main request is not entitled to the priority of PD1 and that documents D4,D8, D10 and D15b thus represent prior art.

During the oral proceedings the parties will have the opportunity to further discuss the question of the validity of the first priority.

2.4 Inventive step

2.4.1 The appellant has contested the finding in the decision under appeal that document D15b represents the closest prior art, because document D15b failed to disclose actual effective treatment of pancreatic cancer. In contrast, document D13 reported actual results of treatment of pancreatic cancer in patients with failed first-line gemcitabine therapy involving liposomal irinotecan and should therefore be considered as closest prior art (see statement of grounds of appeal sections 5.41-5.51).

According to the appellant monotherapy with liposomal irinotecan represented within document D13 the most promising starting point in view of the reported favourable results over triple therapy in a Phase 1

trial and in view of the disclosed subsequent Phase 2 trial, which involved monotherapy only (see statement of grounds of appeal sections 5.7-5.11).

The differences of the claimed subject-matter with this prior art involved the definition of the two weeks cycle, the lower dose of the liposomal irinotecan in the form of irinotecan sucrose octasulfate salt liposome injection and the additional administration of the defined doses of 5-FU and l-leucovorin in the defined order. Document D19 reported the results of a Phase 3 trial carried out in accordance with the design in example 7 of the patent (the NAPOLI-1 trial), which indicated advantageous efficacy with fewer adverse events for the triple therapy with respect to monotherapy. The problem solved was therefore the provision of a safe and effective treatment which is improved with respect to monotherapy (see statement of grounds of appeal sections 5.19-5.21). No prior art lead the skilled person towards the claimed subject-matter as solution to this problem (see statement of grounds of appeal section 5.64).

The differences between the claimed subject-matter and Arm C of the trial protocol described in document D15b concerned the actual effective and safe treatment of the patients, the definition of liposomal irinotecan as irinotecan sucrose octasulfate liposome salt injection instead of the product "MM-398", which remained undefined in document D15b, the order in which the drugs are administered and the distinction in the starting dose of the liposomal irinotecan depending on the patient's status concerning the UGT1A1*28 allele. In case document D15b was considered as starting point, these differences provided for safe and effective treatment which is improved relative to the monotherapy of document D15b (see statement of grounds of appeal sections 5.70-5.72). According to the appellant the

prior art provided the skilled person with no reasonable expectation that the claimed subject-matter would successfully solve such problem. In particular, contrary to the finding in the decision under appeal (see section 5.7.3), in view of documents D23, D24, D37 and D38 the mere announcement in document D15b that the mentioned Arm C was tested in a Phase 3 trial provided no reasonable expectation of success (see statement of grounds of appeal section 5.86 and reply of 30 June 2021 section 6.14). Moreover, document D15b only referred to the product MM-398 and it was not evident that this product was "irinotecan sucrose octasulfate salt liposome injection" as defined in claim 1 of the main request.

Document D5, which was additionally cited as suitable starting point in the prior art in the respondent's reply, described a clinical trial in which patients with pancreatic cancer after failed treatment with gemcitabine were administered non-liposomal irinotecan, leucovirin and 5-FU. The differences between the treatment defined in claim 1 of the main request and the treatment of document D5 concerned the use of liposomal irinotecan and its defined dose and the dose of 5-FU. According to the appellant the prior art provided no reasonable expectation that replacement of the two weekly total dose of 140mg/m^2 of non-liposomal irinotecan a 80mg/m^2 dose of liposomal irinotecan and the 20% increase of the 5-FU dose would allow for safe and effective treatment, let alone treatment resulting in improved therapy as demonstrated by the overall and progression free survival reported in document D19 (see letter of 30 June 2021 sections 6.44 and 6.49).

2.4.2 The Board recalls that the problem solution approach implies that in case an inventive step can be recognized starting from a particular item of prior art which is convincingly identified as most promising

starting point and thus represents the closest prior art, attempts to argue a lack of inventive step starting from less promising starting points are bound to fail. However, in case an inventive step is apparently convincingly denied starting from a promising particular item of prior art, the mere argument that the claimed subject-matter nevertheless involves an inventive step in view of an allegedly closer prior art, may not be persuasive, because in such case the allegedly closest prior art is likely to represent a starting point that is no more promising.

In the decision under appeal the subject-matter of claim 1 of the main request is denied an inventive step starting from document D15b as closest prior art (see sections 5.3 and 5.7.9). As pointed out by the respondent (see reply section 7.26) the patent itself does not present experimental evidence specifically demonstrating the therapeutic effect of the claimed dosage regimen. The circumstance that document D15b relates to a protocol for a Phase 3 trial without report of actual effective treatment does therefore in the Boards preliminary opinion not disqualify this document as suitable alternative starting point in the prior art. Furthermore, as explained in the decision under appeal by reference to document D13 (see section 5.3 page 12 penultimate paragraph) and as sustained by the respondent by reference to *inter alia* document D25 (see reply section 7.84) the product MM-398 mentioned in document D15b apparently concerned a known product at the time of publication of document D15b, which was identifiable as a liposomal formulation containing nano-sized irinotecan crystals complexed with sucrose octasulfate corresponding to the liposomal irinotecan defined in claim 1 of the main request (see D25 page 190 right column under "Investigational Agent"). Having regard to the similarities between the treatment regimen of claim 1 of the main request and the protocol

for treatment in document D15b the Board therefore presently considers that document D15b may not be excluded as suitable starting point in the prior art.

In view of the respondents arguments (see reply items 7.122-7.126) the Board does further not exclude that document D5 may represent a suitable starting point in the prior art, as this document describes effective treatment of pancreatic cancer after failed gemcitabine therapy involving combination treatment with non-liposomal irinotecan, 5-FU and leucovorin.

2.4.3 The differences between the claimed subject-matter and Arm C of the trial protocol described in document D15b seem to concern the actual effective and safe treatment of the patients, the order in which the drugs are administered and possibly the distinction in the starting dose of the liposomal irinotecan depending on the patient's status concerning the UGT1A1*28 allele.

As argued by the respondent (see reply section 7.24) it was already known from documents D10 and D11 that the patient's UGT1A1*28 allele-status was relevant for the irinotecan starting dose (see D10 pages 7-8 section 2.3; see D11 page 1290 right column lines 2-7). Moreover, as further explained in the decision under appeal (see section 5.7.7) and sustained by the respondent (see reply sections 7.30 and 7.31) no particular effect of the order of administration as defined in claim 1 of the main request seems to have been demonstrated, whilst the defined sequence corresponds to the order of administration of irinotecan, leucovorin and 5-FU in known combination treatment as described in documents D2, D4, D6 and D10. The appellant observes that document D15b does not mention the order of administration and the UGT1A1*28 allele-status, but does not seem to specifically contest the respondents arguments in this respect. The

Board therefore doubts whether these differences contribute to any inventive merit.

In view of the difference that document D15b only describes a protocol for treatment without reporting any results the problem to be solved could be formulated as the provision of actually effective and safe treatment of the defined patients suffering from pancreatic cancer.

In this context the Board is presently not convinced that the mere fact that a dosage regimen is reported to be tested in a Phase 3 clinical trial already by itself generally provides the skilled person with a reasonable expectation of success of that dosage regimen. As indicated in the statement of grounds of appeal (see section 5.77) the considerations in T 239/16 (see section 6.5) seem closely linked to the further circumstances of the case decided therein. Similar appears to apply with respect to the considerations in T 2506/12 (see section 3.15).

However, in the present case the patent fails to disclose any results of actual treatment in accordance with claim 1 of the main request and presents in example 7 only a design for a Phase 3 trial. Moreover, whilst the patent refers to promising efficacy and safety of triple combination treatment in the Phase 1 trial of example 6 (see paragraph [0083]), this example 6 does not seem to mention the dose of the administered 5-FU and leucovorin, the two weekly dosing cycle of the defined drugs nor the treatment status of the patients (see the respondent's reply sections 4.8, 5.2, 5.11). At the same time, as pointed out by the respondent (see reply sections 7.100-7.104 and 7.113-7.118), documents D12 and D13 already seem to have reported positive results from administration of MM-398, 5-FU and

leucovorin in treatment of gemcitabine refractory pancreatic cancer in a Phase 1 clinical trial setting.

In line with the introductory arguments of the respondent (see reply sections 5.1-5.4) the Board observes in this context that in as far as the disclosure in the patent itself essentially relies on considerations based on common knowledge and information already available from the prior art for proposing the claimed solution, similar considerations may equally apply in the assessment whether the claimed solution would be obvious to the skilled person..

According to the decision under appeal (see section 5.7.2) any evidence in documents D17-D19 of improved treatment from the defined triple dosage regimen over monotherapy lacked relevance, because the triple treatment in document D15b already represented the closest prior art. The Board recalls in this context that document D15b only describes a protocol for a trial of monotherapy and a triple dosage regimen and does not report actually effective treatment. However, the Board tends to agree with the further finding in the decision under appeal (see also section 5.7.2) that the more ambitiously formulated problem of providing an improvement over monotherapy may not be taken into account, since it seems not evident how such problem and its solution could be plausibly derived from the application as filed. In this context the Boards observes that the technical contribution actually disclosed in the patent is an aspect to be considered in the assessment of inventive step. In the present case there appears to be no disclosure of the technical contribution resulting from the distinguishing features.

2.4.4 The Board observes that the respondent acknowledges that its arguments starting from document D13 are

similar to its arguments based on document D12 (see reply section 7.113). As mentioned in section 2.4.3 above these documents seem to report positive results from administration of MM-398, 5-FU and leucovorin in treatment of gemcitabine refractory pancreatic cancer in a Phase 1 clinical trial setting. In line with the respondents arguments (see sections 7.101-7.103 and 7.118) the Board presently takes the view that the reported triple treatment seems more pertinent than the monotherapy also described in these documents.

As explained in section 2.4.1 the Board further considers that the triple treatment involving non-liposomal irinotecan as described in document D5 may not be excluded as possible starting point in the prior art.

The problem to be solved in view of documents D12/D13 or D5 could be formulated as the provision of further effective and safe treatment of the defined patients suffering from pancreatic cancer.

Faced with this problem the skilled person would likely take note of the trial protocol of document D15b. In line with the respondent's arguments (see reply sections 7.109, 7.118 and 7.127) similar considerations regarding the problem to be solved and its solution as set out in section 2.4.3 above may apply.

2.4.5 During the oral proceedings the parties will have the opportunity to further discuss the issue of inventive step.

3. Auxiliary requests 1-3

Auxiliary requests 1-3 filed with the grounds of appeal correspond to auxiliary requests 1-3 on which the decision under appeal was based.

The Board presently agrees with the decision under appeal (see sections 9.4 and 11.2 and 13.2) that the differences between auxiliary requests 1-3 and the main request do not seem of influence in the assessment of inventive step.

4. Final observations

The attention of the parties is drawn to Article 114(2) EPC and Articles 12 and 13 of the Rules of Procedure of the Boards of Appeal. The revised Rules of Procedure (RPBA 2020) entered into force on 1 January 2020 (Article 24(1) RPBA 2020). For the present case the transitional provisions pursuant to Article 25(2) RPBA apply.

View of NCT01494506 on 2011_12_16

ClinicalTrials Identifier: NCT01494506
Updated: 2011_12_16

Descriptive Information

Brief title Study of MM-398 Versus 5-Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer

Official title A Randomized, Open Label Phase 3 Study of MM-398 Versus 5-Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer

Brief summary

The study is an open label, randomized phase 3 study of MM-398 versus 5-fluorouracil (5-FU) and leucovorin (also known as folinic acid) in metastatic pancreatic cancer patients who have progressed on prior gemcitabine based therapy.

Detailed description

Phase	Phase 3
Study type	Interventional
Study design	Treatment
Study design	Randomized
Study design	Open Label
Study design	Parallel Assignment
Study design	Efficacy Study
Primary outcome	Measure: Overall Survival Time Frame: 24 months Safety Issue? No
Secondary outcome	Measure: Progression Free Survival Time Frame: 24 months Safety Issue? No
Secondary outcome	Measure: Time to treatment failure Time Frame: 24 months Safety Issue? No
Secondary outcome	Measure: Objective response rate Time Frame: 24 months Safety Issue? No
Enrollment	270 (Anticipated)
Condition	Metastatic Pancreatic Cancer
Arm/Group	Arm Label: MM-398 Experimental MM-398 Q3W IV
Arm/Group	Arm Label: 5 Fluorouracil and Leucovorin IV Active Comparator

	5 Fluorouracil and Leucovorin IV
Intervention	Drug: MM-398 Arm Label: MM-398
	MM-398 120 mg/m2 IV Q3W
Intervention	Drug: 5 Fluorouracil Arm Label: 5 Fluorouracil and Leucovorin IV
	5 Fluorouracil 2000 mg/m2 IV for 4 weeks followed by 2 weeks of rest every 6 weeks
Intervention	Drug: Leucovorin Arm Label: 5 Fluorouracil and Leucovorin IV
	Leucovorin 200 mg/m2 IV for 4 weeks followed by 2 weeks of rest every 6 weeks

Recruitment Information

Status	Recruiting
Start date	2011-11
Last follow-up date	2014-06 (Anticipated)
Primary completion date	2013-12 (Anticipated)

Criteria

Inclusion Criteria:
- Histologically or cytologically confirmed adenocarcinoma of the exocrine pancreas
- Metastatic disease
- Documented disease progression after prior gemcitabine based therapy
- KPS >/= 70
- Adequate bone marrow function
- Adequate hepatic function
- Adequate renal function

Exclusion Criteria:
- Prior irinotecan treatment
- Active CNS metastasis
- Clinically significant GI disorders
- Major surgery or radiotherapy within 4 weeks of enrollment
- Severe arterial thromboembolic events less than 6 months before inclusion
- NYHA Class III or IV congestive heart failure, ventricular arrhythmias or uncontrolled blood pressure
- Active infection or uncontrolled fever
- Pregnant or breast feeding patients

Gender	Both
Minimum age	18 Years
Healthy volunteers	No

Administrative Data

Organization name	Merrimack Pharmaceuticals
Organization study ID	MM-398-07-03-01
Sponsor	Merrimack Pharmaceuticals
Health Authority	United States: Food and Drug Administration

COMMENTARY

UGT1A1*28 Genotype and Irinotecan-Induced Neutropenia: Dose Matters

Janelle M. Hoskins, Richard M. Goldberg, Pingping Qu, Joseph G. Ibrahim, Howard L. McLeod

The Food and Drug Administration and Pfizer changed the package insert for irinotecan to include a patient's UGT1A1*28 genotype as a risk factor for severe neutropenia on the basis of the findings of four pharmacogenetic studies, which found that irinotecan-treated patients who were homozygous for the UGT1A1*28 allele had a greater risk of hematologic toxic effects than patients who had one or two copies of the wild-type allele (UGT1A1*1). Findings of subsequent irinotecan pharmacogenetic studies have been inconsistent. In a meta-analysis, we reviewed data presented in nine studies that included a total of 10 sets of patients (for a total of 821 patients) and assessed the association of irinotecan dose with the risk of irinotecan-related hematologic toxicities (grade III–IV) for patients with a UGT1A1*28/*28 genotype. The risk of toxicity was higher among patients with a UGT1A1*28/*28 genotype than among those with a UGT1A1*1/*1 or UGT1A1*1/*28 genotype at both medium (odds ratio [OR] = 3.22, 95% confidence interval [CI] = 1.52 to 6.81; $P = .008$) and high (OR = 27.8, 95% CI = 4.0 to 195; $P = .005$) doses of irinotecan. However, risk was similar at lower doses (OR = 1.80, 95% CI = 0.37 to 8.84; $P = .41$). Low doses of irinotecan (100–125 mg/m^2) are in the commonly used therapeutic range. The risk of experiencing irinotecan-induced hematologic toxicity for patients with a UGT1A1*28/*28 genotype thus appears to be a function of the dose of irinotecan administered.

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Irinotecan (Camptosar), a topoisomerase I poison, is approved for use in combination with 5-fluorouracil and leucovorin chemotherapy for first-line treatment of metastatic colorectal cancer and as a single agent in second-line salvage therapy of 5-fluorouracil refractory metastatic colorectal cancer disease. It is also commonly used to treat esophageal, non–small-cell lung, and breast cancers and other solid tumors in a second- or third-line setting. Irinotecan can be administered weekly, every 2 weeks, or every 3 weeks at doses ranging from 50 to 350 mg/m^2. The principal dose-limiting toxicities are delayed diarrhea and neutropenia; these toxicities are reversible, not cumulative, and related to irinotecan dose (1). Irinotecan is metabolized in vivo by carboxylesterases to the active metabolite SN-38, which is 100- to 1000-fold more potent than irinotecan as a topoisomerase I poison. SN-38 is eliminated predominantly by glucuronidation to SN-38 glucuronide. This glucuronidation reaction is mediated primarily by UDP-glucuronosyltransferase 1 family polypeptide A1, which is encoded by the UGT1A1 gene. Systemic exposure to SN-38 (as measured by area under the concentration–time curve) is related to the number of TA base repeats that a patient carries in the promoter region of each UGT1A1 allele (2–5). The wild-type allele (i.e., allele UGT1A1*1) has six TA repeats, and the variant allele (i.e., allele UGT1A1*28) has seven TA repeats. Patients who are homozygous for the UGT1A1*28 allele glucuronidate SN-38 less efficiently than patients who have one or two wild-type alleles; therefore, homozygous patients are exposed to higher plasma concentrations of SN-38 (3).

In November 2004, the US Food and Drug Administration (FDA) Advisory Committee on Pharmaceutical Sciences considered the findings of four pharmacogenetic trials that had assessed the association between UGT1A1*28 genotype and irinotecan-induced toxicities in a total of 30 patients who were homozygous for the UGT1A1*28 allele (4,6–8). In these studies, associations between the UGT1A1*28/*28 genotype and hematologic toxicity and/or diarrhea were observed. As a result of these findings, the FDA advised Pfizer Pharmaceuticals, the manufacturer of irinotecan, to amend the product information for the drug to include the association between the UGT1A1*28 genotype and hematologic toxicity and to recommend that patients with the UGT1A1*28/*28 genotype receive a lower starting dose of irinotecan. These changes took effect in July 2005. A diagnostic test for the UGT1A1*28 genotype (i.e., Invader UGT1A1 Molecular Assay; Third Wave Technologies, Inc, Madison, WI) for irinotecan dosing was approved in August 2005 by the FDA (9).

Subsequent results have begun to clarify the association between UGT1A1*28 and irinotecan-induced toxicities, particularly for dosing schedules that were not reviewed in the initial FDA committee meeting. Some studies (4,7) found that the UGT1A1*28/*28 genotype predicted grade III–IV neutropenia but not diarrhea, and other studies (6,10) found that the genotype predicted grade III–IV diarrhea but not hematologic toxicity. These results contrast with studies in which the UGT1A1*28

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See "Funding" and "Notes" following "References."

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genotype was not associated with neutropenia or diarrhea (11,12). Moreover, in most studies (4,6,7,10–12), many patients with a UGT1A1*28/*28 genotype did not experience severe toxicity, and in some of the studies (6,10,11), patients with the UGT1A1*1/*1 genotype had high-grade toxicity. Inconsistent relationships are common for genetic association studies and might be explained by false-positive associations or overestimation of the effect size in the initial studies. Whatever the cause, these findings pose the question: How should UGT1A1*28 genotyping be used to determine the optimum treatment regimen for a patient treated with irinotecan?

In a meta-analysis, we assessed the relationships between the incidence of irinotecan-induced hematologic toxicity (grade III–IV) and irinotecan dose among patients with the UGT1A1*28/*28 genotype. We identified nine studies (3,4,6,7,10–14) that assessed the relationship between UGT1A1*28 genotype and irinotecan-induced hematologic toxicity in a total of 821 patients. Two irinotecan-containing regimens were administered to patients in the N9741 study (14), and in our analyses, we analyzed the patients treated with each regimen as two separate samples. A summary of the 10 pharmacogenetic samples included in our analyses is presented in Table 1. Among the samples, patients received a variety of irinotecan-containing regimens, including commonly used higher doses (200–350 mg/m^2) administered every 21 days, an intermediate dose (180 mg/m^2) administered every 2 weeks, or lower doses (80–125 mg/m^2) administered weekly; irinotecan was given either alone or in combination with other anticancer agents. A UGT1A1*28/*28 genotype was associated with severe hematologic toxicity in three (3,4,14) of the 10 samples (P<.05, two-sided Fisher's exact test; see Table 1 for P values) and tended to be associated with toxicity in two of the samples (6,13) (P<.1). In the other five samples, the UGT1A1*28/*28 genotype was not associated with toxicity.

Heterogeneity among samples was tested by use of a chi-square test, and the presence of heterogeneity was not detected (P = .25). Publication bias was assessed by a funnel plot of the log odds ratio (OR) of individual samples against the standard error of the log odds ratios. The plot (Fig. 1) appeared to be symmetrical about the horizontal line (weighted average log OR = 1.35), with the diameter of the funnel decreasing with decreasing standard error (i.e., increasing sample size), indicating no evidence of publication bias. The observation suggests that studies demonstrating non–statistically significant associations between UGT1A1*28/*28 genotype and irinotecan-related hematologic toxicity were ascertained and included in the meta-analytic study.

To assess whether irinotecan dose modulates the association between UGT1A1*28 genotype and the risk of hematologic toxicity, we used a generalized linear mixed model (available in the SAS PROC GLIMMIX program, SAS Institute, Cary, NC) and considered dose as both a continuous and categorical variable. By using a unified regression model, we could account for the sample size of each genotype and of each sample. We first considered dose as a continuous variable and compared the rate of severe hematologic toxicity induced by irinotecan between patients with a UGT1A1*28/*28 genotype and patients with one or two wild-type alleles (UGT1A1*1/*1 or UGT1A1*1/*28 genotype). The results showed that the risk of hematologic toxicity between patients with a UGT1A1*28 and those with a UGT1A1*1/*1 or UGT1A1*1/*28 increased statistically significantly as irinotecan dose increased (slope = 0.012; P = .028). At a low dose level the risk was relatively low, but at a medium to high dose level the risk was higher. For example, at an irinotecan dose of 100 mg/m^2, the odds of hematologic toxicity for UGT1A1*28/*28 patients was 1.28 times higher than that for UGT1A1*1/*1 or UGT1A1*1/*28 patients (OR = 1.28, 95% confidence interval [CI] = 0.42 to 3.91; P = .63), and, at a dose of 250 mg/m^2, it was 8.07 times higher (OR = 8.07, 95% CI = 3.23 to 20.2; P<.001).

In a further analysis, we assessed the association between UGT1A1*28 genotype and hematologic toxicity and their interaction with irinotecan dose as a categorical variable. Irinotecan dose levels were pooled into the following three groups: low (<150 mg/m^2), medium (150–250 mg/m^2), and high (>250 mg/m^2) doses on the basis of the three most commonly used dosage regimens. At medium doses, the risk of toxicity was higher among patients with a UGT1A1*28/*28 genotype than among those with a UGT1A1*1/*1 or UGT1A1*1/*28 genotype (OR = 3.22, 95% CI = 1.52 to 6.81; P = .008); similarly, at high doses, the risk was higher among patients with a UGT1A1*28/*28 genotype than among

CONTEXT AND CAVEATS

Prior knowledge

In four previous studies, a UGT1A1*28 genotype among irinotecan-treated patients was associated with an increased risk of severe neutropenia.

Study design

A meta-analysis of nine studies that included 10 sets of patients (for a total of 821 patients) assessed the association between irinotecan dose and the risk of grade III and IV hematologic toxic effects by UGT1A1*1 or UGT1A1*28 genotype.

Contribution

The risk of hematologic toxic effects at high and medium irinotecan doses was higher among patients with a UGT1A1*28/*28 genotype than among those with a UGT1A1*1/*28 or UGT1A1*1/*1 genotype. However, at lower doses, risk was similar for patients with all genotypes. Low doses of irinotecan (100–125 mg/m^2) are in the commonly used therapeutic range.

Implications

At low doses of irinotecan, decisions about treating individual patients can be made according to standard clinical practice because genotype was not associated with risk. At higher doses, genotype-based decisions are advisable because of the association between the UGT1A1*28/*28 genotype and increased risk of irinotecan-induced toxic effects.

Limitations

There were many sources of heterogeneity among the studies analyzed. Some sources of heterogeneity could have influenced patient participation in a trial and, therefore, the dose of irinotecan that was received. Others could have been related to the dose of irinotecan administered by trials. These factors may also have directly modulated the association observed. Because of limited power or the unavailability of individual data, the relationship between these factors and the association could not be assessed.

Table 1. Summary of 10 samples included in our analyses that assessed the diagnostic value of the homozygous UGT1A1*28 genotype to predict irinotecan-induced grade III–IV hematologic toxicity†

Irinotecan dose, mg/m² (% patients)	Schedule	Concomitant chemotherapy	Trial type	Patient type	Toxicity (grade III–IV)	Grade‡ criteria	No. of patients	Overall incidence of toxicity§	No. of UGT1A1*28/*28 patients (frequency, %)	Toxicity incidence§		Two-sided Fisher's exact *P*	First author	Sample ref.
										UGT1A1 *28/*28	UGT1A1*1/*1 or *1/*28			
350	Every 3 wk	None	Phase I, prospective	Solid tumors, lymphoma	Neutropenia	NCI	61	18 (11/61)	6 (10)	83 (5/6)	11 (6/55)	.0004	Innocenti	(4)
300	Every 3 wk	None	Phase I, prospective	Solid tumors, lymphoma	Neutropenia	NCI	20	10 (2/20)	4 (20)	50 (2/4)	0 (0/16)	.03	Iyer	(3)
200	Every 3 wk	OXA	Prospective	Advanced colorectal	Neutropenia (IV only)	NCI	103	17 (17/103)	11 (11)	55 (6/11)	12 (11/92)	.002	McLeod	(14)
180	Biweekly	5FU	Prospective	Metastatic colorectal	Hematologic	NCI	250	15 (37/250)	22 (9)	18 (4/22)	14 (33/228)	.55	Toffoli	(12)
180	Biweekly	5FU	Prospective	Metastatic colorectal	Neutropenia	WHO	56	25 (14/56)	5 (9)	60 (3/5)	22 (11/51)	.09	Marcuello	(6)‖
180	Biweekly	None	No information	Advanced colorectal	Neutropenia	NCI	58	28 (16/58)	7 (12)	57 (4/7)	24 (12/51)	.08	Chiara	(13)
180	Biweekly	5FU	Retrospective	Advanced colorectal	Neutropenia	NCI	46	33 (15/47)	5 (11)	60 (3/5)	29 (12/41)	.31	Rouits	(7)‖
100	Weekly	5FU	Prospective	Advanced colorectal	Neutropenia (IV only)	NCI	109	10 (11/109)	11 (10)	18 (2/11)	9 (9/98)	.31	McLeod	(14)
80	Weekly	RAL	Prospective	Advanced colorectal	Neutropenia	NCI	56	7 (4/56)	7 (13)	14 (1/7)	6 (3/49)	.42	Massacesi	(10)
100 (22)	Weekly	CAP	Phase II, prospective	Metastatic colorectal	Neutropenia	NCI	64¶	5 (3/64)	6 (9)	0 (0/6)	5 (3/58)	1.00	Carlini	(11)
125 (78)	Weekly	CAP												

† ref. = reference; NCI = National Cancer Institute common toxicity criteria; OXA = oxaliplatin; 5FU = 5-fluorouracil; WHO = World Health Organization; RAL = raltitrexed; CAP = capecitabine.

‡ NCI and WHO systems grade an absolute neutrophil count of less than 1000×10^6 cells per L of blood as grade III–IV neutropenia (http://safetyprofiler-ctep.nci.nih.gov/CTC/CTC.aspx, http://www.who.int/en/).

§ The toxicity incidence (overall or by genotype group) is reported as a percentage. Values in parentheses are number of patients overall or in each genotype group with hematologic toxicity/number of total patients in that group.

‖ More than one irinotecan-containing regimen was administered in these studies. Only results from patients who received irinotecan at 180 mg/m² biweekly with 5-fluorouracil were considered in these analyses.

¶ Patients with TA_5 and TA_6 alleles are included in these results.

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those with a UGT1A1*1/*1 or UGT1A1*1/*28 genotype (OR = 27.8, 95% CI = 4.00 to 195; $P = .005$). In contrast, at low irinotecan doses, the risk of toxicity was not statistically significantly different between patients with a UGT1A1*28/*28 genotype and those with a wild-type allele (OR = 1.80, 95% CI = 0.37 to 8.84; $P = .41$). Results from the categorical-dose and the continuous-dose analyses were similar; i.e., a statistically significant association was found between genotype and toxicity at medium or high doses of irinotecan but not at low doses.

We also assessed whether irinotecan dose modulates the association between UGT1A1*28 genotype and irinotecan-induced diarrhea (grade III–IV). We identified nine studies (3,4,6,7,10,11, 13–15) that assessed the relationship between UGT1A1*28 genotype and toxicity. As noted above, the N9741 study (14) administered two irinotecan-containing regimens to patients, and we treated the patient who was administered the different regimens as two separate samples. In addition, only grade IV diarrhea data weres available for the study (14). Of the 10 samples, UGT1A1*28/*28 genotype was associated with severe diarrhea in only one sample (relative risk = 3.40, 95% CI = 1.76 to 6.59; $P = .02$, two-sided Fisher's exact test), indicating that UGT1A1*28 genotype was not associated with diarrhea (6). We next assessed the relationships between irinotecan dose and the incidence of irinotecan-induced diarrhea (grade III–IV) by genotype. The incidence of severe diarrhea in patients with a UGT1A1*28/*28 genotype was not related to irinotecan dose ($r^2 = .0$; $P = .8$; n = 10 samples) (data not shown); however, the rate of diarrhea among patients with one or two wild-type alleles was inversely associated with dose ($r^2 = .43$; $P = .04$; n = 10 samples) (data not shown). Thus, the risk of diarrhea among patients with a UGT1A1*28/*28 genotype was not associated with irinotecan dose, and so we did not examine this relationship further.

We observed that, at higher irinotecan doses (>150 mg/m^2), the risk of hematologic toxicity was strongly associated with the UGT1A1*28 polymorphism. In contrast, at lower doses (≤150 mg/m^2), the risk of hematologic toxicity among patients with a UGT1A1*28/*28 genotype was not statistically significantly different from that among patients with one or two wild-type alleles (i.e., UGT1A1*1/*28 or UGT1A1*1/*1, respectively). This observation is consistent with a classic gene–environment interaction, in which the association between genotype and outcome depends on the level of exposure to an environmental factor—in this case, the dose of irinotecan (16). To our knowledge, this is the first demonstration of a gene–environment interaction in the context of pharmacogenetics. In contrast, among patients with the UGT1A1*28/*28 genotype, irinotecan dose was not associated with diarrhea. Heterogeneity of irinotecan administration, diarrhea management with loperamide, coadministered chemotherapeutic agents among trials, and difficulty in scoring this toxicity might contribute to the incidence of this adverse event and explain some of the interstudy variation in the incidence of diarrhea among patients with a UGT1A1*28/*28 genotype. The utility of UGT1A1*28 genotype to predict irinotecan-induced diarrhea, therefore, remains unclear and requires further investigation.

We propose two potential strategies for irinotecan dosing to accommodate the modulatory effect of irinotecan dose on the risk of hematologic toxicities among patients with a UGT1A1*28/*28

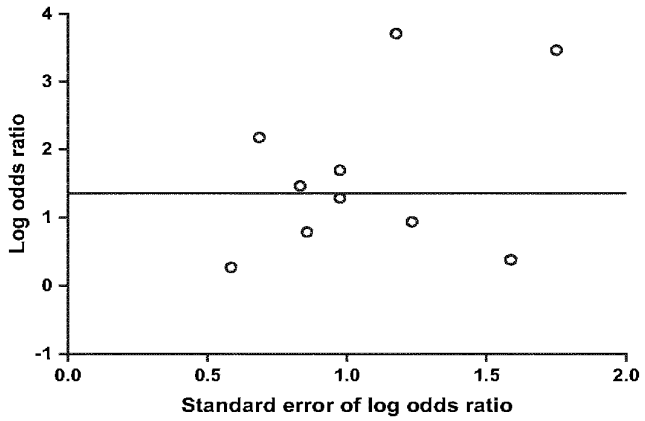

Fig. 1. Funnel plot of log odds ratio against standard error of log odds ratio for the UGT1A1*28/*28 genotype with irinotecan-related grade III–IV hematologic toxicity. Symmetry of the plot about the weighted average of the log odds ratios ($y = 1.3536$) indicates no evidence of publication bias.

genotype. The first is based on a prior selection of the most convenient and appropriate regimen for the individual patient. When regimens with a low dose of irinotecan (<150 mg/m^2) given weekly are being considered, decisions concerning the best irinotecan dose for individual patients could be made on the basis of standard clinical practice rather than genotype because genotype was not associated with an increased risk of toxicity. Low doses of irinotecan (100–125 mg/m^2) are in the commonly used therapeutic range (17). For patients receiving a more convenient high-dose regimen (>250 mg/m^2), however, genotype-based decisions are advisable because UGT1A1*28 genotype was associated with toxicity at higher doses of irinotecan (>150 mg/m^2). For patients with a UGT1A1*28/*28 genotype, a starting irinotecan dose reduction of one level is recommended in the package insert from the manufacturer (18), whereas, for patients with one or two wild-type alleles a standard irinotecan dose can be used. Patients with a UGT1A1*28/*28 genotype had a heightened risk of toxicity at intermediate doses (150–250 mg/m^2) that were given biweekly or every 3 weeks. However, the odds of toxicity at intermediate doses are likely to be within a range acceptable to many patients who do not have other risk factors for neutropenia (e.g., they are not elderly or have not had prior myelotoxic therapy). We suggest that patients and physicians should strongly consider UGT1A1*28 testing for patients with other predictors of irinotecan-induced neutropenia. Alternatively, all patients could initiate therapy at a dose reduction of one level, with doses being increased if toxicity is modest. This approach apparently does not adversely influence outcomes of patients treated with low doses of irinotecan, but clear survival data are not available for high-dose irinotecan regimens (19).

The second potential strategy is to select an irinotecan-containing regimen with a level of toxicity risk that is acceptable to the patient and physician by use of Fig. 2, A, and results of the random effects model for irinotecan as a categorical variable. For irinotecan doses of up to 150 mg/m^2, the absolute risk of severe neutropenia among patients with a UGT1A1*28/*28 genotype is similar to the overall risk for all patients (i.e., ~15%). The absolute

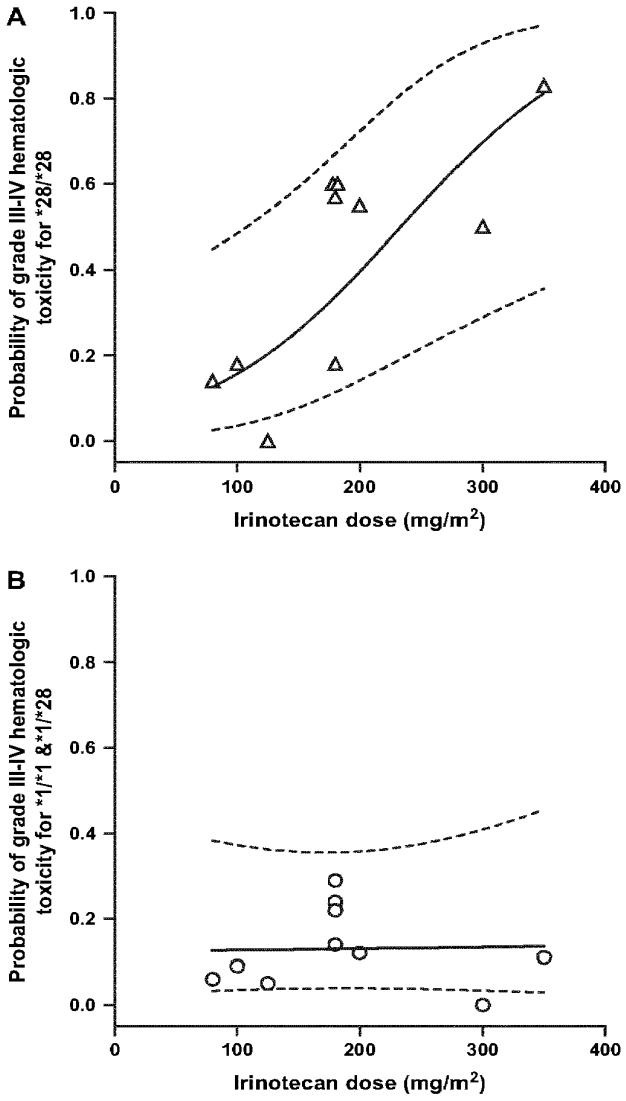

Fig. 2. Relationships between irinotecan dose and3 incidence of hematologic toxicity in patients with a UGT1A1*28/*28 genotype (**A**) and in those with a UGT1A1*1/*1 or UGT1A1*1/*28 genotype (**B**). Results from 10 samples (**triangles** in panel **A** and **circles** in panel **B**) were included in our analysis (3,4,6,7,10–14). **Solid lines** = predicted probabilities of experiencing hematologic toxicity under the generalized linear mixed model at any dose level in the original dose range; **dotted lines** = 95% confidence intervals. For trials in which more than one irinotecan regimen was administered, we considered only the results from patients who received irinotecan at 180 mg/m² biweekly with 5-fluorouracil (6,7). The two different irinotecan-containing regimens in the N9741 trial, which included only grade IV toxicity, were treated as two separate samples in our analyses (14).

risks of toxic effects in these patients increased to 25%–40% for intermediate doses (150–250 mg/m²) and to 50%–70% for higher doses (>250 mg/m²). If patients with a UGT1A1*28/*28 genotype and their physicians are willing to accept the risk of higher levels of toxicity, then the use of more convenient, less frequently administered regimens could be considered. This scenario offers a clear example for the use of patient preference or accepted risk in the selection of a treatment regimen. The greater refinement of the risk of toxicity within a genotype group is also an opportunity for the patient's threshold for risk to help dictate the drug schedule or even specific regimen.

This study has several limitations. We used a meta-analytic approach to combine information from independent trials that had addressed the question whether patients homozygous for the UGT1A1*28 allele have an elevated risk of hematologic toxicity to assess whether the interaction between genotype and toxicity was associated with the administered dose of irinotecan. There were many sources of heterogeneity among the studies, including patient characteristics (e.g., age, ethnicity, sex, performance status, and number of previous chemotherapies), patient eligibility criteria (e.g., type of tumor, stage of disease, and number of previous chemotherapies), treatment schedules (e.g., dose of irinotecan and time between courses and coadministered chemotherapies), and study design (e.g., phase I, phase II, prospective, and retrospective trial). Some sources of heterogeneity (including the stage of tumor, type of tumor, and line of chemotherapy) could have influenced patient participation in a trial and therefore the dose of irinotecan that was received, and other factors (including time between irinotecan doses and coadministered chemotherapies) could have been related to the dose of irinotecan administered by trials. These factors may have directly modulated the association between UGT1A1*28 genotype and irinotecan-related toxicity. Unfortunately, we were unable to assess whether these factors influenced the association between genotype and toxicity among the samples either because of limited power due to the small sample size or because the individual data were not available.

