DETAILED ACTION

Previous Rejections

Applicant's arguments, filed 2/11/19, have been fully considered. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn. The following rejections and/or objections are either reiterated or newly applied. They constitute the complete set presently being applied to the instant application.

Claim Rejections - 35 USC § 103 - Obviousness

The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent for a claimed invention may not be obtained, notwithstanding that the claimed invention is not identically disclosed as set forth in section 102, if the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 1, 5-8, 10 and 19 are rejected under 35 U.S.C. 103 as being unpatentable over Bayever et al (WO 2013/188586), in view of Conroy et al (NEJM, 34(19), 2011, 1817) and further in view of Melis et al (The Society for Surgery of the Alimentary Tract, 2011; http://meetings.ssat.com/abstracts/11ddw/P57.cgi).

Bayever et al disclosed a method for treatment of pancreatic cancer in a patient (e.g., a human, at page 3, 1st paragraph), comprising co-administering to the patient active agents, at a dose of 60 mg/m^2 (e.g., liposomal irinotecan). Bayever further disclosed 5-fluorouracil at a dose of 2400 mg/m^2 and leucovorin (*l* form administered at 200 mg/m^2 or the *l*+*d* racemic form administered at 400 mg/m^2). The method comprised

at least one cycle of administration, wherein the cycle was a period of two weeks (page 3, last full paragraph).

In one embodiment, Bayever's population was patients undergoing treatment for metastatic adenocarcinoma pancreatic cancer (e.g. a patient who has not previously received an antineoplastic agent) (page 12, section V, last embodiment, and claim 10).

Bayever did not disclose oxaliplatin, as recited in claim 9.

Conroy disclosed FOLFIRINOX (oxaliplatin; irinotecan; leucovorin and fluorouracil) treatment of patients having metastatic pancreatic cancer (title and the methods section of the abstract). Conroy disclosed that oxaliplatin has clinical activity against pancreatic cancer only when combined with fluorouracil, and that oxaliplatin and irinotecan have been shown to have synergistic activity *in vitro* (page 1818, left column, second paragraph).

Conroy did not disclose that the irinotecan was liposomal irinotecan.

Since Bayever disclosed treating metastatic pancreatic carcinoma with 5-fluorouracil and irinotecan, it would have been prima facie obvious to one of ordinary skill in the art to include oxaliplatin within Bayever's methods of treatment. An ordinarily skilled artisan would have been motivated because oxaliplatin has clinical activity against pancreatic cancer when combined with fluorouracil, and because oxaliplatin and irinotecan have synergistic activity *in vitro*, as taught by Conroy (Conroy, page 1818, left column, second paragraph).

Regarding the claims 1 and 19 limitation of 60 mg/m^2 oxaliplatin, the combination of Bayever (e.g., Bayever taught 85 mg/m^2 oxlaplatin at the abstract), though not silent the claimed amount of oxaliplatin, does not specifically teach 60 mg/m^2 oxaliplatin.

However, Melis taught [abstract] that a dosage of 60 mg/m^2 oxaliplatin was well tolerated in advanced pancreatic adenocarcinoma patients.

As such, oxlaplatin, and its amount, is recognized to have different effects (treatment of advanced pancreatic adenocarcinoma) with changing amounts used. Thus, the general condition (the dosage) is known and the amount of this ingredient is recognized to be result effective. Therefore, result effective variables can be optimized by routine experimentation, and it would have been prima facie obvious to optimize the dosage of the oxaliplatin present in the combined composition of Bayever and Conroy, as taught by Melis.

The combination of Bayever, Conroy and Melis reads on claims 1 and 19.

Claims 5-6 and 8 are rendered prima facie obvious because Bayever disclosed that 5-fluorouracil was administered intravenously over 46 hours, liposomal irinotecan was administered intravenously over 90 minutes, and that leucovorin was administered prior to 5-FU (page 12, section IV).

Claim 7 is rendered prima facie obvious because Bayever disclosed that active agents were administered on day one of a two-week cycle, where cycles comprised at least one administration. For example, Bayever's method overlaps that which is instantly recited (e.g. administration on days 1 and 15 of a 28-day cycle), because administration on day 1 of at least one 2-week cycle can also be administration on days 1 and 15 of a 28 day cycle (e.g. two 2-week cycles). In the case where the claimed ranges

"overlap or lie inside ranges disclosed by the prior art", a prima facie case of obviousness exists. MPEP 2144.05 A.

Claim 10 is rendered prima facie obvious because Bayever disclosed irinotecan sucrose octasulfate liposomal irinotecan, where the irinotecan was entrapped within the liposome, at page 4, and the last paragraph.

Response to Arguments

Applicant's arguments with respect to claims 1, 5-8, 10 and 19 have been considered but are moot because the arguments do not apply to any of the references being used in the current rejection.

Claims 4, 9, 18 and 23 are rejected under 35 U.S.C. 103 as being unpatentable over Bayever et al (WO 2013/188586), in view of Conroy et al (NEJM, 34(19), 2011,1817) further in view of Melis et al (The Society for Surgery of the Alimentary Tract, 2011; http://meetings.ssat.com/abstracts/11ddw/P57.cgi) and further in view of Fleming et al (http://www.oncologynurseadvisor.com/advisor-forum/importance-of-sequence-in-chemotherapy-administration/article/378072/).

The 35 U.S.C. 103 rejection over Bayever, in view of Conroy and Melis, has been discussed above.

Additionally, Bayever disclosed that prior to each administration of liposomal irinotecan, the patient was pre-medicated with dexamethasone (e.g. corticosteroid) and another anti-emetic (page 4, fourth embodiment from the top of the page).

Further, Conroy disclosed that a second active agent was given two hours after a first active agent (e.g., leucovorin was given two hours after oxaliplatin) (page 1819, 1st paragraph of the section entitled Treatment).

However, the combination of Bayever and Conroy did not specifically disclose oxaliplatin administration after liposomal irinotecan, as recited in claims 4, 18 and 23; liposomal irinotecan administration, followed by oxaliplatin administration, followed by leucovorin administration, followed by 5-fluorouracil administration, as recited in claim 9.

Fleming disclosed that the sequence of various chemotherapy drugs in general does not matter, as the half-life of each drug makes it impossible to determine what drug is at what level at any particular time, based on individual patient pharmacodynamics (last sentence of the first paragraph).

Since the combination of Bayever and Conroy disclosed administration of oxaliplatin, liposomal irinotecan, leucovorin and 5-fluorouracil, it would have been prima facie obvious to one of ordinary skill in the art to have varied the order of administration of the combined methods of Bayever and Conroy, such that the order of administration was liposomal irinotecan, followed by oxaliplatin, followed by leucovorin, followed by 5-fluorouracil administration.

An ordinarily skilled artisan would have been motivated because the sequence of various chemotherapy drugs in general does not matter, as the half-life of each drug makes it impossible to determine what drug is at what level at any particular time, based on individual patient pharmacodynamics, as taught by Fleming (Fleming, last sentence of the first paragraph).

Response to Arguments

Applicant's arguments with respect to claims 4, 9, 18 and 23 have been considered but are moot because the arguments do not apply to any of the references being used in the current rejection.

Claims 11-15 and 21-22 are rejected under 35 U.S.C. 103 as being unpatentable over Bayever et al (WO 2013/188586), in view of Conroy et al (NEJM, 34(19), 2011, 1817), further in view of Melis et al (The Society for Surgery of the Alimentary Tract, 2011; http://meetings.ssat.com/abstracts/11ddw/P57.cgi) and as evidenced by Bayever et al (WO 2016/094402).

The 35 U.S.C. 103 rejection over Bayever (2013), in view of Conroy and Melis, has been discussed above.

Although, Bayever (2013) disclosed MM-398 liposome (at page 4, last paragraph and as discussed above), Bayever was not specific as to the ingredients of the liposome, as recited in claims 11-12 and 21-22.

However, Bayever (2016) evidenced that MM-398 contained irinotecan sucrose octasulfate, DSPC, cholesterol and MPEG-2000-DSPE (page 30, section describing the drug product).

Thus, it is reasonable to assume that Bayever's (2013) MM-398 contained irinotecan, DSPC, cholesterol and MPEG-2000-DSPE, as evidenced by Bayever's (2016) disclosure of the liposomal constituents of MM-398.

Claims 13-15 and 21-22 are rendered prima facie obvious because Bayever disclosed that 5-fluorouracil was administered intravenously over 46 hours,

liposomal irinotecan was administered intravenously over 90 minutes; liposomal irinotecan was administered prior to leucovorin; leucovorin was administered prior to 5-FU (page 12, section IV). Further, Bayever disclosed that active agents were administered on day one of a two-week cycle, where cycles comprised at least one administration.

For example, Bayever's method overlaps that which is instantly recited (e.g. administration on days 1 and 15 of a 28-day cycle) because administration on day 1 of at least one 2-week cycle can also be administration on days 1 and 15 of a 28-day cycle (e.g. two 2-week cycles). A prima facie case of obviousness exists because of overlap, as discussed above.

Response to Arguments

Applicant's arguments with respect to claims 11-15 and 21-22 have been considered but are moot because the arguments do not apply to any of the references being used in the current rejection.

Nonstatutory Double Patenting

A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir.

1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(l)(1) - 706.02(l)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Claims 1, 4-15, 18-19 and 21-23 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-18 of U.S. Patent No. 9,492,442, in view

of Conroy et al (NEJM, 34(19), 2011, 1817) and further in view of Melis et al (The Society for Surgery of the Alimentary Tract, 2011; http://meetings.ssat.com/abstracts/11ddw/P57.cgi)

Although the claims at issue are not identical, they are not patentably distinct from each other. The issued claims recite all of the features instantly recited for the method of treatment except for the administration of oxaliplatin. The instant claims require oxaliplatin, and such an ingredient is not recited by the issued claims.

Conroy disclosed FOLFIRINOX (oxaliplatin; irinotecan; leucovorin and fluorouracil) treatment of patients having metastatic pancreatic cancer (title and the methods section of the abstract). Conroy disclosed that oxaliplatin has clinical activity against pancreatic cancer only when combined with fluorouracil, and that oxaliplatin and irinotecan have been shown to have synergistic activity *in vitro* (page 1818, left column, second paragraph).

Melis taught [abstract] that a dosage of 60 mg/m^2 oxaliplatin was well tolerated in advanced pancreatic adenocarcinoma patients.

Thus, it would have been prima facie obvious to use oxaliplatin in the issued method, because oxaliplatin has clinical activity against pancreatic cancer only when combined with fluorouracil, and because oxaliplatin and irinotecan have been shown to have synergistic activity *in vitro*. It would have been prima facie obvious to use oxaliplatin at 60 mg/m^2 because the said dosage is well tolerated in advanced pancreatic adenocarcinoma patients.

Response to Arguments

Applicant's arguments with respect to claims 11-15 and 21-22 have been considered but are moot because the arguments do not apply to any of the references being used in the current rejection.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to CELESTE A RONEY whose telephone number is (571)272-5192. The examiner can normally be reached on Monday-Thursday; 7 AM-5 PM.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Frederick Krass can be reached on 571-272-0580. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-

217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/CELESTE A RONEY/
Primary Examiner, Art Unit 1612

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15/896,389	02/14/2018	Keelung Hong	263266-426640	3157

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McNeill Baur PLLC/Ipsen
Ipsen Bioscience, Inc.
125 Cambridge Park Drive
Suite 301
Cambridge, MA 02140

EXAMINER	
SHOMER, ISAAC	

ART UNIT	PAPER NUMBER
1612	

NOTIFICATION DATE	DELIVERY MODE
07/18/2019	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docketing@mcneillbaur.com
eofficeaction@appcoll.com
patents.us@ipsen.com

Office Action Summary	Application No. 15/896,389	Applicant(s) Hong et al.	
	Examiner ISAAC SHOMER	Art Unit 1612	AIA (FITF) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 15 July 2019.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 37-57 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 37-57 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

DETAILED ACTION

Notice of Pre-AIA or AIA Status

The present application is being examined under the pre-AIA first to invent provisions.

In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

Claim Interpretation

The instant claims recite the term "lipid matrix." As best understood by the examiner, the term "lipid matrix" is understood to refer to various lipidic structures. These structures include, but are not limited to, liposomal and/or lipid vesicle structures comprising a phospholipid bilayer and an aqueous interior core. See e.g. page 96, paragraph 0242 of the instant specification, which refers to a liposome as a matrix. Both a liposome and a lipid vesicle are understood to comprise an aqueous core surrounded by at least one lipid bilayer.

The claims also recite a fluid pharmaceutical composition. The examiner understands that liposomes dispersed in an aqueous environment read on the required

fluid. This is because the aqueous environment is liquid at room temperature, and liquids (as well as gases) are fluids.

The instant claims also recite that the irinotecan and sucrose octasulfate are associated with a lipid matrix. As best understood by the examiner, a case wherein irinotecan is encapsulated in a liposome is understood to be an association between the irinotecan and the lipid matrix.

Non-Statutory Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory

double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(l)(1) - 706.02(l)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Claims 37-57 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-35 of U.S. Patent No. 8,147,867. Although the claims at issue are not identical, they are not patentably distinct from each other because of the following reasons:

Instant claim 37 is drawn to a method of delivering irinotecan to a tumor. This method comprises intravenously administering a fluid pharmaceutical composition

comprising irinotecan and sucrose octasulfate in a lipid matrix. The claims recite a concentration range of about 500 mg to about 550 mg of irinotecan per mmol of phospholipid.

Conflicting claim 1 is drawn to a liposomal composition comprising irinotecan and sucrose octasulfate. Conflicting claim 24 recites about 550 mg of irinotecan per mmol of neutral phospholipid. Conflicting claim 8 recites intravenous administration. Conflicting claim 5 recites treating a specific type of cancer.

The instant and conflicting claims differ because the instant claims are drawn to method claims, whereas the conflicting claims are drawn to composition claims. Nevertheless, the skilled artisan would have been motivated to have administered the composition of the conflicting claims intravenously for a patient who has cancer in view of the recitations of conflicting claims 5 and 8.

The instant and conflicting claims differ because conflicting claims 1 and 11 recite subject matter not specifically recited by instant claim 35 such as the charging of the lipids and the release time from the liposome. Nevertheless, the subject matter of the conflicting claims, when their recitations are combined, is within the scope of that of the instant claims, resulting in a prima facie case of obviousness-type non-statutory double patenting.

Claims 37-57 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-17 of U.S. Patent No. 8,329,213 in view of Yang et al. (US Patent 6,143,740).

Instant claim 37 is drawn to a method of delivering irinotecan to a tumor. This method comprises intravenously administering a fluid pharmaceutical composition comprising irinotecan and sucrose octasulfate in a lipid matrix. The claims recite a concentration range of about 500 mg to about 550 mg of irinotecan per mmol of phospholipid.

Conflicting claim 11 is drawn to irinotecan liposomes comprising irinotecan and sucrose octasulfate. Conflicting claim 1 is drawn to liposomes with a more generic anti-cancer agent.

While conflicting claim 14 recites parenteral administration, the claim does not recite intravenous administration.

Yang et al. (hereafter referred to as Yang) is drawn to a pharmaceutical composition for treating cancer, as of Yang, title and abstract. Yang teaches that intravenous administration is a form of parenteral administration, as of column 8 lines 22-32.

It would have been prima facie obvious for one of ordinary skill in the art to have administered the composition of the conflicting claims via intravenous administration. The conflicting claims recite parenteral administration, as of conflicting claims 4, 10, and 14. Yang teaches that intravenous administration is a form of parenteral administration. As such, the skilled artisan would have been motivated to have administered the composition of the conflicting claims via intravenous administration in order to have predictably administered the composition in a parenteral manner with a reasonable expectation of success.

The conflicting claims recite a ratio of a maximum of about 1.0 mol of active per mol of lipid, as of conflicting claim 2. Irinotecan has a molecular weight of about 586 g/mole. As such, this is about 586 grams of irinotecan per mole of lipid, or about 586 mg of irinotecan per mmol of lipid. Conflicting claim 2 also recites 0.7 mol of active agent per mol of lipid. This is about 410 mg of active agent per mmol lipid. This overlaps with the claimed range of 500-550 mg of active agent per mmol of lipid. This overlap results in a prima facie case of obviousness-type non-statutory double patenting.

Claims 37-57 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-17 of U.S. Patent No. 8,703,181 in view of Yang et al. (US Patent 6,143,740).

Instant claim 37 is drawn to a method of delivering irinotecan to a tumor. This method comprises intravenously administering a fluid pharmaceutical composition comprising irinotecan and sucrose octasulfate in a lipid matrix. The claims recite a concentration range of about 500 mg to about 550 mg of irinotecan per mmol of phospholipid.

Conflicting claim 1 is drawn to a method of delivering an anti-neoplastic agent, and conflicting claim 11 is drawn to a method of delivering irinotecan. The composition administered in the method of the conflicting claims has sucrose octasulfate, as of conflicting claims 1 and 11, and is a liposome. The conflicting claims recite parenteral administration as of conflicting claims 4, 10, and 14.

While conflicting claim 14 recites parenteral administration, the claim does not recite intravenous administration.

Yang et al. (hereafter referred to as Yang) is drawn to a pharmaceutical composition for treating cancer, as of Yang, title and abstract. Yang teaches that intravenous administration is a form of parenteral administration, as of column 8 lines 22-32.

It would have been prima facie obvious for one of ordinary skill in the art to have administered the composition of the conflicting claims via intravenous administration. The conflicting claims recite parenteral administration, as of conflicting claims 4, 10, and 14. Yang teaches that intravenous administration is a form of parenteral administration. As such, the skilled artisan would have been motivated to have administered the composition of the conflicting claims via intravenous administration in order to have predictably administered the composition in a parenteral manner with a reasonable expectation of success.

The conflicting claims recite a ratio of a maximum of about 1.0 mol of active per mol of lipid, as of conflicting claim 2. Irinotecan has a molecular weight of about 586 g/mole. As such, this is about 586 grams of irinotecan per mole of lipid, or about 586 mg of irinotecan per mmol of lipid. Conflicting claim 2 also recites 0.7 mol of active agent per mol of lipid. This is about 410 mg of active agent per mmol lipid. This overlaps with the claimed range of 500-550 mg of active agent per mmol of lipid. This overlap results in a prima facie case of obviousness-type non-statutory double patenting.

Claims 37-57 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-14 of U.S. Patent No. 8,992,970 in view of Yang et al. (US Patent 6,143,740).

Instant claim 37 is drawn to a method of delivering irinotecan to a tumor. This method comprises intravenously administering a fluid pharmaceutical composition comprising irinotecan and sucrose octasulfate in a lipid matrix. The claims recite a concentration range of about 500 mg to about 550 mg of irinotecan per mmol of phospholipid.

Conflicting claim 1 is drawn to liposomes comprising irinotecan, sucrose octasulfate, and a substituted ammonium cation. Conflicting claim 8 recites parenteral administration.

The conflicting claims do not recite intravenous administration.

Yang et al. (hereafter referred to as Yang) is drawn to a pharmaceutical composition for treating cancer, as of Yang, title and abstract. Yang teaches that intravenous administration is a form of parenteral administration, as of column 8 lines 22-32.

It would have been prima facie obvious for one of ordinary skill in the art to have administered the composition of the conflicting claims via intravenous administration. The conflicting claims recite parenteral administration, as of conflicting claims 4, 10, and 14. Yang teaches that intravenous administration is a form of parenteral administration. As such, the skilled artisan would have been motivated to have administered the composition of the conflicting claims via intravenous administration in order to have

predictably administered the composition in a parenteral manner with a reasonable expectation of success.

The conflicting claims recite a ratio of at least 1.0 mol of irinotecan per mol of lipid, as of conflicting claim 9. Irinotecan has a molecular weight of about 586 g/mole. As such, this is about 586 grams of irinotecan per mole of lipid, or about 586 mg of irinotecan per mmol of lipid. This is greater than the range of about 500 mg to 550 mg of irinotecan per mmol of phospholipids. Nevertheless, one of ordinary skill in the art would have been motivated to have optimized the dosage in order to achieve the claimed dosage of about 500 mg to 550 mg of irinotecan per mmol of phospholipid.

Claims 37-57 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-19 of U.S. Patent No. 9,717,723 in view of Yang et al. (US Patent 6,143,740).

Instant claim 37 is drawn to a method of delivering irinotecan to a tumor. This method comprises intravenously administering a fluid pharmaceutical composition comprising irinotecan and sucrose octasulfate in a lipid matrix. The claims recite a concentration range of about 500 mg to about 550 mg of irinotecan per mmol of phospholipid.

Conflicting claim 1 is drawn to a method of preparing a liposomal irinotecan composition. Said liposomal irinotecan composition comprises sucrose octasulfate, as of conflicting claim 1. Said composition made by the recited method is intended for parenteral administration, as of conflicting claim 5. Said composition made by the

recited method has about 150-550 mg of irinotecan per mmol liposome phospholipids, as of conflicting claim 6.

The conflicting claims do not recite intravenous administration.

Yang et al. (hereafter referred to as Yang) is drawn to a pharmaceutical composition for treating cancer, as of Yang, title and abstract. Yang teaches that intravenous administration is a form of parenteral administration, as of column 8 lines 22-32.

It would have been prima facie obvious for one of ordinary skill in the art to have administered the composition of the conflicting claims via intravenous administration. The conflicting claims recite parenteral administration, as of conflicting claims 4, 10, and 14. Yang teaches that intravenous administration is a form of parenteral administration. As such, the skilled artisan would have been motivated to have administered the composition of the conflicting claims via intravenous administration in order to have predictably administered the composition in a parenteral manner with a reasonable expectation of success.

Regarding the concentration of irinotecan per that of lipids, the conflicting claims recite 150-550 mg of irinotecan per mmol of phospholipids. This overlaps with the claimed requirement of 500-550 mg of irinotecan per mmol of phospholipids. This overlap results in a prima facie case of obviousness-type non-statutory double patenting.

Claims 37-57 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-20 of U.S. Patent No. 9,730,891 in view of Inomata et al. (US Patent 5,756,475).

Instant claim 37 is drawn to a method of delivering irinotecan to a tumor. This method comprises intravenously administering a fluid pharmaceutical composition comprising irinotecan and sucrose octasulfate in a lipid matrix. The claims recite a concentration range of about 500 mg to about 550 mg of irinotecan per mmol of phospholipid.

Conflicting claim 1 is drawn to a method of treatment comprising parenteral administration of irinotecan sucrose octasulfate liposomes. Conflicting claims 2 and 11 recites intravenous administration. Conflicting claims 7 and 14 recite about 500 mg of irinotecan per mmol of total phospholipid in the liposome.

The conflicting claims do not explicitly recite administration to a patient having a tumor.

Inomata et al. (hereafter referred to as Inomata) teaches that irinotecan is an anti-cancer agent, as of Inomata, column 2 lines 6-19.

It would have been prima facie obvious for one of ordinary skill in the art to have administered the composition of the conflicting claims to a patient having cancer and/or a tumor. Irinotecan is a known anti-cancer agent, as of Inomata. As such, the skilled artisan would have been motivated to have conducted the method of administration of the conflicting claims on a patient suffering from a tumor in order to have predictably treated said tumor with a reasonable expectation of success.

Claims 37-57 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-20 of U.S. Patent No. 9,724,303 in view of Slatter et al. (Drug Metabolism and Disposition, Vol. 28 No. 4, 2000, pages 423-433).

Instant claim 37 is drawn to a method of delivering irinotecan to a tumor. This method comprises intravenously administering a fluid pharmaceutical composition comprising irinotecan and sucrose octasulfate in a lipid matrix. The claims recite a concentration range of about 500 mg to about 550 mg of irinotecan per mmol of phospholipid.

Conflicting claim 1 is drawn to an irinotecan sucrose octasulfate lipid vesicle comprising 150-550 mg of irinotecan per mmol of vesicle phospholipids. Conflicting claim 2 recites about 500 mg of irinotecan per mmol of phospholipids.

The conflicting claims do not recite intravenous administration to cancer patients.

Slatter et al. (hereafter referred to as Slatter) teaches that irinotecan (also known as CPT-11) is an anti-cancer agent, as of Slatter, page 423. Slatter teaches intravenous (abbreviated as "i.v.") administration, as of Slatter, page 423, title and abstract. Although Slatter administers radioactive irinotecan, this appears to be used as a model for testing, and Slatter appears to intend that non-radioactive irinotecan is used during normal therapeutic operation.

It would have been prima facie obvious for one of ordinary skill in the art to have administered irinotecan, as of the conflicting claims, in the intravenous manner for treatment of patients suffering from a tumor. Slatter teaches that irinotecan is an anti-cancer agent. As the composition of the conflicting claims comprises irinotecan, the

skilled artisan would have been motivated to have administered the composition of the conflicting claims to a patient suffering from a tumor for predictable treatment of said tumor with a reasonable expectation of success.

Claims 37-57 are rejected on the ground of nonstatutory double patenting as being unpatentable over claim 1 of U.S. Patent No. 9,737,528 in view of Slatter et al. (Drug Metabolism and Disposition, Vol. 28 No. 4, 2000, pages 423-433).

Instant claim 37 is drawn to a method of delivering irinotecan to a tumor. This method comprises intravenously administering a fluid pharmaceutical composition comprising irinotecan and sucrose octasulfate in a lipid matrix. The claims recite a concentration range of about 500 mg to about 550 mg of irinotecan per mmol of phospholipid.

Conflicting claim 1 is drawn to an injectable liquid pharmaceutical composition. Said composition comprises irinotecan and sucrose octasulfate encapsulated within liposomes. The concentration of irinotecan is about 500-550 mg irinotecan per mmol of phospholipids.

While the conflicting claims recite injection, they are silent as to the manner of injection. The conflicting claims also do not recite administration to a patient suffering from a tumor.

Slatter et al. (hereafter referred to as Slatter) teaches that irinotecan (also known as CPT-11) is an anti-cancer agent, as of Slatter, page 423. Slatter teaches intravenous (abbreviated as “i.v.”) administration, as of Slatter, page 423, title and abstract. Although

Slatter administers radioactive irinotecan, this appears to be used as a model for testing, and Slatter appears to intend that non-radioactive irinotecan is used during normal therapeutic operation.

It would have been prima facie obvious for one of ordinary skill in the art to have administered irinotecan, as of the conflicting claims, in the intravenous manner for treatment of patients suffering from a tumor. Slatter teaches that irinotecan is an anti-cancer agent. As the composition of the conflicting claims comprises irinotecan, the skilled artisan would have been motivated to have administered the composition of the conflicting claims to a patient suffering from a tumor for predictable treatment of said tumor with a reasonable expectation of success.

Claims 37-57 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 173-182 of copending Application No. 15/664,976 in view of Slatter et al. (Drug Metabolism and Disposition, Vol. 28 No. 4, 2000, pages 423-433).

Instant claim 37 is drawn to a method of delivering irinotecan to a tumor. This method comprises intravenously administering a fluid pharmaceutical composition comprising irinotecan and sucrose octasulfate in a lipid matrix. The claims recite a concentration range of about 500 mg to about 550 mg of irinotecan per mmol of phospholipid.

Copending claim 173 is drawn to a lipid bilayer liposome comprising irinotecan and sucrose octasulfate. Copending claim 177 recites about 0.15 to 1.5 moles of

irinotecan per moles of total lipid. As irinotecan has a molecular weight of about 568 grams per mole, this is about 88 mg of irinotecan per mmol lipid to about 850 mg of irinotecan per mmol lipid. This overlaps with the claimed amounts of 500 mg to 550 mg of irinotecan per mmol lipid.

The conflicting claims do not teach administration by intravenous injection to a patient suffering from a tumor.

Slatter et al. (hereafter referred to as Slatter) teaches that irinotecan (also known as CPT-11) is an anti-cancer agent, as of Slatter, page 423. Slatter teaches intravenous (abbreviated as "i.v.") administration, as of Slatter, page 423, title and abstract. Although Slatter administers radioactive irinotecan, this appears to be used as a model for testing, and Slatter appears to intend that non-radioactive irinotecan is used during normal therapeutic operation.

It would have been prima facie obvious for one of ordinary skill in the art to have administered irinotecan, as of the conflicting claims, in the intravenous manner for treatment of patients suffering from a tumor. Slatter teaches that irinotecan is an anti-cancer agent. As the composition of the conflicting claims comprises irinotecan, the skilled artisan would have been motivated to have administered the composition of the conflicting claims to a patient suffering from a tumor for predictable treatment of said tumor with a reasonable expectation of success.

This is a provisional nonstatutory double patenting rejection.

Claims 37-57 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 35-55 of copending Application No. 15/896,436 in view of Slatter et al. (Drug Metabolism and Disposition, Vol. 28 No. 4, 2000, pages 423-433).

Instant claim 37 is drawn to a method of delivering irinotecan to a tumor. This method comprises intravenously administering a fluid pharmaceutical composition comprising irinotecan and sucrose octasulfate in a lipid matrix. The claims recite a concentration range of about 500 mg to about 550 mg of irinotecan per mmol of phospholipid.

Copending claim 35 is drawn to a pharmaceutical composition comprising irinotecan and sucrose octasulfate associated with a lipid matrix. An amount of about 500 to 550 mg of irinotecan per mmol phospholipids is recited in the conflicting claims. Copending claim 50 recites an injectable composition.

The copending claims do not explicitly recite intravenous administration to a patient suffering from a tumor.

Slatter et al. (hereafter referred to as Slatter) teaches that irinotecan (also known as CPT-11) is an anti-cancer agent, as of Slatter, page 423. Slatter teaches intravenous (abbreviated as "i.v.") administration, as of Slatter, page 423, title and abstract. Although Slatter administers radioactive irinotecan, this appears to be used as a model for testing, and Slatter appears to intend that non-radioactive irinotecan is used during normal therapeutic operation.

It would have been prima facie obvious for one of ordinary skill in the art to have administered irinotecan, as of the conflicting claims, in the intravenous manner for

treatment of patients suffering from a tumor. Slatter teaches that irinotecan is an anti-cancer agent. As the composition of the conflicting claims comprises irinotecan, the skilled artisan would have been motivated to have administered the composition of the conflicting claims to a patient suffering from a tumor for predictable treatment of said tumor with a reasonable expectation of success.

This is a provisional nonstatutory double patenting rejection.

Close Prior Art – Examiner's Reasons for Not Writing a Prior Art Rejection

As close prior art, the examiner cites Chou et al. (Journal of Bioscience and Bioengineering, Vol. 95, No. 4, 2003, pages 405-408). Chou et al. (hereafter referred to as Chou) is drawn to a liposome comprising irinotecan, which has antitumor activity, as of Chou, page 405, title and abstract. Said liposome includes phospholipids such as phosphatidylcholines, as of Chou, page 405, title.

Chou differs from the claimed invention at least because Chou does not teach sucrose octasulfate. There does not appear to be motivation for the skilled artisan to have included sucrose octasulfate in the liposome of Chou.

Chou also teaches a loading amount of 0.254 mg drug per mg lipid, as of Chou, page 407, right column, last full paragraph. The units of this concentration differ from the recited units. Nevertheless, the examiner has performed a calculation below to estimate the concentration of irinotecan in Chou in mg of irinotecan per mmol of total phospholipids. Assuming, purely *en arguendo*, that the liposome lipids have a molecular

weight of phospholipid of about 790 grams per mole (which is the molecular weight of distearoyl phosphatidylcholine), this is a concentration of

$$\frac{0.254\ mg\ irinotecan}{1\ mg\ lipid} \times \frac{790\ mg\ lipid}{1\ mmol\ lipid} = 200\ \frac{mg\ irinotean}{mmol\ lipid}$$

This amount is significantly less than the recited lower limit of 500 mg irinotecan per mmol phospholipid. As such, the skilled artisan would have understood that the inclusion of sucrose octasulfate loads over twice the amount of irinotecan that would have been loaded in a liposome that lacks sucrose octasulfate.

The examiner notes here that, according to MPEP 2144.05(II)(A), it is generally not inventive to discover the optimum or workable ranges (e.g. of concentration) by routine experimentation. Nevertheless, it is the examiner's position that this provision of the MPEP does not apply to the instant claims. This is because the claims not only require a specific concentration of irinotecan, the claims also require that this concentration of irinotecan be comprised by the lipid matrix (i.e. liposome). Loading a drug such as irinotecan into a lipid matrix would not have been straightforward or predictable to one of ordinary skill in the art, and the skilled artisan would not have been able to have predictably loaded more irinotecan drug simply by increasing the concentration of irinotecan. This is because addition of more irinotecan to the mixture of reagents would not have necessarily and predictably resulted in said irinotecan being loaded in the liposome.

The difficulty of loading irinotecan into a liposome is evident as of Chou, which uses dextran sulfate and a transmembrane gradient loading to load irinotecan, as of Chou, paragraph bridging pages 405 and 406. There would have been no need for such a transmembrane gradient if the concentration of irinotecan in the product could have

been increased simply by increasing the irinotecan amount in the starting material. The difficulty of loading irinotecan would have been further increased by the low water solubility of irinotecan.

As such, the claimed concentration of irinotecan, should **not** be understood as merely a concentration that could have been manipulated at will by one of ordinary skill in the art at the time the invention was made by routine optimization. This is at least due to the lack of a reasonable expectation that the loaded concentration could have been successfully increased to the claimed amount due to the difficulty of loading irinotecan into a liposome.

Additionally, to the extent that the claims read on a case wherein irinotecan and lipids are combined, but the irinotecan is not in the lipid matrix, there would not have been a motivation for the skilled artisan to have added active agent if it was not going to be in the lipid matrix. This is at least because the point of the lipid matrix is to have improved drug delivery of the active agent; however, if the active agent were not in the lipid matrix, the lipid matrix would not have improved delivery of the active agent. Therefore, there would have been no motivation for the skilled artisan to have added additional active agent if it were not to have been in the lipid matrix.

Close Cases Over Which No Double Patenting Rejection Is Written: The examiner notes that US Patent 8,658,203, which has overlapping inventors with the instant application, was considered by the examiner for the purposes of double patenting, but the examiner decided not to write a double patenting rejection over the claims of the ‘203 patent. The claims of the ‘203 patent recite a method of treating a brain tumor comprising administering a liposomal formulation of irinotecan and sucrose

octasulfate. Nevertheless, the claims of the '203 patent recite administration via a conduit placed into brain tissue, which differs from intravenous administration. In fact, claim 14 of the '203 patent recites that this form of administration is better than intravenous administration. As the claims of the '203 patent appear to teach away from intravenous administration, it would not have been obvious to have modified the method of the claims of the '203 patent to provide intravenous administration. The examiner notes that this particular method of the '203 patent may be relevant especially for brain tumors, as the blood brain barrier may make delivery of irinotecan liposomes to brain tumors more difficult than delivery of irinotecan liposomes to other types of tumors. However, the method of the '203 patent may not be needed for delivery to types of tumors other than brain tumors.

The examiner also did not write a double patenting rejection over the claims of US Patent 9,782,349, which has a common inventor with the instant application. The claims of the '349 patent are drawn to an irinotecan liposome comprising a sulfate sugar, as of claim 1 of the '349 patent. Said sulfate sugar may be sucrose octasulfate, as of claim 5 of the '349 patent. Nevertheless, the claims of the '349 patent do not appear to recite the amount of active agent per the amount of phospholipid, which is recited by the instant claims. As explained above in the examiner's analysis of the Chou reference, there would not necessarily have been a reasonable expectation that the skilled artisan could have successfully formulated a composition with 500-550 mg of irinotecan per mmol of phospholipid. As the claims of the '349 patent do not teach the required amounts, there would have been no evidence that the composition claimed by

the '349 patent could have been formulated with 500-550 mg of irinotecan per mmol of phospholipid, as required by the instant claims.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ISAAC SHOMER whose telephone number is (571)270-7671. The examiner can normally be reached on 7:30 AM to 5:00 PM Monday Through Friday.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Frederick F Krass can be reached on (571)272-0580. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see https://ppair-

my.uspto.gov/pair/PrivatePair. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

ISAAC . SHOMER
Primary Examiner
Art Unit 1612

/ISAAC SHOMER/
Primary Examiner, Art Unit 1612

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15/896,436	02/14/2018	Keelung Hong	263266-426641	1952

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McNeill Baur PLLC/Ipsen
Ipsen Bioscience, Inc.
125 Cambridge Park Drive
Suite 301
Cambridge, MA 02140

EXAMINER	
SHOMER, ISAAC	

ART UNIT	PAPER NUMBER
1612	

NOTIFICATION DATE	DELIVERY MODE
07/05/2019	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docketing@mcneillbaur.com
eofficeaction@appcoll.com
patents.us@ipsen.com

Office Action Summary	Application No. 15/896,436	Applicant(s) Hong et al.	
	Examiner ISAAC SHOMER	Art Unit 1612	AIA (FITF) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 28 June 2019.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 35-55 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 35-55 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

DETAILED ACTION

Notice of Pre-AIA or AIA Status

The present application is being examined under the pre-AIA first to invent provisions.

In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

Claim Interpretation

The instant claims recite the term "lipid matrix." As best understood by the examiner, the term "lipid matrix" is understood to refer to various lipidic structures. These structures include, but are not limited to, liposomal and/or lipid vesicle structures comprising a phospholipid bilayer and an aqueous interior core. See e.g. page 96, paragraph 0242 of the instant specification, which refers to a liposome as a matrix. Both a liposome and a lipid vesicle are understood to comprise an aqueous core surrounded by at least one lipid bilayer.

The claims also recite a fluid pharmaceutical composition. The examiner understands that liposomes dispersed in an aqueous environment read on the required fluid. This is because the aqueous environment is liquid at room temperature, and liquids (as well as gases) are fluids.

The instant claims also recite that the irinotecan and sucrose octasulfate are associated with a lipid matrix. As best understood by the examiner, a case wherein irinotecan is encapsulated in a liposome is understood to be an association between the irinotecan and the lipid matrix.

Non-Statutory Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a

result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(l)(1) - 706.02(l)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Claims 35-55 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-35 of U.S. Patent No. 8,147,867. Although the claims at issue are not identical, they are not patentably distinct from each other because of the following reasons:

Instant claim 35 is drawn to a lipid matrix associated with irinotecan and sucrose octasulfate. The claims require a 500-550 mg of irinotecan per mmol of phospholipid.

Conflicting claim 1 is drawn to a liposomal irinotecan composition comprising irinotecan and sucrose octasulfate entrapped within the liposome. Conflicting claim 24 recites about 550 mg of irinotecan per mmol of phospholipid.

The instant and conflicting claims differ because conflicting claims 1 and 11 recite subject matter not specifically recited by instant claim 35 such as the charging of the lipids and the release time from the liposome. Nevertheless, the subject matter of the conflicting claims is within the scope of that of the instant claims, resulting in a prima facie case of anticipatory-type non-statutory double patenting.

Claims 35-55 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-17 of U.S. Patent No. 8,329,213. Although the claims at issue are not identical, they are not patentably distinct from each other because of the following reasons:

Instant claim 35 is drawn to a lipid matrix associated with irinotecan and sucrose octasulfate. The claims require a 500-550 mg of irinotecan per mmol of phospholipid.

Conflicting claim 11 is drawn to a liposome comprising sucrose octasulfate and irinotecan in the aqueous interior space. Conflicting claim 12 recites molar ratios of irinotecan to the totality of lipids.

The instant and conflicting claims differ because the instant claims recite an amount of 500-550 mg of irinotecan per mmol of phospholipids, which is not recited by the conflicting claims. Nevertheless, it would have been prima facie obvious for one of ordinary skill in the art to have modified the composition of the conflicting claims to have achieved the ratio required by the instant claims.

Claims 35-55 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-17 of U.S. Patent No. 8,703,181. Although the claims at issue are not identical, they are not patentably distinct from each other because of the following reasons:

Instant claim 35 is drawn to a lipid matrix associated with irinotecan and sucrose octasulfate. The claims require a 500-550 mg of irinotecan per mmol of phospholipid.

Conflicting claim 11 is drawn to a method of delivering an antineoplastic to a tumor comprising injecting a liposome comprising a sucrose octasulfate salt of irinotecan. Conflicting claim 12 recites a molar ratio of irinotecan to the lipids.

The instant and conflicting claims differ because the conflicting claims are method claims whereas the instant claims are composition claims. Nevertheless, the composition used in the method of the conflicting claims is within the scope of that of the instant claims with the exception of the amount limitations. This results in a prima facie case of non-statutory double patenting.

The instant and conflicting claims differ because the instant claims recite an amount of 500-550 mg of irinotecan per mmol of phospholipids, which is not recited by the conflicting claims. Nevertheless, it would have been prima facie obvious for one of ordinary skill in the art to have modified the composition of the conflicting claims to have achieved the ratio required by the instant claims.

Claims 35-55 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-14 of U.S. Patent No. 8,992,970. Although the claims at issue are not identical, they are not patentably distinct from each other because of the following reasons:

Instant claim 35 is drawn to a lipid matrix associated with irinotecan and sucrose octasulfate. The claims require a 500-550 mg of irinotecan per mmol of phospholipid.

Conflicting claim 1 is drawn to a liposomal composition comprising irinotecan and sucrose octasulfate, as well as various specific lipids.

The instant and conflicting claims differ because the instant claims recite an amount of 500-550 mg of irinotecan per mmol of phospholipids, which is not recited by the conflicting claims. Nevertheless, it would have been prima facie obvious for one of ordinary skill in the art to have modified the composition of the conflicting claims to have achieved the ratio required by the instant claims.

Claims 35-55 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-22 of U.S. Patent No. 8,658,203. Although the claims at issue are not identical, they are not patentably distinct from each other because of the following reasons:

Instant claim 35 is drawn to a lipid matrix associated with irinotecan and sucrose octasulfate. The claims require a 500-550 mg of irinotecan per mmol of phospholipid.

Conflicting claim 1 is drawn to a method for treating a brain tumor comprising administering a liposome comprising irinotecan and sucrose octasulfate to a patient

having a brain tumor. Conflicting claim 3 recites 500 grams of irinotecan per mol phospholipid; this is the same as 500 mg of irinotecan per mmol phospholipid.

The instant and conflicting claims differ because the conflicting claims are method claims whereas the instant claims are composition claims. Nevertheless, the composition used in the method of the conflicting claims is within the scope of that of the instant claims, thereby effectively anticipating the instant claims. This results in a prima facie case of anticipatory-type non-statutory double patenting.

Claims 35-55 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-19 of U.S. Patent No. 9,717,723. Although the claims at issue are not identical, they are not patentably distinct from each other because of the following reasons:

Instant claim 35 is drawn to a lipid matrix associated with irinotecan and sucrose octasulfate. The claims require a 500-550 mg of irinotecan per mmol of phospholipid.

Conflicting claim 1 is drawn to a method for preparing a liposomal irinotecan compound. The method includes adding irinotecan and sucrose octasulfate. Conflicting claim 14 recites 150-550 mg of irinotecan per mmol of liposome phospholipids.

The instant and conflicting claims differ because the conflicting claims are method claims whereas the instant claims are composition claims. Nevertheless, the composition made by the method of the conflicting claims is within the scope of that of the instant claims. As such, the conflicting claims recite all of the subject matter recited by the instant claims with the exception of the limitation regarding the amount of active agent per mmol phospholipid, which is discussed below.

The instant claims recite an amount of about 500-550 mg of irinotecan per total mmol of phospholipids. The conflicting claims recite an amount of about 150-550 mg of irinotecan per mmol of total phospholipids. This overlap results in a prima facie case of obviousness-type non-statutory double patenting.

Claims 35-55 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-17 of U.S. Patent No. 9,782,349. Although the claims at issue are not identical, they are not patentably distinct from each other because of the following reasons:

Instant claim 35 is drawn to a lipid matrix associated with irinotecan and sucrose octasulfate. The claims require a 500-550 mg of irinotecan per mmol of phospholipid.

Conflicting claim 1 is drawn to an irinotecan liposome comprising irinotecan ans a sulfate sugar encapsulated therein. Conflicting claim 4 recites that the sulfate sugar may be sucrose octasulfate.

The instant and conflicting claims differ because the conflicting claims recite various product-by-process limitations not recited by the instant claims. Nevertheless, the subject matter of the conflicting claims is within the scope of the instant claims. As such, the subject matter of the conflicting claims comprises all of the elements of the instant claims, resulting in a prima facie case of non-statutory double patenting.

The instant and conflicting claims differ because the instant claims recite an amount of 500-550 mg of irinotecan per mmol of phospholipids, which is not recited by the conflicting claims. Nevertheless, it would have been prima facie obvious for one of

ordinary skill in the art to have modified the composition of the conflicting claims to have achieved the ratio required by the instant claims.

Claims 35-55 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-20 of U.S. Patent No. 9,730,891. Although the claims at issue are not identical, they are not patentably distinct from each other because of the following reasons:

Instant claim 35 is drawn to a lipid matrix associated with irinotecan and sucrose octasulfate. The claims require a 500-550 mg of irinotecan per mmol of phospholipid.

Conflicting claim 1 is drawn to a method of treatment comprising parenteral administration of a therapeutically effective amount of irinotecan liposomes to a patient, wherein said liposomes comprise irinotecan sucrose octasulfate. Conflicting claim 7 recites about 500 mg of irinotecan per mmol of total phospholipid in the liposome.

The instant and conflicting claims differ because the conflicting claims are method claims whereas the instant claims are composition claims. Nevertheless, the composition used in the method of the conflicting claims is within the scope of that of the instant claims, thereby effectively anticipating the instant claims. This results in a prima facie case of anticipatory-type non-statutory double patenting.

Claims 35-55 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-20 of U.S. Patent No. 9,724,303. Although the

claims at issue are not identical, they are not patentably distinct from each other because of the following reasons:

Instant claim 35 is drawn to a lipid matrix associated with irinotecan and sucrose octasulfate. The claims require a 500-550 mg of irinotecan per mmol of phospholipid.

Conflicting claim 1 is drawn to an irinotecan liposome comprising irinotecan sucrose octasulfate encapsulated therein. Conflicting claim 2 recites about 500 mg of irinotecan per mmol of total vesicle phospholipids.

The instant and conflicting claims differ because the conflicting claims recite limitations not explicitly recited by the instant claims, such as the PEG molecular weight in conflicting claim 6. Nevertheless, the subject matter of conflicting claim 2 is within the scope of that of instant claim 1. This results in a prima facie case of anticipatory-type non-statutory double patenting.

Claims 35-55 are rejected on the ground of nonstatutory double patenting as being unpatentable over claim 1 of U.S. Patent No. 9,737,528. Although the claims at issue are not identical, they are not patentably distinct from each other because of the following reasons:

Instant claim 35 is drawn to a lipid matrix associated with irinotecan and sucrose octasulfate. The claims require a 500-550 mg of irinotecan per mmol of phospholipid.

Conflicting claim 1 is drawn to a pharmaceutical composition comprising irinotecan sucrose octasulfate, as well as specific lipids, and in a specific concentration.

Conflicting claim 1 also recites 500-550 mg of irinotecan per mmol of total liposome phospholipids.

The instant and conflicting claims differ because the conflicting claims recite subject matter not in the instant claims, such as the potency. Nevertheless, the subject matter of the conflicting claims is within the scope of the instant claims, resulting in the subject matter of the conflicting claims effectively anticipating that of the instant claims. This results in a prima facie case of anticipatory-type non-statutory double patenting.

Claims 35-55 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 173-182 of copending Application No. 15/664,976 (reference application). Although the claims at issue are not identical, they are not patentably distinct from each other because of the following reasons.

Instant claim 35 is drawn to a lipid matrix associated with irinotecan and sucrose octasulfate. The claims require a 500-550 mg of irinotecan per mmol of phospholipid.

Copending claim 173 is drawn to a liposome comprising irinotecan as a sucrose octasulfate salt. Claims 177-178 recite an amount of the moles of irinotecan per moles of total lipid.

The instant and copending claims differ because the instant claims recite an amount of 500-550 mg of irinotecan per mmol of phospholipids, which is not recited by the copending claims. Nevertheless, it would have been prima facie obvious for one of ordinary skill in the art to have modified the composition of the copending claims to have achieved the ratio required by the instant claims.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Claims 35-55 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 21-36 of copending Application No. 15/896,389 (reference application). Although the claims at issue are not identical, they are not patentably distinct from each other because of the following reasons.

Instant claim 35 is drawn to a lipid matrix associated with irinotecan and sucrose octasulfate. The claims require a 500-550 mg of irinotecan per mmol of phospholipid.

Copending claim 21 is drawn to a method for delivering irinotecan to a tumor in a liposome comprising irinotecan with sucrose octasulfate. Copending claim 24 recites an amount in moles of irinotecan per moles of total lipid.