Confounding by genotype error, clinical phenotype, or other variables may also be sources of bias. UGT1A1*28 genotyping was conducted in different laboratories that used different methodologies; however, none of the samples in our study departed from the Hardy–Weinberg equilibrium ($P>.05$; chi-square test). Although this test, which assesses the relationship between the frequency of the UGT1A1*28 allele and UGT1A1*28 genotypes in a population, is not the most sensitive measure of assay reliability, it suggests that genotype error was not a large source of bias. Additionally, the genotyping assays for UGT1A1*28 are not especially prone to errors, suggesting that genotype error is an unlikely source of bias among the studies. In our analysis, grade III–IV neutropenia data (absolute neutrophil count nadir of $<1000 \times 10^6$ cells per L) were available for seven samples (3,4,6,7,10,11,13), whereas only grade IV neutropenia information (14) and grade III–IV hematologic toxicity data (12) could be extracted from the literature for other samples. We treated these clinical events as equivalent, which may have introduced bias into our analysis. Data were extracted from publications for some samples (6,7,10) and obtained via correspondence with authors for other samples (3,4,11–14). Possible data errors reported in publications and others introduced by extracting data from publications could be other sources of bias that were not addressed by our methodology.

A diagnostic test that identifies patients at high risk of dose-limiting toxicities to irinotecan would be clinically useful. Although initial studies (4,6–8) found UGT1A1*28 genotype to be strongly associated with risk of toxicity, results of subsequent studies (10–14) were inconsistent. In our meta-analysis, we found that

the irinotecan dose delivered modulated the association between UGT1A1*28 genotype and irinotecan-induced hematologic toxicity and that the interaction was clinically important only at higher irinotecan doses. At lower irinotecan doses, factors other than UGT1A1*28 genotype, either genetic or nongenetic, are likely to determine a patient's risk of hematologic toxicity, whereas at higher drug doses, UGT1A1*28 genotype appears to be an important determinant. We recommend that the product information for irinotecan be amended to describe the association between irinotecan dose and risk of hematologic toxicity among patients with a UGT1A1*28/*28 genotype. We also favor the development of consensus guidelines by national and regional bodies (e.g., the National Cancer Institute, American Society of Clinical Oncologists, European Society of Medical Oncology, or National Comprehensive Cancer Network) for optimal use of UGT1A1*28 genotype information to prescribe irinotecan doses. Finally, we caution that decisions that are based on only a few events may prove to be misleading. Determining the amount of evidence needed to justify the inclusion of black box warnings on product inserts to safeguard patients is a controversial issue that is worthy of further study.

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Oncology

Phase II study of first-line FOLFIRI for progressive metastatic well-differentiated pancreatic endocrine carcinoma

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ABSTRACT

Background: Pancreatic endocrine carcinomas are rare and heterogeneous. Published results concerning treatment of advanced tumours are inconsistent and responses to standard chemotherapy remain unsatisfactory.
Aim: To investigate the ability of the FOLFIRI regimen to manage progressive unresectable metastatic well-differentiated endocrine carcinomas of the pancreas as first-line chemotherapy.
Methods: 20 patients with metastatic or advanced well-differentiated endocrine carcinomas of the pancreas and progressive disease were enrolled in a prospective multicentre phase II trial to receive chemotherapy with FOLFIRI schedule (irinotecan 180 mg/m^2 infusion combined with simplified LV5FU2) every 14 days. The primary end point was the non-progression rate at 6 months.
Results: The 6-month non-progression rate was 80% (95% confidence interval [56–94%]), with stabilisation in 15 patients and 1 objective response. Overall survival at 24 months was 65% [40–82%]. Median progression-free survival was 9.1 months [6.5–17.3 months]. The median number of administered cycles was 12 [range 1–28]. Grade 3/4 haematologic toxicity occurred in 5 patients (25%) and grade 3 digestive toxicity in 11.
Conclusion: The FOLFIRI regimen, as first-line chemotherapy, achieved stabilisation in most patients whose tumours had been progressing and was well-tolerated. It could be an alternative therapy for advanced well-differentiated endocrine carcinomas of the pancreas.

1. Introduction

Endocrine tumours are rare. Their incidence is 5.3/100,000 inhabitants [1]. These tumours constitute a heterogeneous group in terms of histological characteristics, clinical expression, evolution and prognosis. Histological differentiation, grading and disease stage at diagnosis are the main prognostic factors for survival [1–3].

The treatment of well-differentiated endocrine carcinomas depends on the primary site and tumour burden. Radical surgery is the only curative approach and should be considered for patients with potentially resectable disease, even with metastases [4–7]. For unresectable and progressing well-differentiated endocrine carcinomas of the pancreas, anti-cancer treatments, such as chemotherapy, chemoembolisation or biotherapy are recommended. The classical first-line treatment is based on chemotherapy combining doxorubicin and streptozotocin because of the high response rate (69%) obtained by Moertel et al. [8]. However, their results were not confirmed by later studies [9–11]. Numerous chemotherapies and other treatments (biotherapies, targeted biotherapies, radiotherapy and targeted radionucleide radiotherapy) can been given in this setting and have been included in national and international guidelines (www.tncd.org, www.neuroendocrine.net/guidelines_tnm_classifications.html). The rarity of these tumours make recruitment of homogeneous and sufficiently large cohorts of patients, to achieve adequate statistical power in clinical trials, difficult.

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The FOLFIRI regimen (a combination of irinotecan, 5 fluorouracil and leucovorin) was evaluated by Ducreux et al. in a phase II study on 20 patients with pretreated metastatic well-differentiated endocrine carcinomas (including 10 with primary pancreatic tumours). Tumour control was observed in 16 patients with an objective response in 1 patient [12]. Their results were very encouraging because most of the patients had been heavily pretreated and tolerance was good.

The aim of this prospective, multicentre, open, phase II study was to assess the efficacy and toxicity of the FOLFIRI regimen as first line chemotherapy for patients with unresectable and progressing well-differentiated endocrine carcinomas of the duodenopancreatic area.

2. Patients and methods

The protocol was approved by the Regional Ethics Committee of Champagne-Ardenne on 24 June 2003. The study was registered at clinical trial.gov. with reference NCT00416767. Written informed consent was obtained for all patients.

2.1. Patients

Patients with a histologically confirmed unresectable well-differentiated endocrine carcinoma of the pancreas (functional or not) were eligible. Other inclusion criteria were: age between 18 and 80 years, WHO performance status (PS) ≤ 2, measurable locally advanced (>50 mm for primary tumour and/or lymph-node metastases) or metastatic disease (>15 mm for hepatic or extrahepatic metastases), progressive disease (>20% increase of measurable lesions or appearance of new lesions according to RECIST V1.0 criteria) [13] within the 6 months preceding inclusion. Metastases had to be histologically proven or positive on somatostatin-receptor scintigrams.

Histological diagnosis of well-differentiated endocrine carcinoma was based on the 2000 WHO criteria [14]. The Ki-67 index had to be ≤15% and the mitotic count <10 for 10 high-power fields. These cut-off have been determined before the ENETS grading classification has been published [15]. ENETS TNM classification was also retrospectively applied [15].

Biochemical and haematological laboratory tests had to be adequate to receive chemotherapy: neutrophil count ≥ 1500/mm^3, platelets ≥ 100,000/mm^3, creatinine level ≤ 135 μmol/l and total bilirubin ≤ 30 μmol/l.

Patients had to be naive from: chemotherapy, radiotherapy (external or internal) and/or chemoembolisation. External radiotherapy was an exclusion criteria only if it concerned a target. Interferon had to be stopped 3 months before inclusion but somatostatin analogues were allowed for functional tumours.

Non-inclusion criteria were: poorly differentiated endocrine carcinomas, Gilbert's syndrome, pregnancy and breast feeding.

2.2. Clinical and biological work-up

Four weeks before enrolment, pretreatment evaluation included full medical history and physical examination (weight, body surface area, WHO PS), standard haematological and biochemical analyses and dosages of chromogranin A and biomarkers, depending on the clinical history and presentation (gastrin, insulin, C-peptide, glucagon, vasoactive intestinal peptide, somatostatin, thyrocalcitonin, serotonin) and complete morphological evaluation that included chest and abdominal computed-tomography (CT) scans or magnetic resonance imaging (MRI), and somatostatin-receptor scintigraphy.

2.3. Treatment plan

All patients received FOLFIRI chemotherapy, consisting of irinotecan 180 mg/m^2 infusion on day 1 combined with simplified LV5FU2: a single 2-h infusion of leucovorin 200 mg/m^2 on day 1, followed by a 400-mg/m^2 bolus of 5 fluorouracil, then continuous infusion of 5 fluorouracil 2400 mg/m^2 over 46 h. Cycles were scheduled to be repeated every 14 days using a chemotherapy free-interval scheme.

Forty-eight hours before each chemotherapy cycle, haematological and biochemical analyses, physical examination, including body surface area (weight), and WHO PS were done. All toxicities were also assessed using the National Cancer Institute–common toxicity criteria NCI–CTC version 2.0 (available, http://ctep.cancer.gov). Severe adverse events were also recorded within 24 h of their onset.

Treatment was to be stopped when grade 3/4 toxicity persisted after dose reduction or after 3 weeks without treatment because of toxicity, the tumour progressed under chemotherapy, or withdrawal of consent.

When the tumour stabilised, FOLFIRI was prolonged for another 3 months, then a treatment break could be allowed when stabilisation was confirmed and it lasted until progression. During the treatment break, the tumour response was evaluated every 3 months. If the tumour progressed during the chemotherapy free-interval (treatment break), FOLFIRI could be reintroduced and repeated until any toxicity appeared or progression. For a partial response, treatment was continued until stabilisation or progression.

Concomitant supportive and toxicity-preventive treatments (corticosteroids, setrons, atropine and loperamide, haematopoietic-stimulating factors) were allowed.

2.4. Dose adjustment

Treatment adjustment was done as follow: at the first episode of grade 3/4 toxicity, treatment was interrupted until regression ≤ grade 2 for haematological toxicity and to grade 0 for gastrointestinal toxicity. Then chemotherapy was pursued with a 20% reduction of the original dose. A second episode of any grade 3/4 toxicity led to a 50% reduction of the original dose. Treatment was definitively stopped if a third episode grade 3/4 toxicity occurred. No dose escalation was permitted. Treatment was stopped if the patient did not recover grade 2 toxicity or within 3 weeks after the planned date of chemotherapy administration.

2.5. Follow-up assessment

The tumour responses were evaluated every 3 months including MRI or CT-scan measurements of the target lesions and were classified according to RECIST v1.0 criteria [13].

The relevant biomarkers were measured every 3 months if they had been elevated at baseline. Biological complete response was defined as normalisation of chromogranin A or other elevated biomarkers levels, partial response as >50% reduction, stabilisation as variation from 25% to 50%, and progression as a >25% increase.

2.6. Statistical methods

All analyses were performed according to intent-to-treat for all included patients, regardless of eligibility criteria and treatment received. The primary end point was the non-progression rate at 6 months defined as the number of patients free of progression 6 months after treatment initiation. Secondary end points included the tumour and biological responses at 6, 12, 18, and 24

Table 1
Characteristics of the 20 patients with metastatic well-differentiated pancreatic endocrine carcinoma.

Characteristics	Value
Age (mean/SD) years	58.1 (12.3)
Men, *n* (%)	13 (65)
WHO performance status ≤2, *n* (%)	18 (90)
Metastase sites, *n* (%)	
Liver	19 (95)
Lungs	1 (5)
Lymph nodes	6 (30)
Peritoneum	1 (5)
Bones	3 (15)
Other	0 (0)
Functional tumour, *n* (%)	5 (25)
MEN 1, *n* (%)	2 (10)
Prior treatment, *n* (%)	
Chemotherapy	0 (0)
Chemoembolisation	0 (0)
External radiotherapy	1 (5)
Surgery	7 (35)
Somatostatin analogues	5 (25)
KI67 ≥ 15%, *n* (%)	3 (15)
Chromogranin A > normal, *n* (%)	12 (60)

SD: standard deviation, MEN 1: multiple endocrine neoplasia type 1.

months, progression-free survival (PFS), time-to-treatment failure (TTF), disease duration control, overall survival (OS) and safety.

PFS was defined as the time from inclusion until the date of first progression or death (any cause); TTF was defined as the time from inclusion until definitive treatment discontinuation because of progression, toxicity or other reasons; disease duration control was defined as the time interval between response or stability and 1st progression or tumour-related death, OS was defined as the time from inclusion until the date of death (any cause) or the last follow-up visit for a surviving patient. Progression rates were reported using frequency and percent with its 95% confidence intervals (CI). Continuous variables are given as using means ± standard deviation (SD) or medians (range). Survival times were estimated using the Kaplan–Meier method and described as medians [95% CI].

Follow-up was calculated using reverse Kaplan–Meier estimation and reported as medians.

Twenty patients had to be enrolled to use Fleming one-step design (5% unilateral alpha type-one error and 80% power) and the following hypotheses: H0 a non-progression rate at 6 months of 60% is no improvement and H1 a non-progression rate at 6 months of 85% is expected [16]. Fleming's decision rules were the following: if we observed 15 or fewer progression-free patients at 6 months, the treatment will be declared not an improvement; if we observed 16 or more progression-free patients at 6 months, the treatment will be declared promising.

Kurskal–Wallis was used to estimate the distribution of the Ki-67 index according to the best response.

3. Results

3.1. Patient's characteristics

A total of 20 patients from 6 French hospitals were included in the study between May 2004 and July 2005. Median follow-up was 31 months (95% CI 29–35). Patient characteristics at inclusion are summarised in Table 1. All were stage IV according to the TNM ENETS classification. The Ki 67 staining was available for 16 patients (insufficient amount of material in 4) and was ≤ 15% in 13 patients. Tumour was non-functional in 15 patients.

Four patients did not meet the major eligibility criteria: 3 patients had Ki-67 index >15% and 1 patient had received prior radiotherapy to the primary tumour, although that lesion was not used as a measurable target.

Table 2
Maximal grade toxicity observed during FOLFIRI chemotherapy (NCI-CTC version 2.0).

Toxicity	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Overall	0	0	4 (20)	10 (50)	6 (30)
Haematological	3 (15)	4 (20)	8 (40)	2 (10)	3 (15)
Leucopenia	11 (55)	5 (25)	3 (15)	3 (15)	0
Neutropenia	6 (30)	5 (25)	4 (20)	2 (10)	3 (15)
Febrile neutropenia	19 (95)	0	1 (5)	0	0
Non haematological	0	2 (10)	5 (25)	10 (50)	3 (15)
Nausea	9 (45)	4 (20)	3 (15)	4 (20)	0
Vomiting	10 (50)	1 (5)	7 (35)	2 (10)	0
Mucositis	14 (70)	5 (25)	0	1 (5)	0

Results are reported as number of patients (%).

3.2. Treatment and its toxicity

All patients received at least 1 chemotherapy cycle. The median number of cycles was 12 [range 1–28]. Median treatment time was 5.3 [range 0–23] months. Eight patients had at least 1 treatment break (6 had 1 and 2 had 2 breaks). During chemotherapy, the WHO PS remained ≤ 2 for all the patients.

A majority of the patients (80%) experienced grade 3/4 toxicity during treatment (Table 2). However, only 6 (30%) patients had grade 4 toxicity: neutropenia for 3, thrombosis for 1, pain for 1 and rhabdomyolysis for 1; 1 patient had grade 2 febrile neutropenia. Treatment was stopped because of toxicity for 4 patients (20%), and dose was reduced for 5 (25%). Global haematologic grade 3–4 toxicity was 25%. No toxic death was recorded. Eight patients developed severe adverse events: pulmonary embolism for 2, melena and rhabdomyolysis for 1, severe diarrhoea with dehydratation for 2, diabetes decompensation for 2, and gastric perforation requiring surgery for 1.

3.3. Response and survival

The non-progression rate at 6 months was 80% (95% CI: 56–94%): stabilisation for 15 patients, objective response for 1, and disease progression in the remaining 4 (Table 3). Then 16 patients were free of progression at 6 months, meaning that regarding Fleming's decision rules, the non-progression rate was significantly higher than the H0 hypothesis of no improvement at 60% ($P = 0.05$).

The 24-month post-inclusion OS was 65% [40–82%] (Fig. 1), the median PFS was 9.1 [6.5–17.3] months (Fig. 2), the median TTF was 6.5 [3.1–15.5] months, the median disease control duration was 8.6 [3.0–24.8] months. It was calculated for 13 of the 20 patients because 2 patient's disease did not progress, 4 patients had tumour progression at the first evaluation, and the last patient's first tumour evaluation was made at 30 months of follow-up.

Twelve of the 16 patients with progressive disease received second-line therapy: chemotherapy for 9, and somatostatin analogue, chemoembolisation or Lipiocis treatment, for 1 each.

Table 3
Tumour responses according to time after starting treatment.

Date of evaluation	Objective response	Stable disease	Progression
6 months	1 (5%)	15 (75)	4 (20)
12 months	0	9 (45)	11 (55)
18 months	0	5 (25)	15 (75)
24 months	0	4 (20)	16 (80)

Results are expressed as number of patients (%).

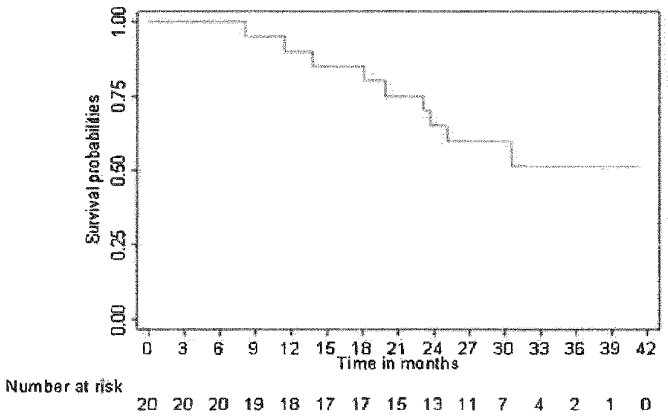

Fig. 1. Overall survival (Kaplan–Meier method).

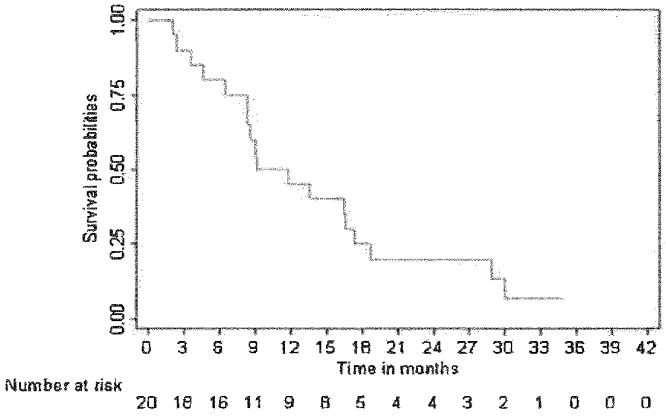

Fig. 2. Progression free survival (Kaplan–Meier method).

At the data cut-off, amongst the 20 patients, 9 died with progression, 9 were alive with progression and 2 were alive without progression.

Chromogranin A levels at baseline were elevated in 12 patients. Partial biological responses were observed in 3, progression in 9 and stability in 4. Radiological responses did not parallel the biological responses. The Ki-67 index, determined for 16 patients, was not predictive of tumour response since Ki-67 distribution did not differ according to the best response during treatment (Kruskal–Wallis $P = 0.29$).

4. Discussion

Progressive unresectable pancreatic NETs have limited treatment options and at the time this study was conducted no chemotherapy has proven high response rate. Moreover data on PFS, duration of stabilisation and OS are heterogeneous when they are available. Thus comparisons of data from these studies with data from the current study are difficult. Although our study included a small number of patients, it was prospective, all patients had histological proven well-differentiated endocrine carcinoma of the pancreas, all were chemotherapy-naive and all had documented disease progression according to the RECIST criteria during the six months preceding enrolment. In previously published studies, these criteria were rarely taken into account.

Our study reached its primary end point: non-progression rate at 6 months was 80% (56–94). Non progression rate is a marker of efficacy because stabililisation leads to improve survival [17–19].

The FOLFIRI regimen has been evaluated in only one previous study to treat endocrine tumours of various primary lesions in pretreated patients [12]. Both studies recorded only 1 objective response amongst their 20 enrolled patients. This result might be considered disappointing compared to the standard chemotherapy regimen (doxorubicin–streptozotocin) with 2 studies that found high response rates (69% and 36%) [8,9], although 2 others showed only 6% objective response rates [10,11], and compared to recent studies with other regimens that gave very enthusiastic results. The combination capecitabine–temozolomide as first-line chemotherapy in patients with advanced neuroendocrine pancreatic tumours gave 70% objective response rates and 27% stabilisation [20]. Two other recent studies performed in patients with different types of advanced neuroendocrine tumours with 5 fluorouracil–cisplatin–streptozotocine [21] and 5 fluorouracil–dacarbazine–epirubicin [22] showed 89–95% tumour control rates in the subgroup of patients with pancreatic tumours, with 38% and 58% objective response rates, respectively.

In our study the PFS was 9.1 months, longer than Ducreux et al.'s [12] (5 months) and within the range obtained with doxorubicin–streptozotocin (from 3.9 months [11] to 15.0 months [9]), but lower than that obtained with other recently evaluated regimens: 17 months to 18 months with 5 fluorouracil–doxorubicin–streptozotocin [23], 5 fluorouracil–dacarbazine–epirubicin [22] and capecitabine–temozolomide [20].

Two recent large studies showed that the targeted therapies sunitinib and everolimus significantly increased PFS as compared to placebo in patients with advanced well-differentiated endocrine carcinomas of the pancreas [19,24]. Although the comparison of our data with the latter studies is debatable, FOLFIRI gave similar results for PFS, 9.1 months versus 11.4 months [19] and 11 months [24], respectively.

Haematological toxicity, especially grade 3/4 neutropenia (25%) was similar to haematological toxicity recorded with doxorubicin–streptozotocin by Delaunoit et al. [9] (24%) and less than that observed in patients given the same regimen for colon cancer [25] (60%) and in the study by Ducreux et al. [12] (40%), but digestive toxicity was similar. We can speculate that the haematological tolerability was better because our patients did not receive chemotherapy previously. Toxicity of targeted therapies is very different [19,24]. The FOLFIRI regimen is easy to use: half-day hospitalisation versus 5 days for the doxorubicin–streptozotocin regimen, and does not lead to renal and cardiac toxicities. Pertinently, the toxicity profile, particularly haematological toxicity, and FOLFIRI regimen efficacy could be improved by the determination of the drug-metabolising enzyme uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) polymorphism [26].

FOLFIRI regimen efficacy against colon cancer has been improved by combining it with bevacizumab [27]. Endocrine tumours are known to be highly vascular and to overexpress vascular endothelium growth factor (VEGF) [28,29]. So the rationale for using VEGF-pathway inhibitors to treat endocrine carcinomas is logical. Some encouraging results have also been obtained with bevacizumab against carcinoid tumours [30]. The combination of FOLFIRI regimen and bevacizumab should be tested against unresectable metastatic endocrine carcinomas, as it has been done with other chemotherapies with encouraging results [31,32].

Our patients had few treatment breaks, perhaps because of the low rate of objective responses or because these time-outs were a novel concept in chemotherapy and had not been applied by investigators.

The main weaknesses of the study concerns Ki67 indexes. It was not possible to measure it in 4 patients because of low amount of material and it was above the predetermined threshold in 3 patients. We cannot exclude that it might have influenced results. However, all tumours were well-differentiated. Ki67 has not been measured or taken into account in the most recently published studies on chemotherapy [20] and on targeted therapies [19,24], probably because its determination has been considered a standard only recently [15]. Furthermore the low amount of tissue material is a frequent drawback. Five patients previously received somatostatin analogues. It has been recently shown in the randomised placebo-controlled PROMID study that octreotide has a significant antitumour effect for well-differentiated endocrine carcinomas of the intestine [33]. Although this effect might also exist for well-differentiated endocrine tumours of the pancreas, it is much improbable that it has influenced the results of our study.

In conclusion, the FOLFIRI regimen induced stabilisation in most patients with progressive, chemotherapy-naive, well-differentiated endocrine carcinomas of the pancreas but only 1 objective response. It can be done on ambulatory hospitalisation. The toxicity profile can be improved by determination of the drug metabolising enzyme. This regimen could be an alternative to other chemotherapies or targeted therapies as a first-line therapy should the other drugs be contraindicated. Combination of targeted therapies and chemotherapies should be further evaluated, FOLFIRI regimen could be a good option.

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Conflict of interest statement

Hedia Brixi-Benmansour, Jean-Louis Jouve, Franck Bonnetain, Bruno Landi, Olivia Hentic, Laurent Bedenne: no conflict of interest that could be perceived as prejudicing the impartiality of the research reported; Guillaume Cadiot and Emmanuel Mitry: fees from Pfizer.

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ORIGINAL ARTICLE

Phase I and pharmacokinetic study of IHL-305 (PEGylated liposomal irinotecan) in patients with advanced solid tumors

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Abstract
Purpose IHL-305 is a novel PEGylated liposome containing irinotecan. This study examined the safety profile and pharmacokinetics of IHL-305 and established the maximum tolerated dose and recommended phase II dose (RP2D).
Patients and methods In a standard 3 + 3 design, IHL-305 was administered IV on day 1 of a 28-day treatment schedule. Subsequently, a 14-day treatment schedule was also explored. Two patient populations were evaluated separately: Patients with at least one wild-type (*wt*) allele of UGT1A1 (UDP glucoronosyltransferase 1A1) *wt/wt* or *wt/*28* as one group (referred to as UGT1A1 *wt* group) and patients with UGT1A1*28 homozygous variant (*28/*28) as another group.
Results Sixty patients were treated: 42 on the 28-day schedule and 18 on the 14-day schedule. Seven patients were homozygous variant (*28/*28). In the UGT1A1 *wt* group, the MTD and RP2D of IHL-305 was 160 mg/m^2 every 28 days and 80 mg/m^2 every 14 days. DLTs included nausea, vomiting, diarrhea, and neutropenia. The most common adverse events were nausea (75 %), vomiting (52 %), diarrhea (62 %), anorexia (57 %), and fatigue (57 %). At the MTD for both schedules, IHL-305 administration resulted in a high and prolonged exposure of sum total irinotecan, released irinotecan, and SN-38 in plasma. One partial response was observed in a patient with breast cancer and eight patients had stable disease for >6 months.
Conclusions IHL-305, a novel preparation of irinotecan encapsulated in liposomes, can be safely given to patients in a repeated fashion on a 4- or 2-week dosing schedule.

Keywords PEGylated liposomal irinotecan · IHL-305 · Phase I · Pharmacokinetic

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Introduction

Irinotecan is a DNA topoisomerase I inhibitor that is FDA approved for the treatment of colon cancer [1]. In order to be clinically effective, irinotecan must be converted to its active metabolite SN-38, which is then converted via UGT1A1 conjugation to its inactive metabolite SN-38 glucuronide (SN-38G) [2, 3]. Biotransformation of SN-38 to SN-38G is protective against gastrointestinal toxicity following irinotecan administration. Previous studies of every 3-week irinotecan demonstrated that patients with the homozygous UGT1A1*28 variant have a higher risk for severe neutropenia due to reduced conversion of SN-38 to SN-38G [4, 5].

IHL-305, a PEGylated liposome containing irinotecan, was developed to achieve improved bioavailability and

antitumor activity. Liposome preparations are selectively transported to tumor tissues due to the effect of enhanced permeability and retention (EPR) and their blood retention time is prolonged [6].

This first-in-human study determined the dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) of IHL-305 in UGT1A1 *wt* patients. Other objectives included evaluation of the pharmacokinetics, antitumor activity, and the potential impact of UGT1A1 genotype on the incidence and severity of adverse events.

Patients and methods

All patients provided written informed consent. The study was approved by the independent ethics committee for each site, and was conducted in accordance with the Declaration of Helsinki. The study was registered with the clinical trials registry (NCT00364143).

Patient selection

Eligibility included the following: histologically confirmed solid tumor with no known regimen of higher efficacy available; Eastern Cooperative Oncology Group (ECOG) performance status of 0–2; normal organ and marrow function; no chemotherapy within 4 weeks; no prior irinotecan; no known brain metastases. Patients with significant cardiac disease, a history of serious ventricular arrhythmias, or a left ventricular ejection fraction (LVEF) $\leq$40 % were excluded.

Study design and treatments

The protocol was designed to determine the DLT and MTD of IHL-305 administered every 28 days in either homozygous wild type (*wt/wt*) or heterozygous variant (*wt/*28*) UGT1A1*28 genotype patients (referred to as UGT1A1 *wt* group). Homozygous variant patients were enrolled at reduced dosages and were not included in the MTD determination. Once the MTD for the 28-day schedule was established, the protocol was amended to change the dosing interval to 14 days and the MTD was re-determined.

A 3 + 3 dose escalation scheme was utilized with dose doubling until $\geq$1 patient experienced a grade 2 toxicity during cycle 1; thereafter, doses were increased in 33 % increments. UGT1A1 genotype testing was performed prior to enrollment. At least three evaluable non-*28/*28 genotype patients were treated at each dose level. Homozygote variant patients were treated at 50 % of the current dose with the option to escalate to 75 and 100 % if $\leq$grade 1 toxicity occurred. The recommended phase II dose was defined as the highest dose level at which $\leq$1 out of 6 UGT1A1 *wt* group patients experienced DLT.

The starting dose was 7 mg/m^2, which corresponded to 1/6 of the highest non-severely toxic dose in dogs (2 mg/kg). IHL-305 was administered as a 60-min infusion. Initially, no antiemetic premedications were given, but the protocol was amended to require premedications at doses >67 mg/m^2.

Assessments

UGT1A1 genotyping, electrocardiogram assessments, and laboratory assessments were performed at baseline. Physical exams were performed on day 1 of every cycle. Laboratory assessments were repeated weekly. Electrocardiograms were obtained prior to the start of infusion, at the infusion midpoint, immediately after and 2 h after the infusion during all cycles. Disease assessments were repeated every 8 weeks. Response Evaluation Criteria in Solid Tumors (RECIST 1.0) criteria were used to assess response.

Safety and tolerability

Toxicity was graded according to National Cancer Institute Common Toxicity Criteria, version 3.0 and acute DLT was determined during the first 28 days. Dose-limiting toxicity was defined as: grade 4 hematologic toxicity lasting $\geq$5 days; grade 3 or 4 febrile neutropenia; grade 4 thrombocytopenia; $\geq$grade 3 non-hematologic toxicities; prolonged QTc >500 ms; or any toxicity resulting in a treatment delay >1 week. If a patient experienced a DLT, the patient was treated at the next lower dose in subsequent cycles.

Pharmacokinetic study design and analytical studies

Heparinized blood samples were collected at the following timepoints: predose, end of infusion, 0.5, 1, 2, 4, 8, 12, and 24 h after the infusion. Once a DLT was observed, additional samples were collected 48, 72, 96, and 192 h after the infusion. Each sample was centrifuged at 3,000×*g* for 15 min at 4 °C. The plasma samples were divided into two aliquots for analysis of released irinotecan, SN-38, SN-38G, APC, and NPC, and for analysis of total irinotecan. The irinotecan total (lactone + hydroxyl acid) form of sum total (encapsulated + released) irinotecan, released irinotecan, SN-38, SN-38G, APC, and NPC concentrations were measured by high-performance liquid chromatography (HPLC) with fluorescence detection.

In the 28-day cohort, urine specimens were collected at the following timepoints: predose, 0–4, 4–8, 8–12, and 12–24 h after the start of infusion. Once a DLT was

observed, samples were also collected during the 24–48, 48–72, and 72–96-h interval after the start of infusion. The total volume of urine collected was recorded and a 1-ml sample was processed and frozen for analysis using the above HPLC assay.

Pharmacokinetic analyses

The pharmacokinetic analyses were performed using non-compartmental methods. The area under the plasma concentration versus time curve (AUC) was calculated for sum total irinotecan, released irinotecan, SN-38, SN-38G, and APC. The percent irinotecan released was calculated as [(irinotecan released AUC/irinotecan sum total AUC) × 100].

Results

Patient characteristics and disposition

Between January 2007 and November 2009, 62 patients were enrolled. Two patients never received treatment and are not included in the analysis. Sixty patients were treated: 42 on the 28-day and 18 on the 14-day schedule. Table 1 describes the patient characteristics for the UGT1A1 *wt* group patients enrolled on both schedules as well as the homozygous variant patients.

Escalation, DLT, and MTD

Thirty-six UGT1A1 *wt* group patients were enrolled across 10 dose levels ranging from 7 to 210 mg/m^2 every 28 days, Table 2. No DLTs were encountered until the 67 mg/m^2 dose level. At this dose level, one patient experienced grade 3 nausea and vomiting, and the dose level was expanded to six with no additional DLTs. All subsequent patients received prophylactic antiemetics. At 160 mg/m^2, one patient experienced grade 3 diarrhea resulting in expansion to six patients, but no additional DLTs were reported. Dose-limiting toxicities consisting of febrile neutropenia and grade 3 nausea/vomiting were reported in two patients enrolled at 210 mg/m^2, respectively. The MTD was exceeded and the RP2D was declared to be 160 mg/m^2 every 28 days.

The starting dose for the 14-day schedule was 80 mg/m^2, Table 2. One of the three patients at the initial dose experienced a 2-week treatment delay due to prolonged neutropenia, which by definition was a DLT. As a result, three additional patients were enrolled with no additional DLTs. The dose was escalated to 105 mg/m^2 and no DLTs were reported. Five patients were enrolled at the highest dose, as two were inevaluable for escalation due to rapidly progressing disease. Dose-limiting toxicities consisting of grade 3 fatigue, nausea, vomiting, and diarrhea in one patient and grade 4 neutropenia in combination with significant grade 2 diarrhea were reported in two of the three evaluable patients at 140 mg/m^2 which exceeded the MTD. The previous dose level of 105 mg/m^2 was expanded to six and DLTs (grade 3 nausea, fatigue, and anorexia as well as grade 2 fatigue warranting a 2-week treatment delay) were encountered in two patients, which exceeded the MTD. As a result, the initial dose of 80 mg/m^2 was the RP2D for the 14-day schedule.

Seven UGT1A1 **28/*28* patients were enrolled at various dose levels throughout the study. No DLTs were reported in this group. Four of the six patients treated on the 28-day schedule were removed from study at the end of cycle 1 due to disease progression (three patients) or patient request (one patient). The other two patients, who initiated treatment at 7 and 80 mg/m^2, were subsequently able to escalate IHL-305 and remained on study for six cycles each. The single UGT1A1 **28/*28* patient on the 14-day schedule received four cycles at 40 mg/m^2, but was unable to dose escalate due to grade 2 toxicities. Overall, the toxicity profile did not appear different among the UGT1A1 **28/*28* patients compared with the UGT1A1 *wt* group.

Safety and tolerability

The total number of 28-day cycles administered was 142: median two cycles/patient (range, 1–12). Nine patients (21 %) received ≥6 cycles, six (14 %) required dose reductions, and eight (19 %) required dose delays of 1–2 weeks. The total number of 14-day cycles administered was 102: median three cycles/patient (range, 1–25). Three patients (17 %) received ≥12 cycles, five (28 %) required dose reductions, and eight (44 %), including the one homozygous variant patient, required dose delays of 1–3 weeks. One patient on the 28-day schedule was switched to the 14-day schedule after 12 cycles and received an additional 10 cycles.

Table 3 describes the treatment-related toxicities for all patients, as well as divided by genotype and dosing schedule. Gastrointestinal toxicities were reported most commonly, were predominantly mild to moderate in intensity, and occurred in a slightly higher percentage of patients on the 14-day schedule. These toxicities were dose limiting in five patients. Other dose-limiting non-hematologic toxicities included fatigue and anorexia. Hematologic toxicities occurred in less than one-third of patients, but febrile neutropenia and neutropenia warranting dose reductions were dose limiting in three patients. One patient on the 28-day schedule experienced a grade 2 hypersensitivity reaction during the initial infusion, but was able to continue treatment following antihistamine and steroid administration. The patient was premedicated with steroids, H1 and

Table 1 Demographic characteristics

Demographic characteristic	UGT1A1 *wt* group (*wt/wt* and *wt/*28*) Every 28 days $N = 36$	UGT1A1 *wt* group (*wt/wt* and *wt/*28*) Every 14 days $N = 17$	Homozygous UGT1A1*28 variant (**28/*28*) Both schedules $N = 7$
Age (years)			
Median	60 (41–75)	52 (35–79)	56 (42–65)
<50	8	7	1
50–69	20	7	6
70+	8	3	0
Gender			
Female	25	12	5
Male	11	5	2
Ethnic origin			
White	31	15	4
Black	4	2	3
Hispanic	1	0	0
ECOG performance status			
0	23	12	3
1	12	5	3
2	1	0	1
Prior chemotherapy			
None	1	0	1
1–3 regimens	12	13	3
≥4 regimens	23	4	3
Prior radiation therapy	17	7	7
Tumor type			
Ovary	8	1	0
Breast	7	3	1
Lung (NSCLC/SCLC)	5/0	1/1	1/1
Unknown primary	0	4	0
Pancreatic	0	3	0
Neuroendocrine	3	0	1
Bladder	2	0	0
Colorectal	2	1	0
Prostate	2	0	0
Head and neck	2	1	1
Other	5	2	2
UGT1A1 genotype			
Homozygous wild type	25	10	0
Heterozygous wild type	12	7	0
Homozygous variant	0	0	7

H2 antagonists for all subsequent cycles with no further incidents.

Efficacy

Sixty patients were treated. One partial response was reported in a metastatic breast cancer patient previously treated with five prior chemotherapy regimens. The patient received 20 cycles on the 14-day regimen and remained on study for 9.5 months. Twenty-four patients (40 %) experienced stable disease as their best response. Twenty-nine patients (48 %) experienced progressive disease at the first disease assessment. Six patients were inevaluable for response.