The instant and copending claims differ because the conflicting claims are method claims whereas the instant claims are composition claims. Nevertheless, the composition made by the method of the copending claims is within the scope of that of the instant claims, thereby effectively anticipating the instant claims. This results in a prima facie case of anticipatory-type non-statutory double patenting.

The instant and copending claims differ because the instant claims recite an amount of 500-550 mg of irinotecan per mmol of phospholipids, which is not recited by the copending claims. Nevertheless, it would have been prima facie obvious for one of

ordinary skill in the art to have modified the composition of the copending claims to have achieved the ratio required by the instant claims.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Close Prior Art – Examiner's Reasons for Not Writing a Prior Art Rejection

As close prior art, the examiner cites Chou et al. (Journal of Bioscience and Bioengineering, Vol. 95, No. 4, 2003, pages 405-408). Chou et al. (hereafter referred to as Chou) is drawn to a liposome comprising irinotecan, as of Chou, page 405, title and abstract. Said liposome includes phospholipids such as phosphatidylcholines, as of Chou, page 405, title.

Chou differs from the claimed invention at least because Chou does not teach sucrose octasulfate. There does not appear to be motivation for the skilled artisan to have included sucrose octasulfate in the liposome of Chou.

Chou also teaches a loading amount of 0.254 mg drug per mg lipid, as of Chou, page 407, right column, last full paragraph. The units of this concentration differ from the recited units. Nevertheless, the examiner has performed a calculation below to estimate the concentration of irinotecan in Chou in mg of irinotecan per mmol of total phospholipids. Assuming, purely *en arguendo*, that the liposome lipids have a molecular weight of phospholipid of about 790 grams per mole (which is the molecular weight of distearoyl phosphatidylcholine), this is a concentration of

$$\frac{0.254\ mg\ irinotecan}{1\ mg\ lipid} \times \frac{790\ mg\ lipid}{1\ mmol\ lipid} = 200\ \frac{mg\ irinotean}{mmol\ lipid}$$

This amount is significantly less than the lower limit of 500 mg irinotecan per mmol phospholipid. As such, the skilled artisan would have understood that the inclusion of sucrose octasulfate loads over twice the amount of irinotecan that would have been loaded in a liposome that lacks sucrose octasulfate.

The examiner notes here that, according to MPEP 2144.05(II)(A), it is generally not inventive to discover the optimum or workable ranges (e.g. of concentration) by routine experimentation. Nevertheless, it is the examiner's position that this provision of the MPEP does not apply to the instant claims. This is because the claims not only require a specific concentration of irinotecan, the claims also require that this concentration of irinotecan be comprised by the lipid matrix (i.e. liposome). Loading a drug such as irinotecan into a lipid matrix would not have been straightforward or predictable to one of ordinary skill in the art, and the skilled artisan would not have been able to have predictably loaded more irinotecan drug simply by increasing the concentration of irinotecan. This is because addition of more irinotecan to the mixture of reagents would not have necessarily and predictably resulted in said irinotecan being loaded inside the liposome.

The difficulty of loading irinotecan into a liposome is evident as of Chou, which uses dextran sulfate and a transmembrane gradient loading to load irinotecan, as of Chou, paragraph bridging pages 405 and 406. There would have been no need for such a transmembrane gradient if the concentration of irinotecan in the product could have been increased simply by increasing the irinotecan amount in the starting material. The difficulty of loading irinotecan would have been further increased by the low water solubility of irinotecan.

As such, the claimed concentration of irinotecan, should **not** be understood as merely a concentration that could have been manipulated at will by one of ordinary skill in the art at the time the invention was made by routine optimization. This is at least due to the lack of a reasonable expectation that the loaded concentration could have been successfully increased to the claimed amount due to the difficulty of loading irinotecan into a liposome.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ISAAC SHOMER whose telephone number is (571)270-7671. The examiner can normally be reached on 7:30 AM to 5:00 PM Monday Through Friday.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Frederick F Krass can be reached on (571)272-0580. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see https://ppair-my.uspto.gov/pair/PrivatePair. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

ISAAC . SHOMER
Primary Examiner
Art Unit 1612

/ISAAC SHOMER/
Primary Examiner, Art Unit 1612

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McNeill Baur PLLC/Ipsen
Ipsen Bioscience, Inc.
125 Cambridge Park Drive
Suite 301
Cambridge, MA 02140

EXAMINER	
STRONG, TORI	

ART UNIT	PAPER NUMBER
1629	

NOTIFICATION DATE	DELIVERY MODE
03/08/2019	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docketing@mcneillbaur.com
eofficeaction@appcoll.com
patents.us@ipsen.com

Office Action Summary	Application No. 16/012,351	Applicant(s) Bayever et al.	
	Examiner TORI STRONG	Art Unit 1629	AIA (FITF) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 10 January 2019.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 1-20 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 1-20 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☑ The drawing(s) filed on 10 January 2019 is/are: a)☑ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

DETAILED ACTION

Notice of Pre-AIA or AIA Status

The present application is being examined under the pre-AIA first to invent provisions.

Status of Claims

Claims 1-20 are pending in the instant application and are the subject of the Office Action below.

Information Disclosure Statement

The information disclosure statements (IDSs) submitted on 1/18/2019 and 1/19/2019 were filed after the mailing date of the application on June 19, 2018. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner. Enclosed with this Office Action are return copies of Form PTO/SB/08B with the Examiner's initials and signature indicating those references that have been considered.

Claim Rejections - 35 USC § 103

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject

matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under pre-AIA 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under pre-AIA 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of pre-AIA 35 U.S.C. 103(c) and potential pre-AIA 35 U.S.C. 102(e), (f) or (g) prior art under pre-AIA 35 U.S.C. 103(a).

Claims 1-5 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Kozuch *et al.* (*The Oncologist*, 2001, 6, pp. 488-495; cited in IDS) and Tsai *et al.* (*Journal of Gastrointestinal Oncology*, 2011, Vol. 2, No. 3, pp. 185-194; cited in IDS) in view of The American Cancer Society (ACS)

(http://www.cancer.org/cancer/pancreaticcancer/detailedguide/pancreatic-cancer-what-is-pancreatic-cancer; cited in IDS).

Applicant's invention, according to **claim 1**, is directed to a method of treating an exocrine pancreatic cancer comprising administering 60-80 mg/m^2 of irinotecan in a liposome injection formulation having a total volume of 500 mL over about 90 minutes, in combination with a therapeutically effective amount of leucovorin and 5-fluorouracil (5-FU); where the irinotecan liposome injection formulation comprises irinotecan encapsulated within a liposome comprising phosphatidylcholine, cholesterol, and a polyethyleneglycol-derivatized phosphatidyl-ethanolamine, and the irinotecan liposome having a diameter of approximately 80-140 nm. It is important to note that the liposomal irinotecan formulation is also referred to as MM-398 (see specification, *Summary*, p.4, para.7).

Kozuch teaches a method of treating metastatic pancreatic cancer refractory to gemcitabine therapy through administration of a combination called G-FLIP which comprises irinotecan (80 mg/m^2), leucovorin and 5-FU (abs). Kozuch teaches adminsitration of irinotecan, referred to as CPT-11, over 90 minutes (p.490, Fig.1). Kozuch provides a similar composition that comprises irinotecan at 80 mg/m^2 and the other therapeutic agents as instantly claimed for treating pancreatic cancer. Kozuch does not teach irinotecan in liposomal form.

Tsai teaches liposomal therapies for advanced pancreatic cancer to enhance drug delivery. Tsai teaches the liposomal irinotecan has superior efficacy over the free form (p.189, col.1, para.3) and further teaches liposomal irinotecan designated as MM-

398 to have a partial response in pancreatic cancer patients (p.189, col.2, para.2). Tsai further teaches the liposomal irinotecan in combination with 5-FU/LV. Tsai provides teaching that one of ordinary skill in the art would substitute the liposomal form of irinotecan and further combine with the instantly claimed therapeutic agents.

The American Cancer Society (ACS) teaches and defines pancreatic cancer for the general public. The ACS teaches that exocrine tumors are the most common type of pancreatic cancer; where 95% are exocrine cell adenocarcinomas. Therefore treatment that is directed towards pancreatic cancer in general refers to the most common form of exocrine pancreatic cancer.

One of ordinary skill in the art would arrive at the instantly claimed invention having a reasonable expectation of success based on the combined teaching of Kozuch, Tsai and the ACS. Kozuch provides clear teaching of treating pancreatic cancer, of which the ACS discloses exocrine pancreatic cancer is the most common, with the drug combination of irinotecan at 80 mg/m^2 with leucovorin and 5-FU. A skilled artisan would readily gleam from Tsai to interchange irinotecan with the more efficacious MM-398 to combine with leucovorin and 5-FU. The combined art provides rationale for the dosing of irinotecan and the expressed combination with other therapeutic agents for treating pancreatic cancer. Therefore at the time of invention, it was *prima facie* obvious to arrive at the instant claim based on the combined teaching of Kozuch, Tai and the ACS.

Applicant's invention, according to **claims 2-5**, limits claim 1 and requires the described liposomal formulation that is also known as MM-398 to have a dose of 60-80 mg/m^2 of irinotecan and that treatment is refractory to gemcitabine therapy.

As expressed supra, a prima facie case of obviousness is established with the combined teaching of Kozuch, Tsai and the ACS. Kozuch provides teaching of arriving at the dose of irinotecan at 80 mg/m^2, which is within the instantly claimed scope for dosing. Kozuch also teaches treatment refractory to gemcitabine therapy. Tsai provides teaching of the liposomal formulation of irinotecan as MM-398; further teaching its superior benefits over the free form. The instantly claimed limitations fall within the scope of the prior art teaching and therefore as whole remains *prima facie* obvious.

Claims 6-20 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Kozuch *et al.* (*The Oncologist*, 2001, 6, pp. 488-495; cited in IDS) and Tsai *et al.* *(Journal of Gastrointestinal Oncology*, 2011, Vol. 2, No. 3, pp. 185-194; cited in IDS) in view of American Cancer Society (ACS) (http://www.cancer.org/cancer/pancreaticcancer/detailedguide/pancreatic-cancer-what-is-pancreatic-cancer; cited in IDS), in further view of Yoo *et al.* (*British Journal of Cancer*, 2009, 101, pp. 1658-1663; cited in IDS).

Applicant's invention, according to **claims 6-9**, limits claims 1 and requires leucovorin (l) at 200 mg/m^2 (or racemic at 400 mg/m^2); 5-FU at 2400 mg/m^2; sequential administration beginning on day 1 of a 2 week cycle.

A *prima facie* case of obviousness is established with the combined teaching of Kozuch, Tsai and the ACS for combining liposomal irinotecan with leucovorin and 5-FU. Kozuch teaches treatment of pancreatic cancer through sequential administration of the therapeutic agents where irinotecan, leucovorin and 5-FU is administered in the respective order (p.490, para.1) on day 1 and teaches administration is repeated every 2 weeks (p.488, abs), implicitly a 2-week cycle. However the combined art does not explicitly teach the instantly claimed dosing for leucovorin and 5-FU.

Yoo teaches a method of treating metastatic pancreatic cancer refractory to gemcitabine therapy (abs) through administration of a drug combination regimen called FOIFIRI.3 which comprises irinotecan (70 mg/m^2), leucovorin (400 mg/m^2) and 5-fluorouracil (2000 mg/m^2) (p.1659, col.2, para.3). Yoo teaches administration of 5-FU over a 46 hour period with the entire regimen repeated every 2 weeks. Yoo provides teaching of a similar composition that comprises the same therapeutic agents in similar amounts as instantly claimed.

One of ordinary skill would arrive at the instant limitations having a reasonable expectation of success based on the combine teaching of Kozuch, Tsai, the ACS and Yoo. Kozuch teaches instantly claimed limitations of sequential administration in a 2 week cycle, where Yoo provides for similar dosing in the chemotherapeutic regimen. As skilled artisan would gleam from Yoo, through routine and conventional means, to optimize the range of dosing for treatment; see MPEP 2144.05 (II) for guidance for optimization of ranges. Therefore, the invention as a whole is *prima facie* obvious with the incorporation of the instant limitations.

Applicant's invention, according to **claims 10-19**, is directed to a method of treating an exocrine pancreatic cancer comprising administering 60-80 mg/m^2 of irinotecan in a liposome injection formulation having a total volume of 500 mL over about 90 minutes, in combination with leucovorin (l) at 200 mg/m^2 (or racemic at 400 mg/m^2) and 5-FU at 1800-2400 mg/m^2; where claims 11-15, 18 and 19 disclose limitations that describe MM-398; where claims 16 and 17 require treatment refractory to gemcitabine treatment.

As expressed *supra*, a case of *prima facie* obviousness is established over the instantly claimed limitations. Kozuch and Yoo teaches the drug regimen in similar dosing and Tsai teaches substituting MM-398 for the free form of irinotecan. Kozuch teaches administration over 90 minutes and the ACS teaches that the most common pancreatic cancer is exocrine pancreatic cancer. As explained *supra*, one of ordinary skill would arrive at the instant claims having a reasonable expectation of success and therefore the invention as whole remains *prima facie* obvious.

Applicant's invention, according to **claim 20**, is directed to a method of treating an exocrine pancreatic cancer comprising administering 60-80 mg/m^2 of irinotecan in a liposome injection formulation having a total volume of 500 mL, in combination with leucovorin (l) at 200 mg/m^2 (or racemic at 400 mg/m2) and 5-FU at 2400 mg/m^2; where the claim discloses limitations that describe MM-398; where administration is sequential administration beginning on day 1 of a 2 week cycle.

As expressed *supra*, a case of *prima facie* obviousness is established over the instantly claimed limitations. Kozuch and Yoo teaches the drug regimen in similar

dosing and Tsai teaches substituting MM-398 for the free form of irinotecan. Kozuch teaches sequential administration on day 1 in a 2 week cycle and the ACS teaches that the most common pancreatic cancer is exocrine pancreatic cancer. As explained supra, one of ordinary skill would arrive at the instant claims having a reasonable expectation of success and therefore the invention as whole remains *prima facie* obvious.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a

result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(l)(1) - 706.02(l)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Claims 1-20 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-11, 19-24 and 30-35 of U.S. Patent No. 9,452,162 B2; claims 1-20 and 23-28 of U.S. Patent No. 9,492,442; claims 1-27 of U.S. Patent No. 9,339,497; claims 1-29 of U.S. Patent No. 9,364,473 B2; and claims 1-24 of U.S. Patent No. 9,717,724 B2. Although the claims at issue are not identical, they are not patentably distinct from each other because each of the disclosures set out to claim a method of treating pancreatic cancer refractory to gemcitabine therapy through intravenous administration of irinotecan as the MM-398 liposome, leucovorin as the (l)-form or

racemic form and 5-fluorouracil. Each of the disclosures claim the combination in either a similar or identical dose for the therapeutic agents where the combination is administered in a two week cycle. The claims are obvious variants of each other.

Claims 1-20 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-20 of copending Application No. 16/012,372. Although the claims at issue are not identical, they are not patentably distinct from each other because each disclosure sets out to claim a method of treating pancreatic cancer refractory to gemcitabine therapy through intravenous administration of liposomal irinotecan in overlapping dosing range with leucovorin as the (l)-form or racemic form and 5-fluorouracil. The claims are obvious variants.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to TORI STRONG whose telephone number is (571)272-6333. The examiner can normally be reached on Monday - Friday 8:00 am - 5:00 pm (EST).

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an

interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffrey Lundgren can be reached on (571)272-5541. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

TORI STRONG
Examiner
Art Unit 1629

/TORI STRONG/
Examiner, Art Unit 1629

/JEFFREY S LUNDGREN/
Supervisory Patent Examiner, Art Unit 1629

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/012,372	06/19/2018	Eliel Bayever	01208-0002-11US	1003

153749 7590 03/08/2019

McNeill Baur PLLC/Ipsen
Ipsen Bioscience, Inc.
125 Cambridge Park Drive
Suite 301
Cambridge, MA 02140

EXAMINER	
STRONG, TORI	

ART UNIT	PAPER NUMBER
1629	

NOTIFICATION DATE	DELIVERY MODE
03/08/2019	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docketing@mcneillbaur.com
eofficeaction@appcoll.com
patents.us@ipsen.com

Office Action Summary	Application No. 16/012,372	Applicant(s) Bayever et al.	
	Examiner TORI STRONG	Art Unit 1629	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 19 June 2018.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 1-20 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 1-20 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☑ The drawing(s) filed on 19 June 2018 is/are: a)☑ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

DETAILED ACTION

Notice of Pre-AIA or AIA Status

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Status of Claims

Claims 1-20 are pending in the instant application and are the subject of the Office Action below.

Information Disclosure Statement

The information disclosure statements (IDSs) submitted on 1/22/2019 and 1/23/2019 were filed after the mailing date of the application on June 19, 2018. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner. Enclosed with this Office Action are return copies of Form PTO/SB/08B with the Examiner's initials and signature indicating those references that have been considered.

Double Patenting

A rejection based on double patenting of the "same invention" type finds its support in the language of 35 U.S.C. 101 which states that "whoever invents or discovers any new and useful process... may obtain a patent therefor..." (Emphasis added). Thus, the term "same invention," in this context, means an invention drawn to

identical subject matter. See *Miller v. Eagle Mfg. Co.*, 151 U.S. 186 (1894); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Ockert*, 245 F.2d 467, 114 USPQ 330 (CCPA 1957).

A statutory type (35 U.S.C. 101) double patenting rejection can be overcome by canceling or amending the claims that are directed to the same invention so they are no longer coextensive in scope. The filing of a terminal disclaimer cannot overcome a double patenting rejection based upon 35 U.S.C. 101.

Claims 1-20 are rejected under 35 U.S.C. 101 as claiming the same invention as that of claims 1-20 of prior U.S. Patent No. 9,717,724 B2. This is a statutory double patenting rejection.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*,

686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(l)(1) - 706.02(l)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Claims 1-20 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-27 of U.S. Patent No. 9,339,497 B2; claims 1-29

of U.S. Patent No. 9,364,473 B2; claims 1-35 of U.S. Patent No. 9,452,162 B2; and claims 1-30 of U.S. Patent No. 9,492,442 B2; and claims 1-24 of U.S. Patent No. 9,717,724 B2. Although the claims at issue are not identical, they are not patentably distinct from each other because each disclosure sets out to claim treatment of pancreatic cancer with the antiproliferative therapeutic regimen that consist of liposomal irinotecan at a dosing of 60, 70 or 80 mg/m^2; leucovorin (*l*-form) at a dose if 200 mg/m^2; and 5-fluorouracil at a dose of 2,400 mg/m^2. Each disclosure teaches and claims treating patients refractory to gemcitabine therapy and treating patients who either are or not homozygous for the UGT1 A1*28 allele. The closest prior art, as indicated in the record of previous case App. No. 14/812,950 (now patented U.S. Patent No. 9,339,497 B2) is found in Yoo *et al.* (*British Journal of Cancer*, 2009, 101, pp. 1658-163); where Yoo teaches treating pancreatic cancer refractory to gemcitabine therapy with the mFOIRIRI.3 regimen that consists of irinotecan, leucovorin and 5-fluorouracil. However, Yoo requires different dosing and that irinotecan is administered twice where the therapy provides the overall survival for the mFOIRIRI.3 regimen to be 16.6 weeks (or 4.2 months). The instantly claimed invention carves out a specific regimen that requires the dosing of the components and administers the drugs only once within a cycle and further provides for the unexpected result of improving clinical benefit of up to 80% and the increasing the patient population survival of at least 6 months. Therefore the claims are free of the art. However, the claims are obvious variants of the previously patented subject matter and therefore the nonstatutory double patenting rejection is applied.

Claims 1-20 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-20 of copending Application No. 16/012,351. Although the claims at issue are not identical, they are not patentably distinct from each other because each disclosure claims treating patients with pancreatic cancer refractory to gemcitabine therapy and treating patients the same general combination with overlapping dosing of liposomal irinotecan. The claims are obvious variants.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to TORI STRONG whose telephone number is (571)272-6333. The examiner can normally be reached on Monday - Friday 8:00 am - 5:00 pm (EST).

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffrey Lundgren can be reached on (571)272-5541. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

TORI STRONG
Examiner
Art Unit 1629

/TORI STRONG/
Examiner, Art Unit 1629

/JEFFREY S LUNDGREN/
Supervisory Patent Examiner, Art Unit 1629

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/036,885	07/16/2018	Eliel Bayever	01208-0003-03US	1094

153749 7590 09/03/2019

McNeill Baur PLLC/Ipsen
Ipsen Bioscience, Inc.
125 Cambridge Park Drive
Suite 301
Cambridge, MA 02140

EXAMINER	
BAEK, BONG-SOOK	

ART UNIT	PAPER NUMBER
1611	

NOTIFICATION DATE	DELIVERY MODE
09/03/2019	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docketing@mcneillbaur.com
eofficeaction@appcoll.com
patents.us@ipsen.com

Office Action Summary	Application No. 16/036,885	Applicant(s) Bayever et al.	
	Examiner BONG-SOOK BAEK	Art Unit 1611	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 36-52 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 36-52 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☑ The drawing(s) filed on 7/16/2018 is/are: a)☑ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

Notice of Pre-AIA or AIA Status

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

DETAILED ACTION

Status of claims

Claims 36-52 are under examination in the instant office action.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent for a claimed invention may not be obtained, notwithstanding that the claimed invention is not identically disclosed as set forth in section 102 of this title, if the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains. Patentability shall not be negated by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103 are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 36-44, 47-48, and 51-52 are rejected under 35 U.S.C. 103 as being unpatentable over US 2014/0170075 (cited in the IDS filed on 2/4/2019) as evidenced by Merrimack Pharmaceuticals, Inc. Pilot study (ClinicalTrials.gov Identifier: NCT01770353, August 9, 2013, cited in the IDS filed on 2/5/2019) and US 2007/0110798 (cited in the IDS filed on 2/4/2019) in view of Chen *et al.* (Journal of Clinical Oncology 26, no. 15_suppl, May 20 2008, 2565-2565).

US 2014/0170075 teaches a method for selecting and providing pharmaceutical treatment to a patient for a localized infectious, inflammatory, or neoplastic condition, the method comprising identifying one or more locations of infection, inflammation or neoplasia in a patient, and subsequently, obtaining at least one contrast-enhanced MRI image of a first location of the one or more locations, and subsequently, selecting an anti-infective, anti-inflammatory, or anti-neoplastic pharmaceutical agent and treating the patient with the selected pharmaceutical agent, wherein the contrast agent is ferumoxytol (FMX) which is intravenously administered at 5 mg/kg up to 510 mg/kg and wherein the pharmaceutical agent is a liposomal anti-neoplastic agent (abstract, [0019], [0020]claims 8-13). US 2014/0170075 also teaches that the liposomal therapeutic agent is MM-398 (irinotecan sucroseoctasulfate liposome injection) and the tumor is a non-small cell lung cancer (NSCLC) tumor, a triple negative breast cancer (TNBC) tumor, a colorectal cancer (CRC) tumor, a pancreatic cancer tumor, a small cell lung cancer tumor, a gastric cancer tumor, a cervical cancer tumor, or Ewing's sarcoma ([0068] and claims 14-16). US 2014/0170075 teaches that as FMX has been demonstrated to be safe for intravenous administration to patients and is shown herein not to interfere with nanoliposome therapies if used as an imaging agent, even within 1-4 hours prior to administration of nanoliposomal therapeutics, these results indicate that FMX MRI allows for selection patients who will (or will not) benefit from nanoliposomal therapy ([0105]). US 2014/0170075 further teaches that patients

identified as having sites of pathology that are predicted to exhibit nanoparticle accumulation would be considered more likely to respond to nanoparticulate therapeutic agents and patients identified as having sites of pathology that are predicted not to exhibit nanoparticle accumulation would be considered less likely to respond to nanoparticulate therapeutic agents and treating patients in accordance with such identifications would avoid the administration of sub-optimal therapeutic treatments to patients in need of therapy ([0011]). MM-398 (irinotecan sucroseoctasulfate liposome) is meeting the limitations of claim 51-52 as evidenced by US 2007/0110798 (see below, [0189], [0190], [0081], [0313], and [0327]).

The reference specifically discloses a human clinical trial (ClinicalTrials.gov Identifier: NCT01770353) wherein patients with advanced solid tumors and multiple metastases were injected with FMX at 5 mg/kg and then were infused with 80 mg/m^2 MM-398 and shows that the patents have tumor lesion with FMX uptake (see[0135], [0139], and Examples 9-10 and Figs. 6-8). As evidenced by the clinical trial (ClinicalTrials.gov Identifier: NCT01770353), the solid tumor includes ER/PR positive breast cancer and triple negative breast cancer (HER2 negative) and MM-398 80 mg/m^2 was intravenously administered over 90 min once every two weeks) (see Condition, Arms, and Criteria sections). The reference further discloses the measured FMX levels at day 2 (24 h) and day 4 (72 h) after FMX injection together with the calculated volumes of four liver lesions at treatment start ("Screening") and after four weeks or two treatment cycles wherein the level of FMX 24 hours after the intravenous administration of FMX is 38.98, 28, 04, 39.94, and 37.35 mcg/ml in lesions 1-4, respectively (Example 10 and table). Also, it discloses that the change in the volume of liver tumor lesion is greater in lesion 1, 3, and 4 (Example 10 and table). Since the patient has a tumor lesion with FMX tumor lesion uptake of 38.98, 39.94, and 37.35 mcg/ml after 24 hours after the intravenous administration, it inherently discloses that

"a FMX tumor lesion uptake is "at least 32.6 mcg/ml one hour after the intravenous administration of 5 mg/kg FMX" as evidenced by Fig. 6B (the FMX uptake level is lower after 24 hrs than after one hour).

The reference does not specifically disclose the treatment of breast cancer with active brain metastasis or HER2-positive breast cancer. Also, the reference does not specifically disclose 60 mg/m^2 dose of irinotecan hydrochloride trihydrate.

US 2007/0110798 discloses unilamellar liposomes (lipid bilayer vesicles) loaded with CPT-11 (irinotecan as a hydrochloride trihydrate salt) in a gelated or precipitated state, yielding a final diameter of 95-110 nm and the drug/lipid ratio of about 500 mg/mmol phospholipid, wherein liposomes with entrapped triethylammonium sucrose octasulfate (0.65 M TEA, pH 6.4, osmolality 485 mmol/kg) and lipid composition of 1,2-Distearoyl-SN-phosphatidylcholine (DSPC), Cholesterol, and N-(omega-methoxy-poly(ethylene glycol)-oxycarbonyl)-1,2-distearoylphosphatidyl ethanolamine (PEG-DSPE) in a molar ratio of 3:2:0.015 were prepared (abstract, [0003], [0081], [0189], [0190], [0172], [0178], [0313], and [0327]). US 2007/0110798 also discloses that the liposomal composition comprising a camptothecin compound such as irinotecan (CPT-11) has an anticancer activity at least two times, four times, or ten times higher than the camptothecin compound similarly administered in the absence of the composition, while the toxicity of the composition does not exceed, is at least two times, or at least four times lower than the toxicity of the camptothecin compound similarly administered in the absence of the composition ([0010]). US 2007/0110798 further discloses antitumor efficacy of CPT-11 liposomes (drug/phospholipid ratio 192 mg/mmol; average liposome size 86.8 nm) in the model of HER2-overexpressing human breast carcinoma (BT-474) and human glioma (U87) wherein greater anti-tumor activity with CPT-11 liposomes treatment were shown compared with free

CPT-11 ([0182], [0184], [0185], [0330] and Fig. 3 and 49). US 20070110798 further discloses the liposome formulation of CPT-11 showed better treatment outcomes against intracranial tumors (brain tumor) due to its significantly lower toxicity and increased persistence in the brain tissue ([0330] and Fig. 49). US 2007/0110798 further discloses that the liposome composition of the present invention can be administered in any way which is medically acceptable which may depend on the condition or injury being treated and possible administration routes include injections, by parenteral routes such as intramuscular, subcutaneous, intravenous, intraarterial, intraperitoneal, intraarticular, intraepidural, intrathecal, or others ([0134]). In addition, US 20070110798 discloses that the mean residence time of the liposomal drug in the healthy brain was at comparable infusate concentration (3 mg/mL) 24 times higher than that of the free CPT-11 (as a hydrochloride trihydrate salt, dissolved) and the mean residence time of the drug in the tumor tissue, at equal drug concentration in the infusate, was 4 times higher than in the normal brain ([0327]).

Chen *et al*. discloses phase I study of liposome encapsulated irinotecan (PEP02), which is a novel nanoparticle liposome formulation of irinotecan aiming to enhance tumor localization and improve pharmacokinetic properties of irinotecan and its active metabolite-SN38, in advanced refractory solid tumor patients wherein PEP02 was given as 90 mins i.v. infusion, repeated every 3 weeks and the doses would have been escalated from 60, 120, 180 to 240 mg/m^2 in a single-patient cohort accelerated titration design (Title, Methods, and Results).

It would have been prima facie obvious to one of ordinary skill in the art before the effective filing date of the claimed invention to use nanoliposomal irinotecan such as MM-398 for treating breast cancer with active brain metastasis or HER2-positive breast cancer because of the following reasons. As stated above, nanoliposomal irinotecan such as MM-398 was known to

be effective for treating breast cancer with active metastatic lesion as evidenced by US 2014/0170075. Also, US 2007/0110798 teaches that nanoliposomal irinotecan has higher antitumor efficacy in the animal model of HER2-positive human breast carcinoma (BT-474) and human glioma (brain cancer) compared with free irinotecan. Thus, one of ordinary skill in the art would have been motivated to use the method taught by US 2014/0170075 in the treatment of breast cancer with active brain metastasis or HER2-postive breast cancer on the reasonable expectation that it would also be useful for treating those cancers based on the antitumor efficacies of nanoliposomal irinotecan in the animal model of HER2-positive human breast carcinoma and human glioma (brain cancer) as evidenced by US 20070110798.

As to the dose of 60 mg/m^2, it was known in the art that the dose of liposomal irinotecan could be titrated from 60, 120, 180 to 240 mg/m^2 as evidenced by Chen *et al.* Thus, it would have been prima facie obvious to one of ordinary skill in the art before the effective filing date of the claimed invention to optimize the dosage for getting desired effects based on known effective dosage. Also, the claimed range falls within the range disclose in the prior art. In the case where the claimed ranges "overlap or lie inside ranges disclosed by the prior art" a prima facie case of obviousness exists. *In re Wertheim*, 541 F.2d 257, 191 USPQ 90 (CCPA 1976). Furthermore, "[A] prior art reference that discloses a range encompassing a somewhat narrower claimed range is sufficient to establish a prima facie case of obviousness." *In re Peterson*, 315 F.3d 1325, 1330, 65 USPQ2d 1379, 1382-83 (Fed. Cir. 2003). In addition, it is well-established that merely selecting proportions and ranges is not patentable absent a showing of criticality. *In re Becket*, 33 USPQ 33; *In re Russell*, 169 USPQ 426. "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955); see also

Peterson, 315 F.3d at 1330, 65 USPQ2d at 1382 ("The normal desire of scientists or artisans to improve upon what is already generally known provides the motivation to determine where in a disclosed set of percentage ranges is the optimum combination of percentages.")

Claims 45-46 are rejected under 35 U.S.C. 103 as being unpatentable over US 2014/0170075 (cited in the IDS filed on 2/4/2019) as evidenced by Merrimack Pharmaceuticals, Inc. Pilot study (ClinicalTrials.gov Identifier: NCT01770353, August 9, 2013, cited in the IDS filed on 2/5/2019) and US 2007/0110798 (cited in the IDS filed on 2/4/2019) in view of Chen *et al.* (Journal of Clinical Oncology 26, no. 15_suppl, May 20 2008, 2565-2565) in further view of US 2007/0219268 and Hayashi *et al.* (Breast Cancer, 20:131-136, 2013, Epub, Nov. 29, 2011, cited in the IDS filed on 2/5/2019).

US 2014/0170075, US 2007/0110798, *and* Chen *et al.* as applied *supra* are herein applied for the same teachings in their entirety.

The prior art does not specifically teach pre-medicating with at least one anti-emetic such as 5-HT3 antagonist as recited in claims 45-46.

However, it was well known in the art that at least one anti-emetic such as 5-HT3 antagonist is pre-medicated before the treatment with a chemotherapeutic agent including irinotecan for preventing vomiting (abstract, [0048], [0119] and [0142]). Thus, it would have been prima facie obvious to one of ordinary skill in the art before the effective filing date of the claimed invention to pre-medicate with at least one anti-emetic such as 5-HT3 antagonist prior to administering liposomal irinotecan in the treatment of breast cancer with active brain metastasis or HER2 positive breast cancer because it was well known practice in the chemotherapy as evidenced by US 2007/0219268 and vomiting is a known adverse event related with irinotecan

treatment as evidenced by Hayashi *et al.* (see p134, Toxicity section and Table 2). One of ordinary skill in the art would have been motivated to do so for preventing vomiting caused by anti-cancer treatment.

Claims 49-50 are rejected under 35 U.S.C. 103 as being unpatentable over US 2014/0170075 (cited in the IDS filed on 2/4/2019) as evidenced by Merrimack Pharmaceuticals, Inc. Pilot study (ClinicalTrials.gov Identifier: NCT01770353, August 9, 2013, cited in the IDS filed on 2/5/2019) and US 2007/0110798 (cited in the IDS filed on 2/4/2019) in view of Chen *et al.* (Journal of Clinical Oncology 26, no. 15_suppl, May 20 2008, 2565-2565) in further view of Hayashi *et al.* (Breast Cancer, 20:131-136, 2013, Epub, Nov. 29, 2011, cited in the IDS filed on 2/5/2019).

US 2014/0170075, US 20070110798, and Chen *et al.* as applied *supra* are herein applied for the same teachings in their entirety.

As to claims 49-50, the prior art does not specifically disclose that the patient has failed at least one prior platinum-based chemotherapy regimen, has failed prior treatment with gemcitabine, and/or has become resistant to gemcitabine. However, Hyashi *et al.* disclose phase II study of bi-weekly irinotecan for patients with previously treated HER2-negative metastatic breast cancer (MBC) wherein eligible patients were HER2-negative, had a performance status of 0 to 2, and had been treated previously with either anthracyclines or taxanes for MBC (title and abstract). Hyashi *et al.* further disclose that patients received irinotecan intravenously at 150 mg/ m^2 on days 1 and 15 every 4 weeks (once every two weeks) and biweekly administration of 150 mg/ m^2 irinotecan was feasible for patients with MBC treated previously with anthracyclines or taxanes (abstract).

Thus, one of ordinary skill in the art would have been motivated to use the liposomal irinotecan as an alternative treatment for patients who failed at least one prior platinum-based chemotherapy regimen or gemcitabine, and/or has become resistant to gemcitabine. It would have obvious to use alternative cancer treatment for MBC such as liposomal irinotecan taught by US 20070110798 and Hyashi *et al.* when the other existing anticancer therapy was not working because irunotecan was taught to be effective for those cancers. This is what a person of ordinary skill in the corresponding art normally does.

Double Patenting Rejections

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the

scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(l)(1) - 706.02(l)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Claims 32-52 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1, 8, 10, and 15-31 of copending application 14/964571 in view of US 2014/0170075.

Although the conflicting claims are not identical, they are not patentably distinct from each other because the claims of '571 application are drawn to a method of treating breast cancer with active brain metastasis, HER2 negative breast cancer, HER2 negative metastatic breast cancer, HER2 negative or HER2 positive metastatic breast cancer with at least one brain lesion.by administering the same liposomal irinotecan in the same dosage as claimed, wherein prior to treatment with the liposomal irinotecan, the patient receives a ferumoxytol infusion followed by an MRI scan. The claims of the '571 application does not specifically teach the dosage amount of ferumoxytol. However, US 2014/0170075 teaches a method for selecting and

providing pharmaceutical treatment to a patient for a localized infectious, inflammatory, or neoplastic condition, the method comprising identifying one or more locations of infection, inflammation or neoplasia in a patient, and subsequently, obtaining at least one contrast-enhanced MRI image of a first location of the one or more locations, and subsequently, selecting an anti-infective, anti-inflammatory, or anti-neoplastic pharmaceutical agent and treating the patient with the selected pharmaceutical agent, wherein the contrast agent is ferumoxytol (FMX) which is intravenously administered at 5 mg/kg up to 510 mg/kg and wherein the pharmaceutical agent is a liposomal anti-neoplastic agent (abstract, [0019], [0020]claims 8-13). US 2014/0170075 also teaches that the liposomal therapeutic agent is MM-398 (irinotecan sucroseoctasulfate liposome injection) and the tumor is a non-small cell lung cancer (NSCLC) tumor, a triple negative breast cancer (TNBC) tumor, a colorectal cancer (CRC) tumor, a pancreatic cancer tumor, a small cell lung cancer tumor, a gastric cancer tumor, a cervical cancer tumor, or Ewing's sarcoma ([0068] and claims 14-16). US 2014/0170075 teaches that as FMX has been demonstrated to be safe for intravenous administration to patients and is shown herein not to interfere with nanoliposome therapies if used as an imaging agent, even within 1-4 hours prior to administration of nanoliposomal therapeutics, these results indicate that FMX MRI allows for selection patients who will (or will not) benefit from nanoliposomal therapy ([0105]). US 2014/0170075 further teaches that patients identified as having sites of pathology that are predicted to exhibit nanoparticle accumulation would be considered more likely to respond to nanoparticulate therapeutic agents and patients identified as having sites of pathology that are predicted not to exhibit nanoparticle accumulation would be considered less likely to respond to nanoparticulate therapeutic agents and treating patients in accordance with such identifications would avoid the administration of sub-optimal therapeutic treatments to patients in need of

therapy ([0011]). The reference further discloses the measured FMX levels at day 2 (24 h) and day 4 (72 h) after FMX injection together with the calculated volumes of four liver lesions at treatment start ("Screening") and after four weeks or two treatment cycles wherein the level of FMX 24 hours after the intravenous administration of FMX is 38.98, 28, 04, 39.94, and 37.35 mcg/ml in lesions 1-4, respectively (Example 10 and table). Also, it discloses that the change in the volume of liver tumor lesion is greater in lesion 1, 3, and 4 (Example 10 and table). Since the patient has a tumor lesion with FMX tumor lesion uptake of 38.98, 39.94, and 37.35 mcg/ml after 24 hours after the intravenous administration, it inherently discloses that "a FMX tumor lesion uptake is "at least 32.6 mcg/ml one hour after the intravenous administration of 5 mg/kg FMX" as evidenced by Fig. 6B (the FMX uptake level is lower after 24 hrs than after one hour).

Thus, it would have been prima facie obvious to one of ordinary skill in the art before the effective filing date of the claimed invention to arrive at the claimed amount of ferumoxytol for administering prior to treatment with the liposomal irinotecan because the claimed amount of ferumoxytol is already taught to be suitable for selecting patients who will (or will not) benefit from nanoliposomal therapy and identifying those patients as having sites of pathology that are predicted to exhibit nanoparticle accumulation as evidenced by US 2014/0170075. As such, the instant claims would have been obvious over the claims of '571 application in view of US 2014/0170075.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to BONG-SOOK BAEK whose telephone number is 571-270-5863. The examiner can normally be reached 9:00AM-6:00PM Monday-Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Bethany Barham can be reached on 571-272-6175. The fax phone number for the organization where this application or proceeding is assigned is (571) 273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/BONG-SOOK BAEK/
Primary Examiner, Art Unit 1611

Institute for the Advancement of Technology for Health

Institute of Biomaterials & Biomedical Engineering
UNIVERSITY OF TORONTO

STTARR
University Health Network

Imaging-Based Assessment of the Treatment Efficacy of Nanoliposomal Irinotecan (nal-IRI) in a Triple Negative Breast Cancer Model of Spontaneous Metastasis

Manuela Ventura[1*], Nicholas Bernards[1*], Helen Lee[2], Bart Hendriks[2], Jonathan Fitzgerald[2], David Jaffray[1,3], Jinzi Zheng[1,3]

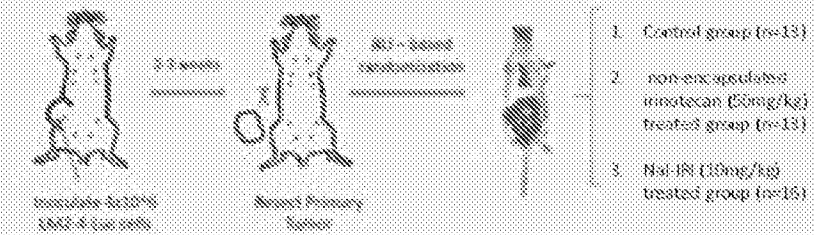
[1]TECHNA Institute for the Advancement of Technology for Health, University Health Network, Toronto, Ontario, Canada
[2]Merrimack Pharmaceuticals, Inc., Cambridge, Massachusetts, USA
[3]Institute of Biomaterial and Biomedical Engineering, University of Toronto, Ontario, Canada
*Equal contribution

Background

Triple negative breast cancer (TNBC) represents a significant treatment challenge because of its aggressive and highly metastatic nature. Non-encapsulated irinotecan has been employed as part of combination treatment regimens for TNBC [1, 2] and pancreatic ductal adenocarcinoma [3] as it demonstrated limited therapeutic activity when administered as a monotherapy. Nanoliposomal irinotecan (nal-IRI) is a nanocarrier formulation of irinotecan and has shown to significantly increase the exposure (i.e. AUC) of irinotecan and its active metabolite SN-38 [4] relative to non-encapsulated irinotecan. The efficacy of nal-IRI in combination with 5-FU/LV has been recently demonstrated by a Phase III trial in patients with advanced pancreatic cancer previously treated with gemcitabine. This study investigates the potential benefit of nal-IRI for the treatment of TNBC in a mouse model of spontaneous metastasis (LM2-4, [5]).

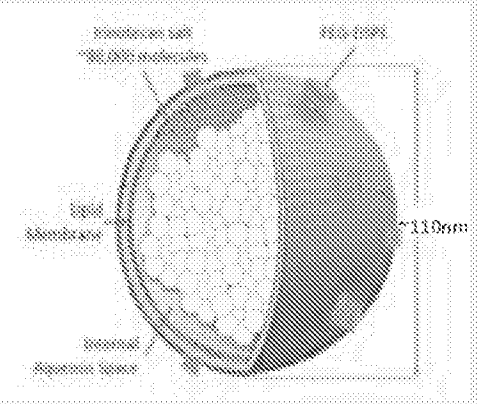
Methods

42 female SCID mice were inoculated with TNB MDA-MB-231 – derived, and metastatic variant, LM2-4-luc cells in their lower right inguinal mammary fat pad. The primary tumors were resected between 2-3 weeks post-inoculation with a resected mean tumor volume of 220 ± 60 mm^3. Post primary tumor resection, bioluminescence imaging (BLI, Xenogen, Perkin Elmer) was used to monitor metastasis formulation. Mice were randomized into 3 groups consisting of (1) control group (n=13), (2) non-encapsulated irinotecan (50 mg/kg salt) treated group (n=13), and (3) nal-IRI (10 mg/kg irinotecan HCl salt) treated group (n=16), when each animal presented with at least one metastasis detected via BLI (in addition to any tumor regrowth at the site of the primary tumor removal). The total BLI photon flux measured prior to treatment initiation showed no statistical differences among the 3 groups (p = 0.82). Treatment with either non-encapsulated irinotecan or nal-IRI was administered IV every 7 days until study endpoint (i.e. when the size of the primary regrowth exceeded 1500 mm^3, or an ulceration of >20% was present at the primary regrowth site, or animals experienced severe difficulties in breathing as a result of lung metastasis, or day 89 post-treatment initiation was reached). Animals were monitored 2-3 times per week using BLI and at the study endpoint using a 1T MRI (M3, Aspect Imaging).



Note: Nal-IRI and IRI concentrations in this poster are specified in salt concentrations. Nal-IRI 10 mg/kg (salt) is approximate to 9 mg/kg (free base).

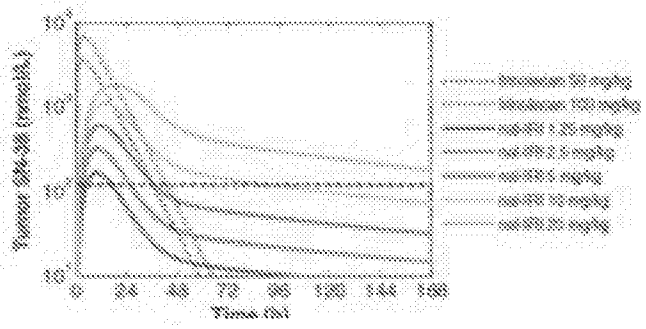
Nanoliposomal Irinotecan (nal-IRI)



Key Attributes:

- Long circulation and sustained release
- Tumor accumulation via the enhanced permeability and retention (EPR) effect
- Improved treatment efficacy and reduced toxicity compared to non-encapsulated irinotecan when administered at comparable SN-38 AUC in plasma and tumor [6].

I



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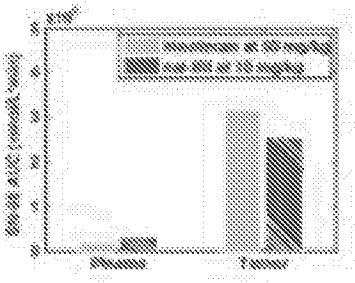


I. Model simulations were used to compare tumor SN-38 (active form of irinotecan) concentration following the administration of varying doses of non-encapsulated irinotecan or nal-IRI. Black dashed line represents threshold concentration of 120 nmol/L to determine tumor SN-38 duration. [6]

II. Model predictions for similar SN-38 AUC in plasma and tumor following non-encapsulated irinotecan (50 mg/kg) and nal-IRI (10 mg/kg) administration.

Results

Nal-IRI provided significant survival benefit achieving 4.7x longer median survival compared to both non-encapsulated irinotecan treated and control animals (66 vs. 14 days, P = 0.0008, **Figure A**). This survival benefit achieved with nal-IRI was supported by, an effective control of the metastatic burden monitored using longitudinal BLI (**Figure B** and **Figure C,** P < 0.0001) as well as a significant delay in tumor regrowth at the site of the excised primary tumor for the animals treated (**Figure D**) and verified at the study endpoint with MRI and histology. Treatment did not induce toxicity based on body weight monitoring over the course of the study (**Figure E**).

Figure A. Nal-IRI increases median survival by 4.7x

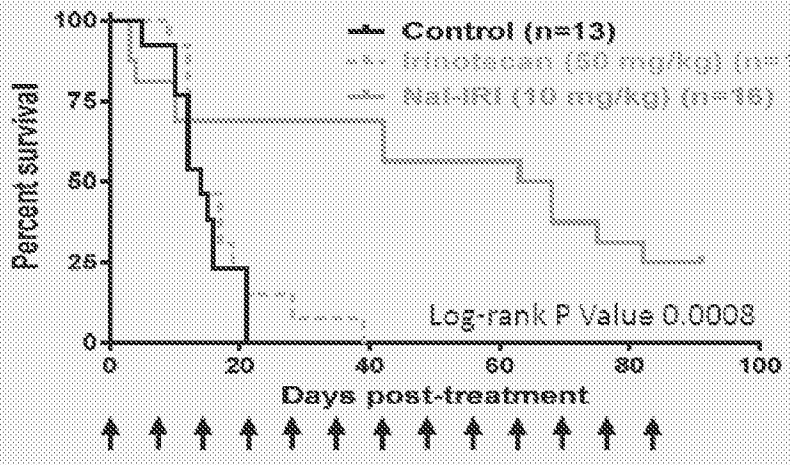


Figure A. Survival data for the 3 groups. Nal-IRI provided significant survival benefit achieving 4.7x longer median survival compared to both non-encapsulated irinotecan treated and control animals (66 vs. 14 days, P = 0.0008, log-rank). Causes of termination include the tumor sizes reaching 1.5 cm in diameter, excessive ulceration (>20% of overall tumor area) and sever breathing difficulty due to lung metastases.

Figure B. Nal-IRI effectively treats distal metastases

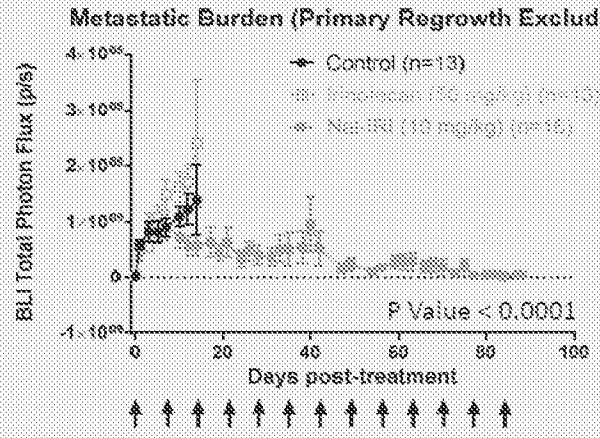


Figure B. Quantification of BLI signal in terms of total whole body photon flux obtained from both prone and supine acquisitions and by excluding the signal measured at the site of the primary tumor regrowth (Figure C). Effective control of the metastatic burden was achieved in the nal-IRI treated group ($P < 0.0001$), as compared to both the control and the non-encapsulated irinotecan treated mice.

Figure C. Longitudinal whole body tumor burden quantification by bioluminescence imaging (BLI)

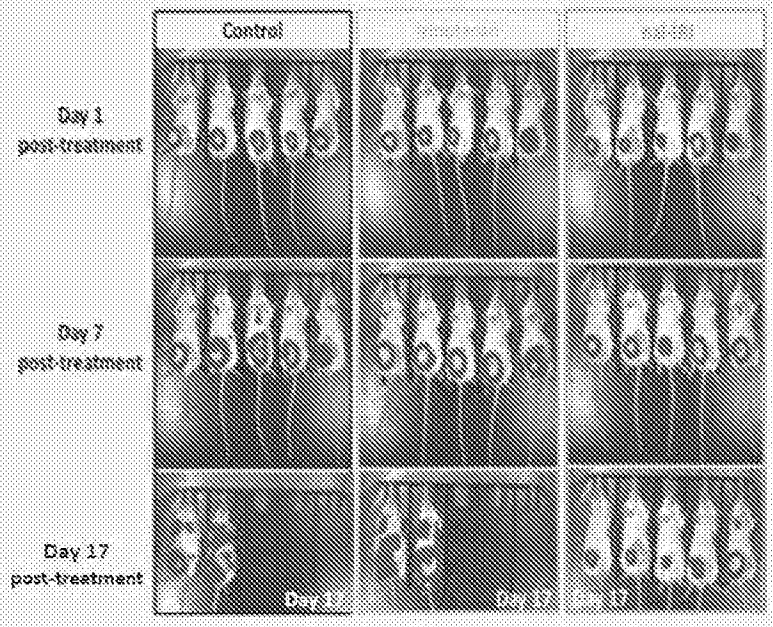


Figure C. BLI – Prone view. Representative animals for each treatment group acquired at day 1, 7, and 17 days post-treatment initiation. The same color scale was used for all images based on total signal flux (p/s). Clear treatment benefit of nal-IRI can be observed both in terms of primary regrowth control and management of metastasis. Each animal is seen at the same position over time. Missing animals indicate lack of survival.

Figure D. Nal-IRI delays and controls tumor regrowth at the primary site post surgery

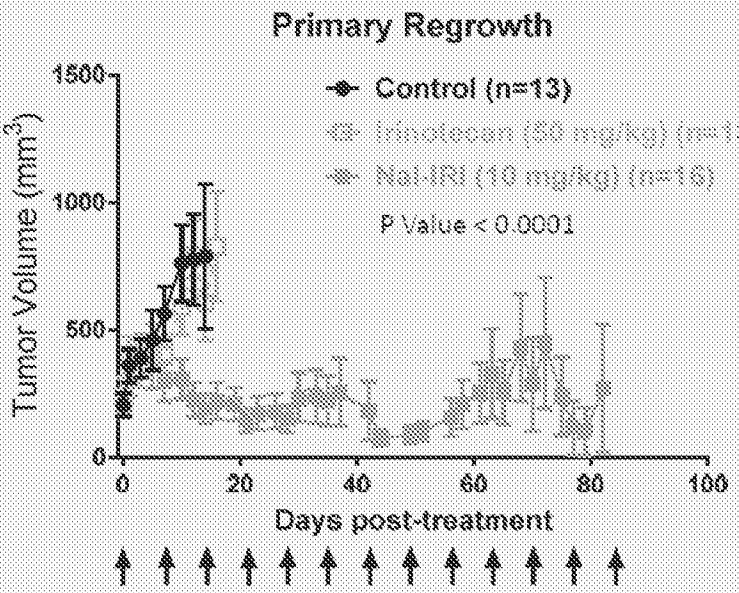


Figure D. Caliper-based measurement of the primary regrowth lesions. The survival benefit achieved with nal-IRI was supported by a significant delay in tumor regrowth at the site of the excised primary tumor for the treated animals (P < 0.0001).

Figure E. No treatment induced weight loss

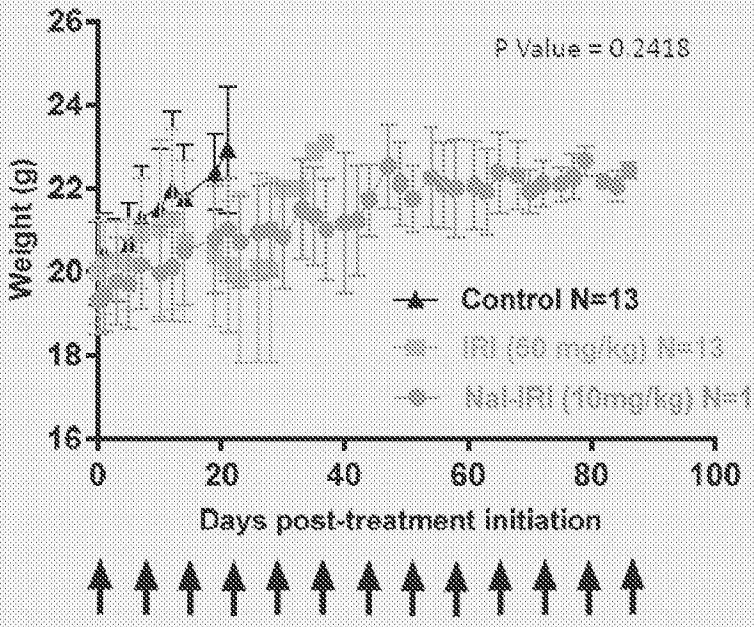


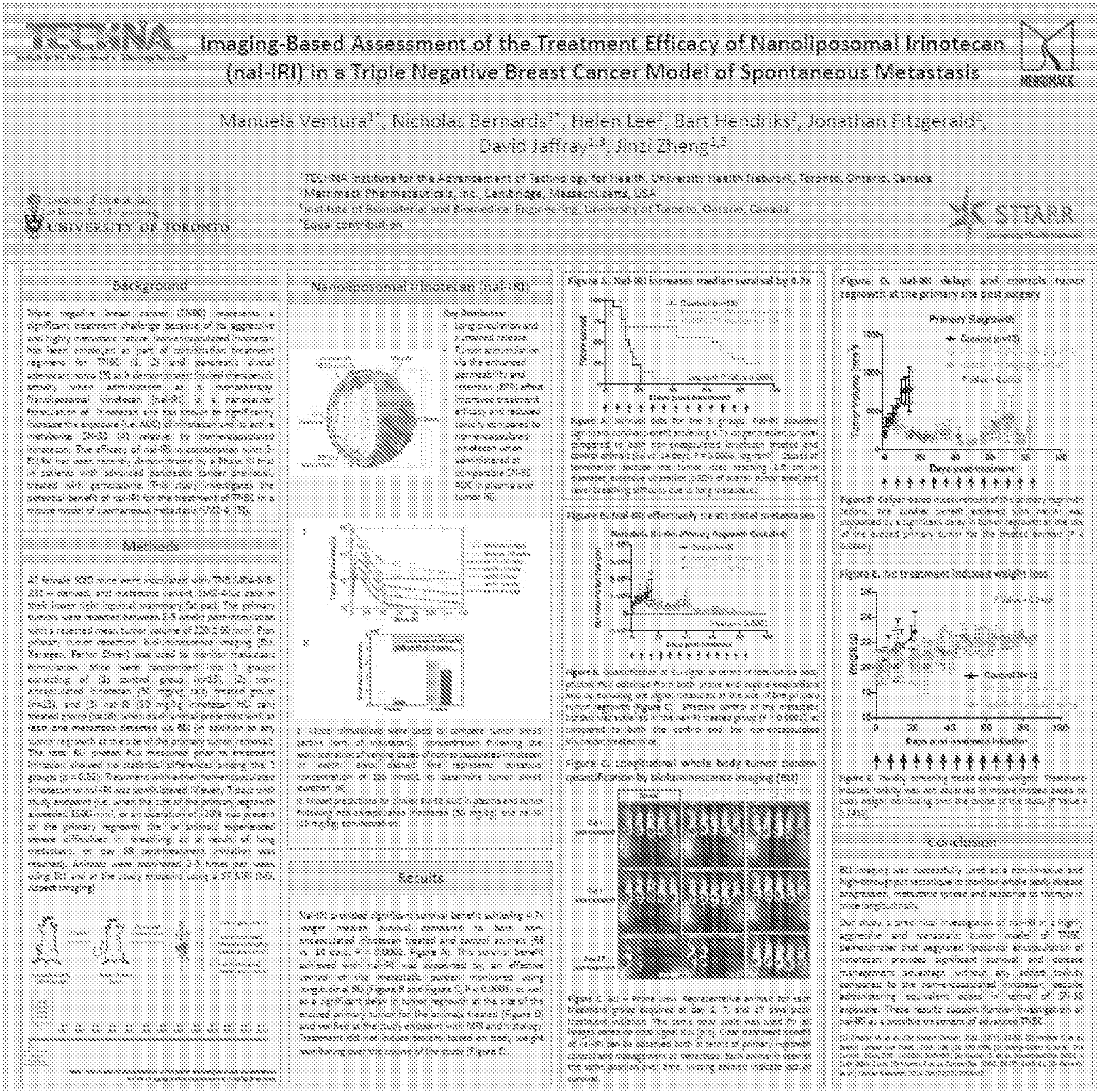
Figure E. Toxicity screening based animal weights. Treatment-induced toxicity was not observed in mouse models based on body weight monitoring over the course of the study (P Value = 0.2418).

Conclusion

BLI imaging was successfully used as a non-invasive and high-throughput technique to monitor whole body disease progression, metastatic spread and response to therapy in mice longitudinally.

Our study, a preclinical investigation of nal-IRI in a highly aggressive and metastatic tumor model of TNBC, demonstrates that pegylated liposomal encapsulation of irinotecan provides significant survival and disease management advantage without any added toxicity compared to the non-encapsulated irinotecan, despite administering equivalent doses in terms of SN-38 exposure. These results support further investigation of nal-IRI as a possible treatment of advanced TNBC.

[1] Crozier JA et al. *Clin Breast Cancer*. 2016, 16(1): 23-30. [2] Anders C et al. *Breast Cancer Res Treat*. 2014, 146 (3): 557-566. [3] Wang-Gillam A et al. The Lancet. 2016, 387 (10018): 545-557. [4] Noble CO et al. *Nanomedicine*. 2014, 9 (14): 2099-2108. [5] Munoz R et al. *Cancer Res*. 2006, 66 (7): 3386-91. [6] Kalra AV et al., Cancer Research. 2014 Oct;74(23):7003–13.


Imaging-Based Assessment of the Treatment Efficacy of Nanoliposomal Irinotecan (nal-IRI) in a Triple Negative Breast Cancer Model of Spontaneous Metastasis
Manuela Ventura, Nicholas Bernards, Helen Lee, Bart Hendriks, Jonathan Fitzgerald, David Jaffray, Jinzi Zheng
UNIVERSITY OF TORONTO
STARR
Background
Nanoliposomal Irinotecan (nal-IRI)
Methods
Results
Conclusion

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ORIGINAL REPORT

Randomized Phase III Trial of Amrubicin Versus Topotecan As Second-Line Treatment for Patients With Small-Cell Lung Cancer

Joachim von Pawel, Robert Jotte, David R. Spigel, Mary E.R. O'Brien, Mark A. Socinski, Jörg Mezger, Martin Steins, Léon Bosquée, Jeffrey Bubis, Kristiaan Nackaerts, José M. Trigo, Philip Clingan, Wolfgang Schütte, Paul Lorigan, Martin Reck, Manuel Domine, Frances A. Shepherd, Shaoyi Li, and Markus F. Renschler

Author affiliations appear at the end of this article.

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Corresponding author: Joachim von Pawel, MD, Asklepios Fachkliniken, München-Gauting, Robert-Koch-Allee 2, 82131 Gauting, Germany; e-mail: j.pawel@asklepios.com.

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ABSTRACT

Purpose
Amrubicin, a third-generation anthracycline and potent topoisomerase II inhibitor, showed promising activity in small-cell lung cancer (SCLC) in phase II trials. This phase III trial compared the safety and efficacy of amrubicin versus topotecan as second-line treatment for SCLC.

Patients and Methods
A total of 637 patients with refractory or sensitive SCLC were randomly assigned at a ratio of 2:1 to 21-day cycles of amrubicin 40 mg/m^2 intravenously (IV) on days 1 to 3 or topotecan 1.5 mg/m^2 IV on days 1 to 5. Primary end point was overall survival (OS); secondary end points included overall response rate (ORR), progression-free survival (PFS), and safety.

Results
Median OS was 7.5 months with amrubicin versus 7.8 months with topotecan (hazard ratio [HR], 0.880; $P = .170$); in refractory patients, median OS was 6.2 and 5.7 months, respectively (HR, 0.77; $P = .047$). Median PFS was 4.1 months with amrubicin and 3.5 months with topotecan (HR, 0.802; $P = .018$). ORR was 31.1% with amrubicin and 16.9% with topotecan (odds ratio, 2.223; $P < .001$). Grade ≥ 3 treatment-emergent adverse events in the amrubicin and topotecan arms were: neutropenia (41% *v* 54%; $P = .004$), thrombocytopenia (21% *v* 54%; $P < .001$), anemia (16% *v* 31%; $P < .001$), infections (16% *v* 10%; $P = .043$), febrile neutropenia (10% *v* 3%; $P = .003$), and cardiac disorders (5% *v* 5%; $P = .759$); transfusion rates were 32% and 53% ($P < .001$), respectively. *NQO1* polymorphisms did not influence safety outcomes.

Conclusion
Amrubicin did not improve survival when compared with topotecan in the second-line treatment of patients with SCLC. OS did not differ significantly between treatment groups, although an improvement in OS was noted in patients with refractory disease treated with amrubicin.

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INTRODUCTION

Small-cell lung cancer (SCLC) is the most aggressive type of lung cancer.[1] Unfortunately, little progress has been made in improving response or survival rates for this malignancy over the last 30 years. Despite encouraging phase II results for many targeted therapies and newer chemotherapeutic agents, current large phase III trials have failed to show improvement compared with standard of care.[2-7] Conversely, many promising newer chemotherapies have yet to be studied as single agents in phase III trials; thus, their potential role in SCLC remains to be established.

First-line SCLC treatment with a combination of cisplatin or carboplatin and etoposide results in 2-year survival of approximately 40% in limited-stage disease, but only 5% in extensive-stage disease.[8] Because most patients experience relapse or disease progression after an initial response to chemotherapy, second-line therapy is often required.[8] In patients with refractory SCLC (relapse < 90 days after end of initial treatment), the response rate to second-line agents is approximately 10%.[8] For patients sensitive to initial treatment (relapse ≥ 90 days after initial treatment), the overall response rate (ORR) is approximately 25%.[8]

The topoisomerase I inhibitor topotecan is the only agent approved for second-line therapy in patients with SCLC sensitive to initial treatment.[8] A study by von Pawel et al[9] compared intravenous

(IV) topotecan with a regimen of cyclophosphamide, doxorubicin, and vincristine (CAV) for patients with recurrent SCLC. Topotecan treatment reduced symptoms to a greater degree than CAV and was associated with less hematologic toxicity, although it failed to provide a survival benefit. The combination of oral topotecan plus best supportive care (BSC) has shown both survival and quality-of-life benefits compared with BSC alone in patients with relapsed SCLC.[10] In a phase II study of topotecan as second-line therapy in both platinum-sensitive and -refractory patients, ORRs of 37.8% and 6.4%, respectively, were observed. Median overall survival (OS) was 6.9 months in platinum-sensitive SCLC and 4.7 months in platinum-refractory patients.[11]

Amrubicin, a third-generation anthracycline and potent topoisomerase II inhibitor, has shown promising activity in SCLC. Gene polymorphisms involved in the metabolism and transport of amrubicin (eg, *NQO1*) may affect the safety and efficacy of amrubicin.[12] The drug has been evaluated in a number of studies in Japanese patients, producing ORRs of 36% to 52% and median survival of 7 to 12 months in the second-line setting.[13-18] Amrubicin was approved in Japan in 2002 for SCLC and non–small-cell lung cancer and is under evaluation in other countries. Two North American/European phase II studies[19,20] demonstrated the clinical activity and safety of amrubicin, supporting further study of its use in SCLC. In the first study, platinum-refractory patients achieved an ORR of 21%, median progression-free survival (PFS) of 3.2 months, and 6-month median OS.[19] In the second study, platinum-sensitive patients were randomly assigned to receive either amrubicin or topotecan. Patients in the amrubicin arm achieved an ORR of 44% versus 15% for topotecan ($P = .021$). Median PFS and OS were 4.5 and 9 months, respectively, for amrubicin versus 3.3 and 7.6 months for topotecan.[20]

Here we report the results of a multicenter, randomized, open-label phase III trial (ACT-1 [Amrubicin Clinical Trial–1]), designed to evaluate the safety and efficacy of amrubicin versus topotecan as second-line therapy for patients with sensitive or refractory SCLC. Pharmacogenomic evaluations were performed in a patient subset to explore the possible impact of *NQO1* on effectiveness or toxicity.

PATIENTS AND METHODS

Patients

Adults (age ≥ 18 years) with histologically or cytologically confirmed SCLC were eligible to participate; those with mixed or combined subtypes of SCLC were ineligible. Patients required documented progression after first-line platinum-based chemotherapy with disease measurable by modified RECIST criteria (version 1.0). Patients with extensive or limited-stage disease either sensitive or refractory to first-line therapy were eligible. Patients had to have Eastern Cooperative Oncology Group performance status of 0 to 1 (amended from 0 to 2) and adequate organ function. Exclusion criteria included chest radiotherapy ≤ 28 days before treatment; prior anthracycline, topotecan, or irinotecan treatment; prior brain metastasis; and symptomatic CNS metastases.

The study was conducted in accordance with the International Conference on Harmonisation Good Clinical Practice guidelines, the Declaration of Helsinki, and applicable local regulatory requirements and laws. Approval from the institutional review board or independent ethics committee of each participating center was required, and patients provided written informed consent.

Study Design

Randomly assigned patients received either amrubicin or topotecan (at ratio of 2:1) and were stratified on the basis of sensitivity to first-line treatment (sensitive: complete response [CR], partial response [PR], or stable disease [SD] after first-line therapy and recurrence- or progression-free interval ≥ 90 days after completion of first-line therapy; refractory: progressive disease [PD] as best response to first-line therapy or progression-free interval < 90 days) and disease stage (extensive or limited). Primary end point was OS (defined as time from random assignment until date of death resulting from any cause). Key secondary end points were investigator-determined ORR based on RECIST (version 1.0), PFS determined by investigator, response duration, and safety.

Patients received amrubicin 40 mg/m^2 administered as 5-minute IV infusion once daily on days 1 to 3 of a 21-day cycle. Topotecan 1.5 mg/m^2 was administered as 30-minute IV infusion once daily on days 1 to 5 of a 21-day cycle. Treatment was continued for six cycles or until PD. Patients with at least SD at cycle six could receive an additional six treatment cycles. Patients were monitored regularly for toxicity, and ≤ two dose reductions per patient were permitted for grade 3 or 4 toxicities. Dose delays ≤ 4 weeks were permitted to allow recovery from treatment-associated toxicities. The protocol was amended to mandate the use of prophylactic hematopoietic growth factors in all cycles for all patients in both treatment arms. Prophylactic antibiotics were recommended for patients at high risk of infectious complications.

Study Procedures

Tumor assessments of the brain, chest, and abdomen were performed at screening and after every second cycle using computed tomography or magnetic resonance imaging. Tumor response was evaluated by investigators every two cycles.

Safety was evaluated by monitoring adverse events (AEs), hematology, blood chemistry, urinalysis, vital signs, and physical examination. AEs were graded using the National Cancer Institute Common Terminology Criteria for AEs (version 3.0). Potential cardiotoxicity was monitored during each cycle using 12-lead ECGs, and left ventricular ejection fraction was assessed at baseline and once between cycles three and four (unless otherwise indicated) using multigated angiography scans or echocardiograms. *NQO1* genotyping was voluntary and performed using peripheral-blood mononuclear cells at Gene Logic (Gaithersburg, MD).

Statistical Analysis

The study was powered based on the assumption of a median OS of 8.7 months for amrubicin and 6.0 months for topotecan (hazard ratio [HR], 0.69); 490 events were required to ensure 97.5% power to detect a statistically significant difference in OS (alternative hypothesis) between the treatment arms (unstratified log-rank test at $\alpha = 0.05$; two-sided overall significance level). Allowing for patients lost to follow-up and administrative censoring, approximately 620 patients would be required.

The intent-to-treat (ITT) population was the primary population for the evaluation of all demographic and efficacy end points. The per-protocol (PP) population was supportive to the analysis of the ITT population for the primary and key secondary efficacy end points at the final analysis. Protocol violations were identified from the clinical database to assess the eligibility of randomly assigned patients for the PP population, which by definition included all ITT patients who experienced no major protocol violations (ie, violation of any inclusion or exclusion criteria, patients who received anticancer therapy at any time during treatment period other than that to which they were randomly assigned or cranial irradiation, treatment cycle delayed > 28 days, and noncompliance with study drug [defined as receiving < 75% of scheduled treatment doses through first four cycles]). The primary population for safety evaluation comprised all patients who received any study medication. Descriptive statistics were used to summarize patient characteristics and treatment administration, tumor response, and safety parameters. Odds ratios (ORs) and HRs are reported as amrubicin to topotecan. OS, PFS, response duration, and time-to-progression (TTP) were summarized using the Kaplan-Meier method; between-treatment comparisons for OS, TTP, and PFS were conducted using the unstratified log-rank test. A supportive analysis using the

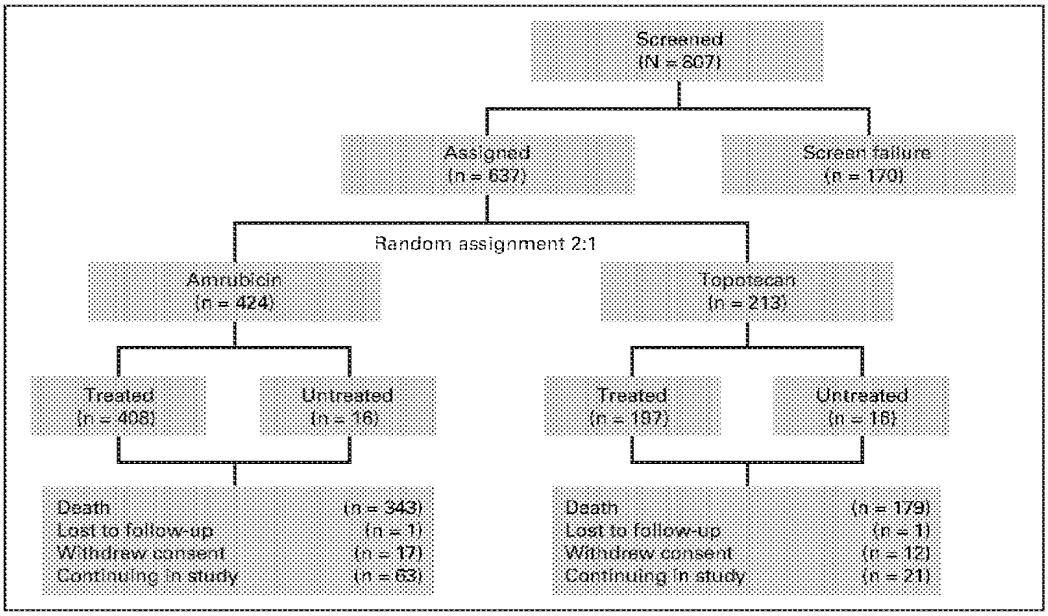

Fig 1. CONSORT flow diagram of patient disposition at time of cutoff for efficacy analysis.

stratified log-rank test was performed for OS and PFS. Categorical variables were analyzed using the χ^2 test. Exploratory analyses using Cox proportional hazards model (CPHM) regression for time-to-event variables and logistic regression for categorical variables were used to explore the effect of various demographic and prognostic factors on efficacy. Two-sided P values were reported for all tests and 95% CIs for all variables.

Exploratory analyses were conducted to examine the effect of genetic variations in *NQO1* genotype polymorphisms. Patients were categorized into one of the following groups: normal enzyme activity, intermediate enzyme activity, or poor enzyme activity. The possible impact of *NQO1* polymorphism was explored for effectiveness (OS and ORR [CR plus PR]) as well as toxicity (AEs and effect on absolute neutrophil count). Until Amendment No. 6, blood for *NQO1* analyses was collected only in the amrubicin arm. For evaluation of the primary efficacy variable at the interim analysis, an O'Brien-Fleming type of group sequential boundary with a Lan-DeMets α-spending function was chosen, preserving the nominal two-sided α level of 0.01.

RESULTS

Patients

Between December 2007 and January 2010, 637 patients from 116 centers in Europe, Canada, Australia, and the United States were assigned to randomly receive amrubicin or topotecan; 605 patients received treatment (Fig 1). Demographic and baseline disease characteristics of the ITT population were balanced between treatment arms; however, several factors showing slightly worse prognostic features were present in the amrubicin arm (Table 1).

Among 341 patients who consented to *NQO1* genotyping, 186 patients receiving amrubicin (64.4%) and 33 receiving topotecan (63.5%) were genotyped as having *NQO1*1/*1* (normal enzyme activity; wild type), 95 patients receiving amrubicin (32.9%) and 16 receiving topotecan (30.8%) were shown to have the *NQO1*1/*2* genotype (intermediate enzyme activity; mutant heterozygotes), and eight patients receiving amrubicin (2.8%) and three receiving topotecan (5.7%) carried the *NQO1*2/*2* genotype (poor enzyme activity; mutant homozygotes; Table 2).

Efficacy

Survival was similar in the ITT populations (median: amrubicin, 7.5 months; 95% CI, 6.8 to 8.5 *v* topotecan, 7.8 months; 95% CI, 6.6 to 8.5; HR demonstrated trend in favor of amrubicin: HR, 0.880; 95% CI, 0.733 to 1.057; unstratified log-rank $P = .170$; Fig 2A). In an exploratory analysis to evaluate the impact of imbalances on prognostic factors using a stratified log-rank test, the survival trend in favor of amrubicin persisted but did not reach statistical significance (HR, 0.853; 95% CI, 0.709 to 1.027; stratified log-rank $P = .094$). The multivariable CPHM, which included well-recognized prognostic factors, identified a statistically significant effect of treatment on OS in favor of amrubicin (HR, 0.82; $P = .036$). In an additional prespecified analysis in the PP population, OS was longer (ie, 2 weeks) with amrubicin versus topotecan (median, 8.0 months; 95% CI, 7.1 to 8.8 *v* 7.5 months; 95% CI, 6.3 to 8.5; HR, 0.806; 95% CI, 0.654 to 0.994; unstratified log-rank $P = .043$). Because the 95% CI upper boundary is < 1.111 for both ITT and PP populations, noninferiority can be concluded with respect to OS. Amrubicin significantly prolonged PFS (median, 4.1 months; 95% CI, 3.5 to 4.3 *v* 3.5 months; 95% CI, 2.9 to 4.2; HR, 0.802; 95% CI, 0.667 to 0.965; unstratified log-rank $P = .018$; Fig 3A) as well as median TTP compared with topotecan in the ITT population (4.6 *v* 4.3 months; HR, 0.758, unstratified log-rank $P = .012$).

In the amrubicin arm, 31.1% of the ITT population responded to treatment compared with 16.9% in the topotecan arm (OR, 2.223; $P < .001$; Table 3). For patients who had at least a PR, median response duration was 0 months for patients receiving either amrubicin or topotecan (HR, 0.680; unstratified log-rank $P = .000$; Fig 3B). There were seven CRs to amrubicin, including one patient who experienced PD after only two cycles of first-line platinum-based chemotherapy and whose response persisted > 1 year.

In the subgroup analysis by sensitivity to first-line therapy, patients with sensitive disease had a median survival of 9.2 versus 9.9 months (amrubicin *v* topotecan: HR, 0.936; $P = .615$; Fig 2B). These patients had a median PFS of 5.5 (95% CI, 4.3 to 5.9) versus 4.3

Table 1. Patient Demographic and Clinical Characteristics at Baseline (ITT population)

Characteristic	Amrubicin (n = 424) No.	%	Topotecan (n = 213) No.	%
Age, years				
Median	62		61	
Range	22-81		30-81	
≥ 65	169	39.9	74	34.7
≥ 75	32	7.5	12	5.6
Male sex	244	57.5	127	59.6
ECOG PS				
0	126	29.7	72	33.8
1	289	68.2	137	64.3
2	9	2.1	4	1.9
Actual disease stage				
Limited	53	12.5	26	12.2
Extensive	371	87.5	187	87.8
Time from SCLC diagnosis, months				
Median	8.4		8.4	
Range	1.2-108		1.6-49.6	
Smoking status				
Current	126	29.7	49	23.0
Former	253	59.7	140	65.7
Never	45	10.6	24	11.3
First-line chemotherapy				
Median No. of cycles	6		5	
Response				
CR	54	12.7	33	15.5
PR	239	56.4	105	49.3
SD	84	19.8	49	23.0
PD	47	11.1	25	11.7
Missing	0	0.0	1	0.5
Prior radiotherapy	200	47.2	106	49.8
Response to prior first-line therapy				
Sensitive	225	53.1	117	54.9
Refractory	199	46.9	96	45.1
Time from end of first-line treatment to PD, days				
Median	96.0		107.0	
< 90	197	46.5	93	43.7
≥ 90	227	53.5	120	56.3

Abbreviations: CR, complete response; ECOG PS, Eastern Cooperative Oncology Group performance status; ITT, intent to treat; PD, progressive disease; PR, partial response; SCLC, small-cell lung cancer; SD, stable disease.

months (95% CI, 3.8 to 5.4; HR, 0.671; 95% CI, 0.518 to 0.869; unstratified log-rank $P = .0023$) with amrubicin and topotecan, respectively. Among sensitive patients, 40.9% responded to amrubicin, compared with 23.1% to topotecan (OR, 2.306; $P = .001$).

Table 2. *NQO1* Genotyping (ITT population)

Genotype	Amrubicin (n = 289) No.	%	Topotecan (n = 52) No.	%
*1/*1	186	64.4	33	63.5
*1/*2	95	32.9	16	30.8
*2/*2	8	2.8	3	5.8

Abbreviation: ITT, intent to treat.

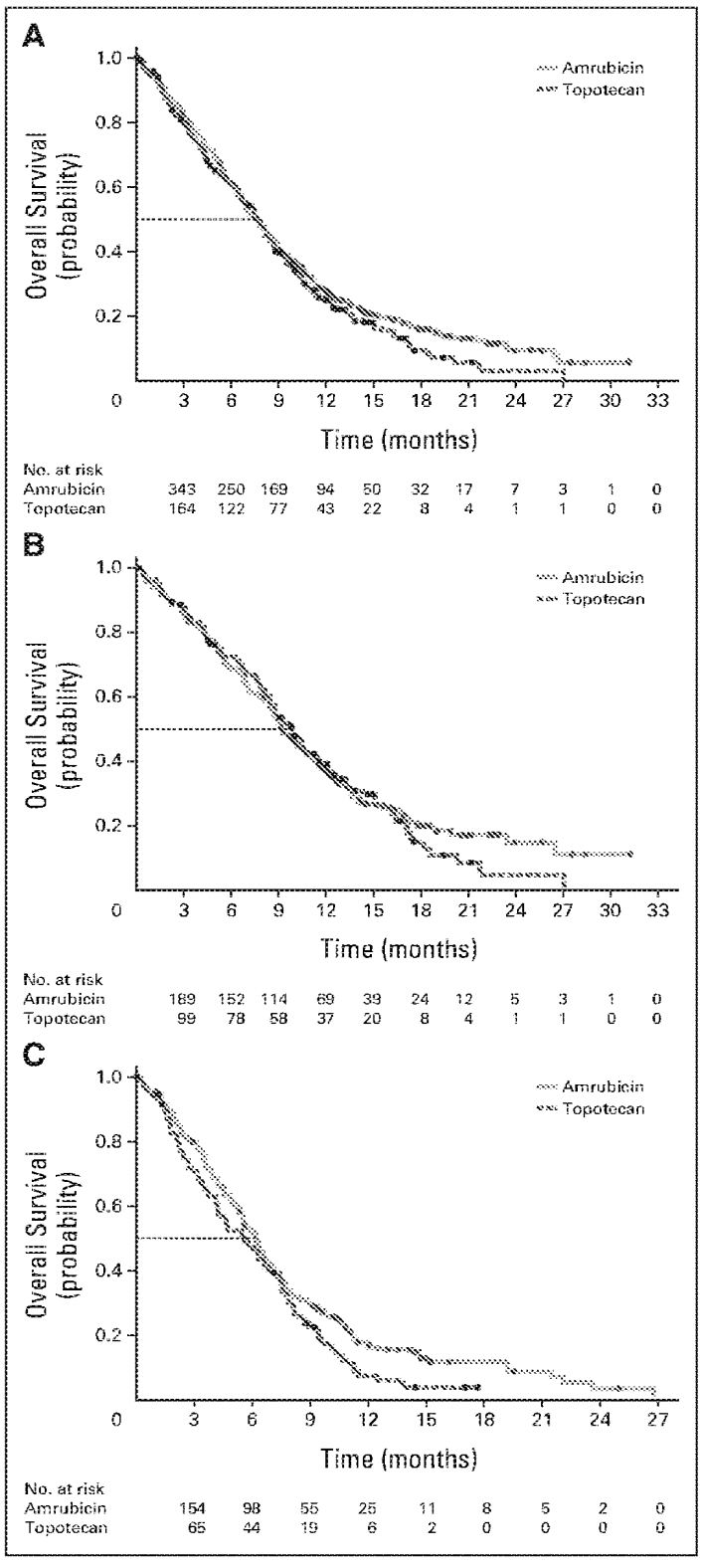

Fig 2. Kaplan-Meier estimates of overall survival for (A) intent-to-treat population receiving amrubicin (median, 7.5 months; 95% CI, 6.8 to 8.5) versus topotecan (median, 7.8 months; 95% CI, 6.6 to 8.5), with hazard ratio (HR) of 0.880 (95% CI, 0.733 to 1.057; $P = .170$); (B) sensitive patients receiving amrubicin (median, 9.2 months; 95% CI, 8.5 to 10.6) versus topotecan (median, 9.9; 95% CI, 8.5 to 11.5), with HR of 0.936 (95% CI, 0.724 to 1.211; $P = .615$); and (C) refractory patients receiving amrubicin (median, 6.2 months; 95% CI, 5.5 to 6.7) versus topotecan (median, 5.7 months; 95% CI, 4.1 to 7.0), with HR of 0.766 (95% CI, 0.589 to 0.997; $P = .047$).

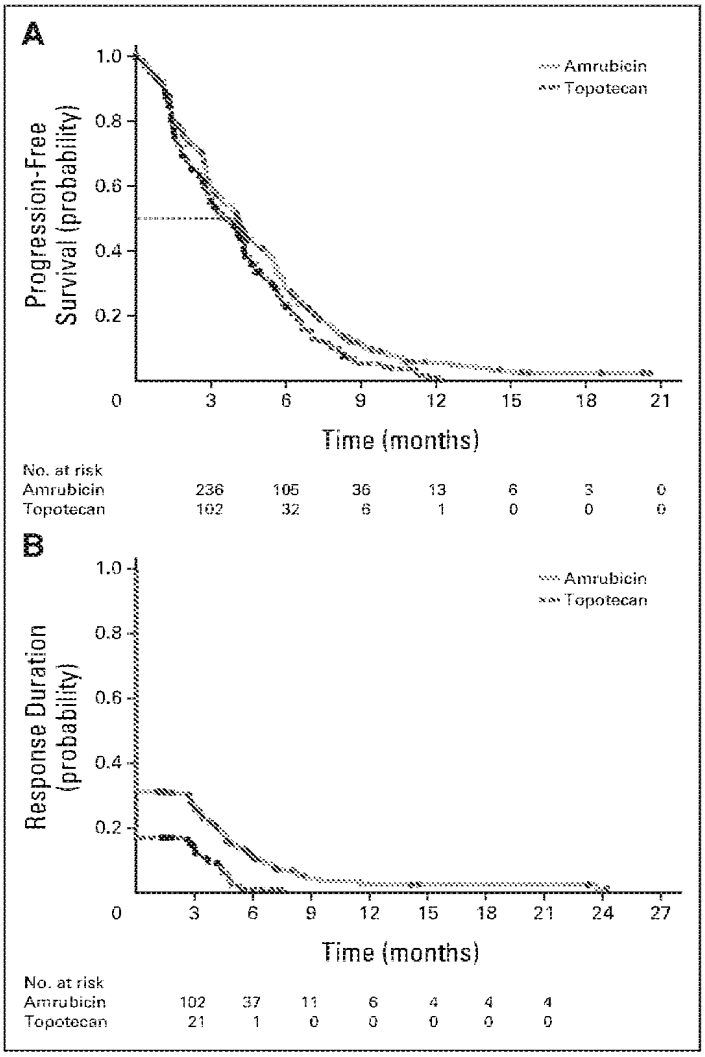

Fig 3. Kaplan-Meier estimates of (A) progression-free survival in intent-to-treat (ITT) amrubicin arm (median, 4.1 months; 95% CI, 3.5 to 4.3) versus ITT topotecan arm (median, 3.5 months; 95% CI, 2.9 to 4.2), with hazard ratio (HR) of 0.802 (95% CI, 0.667 to 0.965; $P = .018$), and (B) response duration in ITT patients receiving amrubicin (median, 0 months; 95% CI, does not exist [DNE] to DNE) versus topotecan (median, 0 months; DNE to DNE), with HR of 0.680 (95% CI, 0.570 to 0.811; $P = .000$). End point for patients without response was assumed at time = 0.

Table 3. Summary of Efficacy Parameters Categorized by Treatment Received (ITT population)

Efficacy Parameter	Amrubicin (n = 424) No.	Amrubicin (n = 424) %	Topotecan (n = 213) No.	Topotecan (n = 213) %
ORR (CR or PR)	132	31.1	36	16.9
CR	7	1.7	1	0.5
PR	125	29.5	35	16.4
Response duration, months	4.8		4.2	
SD	165	38.9	95	44.6
PD	70	16.5	44	20.7
OS, months				
Median	7.5		7.6	
95% CI	6.8 to 8.5		6.6 to 8.5	
OS (PP), months				
Median	8.0		7.5	
95% CI	7.1 to 8.8		6.3 to 8.5	
PFS, months	4.1		3.5	
95% CI	3.5 to 4.3		2.9 to 4.2	
PFS HR	0.802			
95% CI	0.667 to 0.965			

Abbreviations: CR, complete response; HR, hazard ratio; ITT, intent to treat; ORR, overall response rate; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PP, per protocol; PR, partial response; SD, stable disease.

In patients with refractory disease, a statistically significant survival benefit in favor of amrubicin treatment was observed (6.2 *v* 5.7 months; HR, 0.766; $P = .047$; Fig 2C). PFS was similar between amrubicin and topotecan treatments for patients with refractory disease (median, 2.8 months; 95% CI, 2.8 to 3.4 *v* 2.6 months; 95% CI, 1.8 to 3.3; HR, 0.934; 95% CI, 0.716 to 1.220; unstratified log-rank $P = .6110$). For refractory patients, 20.1% of patients responded to amrubicin treatment compared with 9.4% of those receiving topotecan treatment (OR, 2.432; $P = .024$).

Treatment Exposure

For the ITT population, 184 patients (43.4%) in the amrubicin arm and 79 (37.1%) in the topotecan arm completed ≥ six treatment cycles. For the safety population, median number of cycles administered to patients in each treatment arm was four (amrubicin, one to 36 *v* topotecan, one to 13). Relative dose-intensities were 92.7% for amrubicin and 86.9% of the protocol-specified dose for topotecan. Dose modifications were required less frequently with amrubicin (22.3% *v* 44.7% of patients required ≥ one dose reduction and 4.9% *v* 18.8% required ≥ two dose reductions for amrubicin and topotecan, respectively). Cross-over rates from amrubicin to topotecan (follow-up) as one of the treatments occurred in 34.3% of patients (140 of 408); 0% of patients (0 of 197) crossed over from topotecan to amrubicin (follow-up).

Safety

Grade ≥ 3 treatment-emergent adverse events (TEAEs) were reported less frequently with amrubicin (74.0% *v* 89.3%; $P < .001$). The most common grade ≥ 3 hematologic TEAE in each treatment arm was neutropenia (Table 4). Rates of anemia, leukopenia, and thrombocytopenia were lower with amrubicin than with topotecan, whereas febrile neutropenia occurred more frequently with amrubicin. Amrubicin-treated patients experienced significantly more grade ≥ 3 infections; however, significantly fewer patients in this arm required transfusions (32.1% *v* 52.8%; $P < .001$). The most common grade ≥ 3 nonhematologic TEAE in both treatment arms was fatigue.

Grade ≥ 3 cardiac TEAEs were observed in 5.1% and 4.6% of patients receiving amrubicin and topotecan, respectively ($P = .759$). Left ventricular ejection fraction remained stable in patients treated with amrubicin, including those treated with cumulative doses > 1,000 mg/m^2 (Appendix Fig A1, online only).

Gene expression results for *NQO1* were available for 341 of the 637 patients (Table 2). The percentage of patients carrying two mutated alleles was low, so they were not included in this analysis. The percentage of patients with ≥ one AE was similar in patients homozygous for the wild-type *NQO1* allele (*1*1*) and heterozygous patients (*1*2*) in both treatment arms. The majority of patients exhibited grade 0 to 2 absolute neutrophil count and WBC in each treatment cycle, independent of *NQO1* genotype or treatment.

Table 4. Most Common Grade ≥ 3 Treatment-Emergent AEs (occurring in ≥ 5% of patients)

AE	Amrubicin (n = 408)		Topotecan (n = 197)		
	No.	%	No.	%	*P*
Hematologic					
Anemia	65	15.9	60	30.5	< .001
Febrile neutropenia	41	10.0	6	3.0	.003
Leukopenia	62	15.2	43	21.8	.044
Neutropenia	169	41.4	106	53.8	.004
Thrombocytopenia	86	21.1	107	54.3	< .001
Nonhematologic					
Dyspnea	18	4.4	13	6.6	.253
Fatigue	43	10.5	24	12.2	.546
Hyponatremia	21	5.1	11	5.6	.822
Infections	64	15.7	19	9.6	.430
Pneumonia	27	6.6	6	3.0	.070

Abbreviation: AE, adverse event.

DISCUSSION

ACT-1 is the largest reported phase III trial of second-line treatment for SCLC to our knowledge. The primary end point of OS in the ITT population was similar between amrubicin and topotecan treatments. OS for amrubicin was at least comparable to that for topotecan, as shown by the posthoc noninferiority analysis. The study showed a significant improvement in all secondary efficacy end points for amrubicin compared with topotecan.

A CPHM that included well-recognized prognostic factors for SCLC showed a statistically significant impact of treatment on OS ($P = .036$). The results of this model are consistent with the prespecified survival analysis in the PP population, which showed a significant impact of treatment on OS ($P = .043$).

Significant OS improvement was observed for patients with refractory disease treated with amrubicin (median, 6.2 *v* 5.7 months with topotecan; HR, 0.766; $P = .047$). In this setting, responses to second-line chemotherapy are rare, and few studies have investigated this population. Our findings reproduce those from a recent single-arm phase II study of amrubicin in platinum-refractory patients with extensive-stage SCLC, in which a 21% ORR and 6-month median OS were reported for amrubicin—at that time, the longest OS in a refractory population.[19]

In a study by Ardizzoni et al,[11] IV topotecan was investigated as second-line therapy in both platinum-sensitive and -refractory patients. In patients with refractory disease, the ORR was 6.4%, with a median OS of 4.7 months. In another study, 12% of refractory patients had a confirmed response to IV topotecan, with a median OS of only 3.4 months.[21] In a study of oral topotecan versus BSC in which 54% of patients were refractory, the ORR of all patients receiving topotecan was 7%, with a median OS of 6.5 months.[10]

Our findings of a 40.9% ORR and 9.2-month OS in platinum-sensitive patients treated with amrubicin are similar to those reported from a recent phase II study of amrubicin versus topotecan in platinum-sensitive patients.[20] However, topotecan seems to have performed better in our study, with an ORR of 23.1% and OS of 9.9 months, compared with 15% and 7.6 months in the earlier study.[20]

In other studies of topotecan in sensitive-relapsed SCLC, response rates of 22% to 24%[9,22,23] have been documented, with OS times of 5.8 to 8.1 months. In the study by Ardizzoni et al,[11] the platinum-sensitive population included a relatively high proportion of patients with limited-stage disease, and an ORR of 38% was observed in these patients; however, median OS was only 6.9 months.

The safety profile of amrubicin was at least as favorable as that of topotecan. An increased infection trend was noted in the amrubicin arm, but significantly fewer transfusions were required by amrubicin-treated patients. This increased rate of infection led to a protocol amendment requiring prophylactic growth factor support and did not lead to increased mortality; the rate of death during treatment was comparable between arms (11.0% *v* 11.2%). Significant cumulative cardiotoxicity was not observed with amrubicin.

There was no evidence of any effect of *NQO1* gene polymorphism (**1*1 v *1*2*), a metabolic enzyme of amrubicin, on the AE profile in either treatment arm. In a relatively small study enrolling 21 Japanese patients with lung cancer, the *NQO1* polymorphism was correlated with amrubicin clearance and a moderate trend toward grade 4 neutropenia.[24] We could not confirm this correlation in our trial, because the number of patients homozygous for the mutant allele (**2*2*) was low. Further research is needed to confirm this lack of correlation between amrubicin and AEs in Western patients.

In conclusion, amrubicin had demonstrable activity and a safety profile comparable to that of topotecan in patients with SCLC. Amrubicin also demonstrated higher response rates and a minimal survival advantage of 2 weeks in patients with refractory disease, a patient population that currently has no approved treatment options. These results warrant further confirmation, and other studies are being considered.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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AUTHOR CONTRIBUTIONS

Conception and design: Joachim von Pawel, Mary E.R. O'Brien, Mark A. Socinski

Administrative support: Mark A. Socinski

Provision of study materials or patients: David R. Spigel, Mark A. Socinski, Martin Steins, Léon Bosquée, Jeffrey Bubis, Philip Clingan, Martin Reck
Collection and assembly of data: Joachim von Pawel, Robert Jotte, Mary E.R. O'Brien, Mark A. Socinski, Jörg Mezger, Martin Steins, Léon Bosquée, Jeffrey Bubis, Kristiaan Nackaerts, José M. Trigo, Philip Clingan, Wolfgang Schütte, Martin Reck, Frances A. Shepherd, Markus F. Renschler
Data analysis and interpretation: Joachim von Pawel, Robert Jotte, David R. Spigel, Mary E.R. O'Brien, Mark A. Socinski, Jörg Mezger, Kristiaan Nackaerts, Wolfgang Schütte, Paul Lorigan, Martin Reck, Manuel Domine, Frances A. Shepherd, Shaoyi Li, Markus F. Renschler
Manuscript writing: All authors
Final approval of manuscript: All authors

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Affiliations

Joachim von Pawel, Asklepios Fachkliniken München-Gauting, Gauting; Jörg Mezger, St Vincentius-Kliniken Karlsruhe, Karlsruhe; Martin Steins, Thoraxklinik am Universitätsklinikum, Heidelberg; Wolfgang Schütte, Krankenhaus Martha-Maria Halle-Dölau, Halle; Martin Reck, Krankenhaus Großhansdorf, Großhansdorf, Germany; Robert Jotte, Rocky Mountain Cancer Center, Denver, CO, and US Oncology, Houston, TX; David R. Spigel, Sarah Cannon Research Institute, Nashville, TN; Mary E.R. O'Brien, Royal Marsden National Health Service (NHS) Foundation Trust, Surrey; Paul Lorigan, Christie NHS Foundation Trust, Manchester, United Kingdom; Mark A. Socinski, University of Pittsburgh Medical Center, Pittsburgh, PA; Léon Bosquée, Centre Hospitalier Universitaire Sart-Tilman, Liège; Kristiaan Nackaerts, Universitair Ziekenhuis Gasthuisberg, Leuven, Belgium; Jeffrey Bubis, Cancer Specialists of North Florida, Jacksonville, FL; José M. Trigo, Hospital Universitario Virgen de la Victoria, Malaga; Manuel Domine, Fundación Jiménez Diaz, Universidad Autónoma de Madrid, Madrid, Spain; Philip Clingan, Southern Medical Day Care Centre, Wollongong, New South Wales, Australia; Frances A. Shepherd, University Health Network, Princess Margaret Cancer Centre, Toronto, Ontario, Canada; Shaoyi Li and Markus F. Renschler, Celgene, Summit, NJ.