Table 2 Dose escalation schemas (28- and 14-day dosing) for UGT1A1*28 *wt* group

Level	IHL-305 dose (mg/m^2)	Number of patients	Number of cycles[a]	Dose-limiting toxicities (cycle 1 only)
Part I every 28-day dosing				
1	7	3	6	None
2	14	3	11	None
3	28	3	5	None
4	37	3	9	None
5	50	3	9	None
6	67	6	29	1 (grade 3 nausea/vomiting)
7	88	3	18	None
8	120	4	17	None
9	160	6	34	1 (grade 3 diarrhea)
10	210	2	2	2 (febrile neutropenia; grade 3 nausea/vomiting)
Part II every 14-day dosing				
1	80	6	56	1 (Gr 2 neutropenia >2 weeks causing dose delay)
2	105	6	43	2 (Gr 3 anorexia, fatigue, nausea; >2 week treatment delay due to grade 2 fatigue)
3	140	5	8	2 (Gr 3 nausea, vomiting diarrhea; grade 4 neutropenia requiring dose reduction)

[a] Total includes cycles that were administered to patients requiring dose reductions. Three patients in part II received a total of four cycles at a reduced dose of 60 mg/m^2 and one patient received a single cycle at a dose of 52.5 mg/m^2. One patient in part I received two cycles at 90 mg/m^2

Table 3 Treatment-related toxicities for all patients by genotype and schedule ($N = 60$)

Toxicity	UGT1A1 *wt* group ($N = 36$) (*wt/wt* and *wt/*28*) Every 28-day dosing		UGT1A1 *wt* group ($N = 17$) (*wt/wt* and *wt/*28*) Every 14-day dosing		Homozygous UGT1A1*28 variant (*28/*28) ($N = 7$) Both dosing schedules		All patients ($N = 60$)
	Grade 1–2 *n* (%)	Grade 3–4 *n* (%)	Grade 1–2 *n* (%)	Grade 3–4 *n* (%)	Grade 1–2 *n* (%)	Grade 3–4 *n* (%)	Overall *n* (%)
Hematologic toxicities							
Neutropenia	0	2 (6 %)	2 (12 %)	3 (18 %)	0	1(14 %)	8 (13 %)
Febrile neutropenia	0	1 (3 %)	0	0	0	0	1 (2 %)
Anemia	5 (14 %)	4 (11 %)	7 (41 %)	0	3 (43 %)	0	19 (32 %)
Thrombocytopenia	0	0	0	0	0	0	0
Non-hematologic toxicities							
Nausea	21 (58 %)	6 (17 %)	12 (71 %)	2 (12 %)	4 (57 %)	0	45 (75 %)
Diarrhea	20 (56 %)	1 (3 %)	12 (71 %)	1 (6 %)	3 (43 %)	0	37 (62 %)
Vomiting	11 (31 %)	5 (14 %)	8 (47 %)	2(12 %)	5 (72 %)	0	31 (52 %)
Constipation	8 (22 %)	0	3 (18 %)	0	2 (29 %)	0	13 (22 %)
Fatigue	20 (56 %)	1 (3 %)	9 (53 %)	2 (12 %)	2 (29 %)	0	34 (57 %)
Peripheral edema	10 (28 %)	1 (3 %)	1 (6 %)	0	0	0	12 (20 %)
Anorexia	13 (36 %)	8 (22 %)	7 (41 %)	2 (12 %)	3 (43 %)	1 (14 %)	34 (57 %)
Dehydration	3 (8 %)	1 (3 %)	4 (24 %)	0	0	0	8 (13 %)
Alopecia	5 (14 %)	–	3 (18 %)	–	1 (14 %)	–	9 (15 %)

Pharmacokinetics

The mean concentration versus time plot for sum total irinotecan, released irinotecan, SN-38, SN-38G, and APC for the RP2D of each schedule is presented in Fig. 1. There was prolonged exposure of sum total irinotecan, released irinotecan, SN-38, SN-38G, and APC in plasma from 0 to 96 h in both schedules. The sum total form of irinotecan,

which primarily represents the encapsulated form, was 1,000–10,000-fold higher in plasma compared with released irinotecan and other metabolites.

The AUC for sum total irinotecan, released irinotecan, SN-38, SN-38G, and APC for both schedules is presented in Table 4. The inter-patient variability in the pharmacokinetics of sum total and released irinotecan was approximately two- to threefold. The mean percent of irinotecan measured in the plasma as related to sum total irinotecan ranged from 0.35 to 0.95 %. The conversion of released irinotecan to SN-38 and APC and the conversion of SN-38 to SN-38G were similar to after administration of non-liposomal irinotecan [7]. The plasma exposures of sum total and released irinotecan are relatively dose proportional; however, the small number of patients at each dose level and the inter-patient variability make this hard to evaluate.

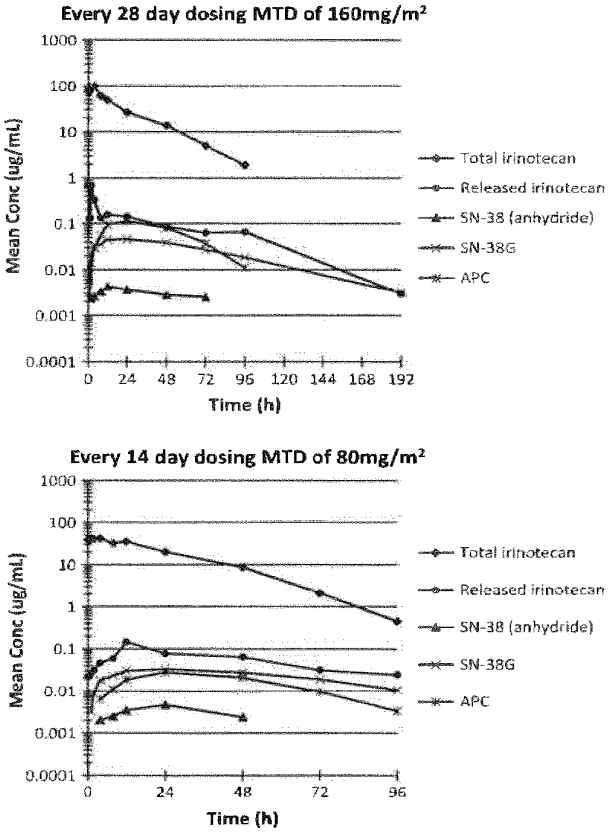

Fig. 1 Mean concentration versus time plot total irinotecan, released irinotecan, SN-38, and SN-38G at MTD for 28-day dosing (160 mg/m^2) and 14-day dosing (80 mg/m^2). NPC concentrations in plasma were below the lower limit of detection

Table 4 PK parameters for IHL-305 administered every 28 and 14 days

	Every 28-day dosing						Every 14-day dosing		
	67 mg/m^2 (0–192 h) $N = 3$	88 mg/m^2 (0–192 h) $N = 2$	120 mg/m^2 (0–192 h) $N = 4$	160 mg/m^2 (0–192 h) $N = 6$	210 mg/m^2 (0–192 h) $N = 2$	80 mg/m^2 (0–96 h) $N = 6$	105 mg/m^2 (0–96 h) $N = 6$	140 mg/m^2 (0–96 h) $N = 5$
Total irinotecan AUC_{0-t}(min–max) (h μg/ml) [mean ± SD, range]	1,250 ± 333 (974–1,620)	1,680 (1,640–1,710)	1,910 ± 824 (1,030–2,970)	2,180 ± 859 (1,370–2,870)	3,570 (1,790–5,340)	1,300 ± 364 (989–1,850)	1,810 ± 603 (1,170–2,570)	2,250 ± 1,016 (1,500–4,020)
Released irinotecan AUC_{0-t} (min–max) (h ng/ml) [mean ± SD, range]	4,380 ± 1,363 (2,910–4,640)	4,460 (2,780–6,130)	13,500 ± 12,865 (4,250–32,100)	13,800 ± 10,457 (5,930–34,700)	7,370 (7,320–7,410)	5,660 ± 3,374 (2,690–11,900)	12,500 ± 16,948 (3,910–46,600)	17,100 ± 14,979 (8,240–42,500)
SN-38 AUC_{0-t}(min–max) (h ng/ml) [mean ± SD, range]	203 ± 98 (117–310)	151 (151)	212 ± 276 (48.8–531)	245 ± 223 (46.0–661)	261 (261)	228 ± 187 (72.3–544)	312 ± 134 (237–529)	478 ± 306 (129–897)
SN-38G AUC_{0-t}(min–max) (h ng/ml) [mean ± SD, range]	2,920 ± 2,484 (1,410–5,790)	1,160 (949–1,370)	3,560 ± 2,092 (1,700–5,640)	4,100 ± 1,690 (1,720–5,910)	2,680 (1,300–4,050)	2,270 ± 1,918 (1,170–6,020)	2,960 ± 3,263 (726–9,470)	6,770 ± 7,878 (3,510–20,700)
APC AUC_{0-t}(min–max) (h ng/ml) [mean ± SD, range]	1,660 ± 719 (1,240–2,490)	1,190 (361–2,020)	2,380 ± 1,015 (1,480–3,670)	6,390 ± 7,804 (1,810–22,000)	1,090 (711–1,470)	1,490 ± 1,802 (522–4,510)	2,730–4,940 (311–12,800)	6,650 ± 5,657 (353–15,800)
Irinotecan released (%) [mean ± SD]	0.35 ± 0.11	0.26 ± 0.13	1.6 ± 0.21	0.67 ± 0.44	0.27 ± 0.2	0.45 ± 0.27	0.95 ± 1.50	0.72 ± 0.42

NPC concentrations in plasma were below the lower limit of detection

The total percent of all forms excreted in the urine over 96 h following IHL-305 administration ranged from 7.6 to 22 %.

Discussion

This first-in-human study evaluated IHL-305, a novel PEGylated liposome containing irinotecan, in patients with advanced solid tumors. In the UGT1A1 *wt* group, the MTD and RP2D of IHL-305 was 160 mg/m^2 every 28 days. When administered every 14 days, the MTD and RP2D is 80 mg/m^2.

Gastrointestinal treatment emergent adverse events were the dominant non-hematologic toxicities, occurring in 83 % of patients, and were grade 3/4 in 12 % of patients irrespective of dose and schedule. Gastrointestinal events also accounted for three of the four DLTs on the 28-day regimen, and one DLT on the 14-day regimen. Though difficult to distinguish the relationship to treatment in advanced cancer patients, fatigue, and anorexia were also observed commonly, sometimes at grade 3–4 levels. As expected with a topoisomerase-1 inhibitor, neutropenia accounted for the remaining dose-limiting toxicities. Interestingly, neutropenia only occurred in 13 % of all patients. Thrombocytopenia was not observed. These data suggest that the liposomal formulation results in predominant GI toxicities and less myelosuppression as compared with non-liposomal irinotecan [1]. Patients with the homozygous UGT1A1*28 variant had similar tolerability of IHL-305, though they were initially treated at half the dose of the UGT1A1 *wt* group. The most frequently reported adverse events remained gastrointestinal disorders (83 %) and no grade ≥3 adverse events were reported.

The patient population included patients with multiple prior therapies from a variety of tumor types. Among the 54 patients with restaging scans, there was one confirmed partial response despite all patients being irinotecan naive. This patient was treated at 140 mg/m^2 in the 14-day cohort (later reduced to 105 mg/m^2). Eight patients (13 %) had stable disease >6 months.

Administration of IHL-305 results in a high and prolonged exposure of sum total irinotecan, released irinotecan, SN-38, and other metabolites. In addition, the release of irinotecan from IHL-305 in plasma is low (<1 %). These results are consistent with other nanoparticle and liposomal formulations of camptothecin analogues and other anticancer agents [8–11]. The inter-patient variability in the sum total irinotecan was significantly lower after IHL-305 (two- to threefold) compared with administration of other PEGylated liposomal agents and nanoparticle agents [8–11]. The factors associated with the lower inter-patient variability in the IHL-305 pharmacokinetics are unknown. The conversion of released irinotecan to SN-38 is similar to the conversion following non-liposomal irinotecan. Thus, administration of IHL-305 does not appear to alter the pharmacokinetics of irinotecan and metabolites once the drug is released from the liposome. However, the exposure of sum total and released irinotecan and SN-38 after IHL-305 is significantly prolonged compared with non-liposomal irinotecan [7]. The prolonged exposure of irinotecan and SN-38 is expected to lead to an increased exposure of SN-38 in tumor. Theoretically, the delivery of encapsulated and released irinotecan to the tumor may result in intra-tumoral conversion of released irinotecan to SN-38; however, proving this in patients has been difficult.

IHL-305 can be safely given to patients in a repeated fashion using an every 4- or 2-week schedule. The liposome formulation resulted in a high and prolonged exposure of both sum total irinotecan and the active metabolite SN-38. Gastrointestinal side effects and neutropenia defined the MTD, but were manageable. The limited responses may be due to the heavily pretreated patient population and the majority of patients being treated at doses below the RP2D. Randomized trials are needed to evaluate if IHL-305 offers clinical benefit compared with non-liposomal irinotecan.

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EUROPEAN
COMMISSION

Bruxelles, 14.10.2016
C(2016) 6778 (final)

COMMISSION IMPLEMENTING DECISION

of 14.10.2016

granting marketing authorisation under Regulation (EC) No 726/2004 of the European Parliament and of the Council for "Onivyde - irinotecan", an orphan medicinal product for human use

(Text with EEA relevance)

(ONLY THE GERMAN TEXT IS AUTHENTIC)

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(Text with EEA relevance)

(ONLY THE GERMAN TEXT IS AUTHENTIC)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency[1], and in particular Article 10(2) thereof,

Having regard to Regulation (EC) No 141/2000 of the European Parliament and of the Council of 16 December 1999 on orphan medicinal products, and in particular Article 5(12) thereof,

Having regard to the application submitted by Baxalta Innovations GmbH, on 28 May 2015, under Article 4(1) of Regulation (EC) No 726/2004,

Having regard to the opinions of the European Medicines Agency, formulated on 21 July 2016 by the Committee for Medicinal Products for Human Use and on 8 September 2016 by the Committee for Orphan Medicinal Products,

Whereas:

(1) Commission Decision C(2011)9419(final), adopted in accordance with Regulation (EC) No 141/2000 of the European Parliament and of the Council of 16 December 1999 on orphan medicinal products[2] designated "Nanoliposomal irinotecan" as an orphan medicinal product.

(2) The orphan medicinal product "Onivyde - irinotecan" complies with the requirements set out in Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use[3] .

(3) It is therefore appropriate to authorise its placing on the market.

(4) The measures provided for in this Decision are in accordance with the opinion of the Standing Committee on Medicinal Products for Human Use,

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1 OJ L 136, 30.4.2004, p. 1.
2 OJ L 18, 22.1.2000, p. 1.
3 OJ L 311, 28.11.2001, p. 67.

HAS ADOPTED THIS DECISION:

Article 1

The marketing authorisation provided for in Article 3 of Regulation (EC) No 726/2004 is granted for the orphan medicinal product "Onivyde - irinotecan", the characteristics of which are summarised in Annex I to this Decision. "Onivyde - irinotecan" shall be registered in the Community register of medicinal products under number EU/1/16/1130.

Article 2

The marketing authorisation concerning the medicinal product referred to in Article 1 shall be subject to compliance with the conditions set out in Annex II and, in particular, with those relating to manufacture and importation, control and issue.

Article 3

The labelling and package leaflet concerning the orphan medicinal product referred to in Article 1 shall conform to Annex III.

Article 4

The period of validity of the authorisation shall be five years from the date of notification of this Decision.

Article 5

This Decision is addressed to Baxalta Innovations GmbH, Industriestrasse 67, A-1221 Wien, Österreich.

Done at Brussels, 14.10.2016

For the Commission

Xavier PRATS MONNÉ
Director-General

ANNEX I

SUMMARY OF PRODUCT CHARACTERISTICS

1. NAME OF THE MEDICINAL PRODUCT

D10

ONIVYDE 5 mg/ml concentrate for solution for infusion

2. QUALITATIVE AND QUANTITATIVE COMPOSITION

One 10 ml vial of concentrate contains the equivalent of 50 mg irinotecan hydrochloride trihydrate (as irinotecan sucrosofate salt in a pegylated liposomal formulation) which corresponds to 43 mg irinotecan.

One ml of concentrate contains the equivalent of 5 mg irinotecan hydrochloride trihydrate (as irinotecan sucrosofate salt in a pegylated liposomal formulation) which corresponds to 4.3 mg irinotecan.

Excipient with known effect

One ml of concentrate contains 0.144 mmol (3.31 mg) sodium.
For the full list of excipients, see section 6.1.

3. PHARMACEUTICAL FORM

Concentrate for solution for infusion.
White to slightly yellow opaque isotonic liposomal dispersion.
The concentrate has a pH of 7.2 and an osmolality of 295 mOsm/kg.

4. CLINICAL PARTICULARS

4.1 Therapeutic indications

Treatment of metastatic adenocarcinoma of the pancreas, in combination with 5-fluorouracil (5-FU) and leucovorin (LV), in adult patients who have progressed following gemcitabine based therapy.

4.2 Posology and method of administration

ONIVYDE (liposomal irinotecan) must only be prescribed and administered to patients by healthcare professionals experienced in the use of anti-cancer therapies.

ONIVYDE (liposomal irinotecan) is not equivalent to non-liposomal irinotecan formulations and should not be interchanged.

Posology

ONIVYDE, leucovorin and 5-fluorouracil should be administered sequentially. The recommended dose and regimen of ONIVYDE is 80 mg/m^2 intravenously over 90 minutes, followed by LV 400 mg/m^2 intravenously over 30 minutes, followed by 5-FU 2,400 mg/m^2 intravenously over 46 hours, administered every 2 weeks. ONIVYDE should not be administered as a single agent.

A reduced starting dose of ONIVYDE (liposomal irinotecan) of 60 mg/ m^2 should be considered for patients known to be homozygous for the UGT1A1*28 allele (see sections 4.8 and 5.1). A dose increase of ONIVYDE to 80 mg/m^2 should be considered if tolerated in subsequent cycles.

Pre-medication

It is recommended that patients receive pre-medication with standard doses of dexamethasone (or an equivalent corticosteroid) together with a 5-HT_3 antagonist (or other antiemetic) at least 30 minutes prior to ONIVYDE infusion.

Dosage adjustments

All dose modifications should be based on the worst preceding toxicity. LV dose does not require adjustment. For Grade 1 and 2 toxicities there are no dose modifications recommended. Dose adjustments, as summarised in Table 1 and Table 2, are recommended to manage Grade 3 or 4 toxicities related to ONIVYDE.

For patients who start treatment with 60 mg/m^2 ONIVYDE and do not dose escalate to 80 mg/m^2, the recommended first dose reduction is to 50 mg/m^2 and the second dose reduction is to 40 mg/m^2. Patients who require further dose reduction should discontinue treatment.

Patients who are known to be homozygous for UGT1A1*28 and without drug related toxicities during the first cycle of therapy (reduced dose of 60 mg/m^2) may have the dose of ONIVYDE increased to a total dose of 80 mg/m^2 in subsequent cycles based on individual patient tolerance.

Table 1: Recommended dose modifications for ONIVYDE+5-FU/LV for Grade 3-4 toxicities for patients not homozygous for UGT1A1*28

Toxicity grade (value) by NCI CTCAE v 4.0[1]	**ONIVYDE/5-FU adjustment (for patients not homozygous for UGT1A1*28)**	
Haematological toxicities		
Neutropenia	A new cycle of therapy should not begin until the absolute neutrophil count is ≥ 1500/mm^3	
Grade 3 or Grade 4 (< 1000/mm^3) **or Neutropenic fever**	***First occurrence***	Reduce ONIVYDE dose to 60 mg/m^2 Reduce 5-FU dose by 25% (1800 mg/m^2).
	Second occurrence	Reduce ONIVYDE dose to 50 mg/m^2 Reduce 5-FU dose by an additional 25% (1350 mg/m^2).
	Third occurrence	Discontinue treatment
Thrombocytopenia **Leukopenia**	A new cycle of therapy should not begin until the platelet count is ≥ 100,000/mm^3 Dose modifications for leukopenia and thrombocytopenia are based on NCI CTCAE toxicity grading and are the same as recommended for neutropenia above.	
Nonhaematological toxicities[2]		
Diarrhoea	A new cycle of therapy should not begin until diarrhoea resolves to ≤ Grade 1 (2-3 stools/day more than pre-treatment frequency).	
Grade 2	A new cycle of therapy should not begin until diarrhoea resolves to ≤ Grade 1 (2-3 stools/day more than pre-treatment frequency).	

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Toxicity grade (value) by NCI CTCAE v 4.0[1]	**ONIVYDE/5-FU adjustment (for patients not homozygous for UGT1A1*28)**	
Grade 3 or 4	***First occurrence***	Reduce ONIVYDE dose to 60 mg/m^2 Reduce 5-FU dose by 25% (1800 mg/m^2)
	Second occurrence	Reduce ONIVYDE dose to 50 mg/m^2 Reduce 5-FU dose by an additional 25% (1350 mg/m^2)
	Third occurrence	Discontinue treatment
Nausea/vomiting	A new cycle of therapy should not begin until nausea/vomiting resolves to ≤ Grade 1 or baseline	
Grade 3 or 4 *(despite antiemetic therapy)*	***First occurrence***	Optimise antiemetic therapy Reduce ONIVYDE dose to 60 mg/m^2
	Second occurrence	Optimise antiemetic therapy Reduce ONIVYDE dose to 50 mg/m^2
	Third occurrence	Discontinue treatment
Hepatic, renal, respiratory or other[2] toxicities ***Grade 3 or 4***	A new cycle of therapy should not begin until the adverse reaction resolves to ≤ Grade 1	
	First occurrence	Reduce ONIVYDE dose to 60 mg/m^2 Reduce 5-FU dose by 25% (1800 mg/m^2)
	Second occurrence	Reduce ONIVYDE dose to 50 mg/m^2 Reduce 5-FU dose by an additional 25% (1350 mg/m^2)
	Third occurrence	Discontinue treatment
Anaphylactic reaction	***First occurrence***	Discontinue treatment

[1] NCI CTCAE v 4.0 = National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0

[2] Excludes asthenia and anorexia; Asthenia and Grade 3 anorexia do not require dose adjustment.

Table 2: Recommended dose modifications for ONIVYDE +5-FU/LV for Grade 3-4 toxicities in patients homozygous for UGT1A1*28

Toxicity grade (value) by NCI CTCAE v 4.0[1]	**ONIVYDE/5-FU adjustment (for patients homozygous for UGT1A1*28 without previous increase to 80 mg/m^2)**	
Adverse reactions[2] ***Grade 3 or 4***	A new cycle of therapy should not begin until adverse event resolves to ≤ Grade 1	
	First occurrence	Reduce ONIVYDE dose to 50 mg/m^2 5-FU dose modification as in Table 1
	Second occurrence	Reduce ONIVYDE dose to 40 mg/m^2 5-FU dose modification as in Table 1
	Third occurrence	Discontinue treatment

[1] NCI CTCAE v 4.0 = National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0

[2] Excludes asthenia and anorexia; asthenia and Grade 3 anorexia do not require dose adjustment.

Hepatic impairment
No dedicated hepatic impairment study has been conducted with ONIVYDE. The use of ONIVYDE should be avoided in patients with bilirubin > 2.0 mg/dl, or aspartate aminotransferase (AST) and alanine aminotransferase (ALT) > 2.5 times upper limit of normal (ULN) or > 5 times ULN if liver metastasis is present (see section 4.4).

Renal impairment
No dedicated renal impairment study has been conducted with ONIVYDE. No dose adjustment is recommended in patients with mild to moderate renal impairment (see sections 4.4 and 5.2). ONIVYDE is not recommended for use in patients with severe renal impairment (CLcr < 30 ml/min).

Elderly
Forty-one percent (41%) of patients treated with ONIVYDE across the clinical program were ≥ 65 years. No dose adjustment is recommended.

Paediatric population
The safety and efficacy of ONIVYDE in children and adolescents aged ≤ 18 years have not yet been established. No data are available.

Method of administration

ONIVYDE is for intravenous use. The concentrate must be diluted prior to administration and given as single intravenous infusion over 90 minutes. For more details see section 6.6.

Precautions to be taken before handling or administering the medicinal product
ONIVYDE is a cytotoxic medicinal product. The use of gloves, goggles and protective clothing when handling or administering ONIVYDE is recommended. Pregnant staff should not handle ONIVYDE.

4.3 Contraindications

History of severe hypersensitivity to irinotecan or to any of the excipients listed in section 6.1.

Breast-feeding (see section 4.6).

4.4 Special warnings and precautions for use

General

ONIVYDE is a liposomal formulation of irinotecan with different pharmacokinetic properties compared to non-liposomal irinotecan. The dose concentration and strength are different in comparison to non-liposomal irinotecans.

ONIVYDE is not equivalent to other non-liposomal irinotecan formulations and should not be interchanged.

In the limited number of patients with prior exposure to non-liposomal irinotecan, no benefit of ONIVYDE has been demonstrated.

Myelosuppression/neutropenia

Complete blood cell count monitoring is recommended during ONIVYDE treatment. Patients should be aware of the risk of neutropenia and the significance of fever. The median time to nadir for ≥ Grade 3 neutropenia is 23 (range 8-104) days post first dose of treatment with ONIVYDE. Febrile neutropenia (body temperature > 38°C and neutrophil count≤ 1,000 cells/mm³) should be urgently treated in the hospital with broad-spectrum intravenous antibiotics. ONIVYDE should be

withheld if neutropenic fever occurs or the absolute neutrophil count drops below 1500/mm^3. Sepsis with neutropenic fever and consequent septic shock with fatal outcome has been observed in patients with metastatic pancreatic adenocarcinoma treated with ONIVYDE.
In patients who experienced severe haematological events, a dose reduction or treatment discontinuation is recommended (see section 4.2). Patients with severe bone marrow failure should not be treated with ONIVYDE.
History of prior abdominal radiation increases the risk of severe neutropenia and febrile neutropenia following ONIVYDE treatment. Close monitoring of blood counts is recommended, and the use of myeloid growth factors should be considered for patients with a history of abdominal radiation. Caution should be exercised in patients receiving concurrent administration of ONIVYDE with irradiation.

Patients with deficient glucuronidation of bilirubin, such as those with Gilbert's syndrome, may be at greater risk of myelosuppression when receiving therapy with ONIVYDE.

Compared to Caucasian patients, Asian patients have an increased risk of severe and febrile neutropenia following treatment with ONIVYDE+5-FU/LV (see sections 4.8 and 5.2).

Immunosuppressive effects and vaccines

Administration of live or live-attenuated vaccines in patients immunocompromised by chemotherapeutic medicinal products including ONIVYDE may result in serious or fatal infections; therefore vaccination with a live vaccine should be avoided. Killed or inactivated vaccines may be administered; however, the response to such vaccines may be diminished.

Interactions with strong CYP3A4 inducers

ONIVYDE should not be administered with strong CYP3A4-enzyme inducers such as anticonvulsants (phenytoin, phenobarbital or carbamazepine), rifampin, rifabutin and St. John's wort unless there are no therapeutic alternatives. The appropriate starting dose for patients taking these anticonvulsants or other strong inducers has not been defined. Consideration should be given to substituting with non-enzyme inducing therapies at least 2 weeks prior to initiation of ONIVYDE therapy (see section 4.5).

Interactions with strong CYP3A4 inhibitors or strong UGT1A1 inhibitors

ONIVYDE should not be administered with strong CYP3A4-enzyme inhibitors (e.g. grapefruit juice, clarithromycin, indinavir, itraconazole, lopinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telaprevir, voriconazole). Strong CYP3A4 inhibitors should be discontinued at least 1 week prior to starting ONIVYDE therapy.
ONIVYDE should not be administered with strong UGT1A inhibitors (e.g. atazanavir, gemfibrozil, indinavir) unless there are no therapeutic alternatives.

Diarrhoea

Diarrhoea can occur early (onset in ≤ 24 hours after starting ONIVYDE) or late (> 24 hours) (see section 4.8).
In patients experiencing early diarrhea, therapeutic and prophylactic atropine should be considered unless contraindicated. Patients should be made aware of the risk of delayed diarrhoea which can be debilitating and, on rare occasions, life threatening since persistent loose or watery stools can result in dehydration, electrolyte imbalance, colitis, gastrointestinal (GI) ulceration, infection or sepsis.
As soon as the first liquid stool occurs, the patient should start drinking large volumes of beverages containing electrolytes. Patients should have loperamide (or equivalent) readily available to begin treatment for late diarrhoea. Loperamide should be initiated at first occurrence of poorly formed or loose stools or at the earliest onset of bowel movements more frequent than normal. Loperamide should be given until patient is without diarrhoea for at least 12 hours.

If diarrhoea persists while patient is on loperamide for more than 24 hours, adding oral antibiotic support (e.g. fluoroquinolone for 7 days) should be considered. Loperamide should not be used for more than 48 consecutive hours due to risk of paralytic ileus. If diarrhoea persists for more than 48 hours, stop loperamide, monitor and replace fluid electrolytes and continue antibiotic support until resolution for accompanying symptoms.
ONIVYDE treatment should be delayed until diarrhoea resolves to ≤ Grade 1 (2-3 stools/day more than pre-treatment frequency). ONIVYDE must not be administered to patients with bowel obstruction, and chronic inflammatory bowel disease, until it is resolved.
Following Grade 3 or 4 diarrhoea, the subsequent dose of ONIVYDE should be reduced, (see section 4.2).

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Cholinergic reactions

Early onset diarrhoea may be accompanied by cholinergic symptoms such as rhinitis, increased salivation, flushing, diaphoresis, bradycardia, miosis and hyperperistalsis. In case of cholinergic symptoms atropine should be administered.

Acute infusion and related reactions

Infusion reactions primarily consisting of rash, urticaria, periorbital oedema or pruritus were reported in patients receiving ONIVYDE treatment. New events (all grade 1 or grade 2) occurred generally early during ONIVYDE treatment, with only 2 out of 10 patients noted with events after the fifth dose. Hypersensitivity reactions, including acute infusion reaction may occur. ONIVYDE should be discontinued in case of severe hypersensitivitiy reactions.

Prior Whipple procedure

Patients with a history of a Whipple procedure have a higher risk of serious infections following ONIVYDE in combination with 5-FU and leucovorin (see section 4.8). Patients should be monitored for signs of infections.

Pulmonary toxicity

Interstitial Lung Disease (ILD)-like events leading to fatalities have occurred in patients receiving non-liposomal irinotecan. No cases of ILD-like events have been reported with ONIVYDE therapy in clinical studies. Risk factors include pre-existing lung disease, use of pneumotoxic medicinal products, colony stimulating factors or having previously received radiation therapy. Patients with risk factors should be closely monitored for respiratory symptoms before and during ONIVYDE therapy. A reticulo-nodular pattern on chest X-ray was observed in a small percentage of patients enrolled in a clinical study with irinotecan. New or progressive dyspnoea, cough, and fever should prompt interruption of ONIVYDE treatment, pending diagnostic evaluation. ONIVYDE should be discontinued in patients with a confirmed diagnosis of ILD.

Hepatic impairment

Patients with hyperbilirubinaemia had higher concentrations for total SN-38 (see section 5.2) and therefore the risk of neutropenia is increased. Regular monitoring of complete blood counts should be conducted in patients with total bilirubin of 1.0-2.0 mg/dl. Caution should be exercised in patients with hepatic impairment (bilirubin > 2 times upper limit of normal [ULN]; transaminases > 5 times ULN). Caution is required when ONIVYDE is given in combination with other hepatotoxic medicinal products, especially in patients with pre-existing hepatic impairment.

Renal impairment

The use of ONIVYDE in patients with significant renal impairment has not been established (see section 5.2).

Underweight patients (body mass index < 18.5 kg/m^2)

In the clinical study evaluating ONIVYDE+5-FU/LV, 5 of 8 underweight patients experienced a Grade 3 or 4 adverse reactions, mostly myelosuppression, while 7 of the 8 patients required dose modification such as dose delay, dose reduction or dose discontinuation. Caution should be exercised when using ONIVYDE in patients with body mass index <18.5 kg/m^2.

Excipients

Each ml of ONIVYDE contains 0.144 mmol (3.31 mg) sodium. This needs to be taken into consideration by patients on a controlled sodium diet.

4.5 Interaction with other medicinal products and other forms of interaction

Information about drug interactions with ONIVYDE is referenced from the published scientific literature for nonliposomal irinotecan.

Interaction affecting the use of ONIVYDE

Strong CYP3A4 inducers
Patients receiving concomitant non-liposomal irinotecan and CYP3A4 enzyme-inducing anticonvulsants phenytoin, phenobarbital or carbamazepine have substantially reduced exposure to irinotecan (AUC reduction by 12% with St John's wort, 57%-79% with phenytoin, phenobarbital, or carbamazepine) and SN-38 (AUC reduction by 42% with St John's wort, 36%-92% with phenytoin phenobarbital, or carbamazepine). Therefore, co-administration of ONIVYDE with inducers of CYP3A4 may reduce systemic exposure of ONIVYDE.

Strong CYP3A4 inhibitors and UGT1A1 inhibitors
Patients receiving concomitant non-liposomal irinotecan and ketoconazole, a CYP3A4 and UGT1A1 inhibitor, have increased SN-38 exposure by 109%. Therefore, co-administration of ONIVYDE with other inhibitors of CYP3A4 (e.g. grapefruit juice, clarithromycin, indinavir, itraconazole, lopinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telaprevir, voriconazole) may increase systemic exposure of ONIVYDE. Based on the drug interaction of non-liposomal irinotecan and ketoconazole, co-administration of ONIVYDE with other inhibitors of UGT1A1 (e.g. atazanavir, gemfibrozil, indinavir) may also increase systemic exposure of ONIVYDE.

Co-administration of ONIVYDE+5-FU/LV does not alter the pharmacokinetics of ONIVYDE based on the population pharmacokinetic analysis.

No interaction of ONIVYDE (liposomal irinotecan) with other medicinal products is known.

4.6 Fertility, pregnancy and lactation

Women of childbearing potential / contraception in males and females

Women of childbearing potential should use effective contraception during ONIVYDE treatment and 1 month thereafter. Males should use condoms during ONIVYDE treatment and 4 months thereafter.

Pregnancy

There are no adequate data on the use of ONIVYDE in pregnant women. ONIVYDE can cause harm to the foetus when administered to the pregnant woman, as the main ingredient irinotecan has been shown to be embryotoxic and teratogenic in animals (see section 5.3). Therefore, based on results from animal studies and the mechanism of action of irinotecan, ONIVYDE should not be used during pregnancy unless clearly necessary. If ONIVYDE is used during pregnancy or if the patient becomes

pregnant while receiving therapy, the patient should be informed about the potential hazard to the foetus. D10

Breast-feeding

It is unknown whether ONIVYDE or its metabolites are excreted into human milk. Because of the potential for serious adverse reactions of ONIVYDE in breast-feeding infants, ONIVYDE is contraindicated during breast-feeding (see section 4.3). Patients should not breast-feed until one month after the last dose.

Fertility

There are no data on the impact of ONIVYDE on human fertility. Non-liposomal irinotecan was shown to cause atrophy of male and female reproductive organs after multiple daily irinotecan doses in animals (see section 5.3).

4.7 Effects on ability to drive and use machines

ONIVYDE has moderate influence on the ability to drive and use machines. During treatment patients should observe caution when driving or using machines.

4.8 Undesirable effects

Summary of the safety profile

The following adverse reactions, considered to be possibly or probably related to the administration of ONIVYDE, were reported in 264 patients with metastatic adenocarcinoma of the pancreas, 147 of whom received ONIVYDE monotherapy (120 mg/m^2) and 117 received ONIVYDE (80 mg/m^2) in combination with 5-FU/LV.
The most common adverse reactions (incidence ≥ 20%) of ONIVYDE+5FU/LV were: diarrhoea, nausea, vomiting, decreased appetite, neutropenia, fatigue, asthenia, anaemia, stomatitis and pyrexia.
The most common serious adverse reactions (≥ 2%) of ONIVYDE therapy were diarrhoea, vomiting, febrile neutropenia, nausea, pyrexia, sepsis, dehydration, septic shock, pneumonia, acute renal failure, and thrombocytopenia.
The rates of adverse reactions leading to permanent treatment discontinuation were 11% for the ONIVYDE+5-FU/LV arm and 12% for the monotherapy arm.
The most frequently reported adverse reactions leading to discontinuation were infection and diarrhoea for ONIVYDE+5-FU/LV arm, and vomiting and diarrhoea for the monotherapy arm.

Tabulated list of adverse reactions

The adverse reactions that may occur during treatment with ONIVYDE are summarised below and are presented by system organ class and frequency category (Table 3). Within each system organ class and frequency category, adverse reactions are presented in order of decreasing seriousness. Frequencies categories used for adverse reactions are: very common (≥ 1/10); common (≥ 1/100 to <1/10); uncommon (≥ 1/1,000 to <1/100) and rare (≥ 1/10,000 to < 1/1,000)**.

Table 3: Adverse reactions reported with ONIVYDE therapy in the NAPOLI-1 clinical study

MedDRA* system organ class	Adverse reaction frequency**
Infections and infestations	*Common:* Septic shock, Sepsis, Pneumonia, Febrile neutropenia, Gastroenteritis, Oral candidiasis *Uncommon:* Biliary sepsis

MedDRA* system organ class	Adverse reaction frequency**
Blood and lymphatic system disorders	*Very common:* Neutropenia, Leukopenia, Anaemia, Thrombocytopenia *Common:* Lymphopenia
Immune system disorders	*Uncommon: Hypersensitivity*
Metabolism and nutrition disorders	*Very common:* Hypokalaemia, Hypomagnesaemia, Dehydration, Decreased appetite *Common:* Hypoglycaemia, Hyponatraemia, Hypophosphataemia
Psychiatric disorders	*Common:* Insomnia
Nervous system disorders	*Very common:* Dizziness *Common:* Cholinergic syndrome, Dysgeusia
Cardiac disorders	*Common:* Hypotension
Vascular disorders	*Common:* Pulmonary embolism, Embolism, Deep vein thrombosis *Uncommon:* Thrombosis
Respiratory, thoracic and mediastinal disorders	*Common:* Dyspnoea, Dysphonia *Uncommon:* Hypoxia
Gastrointestinal disorders	*Very common:* Diarrhoea, Vomiting, Nausea, Abdominal pain, Stomatitis *Common:* Colitis, Haemorrhoids *Uncommon:* Oesophagitis, Proctitis
Hepatobiliary disorders	*Common:* Hypoalbuminaemia
Skin and subcutaneous tissue disorders	*Very common:* Alopecia *Uncommon:* Rash maculo-papular, Nail discolouration
Renal and urinary disorders	*Common:* Acute renal failure
General disorders and administration site conditions	*Very common:* Pyrexia, Peripheral oedema, Mucosal inflammation, Fatigue, Asthenia *Common:* Infusion related reaction, Oedema
Investigations	*Very common:* Weight decrease *Common:* Increased bilirubin, Increased alanine aminotransferase, Increased aspartate aminotransferase, Increased international normalized ratio

* MedDRA version 14.1

** Rare occurrence cannot be estimated from the NAPOLI-1study due to the small sample size

Description of selected adverse reactions

The following adverse reactions were observed in the NAPOLI-1 clinical study:

Myelosuppression

Myelosuppression (neutropenia/leukopenia, thrombocytopenia and, anaemia) was more common in the ONIVYDE+5-FU/LV arm compared to the 5-FU/LV control arm.

Neutropenia/leukopenia
Neutropenia/leukopenia was the most notable important haematological toxicity. Grade 3 or higher neutropenia occurred more frequently in patients treated with ONIVYDE+5-FU/LV (27.4%) compared to patients treated with 5-FU/LV (1.5%). Neutropenic fever/sepsis appeared more frequently in the ONIVYDE+5-FU/LV combination arm [in 4 patients (3.4%)] compared to 5-FU/LV control arm [in 1 patient (0.7%)].