GLOSSARY TERMS

allele: an alternative form of a gene (in diploids, one member of a pair) that is located at a specific position on a specific chromosome.

genotype: the specific genetic makeup of a given individual. Although genotypes give rise to the phenotype of an individual, genotypes and phenotypes are not always correlative. For example, some genotypes are expressed only under specific environmental conditions.

pharmacogenomics: the study of how a person's genome can affect their reaction to medications.

polymorphism: genetic polymorphisms are natural variations in the genomic DNA sequence present in greater than 1% of the population, with single nucleotide polymorphisms (SNPs) representing DNA variations in a single nucleotide. SNPs are being widely used to better understand disease processes, thereby paving the way for genetic-based diagnostics and therapeutics.

prognostic factor: a measurable patient characteristic that is associated with the subsequent course of disease (whether or not therapy is administered). The identification of a prognostic factor does not necessarily suggest a cause-and-effect relationship. However, within a suitable outcome model, the measurement of a prognostic factor contributes to an estimate of an outcome probability (eg, the probability of disease-free survival within a given time interval).

Appendix

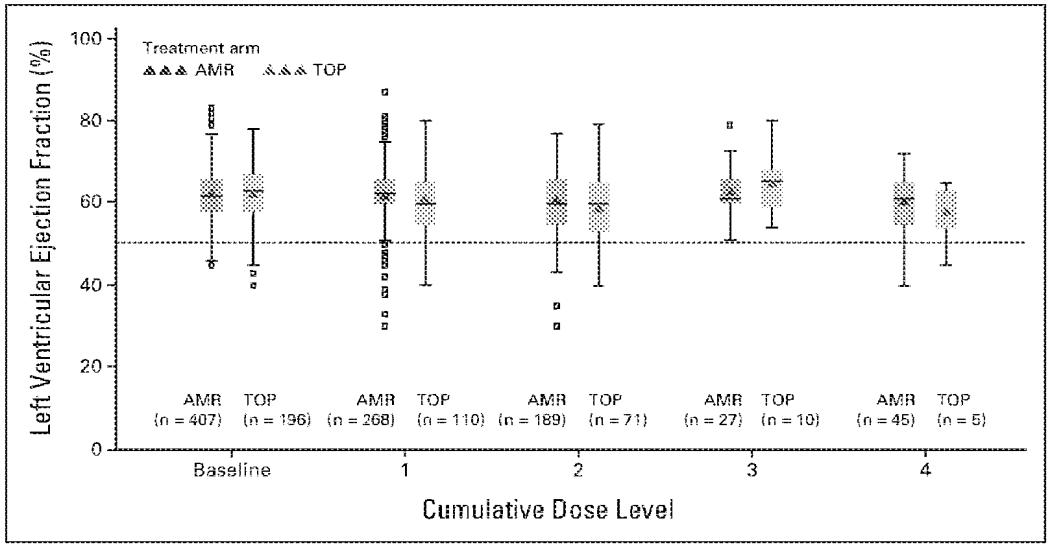

Fig A1. Left ventricular ejection fraction (LVEF) by cumulative dose. Dose levels are as follows: 1, 0 to 400; 2, 400 to 750; 3, 750 to 1,000; and 4, > 1,000 mg/m^2 for amrubicin (AMR) and 1, 1 to 25; 2, 25 to 48; 3, 48 to 82.5; and 4, > 82.5 mg/m^2 for topotecan (TOP). The "n" indicates No. of assessments at each dose level. Patients could have multiple LVEF assessments across dose levels, but only minimum value for each patient is reported for each dose level.

Topotecan Versus Cyclophosphamide, Doxorubicin, and Vincristine for the Treatment of Recurrent Small-Cell Lung Cancer

By Joachim von Pawel, Joan H. Schiller, Frances A. Shepherd, Scott Z. Fields, J.P. Kleisbauer, Nick G. Chrysson, David J. Stewart, Peter I. Clark, Martin C. Palmer, Alain Depierre, James Carmichael, Jacqueline B. Krebs, Graham Ross, Stephen R. Lane, and Richard Gralla

Purpose: Topotecan and cyclophosphamide, doxorubicin, and vincristine (CAV) were evaluated in a randomized, multicenter study of patients with small-cell lung cancer (SCLC) who had relapsed at least 60 days after completion of first-line therapy.

Patients and Methods: Patients received either topotecan (1.5 mg/m^2) as a 30-minute infusion daily for 5 days every 21 days (n = 107) or CAV (cyclophosphamide 1,000 mg/m^2, doxorubicin 45 mg/m^2, and vincristine 2 mg) infused on day 1 every 21 days (n = 104). Eligibility included the following: bidimensionally measurable disease, Eastern Cooperative Oncology Group performance status of less than or equal to 2, and adequate marrow, liver, and renal function. Response was confirmed by blinded independent radiologic review.

Results: Response rate was 26 of 107 patients (24.3%) treated with topotecan and 19 of 104 patients (18.3%) treated with CAV (P = .285). Median times to progression were 13.3 weeks (topotecan) and 12.3 weeks (CAV) (P = .552). Median survival was 25.0 weeks for topotecan and 24.7 weeks for CAV (P = .795). The proportion of patients who experienced symptom improvement was greater in the topotecan group than in the CAV group for four of eight symptoms evaluated, including dyspnea, anorexia, hoarseness, and fatigue, as well as interference with daily activity (P ≤ .043). Grade 4 neutropenia occurred in 37.8% of topotecan courses versus 51.4% of CAV courses (P < .001). Grade 4 thrombocytopenia and grade 3/4 anemia occurred more frequently with topotecan, occurring in 9.8% and 17.7% of topotecan courses versus 1.4% and 7.2% of CAV courses, respectively (P < .001 for both). Nonhematologic toxicities were generally grade 1 to 2 for both regimens.

Conclusion: Topotecan was at least as effective as CAV in the treatment of patients with recurrent SCLC and resulted in improved control of several symptoms.

J Clin Oncol 17:658-667. 1999 by American Society of Clinical Oncology.

SMALL-CELL LUNG CANCER (SCLC) is highly sensitive to initial chemotherapy and radiation treatment. Standard first-line chemotherapy regimens—such as etoposide and cisplatin (EP)[1,2]; cyclophosphamide, doxorubicin, and vincristine (CAV)[3,4]; and alternating EP-CAV[1,2]—yield response rates of 65% to 90%, with 45% to 75% complete remission reported in limited disease and 20% to 30% complete remission reported in extensive disease. However, response durations are short, with a median survival of 10 to 16 months and a 5-year survival of 18% for limited disease, and a median survival of 6 to 12 months and a 5-year survival of 1% to 2% for extensive disease.[5-9]

The prognosis for patients who receive second-line therapy after relapse is poor.[10] Response is influenced by the time to progression after cessation of first-line therapy. Patients who relapse less than 3 months after first-line therapy are commonly termed "refractory" and have response rates that are lower than those of patients who relapse more than 3 months after therapy, who are usually termed "sensitive."[11]

There is no standard second-line therapy for SCLC. CAV is often used after first-line treatment with EP. In two studies that included sensitive and refractory patients, CAV produced second-line response rates of 13% and 28%.[3,4] The toxicity profile for this regimen is well established and acceptable, with myelosuppression being the major toxicity. For these reasons, CAV was chosen as the comparator for this study. Camptothecin analogs offer a promising new treatment option for SCLC. Topotecan HCl (Hycamtin,

From the Zentralkrankenhaus Gauting, Abteilung Onkologie, Gauting bei Muenchen, Germany; University of Wisconsin Comprehensive Cancer Center, Madison, WI; The Toronto Hospital, Toronto, Canada; SmithKline Beecham Pharmaceuticals, Collegeville, PA; Groupe Français de Pneumo Concerologie, Hopital Sainte Morguerite, Marseille, France; Salem Research, Winston-Salem, NC; Ottawa Regional Cancer Centre, Ottawa, Ontario, Canada; Clatterbridge Centre for Oncology, Wirral, Merseyside, United Kingdom; Cross-Cancer Institute, Edmonton, Alberta, Canada; Hopital Saint Jacques, Besancon, France; Nottingham City Hospital, Nottingham, United Kingdom; SmithKline Beecham Pharmaceuticals, Harlow, United Kingdom; and Alton Ochsner Medical Foundation, New Orleans, LA.

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S.Z.F., J.B.K., G.R., and S.R.L. are employees of SmithKline Beecham Pharmaceuticals.

Address reprint requests to Joachim von Pawel, MD, Zentralkrankenhaus Gauting, Abteilung Onkologie, Unterbrunner Str 85, Gauting bei Muenchen, Germany, 82131.

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SK&F 104864-A; SmithKline Beecham Pharmaceuticals, Philadelphia, PA) is a water-soluble, semisynthetic analog of the alkaloid camptothecin, which, like camptothecin, is a specific inhibitor of topoisomerase I. Inhibition of this enzyme results in lethal DNA damage during the course of DNA replication. In preclinical models, topotecan was shown to be effective in a number of solid tumors (eg, Lewis lung carcinoma, colon adenocarcinomas 38 and 51, and subcutaneously implanted B16 melanoma) that were refractory to most of the established anticancer drugs (F. McCabe, personal communication, 1988).

Topotecan, as a single agent, has been studied in several phase II trials as second-line therapy for SCLC. A study conducted by the European Organization for Research and Treatment of Cancer reported a 21.7% overall response rate (38% among sensitive patients, 6.4% among refractory patients) and a 33-week median duration of response.[11] Two other phase II topotecan studies had response rates in sensitive patients of 14% (median survival, 25.7 weeks) and 19% (median survival, 26.6 weeks) and response rates in refractory patients of 2% (median survival, 16.3 weeks) and 3% (median survival, 20.4 weeks).[12,13] A meta-analysis of these three SCLC studies in sensitive patients reported an 18% response rate and median survival of 30 weeks.[14] Based on these results, a randomized trial of topotecan versus CAV was performed in the sensitive population.

PATIENTS AND METHODS

Eligibility Criteria

Patients were required to have documented progressive, limited, or extensive SCLC with the date of progression being at least 60 days after completion of first-line chemotherapy. At least one lesion had to be bidimensionally measurable by computed tomography, magnetic resonance imaging, ultrasound, radiograph, photograph, or physical examination. A minimum of 4 weeks was required between prior surgery or immunotherapy and study entry, and at least 24 hours was required between radiotherapy and initiation of study drugs. Other eligibility criteria included an Eastern Cooperative Oncology Group (ECOG) performance status of less than or equal to 2, hemoglobin more than or equal to 9.0 g/dL, WBC count more than or equal to 3.5×10^9/L, neutrophils more than or equal to 1.5×10^9/L, platelets more than or equal to 100×10^9/L, bilirubin less than or equal to 2.0 mg/dL, transaminase and alkaline phosphatase values less than or equal to 2 times the upper limit of normal (or, if liver metastases were present, less than or equal to 3 times the upper limit of normal), and creatinine less than or equal to 1.5 mg/dL or creatinine clearance more than or equal to 60 mL/min. Patients were ineligible if they had symptomatic brain metastases requiring corticosteroids or pre-existing cardiac disease (including clinical congestive heart failure, arrhythmias requiring treatment, or a myocardial infarction within the preceding 3 months). Also ineligible were patients for whom CAV was contraindicated (including patients with a history of demyelinating polyneuropathy or poliomyelitis), patients whose lifetime cumulative dose of doxorubicin exceeded 270 mg/m^2 or cumulative dose of epirubicin exceeded 540 mg/m,^{2} and patients with prior topotecan therapy or more than one previous chemotherapy regimen.

Written informed consent was obtained from each patient before study entry. The study was conducted in accordance with Good Clinical Practices and the Declaration of Helsinki as amended in Hong Kong (1989).

Randomization

Eligible patients were stratified by extent of disease and performance status at baseline and randomized to one of the arms by a telephone randomization system.

Treatment Schedule

Starting doses were topotecan 1.5 mg/m^2/d administered as a 30-minute infusion for 5 consecutive days every 21 days, or cyclophosphamide 1,000 mg/m^2 (maximum, 2,000 mg), doxorubicin 45 mg/m^2 (maximum, 100 mg), and vincristine 2 mg, administered on day 1 of each course. Full doses were administered if the treatment day neutrophil count was more than or equal to 1.0×10^9/L, the platelet count was more than or equal to 100×10^9/L, and hemoglobin was more than or equal to 9.0 gm/dL. Topotecan could be escalated to a maximum dose of 2.0 mg/m^2 in the absence of grade 2 or higher toxicity.

Dose Modification

Topotecan was reduced by 0.25 mg/m^2/d and cyclophosphamide/doxorubicin was reduced by 25% for the following criteria: grade 4 neutropenia complicated by fever or infection or lasting 7 days or longer, grade 3 neutropenia lasting beyond day 21 of the treatment cycle, or grade 4 thrombocytopenia. The same dose reductions were applied to grade 3 or 4 nonhematologic toxicity (excluding grade 3 nausea), or the patient could be withdrawn from the study. The minimum topotecan dose was 1.0 mg/m^2/d. Doxorubicin was discontinued or the patient was withdrawn from the study once the lifetime maximum-tolerated dose of doxorubicin (450 mg/m^2) or a comparable dose of epirubicin (900 mg/m^2) was reached, or if signs of cardiomyopathy were evident. Doxorubicin and vincristine dose reductions were required for bilirubin or serum transaminase elevations. A vincristine dose reduction of 25% was required for grade 2 neurologic toxicity; vincristine was to be eliminated from the regimen for grade 3 to 4 neurologic toxicity until the toxicity resolved. The minimum doses for cyclophosphamide, doxorubicin, and vincristine were to be set by the administering physician. Patients in both treatment arms were to be withdrawn from the study if there was a delay greater than 2 weeks caused by persistent toxicity at minimum doses. Use of granulocyte colony-stimulating factor (G-CSF) was left to the discretion of the investigator.

Treatment Duration

Patients with a complete or partial response to therapy were to continue treatment until disease progression or unacceptable toxicity occurred, or for at least six courses past the maximal response. Patients whose best response was stable disease after four courses could be removed from the study or continued at the investigator's discretion. Patients whose disease progressed were removed from the study.

Criteria for Efficacy

The primary efficacy parameters were response rate and duration of response. Responses were determined according to the World Health Organization criteria.[15] All claimed responses were reviewed by an

independent radiologist blinded to treatment. Standard response criteria were used, and the duration of response was measured from the time of initial documented response to the first sign of disease progression. All patients who were not fully assessed for efficacy or who were not evaluated were considered to be nonresponders.

The secondary efficacy variables included time to progression, time to response, survival, and improvement of disease-related symptoms. Time to progression was measured from the time of first study drug administration to documented progressive disease (or initiation of subsequent chemotherapy). Time to response and survival were measured from the time of first study drug administration to initial response and death, respectively. Symptom scores were evaluated for dyspnea, cough, chest pain, hemoptysis, anorexia, insomnia, hoarseness, fatigue, and interference with daily activity; improvement had to be sustained for two consecutive courses. Symptom evaluation also included the time to symptom worsening as defined by the interval from the first dose of study medication until the first evidence of worsening in the postbaseline assessment.

Safety Assessment

Complete blood cell counts were performed at least weekly. Blood chemistries were performed on day 15 of each course, and urinalysis was performed each cycle. Electrocardiogram and multiple gated acquisition or echocardiogram were performed before treatment and at the end of treatment. Quantitative hematologic and nonhematologic toxicities were assessed before each cycle according to the National Cancer Institute Common Toxicity Criteria.[16]

Statistical Analysis

Efficacy results were classified by treatment group and summarized. Stratified randomization ensured that the distributions of two prognostic variables, baseline performance status and extent of disease, were comparable between treatment groups. These factors were included in the multivariate analytical models for the time-to-event outcomes. Efficacy results were obtained for all patients treated. Subgroup analyses included response by sex and time to progression relative to first-line chemotherapy.

Response rates and the estimated percentage difference in response rates between treatment groups were determined along with 95% confidence intervals (CI). Traditional Kaplan-Meier survival estimates were used to summarize the time-to-event variables. These included time to response, response duration, time to progression, and survival. Time-to-event outcomes were also compared between treatments using the Cox regression model. In addition, multivariate statistical methodologies were applied to survival and response to determine other possible prognostic factors, namely sex, performance status, extent of disease, age, presence of baseline brain and/or liver metastases, response to first-line therapy (complete or partial response), response duration, and time to progression from first-line therapy. However, because the treatment groups were not balanced with respect to these additional covariates, the main conclusions of this study were based on the results adjusted for the stratification variables only.

"Symptoms of Disease" questionnaires were administered to patients at screening and immediately before each subsequent course of chemotherapy. The questionnaire was not a validated quality-of-life instrument, but rather a symptom-specific questionnaire for SCLC. Patient symptom assessments were scored on a four-point ordinal scale: 1, "Not at All," 2, "A Little Bit," 3, "Quite a Bit," and 4, "Very Much." For each of the symptoms of disease, Pearson's uncorrected χ^2 statistic was used to compare the percentages of patients in each treatment group who experienced sustained improvement over baseline. Patients were required to have both baseline and postbaseline measurements in order to assess a change. In the event that a patient had a missing baseline measurement and at least one nonmissing postbaseline measurement of "A Little Bit" or worse, the baseline value was imputed as "Not at All," and the patient was included in the analysis of that symptom. If symptom assessments were not recorded, algorithms were used to impute scores for the courses with the missing assessments. In addition, Kaplan-Meier estimates were obtained and tested using the log-rank test for the time to worsening for each symptom. The time to symptom worsening (measured in courses) was defined as the interval from the first dose of study medication until the first increase in the postbaseline assessment score. Patients not known to have experienced a worsening in that symptom were censored at their last symptom assessment.

RESULTS

Patient Characteristics

A total of 211 patients (107 treated with topotecan and 104 treated with CAV) were treated in the study. Demographic and baseline disease characteristics for all patients are presented in Table 1. The majority of patients (77% of topotecan patients and 79% of CAV patients) had received a first-line regimen containing both etoposide and platinum (cisplatin or carboplatin), and 97% of both topotecan and CAV patients had received a regimen containing etoposide. Forty-one topotecan patients (38.3%) and 45 CAV patients (43.3%) had a prior treatment regimen that included cyclophosphamide and an anthracycline. Overall, baseline characteristics were comparable between treatment groups, with the exception of sex (43% of topotecan patients were women v 32% of CAV patients; $P = .091$) and documented brain metastases, which were present in 11.2% of topotecan patients and 24.0% of CAV patients ($P = .044$).

Dose-Intensity

Exposure to study drug is presented in Table 2. Dose-intensity was calculated as the sum of the daily doses delivered during the course divided by the duration of the course in weeks. A total of 446 courses of topotecan and 359 courses of CAV were administered to 107 and 104 patients, respectively. The target doses for topotecan and CAV were maintained in 76% and 77% of treatment courses, respectively. Treatment delays beyond 1 week occurred in 7.1% of topotecan and 5.5% of CAV courses.

Efficacy

Response rate. The overall response rates for patients who received topotecan and CAV were 24.3% (estimated 95% CI, 16.2 to 32.4) and 18.3% (estimated 95% CI, 10.8 to 25.7), respectively (Table 3). The 95% CI for the difference in the rates of response (6.0%) was 6 to 18. A single complete response occurred with CAV. Eight patients (three topotecan and five CAV) were reported as responders, but

Table 1. Demographic and Disease Characteristics at Baseline

Baseline Disease Characteristic	Intent to Treat: Topotecan (n = 107) No.	%	CAV (n = 104) No.	%
Extent of disease at study entry				
Limited disease	18	16.8	16	15.4
Extensive disease	89	83.2	88	84.6
Prior anticancer treatment				
Prior radiotherapy	66	61.7	58	55.8
Prior immunotherapy	0	0.0	2	1.9
Prior surgery	15	14.0	29	27.9
Prior brain irradiation				
Yes	27	25.2	24	23.1
No	80	74.8	80	76.9
Performance status at entry				
0	18	16.8	20	19.2
1	64	59.8	64	61.5
2	25	23.4	20	19.2
Liver metastases				
Present	43	40.2	42	40.4
Absent	64	59.8	62	59.6
Brain metastases				
Present	12	11.2	25	24.0
Absent	95	88.8	79	76.0
Prior chemotherapy regimen				
Platinum (cis or carbo)/etoposide	55	51.4	46	44.2
Cyclophosphamide/doxorubicin/vincristine	1	0.9	1	1.0
Both platinum/etoposide + CAV	13	12.1	17	16.3
Cyclophosphamide/doxorubicin/etoposide	20	18.7	16	15.4
Vincristine/platinum (cis or carbo)/etoposide	4	3.7	6	5.8
Other regimens	14	13.1	18	17.3
Best response first-line treatment				
Complete response	47	43.9	43	41.3
Partial response	60	56.1	60	57.7
Stable disease	0	0.0	1	1.0
Time to progression from first-line treatment				
Median, weeks	24.4		22.9	
Range, weeks	7.6-430.6		8.7-156.7	
Maximum lesion diameter, cm				
< 2	2	1.9	1	1.0
2-< 5	53	49.5	49	47.1
5-10	46	43.0	47	45.2
> 10	4	3.7	4	3.8
Missing	2	1.9	3	2.9

Abbreviations: Cis, cisplatin; carbo, carboplatin.

the responses were not confirmed after independent radiologic review.

All patients who received a dose of study medication were included in the efficacy calculations (Table 3). Of all patients treated, 11 topotecan and 18 CAV patients had an overall response of "not assessable" and were classified as nonresponders. Of these, five patients (two topotecan and three CAV) were ineligible, and five patients (one topotecan and four CAV) could not be evaluated for response for the following reasons: one topotecan patient relocated to a nursing home, two CAV patients were lost to follow-up, a third died suddenly as a result of an unrelated cause, and a fourth patient was without lesion assessment after course 2. Sixteen patients (seven topotecan and nine CAV) were withdrawn from the study because of toxicity, either by the investigator or at the patient's request. One topotecan patient requesting withdrawal had tumor lysis syndrome with a 75% reduction in a jugulodigastric node. Additionally, three patients (one topotecan and two CAV) were removed for lack of clinical benefit, but did not have radiologic evidence of disease progression.

Response rates were 30.4% for women compared with 19.7% for men (topotecan) and 30.3% for women compared

Table 2. Exposure to Study Medication

Study Drug Regimen Every 21 Days Dose mg/m²/day	Total Number of Patients	Total Number of Courses	Course Median	Course Range
Topotecan (daily × 5 days)	107	446	4	1-15
1.75	1	6	6	6-6
1.50	107	337	2	1-13
1.38	1	1	1	1-1
1.25	31	69	1	1-10
1.15	4	7	2	1-2
1.00	10	24	2.5	1-5
0.75	2	2	1	1-1
CAV	104	359	3	1-12
Starting dose*	98	277	2	1-7
Modified dose†	32	82	2	1-6

*CAV starting dose is expressed as 1047.00 (cyclophosphamide 1000 mg/m², doxorubicin 45 mg/m², and vincristine 2 mg).

†Modified dose denotes dose adjustment by reduction.

Table 3. Response to Treatment

Response to Treatment	Intent-to-Treat: Topotecan (n = 107) No.	%	CAV (n = 104) No.	%
Responders				
Complete response	0	0.0	1	1.0
Partial response	26	24.3	18	17.3
Total	26	24.3	19	18.3
95% CI	16.2-32.4		10.8-25.7	
Nonresponders				
Stable disease	21	19.6	12	11.5
Progressive disease	49	45.8	55	52.9
Not assessable	11	10.3	18	17.3
Total	81	75.7	85	81.7
Total patients	107	100.0	104	100.0

with 12.7% for men (CAV). Of 41 patients on the topotecan arm and 45 on the CAV arm who had received a first-line regimen that included cyclophosphamide and an anthracycline, the response rates were 26.8% and 20.0%, respectively. No responding CAV patient had to be removed from study as a result of reaching the maximum allowed doxorubicin dose, and only two patients, one with stable disease and one nonassessable patient (not all lesions were assessed after course 2), were reported as discontinuing from the study after five courses of treatment. In patients experiencing relapse 60 to 90 days after completion of first-line chemotherapy, response rates were 3 of 22 patients (13.6%) for topotecan and 1 of 21 patients (4.8%) for CAV. Median duration of response to first-line chemotherapy was 33.5 weeks for topotecan responders compared with 22 weeks in topotecan nonresponders, and 28 weeks in CAV responders compared with 23 weeks in CAV nonresponders. A logistic regression model was applied to evaluate the possible effect of the aforementioned set of baseline characteristics on response rates. The logistic regression model identified presence of baseline liver metastases and sex as the only significant factors of response ($P = .043$ and $P = .008$, respectively); after adjusting for the covariates, patients in the topotecan group still had shown a greater propensity to respond than did patients in the CAV group, although the result was not statistically significant (odds ratio of 1.24; $P = .557$).

Duration of response, time to response, time to progression, and survival. The medians and ranges of these parameters are presented in Table 4. At the time of analysis, 11.2% of topotecan patients and 12.5% of CAV patients were censored for survival. Overall median survival was 25.0 weeks for topotecan patients and 24.7 weeks for CAV patients ($P = .795$). Six- and 12-month survival times for topotecan patients were 46.7% and 14.2%, respectively, and 45.2% and 14.4% for CAV patients, respectively. (Survival curves are displayed in Fig 1).

Table 4. Time-to-Event Parameters

Time-to-Event Parameters, weeks	Topotecan	CAV	P
Response duration	n = 26	n = 19	
Median	14.4	15.3	.300
Range	9.4-50.1	8.6-69.9*	
Time to response	n = 26	n = 19	
Median	6.0	6.1	.953
Range	2.4-15.7	5.1-18.1	
Time to progression	n = 107	n = 104	
Median	13.3	12.3	.552
Range	0.4-55.1	0.1-75.3*	
Survival	n = 107	n = 104	
Median	25.0	24.7	.795
Range	0.4-90.7*	1.3-101.3	

*Estimate corresponds to a censored event.

The Cox regression model for survival did not exhibit a statistically significant result ($P = .795$) between treatments, with a risk ratio of topotecan to CAV of 1.039. As expected, baseline performance status and extent of disease were statistically significant prognostic factors for survival ($P < .001$). An additional Cox model was created to investigate the effect of the aforementioned set of baseline characteristics on survival. In addition to the stratification factors, the model identified sex, baseline liver metastases, and baseline brain metastases as significant factors for survival ($P < .05$); after adjusting for the covariates, the effect of treatment was still not statistically significant (risk ratio of 1.17; $P = .322$).

Improvement in symptoms of disease. Greater symptomatic improvement was seen in patients who received topotecan for symptoms of dyspnea ($P = .002$), anorexia ($P = .042$), hoarseness ($P = .043$), and fatigue ($P = .032$), as well

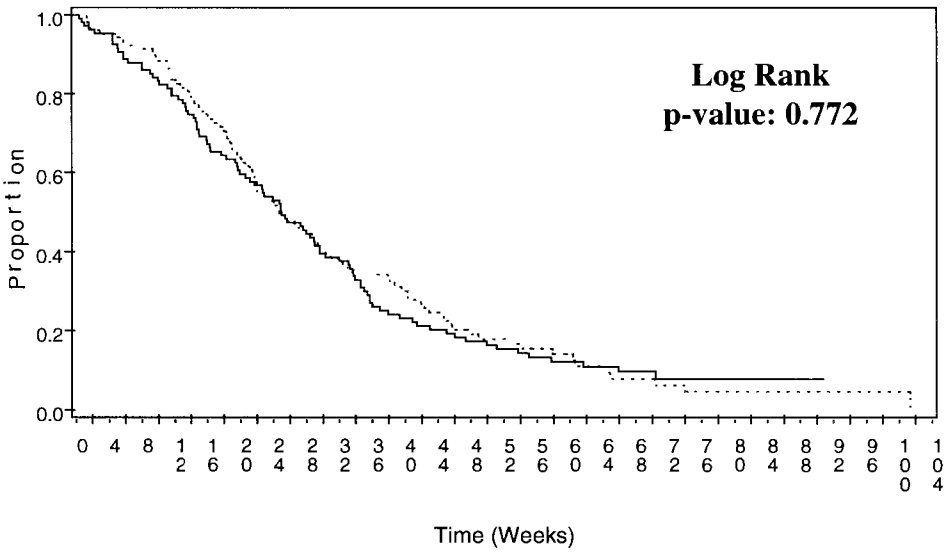

Fig 1. Survival in weeks. Regimen: ——— = Hycamtin; - - - = CAV.

Table 5. Comparison of Improvement in Disease-Related Symptoms

Symptom†	Topotecan (n = 107)			CAV (n = 104)			
	n‡	n	%	n‡	n	%	χ^2P*
Dyspnea	68	19	27.9	61	4	6.6	.002§
Cough	69	17	24.6	61	9	14.8	.160
Chest pain	44	11	25.0	41	7	17.1	.371
Hemoptysis	15	4	26.7	12	4	33.3	.706
Anorexia	56	18	32.1	57	9	15.8	.042§
Insomnia	57	19	33.3	53	10	18.9	.085
Hoarseness	40	13	32.5	38	5	13.2	.043§
Fatigue	70	16	22.9	65	6	9.2	.032§
Interference with daily activity	67	18	26.9	63	7	11.1	.023§

*Pearson χ^2.

†Verbatim terms used in questionnaire: "shortness of breath" (dyspnea), "coughing up blood" (hemoptysis), "loss of appetite" (anorexia), and "interference with sleep" (insomnia).

‡Number of patients with baseline and at least one postbaseline assessment; improvement defined as two consecutive improvements over the baseline assessment.

§$P < .05$.

as for interference with daily activity, ($P = .023$). No symptom improvement was statistically superior for CAV (Table 5). Significant differences in the length of time to worsening of dyspnea ($P = .046$) and anorexia ($P = .003$) were noted, with symptoms progressing more slowly in the topotecan group.

Safety

Hematologic toxicities. Hematologic toxicity is presented in Table 6. Grade 4 neutropenia was experienced by 70.2% of topotecan patients (73 of 104 patients) in 37.8% of treatment courses (166 of 439 courses) compared with 71.7% of CAV patients (71 of 99 patients) in 51.4% of courses (179 of 348 courses) ($P < .001$ for courses). Median nadir of neutrophils with topotecan was 0.6×10^9/L and occurred on a median of day 12; median nadir of neutrophils with CAV was 0.5×10^9/L and occurred on a median of day 15. The median duration of grade 4 neutropenia in both treatment groups was 7 days. The incidence of grade 4 neutropenia was highest during courses 1 and 2 of topotecan therapy (41.2% and 41.4%, respectively), whereas the highest incidence of grade 4 neutropenia (58.6%) was in CAV course 1 ($P = .016$). Granulocyte colony-stimulating factor was used in 5.6% of topotecan courses and in 8.1% of CAV courses.

The incidence of grade 4 thrombocytopenia ($P < .001$) and grade 3/4 anemia ($P < .001$) was significantly higher in patients who received topotecan. Grade 4 thrombocytopenia was observed in 28.8% of topotecan patients (30 of 104) in 9.8% of courses and required platelet transfusions in 19.6% of patients in 5.8% of courses. Grade 4 thrombocytopenia occurred in 5.0% of patients (five of 101) in 1.4% of CAV courses, requiring platelet transfusions in 1.9% of patients in 0.6% of courses. Grade 3/4 anemia occurred in 42.3% of topotecan patients in 17.3% of treatment courses compared with 19.8% of CAV patients in 7.2% of courses. RBC transfusions were administered to 52.3% of topotecan patients in 24.7% of courses versus 26.9% of CAV patients in 11.7% of courses ($P < .001$, patients and courses). Analyses of neutrophil and platelet nadirs for each course of therapy showed no evidence of cumulative toxicity for patients in the topotecan group. Because of the small number of patients in later courses, no conclusions could be reached for patients in the CAV group.

Infectious complications. Suspected or documented infection occurred within 2 days of grade 4 neutropenia in 28% of patients (30 of 107) and 8.7% of courses (39 of 446) in the topotecan group, and in 26% of patients (27 of 104) and 12.8% of courses (46 of 359) in the CAV group. In addition, 4.7% of patients and 1.1% of topotecan courses and 4.8% of patients and 1.4% of CAV courses were

Table 6. Number (%) of Patients and Courses with Grade 3/4 Hematologic Toxicity

	Topotecan						CAV					
	Patients (N = 107)			Courses (N = 446)			Patients (N = 104)			Courses (N = 359)		
	n*	No.	%	n*	No.	%	n*	No.	%	n*	No.	%
Leukopenia	104			441			101			351		
Grade 3		57	54.8		196	44.4		38	37.6		160	45.6
Grade 4		33	31.7		68	15.4		44	43.6		77	21.9
Neutropenia	104			439			99			348		
Grade 3		19	18.3		137	31.2		15	15.2		71	20.4
Grade 4		73	70.2		166	37.8		71	71.7		179	51.4
Thrombocytopenia	104			441			101			350		
Grade 3		30	28.8		83	18.8		10	9.9		17	4.9
Grade 4		30	28.8		43	9.8		5	5.0		5	1.4
Anemia	104			440			101			351		
Grade 3		41	39.4		73	16.6		18	17.8		23	6.6
Grade 4		3	2.9		5	1.1		2	2.0		2	0.6

*Represents the total number of patients and courses with laboratory data available.

associated with sepsis. Seven deaths (four topotecan, three CAV) were associated with therapy-induced myelosuppression with sepsis/infection.

Nonhematologic toxicities. The most frequently reported related or possibly related adverse experiences for both groups were alopecia, fatigue, and gastrointestinal disturbances, including nausea, vomiting, and anorexia (Table 7). Dose reductions for nonhematologic toxicity occurred in one topotecan patient (0.9%) due to grade 3 fatigue and in 11 CAV patients (10.6%) ($P = .003$). Nine of the 11 CAV reductions were due to neurotoxicity. Baseline left ventricular ejection fraction (LVEF) was obtained for 100 topotecan and 97 CAV patients, but only 26 topotecan and 35 CAV patients had LVEF evaluation at the end of study. The incidence of worsening of LVEF, as judged by investigators based on echocardiogram or multiple gated acquisition results, was higher in the CAV population (six of 35 patients or 17.1%) compared with patients who received topotecan (two of 26 patients or 7.7%).

Deaths. The majority of patients in both treatment groups died as a result of progressive disease. In addition to the deaths secondary to treatment-related hematologic toxicity and sepsis previously described, two topotecan deaths were reported as possibly related or related to therapy. One death that was caused by acute respiratory insufficiency occurred 26 days after cycle 1. This occurred in a patient with significant pulmonary involvement, pleural and pericardial effusions, and progressive disease. Although unlikely, the investigator felt that topotecan could not be ruled out as a cause. A second patient died 33 days after cycle 1 as a result of an intracerebral hemorrhage into brain metastases, reported as secondary to topotecan-induced thrombocytopenia. A platelet count of 25×10^9/L was documented 3 weeks before the patient's death, and no further laboratory evaluations were performed, as the patient was declared "do not resuscitate." One patient on CAV died as a result of progressive disease coincident with reported CAV-related renal failure and pancytopenia 8 days after cycle 1.

Reasons for withdrawal from study. Ten topotecan patients (9.3%) and 10 CAV patients (9.6%) were withdrawn from the study because of treatment-related toxicity. The primary reasons for withdrawal in both treatment groups were hematologic toxicity and associated sequelae. Treatment-related withdrawal due to nonhematologic toxicities included a topotecan patient with tumor lysis syndrome and two CAV patients with a decline in cardiac status.

Table 7. Related or Possibly Related Nonhematologic Toxicities Occurring in More Than 10% of Patients

	Common Toxicity Criteria Grade					
	1/2		3/4		Total	
	No.	%	No.	%	No.	%
Topotecan (n = 107)						
Nausea	38	35.5	4	3.7	42	39.3
Alopecia*	38	35.5	0	0.0	38	35.5
Fatigue	23	21.5	5	4.7	28	26.2
Vomiting	24	22.4	2	1.9	26	24.3
Anorexia	19	17.7	1	0.9	20	18.7
Stomatitis	13	12.2	2	1.8	15	14.0
Diarrhea	12	11.2	1	0.9	13	12.1
Fever†	11	10.3	2	1.9	13	12.1
CAV (n = 104)						
Nausea	36	34.6	6	5.8	42	40.4
Fatigue	26	25.0	9	8.7	35	33.7
Vomiting	22	21.1	3	2.9	25	24.0
Anorexia	20	19.2	3	2.9	23	22.1
Alopecia*	23	22.1	0	0.0	23	22.1
Constipation	16	15.4	0	0.0	16	15.4
Asthenia	10	9.6	4	3.8	14	13.5
Stomatitis	12	11.5	1	1.0	13	12.5
Diarrhea	13	12.5	0	0.0	13	12.5

*Reflects the number of patients who developed alopecia on study. Approximately 30% of patients on each arm presented to the study with alopecia secondary to prior chemotherapy.

†Excludes febrile neutropenia.

DISCUSSION

Despite high response rates to initial chemotherapy, the majority of SCLC patients will progress within a year of completing therapy.[17] The outlook for patients who receive second-line therapy is poor. Responses obtained are usually brief and median survival is generally less than 4 months.[18]

Phase II studies indicated that topotecan was active in patients with SCLC, particularly those with sensitive disease.[11-13,19] In the present study, we compared single-agent topotecan with CAV in patients who progressed at least 60 days after initial therapy. The study was originally designed to recruit patients with at least 90 days between completion of first-line therapy and progression, but early in the study the criteria were amended to make topotecan available to a larger proportion of relapsed SCLC patients. The response rates were 24.3% (topotecan) and 18.3% (CAV) with median survival of 25.0 and 24.7 weeks, respectively. Although response in women was greater in this study, the European Organization for Research and Treatment of Cancer study reported by Ardizzoni et al[11] showed a response rate of 25% in men versus 13.8% in women. The value of sex as a prognostic factor in previously treated SCLC remains unclear. Forty-five patients (43.3%) on the CAV arm had received cyclophosphamide and an anthracycline as part of their first-line regimen. Although we were concerned that these patients may have developed resistance to the regimen, the response rate in this group was 20.0%, which was similar to the response rate for the overall population. Time to response, time to progression, and response duration were

similar for both groups. Significant benefit was seen in several symptoms as well as in interference with daily activity. Palliation of disease-related symptoms is an important objective of therapy, as these patients are not potentially curable.

Hematologic toxicity was the predominant toxicity for both regimens, with grade 4 neutropenia occurring more frequently in patients in the CAV group and grade 4 thrombocytopenia and grade 3/4 anemia occurring more frequently in patients on the topotecan arm. The hematologic toxicity in patients on both arms, however, was of short duration, and clinically important sequelae of neutropenia did not increase with subsequent courses of therapy in either group.

For patients in both groups, most nonhematologic toxicities were mild (grades 1 and 2). With the exception of neuropathy secondary to vincristine, these toxicities rarely caused dose reduction in either group. Worsening of LVEF occurred more frequently on the CAV arm, with two patients withdrawn because of cardiotoxicity, but no conclusions were reached due to insufficient LVEF data at the end of study.

There is no standard regimen for SCLC after first-line therapy. CAV has been used in second-line therapy with response rates of 13% and 28% and median response durations of 26 and 24 weeks, respectively, in two studies.[3,4] A 13% response rate was observed in 49 patients, with only 10% classified as "sensitive."[3] Shepherd et al[4] reported a 28% response rate and 15-week median survival in 29 patients, 45% with no response to prior chemotherapy. In a study comparing EP and CAV in patients who had received the alternate regimen as first-line therapy, the response rate was higher with EP, but overall survival was similar.[1]

Early studies with oral etoposide reported response rates as high as 45%, albeit with a modest survival of 14 weeks.[20] Oral etoposide as a single agent has been compared with combination chemotherapy in first-line treatment of patients who were elderly or had poor performance status and was found to be inferior with respect to survival and overall quality of life.[21,22] In one study, 171 patients received oral etoposide and 168 received combination chemotherapy, the majority with CAV. Survival was worse in the oral etoposide arm.[21] A second study compared oral etoposide to alternating cycles of CAV and EP. One-year survival, median progression-free survival, and response rate were all statistically worse for oral etoposide, as was quality of life.[22] Both studies were closed prematurely.

A number of new cytotoxins have recently been shown to be active in SCLC, including taxanes, vinca alkaloids, and antimetabolites. A 34% response rate was observed in a phase II study of 36 previously untreated patients with SCLC who received paclitaxel 250 mg/m^2 over 24 hours every 3 weeks for as many as four cycles.[23] Kirschling et al[24] used the same regimen combined with prophylactic G-CSF in previously untreated patients and reported a 41% response rate with a median survival of 6.6 months in 37 assessable patients. Neither study reported any complete responses. Docetaxel has been studied in both treated and untreated patients. Latreille et al[25] reported a response rate of 8.3% in 12 chemo-naive patients using a dose of 75 mg/m^2 as a 1-hour infusion every 3 weeks. Smyth et al[26,27] used a dose of 100 mg/m^2 of docetaxel and found a response rate of 25% in 28 assessable patients who had received prior chemotherapy. A trial of gemcitabine in 26 previously untreated patients found a 27% response rate.[28] Vinorelbine achieved response rates of 12% and 16% in second-line patients with sensitive disease.[29,30] In two studies from Japan, irinotecan had response rates of 50% in 16 patients, and 47% in 15 patients, but response durations were 46 and 58 days, respectively.[31,32] A European study, in contrast, showed a response rate of 27% in 15 assessable patients.[33] Although these new agents have demonstrated varying degrees of activity in SCLC, randomized studies are needed to determine their role in SCLC.

Topotecan has also been studied as first-line treatment in SCLC. ECOG conducted a "Window of Opportunity" study with single-agent topotecan 2.0 mg/m^2/d intravenously for 5 days every 21 days in patients with extensive disease. Approximately 70% of the patients received G-CSF support. Patients with no response after two cycles or partial response at four cycles were then given standard therapy with cisplatin and etoposide. Of 48 assessable patients, 19 (39%) achieved a partial response on topotecan. Median response duration was 4.8 months and median survival was 10.0 months.[34] The Cancer and Leukemia Group B used combination cisplatin/topotecan and paclitaxel/topotecan in first-line SCLC, but these arms of the study were closed early because of toxicity.[35,36] The paclitaxel/topotecan arm has been reopened with a reduced dose of paclitaxel. Despite the treatment-related deaths at the initial dose level, the median survival in the first group of patients was considered favorable enough to warrant further evaluation of the regimen after dosage adjustment (Mark R. Green, personal communication, 1998). Jett et al[37] used a 5-day schedule of topotecan 1.0 mg/m^2 followed by a 24-hour infusion of paclitaxel 135 mg/m^2 on day 5 with G-CSF in previously untreated patients and obtained a response rate of 92% (11 of 12 patients) with acceptable toxicity. Eastern Cooperative Oncology Group is currently evaluating four cycles of topotecan as consolidation therapy versus observation after four cycles of standard platinum/etoposide therapy. Enrollment for this trial is ongoing.

Of all the new chemotherapy agents, topotecan has undergone the most extensive evaluation in SCLC. It is clearly active in second-line SCLC and shows promise in first-line therapy. In vitro data show synergism of topotecan with many other drugs, including topoisomerase II inhibitors such as etoposide, and with DNA cross-linking agents such as alkylators and platinum compounds. Present development of topotecan in combination with cisplatin or carboplatin, the taxanes, and other agents is focusing on shorter schedules of topotecan, such as 3 days, and use on a weekly basis. Additionally, an oral formulation of topotecan is in development that may increase the ease of administration of the 5-day regimen. Preclinical data suggest that more prolonged exposure to topotecan may increase effectiveness. Hochster et al[38] gave topotecan as a 21-day continuous infusion in second-line ovarian cancer patients and found a response rate of 43%, with considerably less toxicity. Whether prolonged administration will provide a benefit remains to be determined. If prolonged administration is found to be useful, the oral formulation may facilitate this schedule.

In conclusion, we have reported that the efficacy of single-agent topotecan was similar to that of the three-drug combination of CAV, with improved control of several symptoms. Further study with topotecan in SCLC, including combination first-line therapy, is warranted.

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APPENDIX

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Sequential Immunofluorescence Staining and Image Analysis for Detection of Large Numbers of Antigens in Individual Cell Nuclei

Carolina Wählby,[1*] Fredrik Erlandsson,[2] Ewert Bengtsson,[1] and Anders Zetterberg[2]

[1]Centre for Image Analysis at Uppsala University, Uppsala, Sweden

[2]Department of Oncology-Pathology at the Karolinska Institute, Stockholm, Sweden

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Background: Visualization of more than one antigen by multicolor immunostaining is often desirable or even necessary to explore spatial and temporal relationships of functional significance. Previously presented staining protocols have been limited to the visualization of three or four antigens.

Methods: Immunofluorescence staining was performed both on slices of formalin-fixed tissue and on cells in culture. Images of the stained material were recorded using digital imaging fluorescence microscopy. The primary and secondary antibodies, as well as the fluorophores, were thereafter removed using a combination of denaturation and elution techniques. After removal of the fluorescence stain, a new immunofluorescence staining was performed, visualizing a new set of antigens. The procedure was repeated up to three times. A method for image registration combined with segmentation, extraction of data, and cell classification was developed for efficient and objective analysis of the image data.

Results: The results show that immunofluorescence stains in many cases can be repeatedly removed without major effects on the antigenicity of the sample.

Conclusions: The concentration of at least six different antigens in each cell can thus be measured semiquantitatively using sequential immunofluorescence staining and the described image analysis techniques. The number of antigens that can be visualized in a single sample is considerably increased by the presented protocol. Cytometry 47:32–41, 2002. © 2001 Wiley-Liss, Inc.

Key terms: sequential immunofluorescence staining; 3D image analysis; antibody denaturation; antibody elution

In 1941, Albert Coons performed the first successful visualization of an antigen using immunofluorescence (1,2). For many years, fluorescein isothiocyanate (FITC) was the only fluorophore available. In 1957, rhodamine was introduced as an alternative fluorescent label (3). The introduction of narrow-band excitation and emission filters meant that FITC and rhodamine could be studied independently in the same specimen, allowing the exploration of the exact spatial relationship between two antigens (4).

Simultaneous visualization of three or more antigens by immunofluorescence is often desirable. Multicolor immunofluorescence staining is, however, limited by two factors. The first is the spectra of the fluorophores, which have to be separated by band pass filters for the excitation and emission wavelengths. If the spectra of the fluorophores overlap, which is often the case when many fluorophores are used together, it is very difficult to discriminate between signals originating from different fluorophores. The second factor limiting multicolor immunofluorescence staining is the availability of primary antibodies of different origins. The majority of commercially available antibodies are either of rabbit or mouse origin, limiting the number of possible combinations of primary antibodies.

Several techniques have been developed to overcome the lack of primary antibodies from different species. The primary antibodies can be conjugated directly with the fluorophores, so that secondary antibodies directed against primary antibodies of a specific species origin are no longer needed. Unfortunately, the successful detection of most antigens requires the amplification of the signal that is achieved when a secondary fluorophore-conjugated antibody is used, and fluorophore conjugation can change the affinity of the primary antibody. Two primary antibodies from the same species can be used if one of the various blocking techniques using Fab fragments is employed (5) or if the first secondary antibody is replaced by fluorophore-conjugated Fab fragments (6).

The two first authors contributed equally to the presented work. FE developed the staining protocol and CW developed the image analysis procedures.

*Correspondence to: Carolina Wählby, Centre for Image Analysis, Lägerhyddv. 17, Uppsala 752 37, Sweden.

E-mail: carolina@cb.uu.se

An entirely different approach used occasionally in immunostaining is elution of the first applied primary and secondary antibodies using a low pH buffer leaving only the colored end product (7). After elution, another round of staining can be performed, using a second primary antibody from the same species as the first together with a differently colored end product. However, fluorophores cannot withstand elution. More recently, denaturation of the primary antibody by heating of the sample has been used successfully to destroy the first applied primary antibody (8). Again, the method used can only be applied to traditional immunostaining, not immunofluorescence.

We have developed a novel protocol for sequential immunofluorescence staining (SIFS) based on a combination of elution and denaturation of both primary and secondary antibodies, as well as of the fluorophores. The procedure allows us to stain the same tissue slice at least three times, allowing the study of at least six antigens in one slice of tissue. Different primary antibodies from the same species could be applied at different staining rounds, overcoming the limited availability of primary antibodies from different species without the need for blocking the first applied primary antibody. Interesting parts of the tissue were photographed between each round of staining. Through digital registration of the images from each round of staining, an image of the studied area that contained all the information on how each antigen was distributed could be created. After semiautomatic identification of individual cell nuclei in the acquired images, the staining intensity of each stain in each cell nucleus in the tissue slice could be extracted. Based on the relative staining intensities, each cell was classified automatically as positive or negative for each of the investigated antigens. The morphology of the tissue could be visualized using standard HTX-eosin staining after the completion of the SIFS procedure.

MATERIALS AND METHODS

Preparation of Tissue

Tissue sections from routinely fixed and paraffin-embedded samples of cervical carcinoma or carcinoma of the prostate were used to test and optimize the proposed protocol. The tissue sections were cut at a thickness of 2–4 μm. Thin sections are necessary in order to minimize image blurring from multiple layers of cells. The sections were incubated overnight at 47°C to adhere to SuperFrost Plus microscope slides (Menzler Gläser, Braunschweig, Germany). The sections were stored at -20°C and stepwise deparaffinized in graded alcohols prior to staining. Antigenic recovery was performed through microwave cooking for 2 × 5 min in a 0.1 M citrate buffer, pH 6.0.

To visualize the cell nuclei, the tissue sections were incubated in a 10-μM 4,6-diamidino-2-phenylindole, dihydrochloride (DAPI) solution for 5 min. The sections were washed briefly in washing buffer (0.05 mM TRIS-HCl, pH 7.6, 0.3 mM NaCl, and 0.02% Tween 20) and mounted in DABCO mounting medium (25 mg/ml 1,4-diazabicyclo[2,2,2]octane and 0.1 × phosphate-buffered saline [PBS] in spectroglycerol, pH set to 8.6 using HCl). An approximately 170 μm thick (no. 1.5) coverglass was used to optimize the optical conditions for 3D microscopy.

The areas to be studied were chosen based on DAPI morphology. They were photographed digitally before the first round of staining, making it possible to measure separately the amount of autofluorescence emitted from the sample for all filter sets. Filter sets optimized for detection of DAPI, FITC, and Cy3 from Chroma Technology (Brattleboro, VT) were used. The images were acquired using a Delta Vision system (Applied Precision, Seattle, WA) and equipped with a cooled monochrome CCD camera (Photometrics, Tucson, AZ). A Zeiss Plan-Neofluar 63×/NA1.30 lens was used (Carl Zeiss GmbH, Oberkochen, Germany), resulting in a pixel size of 0.1058 μm and an optical resolution of approximately 0.2 μm in the x and y directions. In order to get a 3D image of the studied area, seven z sections 0.5 μm apart were imaged, making the final resolution of the 3D volume in the z direction 1.0 μm. The initial focus was found by visual inspection.

After the background had been documented, the slides were demounted carefully. Each time the slides were demounted, they were washed for at least 15 min in washing buffer before being further processed.

SIFS

Sequential staining on tissue sections has been performed using a variety of primary antibodies. For optimization of the protocol, the mouse monoclonal antibody (mAb) 6E6 against cyclin A (Novocastra Laboratories, Newcastle upon Tyne, United Kingdom) and the rabbit polyclonal antibody sc-528 directed against p27 (Santa Cruz Biotechnology, Santa Cruz, CA) were used. In addition, the rabbit polyclonal antibody H-432 against cyclin A (Santa Cruz Biotechnology) and the mouse mAb K25020 against p27 (Transduction Laboratories, Lexington, KY) were used for the crossover experiments. All secondary antibodies were of donkey origin (Jackson ImmunoResearch Labs, West Grove, PA). The used secondary antibodies included an FITC-conjugated anti-rabbit antibody, a Cy3-conjugated anti-mouse antibody, and a biotin-conjugated anti-mouse antibody. The secondary antibodies were dissolved in blocking buffer with 4% donkey serum. Detection of the biotin was performed using Cy3-conjugated streptavidin (Amersham Life Sciences, Little Chalfont, United Kingdom). The streptavidin was diluted in PBS.

To block nonspecific binding, the tissue sections were immersed in blocking buffer (1% bovine serum albumin [BSA] and 0.5% Tween 20 in PBS) for 30 min. The primary antibodies were then applied. The slides were covered by coverglasses and left overnight at room temperature in a moisture chamber. The tissue sections were washed in washing buffer 3 × 15 min. The tissue sections were immersed in blocking buffer a second time for 30 min, this time using blocking buffer with an addition of 4% donkey serum. The tissue sections were incubated with the secondary antibodies for 30 min, followed by another round

of washing in washing buffer 3 × 15 min. Whenever a biotin-conjugated secondary antibody had been used, the slides were incubated with biotin-Cy3 for 30 min and washed again as described above. Finally, the slides were incubated with DAPI and mounted as described.

After photographing the result of the first round of staining, the antibodies were disposed of using elution, denaturation, or any combination of these (see below). Elution of previously applied secondary and primary antibodies was performed using primarily a 0.1-M lysine buffer, pH 2.0 (7). Denaturation of the applied antibodies was performed in exactly the same way as the antigenic recovery; the slides were cooked in a citrate buffer in a microwave oven (8).

After the denaturation and/or elution of the stain, the areas of interest were again photographed to check the remaining levels of the previously applied antibodies and fluorophores. To measure the amount of remaining fluorophores, the imaging was performed simply without further treatment. To measure both the remaining primary antibodies and the remaining fluorophores, the tissue section was immersed again in blocking buffer with 4% donkey serum, incubated with the same secondary antibody as used previously, and washed again as described above. Any remaining primary antibodies would then cause fluorescence by attracting the newly applied secondary antibodies, and thus be detected.

The subsequent rounds of staining or stain removal were performed in the same manner as described above for the first staining round. Each round of staining or stain removal resulted in one image volume for each of the used (or recently removed) fluorophores and one for the DAPI stain. DAPI was used as a reference stain in the image analysis and therefore had to be reapplied in each round of staining because the stain removal procedure also removed the DAPI stain. Thus, the background fluorescence before the first staining as well as any remaining fluorophores or primary antibodies after each stain removal were documented together with the staining results. By staining the slides for hematoxylin-eosin after the sequential immunostaining procedure, the morphology of the investigated tissue could be investigated.

Image Analysis

In order to quantify the fluorescence emitted by each investigated nucleus at each step of the staining process, the acquired volumes were analyzed using the image analysis methods described previously (9). Expression that extends beyond the nucleus could be analyzed by segmentation of the cytoplasms. This was, however, not investigated in this study. In summary, the image analysis methods can be divided into four steps: image registration, 2D and 3D image segmentation, extraction of image data, and data analysis.

Image Registration

After each round of staining or stain removal, the slides were placed in the microscope and photographed. One monochrome image with each of the filter sets for detection of DAPI, FITC, and Cy3 was acquired before moving the focal plane to the next z-position. This was repeated seven times until one 1024 × 1024 × 7 voxel image volume for each of the investigated wavelengths was created. Each time the slides were reinserted in the microscope, they were repositioned using the automatic repositioning system of the DeltaVision system. The repositioning was not perfect and translation in the x-, y-, and z-direction, as well as z-rotation between the subsequent image sets, remained. Typically, a 5–10-pixel translation in the x- and y-direction, a 1-pixel translation in the z-direction, and a 1–2° z-rotation appeared between subsequent image sets. Exact repositioning, or registration, of the images was necessary for the automatic steps of further analysis based on individual cells.

Because each image set contained a DAPI image and the DAPI morphology did not change between subsequent rounds of staining or stain removal, the DAPI images were used for registration of the whole image set. The DAPI image of each image set was registered automatically to the DAPI image of the first image set using gray-level matching in 3D (9). A smaller iteration step size was used in the z-direction than in the x- and y-direction because of the flat shape of the image volumes (1024 × 1024 × 7 pixels) and the smaller translation error in the z-direction. The transformation matrix of the best registration was then applied to the FITC and Cy3 images within the same set.

2D and 3D Image Segmentation

Once all image volumes were registered, the cell nuclei had to be identified by segmentation so that the fluorescence signal could be extracted. Before segmentation, a common 3D reference image was created from all the registered DAPI images of the cell nuclei. For every voxel, the maximum intensity of all the registered images with respect to a global coordinate system was selected. In this way, only the reference image had to be segmented for every experiment. In other words, for an experiment with three sequential triple stainings, resulting in 18 image volumes, only one image had to be segmented.

A method inspired by the watershed algorithm (10,11) was used for separation of clusters of cells and segmentation of the image into cells and background in one step as described in detail (9). The initial segmentation using this method was always done on a 2D maximum intensity projection along the z-axis of the reference image. This fully automatic initial segmentation results in both oversegmentation and undersegmentation in areas of closely clustered nuclei. Errors were corrected manually using a digital editing tool, making the segmentation step semiautomatic. The manual correction is a minor part of the otherwise fully automatic process because only a single 2D segmentation is needed for complete analysis of each full sequential staining experiment. The extension to 3D was created by gray-level thresholding of the common 3D volume at the same level as was used for the threshold for the background in the 2D segmentation. The 2D labels were transferred automatically to the 3D volume by ex-

tending the 2D segmentation into "3D cylinders" in the z-direction and making a logical AND between the labeled 3D cylinders and the 3D thresholded image, keeping the labels of the 2D segmentation.

Extraction of Image Data

The registration step resulted in a transformation matrix for every image set and the segmentation step resulted in a common segmentation template that could be used on all images after transformation according to the transformation matrix. For extraction of image data, the transformation matrix of each image set was applied to all the images within the set. The voxel intensities were thereafter integrated over each cell nucleus defined by the 3D segmentation template. Because the segmentation templates were created from the DAPI images of the cell nuclei, they found the correct position of the cell nuclei in the tissue sections independent of the fluorescence signal from the other stains.

Data Analysis

A linear relationship between fluorophore concentration and emitted light can be expected (12,13). The response of the camera is also linear (14). The intensity of the detected signal can therefore be expected to be directly proportional to the amount of fluorophore present in the specimen. However, many variables affect the staining intensity of a tissue section. Variations in tissue fixation and thickness, deparaffinization, antigenic recovery, temperature and time at the staining and washing steps, and exposure time all affect the final detected signal. The variability between different tissue sections makes it difficult to compare quantitatively cells from different tissue sections. The staining intensities of cells within the same tissue section can be compared. In order to make comparisons among cells on different slides, it is necessary to classify them according to their intensity values relative to the other cells on the same slide. We have developed an algorithm for the calculation of thresholds for objective classification of a cell nucleus as staining positive or negative for a particular antigen (15). The choice of threshold is based on the shape of a histogram of the staining intensities. The method was developed further by Lindblad (16) and a kernel density estimate was used for approximation of the distributions. Based on the thresholds given by maxima in the second derivative of the kernel density estimate, a fuzzy class membership value was found for each cell (17). Cells were classified as strongly negative, negative, neutral, positive, or strongly positive.

RESULTS

During the development of the described protocol for SIFS of routinely fixed and paraffin-embedded material, a series of experiments were performed. Parameters for elution and denaturation were adjusted to optimize the removal of previously applied antibodies without destroying antigenicity of the tissue sections. The level of success was evaluated using the described image analysis techniques. All experiments were performed two or more times to ensure reproducibility.

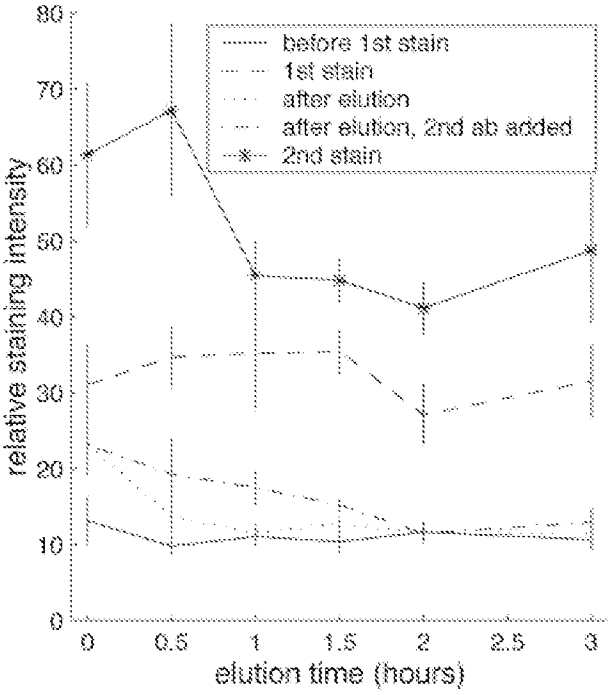

FIG. 1. Stain removal by elution. The staining intensity of a subset of representative positive cells (found by inspection of the slides after the first staining step) from six slides were studied before staining (but with secondary antibodies added), after application of the first stain, after elution for 0, 0.5, 1, 1.5, 2, or 3 h, after the addition of the new FITC-conjugated secondary antibody, and after the application of the new primary and FITC-conjugated secondary antibodies. After 1.5 h, the majority of the previously applied primary and secondary antibodies had been removed. However, when the FITC-conjugated secondary antibody was reapplied, previously strongly staining nuclei still emitted a weak fluorescence. This indicated that a small subset of the primary antibodies remained bound to their antigens, even after 3 h of elution. The antigens were not destroyed by the treatment and the staining intensity increased for the second round of staining, indicating that the antigens are made more available to the antibodies after elution. A very high staining intensity appeared if the elution was shorter than 1 h, probably due to formation of complexes of primary and secondary antibodies.

Elution for Removal of Applied Primary and Secondary Antibodies

In order to study whether elution using a glycine buffer was sufficient to remove all previously applied antibodies, as well as the fluorophores conjugated to the secondary antibodies without disturbing the antigen, slides were washed in glycine buffer, pH 2.0, for 0, 0.5, 1, 1.5, 2, and 3 h. The results are shown in Figure 1. After 1–2 h, the majority of the previously applied fluorophores were removed. However, when the secondary antibody was reapplied, previously strongly staining nuclei still emitted a weak fluorescence, even after 3 h of elution. This indicated that a subset of the primary antibodies remained bound to their antigens.

The primary antigens (cyclin A and p27) were not destroyed by the elution and the staining intensity actually

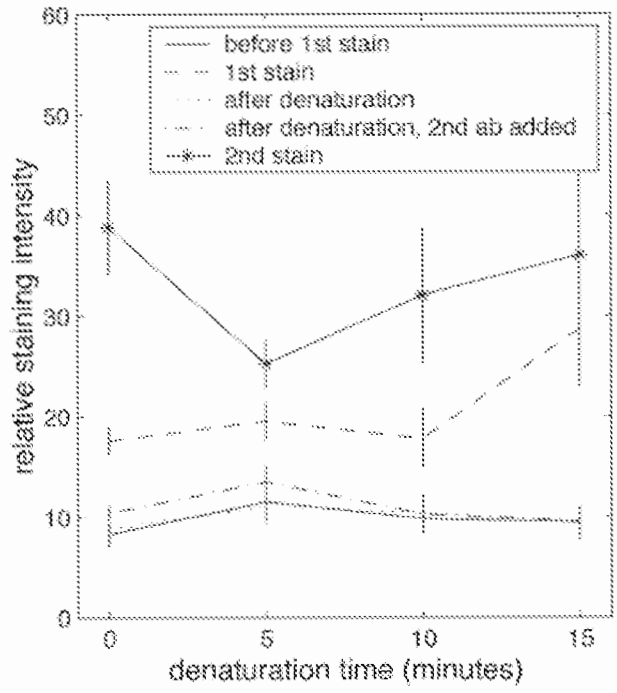

FIG. 2. Stain removal by denaturation. As elution did not remove all the primary antibodies, an attempt was made to denature the remaining antibodies. The staining intensity of a subset of representative positive cells (found by inspection of the slides after the first staining step) from five slides was studied before staining, after application of the first stain, after elution for 2 h followed by denaturation for 0, 5, 2 × 5, or 3 × 5 min, after addition of the new FITC-conjugated secondary antibody, and after application of the new primary and FITC-conjugated secondary antibodies. When the 2 h elution was followed by denaturation for 2 × 5 min, all the primary antibodies were removed or destroyed.

increased in the second round of staining, indicating that the antigens might be more readily available after elution. A very high staining intensity after the second staining round was noted if the elution time was shorter than 1 h, probably due to remaining secondary antibodies binding the subsequently applied primary antibodies. Elution did not fully remove applied biotin-streptavidin-Cy3 complexes. Large aggregates of streptavidin-Cy3 remained even after 6 h of elution (data not shown).

Denaturation Can Destroy Remaining Antibodies

As elution did not remove all the primary antibodies, an attempt was made to destroy the remaining antibodies through heat denaturation. Slides that had been stained and exposed to elution for 2 h were denatured for 0, 5, 2 × 5, or 3 × 5 min. As described previously, 2 × 5 min was sufficient time to denature all the primary antibodies (8; Fig. 2).

Denaturation Alone or Followed by Elution Cannot Remove Fluorophores

The efficiency of denaturation alone, or denaturation followed by a shorter elution, was tested to see whether it would be equally efficient in removing the primary and secondary antibodies as a 2 h elution followed by a 2 × 5 min denaturation. Denaturation alone failed completely in removing the fluorophores (data not shown) and it also seemed to lower considerably the efficiency of a follow-up elution (data not shown).

Crossover Experiments

A crossover experiment was designed to test if the stain is washed away completely without affecting antigenicity, as well as to test whether the same cell will be classified as positive/intermediate/negative if the same stain is applied again. One crossover experiment consisting of three rounds of staining is shown in Figure 3. Only two antigens (cyclin A and p27) were stained throughout the experiment, but two different primary antibodies were used for each antigen. One of the antibodies was of mouse origin and one was of rabbit origin. After each stain removal, the antibody used to detect each of the antigens was switched. Thus, the secondary antibody carrying the fluorophore (FITC or Cy3) shifted between the antigens after each staining step. This setup gives a priori knowledge of the expected result of the second and third rounds of staining, because the result should be the same as after the first round of staining, but in the opposite fluorophore channel. The labeled secondary antibody was reapplied after each stain removal to visualize the remaining primary antibody. Thereby, the removal of the previously applied antibodies could be measured. The experiment provided us with a method to show that the antigenicity of the investigated antigens was not destroyed by elution or denaturation and that the antibodies and fluorophores could be removed satisfactorily after each of the two first rounds of staining.

Any residual fluorescence from previous staining steps was recorded by imaging the specimen after each stain removal. The exposure time was kept constant throughout the experiment for all images acquired with the same filter set. Autofluorescence, as well as residual fluorescence remaining after the stain removal step, could be removed from the subsequent staining step by simple pixel-wise subtraction. The relative antigen concentration per cell was approximated as the mean pixel intensity under the 3D segmentation template as described above. Each cell was then classified as positive (green), weakly positive (cyan), neutral (blue), weakly negative (magenta), or negative (red) using the described classification methods. The classification results are shown in Figure 4 together with the images of the stained cells (after subtraction of the corresponding residual images). The images were scaled for optimal visualization of gray levels. As shown in Figure 4, the cells are classified the same way after each of the three staining rounds for cyclin A (with two minor changes from neutral to weakly positive). This shows that the antigenicity is kept after the subsequent staining steps and that the classification is not affected by previous staining steps. The variations of the classification results are larger when it comes to the p27 stain. The results are similar after the first and third staining steps, when the rabbit polyclonal primary antibody was used, compared with the second staining step, when the mouse

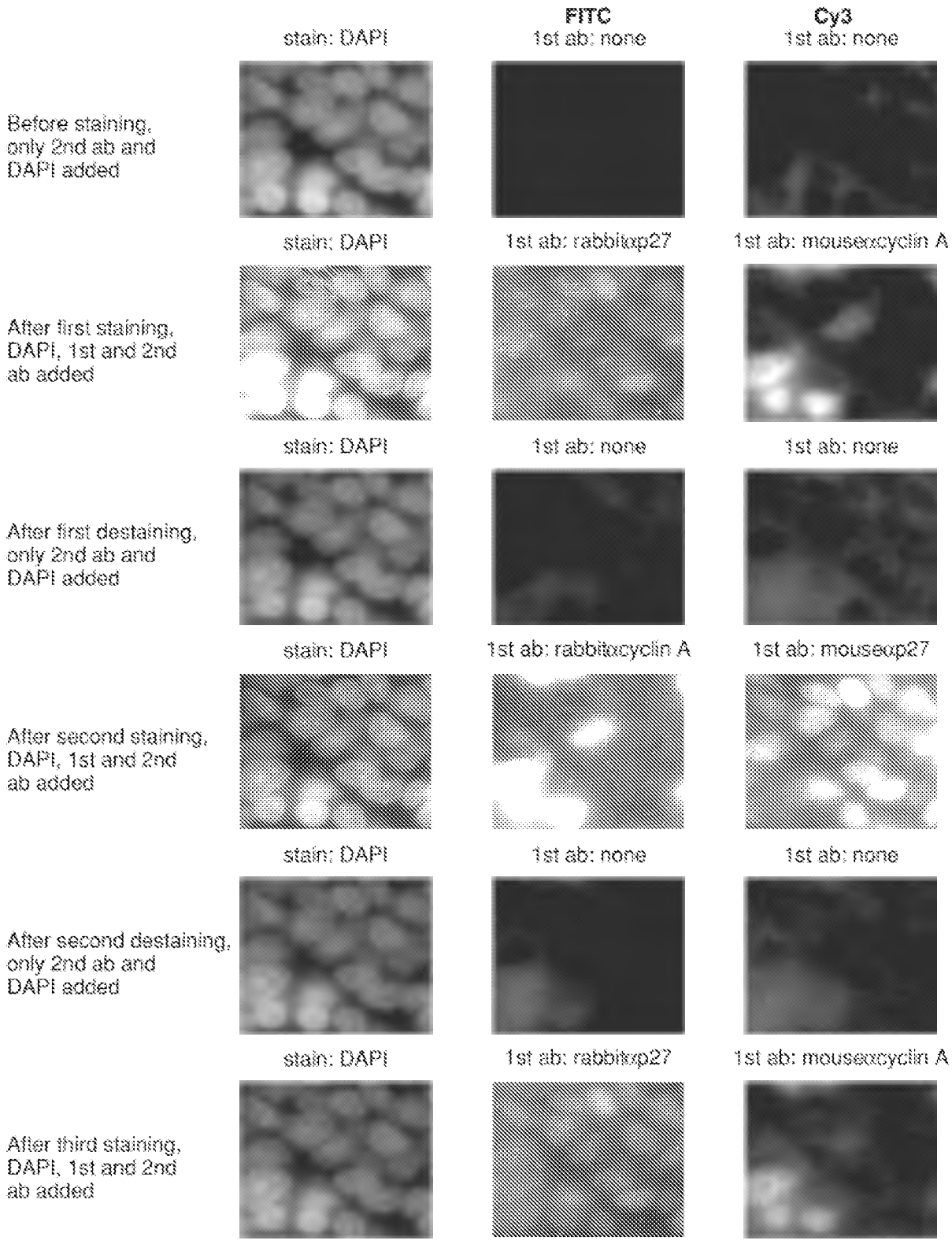

FIG. 3. Set up of the crossover experiment. In each staining or washing step (horizontal rows), the tissue was stained with DAPI and the secondary antibodies FITCαrabbit and Cy3αmouse were applied. The primary antibodies were excluded after each stain removal step and exchanged before the next round of staining. All images are projections of 3D images (explaining the blur). They were acquired using the same exposure time and were subsequently scaled using the same gray-level interval. Histogram equalization or other image enhancement (which would have improved the print quality) was avoided in order to visualize the true variation in fluorescence signal. Only a small part of the full registered image set is shown.

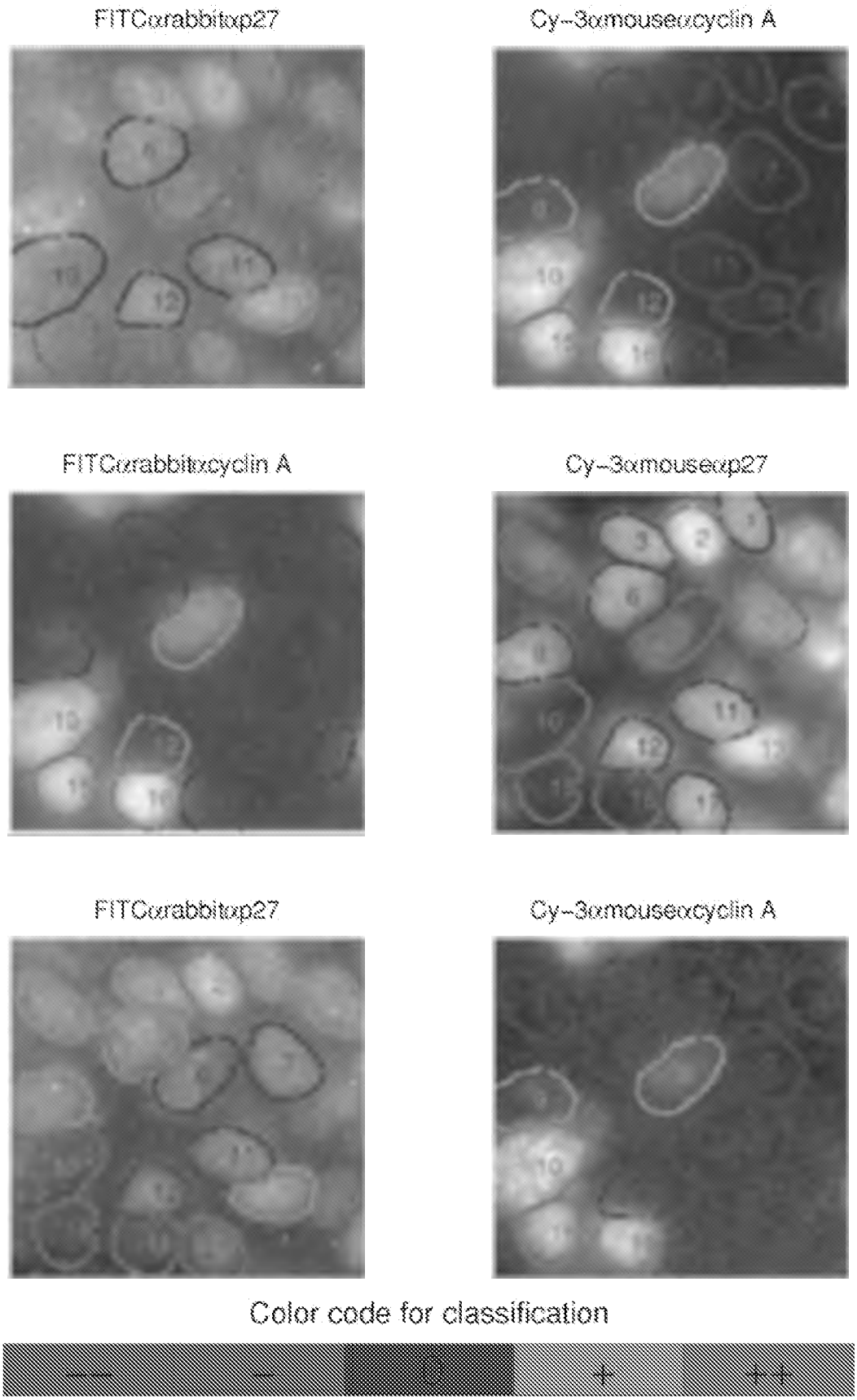
FITC/rabbit/p27
Cy-3/mouse/cyclin A
FITC/rabbit/cyclin A
Cy-3/mouse/p27
FITC/rabbit/p27
Cy-3/mouse/cyclin A
Color code for classification
– – – + + +

FIG. 4.

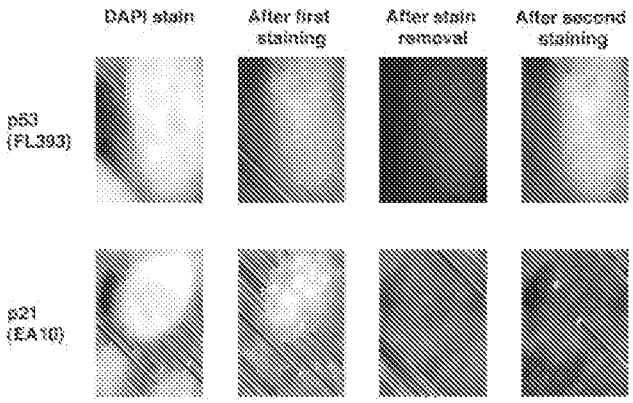

FIG. 5. Two different reactions to elution and denaturation. The antibody FL393 against p53 is well removed by elution for 2 h and a 2 × 5-min denaturation and p53 retains its antigenicity. The majority of the tested antibodies and antigens reacted similarly. p21reacted entirely differently. It lost all of its antigenicity through the elution. It is the only antigen to exhibit this type of behavior. Each set of three images was acquired using the same exposure time. The images were handled exactly the same way with respect to contrast and brightness settings.

monoclonal primary antibody was used. The most likely explanation is the differences in the specificity of the used primary antibodies.

The conclusion of the described crossover experiment is that previously applied stains can be removed at least twice and that at least two rounds of stain removal can be performed without necessarily losing the antigenicity of the investigated antigens. This opens the possibility to stain one slice of tissue for at least six different antigens, using two primary antibodies of different species origin in each staining step.

Some Antigens Lose Their Antigenicity During Elution

The results presented above were acquired using antibodies directed against cyclin A and p27 because these proteins are located primarily in the cell nucleus and because cells generally contain either cyclin A or p27, but not both, which simplified the verification of the method. Other antibodies were also tested in order to see whether a 2 h elution and a 2 × 5 min heat denaturation would affect them and their antigens similarly. Among the tested antibodies, two strikingly different patterns could be distinguished (Fig. 5), which are illustrated by the p53 antibody FL-393 (Santa Cruz Biotechnology) and the p21 antibody EA10 (Oncogene Research Products, Darmstadt, Germany). The antibody detecting p53 was removed or destroyed sufficiently by the treatment and it stained even stronger in the second round of staining than in the first, just like the previously described antibodies against cyclin A and p27. Other antibodies exhibiting the same behavior included another antibody directed against p53 (DO1, Santa Cruz Biotechnology), an antibody against MDM2 (SMP14, Santa Cruz Biotechnology), an antibody against cyclin E (HE12, Santa Cruz Biotechnology), and an antibody directed against p57 (C-20, Santa Cruz Biotechnology). On the other hand, antibody EA10 against p21 did not stain at all in the second round, indicating that the epitope on p21 that it detects was destroyed by elution. Thus, 9 of the 10 tested antibodies (five of the six tested antigens) may be used in a second or later round of staining, whereas the antibody detecting p21 must be used in the first staining round.

FIG. 4. Results of the image analysis and classification procedures. Each cell was classified as positive (green), weakly positive (cyan), neutral (blue), weakly negative (magenta), or negative (red) using the classification methods described in the Materials and Methods. Notice how the cyclin A stain results in almost the exact same classification of the cells after each of the staining steps. The variation in classification is larger for the p27 stain, but this can be explained by differences in the reactivity of the used primary antibodies, because the first and third rounds of staining were almost identical.

DISCUSSION

The results presented show that primary and secondary antibodies, as well as fluorophores, can be removed from a routinely fixed and paraffin-embedded tissue slice. The procedure is based on the elution of antibodies and fluorophores, followed by the denaturation of any remaining primary antibodies. Thus, a tissue slice can be stained repeatedly, allowing the detection of a large number of antigens. Our method for quantification of antigen concentration begins with a fully automatic image registration and the creation of a reference image followed by semi-automatic 2D segmentation. The 2D segmentation is extended to 3D and, finally, image data are extracted and analyzed.

The presented SIFS technique requires the complete removal of previously applied fluorophores and antibodies without disruption of the antigenicity of the sample. We used elution and denaturation, but other means to achieve the same or better results probably exist. The presented protocol is capable of a complete removal of fluorophores and antibodies when only visual inspection is used to evaluate the results. Occasionally, enough primary antibodies remain to be detected when a highly sensitive CCD camera is used. The weak signal emanating from remaining fluorophores and antibodies could be subtracted from the following staining round using the image analysis techniques and the experimental setup described. Another slight drawback of the proposed protocol is that the elution tends to increase nuclear background fluorescence.

Elution and denaturation increase the antigenicity of the tissue for the majority of the tested antigens. The second round of staining is generally stronger than the first and the presented experiments (Figs. 1, 2) indicate that this is not an artifact due to remaining primary and secondary antibodies after the first staining round. The most probable cause of the phenomena is that elution and denaturation actually improve the antigenicity of the tissue by improving the previously performed antigenic recovery. Antigenic recovery performed for 3 or 4 × 5 min instead of the standard 2 × 5 min sometimes yields stronger staining (data not shown).

During the development of the presented procedure, a high variability of staining intensity in different tissues, as well as in material fixed differently, was noticed. It is possible that the described protocol has to be adapted to work with material from other tissues than cervical carcinoma and with material that is sectioned and fixed differently. However, the presented protocol has been used successfully for at least two rounds of staining even on cells grown in monolayer culture and fixed for 1 h in 10% formalin (Hanna-Stina Martinsson, personal communication).

Before designing a full SIFS experiment, the antibodies and their antigens have to be tested extensively in order to evaluate their reaction to elution and denaturation. We have seen two types of reactions. The majority of antibodies are removed readily and their antigens retain their antigenicity (see p53 in Fig. 5). The second type of reaction is illustrated by p21 (Fig. 5). The primary antibody is removed easily, but the elution destroys the antigenicity of p21. Antigens exhibiting this behavior can only be stained in the first staining round. Primary or secondary antibodies that are not removed by elution represent a possible third type of reaction to elution and denaturation. These antibodies can only be applied in the last round of staining. The amount of remaining primary antibody that can be accepted, i.e., the distinction between the first and the third type of reaction, may vary depending on the fluorescence detection system and the availability of image analysis tools to remove any lingering signal from subsequent images.

Each new secondary antibody and each new fluorophore will also require testing before usage. To ensure optimal results, elution pH and duration, denaturation temperature and duration, and staining order may have to be optimized for each individual SIFS experiment.

Several alternative methods to remove or destroy primary antibodies and/or fluorophore-conjugated secondary antibodies have been presented. Elution has been performed with varying degrees of success using glycine buffer (7), dimethylformamide (18), glycine and dimethylformamide in combination with electrophoresis (19), $KMnO_4$-H_2SO_4 (20), and HCl (21). Shorter denaturation at a higher temperature (130°C) can be highly efficient if the tissue is properly shielded by immersion in glycerin for 5–7 days (22). Furthermore, denaturation can be performed by treatment with formaldehyde vapor instead of by heating (23). These methods would have to be evaluated alone and in combination to find the best possible way to remove immunofluorescence stains.

Image analysis of the acquired images was performed in order to remove observer bias and get more quantitative data out of the performed experiments. All the steps are fully automatic except for the manual correction of errors in the segmentation. As all images of an experiment are registered to a common reference image, the segmentation is only done in a single image for a full experiment. The 3D extension of the 2D segmentation results in small errors when cells overlap. A better segmentation algorithm for 3D and correction tools for 3D segmentation are under consideration.

This study concentrated on analysis of antigens located in the cell nucleus. By staining the cytoplasm (and/or the cell membrane) and segmenting the images based on this stain, antigens located in the cytoplasm can be investigated on the single cell level. Methods for cytoplasm segmentation are currently under development.

The future development of SIFS will certainly result in better protocols for stain removal, as well as in equipment allowing highly automated image acquisition, stain removal, and staining. The higher degree of reproducibility acquired by automating the process will be a major advantage and may even allow quantitative measurements of the levels of the investigated antigens. Specialized software will probably be developed, allowing better presentation of acquired data, thus simplifying the analysis. Our vision is a system that automatically performs several rounds of SIFS followed by a classic HTX-eosin staining. The data would be presented as an HTX-eosin image with synthetic colors added to show the relative, or maybe even absolute, levels of the investigated antigens in each cell. With such a system, it would be easy to evaluate the distribution of a very large number of antigens in relationship to each other and to the morphology of the tissue. The status of complicated pathways such as ARF-p53-MDM2-p21-CDK2-pRB-E2F or signaling cascades could then be studied on the individual cell level in tissue sections or on tissue micro arrays.

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PLOS ONE

EZN-2208 (PEG-SN38) Overcomes ABCG2-Mediated Topotecan Resistance in BRCA1-Deficient Mouse Mammary Tumors

Serge A. L. Zander[1], Wendy Sol[1], Lee Greenberger[3¤], Yixian Zhang[3], Olaf van Tellingen[4], Jos Jonkers[2], Piet Borst[1], Sven Rottenberg[1]*

1 Division of Molecular Oncology, The Netherlands Cancer Institute, Amsterdam, The Netherlands, **2** Division of Molecular Pathology, The Netherlands Cancer Institute, Amsterdam, The Netherlands, **3** Department of Pharmacology, Enzon Pharmaceuticals Inc., Piscataway, New Jersey, United States of America, **4** Department of Clinical Chemistry, The Netherlands Cancer Institute, Amsterdam, The Netherlands

Abstract

BRCA1 dysfunction in hereditary breast cancer causes defective homology-directed DNA repair and sensitivity towards DNA damaging agents like the clinically used topoisomerase I inhibitors topotecan and irinotecan. Using our conditional *K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ mouse model, we showed previously that BRCA1;p53-deficient mammary tumors initially respond to topotecan, but frequently acquire resistance by overexpression of the efflux transporter ABCG2. Here, we tested the pegylated SN38 compound EZN-2208 as a novel approach to treat *BRCA1*-mutated tumors that express ABCG2. We found that EZN-2208 therapy resulted in more pronounced and durable responses of ABCG2-positive tumors than topotecan or irinotecan therapy. We also evaluated tumor-specific ABCG2 inhibition by Ko143 in *Abcg2*$^{-/-}$ host animals that carried tumors with topotecan-induced ABCG2 expression. Addition of Ko143 moderately increased overall survival of these animals, but did not yield tumor responses like those seen after EZN-2208 therapy. Our results suggest that pegylation of Top1 inhibitors may be a useful strategy to circumvent efflux transporter-mediated resistance and to improve their efficacy in the clinic.

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* E-mail: s.rottenberg@nki.nl

¤ Current address: Bristol-Myers Squibb, Princeton, New Jersey, United States of America

Introduction

Poisoning topoisomerase I (Top1) is a useful therapeutic approach to target cancer cells with intrinsic defects in the DNA damage response [1]. In particular the camptothecin derivatives topotecan and irinotecan are frequently used to target Top1 in ovarian, colon, and small cell lung cancer patients. Both topotecan and SN38, the active metabolite of irinotecan, stabilize Top1-DNA cleavage complexes (Top1cc), which are subsequently converted into DNA damage during DNA replication and transcription. The conversion of single stranded breaks (SSB) into double stranded breaks (DSB) during stalling of the replication machinery is the primary cytotoxic effect of Top1 poisons [2]. As a consequence, defects of tumor cells in proper repair of DSB provide an Achilles heel that can be targeted using Top1 inhibitors. An example is the increased topotecan sensitivity of cultured cells that are deficient in BRCA1 function [3], which is critical for error-free repair of DSB by homologous recombination (HR) [4].

We have previously studied topotecan responses in a genetically engineered mouse model for BRCA1-deficient breast cancer [5]. Despite high initial sensitivity, tumors were not eradicated and eventually all tumors acquired resistance to the maximum tolerable dose (MTD) of topotecan. About half of the tumors acquired resistance by overexpression of ABCG2 (or Breast Cancer Resistance Protein/BCRP). ABCG2 is an ATP-binding cassette (ABC) efflux transporter [6–8], and its overexpression in cultured cells was found to cause resistance to various clinically used anti-cancer drugs, such as topotecan and irinotecan [2,9]. In our mouse model, we found that tumor-specific ablation of this efflux transporter substantially increased overall survival of tumor-bearing animals [5]. This observation unambiguously confirmed that induction of ABCG2 expression is an effective mechanism of mammary tumors to evade topotecan-induced DNA damage.

At present, useful *in vivo* strategies to reverse ABCG2-mediated drug resistance in patients are lacking. ABCB1/P-gp inhibitors like elacridar or tariquidar also inhibit ABCG2 to some extent [10,11]. However, thus far the clinical benefit of these inhibitors is modest [12–14]. The mycotoxin fumitremorgin C (FTC) was identified as a more specific ABCG2 inhibitor [15,16], but neurotoxicity compromised its clinical potential [17]. Nevertheless, less toxic

FTC analogues were explored, and of these Ko143 was found to be the most potent and specific inhibitor, increasing oral topotecan availability in *Abcb1a/b* knockout mice 4–6-fold [17].

A complication of the systemic application of ABCG2 inhibitors is the fact that ABCG2 is expressed in normal tissues and protects them against xenotoxins [18]. In particular, ABCG2 contributes to the blood-brain barrier [19], and its expression in the liver, gut and kidney results in increased drug clearance. Hence, when combining an ABCG2 inhibitor with topotecan, it may be difficult to distinguish tumor-specific ABCG2 inhibition versus increased drug exposure due to reduced excretion. To be able to make this distinction, the use of ABCG2-deficient mice is helpful. ABCG2-proficient tumors can be grafted into syngeneic mice that lack ABCG2. This allows the analysis of tumor cell-specific effects of the inhibitor. We have shown that the spontaneous mammary tumors of our BRCA1 model can be transplanted orthotopically into syngeneic mice without loss of their genomic profile, morphology, or sensitivity to drug [5,20,21]. By transplanting ABCG2-proficient *Brca1*$^{-/-}$;*p53*$^{-/-}$ mammary tumors derived from FVB/N mice into ABCG2-deficient hosts of the same strain, we show here that Ko143 is indeed useful for reversing ABCG2-mediated topotecan resistance *in vivo*. Nevertheless, the benefit is modest and Ko143 does not result in a clearly detectable increase of intratumoral topotecan concentration. As an alternative approach to overcome ABCG2-mediated resistance, we therefore tested a polyethylene glycol-conjugated ('pegylated') Top1 inhibitor EZN-2208, a SN38 conjugate. In xenografts EZN-2208 results in higher and longer-lasting exposure of tumors to the irinotecan metabolite SN38 compared with irinotecan itself [22–24]. In our model, we found that EZN-2208 results in durable responses of ABCG2-expressing BRCA1-deficient mammary tumors, in contrast to topotecan or irinotecan treatment.

Materials and Methods

Spontaneous Mammary Tumors, Orthotopic Transplantations, Recipient Animals and Drug Intervention Regimens

All animal experimental procedures have been conducted according to the standard operating procedures of the lab animal facility and were approved by the Animal Ethics Committee of The Netherlands Cancer Institute under references 08.001-B44, 08.001-B45, 11.006-B06 and 11.006-B10.

The generation, genotyping, orthotopic transplantation, daily animal weight and mammary tumor caliper measurements as well as the sampling of *Brca1*$^{-/-}$;*p53*$^{-/-}$ mammary tumors were performed as described previously [5,25]. Six- to eight-week-old FVB/N recipient animals were purchased from Harlan, while *Abcg2*$^{-/-}$ host animals of the same age and genetic background were bred within our lab animal facility [26]. The experimental outline of the Ko143 (or vehicle) + topotecan combination therapy interventions in *Abcg2*$^{-/-}$ tumor-bearing animals is described under the results (Fig. 1A). Intervention with topotecan, irinotecan and EZN-2208 in wildtype FVB/N tumor-bearing animals started when a tumor volume of about 200 mm^3 was reached. Animals were either left untreated (control) or received 4 mg topotecan, 40 mg irinotecan or 10 mg EZN-2208 (SN38 equivalents) per kg body weight as a regimen of five consecutive i.v. injections on days 0, 2, 4, 6 and 8. When a tumor volume of about 1500 mm^3 was reached, animals were killed by CO_2 and tumor samples were harvested for further analyzes.

Drug Injection Solutions

Ko143 was purchased from Tocris Bioscience (Minneapolis, MN, USA) and used by diluting 10 mg/mL DMSO stocks in 15% (w/v) 2-hydroxyl-propyl-β-cyclodextrine/PBS to a final volume of 1 mg/mL and administered at 10 μL/g of body weight by i.p. injection. Topotecan was provided by GlaxoSmithKline PLC (London, UK) and dissolved in 5% (w/v) glucose to yield a solution of 0.4 mg/mL (of active compound) and administered at 10 μL/g of body weight by i.p. injection. EZN-2208 was provided by ENZON pharmaceuticals Inc. (Piscataway, NJ, USA) and dissolved in saline to yield a solution of 1 mg/mL (SN38 equivalents) and administered at 10 μL/g of body weight by i.v. injection. Irinotecan was purchased from Pfizer Inc. (New York, NY, USA) and used by diluting 20 mg/mL stocks in saline to yield a solution of 4 mg/mL and administered at 10 μL/g of body weight by i.v. injection.

Immunohistochemical Analysis of ABCG2

Immunohistochemical stainings were performed as described previously [5]. Briefly, ABCG2 was probed with the rat anti-mouse monoclonal (BXP53) from Abcam (ab24115, 1:400) and detected with a biotinylated rabbit anti-rat secondary antibody (Dakocytomation, E0468, 1:100).

Collection of Pharmacokinetics Samples and Quantification of Topotecan Levels

While animals were under isoflurane anesthesia, whole blood was collected by cardiac puncture and transferred into heparinized tubes on ice. Next, the animals were killed by cervical dislocation and their tumors dissected. Blood was centrifuged at 4000 rpm and 4°C for 5 min to separate the plasma fraction. After thawing at 4°C, the mouse tumors were weighed and homogenized in 1% (w/v) BSA in water (equivalent to 500 mg tumor in 2.5 mL volume), using a FastPrep-24 high speed bench top homogenizer (MP-Biomedicals, Santa Ana, CA, USA) at 6.0 M/s for 30 s in 4.5 mL tubes. Homogenized tumor and plasma samples were stored at −20°C until sample pretreatment for reversed-phase high performance liquid chromatography (RP-HPLC) analysis. Total topotecan levels (lactone plus carboxylate form) were determined by using a validated RP-HPLC method, as described previously [27].

Results

Efficacy of Ko143+ Topotecan Combination Therapy in *Abcg2*$^{-/-}$ Tumor-bearing Animals

To study the effect of ABCG2 inhibition on topotecan efficacy in a tumor-specific fashion, we transplanted spontaneous ABCG2-proficient *Brca1*$^{-/-}$;*p53*$^{-/-}$ mammary carcinomas [25], derived on a FVB/N background, orthotopically into ABCG2-deficient recipients [26] of the same mouse strain (Fig. 1A). Since ABCG2 expression in normal tissues is known to contribute to topotecan clearance [19], we had to lower the topotecan dose in *Abcg2*$^{-/-}$ mice substantially (Fig. S1A). In fact, the topotecan MTD of these animals was 4-fold lower than in wild-type animals (0.5 mg instead of 2 mg i.p. per kg body weight on days 0–4 and 14–18). The combination of this topotecan dose with 10 mg Ko143 i.p. per kg body weight on days 0–4 and 14–18 was still tolerable in mice that lack ABCG2 (Fig. S1B). This supports the notion that Ko143 is an ABCG2-specific inhibitor which does not cause serious off-target complications.