Thrombocytopenia
Grade 3 or higher thrombocytopenia occurred in 2.6% of patients treated with ONIVYDE+5-FU/LV and 0% in patients treated with 5-FU/LV.

Anaemia
Grade 3 or higher anaemia occurred in 10.3% of patients treated with ONIVYDE+5-FU/LV and in 6.7% of patients treated with 5-FU/LV.

Acute renal failure
Renal impairment and acute renal failure have been identified, usually in patients who become volume depleted from nausea/vomiting and/or diarrhoea. Acute renal failure was reported in 6 of 117 patients (5.1%) in the ONIVYDE+5-FU/LV arm, 10 of 147 (6.8%) in the ONIVYDE monotherapy arm and 6 of 134 patients (4.5%) in the 5-FU/LV arm.

Diarrhoea and related adverse reactions
Diarrhoea is a very common adverse reaction leading to colitis, ileus, gastroenteritis, fatigue, dehydration, weight loss, renal toxicities, hyponatraemia, and hypokalaemia. Renal impairment and acute renal failure have been identified, usually in patients who became volume depleted from severe vomiting and/or diarrhoea. In the clinical study Grade 3 or Grade 4 diarrhoea occurred in 15 out of 117 patients (12.8%) receiving ONIVYDE+5-FU/LV. For patients experiencing late diarrhoea, the median time to late diarrhoea onset was 8 days from the previous dose of ONIVYDE. Early onset diarrhoea, typically appearing ≤24 hours after dose administration, can occur and is usually transient. Early onset diarrhoea may also be accompanied by cholinergic symptoms that can include rhinitis, increased salivation, flushing, diaphoresis, bradycardia, miosis and hyperperistalsis that can induce abdominal cramping. In the clinical study, early diarrhoea onset occurred in 35 patients (29.9%) and cholinergic events occurred in 4 patients (3.4%) receiving ONIVYDE+5-FU/LV.
Withhold ONIVYDE for Grade 2-4 diarrhoea and initiate treatment for diarrhoea. Following recovery to Grade 1 diarrhoea, resume ONIVYDE at a reduced dose (see section 4.2).

Infusion reaction
Acute infusion reactions were reported in 8 of 117 patients (6.8%) in the ONIVYDE+5-FU/LV arm, 3 of 147 patients (2.0%) in the ONIVYDE monotherapy arm, and 8 of 134 patients (6.0%) in the 5-FU/LV arm.

Other special populations

Elderly
Overall, no major clinical differences in safety or efficacy were reported between patients ≥ 65 years and patients < 65 years, although a higher frequency of discontinuation (14.8% vs 7.9%) was noted in the former group treated with ONIVYDE+5-FU/LV in the NAPOLI-1 study and in some cases the adverse reactions did not resolve. Grade 3 or higher and serious treatment emergent adverse reactions were more frequent in patients < 65 years (84.1% and 50.8%) compared to patients ≥ 65 years (68.5 % and 44.4%). Conversely, patients > 75 years (n=12) experienced more frequent serious adverse reactions, dose delay, dose reduction and discontinuation compared to patients ≤ 75 years (n=105) when treated with ONIVYDE+5-FU/LV in the pancreatic adenocarcinoma study.

Asian population
Compared to Caucasians, Asian patients were observed with a lower incidence of diarrhoea [14 (19.2%) out of 73 Caucasians had a ≥ Grade 3 diarrhoea, and 1 out of 33 (3.3%) Asians had a

≥ Grade 3 diarrhoea], but a higher incidence and higher severity of neutropenia. In patients receiving ONIVYDE+5-FU/LV, the incidence of ≥ Grade 3 neutropenia was higher among Asian patients [18 of 33 (55%)] compared to White patients [13 of 73 (18%)]. Neutropenic fever/neutropenic sepsis was reported in 6% of Asian patients compared to 1% of White patients. This is consistent with the population pharmacokinetic analysis that showed a lower exposure to irinotecan and a higher exposure to its active metabolite SN-38 in Asians than in Caucasians.

Patients with hepatic impairment
In clinical studies of non-liposomal irinotecan administered on a weekly dosage schedule, patients with modestly elevated baseline serum total bilirubin levels (1.0 to 2.0 mg/dl) had a significantly greater likelihood of experiencing first cycle Grade 3 or Grade 4 neutropenia than those with bilirubin levels that were less than 1.0 mg/dl.

Patients with prior Whipple procedure
In the clinical study evaluating ONIVYDE+5-FU/LV, patients with a prior Whipple procedure had a higher risk of serious infections following treatment with ONIVYDE+5-FU/LV [9 of 29 (30%)] compared to 11 of 88 (12.5%) patients with no prior Whipple procedure.

Patients with UGT1A1 allele
Individuals who are 7/7 homozygous for the UGT1A1*28 allele are at increased risk for neutropenia from non-liposomal irinotecan. In the clinical study evaluating ONIVYDE+5-FU/LV, the frequency of ≥ Grade 3 neutropenia in these patients [2 of 7 (28.6%)] was similar to the frequency in patients not homozygous for the UGT1A1*28 allele who received a starting dose of ONIVYDE of 80 mg/m^2 [30 of 110 (27.3%)] (see section 5.1).

Underweight patients (body mass index < 18.5 kg/m^2)
In the clinical study evaluating ONIVYDE+5-FU/LV, 5 of 8 underweight patients experienced a grade 3 or 4 adverse reaction, mostly myelosuppression, while 7 of the 8 patients required dose modification such as dose delay, dose reduction or dose discontinuation (see section 4.4).

Reporting of suspected adverse reactions

Reporting suspected adverse reactions after authorisation of the medicinal product is important. It allows continued monitoring of the benefit/risk balance of the medicinal product. Healthcare professionals are asked to report any suspected adverse reactions via the national reporting system listed in Appendix V.

4.9 Overdose

In clinical trials, ONIVYDE was administered at doses up to 240 mg/m^2 to patients with various cancers. The adverse reactions in these patients were similar to those reported with the recommended dosage and regimen.

There have been reports of overdosage with non-liposomal irinotecan at doses up to approximately twice the recommended therapeutic dose of irinotecan, which may be fatal. The most significant adverse reactions reported were severe neutropenia and severe diarrrhoea.

There is no known antidote for overdose of ONIVYDE. Maximum supportive care should be instituted to prevent dehydration due to diarrhoea and to treat any infectious complications.

5. PHARMACOLOGICAL PROPERTIES

5.1 Pharmacodynamic properties

Pharmacotherapeutic group: antineoplastic agents, other antineoplastic agents, ATC code: L01XX19

Mechanism of action D10

The active substance in ONIVYDE is irinotecan (topoisomerase I inhibitor) encapsulated in a lipid bilayer vesicle or liposome.

Irinotecan is a derivative of camptothecin. Camptothecins act as specific inhibitors of the enzyme DNA topoisomerase I. Irinotecan and its active metabolite SN-38 bind reversibly to the topoisomerase I-DNA complex and induce single-strand DNA lesions which block the DNA replication fork and are responsible for the cytotoxicity. Irinotecan is metabolized by carboxylesterase to SN-38. SN-38 is approximately 1000 times as potent as irinotecan as an inhibitor of topoisomerase I purified from human and rodent tumour cell lines.

Pharmacodynamic effects

In animal models, ONIVYDE has been shown to extend plasma levels of irinotecan and prolong the exposure to the active metabolite SN-38 at the site of the tumour.

Clinical efficacy and safety

The safety and efficacy of ONIVYDE were investigated in a multinational, randomized, open label, controlled clinical trial (NAPOLI–1) that tested two treatment regimens for patients with metastatic pancreatic adenocarcinoma who had documented disease progression after gemcitabine or gemcitabine-containing therapy. The trial was designed to assess the clinical efficacy and safety of ONIVYDE monotherapy or ONIVYDE+5-FU/LV compared to an active control arm of 5-FU/LV.

Patients randomized to ONIVYDE+5-FU/LV received ONIVYDE at 80 mg/m^2 as an intravenous infusion over 90 minutes, followed by LV 400 mg/m^2 intravenously over 30 minutes, followed by 5-FU 2,400 mg/m^2 intravenously over 46 hours, administered every 2 weeks. Patients homozygous for the UGT1A1*28 allele were given a lower initial dose of ONIVYDE (see section 4.2). Patients randomised to 5-FU/LV received leucovorin 200 mg/m^2 intravenously over 30 minutes, followed by 5-FU 2,000 mg/m^2 intravenously over 24 hours, administered on Days 1, 8, 15 and 22 of a 6 week cycle. Patients randomised to ONIVYDE monotherapy received 120 mg/m^2 as an intravenous infusion over 90 minutes every 3 weeks.

Key eligibility criteria for patients with metastatic adenocarcinoma of the pancreas in the NAPOLI-1 clinical study were Karnofsky Performance Status (KPS) $\geq$ 70, normal bilirubin level, transaminase levels $\leq$ 2.5 times the ULN or $\leq$ 5 times the ULN for patients with liver metastases and albumin $\geq$ 3.0 g/dl.

A total of 417 patients were randomised to the ONIVYDE+5-FU/LV arm (N=117), ONIVYDE monotherapy arm (N=151) and 5-FU/LV arm (N=149). Patient demographic and entry disease characteristics were well balanced between trial arms.

In the intent to treat (all randomised) population, the median age was 63 years (range 31-87 years), 57 % were males, and 61% were White and 33% were Asian. Mean baseline albumin level was 3.6 g/dl, and baseline KPS was 90-100 in 55% of patients. Disease characteristics included 68% of patients with liver metastases and 31% with lung metastases; 12% of patients had no prior lines of metastatic therapy, 56 % of patients had 1 prior line of metastatic therapy, 32% of patients had 2 or more prior lines of metastatic therapy.

Patients received treatment until disease progression or unacceptable toxicity. The primary outcome measure was Overall Survival (OS). Additional outcome measures included Progression Free Survival (PFS) and Objective Response Rate (ORR). Results are shown in Table 4. Overall survival is illustrated in Figure 1.

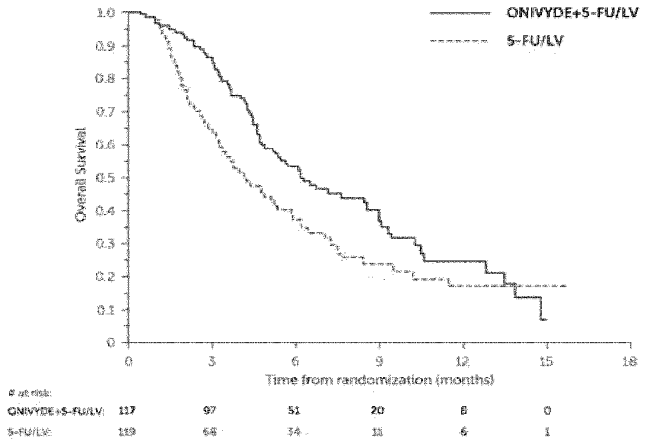
Table 4: Efficacy results from NAPOLI-1 clinical study D10

	ONIVYDE+5-FU/LV (N= 117)	5-FU/LV (N= 119)
Overall Survival[1]		
Number of deaths, n (%)	**75 (64)**	**80 (67)**
Median OS (months)	6.1	4.2
(95% CI)	(4.8, 8.9)	(3.3, 5.3)
Hazard Ratio (95% CI)[3]	0.67 (0.49-0.92)	
p-value[4]	0.0122	
Progression-Free Survival[1,2]		
Death or progression, n (%)	83 (71)	92 (77)
Median PFS (months)	3.1	1.5
(95% CI)	(2.7, 4.2)	(1.4, 1.8)
Hazard Ratio (95% CI)[3]	0.56 (0.41-0.75)	
p-value[4]	0.0001	
Objective Response Rate[2]		
N	19	1
ORR (%)	16.2	0.8
95% CI of Rate[5]	9.6, 22.9	0.0, 2.5
Rate Difference (95% CI)[5]	15.4 (8.5, 22.3)	
p-value[6]	< 0.0001	

1 Median is the Kaplan-Meier estimate of the median survival time
2 Per RECIST guidelines, v 1.1.
3 Cox model analysis
4 Unstratified log-rank test
5 Based on Normal approximation
6 Fisher's exact test

Abbreviations: 5-FU/LV=5-fluorouracil/leucovorin; CI=confidence interval

Figure 1: Overall survival D10



In the limited number of patients with prior exposure to non-liposomal irinotecan, no benefit of ONIVYDE has been demonstrated.

Paediatric population

The European Medicines Agency has waived the obligation to submit the results of studies with ONIVYDE in all subsets of the paediatric population in treatment of adenocarcinoma of the pancreas (see section 4.2 for information on paediatric use).

5.2 Pharmacokinetic properties

Absorption

Liposome encapsulation of irinotecan extends circulation and limits distribution relative to those of the non-liposomal irinotecan.

The plasma pharmacokinetics of total irinotecan and total SN-38 were evaluated in patients with cancer who received ONIVYDE, as a single agent or as part of combination chemotherapy, at doses between 60 and 180 mg/m2. The pharmacokinetic parameters of total irinotecan and SN-38 analytes, following the administration of ONIVYDE 80 mg/m2 are presented in Table 5.

Table 5: Summary of mean (±standard deviation) total irinotecan and total SN-38

Analyte	PK parameters	Unit	ONIVYDE geomean (95% CI)[a] 80 mg/m^2(n=353)[b]	Non-liposomal irinotecan mean (SD) 125 mg/m^2 (n=99)[c]
Total irinotecan	AUC	h ng/ml	919228 (845653-999204)	10529 (3786)
	C_{max}	ng/ml	28353 (27761-28958)	1492 (452)
	Clearance (CL)	l/h/m2	0.087 (0.080-0.094)	13.0 (5.6)
	Volume (V)	l/m2	2.6 (2.6-2.7)	138 (60.9)
	$t_{1/2\ effective}$	h	20.8 (19.4-22.3)	6.07 (1.19)

Analyte	PK parameters	Unit	ONIVYDE geomean (95% CI)[a] 80 mg/m^2(n=353)[b]	Non-liposomal irinotecan mean (SD) 125 mg/m^2 (n=99)[c]
Total SN-38	AUC	h ng/ml	341 (326-358)	267 (115)
	C_{max}	ng/ml	3.0 (2.9-3.1)	27.8 (11.6)
	$t_{1/2\ effective}$	h	40.9 (39.8-42.0)	11.7 (4.29)

SD= standard deviation
AUC= area under the plasma concentration curve (extrapolated to infinity for ONIVYDE and AUC24h for non-liposomal irinotecan)
C_{max}= maximum plasma concentration
$t_{1/2\ effective}$= effective half-lives
[a]Values are estimated from population PK analysis
[b]N=353 refers to all the subjects included in the population PK analysis
[c]Values are obtained from published data [Schaaf LJ et al. *Clin Cancer Res*. 2006 Jun 15;12:3782-91]

Distribution

Direct measurement of liposomal irinotecan shows that 95% of irinotecan remains liposome-encapsulated during circulation. Non-liposomal irinotecan displays a large volume of distribution (138 l/m^2). The volume of distribution of ONIVYDE 80 mg/m^2 was 2.6 l/m^2, which suggests that ONIVYDE is largely confined to vascular fluid.
The plasma protein binding of ONIVYDE is negligible (< 0.44% of total irinotecan in ONIVYDE). The plasma protein binding of non-liposomal irinotecan is moderate (30% to 68%), and SN-38 is highly bound to human plasma proteins (approximately 95%).

Biotransformation

Irinotecan released from liposome encapsulation follows a similar metabolic pathway reported with non-liposomal irinotecan.
The metabolic conversion of irinotecan to the active metabolite SN-38 is mediated by carboxylesterase enzymes. *In vitro* studies indicate that irinotecan, SN-38 and another metabolite aminopentane carboxylic acid (APC) do not inhibit cytochrome P-450 isozymes. SN-38 is subsequently conjugated predominantly by the enzyme UDP-glucuronosyl transferase 1A1 (UGT1A1) to form a glucuronide metabolite. UGT1A1 activity is reduced in individuals with genetic polymorphisms that lead to reduced enzyme activity such as the UGT1A1*28 polymorphism. In the population pharmacokinetic analysis in patients with ONIVYDE using the results of a subset with UGT1A1*28 genotypic testing, in which the analysis adjusted for the lower dose administered to patients homozygous for the UGT1A1*28 allele, patients homozygous (N=14) and non-homozygous (N=244) for this allele had total SN-38 average steady-state concentrations of 1.06 and 0.95 ng/ml, respectively.

Elimination

The disposition of ONIVYDE and non-liposomal irinotecan has not been fully elucidated in humans. The urinary excretion of non-liposomal irinotecan is 11% to 20%; SN-38 <1%; and SN-38 glucuronide is 3%. The cumulative biliary and urinary excretion of irinotecan and its metabolites (SN-38 and SN-38 glucuronide) over a period of 48 hours following administration of irinotecan in two patients ranged from approximately 25% (100 mg/m^2) to 50% (300 mg/m^2).

Renal impairment

No dedicated pharmacokinetic study has been conducted in patients with renal impairment. In a population pharmacokinetic analysis, mild-to-moderate renal impairment had no effect on the exposure of total SN-38 after adjusting for BSA. The analysis included 68 patients with moderate

(CLcr 30-59 ml/min), 147 patients with mild (CLcr 60-89 ml/min) renal impairment, and 135 patients with normal renal function (CLcr > 90 ml/min). There was insufficient data in patients with severe renal impairment (CLcr < 30 ml/min) to assess its effect on pharmacokinetics (see sections 4.2 and 4.4).

Hepatic impairment

No dedicated pharmacokinetic study has been conducted in patients with hepatic impairment. In a population pharmacokinetic analysis, patients with baseline total bilirubin concentrations of 1-2 mg/dl (n=19) had average steady state concentrations for total SN-38 that were increased by 37% (0.98 [95%CI: 0.94-1.02] and 1.29 [95%CI: 1.11-1.5] ng/ml, respectively) compared to patients with baseline bilirubin concentrations of < 1 mg/dl (n=329); however, there was no effect of elevated ALT/AST concentrations on total SN-38 concentrations. No data are available in patients with total bilirubin more than 2 times the ULN.

Other special populations

Age and gender
The population pharmacokinetic analysis in patients aged 28 to 87 years, of whom 11% were ≥75 years suggests that age had no clinically meaningful effect on the exposure to irinotecan and SN-38.
The population pharmacokinetic analysis in 196 male and 157 female patients suggests that gender had no clinically meaningful effect on the exposure to irinotecan and SN-38 after adjusting for body surface area (BSA).

Ethnicity
The population pharmacokinetic analysis suggest that Asians have 56% lower total irinotecan average steady state concentration (3.93 [95%CI: 3.68-4.2] and 1.74 [95%CI: 1.58-1.93] mg/l, respectively) and 8% higher total SN-38 average steady state concentration (0.97 [95%CI: 0.92-1.03] and 1.05 [95%CI: 0.98-1.11] ng/ml, respectively) than Caucasians.

Pharmacokinetic/pharmacodynamic relationship

In a pooled analysis from 353 patients, higher plasma SN-38 C_{max} was associated with increased likelihood of experiencing neutropenia, and higher plasma total irinotecan C_{max} was associated with increased likelihood of experiencing diarrhoea.

In the clinical trial demonstrating effectiveness of ONIVYDE, higher plasma exposures of total irinotecan and SN-38 for patients in the ONIVYDE+5-FU/LV treatment arm were associated with longer OS and PFS as well as with higher ORR (objective response rate).

5.3 Preclinical safety data

In single and repeated dose toxicity studies in mice, rats and dogs, the target organs of toxicity were the gastrointestinal tract and the hematologic system. The severity of effects was dose-related and reversible. The no-observed-adverse-effect level (NOAEL) in rats and dogs following 90 min intravenous infusion of ONIVYDE once every 3 weeks for 18 weeks was at least 180 mg/m^2.
In safety pharmacology studies in dogs, ONIVYDE had no effect on cardiovascular, hemodynamic, electrocardiographic, or respiratory parameters at doses up to 21 mg/kg (420 mg/m^2). No findings indicative of CNS related toxicity were observed in the repeated dose toxicity studies in rats.

Genotoxic and carcinogenic potential

No genotoxicity studies have been performed with ONIVYDE. Non-liposomal irinotecan and SN-38 were genotoxic *in vitro* in the chromosomal aberration test on CHO-cells as well as in the *in vivo* micronucleus test in mice. However, in other studies with irinotecan they have been shown to be devoid of any mutagenic potential in the Ames test.

No carcinogenicity studies have been performed with ONIVYDE. For non-liposomal irinotecan, in rats treated once a week during 13 weeks at the maximum dose of 150 mg/m², no treatment related tumours were reported 91 weeks after the end of treatment. Under these conditions, there was a significant linear trend with dose for the incidence of combined uterine horn endometrial stromal polyps and endometrial stromal sarcomas. Due to its mechanism of action, irinotecan is considered a potential carcinogen.

Reproduction toxicity

No reproductive and developmental toxicity studies have been performed with ONIVYDE.
Non-liposomal irinotecan was teratogenic in rats and rabbits at doses below the human therapeutic dose. In rats, pups born from treated animals and having external abnormalities showed a decrease in fertility. This was not seen in morphologically normal pups. In pregnant rats there was a decrease in placental weight and in the offspring a decrease in foetal viability and increase in behavioural abnormalities.
Non-liposomal irinotecan caused atrophy of male reproductive organs both in rats and dogs after multiple daily doses of 20 mg/kg and 0.4 mg/kg, respectively.These effects were reversible upon cessation of treatment.

6. PHARMACEUTICAL PARTICULARS

6.1 List of excipients

Liposome forming lipids
1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC)
Cholesterol
N-(carbonyl-methoxypolyethylene glycol-2000)-1, 2-distearoly-sn-glycero-3-phosphoethanolamine (MPEG-2000-DSPE)

Other excipients
Sucrose octasulphate
2- [4- (2-Hydroxyethyl)piperazin-1-yl] ethanesulfonic acid (HEPES buffer)
Sodium chloride
Water for injections

6.2 Incompatibilities

ONIVYDE must not be mixed with other medicinal products except those mentioned in section 6.6.

6.3 Shelf life

Unopened vial
30 months.

After dilution
Chemical and physical stability for the diluted solution for infusion has been demonstrated at 15-25°C for up to 6 hours or in the refrigerator (2°C-8°C) for no more than 24 hours.
From a microbiological point of view, the product should be used immediately. If not used immediately, in-use storage times and conditions are the responsibility of the user.

6.4 Special precautions for storage

D10

Store in a refrigerator (2°C-8°C).
Do not freeze.
Keep the vial in the outer carton in order to protect from light.

For storage conditions after dilution of the medicinal product, see section 6.3.

6.5 Nature and contents of container

Type I glass vial with a grey chlorobutyl stopper and an aluminium seal with a flip-off cap, containing 10 ml of concentrate.
Each pack contains one vial.

6.6 Special precautions for disposal and other handling

ONIVYDE is a cytotoxic medicinal product, and caution should be exercised in handling it. The use of gloves, goggles and protective clothing when handling or administering ONIVYDE is recommended. If the solution contacts the skin, the skin should be washed immediately and thoroughly with soap and water. If the solution contacts mucous membranes, they should be flushed thoroughly with water. Pregnant staff should not handle ONIVYDE considering the cytotoxic nature of the medicinal product.

Preparation of the solution and administration

ONIVYDE is supplied as a sterile liposomal dispersion at a concentration of 5 mg/ml and must be diluted prior to administration. Dilute with 5% glucose solution for injection or sodium chloride 9 mg/ml (0.9%) solution for injection to prepare a solution of the appropriate dose of ONIVYDE diluted to a final volume of 500 ml. Mix the diluted solution by gentle inversion. The diluted solution is clear to slightly white to slightly opalescent and free from visible particles.

ONIVYDE should be administered before LV followed by 5-FU. ONIVYDE must not be administered as a bolus injection or an undiluted solution.

Aseptic techniques must be followed during the preparation of the infusion. ONIVYDE is for single use only.

Care should be taken to avoid extravasation, and the infusion site should be monitored for signs of inflammation. Should extravasation occur, flushing the site with sodium chloride 9 mg/ml (0.9%) solution for injection and/or sterile water and applications of ice are recommended.

For storage conditions after dilution of the medicinal product, see section 6.3.

Any unused medicinal product or waste material should be disposed of in accordance with local requirements.

7. MARKETING AUTHORISATION HOLDER

Baxalta Innovations GmbH
Industriestrasse 67
1221 Vienna
Austria

8. MARKETING AUTHORISATION NUMBER(S)

D10

EU/1/16/1130/001

9. DATE OF FIRST AUTHORISATION/RENEWAL OF THE AUTHORISATION

10. DATE OF REVISION OF THE TEXT

Detailed information on this medicinal product is available on the website of the European Medicines Agency http://www.ema.europa.eu.

ANNEX II

A. MANUFACTURER(S) RESPONSIBLE FOR BATCH RELEASE

B. CONDITIONS OR RESTRICTIONS REGARDING SUPPLY AND USE

C. OTHER CONDITIONS AND REQUIREMENTS OF THE MARKETING AUTHORISATION

D. CONDITIONS OR RESTRICTIONS WITH REGARD TO THE SAFE AND EFFECTIVE USE OF THE MEDICINAL PRODUCT

A. MANUFACTURER(S) RESPONSIBLE FOR BATCH RELEASE

D10

Name and address of the manufacturer(s) responsible for batch release
Baxter AG
Industriestrasse 67, 1221 Vienna, Austria

B. CONDITIONS OR RESTRICTIONS REGARDING SUPPLY AND USE

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

C. OTHER CONDITIONS AND REQUIREMENTS OF THE MARKETING AUTHORISATION

- **Periodic safety update reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

D. CONDITIONS OR RESTRICTIONS WITH REGARD TO THE SAFE AND EFFECTIVE USE OF THE MEDICINAL PRODUCT

- **Risk Management Plan (RMP)**

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

ANNEX III

LABELLING AND PACKAGE LEAFLET

A. LABELLING

PARTICULARS TO APPEAR ON THE OUTER PACKAGING D10

OUTER CARTON

1. NAME OF THE MEDICINAL PRODUCT

onivyde 5 mg/ml concentrate for solution for infusion
Pegylated liposomal irinotecan hydrochloride trihydrate

2. STATEMENT OF ACTIVE SUBSTANCE

One 10 ml vial of concentrate contains the equivalent of 50 mg irinotecan hydrochloride trihydrate (as irinotecan sucrosofate salt in a pegylated liposomal formulation) which corresponds to 43 mg irinotecan.

3. LIST OF EXCIPIENTS

Excipients:
DSPC
Cholesterol
MPEG-2000-DSPE
Sucrose octasulphate
HEPES buffer
Sodium chloride
Water for injections
See leaflet for further information.

4. PHARMACEUTICAL FORM AND CONTENTS

Concentrate for solution for infusion.
50 mg/10 ml
1 vial

5. METHOD AND ROUTE OF ADMINISTRATION

For single use only.
Read the package leaflet before use.
Intravenous use after dilution.

6. SPECIAL WARNING THAT THE MEDICINAL PRODUCT MUST BE STORED OUT OF THE SIGHT AND REACH OF CHILDREN

Keep out of the sight and reach of children.

7. OTHER SPECIAL WARNING(S), IF NECESSARY

onivyde (liposomal irinotecan) is not equivalent to non-liposomal formulations. Do not interchange.

8. EXPIRY DATE

EXP:

9. SPECIAL STORAGE CONDITIONS

Store in a refrigerator.
Do not freeze.
Keep the vial in the outer carton in order to protect from light.

10. SPECIAL PRECAUTIONS FOR DISPOSAL OF UNUSED MEDICINAL PRODUCTS OR WASTE MATERIALS DERIVED FROM SUCH MEDICINAL PRODUCTS, IF APPROPRIATE

Cytotoxic: handle with caution and special disposal.

11. NAME AND ADDRESS OF THE MARKETING AUTHORISATION HOLDER

Baxalta Innovations GmbH
Industriestrasse 67
A-1221 Vienna
Austria

12. MARKETING AUTHORISATION NUMBER(S)

EU/1/16/1130/001

13. BATCH NUMBER

Lot:

14. GENERAL CLASSIFICATION FOR SUPPLY

15. INSTRUCTIONS ON USE

16. INFORMATION IN BRAILLE

Justification for not including Braille accepted.

17. UNIQUE IDENTIFIER – 2D BARCODE

D10

2D barcode carrying the unique identifier included.

18. UNIQUE IDENTIFIER – HUMAN READABLE DATA

PC:
SN:
NN:

MINIMUM PARTICULARS TO APPEAR ON SMALL IMMEDIATE PACKAGING UNITS

VIAL LABEL

1. NAME OF THE MEDICINAL PRODUCT AND ROUTE(S) OF ADMINISTRATION

onivyde 5 mg/ml concentrate for solution for infusion
Pegylated liposomal irinotecan hydrochloride trihydrate
IV use after dilution

2. METHOD OF ADMINISTRATION

3. EXPIRY DATE

EXP:

4. BATCH NUMBER

Lot:

5. CONTENTS BY WEIGHT, BY VOLUME OR BY UNIT

50 mg/10 ml

6. OTHER

B. PACKAGE LEAFLET

Package leaflet: Information for the user

ONIVYDE 5 mg/ml concentrate for solution for infusion
Pegylated liposomal irinotecan hydrochloride trihydrate

Read all of this leaflet carefully before you start using this medicine because it contains important information for you.

- Keep this leaflet. You may need to read it again.
- If you have any further questions, ask your doctor or nurse.
- If you get any side effects, talk to your doctor or nurse. This includes any possible side effects not listed in this leaflet. See section 4.

What is in this leaflet

1. What ONIVYDE is and what it is used for

What ONIVYDE is and how it works

ONIVYDE is a cancer medicine that contains the active substance irinotecan. This active substance is held in tiny lipid (fatty) particles called liposomes.

Irinotecan belongs to a group of cancer medicines called 'topoisomerase inhibitors'. It blocks an enzyme called topoisomerase I, which is involved in the division of cell DNA. This prevents the cancer cells from multiplying and growing, and they eventually die.

The liposomes are expected to accumulate within the tumour and release the medicine slowly over time, thereby allowing it to act for longer.

What ONIVYDE is used for

ONIVYDE is used to treat adult patients with metastatic pancreatic cancer (cancer of the pancreas that has already spread elsewhere in the body) whose previously cancer treatment included a medicine called gemcitabine. ONIVYDE is used in combination with other cancer medicines, called 5-fluorouracil and leucovorin.

If you have any questions about how ONIVYDE works or why this medicine has been prescribed for you, ask your doctor.

2. What you need to know before you use ONIVYDE

Follow carefully all instructions given to you by your doctor. They may differ from the general information contained in this leaflet.

Do not use ONIVYDE: D10

- if you have a history of a severe allergy to irinotecan, or any of the other ingredients of this medicine (listed in section 6).
- if you are breastfeeding

Warnings and precautions

Talk to your doctor or nurse before you are given ONIVYDE
- if you have ever had any liver problems or jaundice
- if you have ever had lung disease or have previously received medicines (colony stimulating factors) to increase your blood count or radiation therapy
- if you are taking other medicines (see section "Other medicines and ONIVYDE")
- if you are planning to have a vaccination as many vaccinations must not be given during chemotherapy
- if you are on a controlled sodium diet as this medicine contains sodium.

Talk to your doctor or nurse immediately during treatment with ONIVYDE
- if you feel sudden shortness of breath, flushing, headache, skin rash or hives (itchy rash with swollen red bumps on the skin that appear suddenly), itching, swelling around the eyes, tightness in the chest or throat during or shortly after your infusion
- if you experience fever, chills or other symptoms of infection
- if you get diarrhoea with frequent liquid stools and cannot control this after 12 to 24 hours of treatment (see below)
- if you get breathlessness or cough.

What to do in case of diarrhoea

As soon as the first liquid stool occurs, start drinking large volumes ofrehydration fluids (e.g. water, soda water, fizzy drinks, soup) to avoid losing too much liquid and salts from your body. Contact your doctor immediately to give you a suitable treatment. Your doctor may give you a medicine which contains loperamide to begin treatment at home but it must not be used for longer than 48 consecutive hours. If loose stools persist, contact your doctor.

Blood tests and medical examinations

Before you start treatment with ONIVYDE, your doctor will perform blood tests (or other medical examinations) to determine the best starting dose for you. You will need to have further (blood or other) tests during treatment so that your doctor can monitor your blood cells and assess how you are responding to the treatment. Your doctor may need to adjust the dose or stop treatment.

Children and adolescents

ONIVYDE is not recommended for use in adolescents and children below the age of 18 years.

Other medicines and ONIVYDE

Tell your doctor if you are taking, have recently taken or might take any other medicines.
It is especially important that you tell your doctor if you have been given irinotecan in any form earlier.
ONIVYDE must not be used instead of other medicines containing irinotecan because it behaves differently when it is contained in the liposomes than when it is given in its free form.

It is also especially important that you tell your doctor if you are also taking the following medicines, since they reduce the availability of irinotecan in your body:
- phenytoin, phenobarbital or carbamazepin (medicines used to treat convulsions and falls)
- rifampicin and rifabutin (medicines used to treat tuberculosis)

- St. John´s wort (a plant based medicine used to treat depression and low mood)
- as ONIVYDE should not be given to you together with this medicines.

It is especially important that you tell your doctor if you are also taking the following medicines, since they increase the availability of irinotecan in your body:
- ketoconazole, itraconazole or voriconazole (medicines used to treat fungal infections)
- clarithromycin (an antibiotic medicine used to treat bacterial infections)
- indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, atazanavir (medicines against HIV infection)
- telaprevir (a medicine used to treat a liver disease called hepatitis C)
- nefazodone (a medicine used to treat depression, low mood)
- gemfibrozil (medicine used to treat high fat levels in the blood)

ONIVYDE with food and drink

Avoid eating grapefruits and drinking grapefruit juice while you are receiving ONIVYDE as it may increase the availability of the active substance of ONIVYDE in your body.

Pregnancy and breast-feeding

You should not be given ONIVYDE if you are pregnant as it may harm the baby. Tell your doctor if you are or think you may be pregnant. Ask your doctor for advice if you are planning to have a baby. If you are given ONIVYDE you should not breast-feed until one month after the last dose.

During your ONIVYDE treatment and one month after you should choose an effective birth control method which suits you, to prevent pregnancy in this period of time. Males should use condoms during ONIVYDE treatment and 4 months thereafter.

Tell your doctor if you are breast-feeding. You must not be given ONIVYDE if you are breast-feeding as this may be harmful to your baby.

Driving and using machines

ONIVYDE may influence your ability to drive and use machines (as you may be sleepy, dizzy and exhausted with the use of ONIVYDE). You should avoid driving, using machines or performing other tasks that need full attention if you feel sleepy, dizzy and exhausted.

ONIVYDE contains sodium

One millilitre of this medicine contains 0.144 mmol (3.31 mg) sodium—keep this in mind if you are on a controlled sodium diet.

3. How ONIVYDE is used

ONIVYDE must only be given by healthcare professionals trained in giving anticancer medicines
Carefully follow all instructions given to you by your doctor or nurse.

Your doctor will decide upon the doses you will receive.
ONIVYDE is given as a drip (infusion) into a vein, which should take at least 90 minutes and should be given as a single dose.
After you have been given ONIVYDE you will be given two other medicines, leucovorin and 5-fluorouracil.
The treatment will be repeated every two weeks.

In certain cases, lower doses or longer dosing intervals may be required.

You may receive pre-medication against nausea and vomiting. If you have experienced sweating, D10
abdominal cramping and salivation together with early frequent and liquid stools in previous treatments with ONIVYDE, you may receive additional medicines before ONIVYDE to prevent or reduce this in the following treament cycles.

If you have any further questions on the use of this medicine, ask your doctor or nurse.

4. Possible side effects

Like all medicines, this medicine can cause side effects, although not everybody gets them. It is important that you are aware of what these side effects may be.

Your doctor may also prescribe other medicines to help control your side effects.

Tell your doctor or nurse about any of the following serious side effects straight away:

- if you experience sudden shortness of breath, flushing, nausea, headache, skin rash or hives (itchy rash with swollen red bumps on the skin that appear suddenly), itching, swelling around the eyes, tightness in the chest or throat during the infusion or shortly after it (as the infusion may need to be stopped and you may need to be treated or observed for the side effects)
- if you get fever, chills and signs of an infection (as this might require immediate treatment)
- if you have severe persistent diarrhoea (liquid and frequent stools)—see section 2

The following side effects may occur:

Very common (may affect more than 1 in 10 people)
- Low levels of white blood cells (neutropenia and leukopenia), Low level of red blood cells (anaemia)
- Low level of blood platelets (thrombocytopenia)
- Diarrhoea (loose or watery and frequent stools)
- Nausea and vomiting
- Pain in the stomach or in the gut area
- Sore mouth
- Loss of weight
- Loss of appetite
- Loss of body fluid (dehydration)
- Low level of salts (electrolytes) in the body (e.g. of potassium, magnesium)
- Unusual hair loss
- Tiredness
- Dizziness
- Swelling and fluid retention in the soft tissues (peripheral oedema)
- Soreness and swelling of the digestive tract lining (mucosal inflammation)
- Fever
- Generalised weakness

Common (may affect up to 1 in 10 people)
- Chills
- Infections, for example fungal infections in the mouth (oral candidiasis), fever with low counts of white blood cells (febrile neutropenia), infections related to the administration of the product into a vein
- Inflammation of the stomach and the guts (gastroenteritis)
- Systemic body inflammation, caused by infection (sepsis)
- Potentially life-threatening complication of whole body inflammation (septic shock)
- Infection of the lungs (pneumonia)
- Low level of white blood cells subtype, called lymphocytes with important function for the immune system (lymphopenia)

- Decrease in some salts (electrolytes) in the body (e.g. phosphate, sodium)
- Low blood sugar (hypoglycaemia)
- Sleeplessness
- Bad taste in the mouth
- A syndrome called cholinergic syndrome with sweating, salivation and abdominal cramping
- Low blood pressure (hypotension)
- Formation of a blood clot in a deep vein (deep vein thrombosis) or blockage of the main artery of the lung or one of its branches (pulmonary embolism), or blockage due to a blood clot elsewhere in the blood stream (embolism)
- Voice impairment, hoarse or excessively breathy voice
- Shortness of breath
- Inflammation in the gut
- Piles(haemorrhoids)
- Increases in liver enzymes (alanine aminotransferase or aspartate aminotransferase) in laboratory blood tests
- Increase in bilirubin levels (an orange-yellow pigment, waste product of the normal breakdown of the red blood cells) in other laboratory measurements related to liver function
- Increase in other laboratory measurements (increased international normalized ratio) related to the blood clotting system function
- Abnormally low blood levels of albumin (major protein in the body)
- Sudden problems with kidney function which may lead to rapid deterioration or loss of the kidney function
- Abnormal reaction to the infusion causing symptoms like shortness of breath, flushing, headache, tightness in the chest or throat
- Abnormal fluid retention in the body causing swelling in the affected tissues (oedema)

Uncommon (may affect up to 1 in 100 people)
- Systemic body inflammation, caused by infection of the gall bladder and bile ducts (biliary sepsis)
- Allergic reaction to ONIVYDE (the active substance or the excipients)
- Diminished availability of oxygen to the body tissues
- Inflammation of the oesophagus (food pipe)
- Formation or presence of a blood clot within a blood vessel – vein or artery (thrombosis)
- Inflammation of the lining of the rectum (the end of the large intestine)
- Type of rash, characterised by appearance of a flat, red area on the skin covered with bumps (maculo-papular rash)
- Change in the colour of the nail plates

Reporting of side effects

If you get any side effects, talk to your doctor or nurse. This includes any possible side effects not listed in this leaflet. You can also report side effects directly via the national reporting system listed in Appendix V. By reporting side effects you can help provide more information on the safety of this medicine.