We then investigated the topotecan + Ko143 response of 8 randomly chosen ABCG2-proficient *Brca1*$^{-/-}$;*p53*$^{-/-}$ mammary

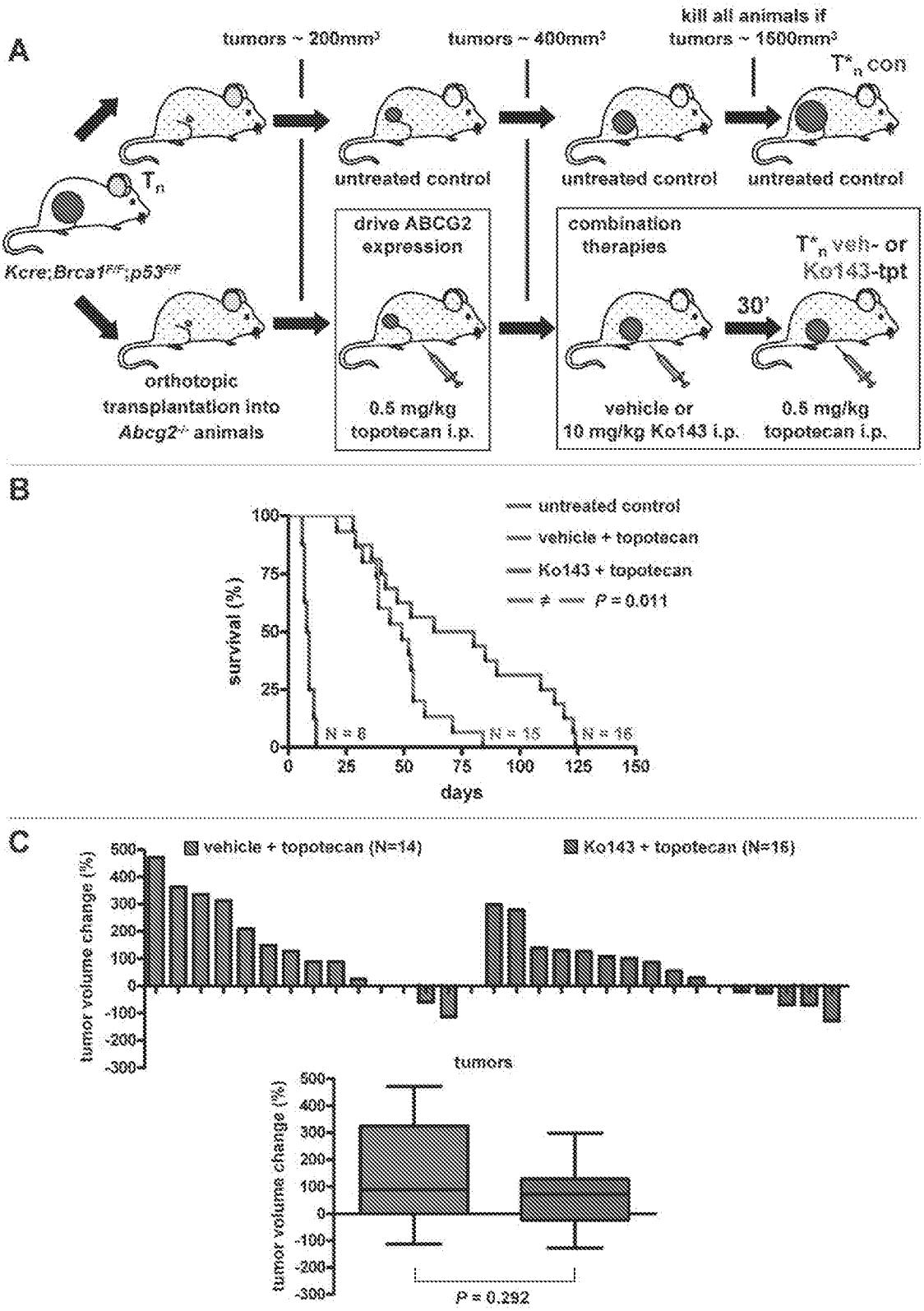

Figure 1. Efficacy of Ko143+ topotecan combination therapy in *Brca1*$^{-/-}$;*p53*$^{-/-}$ mammary tumors. A, Experimental outline. Spontaneous mammary tumors from *K14cre;Brca1*$^{F/F}$;*p53*$^{F/F}$ females were orthotopically transplanted into six- to eight-week-old *Abcg2*$^{-/-}$ syngeneic recipients. When tumors reached a volume of about 200 mm^3, animals were either left untreated (control) or injected i.p. with 0.5 mg topotecan per kg body weight on days 0–4 and 14–18 resulting in elevated ABCG2 expression. When tumors doubled in size (~400 mm^3), animals were injected i.p. with either vehicle +0.5 mg topotecan or 10 mg Ko143+0.5 mg topotecan combination therapy per kg body weight on days 0–4 and 14–18. If the tumor volume shrank to less than 50% of the start volume, treatments were stopped until tumors relapsed to 100%. The experiment was terminated when tumors reached a volume of about 1500 mm^3. B, Kaplan-Meier (K-M) survival curves of untreated and combination therapy-treated tumor-bearing

animals. Eight individual tumors were tested, and per tumor one untreated control (blue line), two vehicle + topotecan- (green line) and two Ko143+ topotecan-treated animals (red line) were included. For the vehicle + topotecan group one tumor did not grow out after transplantation. The *P* value was calculated using the Log-rank test. C, Waterfall plots (upper panel) showing tumor volume change (%) after 5 days of combination therapy per individual mouse, with relative volumes normalized to the treatment start volume (i.e. about 200 mm^3). Box and whiskers plots (lower panel) summarizing the waterfall plot data. Lines represent the median response, while the whiskers show the maximum and minimum values. The *P* values was calculated using the Mann Whitney test.
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donor tumors in ABCG2-deficient hosts (Fig. 1A–C). When tumors reached a volume of 150–250 mm^3 after transplantation, animals were either left untreated (control) or injected with topotecan until the tumor volume doubled to about 400 mm^3 (Fig. 1A). This topotecan selection step was applied to drive ABCG2 expression in these naïve tumors following orthotopic transplantion, as we have reported previously [5]. As expected, there was a clear induction of ABCG2 in the topotecan-resistant tumors also in the donor tumors used for this study (Fig. 2 and Table S2). Once the tumors had doubled in size under topotecan selection, animals were either treated with vehicle + topotecan or Ko143+ topotecan combination therapy. Compared with vehicle + topotecan, Ko143+ topotecan combination therapy significantly ($P = 0.022$, Log-rank test) increased overall survival of the $Abcg2^{-/-}$ animals that carried $Brca1^{-/-};p53^{-/-}$ mammary tumors (Fig. 1B). Nevertheless, the median survival increased only from 52 to 71.5 days, showing that the benefit of adding Ko143 to topotecan therapy was rather modest. Indeed, when tumor volume changes after 5 days of therapy were plotted per individual mouse (Fig. 1C), no significant ($P = 0.292$, Mann Whitney test) increase in tumor shrinkage was found after addition of Ko143.

Ko143 and Topotecan Pharmacokinetics

To quantify the effect of tumor-specific ABCG2 inhibition on topotecan accumulation, we developed a reverse phase high-performance liquid chromatography (RP-HPLC) assay to determine Ko143 concentrations in plasma and tumor matrix, using FTC as an internal standard (Zander *et al.*, submitted for publication). Ko143 was administered to $Abcg2^{-/-}$ tumor-bearing animals by i.p. injections of 15% (w/v) 2-hydroxyl-propyl-β-cyclodextrine/PBS solutions of concentrated Ko143 DMSO stocks. To validate that this formulation yields effective Ko143 plasma and tumor levels, we performed a time course experiment. This confirmed that Ko143 reaches transplanted mammary tumors after i.p. injection, as reported elsewhere (Zander *et al.*, submitted for publication). In contrast to $Abcb1a/b^{-/-}$ animals, in which Ko143 treatment resulted in a 4-fold increase in plasma topotecan concentration [17], in our $Abcg2^{-/-}$ tumor-bearing animals topotecan pharmacokinetics (PK) did not significantly ($P = 0.870$, unpaired t-test) differ between vehicle + topotecan- (Fig. 3A, green line) and Ko143+ topotecan-treated animals (Fig. 3A, red line). This suggests that Ko143 does not block other ABC transporters which may alter topotecan PK. This result is also consistent with the absence of increased toxicity when topotecan is combined with Ko143 (Fig. S1B).

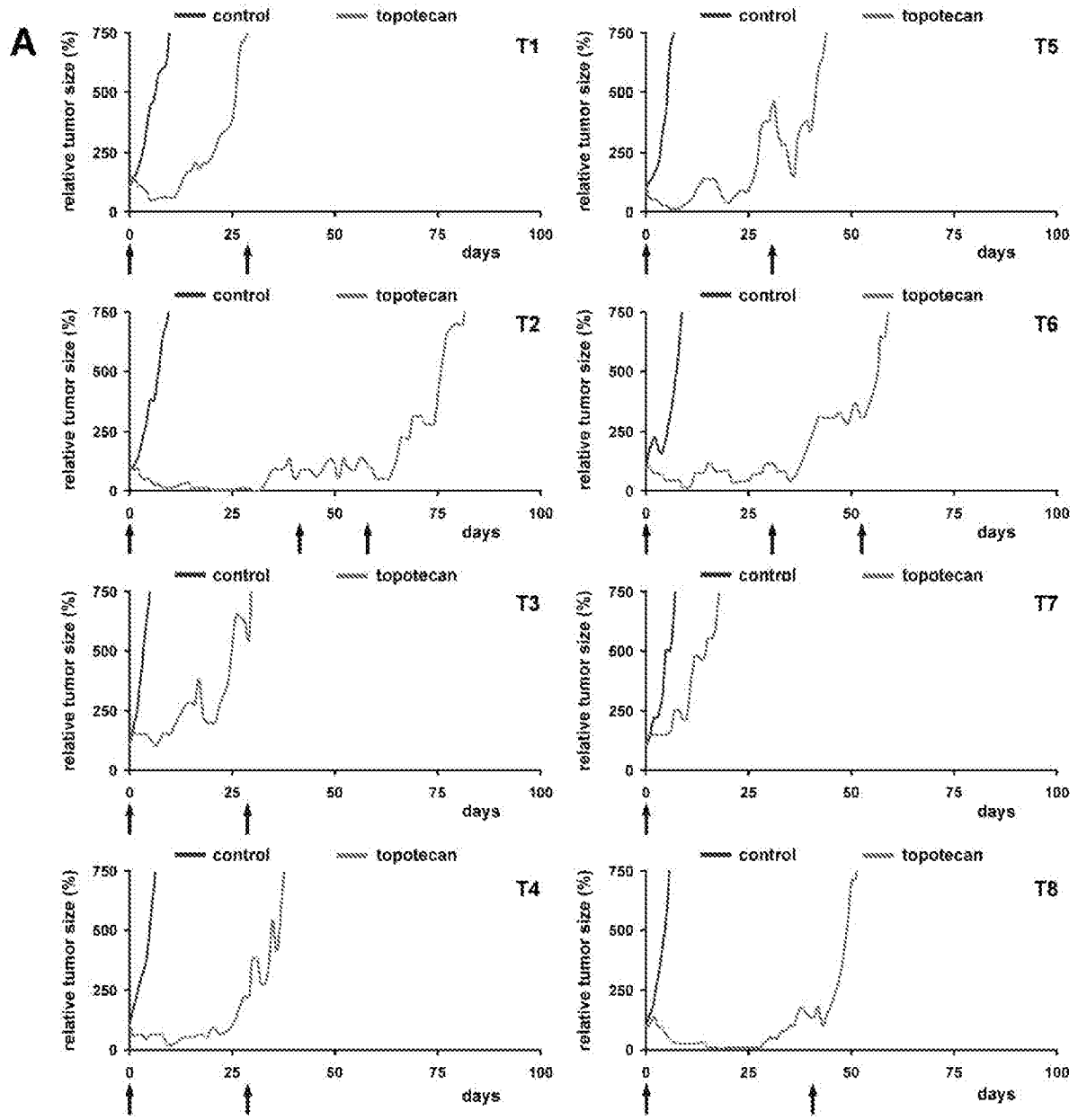
Unexpectedly, when we measured topotecan pharmacokinetics in the ABCG2-positive tumor T1 (Fig. 2C) as described previously [27], we were unable to detect significant differences between the vehicle + topotecan-treated (Fig. 3B, green line) and Ko143+ topotecan-treated animals (Fig. 3B, red line) ($P = 0.713$, unpaired t-test). The short plasma half-life of Ko143 of about 1 hour (Zander *et al.*, submitted for publication) may have contributed to this outcome. Most likely, the increase in topotecan levels within tumors after Ko143 treatment, which must have been responsible for the increased overall survival (Fig. 1B), was rather small. Hence, Ko143 treatment may not be the optimal strategy to increase intratumoral Top1 inhibitor levels.

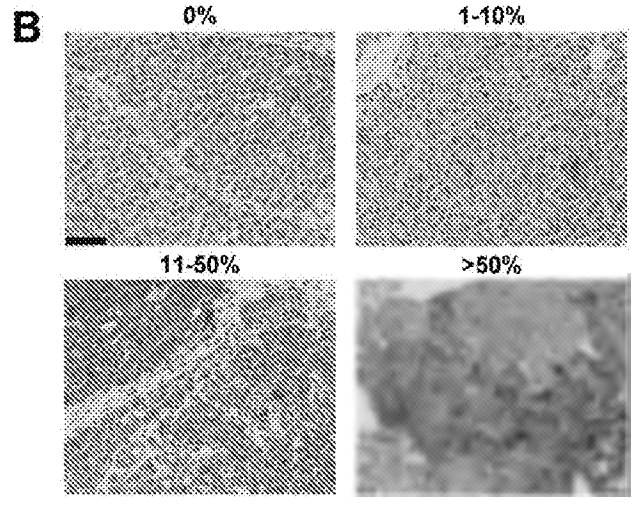
EZN-2208 as Alternative Strategy to Enhance Tumor-specific Drug Accumulation and Reverse ABCG2-mediated Resistance

Given this limited success of the ABCG2 inhibitor Ko143, we tested EZN-2208, a pegylated form of the active irinotecan metabolite SN38 [28], to treat ABCG2-positive $Brca1^{-/-};p53^{-/-}$ mammary tumors. Like topotecan, the topoisomerase I inhibitor SN38 is an ABCG2 substrate [29,30], and therefore drug-naïve tumors with ABCG2-positive nests of tumor cells should quickly acquire resistance during irinotecan treatment. We became interested in EZN-2208, since pegylation promotes tumor-specific drug accumulation by exploiting the phenomenon known as the enhanced permeability and retention effect of solid tumors [31]. Sustained SN38 release from EZN-2208 in the tumor stroma may counteract cellular ABCG2-mediated efflux and maintain a steep diffusion gradient towards the topoisomerase I target within the tumor cell nuclei. Such prolonged tumor-specific drug delivery may help to overcome ABCG2-mediated resistance.

For our analysis, we compared EZN-2208 versus topotecan or irinotecan efficacy in drug-naïve $Brca1^{-/-};p53^{-/-}$ mammary tumors that showed ABCG2 expression in tumor cell subpopulations. In several spontaneous tumors (T1, T3, T4, T7) we observed varying amounts of ABCG2-positive nests of tumor cells (Fig. 2B+C). Obviously, these cells were selected during topotecan treatment, since there is a clear enrichment of ABCG2-positive cells in the resistant tumors (Fig. 2C). Moreover, these tumors acquire topotecan resistance more rapidly (Fig. 2A). In addition to T1 and T7, we found three other spontaneous tumors (named here T9, T10 and T11) with more than 10% ABCG2-positive tumors cells in our panel of 28 $Brca1^{-/-};p53^{-/-}$ mammary tumors derived from FVB/N mice (Zander *et al.*, submitted for publication). Importantly, ABCG2-positive cells were still present after orthotopic transplantation of all 5 donor tumors (Table S1).

Before drug interventions were started using these tumors, we established the maximum tolerable EZN-2208 dose in our mouse model, guided by earlier studies in xenograft models [23]. When EZN-2208 was injected i.v. at 10 mg (SN38 equivalents) per kg body weight on days 0, 2, 4, 6 and 8, the weight loss was still acceptable (about 10%, Fig. S2A). The MTD of topotecan and irinotecan was 4 mg and 40 mg per kg body weight, respectively, using this regimen of five consecutive i.v. injections (Fig. S2B). These doses were then given to mice carrying the orthotopically transplanted ABCG2-positive $Brca1^{-/-};p53^{-/-}$ mammary tumors T1, T7, T9, T10 and T11 (Fig. 4). Compared with both the irinotecan- (pink line) and topotecan-treated tumor-bearing animals (light blue line), we found a dramatic increase in survival until a tumor volume of about 1500 mm^3 was reached after EZN-2208 treatment (Fig. 4A, green line, $P<0.001$, Log-rank test). This result was found for all five ABCG2-positive tumors individually (Fig. S3). Moreover, when tumor volume changes after two weeks of treatment were compared (Fig. 4B+C), EZN-2208 outperformed both irinotecan ($P = 0.013$, Mann Whitney test) and topotecan ($P<0.001$, Mann Whitney test). This was still the case





C

tumor	spon	control	topotecan
T1	11-50%	>50%	>50%
T2	0%	0%	11-50%
T3	1-10%	1-10%	>50%
T4	1-10%	0%	>50%
T5	0%	0%	11-50%
T6	0%	1-10%	11-50%
T7	11-50%	>50%	>50%
T8	0%	0%	>50%
P value	-	0.865	0.020

Figure 2. Topotecan response and ABCG2 immunoreactivity of eight individual *Brca1*$^{-/-}$;*p53*$^{-/-}$ mammary tumors. A, Relative tumor volume (%) of matched control (blue lines) and topotecan-treated (green lines) tumors over time. Each arrow indicates one dosing regimen of i.p. injections of 0.5 mg topotecan per kg body weight on days 0–4 and 14–18. B, Semi-quantification of ABCG2 immunoreactivity. Representative micrographs of four classes of stained tumor cells (0%, 1–10%, 11–50% and more than 50% of counted cells in 10 independent 400x magnification fields are ABCG2-positive) are shown. C, Table indicating ABCG2 immunoreactivity of the spontaneous (spon), untreated control (control) and topotecan-treated samples per individual tumor. *P* values of the spon - control (0.865) and spon - topotecan (0.020) comparisons were calculated using the Wilcoxon rank-sum test.
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for topotecan, but not for irinotecan when tumor volume changes were analysed per individual ABCG2-positive tumor (Fig. S4). Together, these data show that the use of EZN-2208 is an effective strategy to target ABCG2-positive *Brca1*$^{-/-}$;*p53*$^{-/-}$ mammary tumors.

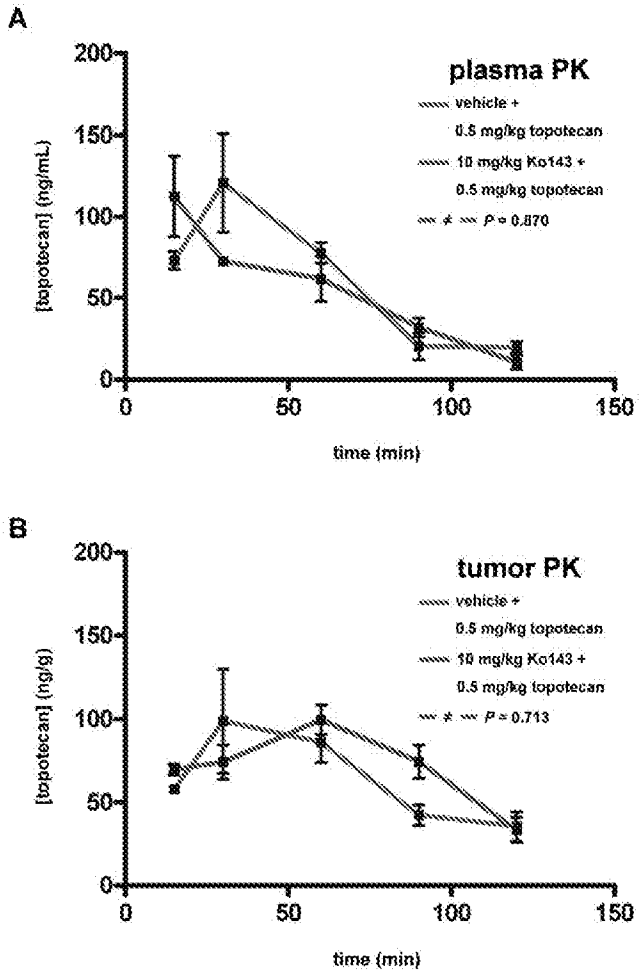

Figure 3. Plasma and tumor topotecan pharmacokinetics (PK). A, Plasma topotecan PK. *Abcg2*$^{-/-}$ animals, carrying ABCG2-positive tumor T1 (Fig. 2C), were treated with either vehicle +0.5 mg topotecan (green line) or 10 mg Ko143+0.5 mg topotecan (red line) per kg body weight and plasma was collected at 15, 30, 60, 90 and 120 minutes following i.p. injection. Error bars indicate standard deviations of the mean of at least 3 animals per time point. B, Tumor topotecan PK. The tumors of the animals in A were harvested and homogenized to determine topotecan concentrations at the same time points. Error bars indicate standard deviations of the mean of at least 3 animals per time point. *P* values were calculated using the unpaired t-test.
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Discussion

Using a genetically engineered mouse model for BRCA1-deficient breast cancer, we investigated therapeutic strategies to overcome ABCG2-mediated resistance to clinically used topoisomerase I inhibitors. We found that ABCG2-positive *Brca1*$^{-/-}$;*p53*$^{-/-}$ mammary tumors are highly sensitive to EZN-2208, a pegylated SN38 compound. Compared with the MTD of irinotecan or topotecan, ABCG2-positive tumors shrank substantially in size and the time until relapse was greatly increased. Previously, EZN-2208 was also shown to be superior compared with irinotecan in xenograft models of neuroblastoma, B-cell non-Hodgkin's lymphoma, breast, colon and pancreatic cancer [22–24]. Polyethylene glycol (PEG)-conjugation of the active irinotecan metabolite SN38 generates a water soluble camptothecin analogue [28] with enhanced pharmacokinetics profile as shown by Sapra *et al.* [23] in plasma and tumors of MX-1 breast cancer xenografted nude mice. Pegylation shields a conjugated compound from clearance by the liver reticulo-endothelial system and impairs glomerular filtration [32,33], resulting in a considerably prolonged plasma half-life compared with irinotecan [23]. Our results suggest that EZN-2208 is capable of overcoming ABCG2-mediated drug resistance *in vivo*. This may be due to better drug delivery towards tumors by the enhanced permeability and retention (EPR) effect, as suggested previously [31,34]. The EPR effect is based on the presence of fenestrated blood vessels in tumors, which allow pegylated molecules to enter the tissue. Lack of effective lymphatic drainage within tumors further increases local drug concentration. The poor penetration of pegylated drug into normal tissues may also explain the absence of increased toxicity despite higher and longer-lasting plasma levels [23]. Local esterase activity within the tumor cleaves off the bulky PEG moiety and releases SN38, which passes the plasma membrane and inhibits nuclear topoisomerase I. ABCG2 is apparently not a sufficiently effective defense mechanism against sustained SN38 delivery by EZN-2208. The PEG-liposome encapsulated formulation of the topoisomerase II inhibitor doxorubicin (Doxil or Caelyx) [35] also showed superior efficacy in preclinical mouse models compared with free doxorubicin [36,37], and was also found to bypass P-glycoprotein-mediated drug efflux [38].

We show here that adding the specific ABCG2 inhibitor Ko143 to topotecan therapy increases the overall survival of animals carrying *Brca1*$^{-/-}$;*p53*$^{-/-}$ mammary tumors. This is not unexpected, given our recent finding that the survival of topotecan-treated animals carrying ABCG2-deficient *Brca1*$^{-/-}$;*p53*$^{-/-}$ mammary tumors is markedly increased over that of animals with ABCG2-proficient tumors. By using ABCG2-proficient tumors in ABCG2-deficient hosts, we ensured that the Ko143 effect was indeed tumor-specific and not due to drug clearance differences. Despite the increase in overall survival, the actual benefit of Ko143 was rather modest. Moreover, we failed to detect increased topotecan accumulation in these tumors. We have shown that dosing animals with 10 mg Ko143 per kg body weight resulted in tumor Ko143 levels of more than 250 ng/g for at least two hours following i.p. injection (Zander *et al.*, submitted for publication). Such levels are well above the EC_{90} concentration of 25 nM as

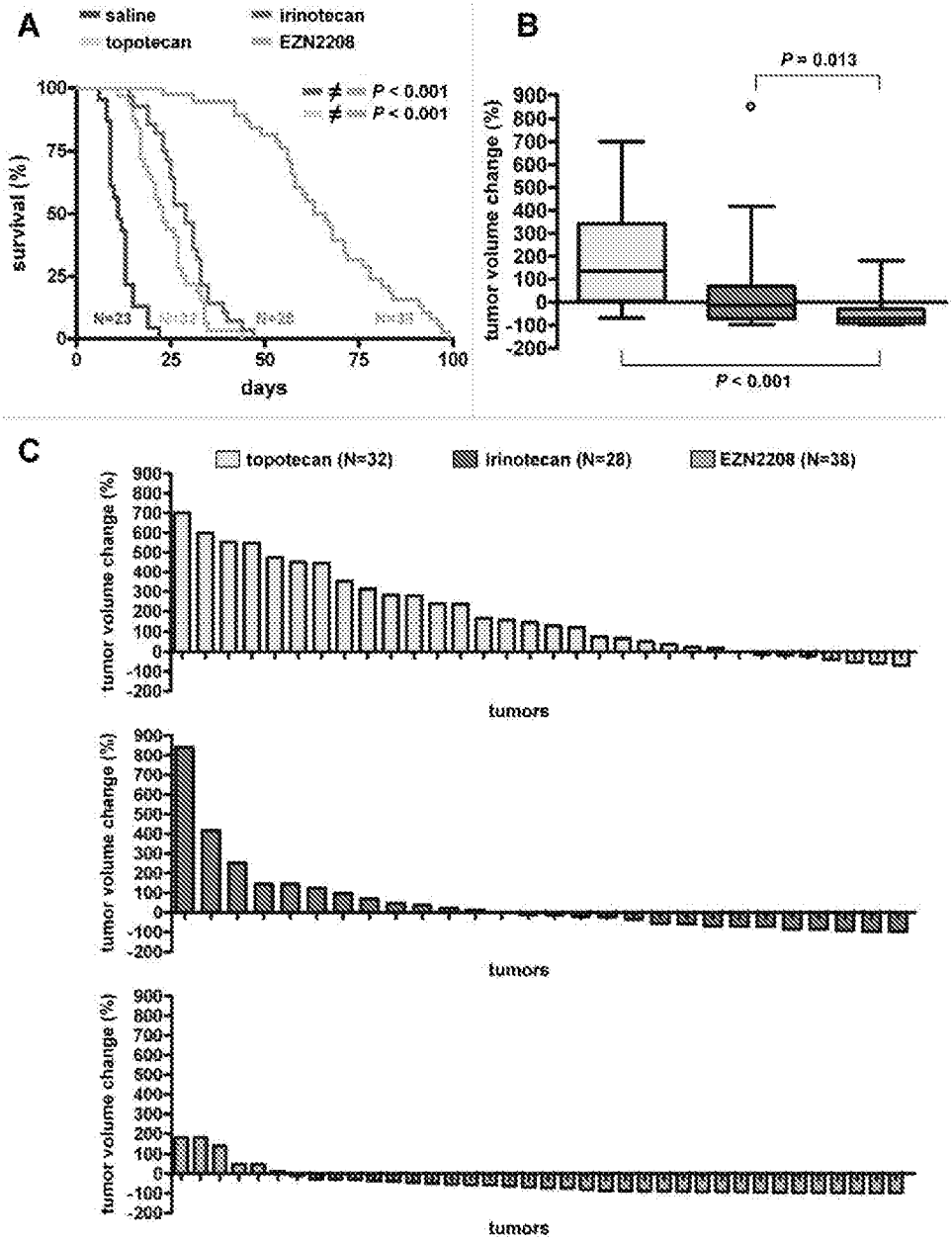

Figure 4. Efficacy of topotecan, irinotecan and EZN-2208 in five ABCG2-positive *Brca1*$^{-/-}$;*p53*$^{-/-}$ mammary tumors. A, K–M curves showing survival (%) until a tumor volume of about 1500 mm^3 was reached after one regimen of five consecutive i.v. injections on days 0, 2, 4, 6 and 8 of saline- (dark blue line, N = 23), topotecan- (light blue line, 4 mg i.v., N = 32), irinotecan- (pink line, 40 mg i.v., N = 28) and EZN-2208-treatments (green line, 10 mg SN38 equivalents i.v., N = 38) per kg body weight. *P* values were calculated using the Log-rank test. B, Box and whiskers plots indicating per drug the tumor volume change (%) after two weeks of treatment. Lines represent the median response, while the whiskers show the maximum and minimum values; the small circle is an outlier. *P* values were calculated using the Mann Whitney test. C, Waterfall plots showing tumor volume change (%) after two weeks of treatment per individual mouse, with relative volumes normalized to the treatment start volume.
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determined by Allen *et al.* [17] in tumor cell cultures with *Abcg2* amplifications [17,39,40]. ABCG2 inhibition by Ko143 in the mouse tumors should therefore be effective during at least this period following administration. However, the differences in tumor topotecan levels were probably small and our RP-HPLC quantification method did not detect them, given the variation between individual animals.

Since the initial report of Ko143 in the literature, several new inhibitors of ABCG2 have been identified [41]. PZ-39, for example, seems to be an attractive candidate, not only inhibiting ABCG2, but also accelerating its lysosome-dependent degradation [42,43]. This two-pronged action may disrupt tumor ABCG2 function in a more profound and longer-lasting way than treatment with Ko143. However, these newer ABCG2 inhibitors are still at an early stage of development and, in contrast to Ko143, toxicity data are lacking.

Since ABC transporters have been shown to play a critical role in chemoresistance of cultured tumor cells, it has been a long-standing goal to use inhibitors of these transporters as a strategy to overcome resistance in cancer patients [44]. However, successful

clinical application of combination therapies has proven to be difficult, often only increasing toxicity of the chemotherapeutic agent without actually improving its efficacy [12,13,45]. Since ABC transporters are also involved in the disposition of most of these drugs, pharmacokinetic interactions may considerably complicate successful application of this therapy strategy in the clinic. To avoid intolerable toxicities, dosing of the cytostatic agents has often to be lowered in such combinations. Indeed, we also found that the topotecan MTD was 4-fold lower in *Abcg2*$^{-/-}$ tumor-bearing animals compared with wild-type animals.

Given these disadvantages, EZN-2208 may be a helpful alternative to the use of ABCG2 inhibitors in combination with topoisomerase inhibitors. Moreover, improved drug delivery to tumors without increased toxicity in other tissues is attractive in combination with other therapeutics that target BRCA-deficient cancer, such as the PARP inhibitor olaparib (AZD2281) [21]. Previously, we treated ABCG2-deficient mammary tumors with topotecan-olaparib combination therapy and observed improved tumor response compared with topotecan monotherapy [5]. However, the topotecan dose had to be lowered 8-fold in this combination, because of the intestinal toxicity. Indeed, dose-limiting haematological toxicity was also reported for patients with advanced solid tumors in phase I clinical trials of the topotecan-olaparib and -ABT-888 combinations [46,47]. Another reason for dose reduction of irinotecan are glucuronidation-impairing UGT1A1 polymorphisms, such as the UGT1A1*28 genotype, which is known to increase the risk of diarrhea [48]. Mild intestinal toxicity was observed in phase I and II trails with EZN-2208, but this was not dose-limiting [49,50]. Combination of olaparib with EZN-2208 may therefore be tolerated better, potentially achieving tumor eradication by dose escalation.

In summary, two different therapeutic strategies were tested to overcome ABCG2-mediated topotecan resistance in *Brca1*$^{-/-}$;*p53*$^{-/-}$ mouse mammary tumors. Although tumor-specific ABCG2 inhibition by Ko143 resulted in improved overall survival of tumor-bearing animals, the benefit was rather modest. In contrast, ABCG2-expressing tumors are highly sensitive to EZN-2208, resulting in a substantial increase in survival. Application of this pegylated SN38 formulation may therefore be a useful clinical approach to bypass ABCG2-mediated resistance to conventional camptothecin analogues in cancers with defects in homology-directed DNA repair.

Supporting Information

Figure S1 Relative animal weights in response to topotecan mono- or Ko143+ topotecan combination therapy. A, Six- to eight-week-old *Abcg2*$^{-/-}$ females were injected i.p. with either 1 (red line), 0.75 (green line) or 0.5 mg topotecan (blue line) per kg body weight on days 0–4 and 14–18 (arrows) and weighed daily for 28 days. The average relative weight (%) of five animals per treatment is plotted and error bars indicate standard deviations. If weight loss approached 20%, animals were killed by CO_2. B, Six- to eight-week-old *Abcg2*$^{-/-}$ females were injected i.p. with 10 mg Ko143 and 0.5 mg topotecan per kg body weight on days 0–4 and 14–18 (arrows). There was a 30 minute interval between Ko143 and topotecan i.p. injections. The average relative weight (%) of five animals is plotted and error bars indicate standard deviations.
(TIFF)

Figure S2 Relative animal weights in response to EZN-2208, irinotecan and topotecan therapy. A, Six- to eight-week-old FVB/N females were treated with one regimen of five consecutive i.v. injections on days 0, 2, 4, 6 and 8 of either saline (black line) or 15 mg (red line) and 10 mg (SN38 equivalents) EZN-2208 (green line) per kg body weight as indicated by the arrows and weighed daily for 28 days. The average relative weight (%) of five animals per treatment is plotted and error bars indicate standard deviations. If weight loss approached 20%, animals were killed by CO_2. B, Six- to eight-week-old FVB/N females were treated with one regimen of five consecutive i.v. injections on days 0, 2, 4, 6 and 8 of either saline (black line) or 4 mg topotecan (light blue line) and 40 mg irinotecan (pink line) per kg body weight as indicated by the arrows. The average relative weight (%) of five animals per treatment is plotted and error bars indicate standard deviations.
(TIFF)

Figure S3 Survival of topotecan-, irinotecan- and EZN-2208-treated animals per individual ABCG2-positive donor tumor. K-M curves showing survival (%) until a tumor volume of about 1500 mm^3 was reached after one regimen of five consecutive i.v. injections on days 0, 2, 4, 6 and 8 of saline- (dark blue lines), topotecan- (light blue lines, 4 mg i.v.), irinotecan- (pink line, 40 mg i.v.) and EZN-2208-treatments (green line, 10 mg SN38 equivalents i.v.) per kg body weight. The number of experimental animals per treatment are indicated next to each K-M curve. *P* values were calculated using the Log-rank test.
(TIFF)

Figure S4 Topotecan, irinotecan and EZN-2208 response per individual ABCG2-positive donor tumor. Waterfall plots showing tumor volume change (%) after two weeks of treatments per individual mouse, with relative volumes normalized to the treatment start volume. Box and whiskers plots summarizing the waterfall plot data. Lines represent the median response, while the whiskers show the maximum and minimum values; the small circles are outliers. *P* values were calculated using the Mann Whitney test.
(TIFF)

Table S1 ABCG2 immunoreactivity of EZN-2208 intervention tumors. In addition to the two ABCG2-positive tumors of the Ko143+ topotecan intervention study (Fig. 2C, T1 and T7), three additional spontaneous (spon) *Brca1*$^{-/-}$;*p53*$^{-/-}$ mammary tumors (T9, T10 and T11) were selected with high ABCG2 expression and orthotopically transplanted into wild-type recipients to test EZN-2208 efficacy. Semi-quantified ABCG2 immunoreactivity of the untreated controls is indicated per individual donor tumor.
(TIFF)

Table S2 ABCG2 immunoreactivity of Ko143 intervention tumors. Semi-quantified ABCG2 immunoreactivity of the untreated controls, vehicle + topotecan- and Ko143+ topotecan-treated animals is indicated per individual donor tumor.
(TIF)

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Author Contributions

Conceived and designed the experiments: SALZ SR PB JJ OvT. Performed the experiments: SALZ WS SR. Analyzed the data: SALZ PB SR. Contributed reagents/materials/analysis tools: LG YZ OvT. Wrote the paper: SALZ SR.

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Poly(ADP-ribose) polymerase and XPF–ERCC1 participate in distinct pathways for the repair of topoisomerase I-induced DNA damage in mammalian cells

Yong-Wei Zhang, Marie Regairaz, Jennifer A. Seiler, Keli K. Agama, James H. Doroshow and Yves Pommier*

Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, National Institute of Health, Bethesda, MD 20892, USA

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ABSTRACT

Poly(ADP-Ribose) (PAR) polymerase (PARP) inhibitors represent a promising class of novel anticancer agents. The present study explores the molecular rationale for combining veliparib (ABT-888) with camptothecin (CPT) and its clinical derivatives, topotecan and irinotecan. ABT-888 inhibited PAR induction by CPT and increased CPT-induced cell killing and histone γH2AX. Increased DNA breaks by ABT-888 were not associated with a corresponding increase of topoisomerase I cleavage complexes and were further increased by inactivation of tyrosyl-DNA phosphodiesterase 1. SiRNA knockdown for the endonuclease *XPF–ERCC1* reduced the ABT-888-induced γH2AX response in non-replicating and replicating cells but enhanced the antiproliferative effect of ABT-888 in CPT-treated cells. Our findings indicate the involvement of XPF–ERCC1 in inducing γH2AX response and repairing topoisomerase I-induced DNA damage as an alternative pathway from PARP and tyrosyl-DNA phosphodiesterase 1.

INTRODUCTION

DNA topoisomerase I (Top1) is the target of clinically approved anticancer agents (topotecan, irinotecan and belotecan) derived from the plant alkaloid camptothecin (CPT) (1–4). It is essential in metazoans for the relaxation of DNA supercoiling generated during transcription and replication. Relaxation proceeds by formation of Top1 cleavage complexes (Top1cc), in which one DNA strand is cleaved by the covalent linkage of Top1 to the 3′-end of a DNA phosphodiester bond [reviewed in (3–6)]. Top1cc are normally very transient. Following DNA relaxation, Top1 is released by religation of the DNA. Top1cc can be stabilized (or 'trapped') under at least three conditions (2): (i) by drugs such as CPTs and non-CPT Top1 inhibitors (3,7); (ii) when the DNA template is altered (2); and (iii) during apoptosis (8). Abnormally stabilized Top1cc can be highly damaging when they interfere with the movement of replication and transcription complexes (9–12). Such collisions convert the Top1cc into DNA double-strand ends (DSE) with Top1 remaining covalently attached to the 3′-end of the broken DNA.

The repair of Top1-associated DNA damage in human cells is not fully understood (2). In budding yeast, two main pathways can remove Top1 adducts: hydrolysis of the Top1–DNA bond by tyrosyl–DNA phosphodiesterase 1 (TDP1) (13–15), and endonucleolytic excision of the Top1cc along with a section of the covalently attached DNA segment by different endonuclease complexes including Rad1–Rad10, Mre11–Rad50–Xrs2, Mus81–Mms4 and Slx1–Slx4 (2,16–18). The redundancy between the TDP1 and the endonuclease pathways has been demonstrated in yeast where inactivation of TDP1 has minimal impact on CPT action unless the Rad1–Rad10 endonuclease is simultaneously inactivated (16–18). Rad1–Rad10 is the heterodimeric ortholog of the human endonuclease XPF–ERCC1 (19). Those endonucleases cleave the duplex DNA segment immediately 5′ from the damaged region where the two DNA strands are separated (3′-flap, splayed arm or bubble) (19). XPF–ERCC1 is also a critical 5′-endonuclease in nucleotide excision repair (NER) both for global and transcription-coupled repair (TCR) (20).

A role for poly(ADP-ribose) polymerase (PARP) in the repair of Top1-associated DNA damage is

*To whom correspondence should be addressed. Tel: +301 496 5944; Fax: +301 402 0752; Email: pommier@nih.gov

well-established. Genetic inactivation of PARP sensitizes mammalian cells to CPT (21–23); PARP inhibitors enhance the effects of CPT and its clinical derivatives both in cell culture (22,24–30) and in xenograft systems (29,30). However, the molecular mechanisms by which PARP acts in the repair of Top1-induced DNA damage have not been elucidated and yeast cannot be used because the PARP pathway is not present in yeast cells. In mammalian cells, PARP inhibitors increase DNA breaks in response to Top1cc (22,24,27) but without concomitant increase in Top1–DNA complexes (27). PARP inactivation is associated with Tdp1 deficiency (2) and with toxic interference of Ku and DNA-PK in the homologous recombination (HR) pathway (23), which is critical for the repair of Top1cc (17,23,31–36).

The purpose of the present study is to elucidate the molecular mechanisms involved in the sensitization of cancer cells to CPT by PARP inhibitors. For this purpose, we used veliparib (ABT-888), one of the leading PARP inhibitors in clinical development (37,38). ABT-888 is a benzimidazole derivative with high potency against both PARP-1 and PARP-2 enzymes (Ki = 5 nM) (28). ABT-888 is orally bioavailable (39) and in clinical trials in combination with temozolomide, cyclophosphamide, platinum derivatives (*cis*-platin and oxaliplatin), mitomycin, radiation therapy and CPT derivatives (irinotecan and topotecan) (http://clinicaltrialsfeeds.org/clinical-trials/results/term=Drug:+veliparib+(ABT-888)?recr=Open). Our study examines the molecular effects of ABT-888 on CPT-induced cytotoxicity and γH2AX response, and the role of XPF–ERCC1 in the repair of Top1cc-induced DNA damage, which is relevant to the ongoing clinical trials combining ABT-888 with CPT derivatives.

MATERIALS AND METHODS

Cell lines and drugs

Human U2OS osteosarcoma cells and human HT29 colon carcinoma cells were obtained from the Developmental Therapeutics Program (DTP, National Cancer Institute, Bethesda, MD, USA) and maintained in RPMI 1640 medium complemented with 10% fetal bovine serum (FBS) at 37°C, 5% CO_2. Human peripheral lymphocytes were obtained from the Blood Bank at the National Institutes of Health and maintained in RPMI 1640 supplemented with 10% FBS. CPT, veliparib (ABT-888, Abbott Laboratories, Abbott Park, IL, USA) and flavopiridol (FLV) were obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment (National Cancer Institute, Bethesda, MD, USA). 5,6-dichlorobenzimidazole 1-β-D-ribofuranoside (DRB) and the proteasome inhibitor, MG-132 were obtained from Sigma (St Louis, MO, USA). $TDP1^{+/+}$ and $TDP1^{-/-}$ murine embryonic fibroblasts (MEFs) (40) were a kind gift from Dr. Cornelius F. Boerkoel (Center for Molecular Medicine and Therapeutics; University of British Columbia, Canada).

Cytotoxicity assay

The ATPlite assay (PerkinElmer Life Sciences, Waltham, MA, USA) was used to determine the cytotoxicity of CPT in the absence or presence of ABT-888. The ATP level in untreated cells was defined as 100%. Survival of treated cells was defined as $ATP_{\text{treated cells}}/ATP_{\text{untreated cells}} \times 100$.

Western blotting

Proteins were detected by western blotting with corresponding specific primary antibodies. XPF monoclonal antibody (Ab-1) was purchased from Lab Vision, Thermo Fisher Scientific, Fremont, CA, USA, ERCC1 monoclonal antibody (D-10) from Santa Cruz Biotechnology, Santa Cruz, CA, β-actin monoclonal antibody (A5441) from Sigma, anti-phosphorylated-H2AX (γH2AX, clone JBW301) from Upstate Biotech, Millipore, Billerica, NY, USA and GAPDH monoclonal antibody (14C10) from Cell signaling Technology, Danvers, MA, USA. The figures show representative data that were reproducible in separate experiments.

COMET assays

DNA double-strand breaks (DSBs) were evaluated using the neutral single cell gel electrophoresis (neutral COMET assay). The COMET assays were performed according to the manufacturer's instructions (Trevigen, Gaithersburg, MD, USA). Data are expressed as mean ± SD.

Immunofluorescence assays

Cells plated in 4-well chamber slides (Nalgene Nunc International, Rochester, NY, USA) and cytospins of human lymphocytes were processed for immunofluorescence microscopy as described (41). For the simultaneous detection of γ-H2AX and replication foci, cells were labeled with 30 μmol/l 5-ethenyl-2′-deoxyuridine (EdU, Invitrogen, Carlsbad, CA, USA) for 90 min. During the last 30 min, cells were treated with CPT in the absence or presence of ABT-888. Following treatment, the medium was aspirated out and the cells were washed in phosphate buffer saline (PBS). Cells were immediately fixed and permeabilized by a 20-min incubation at room temperature with 4% paraformaldehyde and an overnight incubation in ice-cold 70% ethanol at 4°C. The staining for γH2AX was done first as described (41). EdU staining was then done with the Click-iT EdU Alexa Fluor® 647 flow cytometry assay kit from Invitrogen following the manufacturer's instructions. The primary antibody for γH2AX (clone JBW301) was from Upstate (Millipore). The anti-PAR polymer rabbit polyclonal antibody was the product of Trevigen (Gaithersburg, MD, USA). The anti-53BP1 antibody (NB100-904) was from Novus Biologicals (Littleton, CO, USA).

γH2AX flow cytometry

Human lymphocytes were processed for flow cytometry as described (12) using the anti-γH2AX antibody (ab11174) from Abcam (Cambridge, MA, USA) and a Becton Dickinson FACScan flow cytometer (BD Biosciences, San Jose, CA, USA). Percentages of γH2AX positive

cells were determined using CellQuest software (BD Biosciences).

Alkaline elution assay

DNA damage was detected using alkaline elution assays as described earlier (42,43). Briefly, cells were radiolabeled with [^{3}H]-thymidine (1.0 μCi/ml) for 72 h and chased overnight (16 h) with radioisotope-free medium before receiving drug treatments. Cells were treated as indicated; after which, they were harvested by scraping into ice-cold Hank's balanced salt solution (HBSS). Total DNA breaks [DNA strand breaks (SB)] were detected using DNA-denaturing conditions (pH 12.1) under deproteinizing conditions. To assess DNA–protein cross-links (DPC), cells were treated as indicated followed by irradiation with 30 Gy to break the DNA. Samples were lysed and evaluated for binding of the protein-crosslinked DNA by its retention on a 0.8 μm polyvinyl chloride/acrylic copolymer. Following alkaline elution, filters were incubated at 65°C with 1 M HCl for 45 min and 0.04 M NaCl was added for an additional 45 min of incubation. Radioactivity in each fraction was measured by liquid scintillation (Packard Instruments, Meridien, CT, USA).

Cellular Top1–DNA complexes detection (ICE Bioassay)

Top1–DNA complexes were detected as described earlier (43). Briefly, cells were pelleted and immediately lysed in 1% sarkosyl after drug treatment. Following homogenization with a Dounce homogenizer and pestle B, cell lysates were gently layered on cesium chloride step gradients and centrifuged at 165 000*g* for 20 h at 20°C. Half-milliliter fractions were collected and the fractions 6–10 were pooled together. The pooled fractions were then diluted with 25 mmol/l sodium phosphate buffer (pH 6.5) to make a 1×, 2×, 4×, or 8× scaled dilution for better resolution of differences and applied to Immobilon-P membranes (Millipore) in a slot-blot vacuum manifold. Top1–DNA complexes were detected using the C21 Top1 monoclonal antibody (gift from Yung-Chi Cheng, Yale University, New Haven, CT, USA) using standard western blotting procedures.

siRNA transfection

Gene-specific siRNAs for XPF (L-019946-00) or ERCC1 (L-006311-00) were products of Dharmacon (Lafayette, CO, USA). About 50 nM siRNAs were transfected to U2OS cells with Dharmafect transfection reagent (Dharmacon) for 48 h according to manufacturer's instructions. Then culture medium was removed and cells were treated with CPT in the absence or presence of ABT-888. Cells transfected with negative control siRNA (D-001810-10, Dharmacon) were used as control.

Clonogenic assay

After drug treatment, cells were plated at a density of 100, 1000 and 10 000 per well in six-well plates and incubated for 10 days to allow formation of colonies. Cells were fixed with methanol, stained with 0.1% crystal violet (Sigma) for 30 min and washed with distilled water. Colonies were counted after air drying. Plating efficiency (PE) was defined as the number of colonies counted/the number of cells seeded. The survival fraction (SF) of untreated negative siRNA-transfected cells was defined as 100. SF were calculated as: $PE_{\text{treated}}/PE_{\text{untreated}} \times 100$.

Statistical analyses

The data are represented as mean ± SD or mean ± SEM. The significance of differences between means was assessed by the Student's *t*-test, with $P < 0.05$ being considered statistically significant. Three-way ANOVA tests were performed to compare the difference between γH2AX levels in individual CPT-treated cells in the presence or absence of ABT-888 (Figure 3 and Supplementary Figure S4A). Four-way ANOVA tests were used to compare the difference of γH2AX enhancement in si-XPF and si-Negative cells (Figure 7 and Supplementary Figure S4B).

RESULTS

Induction of poly(ADP-ribosyl)ation by CPT

First, we tested PAR levels in CPT-treated human cancer cells and the inhibitory effect of ABT-888 on CPT-treated cells. In both human colon cancer HT-29 cells (Figure 1A) and human osteosarcoma U2OS cells (Supplementary Figure S1), PAR polymer levels increased after 30-min CPT treatments. This PAR response was inhibited by ABT-888, which demonstrates rapid PAR induction by Top1 inhibition with CPT and efficient inhibition by ABT-888.

The PARP inhibitor ABT-888 (Veliparib) increases CPT-induced cytotoxicity and DNA breaks without increasing CPT-induced Top1cc

Next, we determined the effect of ABT-888 on the cytotoxicity of CPT by treating HT-29 cells with CPT in the presence or absence of ABT-888. The results shown in Figure 1B demonstrate that ABT-888 potentiates the cytotoxicity of CPT under conditions where ABT-888 (0.5 μM) had no detectable cytotoxicity (data not shown). With 30-min exposure of CPT in the presence of ABT-888, the molecular marker of DNA DSB (44), γH2AX was increased by ABT-888 in CPT-treated cells (Fig.1C). Neutral COMET assays also showed increased CPT-induced DSBs in the presence of ABT-888 (Figure 1D). Upon CPT removal, those DSBs were more persistent than in the absence of ABT-888 (Figure 1D). Taken together, these experiments demonstrate increased DSBs induced by CPT in the presence of the PARP inhibitor ABT-888. They also show enhancement of the CPT-induced γH2AX response.

To investigate the mechanism(s) by which ABT-888 enhances CPT-induced DNA damage, we measured CPT-induced Top1cc as DNA–protein crosslinks (DPC) by alkaline elution (42,43) and by the ICE-bioassay (43). Figure 2A shows a representative experiment in which DPC and DNA strand breaks (SB) were measured in the same cells. Consistent with the COMET assays, ABT-888

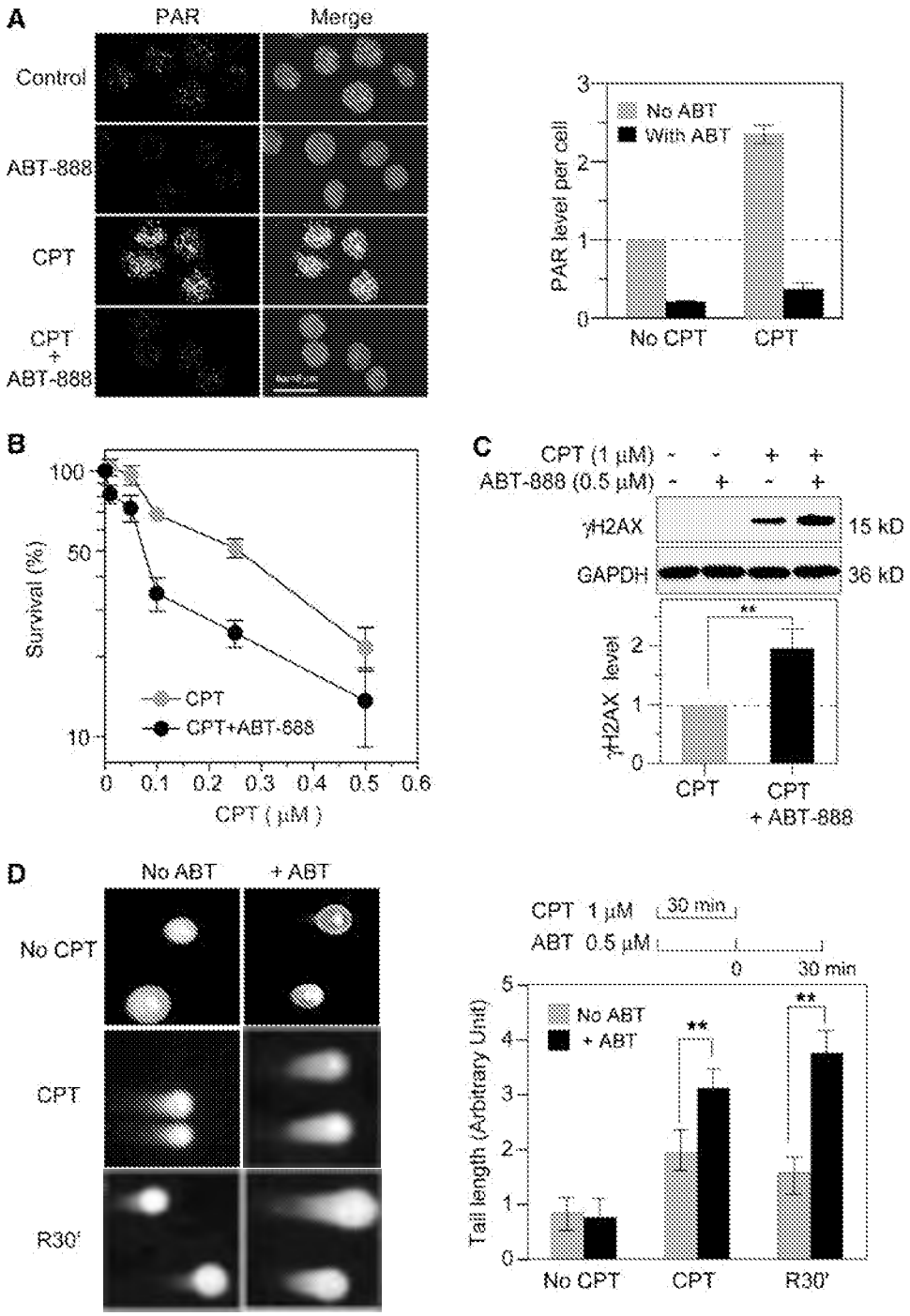

Figure 1. Potentiation of CPT-induced DNA damage by the PARP inhibitor ABT-888 in human colon cancer HT-29 cells. (**A**) Fluorescence images of PAR. Cells were treated with 1 μM CPT for 30 min in the absence or presence of ABT-888 (0.5 μM). Nuclei were labeled with PI (gray signals in the Merge images) and PAR polymers are shown as white dots. Bar = 8 μm. Right, PAR quantitation (mean values ± SD) using untreated Control set as one. (**B**) Cytotoxicity assays. Cells were treated with CPT for 72 h in the absence or presence of ABT-888 (0.5 μM). The survival of untreated cells was defined as 100%. Data are shown as mean values ± SD ($n = 3$). (**C**) Enhancement of CPT-induced γH2AX by ABT-888. Cells were treated with CPT for 30 min in the presence or absence of ABT-888 (0.5 μM). γH2AX was determined by Western blotting: Top: representative experiment; Bottom: γH2AX levels were quantified from three independent experiments (mean values ± SD) using CPT-induced γH2AX levels set as one (**$P < 0.01$). (**D**) DSB measured by neutral COMET assays. Cells were co-treated with CPT and ABT-888 for 30 min; R30′: cells were examined 30 min after CPT removal in the absence or presence of ABT-888 (0.5 μM). Left, representative images; Right top: treatment schedule; Right bottom: quantitation of average tail lengths. At least 50 cells were quantified in each data set. Data are shown as mean values ± SD. Standard *t*-tests were used for statistical analyses; **$P < 0.01$.

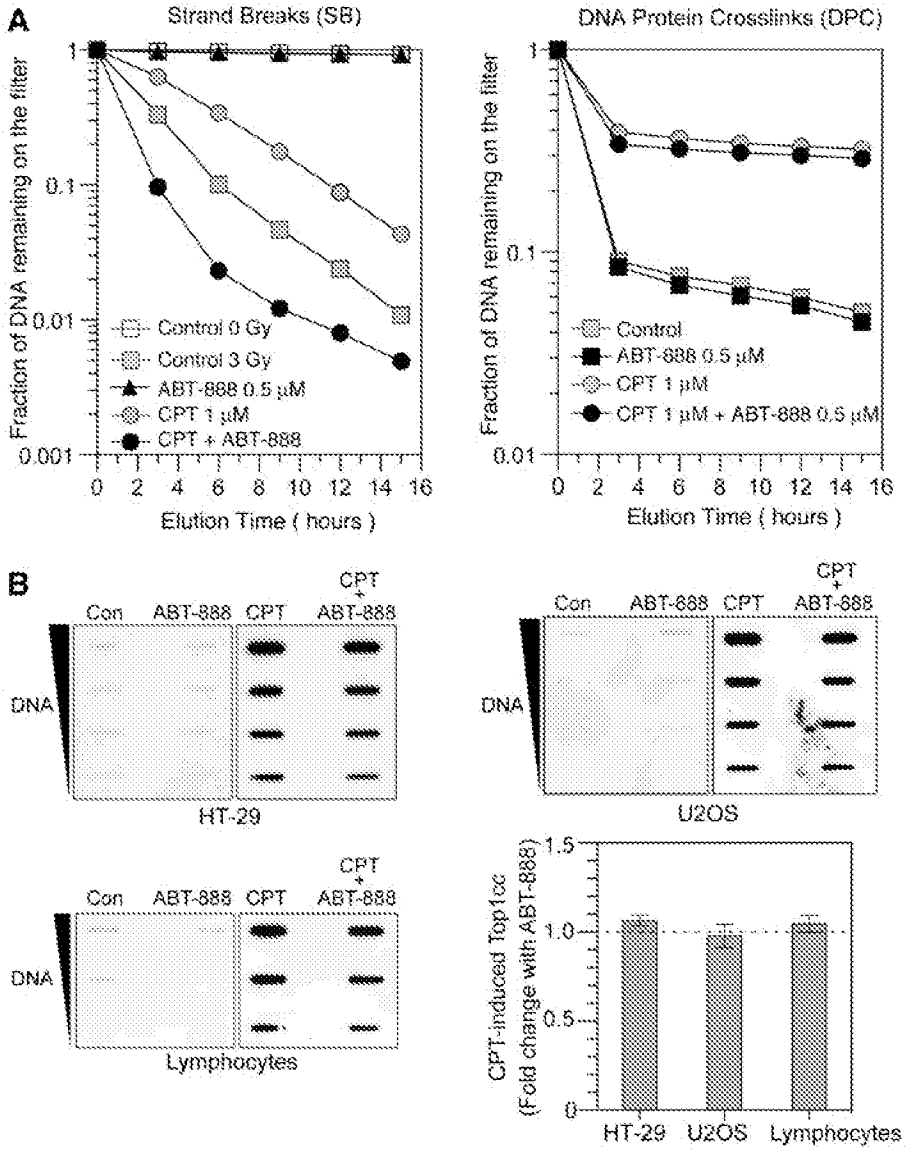

Figure 2. ABT-888 induces DNA breaks without increasing CPT-induced Top1cc. (**A**) Representative alkaline elution assays in HT-29 cells; Left, CPT-induced SB under DNA denaturing conditions. Ionizing radiation (3 Gy) was used as positive control. Cells were exposed to either ionizing radiation or 1 μM CPT in the presence or absence of ABT-888 (0.5 μM), or were left untreated; Right, CPT-induced DPC. Cells were treated with 1 μM CPT for 30 min in the presence or absence of ABT-888 (0.5 μM) followed by irradiation with 30 Gy. DPC were measured by DNA retention on protein-adsorbing filters. (**B**) Top1cc measured by ICE-bioassay in three different cell lines. HT-29 cells (top left) or U2OS cells (top right) were treated with 1 μM CPT for 30 min in the presence or absence of ABT-888 (0.5 μM). Human peripheral lymphocytes require higher drug concentrations to elicit signals and were treated with 20 μM CPT for 2 h with or without ABT-888 (5 μM) (bottom left). Quantitation of the lack of significant effect of ABT-888 on CPT-induced Top1cc in three cell lines (bottom right). For each cell line, fold change was defined as Top1cc (CPT + ABT-888)/Top1cc (CPT alone). Data are mean values ± SD (n = 3).

increased the frequency of DNA SB (Figure 2A, left panel). Noticeably, under these conditions, the DPC remained at the same frequency in the presence of ABT-888 as in its absence (DPC; Figure 2A, right panel). Similarly, ICE-bioassays showed similar levels of Top1cc in the absence and presence of ABT-888 not only in HT29 cells, but also in U2OS osteosarcoma cells, and normal human peripheral lymphocyte cells (Figure 2B). Together these results demonstrate that PARP inhibition by ABT-888 induces the formation of additional DNA breaks in response to CPT without increasing Top1cc.

Enhancement of both replication-dependent and independent γH2AX by ABT-888

Because CPT-induced DNA damage results from both replication (10,11,45,46) and transcription interference (2,8,12,47–49), we tested the relationship between the ABT-888-induced γH2AX and DNA replication. Immunofluorescence microscopy was used to quantitate the γH2AX fluorescent signals in individual cells (Supplementary Figure S2A) (45,50). The distribution of γH2AX levels showed two groups of cells with low and high γH2AX levels, respectively. In the CPT + ABT-888

treated-cells, both peaks were shifted to the right, demonstrating the levels of γH2AX were increased by PARP inhibition in both groups (Supplementary Figure S2B). To clarify whether those two groups were related to DNA replication, EdU incorporation (51) was used to detect the replicating cells and γH2AX co-staining was used to observe the relationship between DNA synthesis and γH2AX induction (52). Figure 3A demonstrates the induction of γH2AX foci both in replicating cells (EdU positive) and in non-replicating cells (EdU negative cells, indicated by arrows). EdU positive cells presented higher levels of γH2AX than EdU negative cells (Figure 3B), suggesting high γH2AX levels correspond to replicating cells. ABT-888 induced more and larger CPT-induced γH2AX foci in both EdU positive (replicating) and EdU negative (non-replicating) cells (Figure 3A and B). These results were reproducible in an independent experiment (Supplementary Figure S4A). ANOVA analysis of the data from two independent experiments indicated significant difference between CPT and CPT + ABT-888 in EdU negative cells ($P < 0.001$), and EdU positive cells ($P < 0.001$). Noticeably, CPT-induced

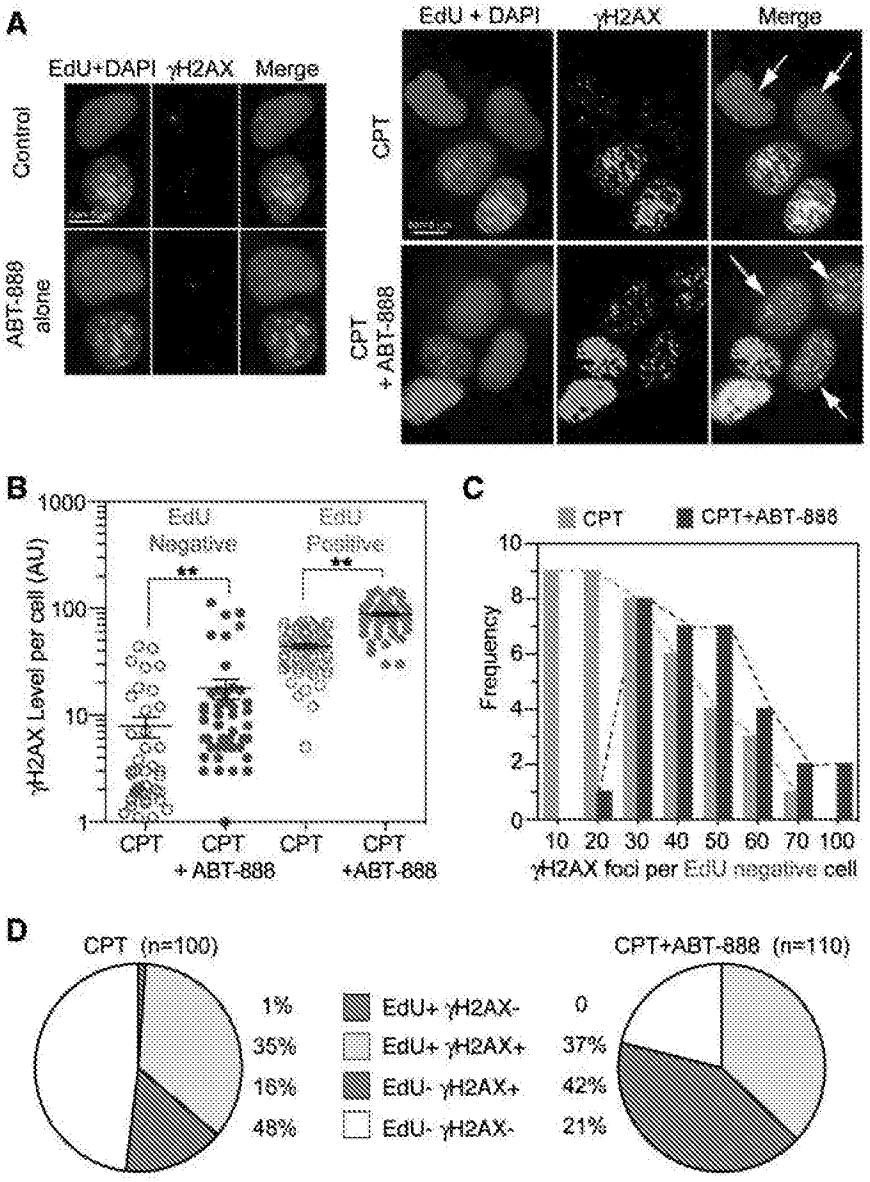

Figure 3. ABT-888 enhances both replication-dependent and independent γH2AX induced by CPT. Human osteosarcoma U2OS cells were treated with CPT (1 μM) for 30 min in the presence or absence of ABT-888 (0.5 μM). (**A**) The fluorescent thymidine analog EdU was used to identify S-phase cells by labeling their DNA (red signal). γH2AX is shown in green signal and nuclei labeled with DAPI are in blue. Representative immunofluorescence images (bar = 5 μm). White arrows indicate EdU negative but γH2AX positive cells. (**B**) Scattered-dot plot derived from the analyses of γH2AX level in individual cells in one representative experiment (see Supplementary Figure S4 for an independent experiment). Mean values ± SEM are shown as black bars. Number of cells analyzed: EdU-/CPT: $n = 67$; EdU+/CPT: $n = 42$; CPT + ABT: $n = 46$ (EdU+), $n = 60$ (EdU-). Standard *t*-tests were used for statistical analyses of the data from the representative experiment, $^{**}P < 0.01$; (**C**) Distribution of γH2AX foci numbers per EdU negative cells in the presence and absence of ABT-888. Dotted curves link the edges of columns (Light blue, CPT alone; Dark blue, CPT + ABT-888). (**D**) Percentage distribution based on EdU and γH2AX showing γH2AX enhancement in EdU negative cells.

γH2AX in the EdU negative cell population was augmented from 16% (16 out of 100 cells) for CPT alone to 42% (46 out of 110 cells) for the combination CPT + ABT-888 (Figure 3C). Moreover, ABT-888 almost doubled the average CPT-induced γH2AX foci per cell (26 ± 16 for CPT alone versus 45 ± 13 for CPT + ABT-888) (Figure 3D). Taken together, those data demonstrate that PARP inhibition enhances CPT-induced γH2AX both in replicating cells and non-replicating cells.

Enhancement of transcription-related γH2AX by ABT-888 in non-replicating cells

To further elucidate the replication-independent effects of ABT-888, we tested human peripheral lymphocytes treated with CPT, which we previously reported induce transcription-induced DSBs in response to CPT (12,49). Immunofluorescence imaging of γH2AX and analyses of the number of γH2AX foci, showed a significant increase in γH2AX foci in the presence of ABT-888 (5.1 ± 3.2 foci/cell versus 1.3 ± 1.2 foci/cell for CPT alone) (Figure 4A). Those γH2AX foci were co-localized with p53 binding protein 1 (53BP1), consistent with the formation of DSBs (Figure 4B). Flow cytometry also showed ~2-fold increased γH2AX in the presence of ABT-888 (Figure 4C). These data, together with those obtained in Figure 3 indicate that PARP inhibition enhances both CPT-induced replication-dependent and independent DNA damage.

Next we tested whether the effect of ABT-888 in non-replicating cells was transcription-linked. Lymphocytes were pretreated with transcription inhibitors before the addition of CPT with or without ABT-888. The transcription inhibitors DRB and FLV (8,12,49) not only

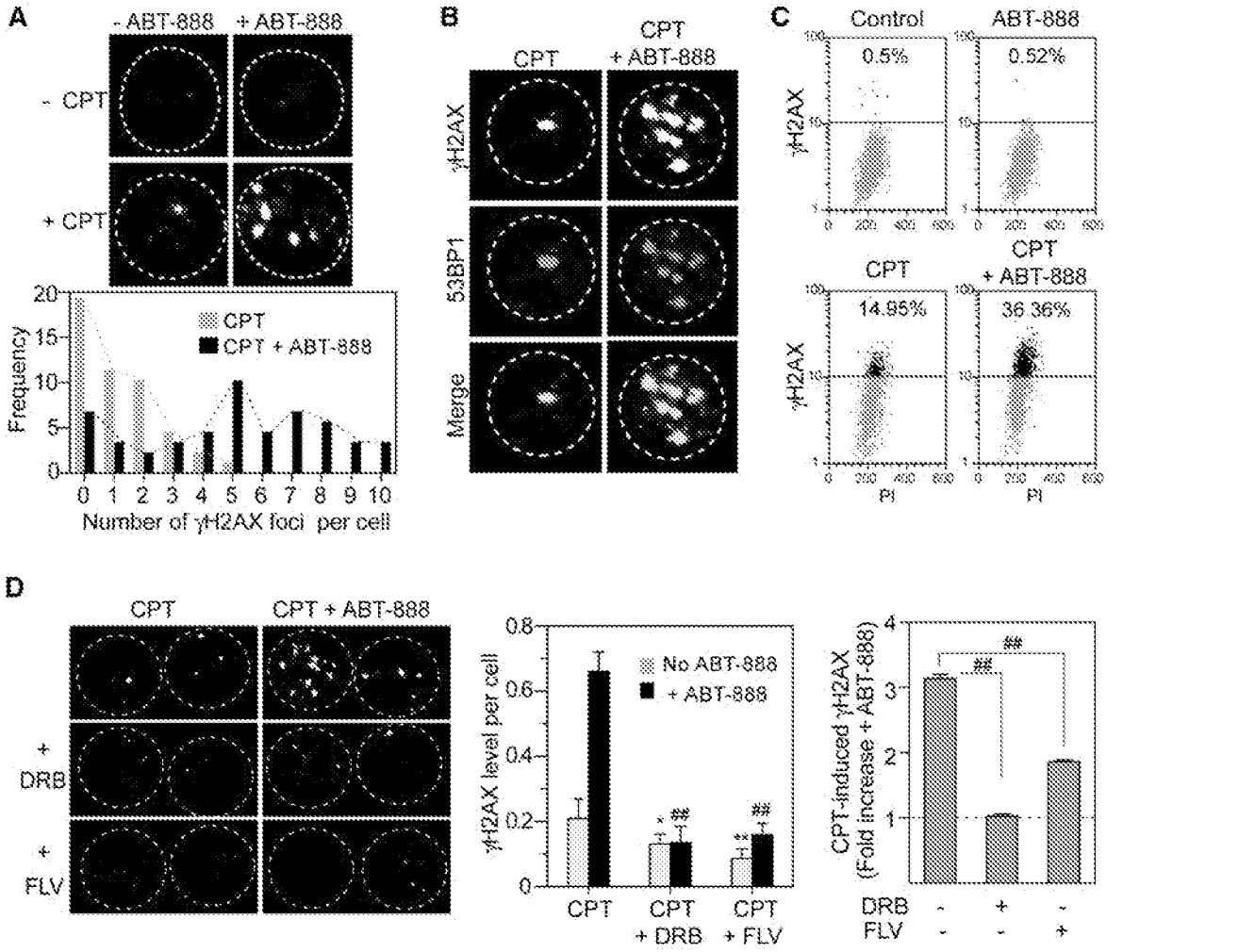

Figure 4. Enhancement of γH2AX by ABT-888 in non-replicating human peripheral blood lymphocytes. Lymphocytes were treated with CPT (20 μM) for 2 h in the absence or presence of ABT-888 (5 μM). (**A**) Top: γH2AX foci induction observed by confocal microscope; Bottom: single cell analysis of the distribution of γH2AX foci numbers. (**B**) Co-localization of γH2AX and 53BP1. (**C**) γH2AX response determined by 2D flow-cytometry. (**D**) Inhibition of the γH2AX enhancement by transcription inhibitors. Lymphocytes were pretreated for 1 h with DRB (100 μM) or FLV (1 μM) before the addition of CPT and ABT-888. Left: representative images of γH2AX foci by confocal microscopy. Middle: average γH2AX level per nucleus. Mean and SD from three experiments are shown. Standard *t*-tests were used for statistical analyses; $*P < 0.05$ versus CPT, $**P < 0.01$ versus CPT, $\#\#P < 0.01$ versus CPT + ABT-888. Right: fold induction of CPT-induced γH2AX by ABT-888. Fold change was defined as γH2AX (CPT + ABT-888)/γH2AX (CPT). $\#\#P < 0.01$.

prevented CPT-induced γH2AX (12), but also reduced the enhancement of the CPT-induced γH2AX by ABT-888 (Figure 4D). These results demonstrate the involvement of PARP in the repair of transcription-linked DNA damage induced by CPT.

PARP is involved in a common repair pathway with TDP1

Because TDP1 represents a major pathway for the repair of Top1cc (2,15), the effects of ABT-888 were examined in TDP1 knockout cells (40). Figure 5 (panels A and B) shows a more intense CPT-induced γH2AX response in the TDP1 knockout cells than the corresponding wild-type cells (53), which is consistent with the defective repair of Top1cc and increased DNA breaks in TDP1-deficient cells (40,43,54,55). Notably, ABT-888 failed to further enhance the γH2AX response in the TDP1$^{-/-}$ cells, which indicates that PARP inhibition no longer enhances Top1-induced DNA damage once TDP1 is inactivated. The effects of ABT-888 on CPT-induced cytotoxicity were also measured in TDP1$^{-/-}$ cells. Figure 5C shows that ABT-888 was unable to potentiate the cytotoxicity of CPT in the TDP1$^{-/-}$ cells. These data suggest that PARP acts in the same pathway as TDP1 for the repair of Top1cc.

Because the repair of Top1cc requires the proteasomal degradation of Top1 following its ubiquitination (46,48), we tested whether the enhancement of γH2AX by ABT-888 was dependent on the proteasome. Cells treated with CPT and ABT-888 in the presence of the proteasome inhibitor, MG-132, failed to induce γH2AX (Supplementary Figure S3) demonstrating that PARP acts downstream from the proteasome for the repair of Top1–DNA complexes.

The XPF–ERCC1 complex is involved in the repair of CPT-induced DNA damage

Based on our finding that ABT-888 induces the formation of DNA breaks in response to CPT (see above), and on the genetic data from yeast showing the importance of the endonuclease Rad1–Rad10 complex as an alternative pathway for the repair of Top1-induced DNA damage ('Introduction' section), we tested the possible implication of the mammalian Rad1–Rad10 ortholog XPF–ERCC1 complex in the repair of CPT-induced DNA damage. Knocking-down *XPF* by siRNA resulted in a marked attenuation of the γH2AX enhancement by ABT-888 (Figure 6A, C and D). Similar results were observed by knocking down the DNA binding partner of *XPF*, *ERCC1* (Figure 6B). Notably, we found that knocking

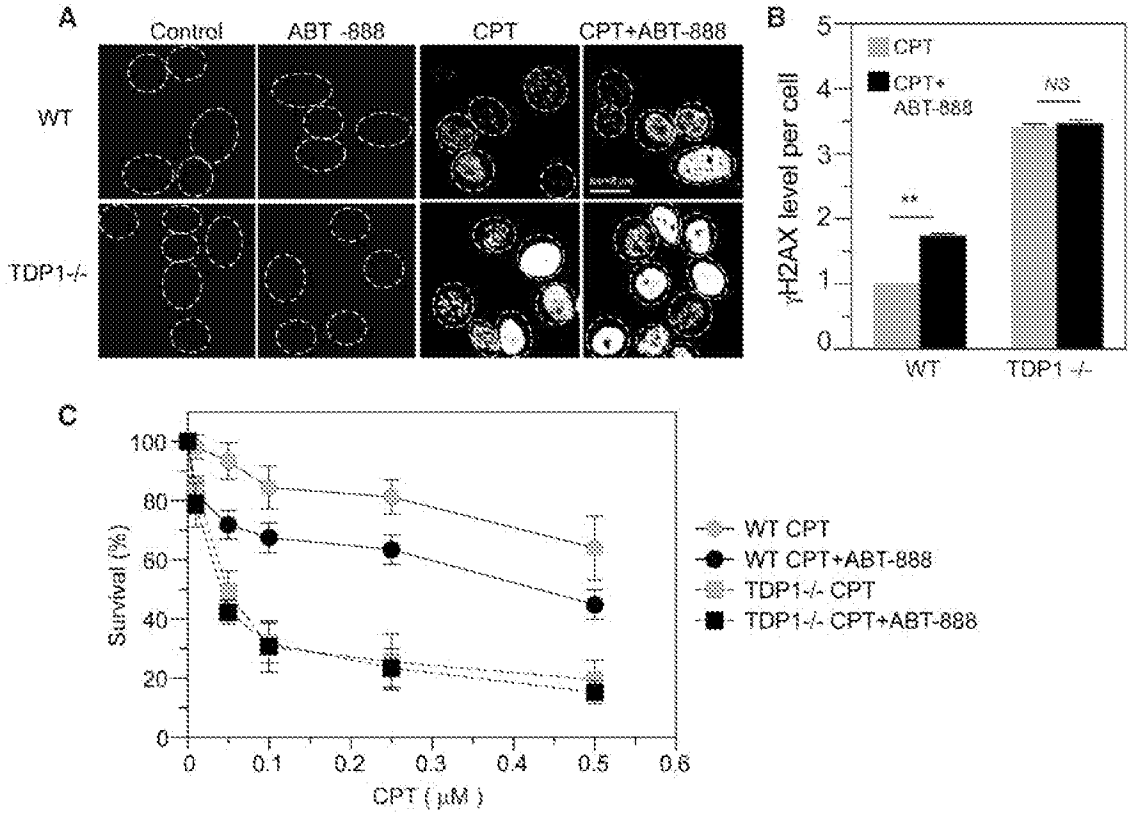

Figure 5. TDP1-dependent enhancement of γH2AX by ABT-888. (**A**) Representative immunofluorescence images of γH2AX in wild-type (WT) MEF or TDP1$^{-/-}$ MEF cells treated with CPT (1 μM) for 30 min with or without ABT-888 (0.5 μM). (**B**) Quantitation of γH2AX levels per cell. The average γH2AX level in CPT-treated WT cells was defined as one. Data are mean values ± SD. Standard *t*-tests were used for statistical analyses; $^{**}P < 0.01$; NS, no significant difference. (**C**) Cytotoxicity of CPT with or without ABT in WT and TDP1$^{-/-}$ cells. MEF cells were treated with CPT for 72 h in the absence or presence of ABT-888 (0.5 μM). The survival of untreated WT cells was defined as 100%. Data are mean values ± SD ($n = 3$ independent experiments).

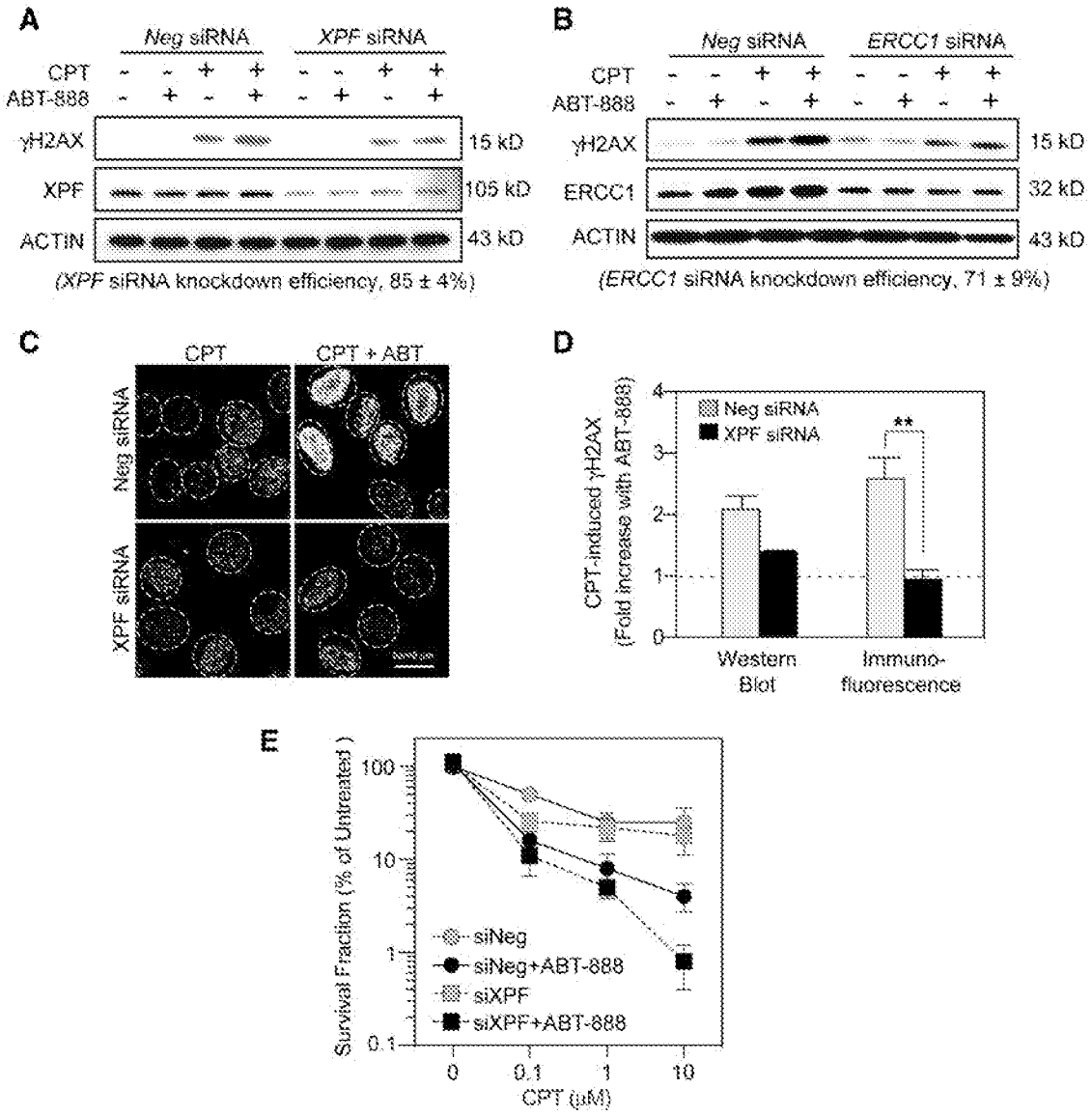

Figure 6. Involvement of XPF–ERCC1 and PARP in CPT-induced γH2AX and cytotoxicity. *XPF* or *ERCC1* siRNAs were transfected into U2OS cells for 48 h before CPT treatment (1 μM for 30 min) in the presence or absence of ABT-888 (0.5 μM). (**A**) Effect of *XPF* knockdown on the γH2AX enhancement by ABT-888. γH2AX levels were detected by western blotting. *XPF* siRNA knockdown efficiency was determined as mean values ± SD ($n = 4$). (**B**) Effect of *ERCC1* knockdown on the γH2AX enhancement by ABT-888. γH2AX levels were detected by western blotting. *ERCC1* siRNA knockdown efficiency was determined as mean values ± SD ($n = 4$). (**C**) Representative γH2AX immunofluorescence images in *XPF* siRNA-transfected cells. (**D**) Fold induction of CPT-induced γH2AX by ABT-888. Results were from western blotting and immunofluorescence assays, respectively. Data are mean values ± SD ($n = 2$ independent experiments for western blotting, $n = 3$ independent experiments for immunofluorescence assay). Standard *t*-tests were used for statistical analyses of immunofluorescence data; $^{**}P < 0.01$). (**E**) Effect of *XPF* knockdown on the survival of cells treated with CPT in the presence or absence of ABT-888. U2OS cells transfected with *XPF* siRNA or negative siRNA for 48 h were treated with CPT for 30 min in the absence or presence of ABT-888 (0.5 μM). Cells were cultured for 10 days following drug removal to allow colony formation. The SF of untreated cells transfected with negative siRNA (Neg siRNA) was defined as 100%. Data are mean values ± SD ($n = 3$ independent experiments).

down XPF also reduced ERCC1 expression and vice versa (data not shown) suggesting the cross-stabilization of XPF and ERCC1. Immunofluorescence microscopy confirmed that *XPF* knockdown blocked the enhancing effect of ABT-888 on the CPT-induced γH2AX formation (Fig. 6C and D). *XPF* knockdown had no effect on the γH2AX level of untreated or ABT-alone-treated cells (data not shown). Together, these experiments demonstrate that the enhancement of CPT-induced γH2AX by PARP inhibition is dependent on XPF–ERCC1.

Clonogenic assays were performed to determine the functional implication of XPF–ERCC1 in the repair of CPT-induced DNA damage in the absence and presence of ABT-888. Figure 6E shows that XPF-inactivation sensitized cells to CPT and that ABT-888 increased the

cytotoxicity of CPT both in the presence or absence of *XPF* (Figure 6E). These results demonstrate that ABT-888 further enhances the killing of XPF–ERCC1-deficient cells in response to CPT.

Involvement of XPF–ERCC1 in the replication-independent γH2AX induced by ABT-888 in CPT-treated cells

Next we wished to determine the γH2AX response as a function of DNA replication in *XPF*-knockdown cells in the absence or presence of ABT-888. As in Figure 3, EdU co-staining was used to differentiate the non-replicating and replicating cells. *XPF* siRNA reduced the γH2AX enhancement by ABT-888 in both cell populations (Figure 7A). Further analyses of γH2AX levels in individual cells also showed that *XPF* siRNA reduced γH2AX levels enhancement both in the EdU negative and EdU positive cells (Figure 7B). Four-way ANOVA analyses of γH2AX data from two independent experiments demonstrated significant difference of γH2AX enhancement between *XPF* siRNA- and Negative siRNA-transfected cells ($P < 0.05$). The dependence on XPF was further analyzed in the EdU negative cells (Fig. 7C). In those cells, *XPF* knockdown significantly attenuated the γH2AX response to ABT-888. Together these results demonstrate the involvement of XPF–ERCC1 both in replication-dependent and independent γH2AX activation by PARP inhibition in CPT-treated human cells.

DISCUSSION

The present study provides new insights into alternative pathways for the repair of Top1-induced DNA damage in human cells and on the rationale for combining Top1 and PARP inhibitors. Our experiments demonstrate that ABT-888 enhances the DNA damaging activities of CPT (cytotoxicity, DNA breaks, γH2AX induction) at concentrations where ABT-888 does not have detectable effects on its own. Our results are consistent with the reported effects of other PARP inhibitors (NU1025, AG14361) in combination with CPT derivatives (25,27) and provide mechanistic insights for the recently initiated clinical trials combining topotecan or irinotecan and veliparib (ABT-888). Our data also suggest the usefulness of γH2AX as a clinical pharmacodynamic biomarker in such combination therapies (44,56).

Although PARP has been reported to directly activate Top1 (57–59), our study shows that ABT-888 increases the cytotoxicity of CPT without affecting the levels of Top1cc (Figure 2). Similarly, another PARP inhibitor, AG14361 was reported to synergize with topotecan without affecting the Top1cc levels (27). Noticeably, our study also shows that PARP inhibition increases the levels of 'frank breaks' (i.e. non-Top1-associated) (Figures 1 and 2), which is in agreement with independent studies with different PARP inhibitors (24,27). Our interpretation is that these breaks correspond to new 'repair lesions' introduced by endonucleases such as XPF–ERCC1 in order to remove the Top1cc and process the damaged DNA into a suitable substrate for HR (Figure 8).

Our data indicate that PARP acts downstream from the proteasome. Indeed, ABT-888 was unable to enhance the γH2AX response to CPT when cells were simultaneously treated with MG-132 (Supplementary Figure S3). Our results along with recent studies (46,48,60,61) place the proteasome as an early effector in the repair of Top1cc (Figure 8). It is plausible that Top1, which is a 100 kDa polypeptide encircling the DNA to which it is covalently bound (5) needs to be proteolyzed for the repair enzymes (including TDP1) to access the broken DNA ends (13,62–64). PARP is also a known cofactor of XRCC1, and XRCC1 is an established repair factor for Top1cc (54,63,65,66). XRCC1 forms repair complexes with TDP1 (53,63,67), and PARP1 knockout cells tend to be deficient in TDP1 activity [Figure 7 in (2)]. Together with our finding that ABT-888 failed to enhance the γH2AX and cytotoxic responses to CPT in TDP1$^{-/-}$ cells (53) (see Figure 5), these results suggest that PARP functions together with TDP1 in a common repair pathway (Figure 8A).

The present study provides the first evidence for a role of XPF–ERCC1 in the repair of Top1-induced DNA damage in human cells (Figure 8). This conclusion is consistent with genetic data in budding yeast where inactivation of Rad1–Rad10 (the yeast XPF–ERCC1 orthologs) sensitizes cells to CPT, especially when TDP1 is also inactivated (17,32) [reviewed in (2)]. Thus, we propose that XPF–ERCC1 functions as an alternative repair pathway besides the PARP-TDP1 pathway in mammalian cells (Figure 8). This can explain why inactivation of XPF–ERCC1 can further increase the cytotoxicity of ABT-888 in CPT-treated cells (Figure 6E). However, because we also found that inactivation of XPF–ERCC1 inhibits the γH2AX response (Figure 6A–D), it is plausible that XPF–ERCC1 repairs Top1cc by cleaving the DNA upstream from the Top1cc and generating 'frank breaks' (see beginning of 'Discussion' section). These breaks could then be responsible for the XPF–ERCC1-dependent activation of γH2AX (Figure 8B).

Our data suggest that XPF–ERCC1 is involved not only in the repair of replication-associated DNA damage but also in the repair of replication-independent DNA damage generated by Top1cc. This adds XPF–ERCC1 to the cellular responses to Top1cc-induced transcription-associated damage in addition to RNA polymerase II hyperphosphorylation, BRCA1-dependent proteolysis of Top1 (8), RNaseH1-dependent DSB induction with ATM activation (12) and altered RNA splicing (68). The increase in DSB and γH2AX by ABT-888 in normal peripheral lymphocytes raises the question as to whether the synergistic effect of PARP inhibitors is selective for cancer cells. The ongoing clinical trials with veliparib in association with topotecan or irinotecan should provide an answer to this question. Finally, our experiments suggest that maximum benefit for combining PARP and Top1 inhibitors might be achieved in tumors with XPF–ERCC1 deficiency.

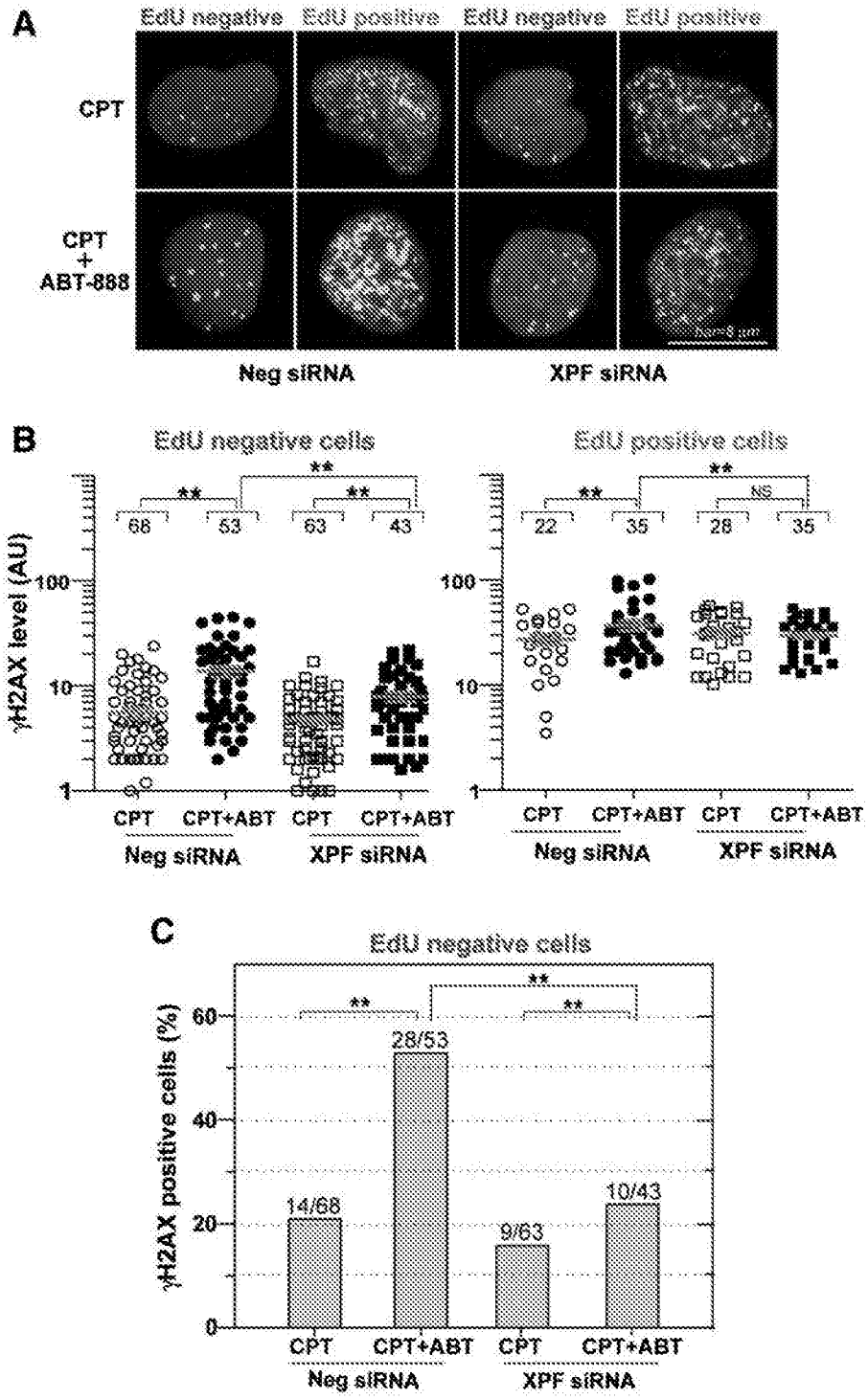

Figure 7. XPF–ERCC1-dependent γH2AX enhancement by ABT-888 in non-replicating cells. U2OS cells were transfected with *XPF* siRNAs or control siRNAs (Neg siRNA). Two days later, the cells were labeled with EdU and treated with CPT (1 μM) for 30 min in the absence or presence of ABT-888 (0.5 μM). Cells were fixed and stained for immunofluorescence assays. (**A**) Representative confocal microscopy images (red signal: EdU; green signal: γH2AX; blue signal: DAPI to stain nuclei); bar = 8 μm. (**B**) Quantitation of γH2AX signals in individual cells (represented as scattered dots) from one representative experiment; Mean values ± SEM are shown as red lines. Numbers above each cluster indicate the number of cells counted. Standard *t*-tests were used for statistical analyses of the data from the representative experiment, $^{**}P < 0.01$; NS, no significant difference. (**C**) XPF-dependent effect of ABT-888 in non-replicating (EdU negative) cells. Percentages of γH2AX-positive cells were scored based on drug treatment and *XPF* knockdown. The number of γH2AX positive cells and total number of cells scored in each group are indicated above each bar.

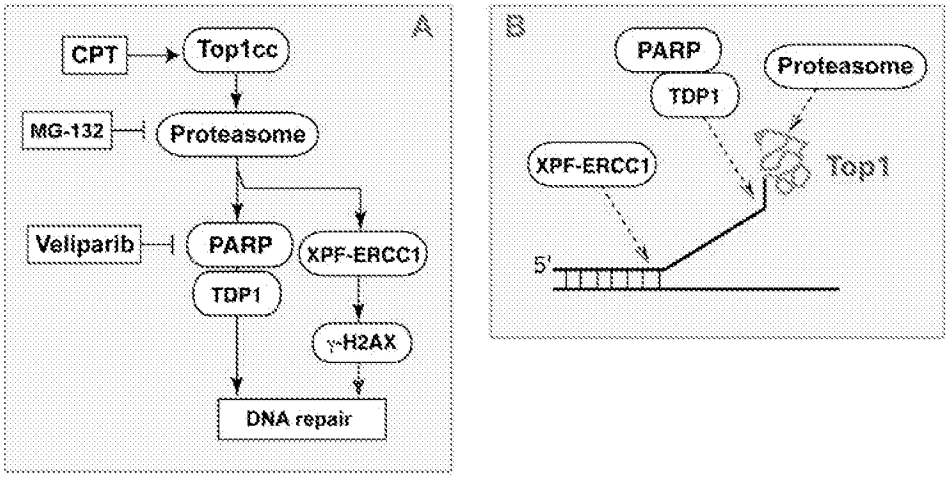

Figure 8. Role of XPF–ERCC1 and PARP in parallel pathways for the repair of Top1cc. (**A**) Scheme illustrating the alternative repair pathways involving the proteasome, PARP, TDP1 and XPF–ERCC1 for the repair of Top1-induced DNA damage. Other alternative pathways involving Mus81–Eme1 and Mre11–Nbs1 and Rad50 (2) are not represented. (**B**) Hypothetical biochemical mechanisms for Top1cc excision by XPF–ERCC1 (endonuclease pathway) and TDP1 (PARP–TDP1 excision pathway).