5. How to store ONIVYDE

Keep this medicine out of the sight and reach of children.

Do not use this medicine after the expiry date which is stated on the carton and vial after "EXP". The expiry date refers to the last day of that month.

Store in a refrigerator (2°C - 8°C).
Do not freeze.
Keep the vial in the outer carton in order to protect from light.

Once the concentrate has been diluted for infusion with 5% glucose solution for injection or sodium chloride 9 mg/ml (0.9%) solution for injection, the solution should be used as soon as possible, but may be stored at ambient temperature (15°C to 25°C) for up to 6 hours. The diluted solution for infusion can be stored in the refrigerator (2°C - 8°C) for no more than 24 hours prior to use. It must be protected from light, and it must not be frozen.

Do not throw away this medicine via wastewater or household waste. Ask your pharmacist how to throw away medicines you no longer use. These measures will help protect the environment.

6. Contents of the pack and other information

What ONIVYDE contains

- The active substance is irinotecan hydrochloride trihydrate. One 10 ml vial of concentrate contains the equivalent of 50 mg irinotecan hydrochloride trihydrate (as the sucrosofate salt irinotecan sucrose octasulphate, in a pegylated liposomal formulation) which corresponds to 43 mg irinotecan.
- The other ingredients are: 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC); cholesterol, N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoly-sn-glycero-3-phosphoethanolamine (MPEG-2000-DSPE); Sucrose octasulphate; 2- [4- (2-Hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES buffer); sodium chloride and water for injections. ONIVYDE contains sodium, if you are on a controlled sodium diet, see section 2.

What ONIVYDE looks like and contents of the pack

ONIVYDE is supplied as a white to slightly yellow opaque isotonic liposomal dispersion in a glass vial.

Each pack contains one vial with 10 ml of concentrate.

Marketing Authorisation Holder
Baxalta Innovations GmbH
Industriestrasse 67
1221 Vienna
Austria
Tel.: +44(0)1256 894 959
E-mail: medinfoEMEA@shire.com

Manufacturer
Baxter AG
Industriestrasse 67
1221 Vienna
Austria

This leaflet was last revised in

Detailed information on this medicine is available on the European Medicines Agency web site: http://www.ema.europa.eu.

The following information is intended for healthcare professionals only:

How to prepare and administer ONIVYDE

- ONIVYDE is supplied as a sterile liposomal dispersion at a concentration of 5 mg/ml and must be diluted prior to administration. Dilute with 5% glucose solution for injection or sodium chloride 9 mg/ml (0.9%) solution for injection to prepare a solution of the appropriate dose of ONIVYDE diluted to a final volume of 500 ml. Mix diluted solution by gentle inversion.
- ONIVYDE should be administered before leucovorin followed by 5-fluorouracil. ONIVYDE must not be administered as a bolus injection or an undiluted solution.
- Aseptic techniques must be followed during the preparation of the infusion. ONIVYDE is for single use only.
- From a microbiological point of view, the product should be used as soon as possible after dilution. The diluted solution for infusion can be stored at ambient temperature (15°C to 25°C)for up to 6 hours or in the refrigerator (2°C - 8°C) for no more than 24 hours prior to use. It must be protected from light, and it must not be frozen.
- Care should be taken to avoid extravasation, and the infusion site should be monitored for signs of inflammation. Should extravasation occur, flushing the site with sodium chloride 9 mg/ml (0.9%) solution for injection and/or sterile water and applications of ice are recommended.

How to handle and dispose of ONIVYDE

- ONIVYDE is a cytotoxic medicinal product and caution should be exercised in handling it. The use of gloves, goggles and protective clothing when handling or administering ONIVYDE is recommended. If the solution contacts the skin, the skin should be washed immediately and thoroughly with soap and water. If the solution contacts mucous membranes, they should be flushed thoroughly with water. Pregnant staff should not handle ONIVYDE considering the cytotoxic nature of the medicinal product.
- Any unused medicinal product or waste material should be disposed of in accordance with local requirements.

Nanoliposomal irinotecan with fluorouracil and folinic acid in metastatic pancreatic cancer after previous gemcitabine-based therapy (NAPOLI-1): a global, randomised, open-label, phase 3 trial

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Summary

Background Nanoliposomal irinotecan showed activity in a phase 2 study in patients with metastatic pancreatic ductal adenocarcinoma previously treated with gemcitabine-based therapies. We assessed the effect of nanoliposomal irinotecan alone or combined with fluorouracil and folinic acid in a phase 3 trial in this population.

Methods We did a global, phase 3, randomised, open-label trial at 76 sites in 14 countries. Eligible patients with metastatic pancreatic ductal adenocarcinoma previously treated with gemcitabine-based therapy were randomly assigned (1:1) using an interactive web response system at a central location to receive either nanoliposomal irinotecan monotherapy (120 mg/m² every 3 weeks, equivalent to 100 mg/m² of irinotecan base) or fluorouracil and folinic acid. A third arm consisting of nanoliposomal irinotecan (80 mg/m², equivalent to 70 mg/m² of irinotecan base) with fluorouracil and folinic acid every 2 weeks was added later (1:1:1), in a protocol amendment. Randomisation was stratified by baseline albumin, Karnofsky performance status, and ethnic origin. Treatment was continued until disease progression or intolerable toxic effects. The primary endpoint was overall survival, assessed in the intention-to-treat population. The primary analysis was planned after 305 events. Safety was assessed in all patients who had received study drug. This trial is registered at ClinicalTrials.gov, number NCT01494506.

Findings Between Jan 11, 2012, and Sept 11, 2013, 417 patients were randomly assigned either nanoliposomal irinotecan plus fluorouracil and folinic acid (n=117), nanoliposomal irinotecan monotherapy (n=151), or fluorouracil and folinic acid (n=149). After 313 events, median overall survival in patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid was 6·1 months (95% CI 4·8–8·9) *vs* 4·2 months (3·3–5·3) with fluorouracil and folinic acid (hazard ratio 0·67, 95% CI 0·49–0·92; p=0·012). Median overall survival did not differ between patients assigned nanoliposomal irinotecan monotherapy and those allocated fluorouracil and folinic acid (4·9 months [4·2–5·6] *vs* 4·2 months [3·6–4·9]; 0·99, 0·77–1·28; p=0·94). The grade 3 or 4 adverse events that occurred most frequently in the 117 patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid were neutropenia (32 [27%]), diarrhoea (15 [13%]), vomiting (13 [11%]), and fatigue (16 [14%]).

Interpretation Nanoliposomal irinotecan in combination with fluorouracil and folinic acid extends survival with a manageable safety profile in patients with metastatic pancreatic ductal adenocarcinoma who previously received gemcitabine-based therapy. This agent represents a new treatment option for this population.

Funding Merrimack Pharmaceuticals.

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Introduction

Pancreatic ductal adenocarcinoma is typically diagnosed late, when curative resection is impossible and prognosis is poor, with only 1–2% of patients surviving at 5 years.[1,2] Gemcitabine-based therapies have been the standard of care for patients with locally advanced or metastatic pancreatic ductal adenocarcinoma for the past two decades.[3–5] However, two combination regimens—FOLFIRINOX (a combination of oxaliplatin, folinic acid, irinotecan, and fluorouracil) and albumin-bound paclitaxel in combination with gemcitabine—have gained acceptance as front-line treatments.[6,7] Despite these advances, progression after front-line therapy is inevitable, leaving patients and clinicians with few options and no universally accepted standard treatment—showing the unmet need in this population.[8]

Irinotecan has been investigated in several small monotherapy[9] and combination therapy[10–20] studies. The findings have provided initial evidence of the activity of irinotecan in the second-line setting. 1 mg of irinotecan hydrochloride trihydrate salt is equivalent to 0·86 mg of irinotecan free base. Nanoliposomal irinotecan comprises irinotecan free base encapsulated in liposome nanoparticles. The liposome is designed to keep irinotecan in the circulation—sheltered from conversion

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Research in context

Evidence before this study

There is no consensus on the standard of care in patients with metastatic pancreatic cancer whose disease progressed after gemcitabine-based therapy despite the availability of more effective front-line treatments. At the time this study was designed, guidelines recommended clinical trials in this setting. Nanoliposomal irinotecan has shown activity in phase 2 studies in solid tumours, including metastatic pancreatic cancer, previously treated with gemcitabine-based therapy.

Added value of this study

In patients with metastatic pancreatic cancer previously treated with gemcitabine-based therapy, nanoliposomal irinotecan in combination with fluorouracil and folinic acid increased overall survival, progression-free survival, and time to treatment failure, reduced carbohydrate antigen 19-9 (a pancreatic tumour biomarker), and amplified the number of patients achieving an objective response. In a population with few treatment options, this drug combination was tolerable and did not have a negative effect on quality of life, which are important factors for this population.

Implications of all the available evidence

Nanoliposomal irinotecan in combination with fluorouracil and folinic acid represents a potential treatment option for patients with metastatic pancreatic cancer that progressed after a gemcitabine-based regimen. Future research will assess its use in front-line therapy.

to its active metabolite (SN-38)—longer than free (unencapsulated) irinotecan, which would increase and prolong intratumoral levels of both irinotecan and SN-38 compared with free irinotecan.[21–23] The roughly 5·6-fold higher level of SN-38 found in tumours compared with plasma at 72 h suggests local metabolic activation of irinotecan, which was contained in the liposomal nanoparticles, to SN-38.[21] In a phase 2 study of 40 patients with metastatic pancreatic ductal adenocarcinoma previously treated with gemcitabine-based therapy, nanoliposomal irinotecan at 120 mg/m² every 3 weeks resulted in a median overall survival of 5·2 months, 1-year survival of 25%, and a manageable toxicity profile.[24] The aim of this study (NAPOLI-1) was to assess the effect of nanoliposomal irinotecan, alone and in combination with fluorouracil and folinic acid, compared with a common control (fluorouracil and folinic acid), for patients with metastatic pancreatic ductal adenocarcinoma previously treated with gemcitabine-based therapy.

Methods

Study design and participants

We designed a global, multicentre, open-label, phase 3 study conducted at 76 sites in 14 countries (Argentina, Australia, Brazil, Canada, Czech Republic, France, Germany, Hungary, Italy, South Korea, Spain, Taiwan, the UK, and USA). We included patients aged 18 years or older with histologically or cytologically confirmed pancreatic ductal adenocarcinoma and documented measurable or non-measurable distant metastatic disease. The disease must have progressed after previous gemcitabine-based therapy given in a neoadjuvant, adjuvant (only if distant metastases occurred within 6 months of completing adjuvant therapy), locally advanced, or metastatic setting. Other key inclusion criteria were a Karnofsky performance status score of 70 or more and adequate haematological (including absolute neutrophil count >1·5×10⁹ cells per L), hepatic (including normal serum total bilirubin, according to local institutional standards, and albumin levels ≥30 g/L), and renal function. We also enrolled patients who had previously received irinotecan or fluorouracil, or both.

All versions of the protocol and informed consent form were approved by the institutional review board or ethics committees for every site. The study was done according to the principles of the Declaration of Helsinki, the International Conference on Harmonisation Guidance on Good Clinical Practice, and the requirements of the US Food and Drug Administration and local regulatory authorities regarding the conduct of human clinical trials. All patients provided written informed consent.

Randomisation and masking

We initially randomly assigned patients in a 1:1 ratio to receive either nanoliposomal irinotecan monotherapy or a control of fluorouracil and folinic acid (protocol version 1). Following clinical interest in the combination of nanoliposomal irinotecan with other agents, we amended the protocol to add a third arm (1:1:1 ratio) of nanoliposomal irinotecan plus fluorouracil and folinic acid (protocol version 2) after safety data on this combination became available from an ongoing study in metastatic colorectal cancer.[25] Sites continued to enrol patients under protocol version 1 until protocol version 2 was approved at that site.

We randomised patients according to a prespecified scheme generated by an independent statistician within the funder-designated contract research organisation. On confirmation of a patient's eligibility, investigators used a computerised interactive web response system to obtain a patient number, which was associated with a random assignment. We stratified the randomisation by baseline albumin levels (≥40 g/L *vs* <40 g/L), Karnofsky performance status (70 and 80 *vs* ≥90), and ethnic origin (white *vs* east Asian *vs* all others).

Procedures

All patients underwent *UGT1A1* genotype testing. Patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid (combination therapy arm) received an intravenous infusion of nanoliposomal irinotecan over 90 min at a dose of 80 mg/m² (equivalent to 70 mg/m² of irinotecan free base), followed by folinic acid 400 mg/m² over 30 min, then fluorouracil 2400 mg/m² over 46 h, every 2 weeks. For those allocated to the monotherapy arm, nanoliposomal irinotecan was administered at a dose of 120 mg/m² (equivalent to 100 mg/m² of irinotecan free base), every 3 weeks.[24,26] We reduced the initial nanoliposomal irinotecan dose for patients homozygous for the *UGT1A1*28* allele by 20 mg/m² then increased it to the standard dose after the first cycle in the absence of drug-related toxic effects.[27] Patients who were assigned fluorouracil and folinic acid (control arm) received 200 mg/m² of folinic acid as a 30-min infusion followed by an infusion of 2000 mg/m² fluorouracil over 24 h, every week for the first 4 weeks of each 6-week cycle. We based the fluorouracil and folinic acid schedule of the control arm on that used in the CONKO-003 trial[28] and of the combination therapy arm on that used in the PEPCOL study,[25] with expected dose intensities of fluorouracil over a 6-week period of 8000 mg/m² in the control arm and 7200 mg/m² in the combination therapy arm. Treatment continued until disease progression or intolerable toxic effects arose.

We did serial imaging studies and measured amounts of carbohydrate antigen 19-9 (CA19-9) at baseline and every 6 weeks until either disease progression, a new antineoplastic treatment was started, or withdrawal of consent. We did radiographic tumour response assessment according to Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1, and we assessed safety by grading adverse events according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. We measured quality of life at baseline and every 6 weeks with the European Organization for Research and Treatment of Cancer Quality-of-Life Core Questionnaire (EORTC-QLQ-C30). We assessed clinical benefit response as described elsewhere.[3] We followed up patients every month after treatment termination for survival until death or study completion. An independent data safety monitoring board assessed cumulative safety and other trial-related data at regular intervals.

Outcomes

The primary efficacy endpoint was overall survival. Secondary endpoints included progression-free survival; time to treatment failure; the proportion of patients achieving an objective response; serum CA19-9 response (ie, ≥50% decrease in amount of CA19-9 from baseline at least once during the treatment period); clinical benefit response (ie, either achievement of pronounced and sustained [≥4 weeks contiguous] improvement in pain intensity, analgesic consumption, or performance status, or a combination of these, without any worsening in any of the other factors, or stability in pain intensity, analgesic consumption, and performance status with pronounced and sustained [≥4 weeks contiguous] weight gain); quality of life; and safety. A secondary objective, the pharmacokinetics of nanoliposomal irinotecan as a single agent and in combination with fluorouracil and folinic acid, will be reported separately.

Statistical analysis

We calculated the sample size for the three-arm study through a simulation as part of this study. In the protocol, we planned to enrol 405 patients, for a primary analysis of overall survival after 305 events, to provide at least 98% power to detect a hazard ratio (HR) for death with nanoliposomal irinotecan plus fluorouracil and folinic acid relative to fluorouracil and folinic acid of 0·5, and at least 85% power to detect a HR for death with nanoliposomal irinotecan monotherapy relative to fluorouracil and folinic acid of 0·67.

We did efficacy analyses in the intention-to-treat population (ie, all randomised patients). We analysed safety in patients who received one dose or more (including a partial dose) of study treatment. For the primary efficacy analysis, the null hypotheses tested were: no effect of nanoliposomal irinotecan monotherapy on overall survival relative to control; and no effect of nanoliposomal irinotecan plus fluorouracil and folinic acid on overall survival relative to control. We controlled the family-wise error at a two-sided 0·05 level with the Bonferroni-Holm procedure. For all efficacy and quality-of-life comparisons, the patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid were compared with those allocated the fluorouracil and folinic acid control under the amended protocol (version 2), whereas patients assigned nan] oliposomal irinotecan monotherapy were compared with those allocated the fluorouracil and folinic acid control under either version of the protocol (versions 1 and 2).

We did Kaplan-Meier analyses on each treatment group to obtain non-parametric estimates of median overall survival and progression-free survival and time to treatment failure. We calculated corresponding 95% CIs with the log–log method. We used unstratified Cox proportional hazards regression to estimate HRs and their corresponding 95% CIs. We did two pairwise comparisons of overall survival and progression-free survival between the study treatments by unstratified log-rank test. To assess the robustness of the primary endpoint results, we used a Cox regression model with stepwise selection (p to enter <0·25, p to remain <0·15), with treatment and baseline potential prognostic factors as candidates for inclusion in the model for overall survival. We also did a supportive stratified analysis for overall survival, accounting for randomisation strata.

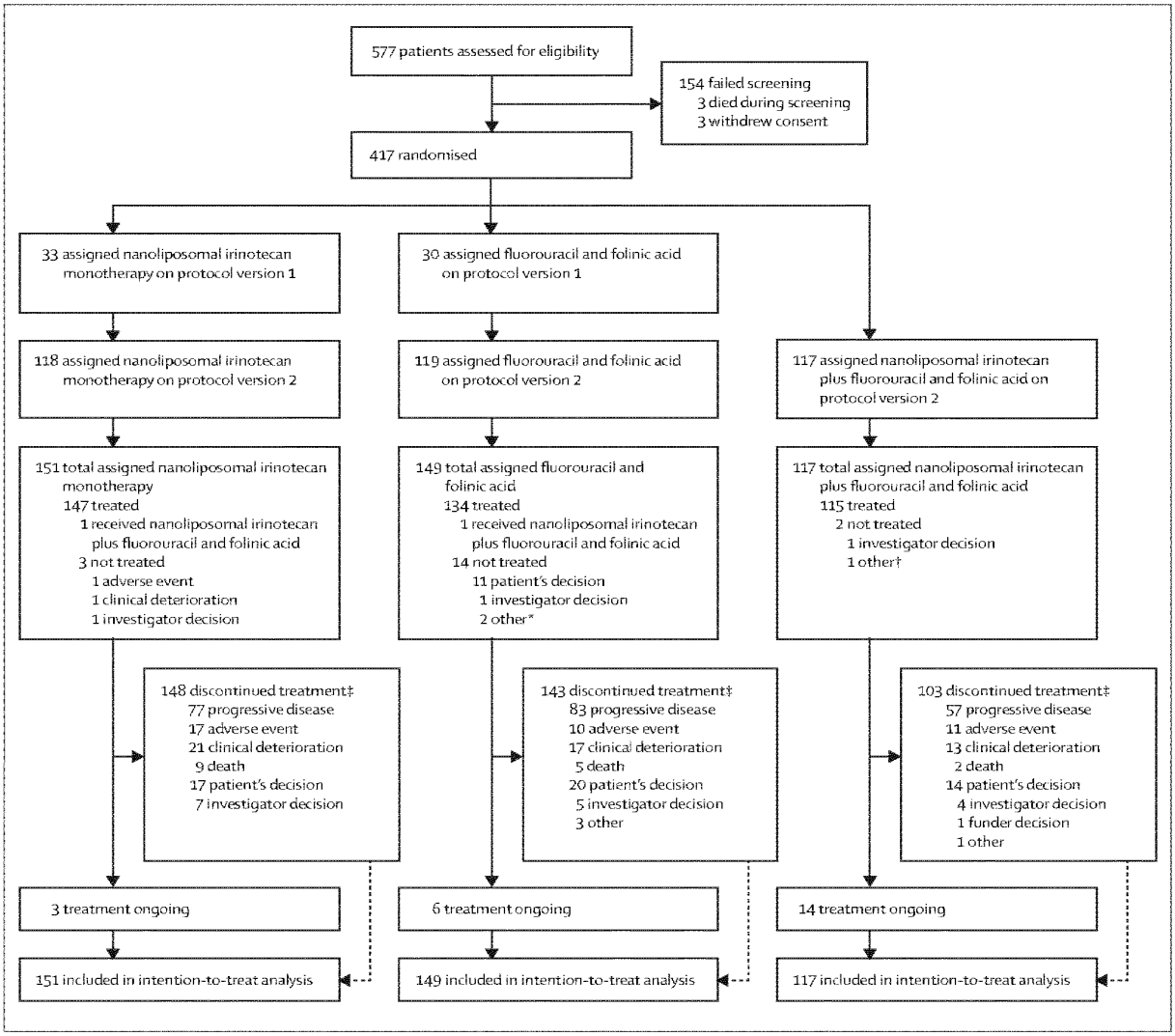

Figure 1: **Trial profile**

*One patient became ineligible after randomisation; one patient had an adverse event that delayed dosing more than 7 days from randomisation. †One patient became ineligible after randomisation. ‡The primary reason for discontinuation was at the discretion of the investigator.

We used Fisher's exact test for pairwise comparisons of objective response, clinical benefit response (we only included treated patients with a baseline pain intensity ≥20/100, baseline opioid pain medication consumption ≥10 mg/day of oral morphine equivalents, and a baseline Karnofsky performance status score of 70–90), and CA19-9 response (we only included treated patients with a baseline CA19-9 value >30 U/mL). We based analyses of progression-free survival and response on tumour and disease progression assessments per the investigator. We did pairwise treatment group comparisons for response classification for each quality-of-life subscale with the Cochran-Mantel-Haenszel test, and we used the Benjamini-Hochberg method to control type I error for comparisons on multiple subscales. We did all analyses and summaries with SAS, version 9.2 (or higher).

This study is registered with ClinicalTrials.gov, number NCT01494506.

Role of the funding source

This study was funded by Merrimack Pharmaceuticals. The study protocol was designed by the funder and external consultants, and data were analysed by a statistician employed by the funder (BB). All authors gathered data and were assisted in writing of the report by a medical writer employed by the funder. L-TC, AW-G, and DDVH had full access to all data in the study, participated in data interpretation, and had final responsibility for the

	Nanoliposomal irinotecan plus fluorouracil and folinic acid combination therapy (n=117)	Fluorouracil and folinic acid combination therapy control (n=119*)	Nanoliposomal irinotecan monotherapy (n=151)	Fluorouracil and folinic acid monotherapy control (n=149)
Men	69 (59%)	67 (56%)	87 (58%)	81 (54%)
Women	48 (41%)	52 (44%)	64 (42%)	68 (46%)
Age (years)	63 (57–70)	62 (55–69)	65 (58–70)	63 (55–69)
Ethnic origin				
East Asian	34 (29%)	36 (30%)	52 (34%)	50 (34%)
Black or African American	4 (3%)	3 (3%)	3 (2%)	3 (2%)
White	72 (62%)	76 (64%)	89 (59%)	92 (62%)
Other	7 (6%)	4 (3%)	7 (5%)	4 (3%)
Region				
Asia	34 (29%)	35 (29%)	50 (33%)	48 (32%)
Europe	47 (40%)	49 (41%)	54 (36%)	55 (37%)
North America	19 (16%)	19 (16%)	26 (17%)	25 (17%)
Other	17 (15%)	16 (13%)	21 (14%)	21 (14%)
Karnofsky performance status score†				
100	18 (15%)	17 (14%)	22 (15%)	22 (15%)
90	51 (44%)	40 (34%)	64 (42%)	54 (36%)
80	38 (32%)	51 (43%)	50 (33%)	61 (41%)
70	7 (6%)	10 (8%)	15 (10%)	11 (7%)
50–60	3 (3%)	0	0	0
Pancreatic tumour location				
Head	76 (65%)	69 (58%)	99 (66%)	81 (54%)
Other	41 (35%)	50 (42%)	52 (34%)	68 (46%)
Amount of CA19-9‡				
≥40 U/mL	92/114 (81%)	91/114 (80%)	125/146 (86%)	116/144 (81%)
<40 U/mL	22/114 (19%)	23/114 (20%)	21/146 (14%)	28/144 (39%)
Site of metastatic lesions§				
Liver	75 (64%)	83 (70%)	101 (67%)	108 (72%)
Lung	36 (31%)	36 (30%)	49 (32%)	44 (30%)
Lymph node, distant	32 (27%)	31 (26%)	44 (29%)	40 (27%)
Lymph node, regional	13 (11%)	14 (12%)	19 (13%)	20 (13%)
Pancreas	75 (64%)	72 (61%)	99 (66%)	97 (65%)
Peritoneum	28 (24%)	32 (27%)	48 (32%)	39 (26%)
Other	27 (23%)	39 (33%)	38 (25%)	48 (32%)

(Table 1 continues on next page)

decision to submit for publication. All authors agreed to submit the report.

Results

Between Jan 11, 2012, and Sept 11, 2013, 417 patients at 76 sites from 14 countries worldwide were randomly assigned to nanoliposomal irinotecan plus fluorouracil and folinic acid (n=117), nanoliposomal irinotecan monotherapy (n=151), or fluorouracil and folinic acid (n=149). 63 patients were enrolled under protocol version 1 before all sites switched to version 2 (figure 1). Patients' demographics and baseline clinical characteristics were similar among the three treatment groups (table 1). 51 (12%) patients received gemcitabine-based therapy in the adjuvant, neoadjuvant, or locally advanced setting but had not had previous treatment for metastatic disease, 234 (56%) had received one previous line of metastatic treatment, and 132 (32%) patients had previously received two or more lines of metastatic treatment.

Seven patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid and seven individuals allocated nanoliposomal irinotecan as monotherapy were homozygous for the *UGT1A1*28* allele. Three patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid were able to escalate to the standard starting dose of 80 mg/m² without need for subsequent dose reduction. One additional patient needed a dose reduction to 40 mg/m², and one discontinued because of an adverse event (grade 3 vomiting). Two of seven patients allocated nanoliposomal irinotecan were able to increase the dose, to 100 mg/m² and 120 mg/m². One patient needed a dose

	Nanoliposomal irinotecan plus fluorouracil and folinic acid combination therapy (n=117)	Fluorouracil and folinic acid combination therapy control (n=119*)	Nanoliposomal irinotecan monotherapy (n=151)	Fluorouracil and folinic acid monotherapy control (n=149)
(Continued from previous page)				
Measurable metastatic sites (n)				
1	19 (16%)	22 (18%)	36 (24%)	26 (17%)
2	49 (42%)	58 (49%)	63 (42%)	72 (48%)
3	22 (19%)	15 (13%)	22 (15%)	21 (14%)
≥4	7 (6%)	8 (7%)	7 (5%)	10 (7%)
Previous therapies or procedures				
Radiotherapy	24 (21%)	27 (23%)	40 (26%)	33 (22%)
Whipple procedure	30 (26%)	33 (28%)	47 (31%)	36 (24%)
Biliary stent	15 (13%)	8 (7%)	13 (9%)	9 (6%)
Previous lines of metastatic therapy				
0¶	15 (13%)	15 (13%)	17 (11%)	19 (13%)
1	62 (53%)	67 (56%)	86 (57%)	86 (58%)
≥2	40 (34%)	37 (31%)	48 (32%)	44 (30%)
Previous anticancer therapy‖				
Gemcitabine alone	53 (45%)	55 (46%)	67 (44%)	66 (44%)
Gemcitabine combination	64 (55%)	64 (54%)	84 (56%)	83 (56%)
Fluorouracil based	50 (43%)	52 (44%)	70 (46%)	63 (42%)
Irinotecan based	12 (10%)	17 (14%)	17 (11%)	17 (11%)
Platinum based	38 (32%)	41 (34%)	54 (36%)	45 (30%)

Data are number of patients (%) or median (IQR). CA19-9=carbohydrate antigen 19-9. *Fluorouracil and folinic acid combination control group based on protocol version 2. †Baseline Karnofsky performance status score was missing for one patient in the fluorouracil and folinic acid group (enrolled under protocol 2) who was subsequently stratified as having a score ≥90. ‡Data were missing for three patients in the nanoliposomal irinotecan plus fluorouracil and folinic acid group and in five patients each in the nanoliposomal irinotecan monotherapy and fluorouracil and folinic acid groups (enrolled under protocol 2). §Investigator-reported with review by the funder's medical team. Some patients had multiple metastatic sites and are listed in more than one group. ¶Patients received neoadjuvant, adjuvant, or locally advanced treatment, but no previous therapy for metastatic disease. ‖Columns add up to greater than 100% because some patients received more than one line of therapy and are listed in more than one group, and regimens might include multiple drug classes, but at least one gemcitabine based.

Table 1: **Baseline characteristics**

reduction to 40 mg/m², but none discontinued because of an adverse event.

The survival analysis was based on 313 deaths, with a cutoff date of Feb 14, 2014. Deaths were recorded in 75 (64%) of 117 patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid, 80 (67%) of 119 individuals allocated the fluorouracil and folinic acid combination control, 129 (85%) of 151 patients assigned nanoliposomal irinotecan monotherapy, and 109 (73%) of 149 individuals allocated the fluorouracil and folinic acid monotherapy control. Median overall survival was 6·1 months (95% CI 4·8–8·9) in patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid and 4·2 months (3·3–5·3) in those allocated fluorouracil and folinic acid (unstratified HR 0·67, 95% CI 0·49–0·92; p=0·012; figure 2A). Median overall survival was 4·9 months (95% CI 4·2–5·6) for patients allocated nanoliposomal irinotecan monotherapy compared with 4·2 months (3·6–4·9) for those assigned fluorouracil and folinic acid (unstratified HR 0·99, 95% CI 0·77–1·28; p=0·94; figure 2B).

Preplanned subgroup analyses showed that the survival benefit of nanoliposomal irinotecan plus fluorouracil and folinic acid was homogeneous across most subgroups (figure 3). In the stepwise Cox regression analysis of nanoliposomal irinotecan plus fluorouracil and folinic acid versus fluorouracil and folinic acid, an association of overall survival was identified between treatment and the following prognostic factors: baseline Karnofsky performance status, albumin, time since receiving most recent anticancer therapy, tumour stage at diagnosis, status of liver metastases, and baseline CA19-9. Adjusting for these prognostic factors, the combination of nanoliposomal irinotecan plus fluorouracil and folinic acid maintained a strong treatment effect on overall survival (HR 0·58, 95% CI 0·42–0·81).

In patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid, median progression-free survival was 3·1 months (95% CI 2·7–4·2) compared with 1·5 months (1·4–1·8) in those allocated fluorouracil and folinic acid (unstratified HR 0·56, 95% CI 0·41–0·75; p=0·0001; figure 2C). In patients allocated nanoliposomal irinotecan monotherapy, median progression-free survival was 2·7 months (95% CI 2·1–2·9) versus 1·6 months (1·4–1·8) for those assigned fluorouracil and

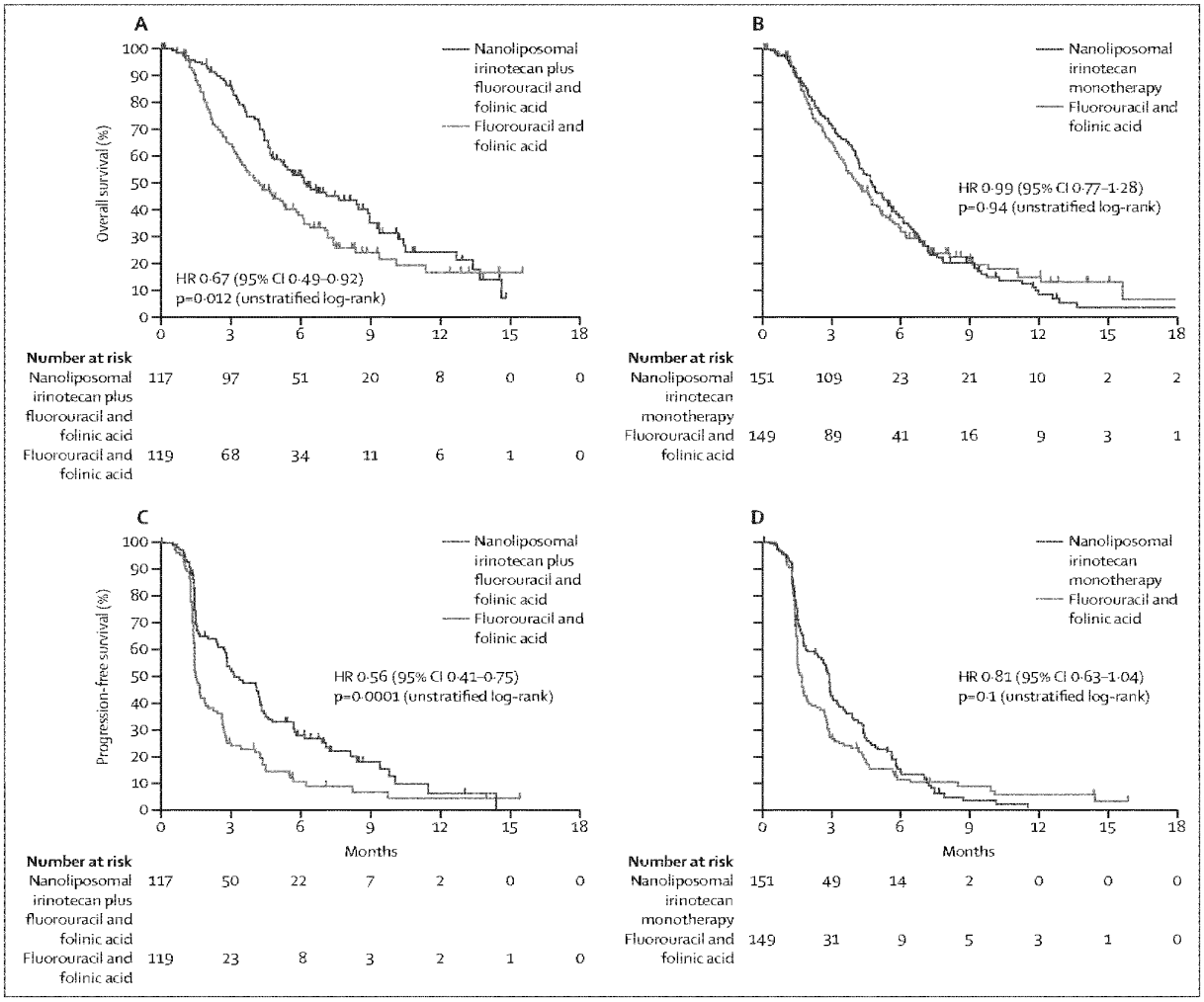

Figure 2: **Kaplan-Meier survival analyses**

HR=hazard ratio. (A) Overall survival with nanoliposomal irinotecan plus fluorouracil and folinic acid versus fluorouracil and folinic acid. (B) Overall survival with nanoliposomal irinotecan monotherapy versus fluorouracil and folinic acid. (C) Progression-free survival with nanoliposomal irinotecan plus fluorouracil and folinic acid versus fluorouracil and folinic acid. (D) Progression-free survival with nanoliposomal irinotecan monotherapy versus fluorouracil and folinic acid.

folinic acid (unstratified HR 0·81, 95% CI 0·63–1·04; p=0·1; figure 2D).

Median time to treatment failure was 2·3 months (95% CI 1·6–2·8) in patients allocated nanoliposomal irinotecan plus fluorouracil and folinic acid compared with 1·4 months (1·3–1·4) in those assigned fluorouracil and folinic acid (HR 0·6, 95% CI 0·45–0·78; p=0·0002). Time to treatment failure did not differ significantly between patients assigned nanoliposomal irinotecan monotherapy and those allocated fluorouracil and folinic acid (1·7 months [95% CI 1·5–2·7] *vs* 1·4 months [1·3–1·4]; HR 0·82, 95% CI 0·65–1·03; p=0·1).

19 (16%) of 117 patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid achieved an objective response compared with one (1%) of 119 individuals allocated fluorouracil and folinic acid (difference 15·4 percentage points, 95% CI 8·5–22·3; p<0·0001). Nine (6%) of 151 patients allocated nanoliposomal irinotecan monotherapy achieved an objective response compared with one (1%) of 149 assigned fluorouracil and folinic acid (difference 5·3 percentage points, 95% CI 1·3–9·3; p=0·02).

28 (29%) of 97 patients allocated nanoliposomal irinotecan plus fluorouracil and folinic acid achieved a CA19-9 response (≥50% decrease from abnormal baseline) versus seven (9%) of 81 assigned fluorouracil and folinic acid (p=0·0006). 29 (24%) of 123 patients allocated nanoliposomal irinotecan monotherapy had a CA19-9 response versus 12 (11%) of 105 assigned fluorouracil and folinic acid (p=0·024).

At baseline, median scores for quality-of-life measures (global health status, functional scale, and symptoms

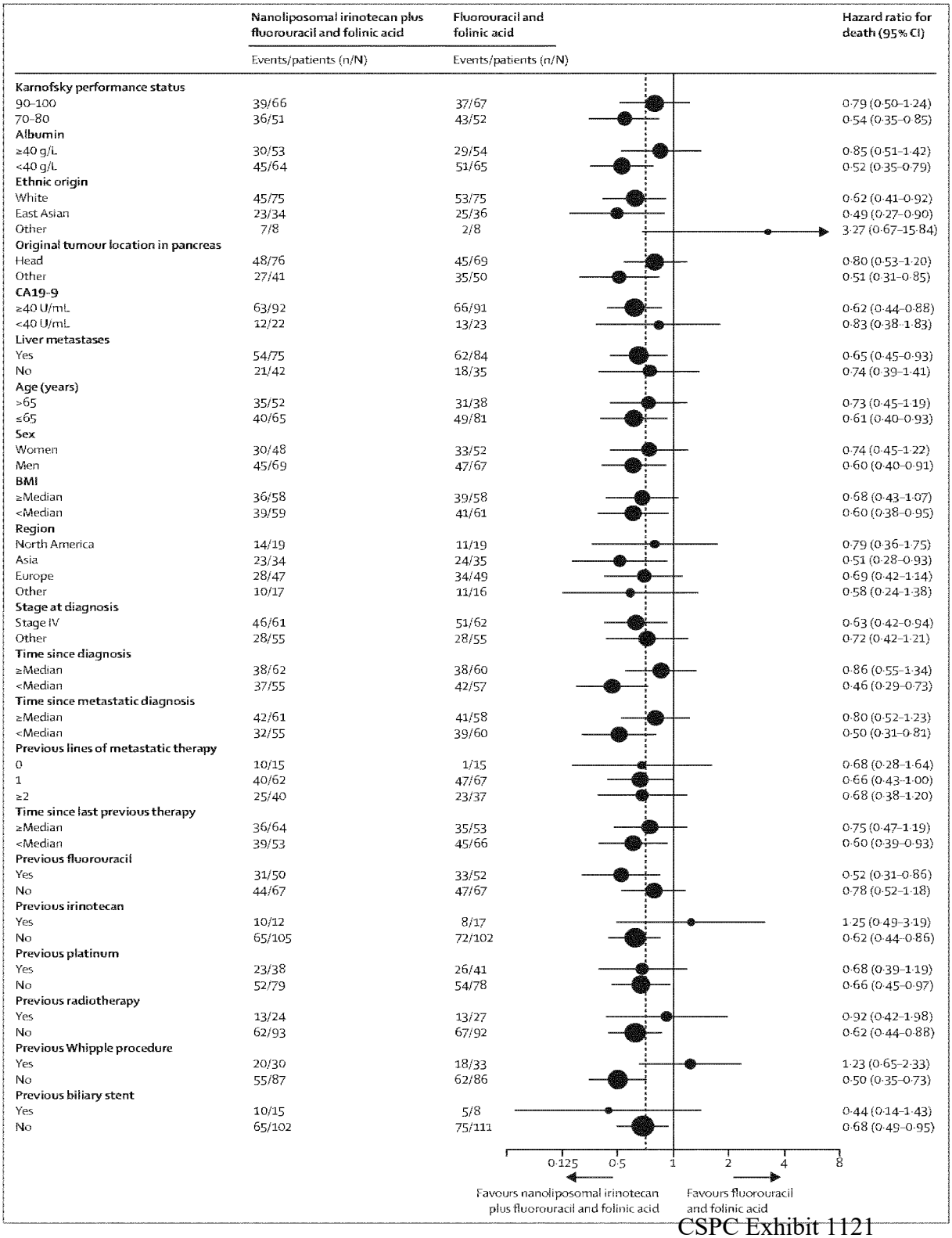

Figure 3: **Forest plot of treatment effect on survival in prespecified subgroups**
Hazard ratios are depicted by filled circles and 95% CIs by horizontal lines. The size of the circle reflects the size of the subgroup relative to the intention-to-treat population. BMI=body-mass index. CA19-9=carbohydrate antigen 19-9.

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scores) were similar between groups. At 6 and 12 weeks, the median functional scale scores did not differ appreciably from baseline, suggesting that the effects of the treatments on functional scale scores were negligible. Clinical benefit response was less than 20% and did not differ significantly between treatment groups (appendix pp 1–3).

In patients allocated nanoliposomal irinotecan plus fluorouracil and folinic acid and nanoliposomal irinotecan monotherapy, median duration of exposure to nanoliposomal irinotecan was 8·7 weeks (IQR 5·4–22·0) and 8·9 weeks (6·0–16·0), respectively, and mean dose intensities were 167·5 mg/m² (SD 44·8) over 6 weeks and 188·0 mg/m² (52·0) over 6 weeks, respectively. Median exposure to fluorouracil was 8·7 weeks (IQR 5·4–22·0) in patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid and 6·0 weeks (5·9–12·1) in those allocated fluorouracil and folinic acid (n=105, protocol version 2); mean dose intensities of fluorouracil were, respectively, 5065·0 mg/m² (SD 1539·1) over 6 weeks and 6710·2 mg/m² (1719·2) over 6 weeks.

Post-progression anticancer therapy was given to 36 (31%) of 117 patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid and 45 (38%) of 119 patients allocated fluorouracil and folinic acid. The use of treatment after progression and the type of therapy used was generally similar between groups. Eight patients assigned the combination therapy and nine patients allocated the control received irinotecan as part of post-progression therapy.

398 (95%) of the 417 patients randomly assigned received at least one dose of study drug and were included in the safety analysis population. The most common treatment-emergent adverse events of all grades in patients whose treatment included nanoliposomal irinotecan were diarrhoea, nausea, and vomiting (table 2). Alopecia occurred in 16 (14%) of 117 patients in the nanoliposomal irinotecan plus fluorouracil and folinic acid group, 32 (22%) of 147 individuals who received nanoliposomal irinotecan as monotherapy, and six (5%) of 134 patients who received fluorouracil and folinic acid. Adverse events that resulted in a dose reduction occurred in 39 (33%) patients in the nanoliposomal irinotecan plus fluorouracil and folinic acid group, 46 (31%) individuals given nanoliposomal irinotecan monotherapy, and five (4%) patients who received fluorouracil and folinic acid. Grade 3 or 4 neutropenic sepsis (including febrile neutropenia) was noted in three (3%) patients in the combination therapy group and six (4%) individuals who had monotherapy, with no events of this type reported in the control group. Granulocyte colony-stimulating factor was administered to 20 (17%) patients receiving nanoliposomal irinotecan plus fluorouracil and folinic acid and 17 (12%) of those treated with nanoliposomal irinotecan monotherapy, compared with one (1%) patient in the fluorouracil and folinic acid group. Grade 4 treatment-emergent adverse events were reported in 12 (10%) patients given nanoliposomal irinotecan plus fluorouracil and folinic acid, 24 (16%) individuals who received nanoliposomal irinotecan monotherapy, and nine (7%) of those in the fluorouracil and folinic acid group. Of these, only three patients in the monotherapy group and one individual receiving control had a gastrointestinal event. 30-day mortality was low in all groups (three [3%] of 117 in the nanoliposomal irinotecan plus fluorouracil and folinic acid group, three [2%] of 151 in the monotherapy group, and four [3%] of 149 with control). Adverse events leading to discontinuation of study drug arose in 13 (11%) patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid, 17 (12%) individuals allocated nanoliposomal irinotecan monotherapy, and ten (7%) patients assigned fluorouracil and folinic acid.

Of 47 patients who died during the study or within 30 days from the last dose of study drug, 30 deaths were attributed to pancreatic cancer, 16 were due to an adverse event (five related to treatment, according to the investigator), and the cause was unknown for one. The treatment-related adverse events that resulted in death were gastrointestinal toxic effects (n=1, nanoliposomal irinotecan monotherapy), infectious enterocolitis (n=1, nanoliposomal irinotecan monotherapy), septic shock (n=1, nanoliposomal irinotecan monotherapy; n=1, nanoliposomal irinotecan plus fluorouracil and folinic acid), and disseminated intravascular coagulation with pulmonary embolism (n=1, nanoliposomal irinotecan monotherapy). 90 (61%) of 147 patients assigned nanoliposomal irinotecan monotherapy had a treatment-emergent serious adverse event compared with 56 (48%) of 117 individuals allocated nanoliposomal irinotecan plus fluorouracil and folinic acid and 60 (45%) of 134 patients assigned fluorouracil and folinic acid.

	Nanoliposomal irinotecan plus fluorouracil and folinic acid combination therapy (n=117)		Nanoliposomal irinotecan monotherapy (n=147)		Fluorouracil and folinic acid control (n=134)	
	Any grade	Grades 3–4	Any grade	Grades 3–4	Any grade	Grades 3–4
Diarrhoea	69 (59%)	15 (13%)	103 (70%)	31 (21%)	35 (26%)	6 (4%)
Vomiting	61 (52%)	13 (11%)	80 (54%)	20 (14%)	35 (26%)	4 (3%)
Nausea	60 (51%)	9 (8%)	89 (61%)	8 (5%)	46 (34%)	4 (3%)
Decreased appetite	52 (44%)	5 (4%)	72 (49%)	13 (19%)	43 (32%)	3 (2%)
Fatigue	47 (40%)	16 (14%)	54 (37%)	9 (6%)	37 (28%)	5 (4%)
Neutropenia*	46 (39%)	32 (27%)	37 (25%)	22 (15%)	7 (5%)	2 (1%)
Anaemia	44 (38%)	11 (9%)	48 (33%)	16 (11%)	31 (23%)	9 (7%)
Hypokalaemia	14 (12%)	4 (3%)	32 (22%)	17 (12%)	12 (9%)	3 (2%)

Data are number of patients (%). The table shows grade 3 and 4 adverse events reported in ≥5% of patients whose treatment included nanoliposomal irinotecan with ≥2% incidence versus fluorouracil and folinic acid. *Includes agranulocytosis, febrile neutropenia, granulocytopenia, neutropenia, neutropenic sepsis, decreased neutrophil count, and pancytopenia.

Table 2: Adverse events

See Online for appendix

Discussion

The results of this international, multicentre, randomised, phase 3 study (NAPOLI-1) showed that nanoliposomal irinotecan plus fluorouracil and folinic acid significantly improved the overall survival of patients with metastatic pancreatic ductal adenocarcinoma previously treated with gemcitabine-based therapy. Progression-free survival, objective tumour response, time to treatment failure, and CA19-9 tumour marker response in patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid were also significantly superior to fluorouracil and folinic acid control. In the preplanned analyses of each subgroup, overall survival was increased in patients with a Karnofsky performance score less than 90, a concentration of albumin less than 40 g/L, CA19-9 greater than 40 IU/mL, and liver metastases who were assigned nanoliposomal irinotecan plus fluorouracil and folinic acid, versus those allocated fluorouracil and folinic acid. Furthermore, patients with unfavourable prognostic factors who were assigned nanoliposomal irinotecan plus fluorouracil and folinic acid achieved lower HRs compared with patients assigned fluorouracil and folinic acid, supporting the possible use of nanoliposomal irinotecan plus fluorouracil and folinic acid in this population. Use of post-progression anticancer therapy was generally similar between treatment groups.

Patients assigned nanoliposomal irinotecan monotherapy achieved a median overall survival of 4·9 months, which was consistent with the 5·2 months recorded in a previous phase 2 study of nanoliposomal irinotecan in 40 patients with similar demographics and baseline disease characteristics.[24] Although patients assigned nanoliposomal irinotecan monotherapy did not show superiority in overall survival or progression-free survival compared with those allocated fluorouracil and folinic acid, they had better objective and CA19-9 responses, suggesting that nanoliposomal irinotecan alone has some activity against pancreatic cancer. However, nanoliposomal irinotecan as monotherapy was administered at a higher dose and a lower frequency, which resulted in patients assigned to this group having a higher incidence of gastrointestinal adverse events compared with those allocated the nanoliposomal irinotecan combination regimen.

The choice of fluorouracil and folinic acid as control was based on several factors. First, there is no universally accepted standard treatment for metastatic pancreatic ductal adenocarcinoma following gemcitabine-based therapy. Second, there is a preference for using approved drugs as controls in registration trials, and fluorouracil is an approved agent for treatment of pancreatic cancer. Third, fluorouracil and folinic acid served as the control in CONKO-003,[28] a controlled study in patients with advanced and metastatic pancreatic ductal adenocarcinoma following gemcitabine therapy, thereby setting a precedent. We did not change the control after adding the third arm of nanoliposomal irinotecan plus fluorouracil and folinic acid to our study because 63 patients had been treated with the original schedule and changing it would render the data not available for inclusion in the final analysis. Fourth, no data existed on use of the fluorouracil and folinic acid dose and schedule (FOLFIRI.3) given without irinotecan for treatment of metastatic pancreatic ductal adenocarcinoma, so although it would have been an ideal control arm it would not have historical precedence. Although we acknowledge that the fluorouracil and folinic acid regimen used in the combination arm was different from the control, which is not a standard design with the study drug being added to the same control regimen, the fluorouracil and folinic acid regimen given with nanoliposomal irinotecan was optimised for the combination. It is highly unlikely that the difference in dosing created bias in favour of the investigational arm, because the planned and recorded fluorouracil dose intensities were lower in the investigational arm compared with the control arm. The significantly improved overall survival in patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid, particularly in view of the lower fluorouracil dose intensity compared with the fluorouracil and folinic acid control, supports the benefit of combining nanoliposomal irinotecan with fluorouracil and folinic acid. Finally, it should be noted that the control arm in NAPOLI-1 performed better than did the historical control (CONKO-003)[28] with respect to overall survival (3·3 months in CONKO-003 *vs* 4·2 months in NAPOLI-1).

The NAPOLI-1 and CONKO-003 studies used the same dose and schedule of fluorouracil and folinic acid as control. Moreover, relatively similar median overall survival was reported in both studies (6·1 months with nanoliposomal irinotecan plus fluorouracil and folinic acid in NAPOLI-1 *vs* 5·9 months with oxaliplatin plus fluorouracil and folinic acid in CONKO-003). However, findings of a more recent study comparing an oxaliplatin plus fluorouracil and folinic acid regimen failed to show superiority of overall survival over fluorouracil and folinic acid (n=54 in each arm; 6·1 months *vs* 9·9 months; p=0·02).[29] The reasons for these contradictory results are not clear, but they show the hazards of cross-study comparisons. Despite the similarities between NAPOLI-1 and CONKO-003, there are many differences. For example, the study population in NAPOLI-1 consisted of patients with metastatic pancreatic ductal adenocarcinoma who had progressed after previous gemcitabine-based therapy in a neoadjuvant, adjuvant, locally advanced, or metastatic setting. Patients in CONKO-003 had advanced cancer, which included a heterogeneous group of both metastatic and locally advanced disease (12%)—those with locally advanced disease having a historically documented better survival.[28] Moreover, patients in CONKO-003 had disease that had progressed with first-line gemcitabine monotherapy, and they had to start on the study within 4 weeks of disease

progression. On the other hand, patients in NAPOLI-1 had received gemcitabine either as monotherapy or in combination, for any stage of disease at any time in the past, in many cases having received multiple lines of different treatments; their disease had to be progressing with distant metastases only, irrespective of the previous line of therapy for inclusion. Patients' enrolment for CONKO-003 (n=168) occurred between 2004 and 2007 at 16 sites in Germany.[28] The larger, global, NAPOLI-1 study (n=117 study arm, n=119 control arm after protocol amendment) was conducted between 2012 and 2013 at 76 sites worldwide: more than a third of patients in NAPOLI-1 were not of white ethnic origin. The consistency of the results of NAPOLI-1 in a diverse population at multiple medical centres supports the robustness of the positive outcome. The biggest difference between CONKO-003 and NAPOLI-1 with respect to safety is that, unlike with the oxaliplatin plus fluorouracil and folinic acid regimen, nanoliposomal irinotecan plus fluorouracil and folinic acid is not associated with neuropathy and should be considered as a treatment option for patients who fail the albumin-bound paclitaxel and gemcitabine combination.

With respect to combination therapies containing unencapsulated irinotecan plus fluorouracil and folinic acid in second-line pancreatic cancer, Yoo and colleagues[10] did a randomised phase 2 trial comparing modified versions of FOLFOX (folinic acid, fluorouracil, and oxaliplatin) and FOLFIRI (folinic acid, fluorouracil, and irinotecan) regimens for treatment of gemcitabine-refractory advanced pancreatic cancer. However, in that study, the median overall survival was short (3·5 months with FOLFOX and 3·9 months with FOLFIRI). Various other prospective and retrospective studies of FOLFIRI had small sample sizes and were of single-arm design. Of these, the longest median overall survival—6·6 months—was reported in a small (n=63) non-randomised study that included use of either the FOLFIRI.1 or FOLFIRI.3 regimens.[14] These two regimens differ in that FOLFIRI.3 does not include a fluorouracil bolus and divides the irinotecan dose in two, with the second irinotecan dose being given after fluorouracil and folinic acid administration. The next most effective study was a retrospective analysis of a small population (n=40) of patients with gemcitabine-refractory locally advanced and metastatic cancer, which showed an overall survival of 6·0 months.[11] Although promising, these studies show the need for large randomised, multicentre studies to clearly identify optimum therapy for patients with previously treated metastatic pancreatic cancer. Irinotecan-containing regimens have not been a standard until the advent of the FOLFIRINOX regimen in front-line metastatic pancreatic cancer.

Despite additional toxicity, the quality of life of patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid was not appreciably different from those allocated the fluorouracil and folinic acid control, which is an important measure in patients with metastatic pancreatic ductal adenocarcinoma, who are generally in poor health from the effects of the underlying disease and previous treatments. Little difference between study treatments was reported in clinical benefit response; however, assessment of this outcome is limited because of the burdensome requirements of data collection from these very sick patients. The pain component, based on patient-reported daily diary data, had low compliance (60% [250/417] of intention-to-treat patients eligible). The precision of the clinical benefit response classification rules, which call for 4 consecutive weeks with robust criteria for improvement and less robust criteria for negative clinical benefit, also restricted the assessment.

Adverse events in our study were consistent with those in previous studies of nanoliposomal irinotecan.[22,24,26] Despite a lower delivered dose per cycle and lower observed mean dose intensity of nanoliposomal irinotecan compared with the monotherapy arm, patients in the nanoliposomal irinotecan plus fluorouracil and folinic acid group had a higher incidence of grade 3 or 4 neutropenia than did those receiving nanoliposomal irinotecan monotherapy, which could be attributable to the addition of fluorouracil and folinic acid. However, the incidence of neutropenic sepsis was low in all treatment groups. Conversely, patients in the nanoliposomal irinotecan plus fluorouracil and folinic acid group had less frequent severe diarrhoea than did those receiving nanoliposomal irinotecan monotherapy. The incidence of alopecia was also lower in patients receiving nanoliposomal irinotecan plus fluorouracil and folinic acid than in those in the nanoliposomal irinotecan monotherapy group. The lower nanoliposomal irinotecan dose every 2 weeks—even in combination with fluorouracil and folinic acid—showed a better therapeutic index for severe gastrointestinal events than did nanoliposomal irinotecan at a higher dose every 3 weeks. Most patients tolerated the gastrointestinal adverse events, with around 11% of patients in each nanoliposomal irinotecan-containing treatment group discontinuing treatment because of any adverse event. Of note, there were no reports of hand-foot syndrome, which can be associated with irinotecan and pegylated liposomal doxorubicin therapy, in any study group.

The value of using this nanoliposomal irinotecan-containing regimen immediately after FOLFIRINOX treatment is still not clear, because very few patients received previous irinotecan in this study. Further investigation is needed to answer this question with confidence. Aside from this consideration, nanoliposomal irinotecan plus fluorouracil and folinic acid could potentially become a new standard of care for patients with metastatic pancreatic ductal adenocarcinoma whose disease has progressed following treatment with gemcitabine-based therapy.

In conclusion, the results of this phase 3 study show that nanoliposomal irinotecan in combination with

fluorouracil and folinic acid extends survival and improves other efficacy variables in patients with metastatic pancreatic ductal adenocarcinoma previously treated with gemcitabine-based regimens, and has a manageable and mostly reversible safety profile. However, it is not possible to generalise the results of this study to patients with low performance status, as shown by a Karnofsky performance status less than 70 and albumin less than 30 g/L, and those with increased bilirubin, all of which occur in this disease. Future studies will assess the use of nanoliposomal irinotecan in other settings, including first-line therapy and the role of sequencing various regimens for pancreatic cancer.

Contributors

DDVH, L-TC, AW-G, VM, BB, ND, and EB led and coordinated the study design. All authors recruited patients and contributed to data collection. BB and EB analysed data, which was interpreted by all authors. L-TC, AW-G, DDVH, GB, AD, BB, and EB drafted the manuscript with input from all other authors. All authors have seen and approved the final report.

Declaration of interests

GB, C-FC, JFB, RAH, K-HL, C-PL, GS, TM, and Y-SS declare no competing interests. FB and JTS report personal fees from Merrimack Pharmaceuticals advisory boards, outside the submitted work. L-TC reports other funding from Merrimack Pharmaceuticals, during the conduct of the study; and personal fees from PharmaEngine, outside the submitted work. DC reports grants from AstraZeneca, Amgen, Celgene, Merck Serono, Sanofi, Merrimack Pharmaceuticals, and Medimmune, outside the submitted work. AD reports personal fees from AstraZeneca and Specialized Therapeutics, outside the submitted work; grants and personal fees from Roche, outside the submitted work; and grants from Boehringer Ingelheim, outside the submitted work. GJ reports grants from Merrimack Pharmaceuticals, during the conduct of the study. DDVH reports grants from Merrimack Pharmaceuticals, during the conduct of the study; and personal fees from AlphaMed Consulting, outside the submitted work. AW-G reports grants from Newlink, EMD, Pfizer, AstraZeneca, Precision Biological, BioMed Valley, Halozyme, ChemoCentryx, OncoMEd, ADURO, and Millennium, outside the submitted work; other fees from Pfizer and Merrimack Pharmaceuticals, outside the submitted work; and grants from Merrimack Pharmaceuticals, Prometheus, and CTI, outside the submitted work. EB, ND, and VM are employees of Merrimack Pharmaceuticals and have a patent (Methods for treating pancreatic cancer using combination therapies comprising liposomal irinotecan) issued to Merrimack Pharmaceuticals. BB is employed as statistician at Merrimack Pharmaceuticals.

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U.S. Food and Drug Administration
Protecting and Promoting *Your* Health

FDA News Release

FDA approves new treatment for advanced pancreatic cancer

For Immediate Release

October 22, 2015

Release

The U.S. Food and Drug Administration today approved Onivyde (irinotecan liposome injection), in combination with fluorouracil and leucovorin, to treat patients with advanced (metastatic) pancreatic cancer who have been previously treated with gemcitabine-based chemotherapy.

According to the National Cancer Institute, there will be 48,960 new cases of pancreatic cancer diagnosed in the U.S. in 2015, and nearly the same number of deaths caused by the disease (40,560). Pancreatic cancer can be difficult to diagnose early and treatment options are limited, especially when the disease has spread to other parts of the body (metastatic disease) and surgery to remove the tumor is not possible.

"Many FDA staff who review drug applications are clinicians as well, so it's especially rewarding when we are able to expedite access to new treatments for patients with unmet needs," said Richard Pazdur, M.D., director of the Office of Hematology and Oncology Products in the FDA's Center for Drug Evaluation and Research. "By using the Priority Review designation for the application for Onivyde, patients will have earlier access to a drug that helps extend survival."

The FDA granted Priority Review and orphan drug designations for Onivyde. **Priority review (http://www.fda.gov/ForPatients/Approvals/Fast/ucm405405.htm)** status is granted to applications for drugs that, if approved, would be a significant improvement in safety or effectiveness in the treatment of a serious condition. **Orphan drug designation (http://www.fda.gov/ForIndustry/DevelopingProductsforRareDiseasesConditions/ucm2005525.htm)** provides incentives such as tax credits, user fee waivers, and eligibility for orphan drug exclusivity to assist and encourage the development of drugs for rare diseases.

The effectiveness of Onivyde was demonstrated in a three-arm, randomized, open label study of 417 patients with metastatic pancreatic adenocarcinoma whose cancer had grown after receiving the chemotherapeutic drug gemcitabine or a gemcitabine-based therapy. The study was designed to determine whether patients receiving Onivyde plus fluorouracil/leucovorin or Onivyde alone lived longer than those receiving fluorouracil/leucovorin. Patients treated with Onivyde plus

fluorouracil/leucovorin lived an average of 6.1 months, compared to 4.2 months for those treated with only fluorouracil/leucovorin. There was no survival improvement for those who received only Onivyde compared to those who received fluorouracil/leucovorin.

In addition, patients receiving Onivyde plus fluorouracil/leucovorin had a delay in the amount of time to tumor growth compared to those who received fluorouracil/leucovorin. The average time for those receiving Onivyde plus fluorouracil/leucovorin was 3.1 months compared to 1.5 months for those receiving fluorouracil/leucovorin.

The safety of Onivyde was evaluated in 398 patients who received either Onivyde with fluorouracil/leucovorin, Onivyde alone or fluorouracil/leucovorin. The most common side effects of treatment with Onivyde included diarrhea, fatigue, vomiting, nausea, decreased appetite, inflammation in the mouth (stomatitis) and fever (pyrexia). Onivyde was also found to result in low counts of infection-fighting cells (lymphopenia and neutropenia). Death due to sepsis following neutropenia has been reported in patients treated with Onivyde.

The labeling for Onivyde includes a boxed warning to alert health care professionals about the risks of severe neutropenia and diarrhea. Onivyde is not approved for use as a single agent for the treatment of patients with metastatic pancreatic cancer.

Onivyde is marketed by Merrimack Pharmaceuticals Inc. of Cambridge, Massachusetts.

The FDA, an agency within the U.S. Department of Health and Human Services, promotes and protects the public health by, among other things, assuring the safety, effectiveness, and security of human and veterinary drugs, vaccines and other biological products for human use, and medical devices. The agency also is responsible for the safety and security of our nation's food supply, cosmetics, dietary supplements, products that give off electronic radiation, and for regulating tobacco products.

###

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Consumers

888-INFO-FDA

Related Information

- **FDA: Office of Hematology and Oncology Products (/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ucm091745.htm)**

- **FDA Approved Drugs: Questions and Answers (/Drugs/ResourcesForYou/Consumers/ucm054420.htm)**
- **NCI: Pancreatic Cancer (http://www.cancer.gov/types/pancreatic/patient/pancreatic-treatment-pdq)**

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Safeguarding public health

MHRA

Public Assessment Report

Fluorouracil 50mg/ml Solution for Injection or Infusion

PL 20851/0010
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Medicines and Healthcare products Regulatory Agency

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

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FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

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LAY SUMMARY

The Medicines and Healthcare products Regulatory Agency (MHRA) has granted Wockhardt UK Limited Marketing Authorisations (licences) for the medicinal products Fluorouracil 50mg/ml Solution for Injection or Infusion (PLs 20851/0010-3). These are prescription only medicines [POMs] used to treat various types of cancer, including breast cancer and lung cancer.

The active ingredient fluorouracil interferes with the production of DNA in cells.

The clinical data presented to the MHRA, before licensing, demonstrated that Fluorouracil 50mg/ml Solution for Injection or Infusion is essentially similar or equivalent to the approved product, Fluorouracil 50mg/ml Injection, and as such can be used interchangeably.

No new or unexpected safety concerns arose from these applications and it was decided that the benefits of using Fluorouracil 50mg/ml Solution for Injection or Infusion outweigh the risks, hence Marketing Authorisations have been granted.

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

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SCIENTIFIC DISCUSSION

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INTRODUCTION

Based on the review of the data on quality, safety and efficacy, the UK granted marketing authorisations for the medicinal product Fluorouracil 50mg/ml Solution for Injection or Infusion (PLs 20851/0010-3) to Wockhardt UK Limited on 19 September 2006. The product is a prescription only medicine.

The applications were submitted as abridged applications according to Article 10.1(a)(iii) of Directive 2001/83/EC, claiming essential similarity to Fluorouracil 50mg/ml Injection (PL 04515/0088), which was authorised in January 1996.

Fluorouracil 50mg/ml Solution for Injection or Infusion may be used alone, or in combination, for its palliative action in the management of common malignancies, particularly cancer of the colon and breast, either as a single agent or in combination with other cytotoxic agents.

Fluorouracil is an analogue of uracil, a component of ribonucleic acid. The drug is believed to function as an antimetabolite. After intracellular conversion to the active deoxynucleotide, it interferes with the synthesis of DNA by blocking the conversion of deoxyuridylic acid to thymidylic acid by the cellular enzyme thymidylate synthetase. Fluorouracil may also interfere with RNA synthesis.

PHARMACEUTICAL ASSESSMENT

PL NUMBER:	**PLs 20851/0010-0013**
PRODUCT:	**Fluorouracil 50mg/ml Solution for Injection or Infusion**
ACTIVE:	**Fluorouracil**
COMPANY:	**Wockhardt UK Limited**
E.C. ARTICLE:	**10.1(a)(iii) of Directive 2001/83/EC**
LEGAL STATUS:	**POM**

INTRODUCTION

These are generic applications for Marketing Authorisations in the UK submitted under Article 10.1(a)(iii) of Directive 2001/83/EC, as amended, first paragraph (so-called generic application). The UK reference product is Fluorouracil 50mg/ml Injection, PL 04515/0088 licensed to Mayne Pharma plc on 4 January 1996.

DRUG SUBSTANCE

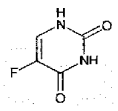
General information

Nomenclature

Chemical name: 5-fluoropyrimidine-2,4(1H,3H)-dione

INN: Fluorouracil

Structure



Molecular formula: $C_4H_3FN_2O_2$

Relative molecular mass: 130.1

General properties

Description: White to almost white crystalline powder

Manufacture

Manufacturer

Suitable manufacturing sites of the active substance have been named.

Manufacturing process

The manufacturing process is referenced to the Certificates of Suitability.

Characterisation

Referenced to the Certificates of Suitability.

Control of active substance

Specification

The finished product manufacturer specification provided covers the requirements of the European Pharmacopoeia. Additional limits are also included in the respective Certificates of Suitability.

Analytical test methods

Relevant details have been provided on the pharmacopoeial and non-pharmacopoeial test methods used.

Analytical test method validation

No validation data has been provided for the pharmacopoeial methods. Other methods are suitably validated.

Batch analyses

Reference has been made to the Certificate of Suitability. In addition Certificates of Analysis has been provided on a batch from each supplier as tested by the finished product manufacturer. All parameters are within specification and show a reasonable degree of comparability.

Reference standards

Details of appropriate reference standards have been provided.

Container closure system

Referenced to the Certificates of Suitability.

Stability

Stability data have been provided from batches manufactured at each of the active substance manufacturing sites. The batches show acceptable stability and support the proposed re-test period and shelf-life.

DRUG PRODUCT

Description and composition of the drug product

The composition of the product is summarised in the table below. The product is a sterile solution filled into colourless glass (type I) vials with a nominal volume of 6ml (250mg/5ml), 10ml (500mg/10ml), 20ml (1000mg/20ml), 50ml (2500mg/50ml) and 100ml (5000mg/100ml). The vials are closed with grey halobutyl rubber stoppers.

Ingredient	Function	Reference
Fluorouracil	Active	Ph.Eur.
Sodium hydroxide	pH adjustment	Ph.Eur.
Water for injections	Solvent	Ph.Eur.
Nitrogen	Inert gas	Ph.Eur.

Pharmaceutical development

Physicochemical and biological properties

It has been stated that the physicochemical and biological parameters are controlled with the specification. Sterility has been identified as an important biological property with data presented on seven batches covering the different presentations demonstrating compliance to sterility.

Manufacturing development

Data has been provided to justify the method of manufacture.

Manufacture

Batch formula

The batch formula is provided for suitable batch sizes.

Manufacturing process

A flow diagram detailing the manufacturing process and in-process control testing has been provided. A written summary of the process has been included.

Control of critical steps

The critical steps are controlled by the proposed in-process controls.

Process validation or evaluation

Satisfactory data provided.

Control of excipients

Specification

Sodium hydroxide, water for injections and nitrogen have monographs in the European Pharmacopoeia. Batch analysis shows compliance with the respective monographs.

No excipients of human or animal origin have been used in the manufacture of the finished product.

Control of drug product

Specification

An acceptable finished product specification has been provided.

Analytical procedures

Details have been provided for the pharmacopoeial and non-pharmacopoeial methods.

Validation

Relevant validation data has been provided and is satisfactory.

Batch analyses

Batch analyses have been provided for six batches. All parameters are within specification and comparable.

Reference standards

Details of appropriate reference standards have been provided.

Container closure system

The injection vials are colourless glass, hydrolytic type I complying with the European Pharmacopoeia and the manufacturer's specification. The vials used have a 6ml, 10ml, 20ml, 50ml and 100ml capacity.

The closures are grey halobutyl rubber. The quality is in compliance with the European Pharmacopoeia and the manufacturer's specification.

The metallic cap is aluminium sheet. This item is not in contact with the finished product.

Relevant specifications have been provided which are considered acceptable. Relevant details of the methods have been provided. Drawings have also been supplied.

Stability

All dosage strengths have the same composition and only differ on fill volume. Consequently, data from one strength can be used as supporting data for the others.

Stability data has been presented on batches of the product packed in the proposed packaging. All vials have been stored upside down at 25°C/60%RH and at 40°C/75%RH. The data support a two year shelf-life with storage below 25°C.

Stability in the infusion fluids has been determined in glucose 5% and sodium chloride 0.9% in glass bottles and polyethylene bags at concentrations of 0.35mg/ml and 15.0mg/ml. All parameters remained constant and within specification under all conditions in all packaging for up to 28 days at 25°C.

ESSENTIAL SIMILARITY

Comparable impurity profiles have been provided for the finished product in comparison to the UK reference product.

SUMMARY OF PRODUCT CHARACTERISTICS
LABELLING
PACKAGE LEAFLETS

Satisfactory.

ASSESSOR'S OVERALL CONCLUSIONS ON QUALITY

Marketing authorisations can be granted.

PRECLINICAL ASSESSMENT

No new preclinical data have been supplied with these applications and none are required.

CLINICAL ASSESSMENT

PL NUMBER:	**PLs 20851/0010-0013**
PRODUCT:	**Fluorouracil 50mg/ml Solution for Injection or Infusion**
ACTIVE:	**Fluorouracil**
COMPANY:	**Wockhardt UK Limited**
E.C. ARTICLE:	**10.1(a)(iii) of Directive 2001/83/EC**
LEGAL STATUS:	**POM**

INTRODUCTION

These are generic applications for UK marketing authorisations.

Fluorouracil injection has been available on the UK market for decades. The product was first licensed in September 1972 to Roche Products Ltd. This licence has since been cancelled although there are a number of generic products currently available.

The applicant has submitted these applications under Article 10.1(a)(iii) of Directive 2001/83/EC, claiming essential similarity to one of the generic products as the reference medicinal product. The reference medicinal product in the UK is:

Product Name:	Fluorouracil 50mg/ml Injection
MAH:	Mayne Pharma plc
MA Number:	PL 04515/0088
Date approved:	4 January 1996

Fluorouracil is a fluorinated derivative of the pyrimidine base, uracil, and belongs to the antimetabolite group of cytostatic agents. This current application is indicated for use either alone or in combination, for its palliative action in the management of common malignancies particularly cancer of the colon and breast, either as single agent or in combination with other cytotoxic agents.

ASSESSMENT

The Summaries of Product Characteristics are satisfactory and compare well with the Summary of Product Characteristics of the generic reference product in the UK.

BIOEQUIVALENCE

Since these products are for parenteral (intravenous or intra-arterial) administration, there are no issues relevant to bioequivalence.

PATIENT INFORMATION LEAFLETS

These are satisfactory and in compliance with Directive 2001/83/EEC, as amended.

RECOMMENDATION

The recommendation is to grant marketing authorisations for these preparations.

OVERALL CONCLUSION AND RISK-BENEFIT ASSESSMENT

QUALITY

The important quality characteristics of Fluorouracil 50mg/ml Solution for Injection or Infusion are well defined and controlled. The specifications and batch analytical results indicate consistency from batch to batch. There are no outstanding quality issues that would have a negative impact on the benefit/risk balance.

PRECLINICAL

No new preclinical data were submitted and none are required for applications of this type.

EFFICACY

No clinical pharmacology data or clinical trials data have been submitted to directly support the claim of essential similarity of the proposed product to the reference product Fluorouracil 50mg/ml Injection (PL 04515/0088). This is acceptable as the formulations are similar and the same routes of administration are proposed.

No new or unexpected safety concerns arise from these applications.

The SPC, PIL and labelling are satisfactory and consistent with those of Fluorouracil 50mg/ml Injection.

RISK-BENEFIT ASSESSMENT

The quality of the products is acceptable and no new preclinical or clinical safety concerns have been identified. The risk-benefit assessment is therefore considered to be favourable.

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

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STEPS TAKEN FOR ASSESSMENT

1	The MHRA received the marketing authorisation applications for Fluorouracil 50mg/ml Solution for Injection or Infusion on 19 August 2005.
2	The MHRA's assessment of the submitted quality data was completed on 25 February 2006.
3	Further information (quality) was requested from the company on 27 February 2006.
4	The MHRA's assessment of the submitted clinical data was completed on 2 March 2006.
5	Further information (clinical) was requested from the company on 2 March 2006.
6	The applicant's response to further information request (clinical) was received on 6 March 2006.
7	The applicant's response to further information request (quality) was sent in a letter dated 7 July 2006.
8	The MHRA completed its assessment of the applications on 19 September 2006.
9	The applications were determined on 19 September 2006.

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

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STEPS TAKEN AFTER AUTHORISATION - SUMMARY

Date submitted	Application type	Scope	Outcome

SUMMARY OF PRODUCT CHARACTERISTICS

1 NAME OF THE MEDICINAL PRODUCT

Fluorouracil 50mg/ml Solution for Injection or Infusion.

2 QUALITATIVE AND QUANTITATIVE COMPOSITION

Each 1ml contains 50 mg of fluorouracil.

Each vial contains 250mg of fluorouracil in 5ml of solution

For full list of excipients, see section 6.1

3 PHARMACEUTICAL FORM

Solution for injection or infusion.

Clear and colourless solution.

4 CLINICAL PARTICULARS

4.1 Therapeutic indications

Fluorouracil may be used alone, or in combination for its palliative action in the management of common malignancies, particularly cancer of the colon and breast, either as a single agent or in combination with other cytotoxic agents.

4.2 Posology and method of administration

Selection of an appropriate dose and treatment regime will depend upon the condition of the patient, the type of carcinoma being treated and whether fluorouracil is to be administered alone or in combination with other therapy. Initial treatment should be given in hospital and the total daily dose should not exceed one gram. It is customary to calculate the dose in accordance with patient's actual weight unless there is obesity, oedema or some other form of abnormal fluid retention such as ascites. In this case, ideal weight should be used as the basis for the calculation. Reduction of the dose is advisable in patients with any of the following:

1) Cachexia

2) Major surgery within preceding 30 days

3) Reduced bone marrow function

4) Impaired hepatic or renal function

Fluorouracil injection can be given by intravenous injection or, intravenous or intra-arterial infusion.

Adult Dose:

The following regimen have been recommended for use as a single agent:

Initial Treatment: This may be in the form of an infusion or an injection, the former usually being preferred because of lesser toxicity.

Intravenous infusion: 15mg/kg bodyweight but not more than 1g per infusion, diluted in 300 - 500ml of 5% glucose or 9% sodium chloride injection and given by intravenous infusion at a rate of 40 drops per minute over four hours. Alternatively the daily dose may be infused over 30 - 60 minutes or may be given as a continuous infusion over 24 hours. The infusion may be repeated daily until there is evidence of toxicity or a total dose of 12 - 15g has been reached.

Intravenous Injection: 12mg/kg bodyweight may be given daily for three days and then, if there is no evidence of toxicity, 6mg/kg on alternate days for three further doses. An alternative regimen is 15mg/kg as a single intravenous injection once a week throughout the course.

Intra-arterial Infusion: 5/7.5mg/kg may be given by 24 hour continuous intra-arterial infusion.

Maintenance Therapy: An initial intensive course may be followed by maintenance therapy providing there are no significant toxic effects. In all instances, toxic side effects must disappear before maintenance therapy is started.

The initial course of fluorouracil can be repeated after an interval of four to six weeks from the last dose or, alternatively, treatment can be continued with intravenous injections of 5-15mg/kg bodyweight at weekly intervals.

This sequence constitutes a course of therapy. Some patients have received up to 30g at a maximum rate of 1g daily. A more recent alternative method is to give 15mg/kg IV once a week throughout the course of treatment. This obviates the need for an initial period of daily administration.

In combination with Irradiation: Irradiation combined with fluorouracil has been found to be useful in the treatment of certain types of metastatic lesions in the lungs and for the relief of pain caused by recurrent, inoperable growth. The standard dose of fluorouracil should be used.

Children:

No recommendations are made regarding the use of fluorouracil in children.

Elderly:

Fluorouracil should be used in the elderly with similar considerations as with normal adult dosages.

4.3 Contraindications

Fluorouracil is contraindicated in seriously debilitated patients or those with bone marrow depression after radiotherapy or treatment with other antineoplastic agents.

Fluorouracil is strictly contraindicated in pregnant or breast feeding women.

Fluorouracil should not be used in the management of non-malignant disease.

4.4 Special warnings and precautions for use

It is recommended that fluorouracil be given only by, or under the strict supervision of, a qualified physician who is conversant with the use of potent antimetabolites.

All patients should be admitted to hospital for initial treatment.

Adequate treatment with fluorouracil is usually followed by leucopenia, the lowest white blood cell (W.B.C) count commonly being observed between the seventh and fourteenth day of the first course, but occasionally being delayed for as long as 20 days. The count usually returns to normal by the thirtieth day. Daily monitoring of platelet and W.B.C count is recommended and treatment should be stopped if platelets fall below 100,000 per mm^3 or the W.B.C. count falls below 3,500 per mm^3. If the total count is less than $2000mm^3$, and especially if there is granulocytopenia, it is recommended that the patient be placed in protective isolation in the hospital and treated with appropriate measures to prevent systemic infection.

Treatment should be stopped at the first sign of oral ulceration or if there is evidence of gastrointestinal side effects such as stomatitis, diarrhoea, bleeding from the G.I. tract or haemorrhage at any site. The ratio between effective and toxic dose is small and therapeutic response is unlikely without some degree of toxicity. Care must be taken therefore, in the selection of patients and adjustment of dosage.

Fluorouracil should be used with caution in patients with reduced renal or liver function or jaundice. Isolated cases of angina, ECG abnormalities and rarely, myocardial infarction have been reported following administration of fluorouracil. Care should be therefore be exercised in treating patients who experience chest pain during courses of treatment, or patients with a history of heart disease.

There have been reports of increased toxicity in patients who have reduced activity/deficiency of the enzyme dihydropyrimidine dehydrogenase (DPD).

4.5 Interaction with other medicinal products and other forms of interaction

Various agents have been reported to biochemically modulate the antitumour efficacy or toxicity of fluorouracil - common drugs include methotrexate, metronidazole, leucovorin as well as allopurinol and cimetidine which can affect the availability of the active drug.

Marked elevations of prothrombin time and INR have been reported in a few patients stabilised on warfarin therapy following initiation of fluorouracil regimes.

A clinically significant interaction has been reported between the antiviral sorivudine and fluorouracil , resulting from inhibition of dihydropyrimidine dehydrogenase by sorivudine or chemically related analogues. Caution should be taken when using fluorouracil in conjunction with medications which may affect dihydropyrimidine dehydrogenase activity.

4.6 Pregnancy and lactation

Fluorouracil is strictly contraindicated in pregnant and breast feeding women.

4.7 Effects on ability to drive and use machines

Not applicable.

4.8 Undesirable effects

Diarrhoea, nausea and vomiting are observed quite commonly during therapy and may be treated symptomatically. An anti-emetic may be given for nausea and vomiting.

Alopecia may be seen in a substantial number of cases, particularly females, but is reversible. Other side effects include dermatitis, pigmentation, changes in nails, ataxia and fever.

There have been reports of chest pain, tachycardia, breathlessness and E.C.G. changes after administration of Fluorouracil. Special attention is advisable in treating patients with a history of heart disease or those who develop chest pain during treatment.

Leucopenia is common and the precautions described above should be followed.

Systemic fluorouracil treatment has been associated with various types of ocular toxicity.

A transient reversible cerebellar syndrome has been reported following fluorouracil treatment. Rarely, a reversible confusional state may occur. Cases of leucoencephalopathy have also been reported.

Additionally, several other reports have been noted including:

Incidences of excessive lacrimation, dacryostenosis, visual changes and photophobia.

Palmar-Plantar Erythrodysesthesia Syndrome has been reported as an unusual complication of high dose bolus or protracted continuous therapy for 5-fluorouracil.

Thrombophlebitis / Vein Tracking.

4.9 Overdose

The symptoms and signs of overdosage are qualitatively similar to the adverse reactions and should be managed as indicated under "Other Undesirable Effects" and "Special Warnings and Precautions".

5 PHARMACOLOGICAL PROPERTIES

5.1 Pharmacodynamic properties

ATC Code: L01B C02, Pyrimidine Analogue

Fluorouracil is an analogue of uracil, a component of ribonucleic acid. The drug is believed to function as an antimetabolite. After intracellular conversion to the active deoxynucleotide, it interferes with the synthesis of DNA by blocking the conversion of deoxyuridylic acid to thymidylic acid by the cellular enzyme thymidylate synthetase. Fluorouracil may also interfere with RNA synthesis.

5.2 Pharmacokinetic properties

After intravenous administration, fluorouracil is distributed through the body water and disappears from the blood within three hours. It is preferentially taken up by actively dividing tissues and tumours after conversion to its nucleotide. Fluorouracil ready enters the C.S.F and brain tissue.

Following IV administration, the plasma elimination half-life averages about 16 minutes and is dose dependent. Following a single intravenous dose of fluorouracil approximately 15% of the dose is excreted unchanged in the urine within six hours; over 90% of this is excreted in the first hour. The remainder is mostly metabolised in the liver by the usual body mechanisms for uracil.

5.3 Preclinical safety data

Preclinical information has not been included because the toxicity profile of fluorouracil has been established after many years of clinical use. Please refer to section four.

6 PHARMACEUTICAL PARTICULARS

6.1 List of excipients

Sodium hydroxide (for pH adjustment)
Water for injections

6.2 Incompatibilities

Fluorouracil is incompatible with carboplatin, cisplatin, cytarabine, diazepam, doxorubicin, other anthracyclines and possibly methotrexate.

Formulated solutions are alkaline and it is recommended that admixture with acidic drugs or preparations should be avoided.

6.3 Shelf life

24 months

After dilution, see section 6.4 Special precautions for storage.

Discard any unused solution immediately after use.

6.4 Special precautions for storage

Unopened: Do not store above 25°C
After dilution: Do not store above 25°C (see 6.3 Shelf Life).

Chemical and physical in use stability of fluorouracil solutions with a concentration of 0.35mg/ml and 15.0mg/ml prepared in glucose 5% or sodium chloride 0.9% has been demonstrated for 28 days at 25°C.

From a microbiological point of view, the product should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would not normally be longer than 24 hours at 2-8°C, unless opening and dilution has taken place in controlled and validated aseptic conditions.

6.5 Nature and contents of container

Packs* of one, five or ten Type I colourless glass 6ml vials stoppered with a halobutyl stopper sealed with an aluminium cap.

*Not all pack sizes may be marketed

6.6 Special precautions for disposal

Cytotoxic Handling Guidelines

Should be administered only by or under the direct supervision of a qualified physician who is experienced in the use of cancer chemotherapeutic agents.

Fluorouracil injection should only be prepared for administration by professionals who have been trained in the safe use of the preparation. Preparation should only be carried out in an aseptic cabinet or suite dedicated for the assembly of cytotoxics.

In the event of spillage, operators should put on gloves, face mask, eye protection and disposable apron and mop up the spilled material with a absorbent material kept in the area for that purpose. The area should then be cleaned and all contaminated material transferred to a cytotoxic spillage bag or bin and sealed for incineration.

Contamination

Fluorouracil is an irritant, contact with skin and mucous membranes should be avoided.

In the event of contact with the skin or eyes, the affected area should be washed with copious amounts of water or normal saline. A bland cream may be used to treat the transient stinging of the skin. Medical advice should be sought if the eyes are affected or if the preparation is inhaled or ingested.

Preparation Guidelines

a) Chemotherapeutic agents should be prepared for administration only by professionals who have been trained in the safe use of the preparation.

b) Operations such as reconstitution of powder and transfer to syringes should be carried out only under aseptic conditions in a suite or cabinet dedicated for the assembly of cytotoxics.

c) The personnel carrying out these procedures should be adequately protected with clothing, gloves and eye shield.

d) Pregnant personnel are advised not to handle chemotherapeutic agents.

Disposal

Syringes, vials and adaptors containing remaining solution, absorbent materials, and any other contaminated material should be placed in a thick plastic bag or other impervious container and incinerated at 700°C.

Diluents

Fluorouracil injection may be diluted with glucose 5% injection or sodium chloride 0.9% injection or water for injections immediately before parenteral use.

7 MARKETING AUTHORISATION HOLDER

Wockhardt UK Limited
Ash Road North
Wrexham LL13 9UF
United Kingdom

8 MARKETING AUTHORISATION NUMBER(S)

PL 20851/0010

9 DATE OF FIRST AUTHORISATION/RENEWAL OF THE AUTHORISATION

19/09/2006

10 DATE OF REVISION OF THE TEXT

19/09/2006

SUMMARY OF PRODUCT CHARACTERISTICS

1 NAME OF THE MEDICINAL PRODUCT

Fluorouracil 50mg/ml Solution for Injection or Infusion.

2 QUALITATIVE AND QUANTITATIVE COMPOSITION

Each 1ml contains 50 mg of fluorouracil.

Each vial contains 500mg of fluorouracil in 10ml of solution

For full list of excipients, see section 6.1

3 PHARMACEUTICAL FORM

Solution for injection or infusion.

Clear and colourless solution.

4 CLINICAL PARTICULARS

4.1 Therapeutic indications

Fluorouracil may be used alone, or in combination for its palliative action in the management of common malignancies, particularly cancer of the colon and breast, either as a single agent or in combination with other cytotoxic agents.

4.2 Posology and method of administration

Selection of an appropriate dose and treatment regime will depend upon the condition of the patient, the type of carcinoma being treated and whether fluorouracil is to be administered alone or in combination with other therapy. Initial treatment should be given in hospital and the total daily dose should not exceed one gram. It is customary to calculate the dose in accordance with patient's actual weight unless there is obesity, oedema or some other form of abnormal fluid retention such as ascites. In this case, ideal weight should be used as the basis for the calculation. Reduction of the dose is advisable in patients with any of the following:

1) Cachexia

2) Major surgery within preceding 30 days

3) Reduced bone marrow function

4) Impaired hepatic or renal function

Fluorouracil injection can be given by intravenous injection or, intravenous or intra-arterial infusion.

Adult Dose:

The following regimen have been recommended for use as a single agent:

Initial Treatment: This may be in the form of an infusion or an injection, the former usually being preferred because of lesser toxicity.

Intravenous infusion: 15mg/kg bodyweight but not more than 1g per infusion, diluted in 300 - 500ml of 5% glucose or 9% sodium chloride injection and given by intravenous infusion at a rate of 40 drops per minute over four hours. Alternatively the daily dose may be infused over 30 - 60 minutes or may be given as a continuous infusion over 24 hours. The infusion may be repeated daily until there is evidence of toxicity or a total dose of 12 - 15g has been reached.

Intravenous Injection: l2mg/kg bodyweight may be given daily for three days and then, if there is no evidence of toxicity, 6mg/kg on alternate days for three further doses. An alternative regimen is 15mg/kg as a single intravenous injection once a week throughout the course.

Intra-arterial Infusion: 5/7.5mg/kg may be given by 24 hour continuous intra-arterial infusion.

Maintenance Therapy: An initial intensive course may be followed by maintenance therapy providing there are no significant toxic effects. In all instances, toxic side effects must disappear before maintenance therapy is started.

The initial course of fluorouracil can be repeated after an interval of four to six weeks from the last dose or, alternatively, treatment can be continued with intravenous injections of 5-15mg/kg bodyweight at weekly intervals.

This sequence constitutes a course of therapy. Some patients have received up to 30g at a maximum rate of 1g daily. A more recent alternative method is to give 15mg/kg IV once a week throughout the course of treatment. This obviates the need for an initial period of daily administration.

In combination with Irradiation: Irradiation combined with fluorouracil has been found to be useful in the treatment of certain types of metastatic lesions in the lungs and for the relief of pain caused by recurrent, inoperable growth. The standard dose of fluorouracil should be used.

Children:

No recommendations are made regarding the use of fluorouracil in children.

Elderly:

Fluorouracil should be used in the elderly with similar considerations as with normal adult dosages.

4.3 Contraindications

Fluorouracil is contraindicated in seriously debilitated patients or those with bone marrow depression after radiotherapy or treatment with other antineoplastic agents.

Fluorouracil is strictly contraindicated in pregnant or breast feeding women.

Fluorouracil should not be used in the management of non-malignant disease.

4.4 Special warnings and precautions for use

It is recommended that fluorouracil be given only by, or under the strict supervision of, a qualified physician who is conversant with the use of potent antimetabolites.

All patients should be admitted to hospital for initial treatment.

Adequate treatment with fluorouracil is usually followed by leucopenia, the lowest white blood cell (W.B.C) count commonly being observed between the seventh and fourteenth day of the first course, but occasionally being delayed for as long as 20 days. The count usually returns to normal by the thirtieth day. Daily monitoring of platelet and W.B.C count is recommended and treatment should be stopped if platelets fall below 100,000 per mm^3 or the W.B.C. count falls below 3,500 per mm^3. If the total count is less than $2000mm^3$, and especially if there is granulocytopenia, it is recommended that the patient be placed in protective isolation in the hospital and treated with appropriate measures to prevent systemic infection.

Treatment should be stopped at the first sign of oral ulceration or if there is evidence of gastrointestinal side effects such as stomatitis, diarrhoea, bleeding from the G.I. tract or haemorrhage at any site. The ratio between effective and toxic dose is small and therapeutic response is unlikely without some degree of toxicity. Care must be taken therefore, in the selection of patients and adjustment of dosage.

Fluorouracil should be used with caution in patients with reduced renal or liver function or jaundice. Isolated cases of angina, ECG abnormalities and rarely, myocardial infarction have been reported following administration of fluorouracil. Care should be therefore be exercised in treating patients who experience chest pain during courses of treatment, or patients with a history of heart disease.

There have been reports of increased toxicity in patients who have reduced activity/deficiency of the enzyme dihydropyrimidine dehydrogenase (DPD).

4.5 Interaction with other medicinal products and other forms of interaction

Various agents have been reported to biochemically modulate the antitumour efficacy or toxicity of fluorouracil - common drugs include methotrexate, metronidazole,

leucovorin as well as allopurinol and cimetidine which can affect the availability of the active drug.

Marked elevations of prothrombin time and INR have been reported in a few patients stabilised on warfarin therapy following initiation of fluorouracil regimes.

A clinically significant interaction has been reported between the antiviral sorivudine and fluorouracil , resulting from inhibition of dihydropyrimidine dehydrogenase by sorivudine or chemically related analogues. Caution should be taken when using fluorouracil in conjunction with medications which may affect dihydropyrimidine dehydrogenase activity.

4.6 Pregnancy and lactation

Fluorouracil is strictly contraindicated in pregnant and breast feeding women.

4.7 Effects on ability to drive and use machines

Not applicable.

4.8 Undesirable effects

Diarrhoea, nausea and vomiting are observed quite commonly during therapy and may be treated symptomatically. An anti-emetic may be given for nausea and vomiting.

Alopecia may be seen in a substantial number of cases, particularly females, but is reversible. Other side effects include dermatitis, pigmentation, changes in nails, ataxia and fever.

There have been reports of chest pain, tachycardia, breathlessness and E.C.G. changes after administration of Fluorouracil. Special attention is advisable in treating patients with a history of heart disease or those who develop chest pain during treatment.

Leucopenia is common and the precautions described above should be followed.

Systemic fluorouracil treatment has been associated with various types of ocular toxicity.

A transient reversible cerebellar syndrome has been reported following fluorouracil treatment. Rarely, a reversible confusional state may occur. Cases of leucoencephalopathy have also been reported.

Additionally, several other reports have been noted including:

Incidences of excessive lacrimation, dacryostenosis, visual changes and photophobia.

Palmar-Plantar Erythrodysesthesia Syndrome has been reported as an unusual complication of high dose bolus or protracted continuous therapy for 5-fluorouracil.

Thrombophlebitis / Vein Tracking.

4.9 Overdose

The symptoms and signs of overdosage are qualitatively similar to the adverse reactions and should be managed as indicated under "Other Undesirable Effects" and "Special Warnings and Precautions".

5 PHARMACOLOGICAL PROPERTIES

5.1 Pharmacodynamic properties

ATC Code: L01B C02, Pyrimidine Analogue

Fluorouracil is an analogue of uracil, a component of ribonucleic acid. The drug is believed to function as an antimetabolite. After intracellular conversion to the active deoxynucleotide, it interferes with the synthesis of DNA by blocking the conversion of deoxyuridylic acid to thymidylic acid by the cellular enzyme thymidylate synthetase. Fluorouracil may also interfere with RNA synthesis.

5.2 Pharmacokinetic properties

After intravenous administration, fluorouracil is distributed through the body water and disappears from the blood within three hours. It is preferentially taken up by actively dividing tissues and tumours after conversion to its nucleotide. Fluorouracil ready enters the C.S.F and brain tissue.

Following IV administration, the plasma elimination half-life averages about 16 minutes and is dose dependent. Following a single intravenous dose of fluorouracil approximately 15% of the dose is excreted unchanged in the urine within six hours; over 90% of this is excreted in the first hour. The remainder is mostly metabolised in the liver by the usual body mechanisms for uracil.

5.3 Preclinical safety data

Preclinical information has not been included because the toxicity profile of fluorouracil has been established after many years of clinical use. Please refer to section four.

6 PHARMACEUTICAL PARTICULARS

6.1 List of excipients

Sodium hydroxide (for pH adjustment)
Water for injections

6.2 Incompatibilities

Fluorouracil is incompatible with carboplatin, cisplatin, cytarabine, diazepam, doxorubicin, other anthracyclines and possibly methotrexate.

Formulated solutions are alkaline and it is recommended that admixture with acidic drugs or preparations should be avoided.

6.3 Shelf life

24 months

After dilution, see section 6.4 Special precautions for storage.

Discard any unused solution immediately after use.

6.4 Special precautions for storage

Unopened: Do not store above 25°C
After dilution: Do not store above 25°C (see 6.3 Shelf Life).

Chemical and physical in use stability of fluorouracil solutions with a concentration of 0.35mg/ml and 15.0mg/ml prepared in glucose 5% or sodium chloride 0.9% has been demonstrated for 28 days at 25°C.

From a microbiological point of view, the product should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would not normally be longer than 24 hours at 2-8°C, unless opening and dilution has taken place in controlled and validated aseptic conditions.

6.5 Nature and contents of container

Packs* of one, five or ten Type I colourless glass 10ml vials stoppered with a halobutyl stopper sealed with an aluminium cap.

*Not all pack sizes may be marketed

6.6 Special precautions for disposal

Cytotoxic Handling Guidelines

Should be administered only by or under the direct supervision of a qualified physician who is experienced in the use of cancer chemotherapeutic agents.

Fluorouracil injection should only be prepared for administration by professionals who have been trained in the safe use of the preparation. Preparation should only be carried out in an aseptic cabinet or suite dedicated for the assembly of cytotoxics.

In the event of spillage, operators should put on gloves, face mask, eye protection and disposable apron and mop up the spilled material with a absorbent material kept in the area for that purpose. The area should then be cleaned and all contaminated material transferred to a cytotoxic spillage bag or bin and sealed for incineration.

Contamination

Fluorouracil is an irritant, contact with skin and mucous membranes should be avoided.

In the event of contact with the skin or eyes, the affected area should be washed with copious amounts of water or normal saline. A bland cream may be used to treat the transient stinging of the skin. Medical advice should be sought if the eyes are affected or if the preparation is inhaled or ingested.

Preparation Guidelines

a) Chemotherapeutic agents should be prepared for administration only by professionals who have been trained in the safe use of the preparation.

b) Operations such as reconstitution of powder and transfer to syringes should be carried out only under aseptic conditions in a suite or cabinet dedicated for the assembly of cytotoxics.

c) The personnel carrying out these procedures should be adequately protected with clothing, gloves and eye shield.

d) Pregnant personnel are advised not to handle chemotherapeutic agents.

Disposal

Syringes, vials and adaptors containing remaining solution, absorbent materials, and any other contaminated material should be placed in a thick plastic bag or other impervious container and incinerated at 700°C.

Diluents

Fluorouracil injection may be diluted with glucose 5% injection or sodium chloride 0.9% injection or water for injections immediately before parenteral use.

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SUMMARY OF PRODUCT CHARACTERISTICS

1 NAME OF THE MEDICINAL PRODUCT

Fluorouracil 50mg/ml Solution for Injection or Infusion.

2 QUALITATIVE AND QUANTITATIVE COMPOSITION

Each 1ml contains 50 mg of fluorouracil.

Each vial contains 1000mg of fluorouracil in 20ml of solution

For full list of excipients, see section 6.1

3 PHARMACEUTICAL FORM

Solution for injection or infusion.

Clear and colourless solution.

4 CLINICAL PARTICULARS

4.1 Therapeutic indications

Fluorouracil may be used alone, or in combination for its palliative action in the management of common malignancies, particularly cancer of the colon and breast, either as a single agent or in combination with other cytotoxic agents.

4.2 Posology and method of administration

Selection of an appropriate dose and treatment regime will depend upon the condition of the patient, the type of carcinoma being treated and whether fluorouracil is to be administered alone or in combination with other therapy. Initial treatment should be given in hospital and the total daily dose should not exceed one gram. It is customary to calculate the dose in accordance with patient's actual weight unless there is obesity, oedema or some other form of abnormal fluid retention such as ascites. In this case, ideal weight should be used as the basis for the calculation. Reduction of the dose is advisable in patients with any of the following:

1) Cachexia

2) Major surgery within preceding 30 days

3) Reduced bone marrow function

4) Impaired hepatic or renal function

Fluorouracil injection can be given by intravenous injection or, intravenous or intra-arterial infusion.

Adult Dose:

The following regimen have been recommended for use as a single agent:

Initial Treatment: This may be in the form of an infusion or an injection, the former usually being preferred because of lesser toxicity.

Intravenous infusion: 15mg/kg bodyweight but not more than 1g per infusion, diluted in 300 - 500ml of 5% glucose or 9% sodium chloride injection and given by intravenous infusion at a rate of 40 drops per minute over four hours. Alternatively the daily dose may be infused over 30 - 60 minutes or may be given as a continuous infusion over 24 hours. The infusion may be repeated daily until there is evidence of toxicity or a total dose of 12 - 15g has been reached.

Intravenous Injection: 12mg/kg bodyweight may be given daily for three days and then, if there is no evidence of toxicity, 6mg/kg on alternate days for three further doses. An alternative regimen is 15mg/kg as a single intravenous injection once a week throughout the course.

Intra-arterial Infusion: 5/7.5mg/kg may be given by 24 hour continuous intra-arterial infusion.

Maintenance Therapy: An initial intensive course may be followed by maintenance therapy providing there are no significant toxic effects. In all instances, toxic side effects must disappear before maintenance therapy is started.

The initial course of fluorouracil can be repeated after an interval of four to six weeks from the last dose or, alternatively, treatment can be continued with intravenous injections of 5-15mg/kg bodyweight at weekly intervals.

This sequence constitutes a course of therapy. Some patients have received up to 30g at a maximum rate of 1g daily. A more recent alternative method is to give 15mg/kg IV once a week throughout the course of treatment. This obviates the need for an initial period of daily administration.

In combination with Irradiation: Irradiation combined with fluorouracil has been found to be useful in the treatment of certain types of metastatic lesions in the lungs and for the relief of pain caused by recurrent, inoperable growth. The standard dose of fluorouracil should be used.

Children:

No recommendations are made regarding the use of fluorouracil in children.

Elderly:

Fluorouracil should be used in the elderly with similar considerations as with normal adult dosages.

4.3 Contraindications

Fluorouracil is contraindicated in seriously debilitated patients or those with bone marrow depression after radiotherapy or treatment with other antineoplastic agents.

Fluorouracil is strictly contraindicated in pregnant or breast feeding women.

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All patients should be admitted to hospital for initial treatment.

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Treatment should be stopped at the first sign of oral ulceration or if there is evidence of gastrointestinal side effects such as stomatitis, diarrhoea, bleeding from the G.I. tract or haemorrhage at any site. The ratio between effective and toxic dose is small and therapeutic response is unlikely without some degree of toxicity. Care must be taken therefore, in the selection of patients and adjustment of dosage.

Fluorouracil should be used with caution in patients with reduced renal or liver function or jaundice. Isolated cases of angina, ECG abnormalities and rarely, myocardial infarction have been reported following administration of fluorouracil. Care should be therefore be exercised in treating patients who experience chest pain during courses of treatment, or patients with a history of heart disease.

There have been reports of increased toxicity in patients who have reduced activity/deficiency of the enzyme dihydropyrimidine dehydrogenase (DPD).

4.5 Interaction with other medicinal products and other forms of interaction

Various agents have been reported to biochemically modulate the antitumour efficacy or toxicity of fluorouracil - common drugs include methotrexate, metronidazole, leucovorin as well as allopurinol and cimetidine which can affect the availability of the active drug.

Marked elevations of prothrombin time and INR have been reported in a few patients stabilised on warfarin therapy following initiation of fluorouracil regimes.

A clinically significant interaction has been reported between the antiviral sorivudine and fluorouracil , resulting from inhibition of dihydropyrimidine dehydrogenase by sorivudine or chemically related analogues. Caution should be taken when using fluorouracil in conjunction with medications which may affect dihydropyrimidine dehydrogenase activity.

4.6 Pregnancy and lactation

Fluorouracil is strictly contraindicated in pregnant and breast feeding women.

4.7 Effects on ability to drive and use machines

Not applicable.

4.8 Undesirable effects

Diarrhoea, nausea and vomiting are observed quite commonly during therapy and may be treated symptomatically. An anti-emetic may be given for nausea and vomiting.

Alopecia may be seen in a substantial number of cases, particularly females, but is reversible. Other side effects include dermatitis, pigmentation, changes in nails, ataxia and fever.

There have been reports of chest pain, tachycardia, breathlessness and E.C.G. changes after administration of Fluorouracil. Special attention is advisable in treating patients with a history of heart disease or those who develop chest pain during treatment.

Leucopenia is common and the precautions described above should be followed.

Systemic fluorouracil treatment has been associated with various types of ocular toxicity.

A transient reversible cerebellar syndrome has been reported following fluorouracil treatment. Rarely, a reversible confusional state may occur. Cases of leucoencephalopathy have also been reported.

Additionally, several other reports have been noted including:

Incidences of excessive lacrimation, dacryostenosis, visual changes and photophobia.

Palmar-Plantar Erythrodysesthesia Syndrome has been reported as an unusual complication of high dose bolus or protracted continuous therapy for 5-fluorouracil.

Thrombophlebitis / Vein Tracking.

4.9 Overdose

The symptoms and signs of overdosage are qualitatively similar to the adverse reactions and should be managed as indicated under "Other Undesirable Effects" and "Special Warnings and Precautions".

5 PHARMACOLOGICAL PROPERTIES

5.1 Pharmacodynamic properties

ATC Code: L01B C02, Pyrimidine Analogue

Fluorouracil is an analogue of uracil, a component of ribonucleic acid. The drug is believed to function as an antimetabolite. After intracellular conversion to the active deoxynucleotide, it interferes with the synthesis of DNA by blocking the conversion of deoxyuridylic acid to thymidylic acid by the cellular enzyme thymidylate synthetase. Fluorouracil may also interfere with RNA synthesis.

5.2 Pharmacokinetic properties

After intravenous administration, fluorouracil is distributed through the body water and disappears from the blood within three hours. It is preferentially taken up by actively dividing tissues and tumours after conversion to its nucleotide. Fluorouracil ready enters the C.S.F and brain tissue.

Following IV administration, the plasma elimination half-life averages about 16 minutes and is dose dependent. Following a single intravenous dose of fluorouracil approximately 15% of the dose is excreted unchanged in the urine within six hours; over 90% of this is excreted in the first hour. The remainder is mostly metabolised in the liver by the usual body mechanisms for uracil.

5.3 Preclinical safety data

Preclinical information has not been included because the toxicity profile of fluorouracil has been established after many years of clinical use. Please refer to section four.

6 PHARMACEUTICAL PARTICULARS

6.1 List of excipients

Sodium hydroxide (for pH adjustment)
Water for injections

6.2 Incompatibilities

Fluorouracil is incompatible with carboplatin, cisplatin, cytarabine, diazepam, doxorubicin, other anthracyclines and possibly methotrexate.

Formulated solutions are alkaline and it is recommended that admixture with acidic drugs or preparations should be avoided.

6.3 Shelf life

24 months

After dilution, see section 6.4 Special precautions for storage.

Discard any unused solution immediately after use.

6.4 Special precautions for storage

Unopened: Do not store above 25°C
After dilution: Do not store above 25°C (see 6.3 Shelf Life).

Chemical and physical in use stability of fluorouracil solutions with a concentration of 0.35mg/ml and 15.0mg/ml prepared in glucose 5% or sodium chloride 0.9% has been demonstrated for 28 days at 25°C.

From a microbiological point of view, the product should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would not normally be longer than 24 hours at 2-8°C, unless opening and dilution has taken place in controlled and validated aseptic conditions.

6.5 Nature and contents of container

Packs* of one, five or ten Type I colourless glass 20ml vials stoppered with a halobutyl stopper sealed with an aluminium cap.

*Not all pack sizes may be marketed

6.6 Special precautions for disposal

Cytotoxic Handling Guidelines

Should be administered only by or under the direct supervision of a qualified physician who is experienced in the use of cancer chemotherapeutic agents.

Fluorouracil injection should only be prepared for administration by professionals who have been trained in the safe use of the preparation. Preparation should only be carried out in an aseptic cabinet or suite dedicated for the assembly of cytotoxics.

In the event of spillage, operators should put on gloves, face mask, eye protection and disposable apron and mop up the spilled material with a absorbent material kept in the area for that purpose. The area should then be cleaned and all contaminated material transferred to a cytotoxic spillage bag or bin and sealed for incineration.

Contamination

Fluorouracil is an irritant, contact with skin and mucous membranes should be avoided.

In the event of contact with the skin or eyes, the affected area should be washed with copious amounts of water or normal saline. A bland cream may be used to treat the transient stinging of the skin. Medical advice should be sought if the eyes are affected or if the preparation is inhaled or ingested.

Preparation Guidelines

a) Chemotherapeutic agents should be prepared for administration only by professionals who have been trained in the safe use of the preparation.

b) Operations such as reconstitution of powder and transfer to syringes should be carried out only under aseptic conditions in a suite or cabinet dedicated for the assembly of cytotoxics.

c) The personnel carrying out these procedures should be adequately protected with clothing, gloves and eye shield.

d) Pregnant personnel are advised not to handle chemotherapeutic agents.

Disposal

Syringes, vials and adaptors containing remaining solution, absorbent materials, and any other contaminated material should be placed in a thick plastic bag or other impervious container and incinerated at 700°C.

Diluents

Fluorouracil injection may be diluted with glucose 5% injection or sodium chloride 0.9% injection or water for injections immediately before parenteral use.

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10 DATE OF REVISION OF THE TEXT

19/09/2006

SUMMARY OF PRODUCT CHARACTERISTICS

1. NAME OF THE MEDICINAL PRODUCT

Fluorouracil 50mg/ml Solution for Injection or Infusion.

2. QUALITATIVE AND QUANTITATIVE COMPOSITION

Each 1ml contains 50 mg of fluorouracil.

Each vial contains 5000mg of fluorouracil in 100ml of solution

For full list of excipients, see section 6.1

3. PHARMACEUTICAL FORM

Solution for injection or infusion.

Clear and colourless solution.

4 CLINICAL PARTICULARS

4.1. Therapeutic indications

Fluorouracil may be used alone, or in combination for its palliative action in the management of common malignancies, particularly cancer of the colon and breast, either as a single agent or in combination with other cytotoxic agents.

4.2 Posology and method of administration

Selection of an appropriate dose and treatment regime will depend upon the condition of the patient, the type of carcinoma being treated and whether fluorouracil is to be administered alone or in combination with other therapy. Initial treatment should be given in hospital and the total daily dose should not exceed one gram. It is customary to calculate the dose in accordance with patient's actual weight unless there is obesity, oedema or some other form of abnormal fluid retention such as ascites. In this case, ideal weight should be used as the basis for the calculation. Reduction of the dose is advisable in patients with any of the following:

1) Cachexia

2) Major surgery within preceding 30 days

3) Reduced bone marrow function

4) Impaired hepatic or renal function

Fluorouracil injection can be given by intravenous injection or, intravenous or intra-arterial infusion.

Adult Dose:

The following regimen have been recommended for use as a single agent:

Initial Treatment: This may be in the form of an infusion or an injection, the former usually being preferred because of lesser toxicity.

Intravenous infusion: 15mg/kg bodyweight but not more than 1g per infusion, diluted in 300 - 500ml of 5% glucose or 9% sodium chloride injection and given by intravenous infusion at a rate of 40 drops per minute over four hours. Alternatively the daily dose may be infused over 30 - 60 minutes or may be given as a continuous infusion over 24 hours. The infusion may be repeated daily until there is evidence of toxicity or a total dose of 12 - 15g has been reached.

Intravenous Injection: 12mg/kg bodyweight may be given daily for three days and then, if there is no evidence of toxicity, 6mg/kg on alternate days for three further doses. An alternative regimen is 15mg/kg as a single intravenous injection once a week throughout the course.

Intra-arterial Infusion: 5/7.5mg/kg may be given by 24 hour continuous intra-arterial infusion.

Maintenance Therapy: An initial intensive course may be followed by maintenance therapy providing there are no significant toxic effects. In all instances, toxic side effects must disappear before maintenance therapy is started.

The initial course of fluorouracil can be repeated after an interval of four to six weeks from the last dose or, alternatively, treatment can be continued with intravenous injections of 5-15mg/kg bodyweight at weekly intervals.

This sequence constitutes a course of therapy. Some patients have received up to 30g at a maximum rate of 1g daily. A more recent alternative method is to give 15mg/kg IV once a week throughout the course of treatment. This obviates the need for an initial period of daily administration.

In combination with Irradiation: Irradiation combined with fluorouracil has been found to be useful in the treatment of certain types of metastatic lesions in the lungs and for the relief of pain caused by recurrent, inoperable growth. The standard dose of fluorouracil should be used.

Children:

No recommendations are made regarding the use of fluorouracil in children.

Elderly:

Fluorouracil should be used in the elderly with similar considerations as with normal adult dosages.

4.3 Contraindications

Fluorouracil is contraindicated in seriously debilitated patients or those with bone marrow depression after radiotherapy or treatment with other antineoplastic agents.

Fluorouracil is strictly contraindicated in pregnant or breast feeding women.

Fluorouracil should not be used in the management of non-malignant disease.

4.4. Special warnings and precautions for use

It is recommended that fluorouracil be given only by, or under the strict supervision of, a qualified physician who is conversant with the use of potent antimetabolites.

All patients should be admitted to hospital for initial treatment.

Adequate treatment with fluorouracil is usually followed by leucopenia, the lowest white blood cell (W.B.C) count commonly being observed between the seventh and fourteenth day of the first course, but occasionally being delayed for as long as 20 days. The count usually returns to normal by the thirtieth day. Daily monitoring of platelet and W.B.C count is recommended and treatment should be stopped if platelets fall below 100,000 per mm^3 or the W.B.C. count falls below 3,500 per mm^3. If the total count is less than $2000mm^3$, and especially if there is granulocytopenia, it is recommended that the patient be placed in protective isolation in the hospital and treated with appropriate measures to prevent systemic infection.

Treatment should be stopped at the first sign of oral ulceration or if there is evidence of gastrointestinal side effects such as stomatitis, diarrhoea, bleeding from the G.I. tract or haemorrhage at any site. The ratio between effective and toxic dose is small and therapeutic response is unlikely without some degree of toxicity. Care must be taken therefore, in the selection of patients and adjustment of dosage.

Fluorouracil should be used with caution in patients with reduced renal or liver function or jaundice. Isolated cases of angina, ECG abnormalities and rarely, myocardial infarction have been reported following administration of fluorouracil. Care should be therefore be exercised in treating patients who experience chest pain during courses of treatment, or patients with a history of heart disease.

There have been reports of increased toxicity in patients who have reduced activity/deficiency of the enzyme dihydropyrimidine dehydrogenase (DPD).

4.5. Interaction with other medicinal products and other forms of interaction

Various agents have been reported to biochemically modulate the antitumour efficacy or toxicity of fluorouracil - common drugs include methotrexate, metronidazole, leucovorin as well as allopurinol and cimetidine which can affect the availability of the active drug.

Marked elevations of prothrombin time and INR have been reported in a few patients stabilised on warfarin therapy following initiation of fluorouracil regimes.

A clinically significant interaction has been reported between the antiviral sorivudine and fluorouracil , resulting from inhibition of dihydropyrimidine dehydrogenase by sorivudine or chemically related analogues. Caution should be taken when using fluorouracil in conjunction with medications which may affect dihydropyrimidine dehydrogenase activity.

4.6 Pregnancy and lactation

Fluorouracil is strictly contraindicated in pregnant and breast feeding women.

4.7 Effects on ability to drive and use machines

Not applicable.

4.8 Undesirable effects

Diarrhoea, nausea and vomiting are observed quite commonly during therapy and may be treated symptomatically. An anti-emetic may be given for nausea and vomiting.

Alopecia may be seen in a substantial number of cases, particularly females, but is reversible. Other side effects include dermatitis, pigmentation, changes in nails, ataxia and fever.

There have been reports of chest pain, tachycardia, breathlessness and E.C.G. changes after administration of Fluorouracil. Special attention is advisable in treating patients with a history of heart disease or those who develop chest pain during treatment.

Leucopenia is common and the precautions described above should be followed.

Systemic fluorouracil treatment has been associated with various types of ocular toxicity.

A transient reversible cerebellar syndrome has been reported following fluorouracil treatment. Rarely, a reversible confusional state may occur. Cases of leucoencephalopathy have also been reported.

Additionally, several other reports have been noted including:

Incidences of excessive lacrimation, dacryostenosis, visual changes and photophobia.

Palmar-Plantar Erythrodysesthesia Syndrome has been reported as an unusual complication of high dose bolus or protracted continuous therapy for 5-fluorouracil.

Thrombophlebitis / Vein Tracking.

4.9 Overdose

The symptoms and signs of overdosage are qualitatively similar to the adverse reactions and should be managed as indicated under "Other Undesirable Effects" and "Special Warnings and Precautions".

5 PHARMACOLOGICAL PROPERTIES

5.1. Pharmacodynamic properties

ATC Code: L01B C02, Pyrimidine Analogue

Fluorouracil is an analogue of uracil, a component of ribonucleic acid. The drug is believed to function as an antimetabolite. After intracellular conversion to the active deoxynucleotide, it interferes with the synthesis of DNA by blocking the conversion of deoxyuridylic acid to thymidylic acid by the cellular enzyme thymidylate synthetase. Fluorouracil may also interfere with RNA synthesis.

5.2 Pharmacokinetic properties

After intravenous administration, fluorouracil is distributed through the body water and disappears from the blood within three hours. It is preferentially taken up by actively dividing tissues and tumours after conversion to its nucleotide. Fluorouracil ready enters the C.S.F and brain tissue.

Following IV administration, the plasma elimination half-life averages about 16 minutes and is dose dependent. Following a single intravenous dose of fluorouracil approximately 15% of the dose is excreted unchanged in the urine within six hours; over 90% of this is excreted in the first hour. The remainder is mostly metabolised in the liver by the usual body mechanisms for uracil.

5.3 Preclinical safety data

Preclinical information has not been included because the toxicity profile of fluorouracil has been established after many years of clinical use. Please refer to section four.

6 PHARMACEUTICAL PARTICULARS

6.1. List of excipients

Sodium hydroxide (for pH adjustment)
Water for injections

6.2. Incompatibilities

Fluorouracil is incompatible with carboplatin, cisplatin, cytarabine, diazepam, doxorubicin, other anthracyclines and possibly methotrexate.

Formulated solutions are alkaline and it is recommended that admixture with acidic drugs or preparations should be avoided.

6.3 Shelf life

24 months

After dilution, see section 6.4 Special precautions for storage.

Discard any unused solution immediately after use.

6.4 Special precautions for storage

Unopened: Do not store above 25°C
After dilution: Do not store above 25°C (see 6.3 Shelf Life).

Chemical and physical in use stability of fluorouracil solutions with a concentration of 0.35mg/ml and 15.0mg/ml prepared in glucose 5% or sodium chloride 0.9% has been demonstrated for 28 days at 25°C.

From a microbiological point of view, the product should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would not normally be longer than 24 hours at 2-8°C, unless opening and dilution has taken place in controlled and validated aseptic conditions.

6.5 Nature and contents of container

Packs* of one, five or ten Type I colourless glass 20ml vials stoppered with a halobutyl stopper sealed with an aluminium cap.

*Not all pack sizes may be marketed

6.6 Special precautions for disposal

Cytotoxic Handling Guidelines

Should be administered only by or under the direct supervision of a qualified physician who is experienced in the use of cancer chemotherapeutic agents.

Fluorouracil injection should only be prepared for administration by professionals who have been trained in the safe use of the preparation. Preparation should only be carried out in an aseptic cabinet or suite dedicated for the assembly of cytotoxics.

In the event of spillage, operators should put on gloves, face mask, eye protection and disposable apron and mop up the spilled material with an absorbent material kept in the area for that purpose. The area should then be cleaned and all contaminated material transferred to a cytotoxic spillage bag or bin and sealed for incineration.

Contamination

Fluorouracil is an irritant, contact with skin and mucous membranes should be avoided.

In the event of contact with the skin or eyes, the affected area should be washed with copious amounts of water or normal saline. A bland cream may be used to treat the transient stinging of the skin. Medical advice should be sought if the eyes are affected or if the preparation is inhaled or ingested.

Preparation Guidelines

a) Chemotherapeutic agents should be prepared for administration only by professionals who have been trained in the safe use of the preparation.

b) Operations such as reconstitution of powder and transfer to syringes should be carried out only under aseptic conditions in a suite or cabinet dedicated for the assembly of cytotoxics.

c) The personnel carrying out these procedures should be adequately protected with clothing, gloves and eye shield.

d) Pregnant personnel are advised not to handle chemotherapeutic agents.

Disposal

Syringes, vials and adaptors containing remaining solution, absorbent materials, and any other contaminated material should be placed in a thick plastic bag or other impervious container and incinerated at 700°C.

Diluents

Fluorouracil injection may be diluted with glucose 5% injection or sodium chloride 0.9% injection or water for injections immediately before parenteral use.

7 MARKETING AUTHORISATION HOLDER

Wockhardt UK Limited
Ash Road North
Wrexham LL13 9UF
United Kingdom

8 MARKETING AUTHORISATION NUMBER(S)

PL 20851/0013

9 DATE OF FIRST AUTHORISATION/RENEWAL OF THE AUTHORISATION

19/09/2006

10 DATE OF REVISION OF THE TEXT

19/09/2006

Patient Information Leaflet

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

PL 20851/0010
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PACKAGE LEAFLET: INFORMATION FOR THE USER

Fluorouracil 50mg/ml Solution for Injection or Infusion

Read all of this leaflet carefully before you start using this medicine.
- Keep this leaflet. You may need to read it again.
- If you have any further questions, ask your doctor or pharmacist.
- This medicine has been prescribed for you. Do not pass it on to others. It may harm them, even if their symptoms are the same as yours.
- If any of the side effects get serious, or if you notice any side effects not listed in this leaflet, please tell your doctor or pharmacist.

In this leaflet:

1. WHAT FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION IS AND WHAT IT IS USED FOR

Fluorouracil belongs to a group of medicines known as cytotoxics, which are used in the treatment of cancer.

Fluorouracil is usually used to treat breast cancer and colon cancer. However, it may also be given to treat other types of cancer.

2. BEFORE YOU USE FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

Do not use Fluorouracil 50mg/ml Solution for Injection or Infusion

- if you are allergic to fluorouracil or any of the other ingredients
- if you are weakened after a long illness
- if your bone marrow has been damaged by other cytotoxic drugs or radiotherapy
- if you are pregnant, breast-feeding or trying for a baby
- if you have a tumour that is not malignant

Take special care with Fluorouracil 50mg/ml Solution for Injection or Infusion

Your doctor will take special care when giving you fluorouracil:

- if you have a low white blood cell count (you will have blood tests to check this)
- if you have liver or kidney problems
- if you have jaundice (yellowing of the skin)
- if you have angina or a history of heart disease (you should let your doctor know if you experience chest pain while you are receiving your treatment)
- if you have been told by your doctor that you have a low level of the enzyme dihydropyrimidine dehydrogenase (DPD)

Consult your doctor if any of the above warnings applies to you or has applied to you in the past.

Your doctor will also check your blood before, during and after every treatment. If the results of any of these tests are abnormal, treatment will only be resumed when all readings are back to normal.

Using other medicines

Please tell your doctor or pharmacist if you are taking or have recently taken any other medicines, including medicines obtained without a prescription.

Taking or being given another medicine while you are receiving fluorouracil can affect how it or the other medicine works. Please inform your doctor or pharmacist if you are taking or have recently taken any other medicines, even those you may have bought yourself without a prescription. Please check with your doctor if you are taking any of the following (or any other medication):

- Methotrexate, another cytotoxic drug
- Metronidazole, an antibiotic
- Calcium leucoverin (calcium folinate), used to reduce the harmful effects of cytotoxic drugs
- Allopurinol, used to treat gout
- Cimetidine, used to treat stomach ulcers
- Warfarin, used to treat blood clots
- Sorivudine, an antiviral drug

Pregnancy

Fluorouracil should not be given to you if you are pregnant, because it can cause serious birth defects.

Female patients should also avoid getting pregnant while being treated with fluorouracil and for at least six months afterwards. Male patients receiving fluorouracil should take adequate precautions to ensure that their partner does not become pregnant for the same period. If you are considering becoming parents after the treatment, you should discuss this with your doctor.

Men who wish to father children in the future should seek advice about freezing sperm before the fluorouracil treatment is started.

Breast-feeding

Fluorouracil should not be given to you if you are breast-feeding, as fluorouracil might pass into breast milk and affect the baby.

Driving and using machines:

Fluorouracil treatment should not affect your ability to drive, but if you feel unwell, you should not drive or operate machinery.

3. HOW TO USE FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

Fluorouracil injection can be given by intravenous injection (the solution is given directly into a vein) or, intravenous or intra-arterial infusion (the solution is diluted and given by a drip into a vein or artery).

Fluorouracil 50mg/ml Solution for Injection or Infusion will only be given to you under the supervision of a doctor specialised in this type of treatment, which should be started in hospital. It may be diluted with glucose solution, sodium chloride solution or water for injections before use. It is injected into a vein or artery. If it is given into an artery it must be diluted first.

The dosage of fluorouracil depends on the condition you are being treated for, your bodyweight, if you have had recent surgery, how well your liver and kidneys are working and results of your blood tests.

Your general condition and your response to the treatment will be closely observed before, during and after the fluorouracil treatment. This will include blood tests.

4. POSSIBLE SIDE EFFECTS

Like all medicines, Fluorouracil 50mg/ml Solution for Injection or Infusion can cause side effects, although not everybody gets them.

These include nausea, vomiting, temporary hair loss, skin problems, changes in your nails, feeling unsteady on your feet, quickening of the heart and breathlessness (following injection), painful and watery eyes, changes in vision and sensitivity to light, feeling confused and reddening of the palms of the hands and soles of the feet.

If you develop any of the following symptoms, **tell your doctor immediately:**

- chest pain
- diarrhoea
- blood stained or black bowel motions
- sore throat, sore mouth or mouth ulcers
- feeling generally unwell
- fever
- aching muscles and joints
- weakness,
- confusion
- difficulties with co-ordination, memory, thinking, or talking
- fits
- severe headache

Pain may occur temporarily at the injection site.

Allergic reactions to fluorouracil can occur, with wheezing, a skin rash or swelling of your lips, eyes or tongue. You should contact your doctor **immediately** if you develop such symptoms.

If any of the side effects gets serious, or if you notice any side effects not listed in this leaflet, please tell your doctor or pharmacist.

5. HOW TO STORE FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

Keep out of the reach and sight of children.

Do not use Fluorouracil 50mg/ml Solution for Injection or Infusion after the expiry date which is stated on the label or carton. The expiry date refers to the last day of that month.

<o not use Fluorouracil 50mg/ml Solution for Injection or Infusion if you notice signs of discoloration {description of the visible signs of deterioration}.

After first opening or following dilution, from a microbiological point of view, the product should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would not normally be longer than 24 hours at 2-8°C, unless opening and dilution has taken place in controlled and validated aseptic conditions.

Medicines should not be disposed of via wastewater or household waste. Ask your pharmacist how to dispose of medicines no longer required. These measures will help to protect the environment.

6. FURTHER INFORMATION

What Fluorouracil 50mg/ml Solution for Injection or Infusion contains

- The active substance is fluorouracil
  The other ingredients are sodium hydroxide and water for injections.

What X looks like and contents of the pack

Fluorouracil 50mg/ml Solution for Injection or Infusion is a clear and colourless solution free from particles.

Fluorouracil 50mg/ml Solution for Injection or Infusion is available in single packs containing:-

- 250mg of fluorouracil in 5ml of solution
- 500mg of fluorouracil in 10ml of solution
- 1000mg of fluorouracil in 20ml of solution
- 5000mg of fluorouracil in 100ml of solution

Marketing Authorisation Holder and Manufacturer

Fluorouracil 50mg/ml Solution for Injection or Infusion is manufactured by:-

EBEWE Pharma
Ges.m.b.H. Nfg. KG, A-4866
Unterach
Austria

for the Marketing Authorisation holder

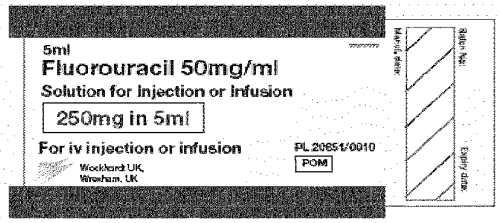
Wockhardt UK Limited
Ash Road North
Wrexham
LL13 9UF

This leaflet was last approved on .....

Labels/Packaging

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

PL 20851/0010



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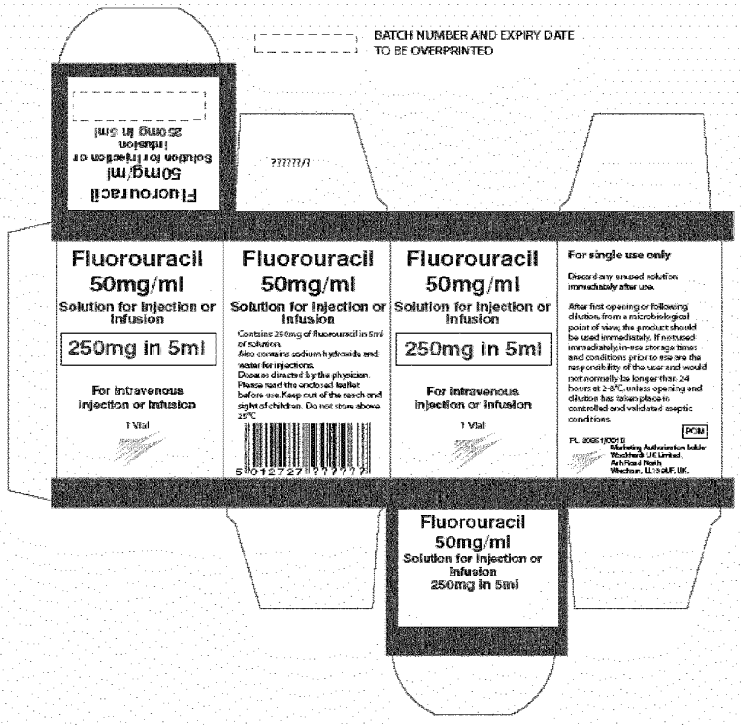
FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

PL 20851/0010

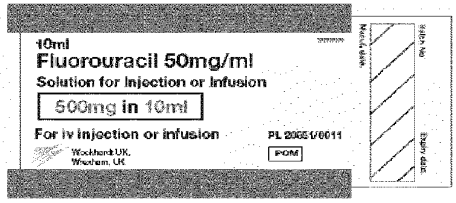


Fluorouracil 50mg/ml
1 Vial
(250mg in 5ml)
Colour: Red 221, Black

Mock-up 07/07/06

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

PL 20851/0011



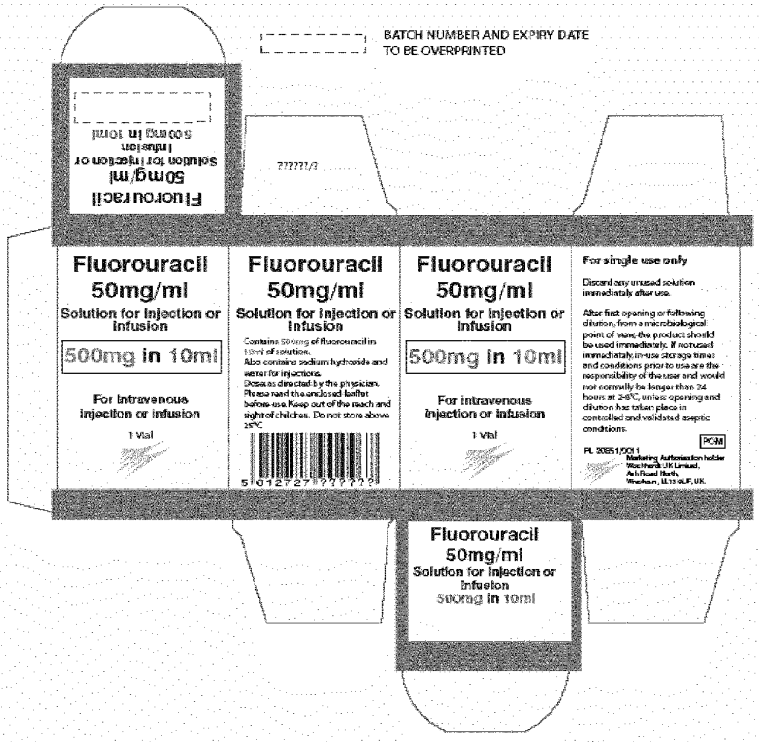
100% size

Fluorouracil 50mg/ml
1 Vial
(500mg in 10ml)
Colour: Green 370, Black

Mock-up 29/06/06

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

PL 20851/0011

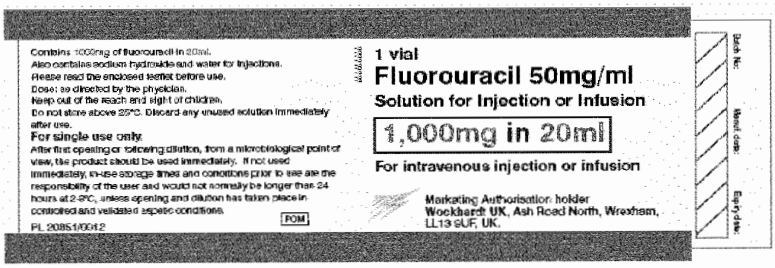


Fluorouracil 50mg/ml
1 Vial
(500mg in 10ml)
Colour: Green 370, Black

Mock-up 07/07/06

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

PL 20851/0012



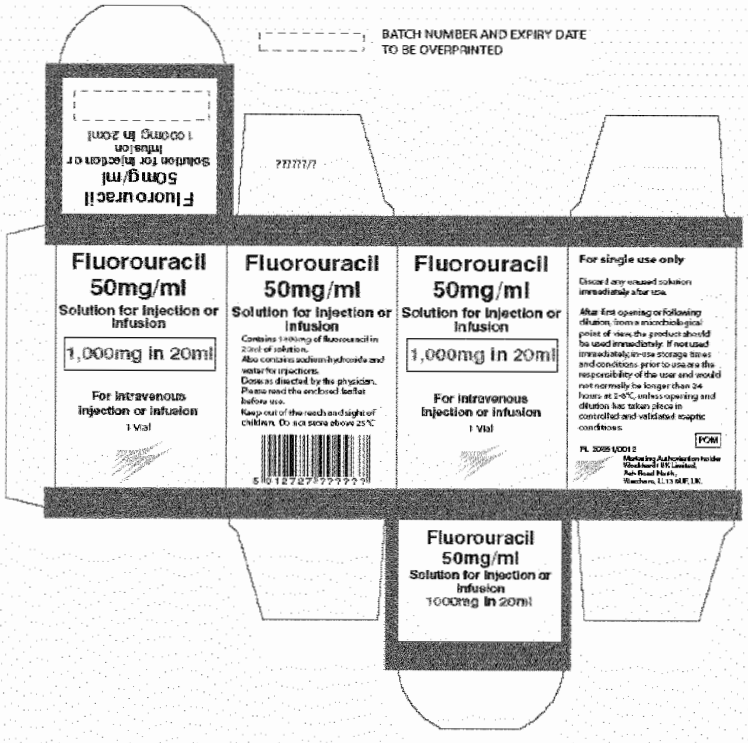
100%

Fluorouracil 50mg/ml
1 Vial
(1000mg in 20ml)
Colour: Blue 285, Black

Mock-up 07/07/06

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

PL 20851/0012

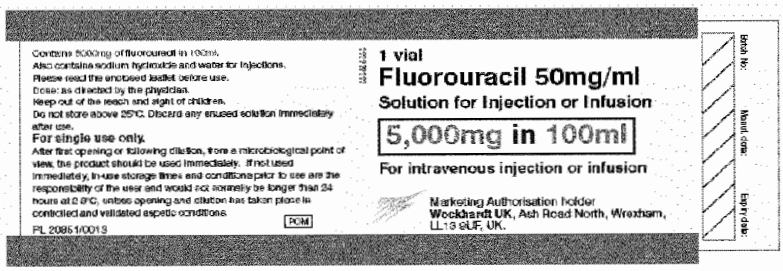


Fluorouracil 50mg/ml
1 Vial
(1000mg in 20ml)
Colour: Blue 285, Black

Mock-up 07/07/56

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

PL 20851/0013



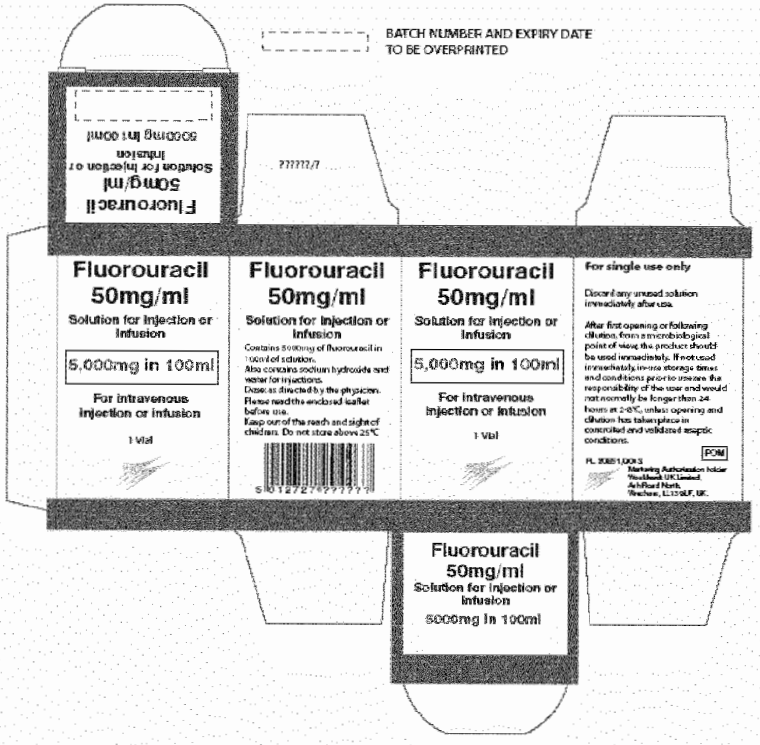
100%

Fluorouracil 50mg/ml
1 Vial
(5000mg in 100ml)
Colour: Red 186, Black

Mock-up 07/07/06

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

PL 20851/0013



Fluorouracil 50mg/ml
1 Vial
(5000mg in 100ml)
Colour: Red 186, Black

Mock-up 07/07/06

Irinotecan Plus Bolus/Infusional 5-Fluorouracil and Leucovorin in Patients With Pretreated Advanced Pancreatic Carcinoma

A Multicenter Experience of the Gruppo Oncologico Italia Meridionale

Vittorio Gebbia, MD, PhD, Evaristo Maiello, MD,† Francesco Giuliani, MD,‡ Nicolò Borsellino, MD,§ Carlo Arcara, MD,* and Giuseppe Colucci, MD‡*

Background: Patients with advanced pancreatic cancer failing gemcitabine-based first-line chemotherapy are still in relatively good clinical conditions and may still require second-line chemotherapy, which is frequently administered in daily clinical practice given to without solid scientific support.

Patients and Methods: A retrospective survey was carried out including 40 patients with stage III or IV gemcitabine-refractory pancreatic carcinoma. Patients received standard FOLFIRI regimen biweekly until progression or unacceptable toxicity. Response evaluation criteria in solid tumors and National Cancer Institute common toxicity criteria were employed respectively for response and toxicity assessment.

Results: Six partial responses (15%) and 14 stabilizations of disease (35%) were recorded for a tumor growth control rate of 50%. The median time to progression was 3.7 (range, 1–6.5 months), and median overall survival was 6 months (range, 2–8.2 months). A stabilization of performance status and a subjective improvement of cancer-related symptoms were recorded in 21 patients (52.5%). No correlation has been found between length of time to progression during first-line chemotherapy and length of that reported in the second-line setting or objective response. Grade 3–4 diarrhea and mucositis was observed in 15% and 10% of cases, respectively.

Conclusions: Data presented in this article demonstrate that the second-line FOLFIRI regimen are able to induce an objective response in a relatively small fraction of patients with gemcitabine-refractory adenocarcinoma of the pancreas. The use of second-line chemotherapy should be carefully proposed to patients with good performance status or those who had a good response to first-line therapy.

Key Words: FOLFIRI regimen, pancreatic carcinoma, second-line chemotherapy

(*Am J Clin Oncol* 2010;33: 461–464)

In Western countries, pancreatic carcinoma represents the fourth leading cause of death by cancer, with most patients being diagnosed at a locally advanced and/or metastatic stage.[1] Although most studies have reported a 1-year survival rate is lower than 10% for patients with advanced pancreatic carcinoma (APC); however, the availability of gemcitabine (GEM) has represented a small but significant progress in the medical management of advanced disease.[2] In fact, GEM-based chemotherapy represents the standard systemic treatment of APC for the majority of patients, being able to improve cancer-related symptoms and patient's performance status (PS) albeit conferring only a modest survival advantage.[2–4]

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DOI: 10.1097/COC.0b013e3181b4e3b0

Phase III trials have shown that combination regimens incorporating GEM, cisplatin, 5-fluorouracil (5-FU), oxaliplatin, pemetrexed, or irinotecan (CPT-11) generally improve patient outcomes in terms of objective response rates but with little or no improvement in survival parameters.[3–9] The addition of newer biologic agents, such as marimastat, bevacizumab, or cetuximab, to GEM has not improved the results in terms of survival with an increase in toxicity,[10–12] whereas the addition of erlotinib yielded a very modest improvement in survival as compared with GEM alone.[13] Moreover, the use of sequential chemotherapy, ie, 5-FU plus folinic acid and cisplatin followed by GEM versus the reverse sequence, has also yielded poor results.[14]

In clinical practice, APC patients progressing after GEM-based chemotherapy often are in relatively good clinical conditions and may require a second- and even a third-line therapy. Phase II trials evaluating second-line chemotherapy in patients failing GEM-based chemotherapy are relatively scarce in medical literature. Some agents, such as paclitaxel, oxaliplatin, CPT-11, capecitabine, rubitecan, pemetrexed, and flutamide, have been tested as single agents.[2–4] There are no standard second-line regimens for advanced pancreatic cancer, after gemcitabine failure,[15] even if 2 recently reported studies have indicated that the FOLFOX regimen is superior to both best supportive Care and 5-FU plus folinic acid in the second-line setting.[2,16] Therefore, several off-label drugs shown to be active in advanced APC are employed regularly in daily clinical practice even if no specifically addressed trial has scientifically demonstrated their efficacy in terms of symptoms palliation and survival parameters.

Preclinical studies have shown significant activity of CPT-11 both in cultured pancreatic carcinoma cells and xenograft model with a synergism between if given with 5-FU.[17–20] Single agent CPT-11 has been tested in 2 phase II trials on previously untreated patients with APC yielding response rates of 9% and 27%, respectively.[21,22] In patients pretreated with GEM-based regimens single-agent CPT-11 has been reported to yield a <10% overall response rate with reduction of serum tumor markers in 25% of a small series of patients who showed a 4-months median progression-free survival.[23] A second study employed CPT-11 in combination with raltitrexed with better results.[24]

In this article, we report a retrospective survey of the efficacy and toxicity of CPT-11 in combination with 5-FU and folinic acid (FOLFIRI regimen) administered in the same schedule employed in colon carcinoma in a series of unselected patients affected by APC progressing after GEM-based first-line treatment.[25]

PATIENTS AND METHODS

Patient Population

Patients included in this study showed APC progressing after GEM-based first-line treatment. Patients were enrolled into the study if they satisfied the following inclusion criteria: histologic diagnosis of APC; measurable disease according to the response evaluation criteria in solid tumors[26]; age between 18 and 75 years; Eastern

Cooperative Oncology Group PS ≤2; absence of severe uncontrolled cardiovascular, metabolic, infectious, or neurologic diseases; and informed written consent.[5] Other requirements were adequate bone marrow (platelets count ≥100.000/mm^3, WBC count ≥4000/mm^3, granulocyte count 500/mm^3, a hemoglobin level of ≥10.0 g/mm^3), renal (serum creatinine concentration <2.0 mg/dL), and hepatic functions (serum bilirubin level <2.0 mg/dL and AST 3 or less times the normal level in the absence of liver involvement with cancer or up to 5 times the institutional normal level when cancer was present in the liver).

Pretreatment Evaluation

Staging procedures consisted of medical history, physical examination, Electrocardiogram, peripheral blood cell counts, serum chemistry panel, carcinoembryonic antigen, and CA 19-9. Extent of disease was determined by chest x-rays, computed tomography and/or nuclear magnetic resonance, and endoscopy, as needed. Patients underwent follow-up examinations until death.

Efficacy Assessment

Partial response (PR), stable disease (SD), and progressive disease (PD) were determined according to the response evaluation criteria in solid tumors every 6 cycles.[26] The sum of PR and SD was reported as tumor growth control rate (TGCR). Time to progression (TTP) was estimated from the date of first treatment to the first evidence of PD. Overall survival (OS) was estimated from the date of first cycle of second-line chemotherapy to the date of death or the last follow-up. Clinical benefit assessment was based on patients and physician-reported improvement of cancer-related symptoms and/or stabilization of improvement of PS. This procedure is standard clinical practice at all participating institutions.

Treatment Schedule

The chemotherapy schedules was as follows: CPT-11 180 mg/m^2 on day 1 with levofolinic acid 100 mg/m^2 administered as a 2-hour infusion before 5-FU at 400 mg/m^2 as an i.v. bolus, and 5-FU at 600 mg/m^2 as a 22-hour infusion immediately after 5-FU bolus injection on days 1 and 2.

Treatment was given biweekly until PD or unacceptable toxicity, withdrawal of consent, and physicians decision or treatment interruption for >2 weeks.

Toxicity

Adverse events were graded according to the National Cancer Institute common toxicity criteria (version 3.0). A full blood count was carried out each week to assess hematological toxicity, and the patients had a complete physical examination and serum bilirubin, transaminases, alkaline phosphatase, and creatinine assays before each treatment cycle. The patients were interviewed before each session, focusing on pain, nausea, vomiting, mucositis, diarrhea, asthenia, weight loss, and neurologic disorders. All patients who received at least one treatment session were considered assessable for toxicity. If multiple toxic effects were observed, the dose administered was based on the most severe toxicity experienced. The dose adjustment schedule was evaluated at the beginning of a new administration. Dose reductions were carried out as described previously.[25]

Statistical Analysis

Objective responses were reported as their relative rates adjusted to the nearest unit with 95% confidence interval. TTP and OS were calculated employing a GraphPad statistical software.[27]

RESULTS

Patient Characteristics

As shown in Table 1, 40 patients of 255 screened cases were collected from centers belonging to the Gruppo Oncologico Italia Meridionale (Table 1). Patients were GEM-pretreated and received second-line FOLFIRI regimen between January 2003 and June 2008. There were 24 males (60%) and 16 females (40%) with a median age of 63 years and a median PS of 1, according to the Eastern Cooperative Oncology Group scale. Five patients (12.5%) had a PR to front-line treatment, and 12 patients (30%) had SD. All patients except 7 had metastatic stage IV APC, and 60% had multiple sites of disease. Liver metastases were present in 70% of patients, locoregional lymph nodes in 67.5%, lung metastases in 15%, and peritoneal carcinomatosis in 22.5% of patients.

TABLE 1. Patients Clinical and Demographical Characteristics

No. patients	40 (100%)
Median age (range)	63 (38–73)
Sex	
Male	24 (60%)
Female	16 (40%)
PS (ECOG)	
Median	1
PS 0	2 (0.5%)
PS 1	31 (77%)
PS 2	7 (17.5%)
Stage	
III	7 (17.5%)
IV	33 (82.5%)
Previous surgery	4 (10%)
Previous RT	0
Previous CT	
GEM	22 (55%)
GEMOX	3 (0.7%)
GEM/CDDP	15 (37.5%)
Response to previous chemotherapy	
Partial response	5 (12.5%)
Stable disease	12 (30%)
Progression	23 (57.5%)
Clinical benefit response to previous chemotherapy	
Yes	13 (32.5%)
No	27 (67.5%)
Site of disease	
Pancreas	38 (95%)
Lymph node	27 (67.5%)
Liver	28 (70%)
Peritoneum	9 (22.5%)
Lung	6 (15%)
Other	3 (0.7%)

CDDP indicates cisplatin; CT, chemotherapy; ECOG, Eastern Cooperative Oncology Group; GEM, gemcitabine; GEMOX, gemcitabine and oxaliplatin

Antineoplastic Activity and Survival

As shown in Table 2, there were 6 objective PR (15%), and 14 patients (35%) had an SD for a TGCR of 50%. No complete response was recorded. Median duration of PR was 4.9 months (range, 2–6.5 months). Median TTP from the start of second-line treatment was 3.7 (range, 1–6.5 months). No correlation has been found between length of TTP during first-line chemotherapy and length of TTP in the second-line setting or objective response.

TABLE 2. Activity and Survival Parameters

No. patients	40 (100%)
Partial response	6 (15%)
Stable disease	14 (35%)
Progressive disease	20 (50%)
TGCR	29 (50%)
Clinical benefit	20 (50%)
Median TTP (mo)	3.7 (range, 1–6.5)
GMI (>1.33)	9 (22.5%)
Median OS (mo)	6 (range, 2–8.2)

TABLE 3. Toxicity

Toxicity (NCI-CTC)	Grade 1	Grade 2	Grade 3	Grade 4
Neutropenia	8 (20%)	10 (25%)	4 (10%)	3 (7.5%)
Thrombocytopenia	3 (7.5%)	2 (5%)	1 (2.5%)	0
Anemia	9 (22.5%)	8 (20%)	3 (7.5%)	0
Alopecia	14 (35%)	11 (27.7%)	4 (10%)	0
Diarrhea	13 (32.5%)	10 (25%)	5 (12.5%)	1 (2.5%)
Nausea-vomiting	12 (30%)	9 (22.5%)	3 (7.5%)	0
Mucositis	8 (20%)	8 (20%)	4 (10%)	0
Hand-foot syndrome	3 (7.5%)	0	0	0

NCI-CTC indicates National Cancer Institute common toxicity criteria.

It had been proposed that the activity of a second-line chemotherapy could be documented by showing that the TTP after second-line treatment is longer than the TTP after front-line therapy in each patient. The ratio of TTPs has been defined as the growth modulation index (GMI) with each patient being his own control.[28] A GMI >1 means that TTP was longer with the second-line chemotherapy, and treatment that produces a GMI ≥1.33 (33% improvement) should be considered to have excellent activity.[29] In this series, 9 patients (22.5%) had a GMI >1.33, and 3 further patients had a GMI = 1. Median OS from the start of second-line therapy was 6 months (range, 2–8.2 months). About 21 patients (52.5%) had a stabilization of PS or a subjective improvement of cancer-related symptoms.

Tolerability and Safety

Patients received between 2 and 12 cycles. Most patients received full treatment and median delivered dose intensity was higher than 80% in the whole series. Sixteen patients (40%) had some treatment delay with a median of 3.5 days, but only 3 patients (7.5%) experienced more than 2 cycles of delay. Reasons for delay were not treatment related in 5 cases. Overall, side-effects were moderate and easily manageable with no case of toxic death (Table 3). However, both hematological and gastrointestinal side-effects were commonly observed and managed aggressively, according to the participating institution protocols. Grade 3 anemia was recorded in 7.5% of patients, and grade 3–4 neutropenia occurred in 17.5% of patients with 2 cases of febrile neutropenia, which required hospitalization. G-CSF was employed in 13 cases. Thrombocytopenia occurred in 6 patients (12.5%), but was severe only in 1 case with massive liver disease. Most of the nonhematological symptoms were mild. Grade 3 vomiting occurred in 3 patients (7.5%), even if grade 1–2 vomiting was recorded in 50.5% of cases. Grade 3 mucositis was experienced by 10% of patients, whereas 21 patients had grade 1–2 mucositis. Severe grade 3–4 diarrhea was observed in 6 patients (15%), but mild grade 1–2 diarrhea was quite common, being recorded in more than 50% of cases. Mild hand-foot syndrome was observed in 3 cases (7.5%). When grade 3–4 side-effects are plotted according to PS, 5 of 7 patients with a PS2 had some grade 3 toxicity (71%), whereas grade 3 side-effects were observed in 45% of PS 0–1 patients.

Reasons for treatment discontinuation were progressive cancer in all patients but 3 cases. Two patients had grade 3–4 toxicity, which precluded continuation of chemotherapy, and 1 patient refused to continue for psychologic distress.

DISCUSSION

Despite the disappointing clinical results, systemic chemotherapy has, in the last decade, improved the survival and quality of life of a fraction patients with APC.[30] Systemic chemotherapy with single-agent GEM or a GEM-based regimen still remains a standard of care for the treatment of patients with locally advanced and metastatic pancreatic cancer.[31,32]

To date, there is no defined second-line standard treatment for patients progressing during GEM.[15,31] Several clinical trials have evaluated the efficacy and tolerability of different combination chemotherapy regimens as second-line chemotherapy after GEM failure. Currently available data provide increasing evidence that selected patients with GEM-refractory APC may yield clinical benefit and slight survival improvement from second-line chemotherapy. However, sufficient results from large randomized phase III trials are still lacking and therefore no evidence-based treatment recommendation can be given for patients with APC after failure of first-line GEM.

In this article, we reported the activity and tolerability of the FOLFIRI regimen in a retrospective series of 40 patients with GEM-refractory APC. We reported a 50% TGCR and a 15% PR rate with a median duration of 4.9 months. Median TTP from the start of second-line treatment was 3.7 and median overall survival of 6 months. In this series, 9 patients (22.5%) had a GMI >1.33, and 21 patients (52.5%) had a stabilization of PS or a subjective improvement of cancer-related symptoms. The FOLFIRI regimen has acceptable tolerability, despite grade 3 hematological and gastrointestinal toxicity may occur in up to 18% of cases. Patients with PS2 and/or other factors of poor prognosis may not benefit from this regimen and be exposed to a higher incidence of severe side-effects. Even if no formal comparison has been made, however, the FOLFIRI regimen seems to be associated with higher gastrointestinal side-effects as compared to the toxicity reported in other series of patients with similar characteristics treated with the FOLFOX regimen. On the other hand, antineoplastic data reported in this article, with the FOLFIRI regimen, do not show clinical meaningful differences with results reported by our group in a series of APC patients treated with the FOLFOX regimen.[33] In our hands, the latter regimen achieved a 14% PR rate and a SD in 38% of cases with a median TTP of 4 months and a median OS of 6.7 months. The use of FOLFOX regimen in the second-line treatment of APC patients is supported by 2 studies. Oettle et al compared the FOLFOX regimen with best supportive care in a phase III study on GEM-refractory patients achieving a median overall survival of 21 weeks in the treatment arm compared with 10 weeks in the best supportive care one (P = 0.007) leading to early closure of the study.[34] The CONKO-3 study randomized 168 patients who had GEM-refractory pancreatic cancer to FOLFOX or 5-FU/levofolinic acid.[16] The study was powered at 90% to detect an improved OS by 2 months in the oxaliplatin arm. The median OS of the oxaliplatin arm was 28 weeks as compared with 13 weeks recorded in the 5-FU/levofolinic acid arm, thereby fulfilling the study hypothesis. There was also a significant prolongation of progression-free survival in the FOLFOX arm (13 weeks vs. 9 weeks). Both regimens were tolerable, with the exception of higher neuropathy in the oxaliplatin arm. Therefore, it

has been suggested that the FOLFOX regimen could be regarded as a standard second-line regimen for APC.

In conclusion, data presented in this article support the use of FOLFIRI regimen in the second-line treatment of APC patients. Data from medical literature and our experience support the careful use of second-line chemotherapy in patients with adequate PS or those who had a good response to first-line therapy. Future trials may be needed to validate the role of the FOLFIRI regimen in the second-line treatment of progressing APC.

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