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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伊立替康与拓扑替康二线治疗43例小细胞肺癌的临床观察

赵明利 毕清 任宏轩 田青 鲍明亮

云南省肿瘤医院(昆明医学院第三附属医院)化疗中心二病区，云南 昆明 650118

[关键词] 伊立替康； 拓扑替康； 小细胞肺癌； 化疗

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目前，肺癌已经成为严重威胁人类健康的常见恶性肿瘤之一，其中小细胞肺癌占原发性支气管肺癌的15%~20%[1]。小细胞肺癌肿瘤细胞倍增时间短的特性决定了其分化程度低、恶性程度高，通常伴有内分泌异常或类癌综合征。由于患者早期即发生血行转移且对放化疗敏感，故小细胞肺癌系全身性疾病已成为不争的事实[1]。虽然小细胞肺癌对放化疗敏感、初治效果尚佳，但一旦复发转移，即获继发性耐药，5年生存率低于初发的非小细胞肺癌。目前，对于复发性小细胞肺癌患者的二线治疗无标准方案，单药伊立替康(CPT-11)及拓扑替康(TPT)被认为是有效的二线治疗方案。本研究回顾性分析本科室应用CPT-11及TPT治疗43例复发性小细胞肺癌的疗效及不良反应。

1 资料和方法

1.1 病例选择

选取2005年1月—2010年1月在本科室治疗的43例复发性小细胞肺癌患者，其中男性30例，女性13例，中位年龄55岁(27~68岁)，所有患者均经组织病理学确诊。患者既往均接受过化疗，采用EP(鬼臼乙叉甙+顺铂)或EC(鬼臼乙叉甙+卡铂)方案治疗4~6个周期，其中16例患者曾接受过放疗；所有患者均为广泛期，ECOG评分≤2，血常规、肝肾功能、心电图无明显化疗禁忌，既往治疗已经结束1个月或以上，有可测量病灶，且预计生存期长于2个月。

1.2 治疗方法

43例小细胞肺癌患者中22例接受CPT-11治疗(CPT-11组)，21例接受TPT治疗(TPT组)，CPT-11组予以CPT-11 300 mg/m^{2}第1天静脉注射，TPT组予以TPT 1.25~1.5 mg/m^{2}第1~5天静脉注射，均以3周为1个周期。两组患者分别予化疗2~6个周期，平均化疗3.4个周期。化疗前均给予5-HT$_3$阻滞剂镇吐治疗，化疗期间必要时予重组人粒细胞集落刺激因子及其他对症治疗。对使用CPT-11出现迟发性腹泻的患者，及时并按时、按量使用盐酸洛哌丁胺治疗，直至最后一次腹泻结束后12 h。两组临床资料见表1。

表1 两组患者临床资料比较

Tab.1 The clinical data between the two groups

Parameter	CPT-11(n=22)	TPT(n=21)	P
Age/year			0.893
Range	27-68	29-65	
Mean	55	53	
Gender			0.097
Male	15	15	
Female	7	6	
The number of cycles	3.5	3.3	0.052
Time to second-line/week	3-130	3.1-130	0.752

1.3 疗效评价标准

参照RECIST关于可测量病灶标准进行评价，疗效分为完全缓解(CR)、部分缓解(PR)、稳定(SD)以及进展(PD)。临床有效率(RR)为CR+PR，疾病控制率(DCR)为CR+PR+SD，疾病进展时间(TTP)为从化疗开始至病情进展的时间；生存时间指从化疗开始至死亡或末次随诊时间。

1.4 统计学处理

运用SPSS 11.5统计软件包，组间率和构成比的比较采用χ^2检验，生存时间和TTP采用Kaplan-Meier法计算，以P<0.05为差异有统计学意义。

2 结 果

2.1 疗效评价

43例患者均可评价疗效，每例接受最少2个周期、最多6个周期的化疗，所有患者共接受化疗134个周期，中位化疗3.1个周期。两组RR及DCR差异无统计学意义(31.2% *vs* 28.6%、72.7% *vs* 71.4%，P>0.05)。CPT-11组中位TTP为12.2周，中位生存期为30周；TPT组中位TTP为12.5周，中位生存期为28.6周，差异无统计学意义(P<0.05，表2)。生存曲线见图1。

2.2 不良反应

两组患者的主要不良反应均为骨髓抑制和消化道反应。TPT组白细胞及血小板减少高于CPT-11组，差异有统计学意义(42.9% *vs* 21.7%、14.3% *vs* 5%，P<0.05)；而延迟

通讯作者：毕清 E-mail:kmbiqing@163.com

表2 CPT-11及TPT治疗小细胞肺癌疗效比较

Tab. 2 The efficacy between irinotecan and topotecan group in treating small cell lung cancer patients

Group	Case	CR	PR	SD	PD	RR/%	DCR/%	mTTP/week	MST/week
CPT-11	22	1	6	9	6	31.2	72.7	12.2	30.0
TPT	21	0	6	9	6	28.6	71.4	12.5	28.6

χ^2=0.285, P=0.593.

表3 CPT-11及TPT治疗小细胞肺癌的不良反应比较

Tab. 3 The toxicity between irinotecan and topotecan in treating small cell lung cancer patients

[n(%)]

Toxicity	Grade					P
	0	Ⅰ	Ⅱ	Ⅲ	Ⅳ	
Leucocytopenia						
CPT-11 group	2(9)	5(22.8)	10(45.5)	3(13.7)	2(9)	0.042
TPT group	1(4.6)	5(23.9)	6(28.6)	5(23.9)	4(19)	
Anemia						
CPT-11 group	17(78)	5(22)	0(0)	0(0)	0(0)	0.808
TPT group	17(80)	4(20)	0(0)	0(0)	0(0)	
Thrombocytopena						
CPT-11 group	17(78)	3(13.7)	1(5)	1(5)	0(0)	0.039
TPT group	13(61.9)	3(14.2)	29(9.6)	2(9.6)	1(4.7)	
Nause and vomitting						
CPT-11 group	7(31.8)	8(36.3)	6(27.3)	1(4.5)	0(0)	0.930
TPT group	6(28.6)	8(38.1)	6(28.6)	1(4.7)	0(0)	
Acute diarrhea						
CPT-11 group	18(81.8)	4(18.2)	0(0)	0(0)	0(0)	0.680
TPT group	20(95.3)	1(4.7)	0(0)	0(0)	0(0)	
Tardily diarrhea						
CPT-11 group	14(63.6)	4(18.2)	3(13.7)	1(4.5)	0(0)	0.025
TPT group	20(95.2)	1(4.8)	0(0)	0(0)	0(0)	
Hepatic disfunction						
CPT-11 group	20(91)	1(4.5)	1(4.5)	0(0)	0(0)	0.279
TPT group	19(90.5)	1(4.75)	1(4.75)	0(0)	0(0)	
Weary						
CPT-11 group	17(78)	3(13.7)	1(4.15)	1(4.15)	0(0)	0.867
TPT group	14(67)	3(14.3)	2(9.35)	2(9.35)	0(0)	

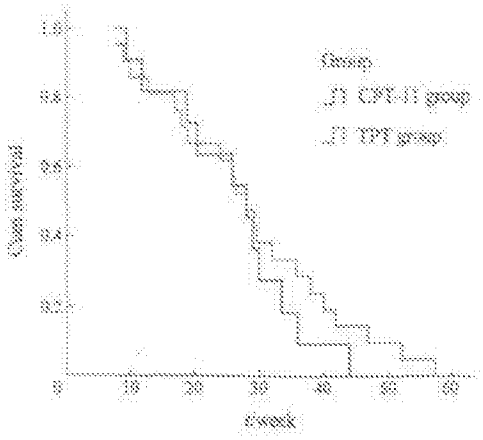

图1 两组患者生存曲线

Fig. 1 Survival curves of the two groups

性腹泻CPT-11组高于TPT组，差异有统计学意义(36.4% *vs* 4.8%，P<0.05，表3)。

3 讨 论

小细胞肺癌是肺癌中恶性程度最高的病理类型，对放化疗敏感[2-4]。多年来，对于接受标准化疗方案治疗的广泛期小细胞肺癌患者，其中位生存时间为8～10个月，2年生存率约为10%～15%。而对于局限期小细胞肺癌患者在诱导化放疗后仍有75%～80%出现复发，复发性小细胞肺癌患者二线治疗的RR主要取决于一线治疗后缓解至肿瘤复发的时间。一线治疗无效或一线治疗后缓解期小于3个月的患者具有高度耐药性，通常对多种细胞毒性药物均无作用，称为难治性小细胞肺癌，其药物的RR低于10%，生存期常为二线治疗后数周。如肿瘤缓解至进展时间超过3个月，此类肿瘤称为敏感性肿瘤，二线治疗疗效可以提高[5]，且RR可能随肿瘤缓解至进展时间的延长而升高。目前，对于复发性小细胞肺癌的二线治疗方案仍无“金标准”，且联合化疗是否优于单药化疗仍

有争论[6]。

CPT-11和TPT均为DNA拓扑异构酶Ⅰ(Topo Ⅰ)抑制剂，是半合成的喜树碱衍生物，通过抑制人体细胞DNA复制所必需的Topo Ⅰ，诱导DNA单链损伤、阻断DNA复制而产生细胞毒性。TPT早在1998年被美国FDA批准用于复发转移性小细胞肺癌的二线治疗，近期公布的资料表明，TPT使复发的小细胞肺癌患者生存受益，并明显改善了患者的症状和生活质量[7]。2002年日本临床肿瘤学会报道，CPT-11对复发性小细胞肺癌治疗的RR约为41%[8]。

本研究观察了CPT-11及TPT治疗复发性小细胞肺癌的近远期疗效及不良反应。结果显示，两药治疗复发性小细胞肺癌的疗效相同。在不良反应方面，TPT的不良反应主要为粒细胞及血小板减少，且为该药的剂量限制性毒性。CPT-11的Ⅲ～Ⅳ粒细胞抑制及血小板减少较TPT发生率低，在集落刺激因子的作用下很快可以恢复。迟发性腹泻则是由于CPT-11的活性代谢产物SN-38导致肠结构及功能的损害，引起肠黏膜损伤相关性腹泻，在对患者进行宣传教育、饮食指导并对症治疗后可以缓解。

综上所述，CPT-11与TPT治疗复发性晚期小细胞肺癌是一种安全有效的方案，近期及远期疗效相似，两药的不良反应不完全相同，但患者均可耐受，可根据患者的机体状况决定治疗策略，骨髓功能相对不良的可考虑选择CPT-11化疗，而消化道功能不良的可以选择TPT化疗。小细胞肺癌二线化疗的疗效还取决于肿瘤对初次化疗的反应、初次治疗停止到复发的间隔时间，一线治疗反应越差，间隔时间越短，二线化疗疗效越差[9]。然而，关于晚期小细胞肺癌二线化疗的更佳治疗方案仍有待近一步的临床研究证据。

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[^{18}F]FAZA-PET Detection of Hypoxia Changes following Anti-cancer Therapy

Jinzi Zheng[1], Stephan Klinz[2], Raquel De Souza[1], Michael Dunne[1], Jonathan Fitzgerald[2], David Jaffray[1, 3, 4]

1. STTARR Innovation Centre, Radiation Medicine Program, Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada
2. Merrimack Pharmaceuticals, Inc., Cambridge, MA, USA
3. Techna Institute for the Advancement of Technology for Health, University Health Network, Toronto, Ontario, Canada
4. Radiation Oncology, University of Toronto, Toronto, Ontario, Canada

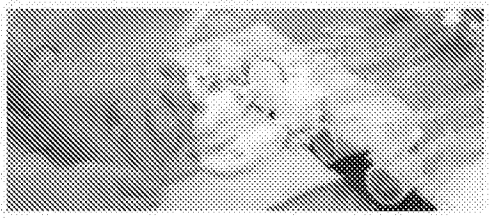
Rationale:

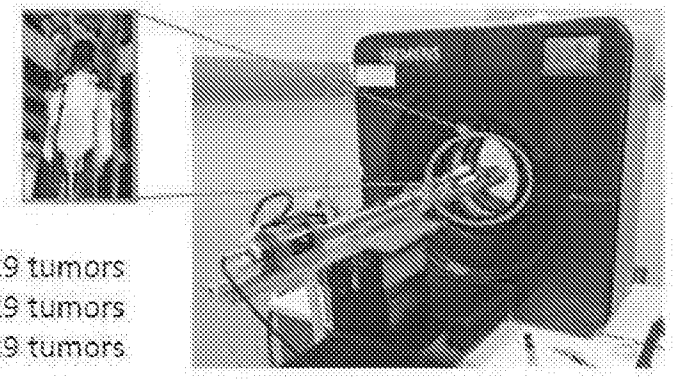
- Tumor hypoxia is strongly linked to aggressive disease progression and resistance to therapy
- Chemotherapy can modify tumor microenvironment
- Investigation of the effect of a liposome formulation of irinotecan (irinotecan sucrosofate liposome injection – MM-398) compared to the free drug (irinotecan, CPT-11) in modulating tumor hypoxia

Study Aims:

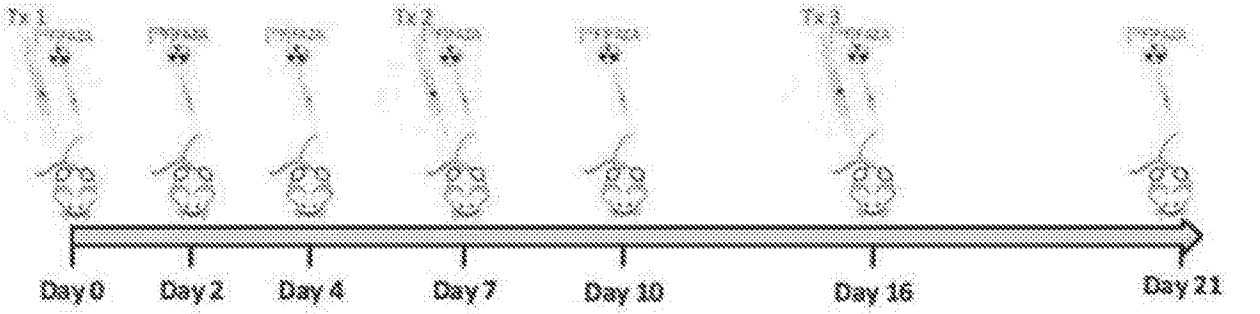
1. Establish high-throughput longitudinal imaging method
2. Characterize imaging data output
   - Tumor and healthy tissue signal levels
   - Baseline vs. treatment time-course FAZA uptake
   - Single dose vs. multi-dose hypoxia response
3. Gain insight in how hypoxia changes with chemotherapy

Methods:



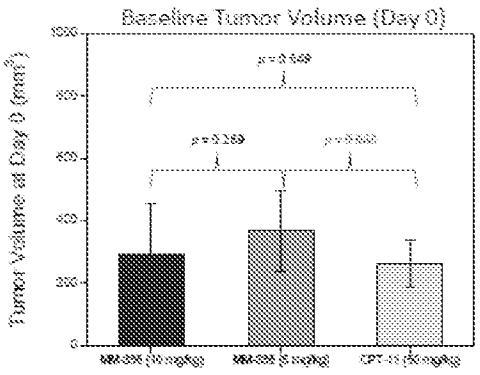


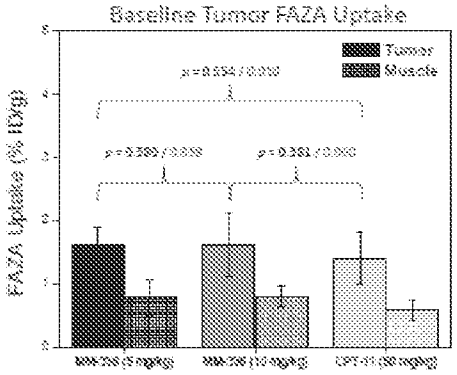
CPT-11 (50 mg/kg): 5 mice, bilateral HT29 tumors
MM-398 (5 mg/kg): 5 mice, bilateral HT29 tumors
MM-398 (10 mg/kg): 5 mice, bilateral HT29 tumors

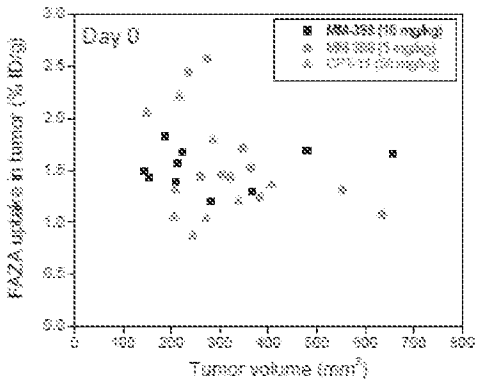


[^{18}F]FAZA injection (~ 8 MBq/g) → 2 h (awake) → PET/CT imaging (air / 2% isofluorane)

Study baseline:



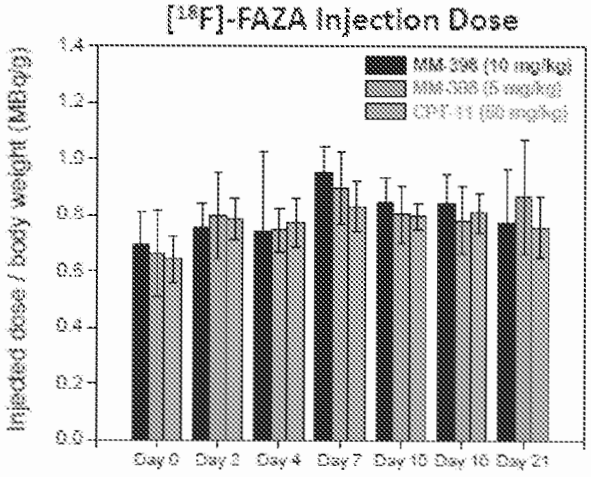




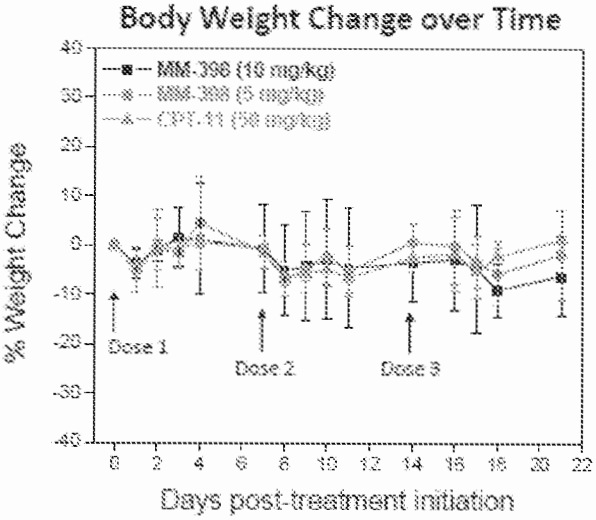
- No difference in initial tumor volume or baseline FAZA uptake among groups

- No correlation on day 0 between tumor FAZA uptake and tumor volume for individual tumors

Time-course information:



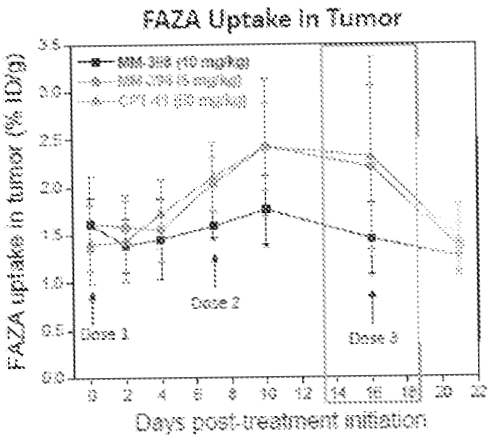
[18F]FAZA radiochemical purity: 95.7 ± 3.7%
(7 productions over 21 days)

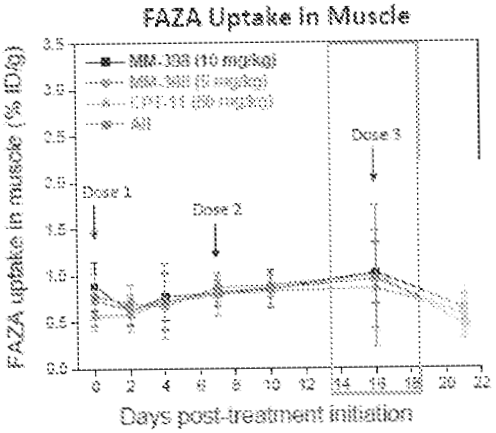


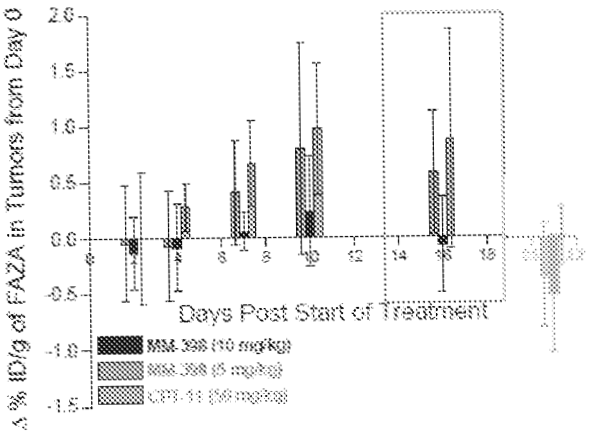
Challenges with longitudinal studies:

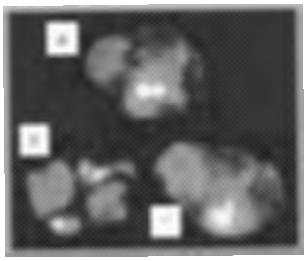
- Variability in radiotracer production
- Fluctuation in weight and health
- Repeated *i.v.* cannulation and anesthesia
- Study length dictated by tumor endpoint (*i.e.* different for each institution)
- Data normalization (*i.e.* repeated blood sampling not feasible)

Time-course [^{18}F]FAZA uptake in tumor and muscle:







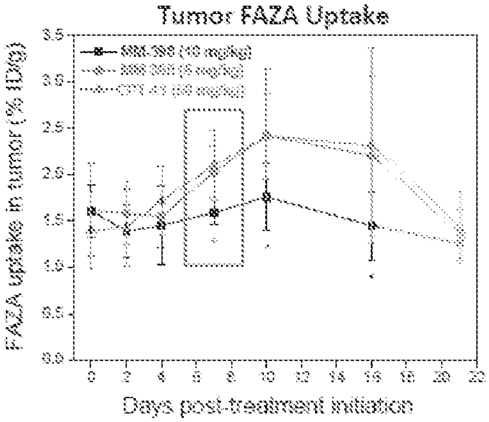


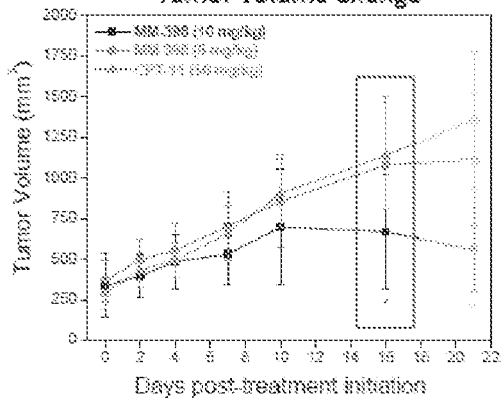
A: MM-398 (10 mg/kg)

B: MM-398 (5 mg/kg)

C: CPT-11 (50 mg/kg)

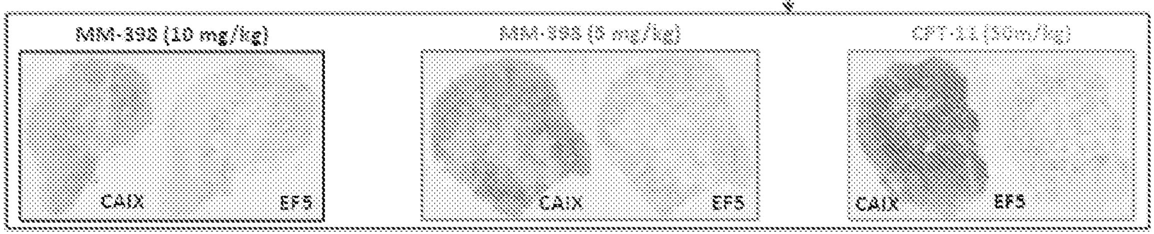
Early assessment of treatment response:



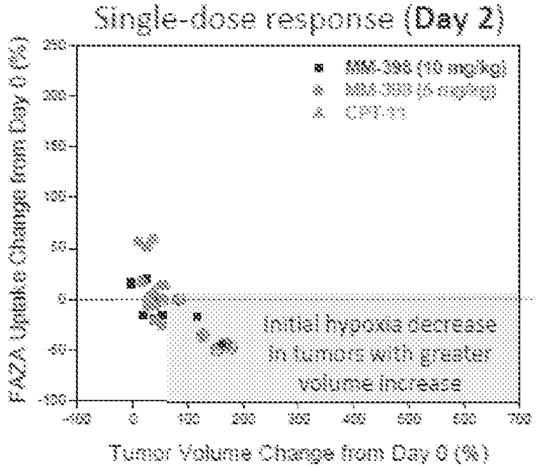


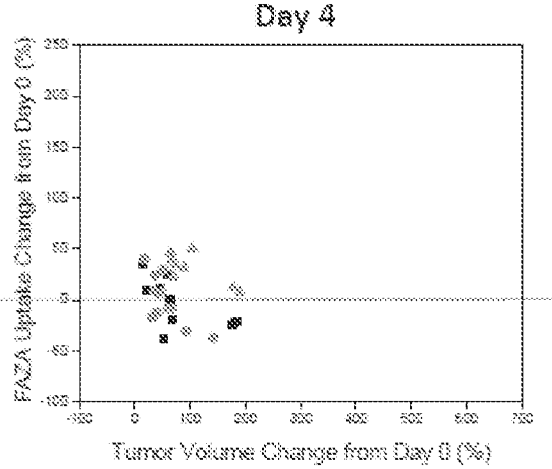
Independent samples *t*-test, two-tailed, equal variance not assumed

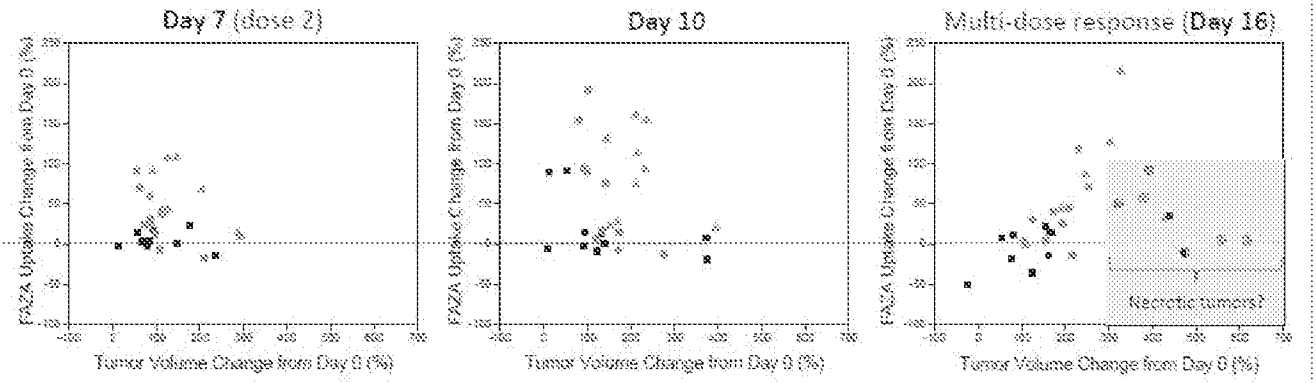
	p value (red indicates $p < 0.05$)									
	Day 4		Day 7		Day 10		Day 16		Day 21	
	Tumor volume (mm^3)	FAZA Uptake (% ID/g)	Tumor volume (mm^3)	FAZA Uptake (% ID/g)	Tumor volume (mm^3)	FAZA Uptake (% ID/g)	Tumor volume (mm^3)	FAZA Uptake (% ID/g)	Tumor volume (mm^3)	FAZA Uptake (% ID/g)
A versus B	0.964	0.509	0.151	0.002	0.264	0.019	0.073	0.025	0.103	0.366
A versus C	0.884	0.07	0.176	0.011	0.597	0.006	0.081	0.053	0.022	0.435



Longitudinal changes in tumor [^{18}F]FAZA uptake:







Conclusion:

- Demonstrated feasibility in performing longitudinal and repeated tumor hypoxia assessment with [^{18}F]FAZA-PET
- Detected differences in tumor hypoxia levels within tumor-size matched groups and in response to treatment
- Hypoxia changes following anti-cancer therapy may allow early assessment of treatment activity

[^{18}F]FAZA-PET Detection of Hypoxia Changes following Anti-cancer Therapy

Jinzi Zheng[1], Stephan Klinz[2], Raquel De Souza[1], Michael Dunne[1], Jonathan Fitzgerald[2], David Jaffray[1, 3, 4]

1. STTARR Innovation Centre, Radiation Medicine Program, Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada
2. Merrimack Pharmaceuticals, Inc., Cambridge, MA, USA
3. Techna Institute for the Advancement of Technology for Health, University Health Network, Toronto, Ontario, Canada
4. Radiation Oncology, University of Toronto, Toronto, Ontario, Canada

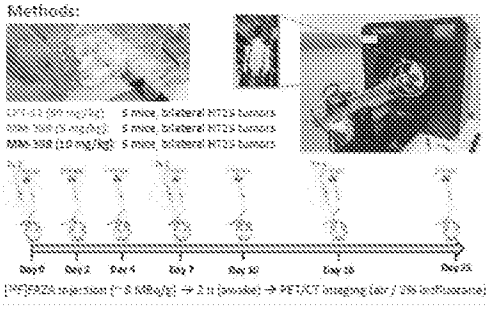
Rationale:

- Tumor hypoxia is strongly linked to aggressive disease progression and resistance to therapy
- Chemotherapy can modify tumor microenvironment
- Investigation of the effect of a liposome formulation of irinotecan (irinotecan sucrosofate liposome injection – MM-398) compared to the free drug (irinotecan, CPT-11) in modulating tumor hypoxia

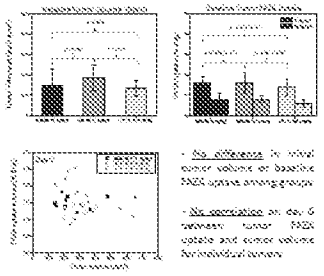
Study Aims:

1. Establish high-throughput longitudinal imaging method
2. Characterize imaging data output
   - Tumor and healthy tissue signal levels
   - Baseline vs. treatment time-course FAZA uptake
   - Single dose vs. multi-dose hypoxia response
3. Gain insight in how hypoxia changes with chemotherapy

Methods:

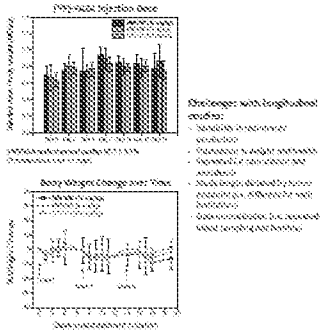


Study baseline:

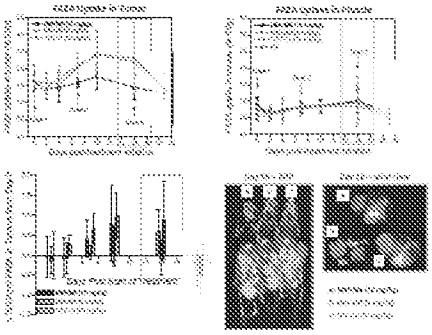


- No difference in initial tumor volume or baseline FAZA uptake among groups
- No correlation on day 0 between tumor FAZA uptake and tumor volume for individual tumors

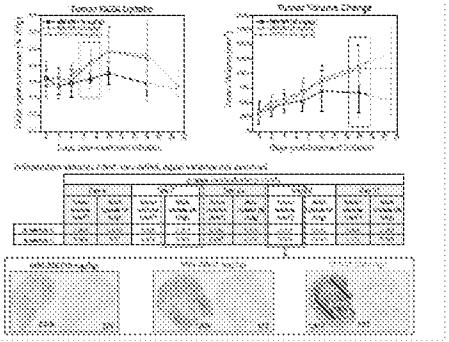
Time-course information:



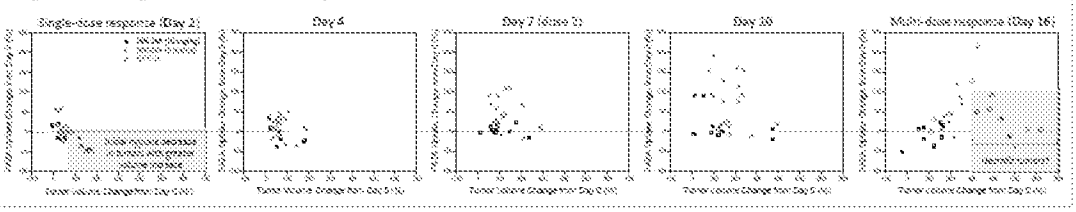
Time-course [^{18}F]FAZA uptake in tumor and muscle:



Early assessment of treatment response:



Longitudinal changes in tumor [^{18}F]FAZA uptake:



Conclusion:

- Demonstrated feasibility in performing longitudinal and repeated tumor hypoxia assessment with [^{18}F]FAZA-PET
- Detected differences in tumor hypoxia levels within tumor-size matched groups and in response to treatment
- Hypoxia changes following anti-cancer therapy may allow early assessment of treatment activity

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ORIGINAL RESEARCH Open Access

Longitudinal tumor hypoxia imaging with [^{18}F]FAZA-PET provides early prediction of nanoliposomal irinotecan (nal-IRI) treatment activity

Jinzi Zheng[1,2,3*], Stephan G. Klinz[4], Raquel De Souza[2], Jonathan Fitzgerald[4] and David A. Jaffray[1,2,3,5]

Abstract

Background: Non-invasive measurement of tumor hypoxia has demonstrated potential for the evaluation of disease progression, as well as prediction and assessment of treatment outcome. [^{18}F]fluoroazomycin arabinoside (FAZA) positron emission tomography (PET) has been identified as a robust method for quantification of hypoxia both preclinically and clinically. The goal of this investigation was to evaluate the feasibility and value of repeated FAZA-PET imaging to quantify hypoxia in tumors that received multi-dose chemotherapy.

Methods: FAZA-PET imaging was conducted over a 21-day period in a mouse xenograft model of HT-29 human colorectal carcinoma, following multi-dose chemotherapy treatment with irinotecan (CPT-11) or nanoliposomal irinotecan (nal-IRI, MM-398).

Results: Tumors treated with 10 mg/kg nal-IRI maintained significantly lower levels of hypoxia and smaller hypoxic fractions compared to tumors that received 50 mg/kg CPT-11. Specifically, differences in FAZA uptake were detectable 9 days before any significant differences in tumor volume were observed between the treatment groups.

Conclusions: These findings highlight the potential use of FAZA-PET as an early marker of treatment response following multi-dose chemotherapy.

Keywords: Hypoxia; FAZA; PET; Irinotecan; Liposome

Background

Tumor hypoxia is strongly linked to aggressive disease progression and resistance to therapy [1]. Specifically, hypoxia-induced chemoresistance is associated with (1) reduced intratumoral perfusion, which hinders drug access to hypoxic areas; and (2) the quiescent state of hypoxic cells, which render DNA structure modifying chemo agents ineffective. The degree of hypoxia is a dynamic quantity that is influenced by physiological factors. It is therefore important to assess hypoxia in tumors before, during, and after therapy.

Advances in non-invasive imaging have resulted in the development and clinical exploration of a number of hypoxia targeted agents for positron emission tomography (PET), including [^{18}F]fluoromisondazole (FMISO) [2, 3], [^{18}F]fluoroazomycin arabinoside (FAZA) [4–11], [^{64}Cu]diacetyl-bis(N4-methylthiosemicarbazone (ATSM) [12–14], and [^{18}F]flortanidazole (HX4) [15–19]. Recent reports have shown that [^{18}F]FAZA may offer superior sensitivity in the detection of hypoxic regions due to faster systemic clearance from non-hypoxic tissues and, therefore, lower non-specific background activity compared to [^{18}F]FMISO [20]. In addition, preclinical validation has demonstrated a good agreement between intratumoral FAZA uptake, Eppendorf electrode measurements, and

* Correspondence: jinzi.zheng@rmp.uhn.on.ca
[1]TECHNA Institute for the Advancement of Technology for Health, University Health Network, 101 College Street, Rm 7-302, Toronto, Ontario M5G 1L7, Canada
[2]Department of Radiation Physics, Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada
Full list of author information is available at the end of the article

pimonidazole staining [5], while [^{64}Cu]ATSM failed to show good correlation with carbonic anhydrase IX (CAIX) immunostaining [21]. Furthermore, FAZA-based PET quantification of hypoxia proved to be highly reproducible in untreated animals when imaged 24 h apart [6]. Both preclinical and clinical reports have shown encouraging prognostic and predictive power of FAZA-PET-based hypoxia imaging, particularly when used in conjunction with radiotherapy [4, 8, 9]. These findings support the employment of FAZA-PET as an effective imaging technique to quantify hypoxia. Although other PET-based tracers such as [^{18}F]-fluorodeoxygluocose (FDG) and [^{18}F]-fluorothymidine (FLT) have shown potential in predicting early treatment response in patients and animal models of cancer [22, 23], they do not directly provide information on the hypoxia status of a tumor.

Nanoliposomal irinotecan (nal-IRI, MM-398) is a highly stable liposomal nanocarrier formulation of irinotecan hydrochloride (CPT-11) that significantly prolongs the pharmacokinetics and tumor bio-distribution of the free drug [24, 25]. Nal-IRI greatly increases the duration of the therapeutically active metabolite, SN-38, within tumors, which becomes a better correlate to in vivo activity of either free or nanoliposomal irinotecan than SN-38 exposure when measured as the area-under-the-curve (AUC) [24]. Nal-IRI has shown activity in a number of preclinical tumor models [24, 25] and has met its clinical endpoint in a phase III clinical trial in gemcitabine-refractory pancreatic cancer [26], a tumor indication that is characterized by low vascular density as well as numerous and severe hypoxic regions [27].

We and others have observed that sustained exposure as provided by nanoformulations of irinotecan can reduce levels of hypoxia or hypoxia-regulated protein markers relative to untreated tumors after either prolonged treatment [28] or as immediate as following a single-dose administration. However, such endpoint assessments do not provide a comprehensive description of the dynamic changes in tumor hypoxia characteristics during exposure to a course of irinotecan-based chemotherapy, and no investigation to date has explored the feasibility and performance of hypoxia imaging for quantification of acute (i.e., hours) and chronic (i.e., days) hypoxia changes in such a setting. Here, we report the use of FAZA-PET for repeated and longitudinal monitoring of tumor hypoxia changes in a mouse xenograft model of HT-29 colorectal cancer before, during, and after three weekly chemotherapy administrations of either free irinotecan or nal-IRI.

Methods

Animal model

Studies were approved by the University Health Network Animal Care Committee and adhered to the ethical guidelines of the Canadian Council on Animal Care. Female, 6- to 8-week-old NOD/SCID mice (Ontario Cancer Institute, Toronto, Canada) were inoculated subcutaneously with 1×10^7 HT-29 human colorectal adenocarcinoma cells (ATCC, Manassas, VA, USA), in a 100 μL injection volume, at both dorsal flank sites such that each mouse bore bilateral tumors. HT-29 cells represent a goblet-like subtype of colorectal adenocarcinoma [29]. Tumor growth was monitored using caliper-based measurements. Studies began 17 days post-inoculation, when tumors reached a mean volume of 307.5 ± 131.7 mm^3 (15 mice, 30 tumors).

Chemotherapy treatment

Animals (n = 15) were randomized into three treatment groups: (1) irinotecan hydrochloride (referred to as irinotecan hereafter) administered at 50 mg/kg, (2) nal-IRI at 5 mg/kg, and (3) nal-IRI at 10 mg/kg. Based on a mechanistic pharmacokinetic model, 10 mg/kg of nal-IRI or 50 mg/kg irinotecan was estimated to result in similar AUC exposure to SN-38 in both plasma and tumor, while 5 mg/kg nal-IRI and 50 mg/kg irinotecan showed a comparable duration of SN-38 levels above a critical intratumoral threshold of 120 nmol/L [24]. All treatment doses were known to suboptimally control tumor growth in the bilateral subcutaneous HT-29 tumor model. Each treatment group was composed of a total of 10 tumors (five animals bearing two tumors each). A total of four weekly administrations were given *i.v.* on day 0, 7, 15, and 21.

In vivo imaging

A total of seven FAZA-PET/CT imaging sessions were performed on day 0, 2, 4, 7, 10, 16, and 21 following initiation of treatment using a triple mouse imaging bed (Fig. 1). [^{18}F]FAZA was produced by CanProbe (Ontario, Canada) with a radiochemical purity of 95.7 ± 3.7 % (calculated over seven productions). PET imaging (Focus 220, Siemens) was performed at 2-h post-FAZA administration (0.79 ± 0.06 MBq/g of body weight, Additional file 1: Figure S1). Imaging at 2-h post-FAZA injection was reported to be a desirable imaging time point based on tracer kinetics at the tumor site (i.e., reaching steady state) and on the fact that at this time, post-injection, the tumor tracer uptake levels correlated well with tissue hypoxia [5, 30]. Each PET acquisition consisted of a 20-min emission scan followed by an 8-min ^{57}Co transmission scan for attenuation and scatter correction. Then, a CT scan (GE Locus Ultra, 80 kVp, 50 mA) was performed with animals in the same position in order to provide anatomical data for image registration. Treatment response was quantified based on CT tumor volumes.

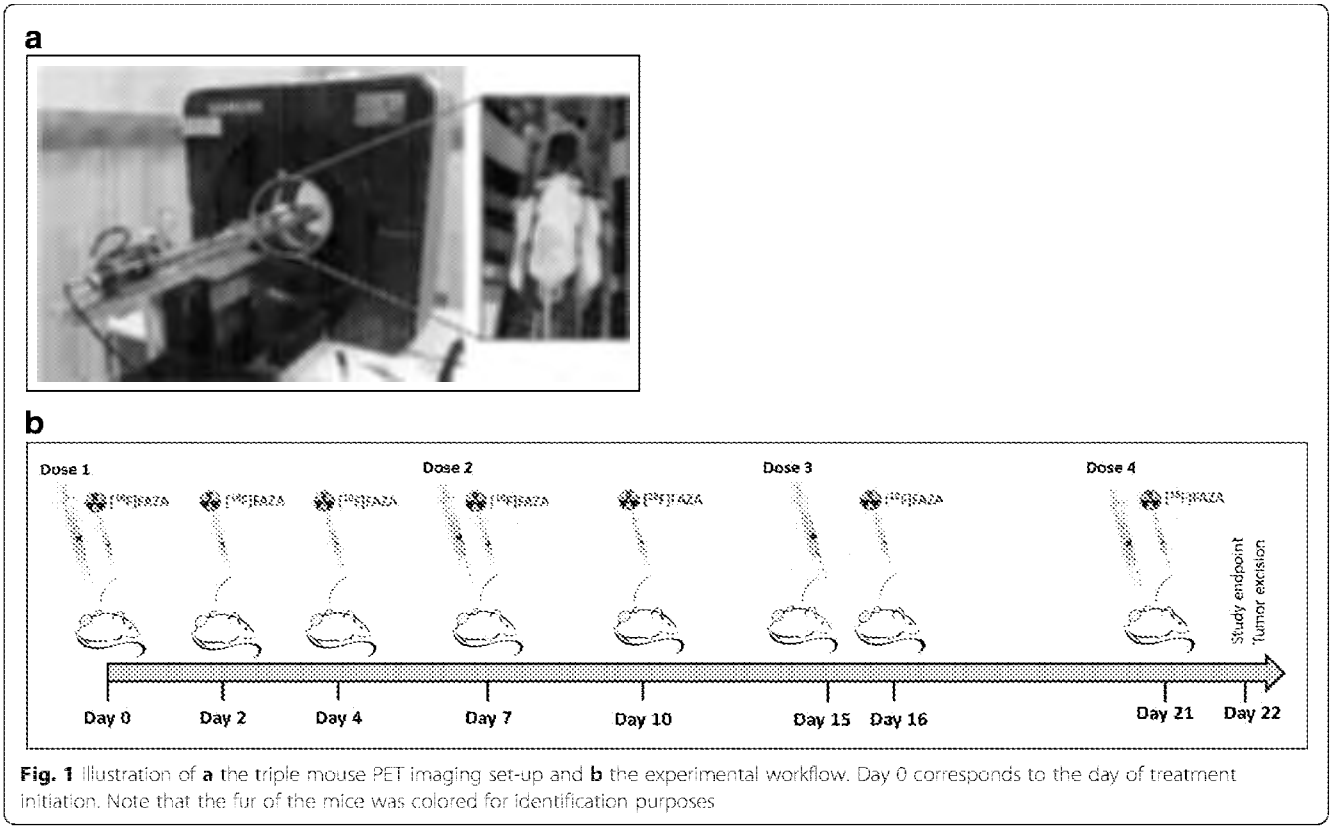

Fig. 1 Illustration of **a** the triple mouse PET imaging set-up and **b** the experimental workflow. Day 0 corresponds to the day of treatment initiation. Note that the fur of the mice was colored for identification purposes

Image analysis

The PET and CT datasets were registered, contoured, and analyzed using the Inveon Research Workplace software (IRW 4.0, Siemens). The hypoxic fraction is defined as the number of tumor voxels with FAZA-PET signal values above a set threshold over the total number of tumor voxels. The hypoxia signal threshold value was defined as the mean FAZA-PET signal value measured in the upper leg muscle of the same mouse + 3 standard deviations [31].

Histology

Tumor-specific hypoxia status at the study endpoint (day 22) was confirmed by immunohistochemistry. Animals received an intraperitoneal administration of EF5 (0.1 mM EF5/g body weight) 2 h before euthanasia, and portions of the excised tumors were fixed, sectioned, and stained for hematoxylin and eosin (H&E), EF5 (anti-EF5 ELK3-51), and CAIX (anti-CAIX M75). Image acquisition was done with an Aperio Scanscope AT. Analysis of the histology images were performed using Definiens Tissue Studio (Definiens AG, Munich, Germany).

Statistical analysis

Differences between means for the different treatment groups were compared using one-way ANOVA or an independent samples t test where equal variances are not assumed and with a confidence interval of 95 %. Differences between means for the same treatment group on different days were compared using a paired-samples t test with a confidence interval of 95 %. All statistical calculations were performed using SPSS version 22 (IBM, Armonk, NY, USA).

Results and discussion

Treatment group randomization and baseline FAZA uptake

In order to ensure that the response is consistent across animals and tumors that received the same treatment, mice bearing bilateral tumors were randomized into three treatment groups (irinotecan at 50 mg/kg, nal-IRI at 5 mg/kg, and nal-IRI at 10 mg/kg) based on pre-treatment growth kinetics (Fig. 2a) and caliper-measured tumor volume at treatment day 0 (Fig. 2b). Figure 2a shows no significant difference in the tumor growth curves from day 7 to day 17 post-tumor inoculation. Figure 2b illustrates the mean CT tumor volumes for each group (a total of 10 tumors in 5 mice per group), assessed after animals were already randomized on the day of treatment initiation. No statistically significant difference was found among groups ($p = 0.202$ for CT-measured tumor volumes, $p = 0.539$ for caliper-measured tumor volumes). The correlation ($R^2 = 0.904$, Additional file 1: Figure S2)

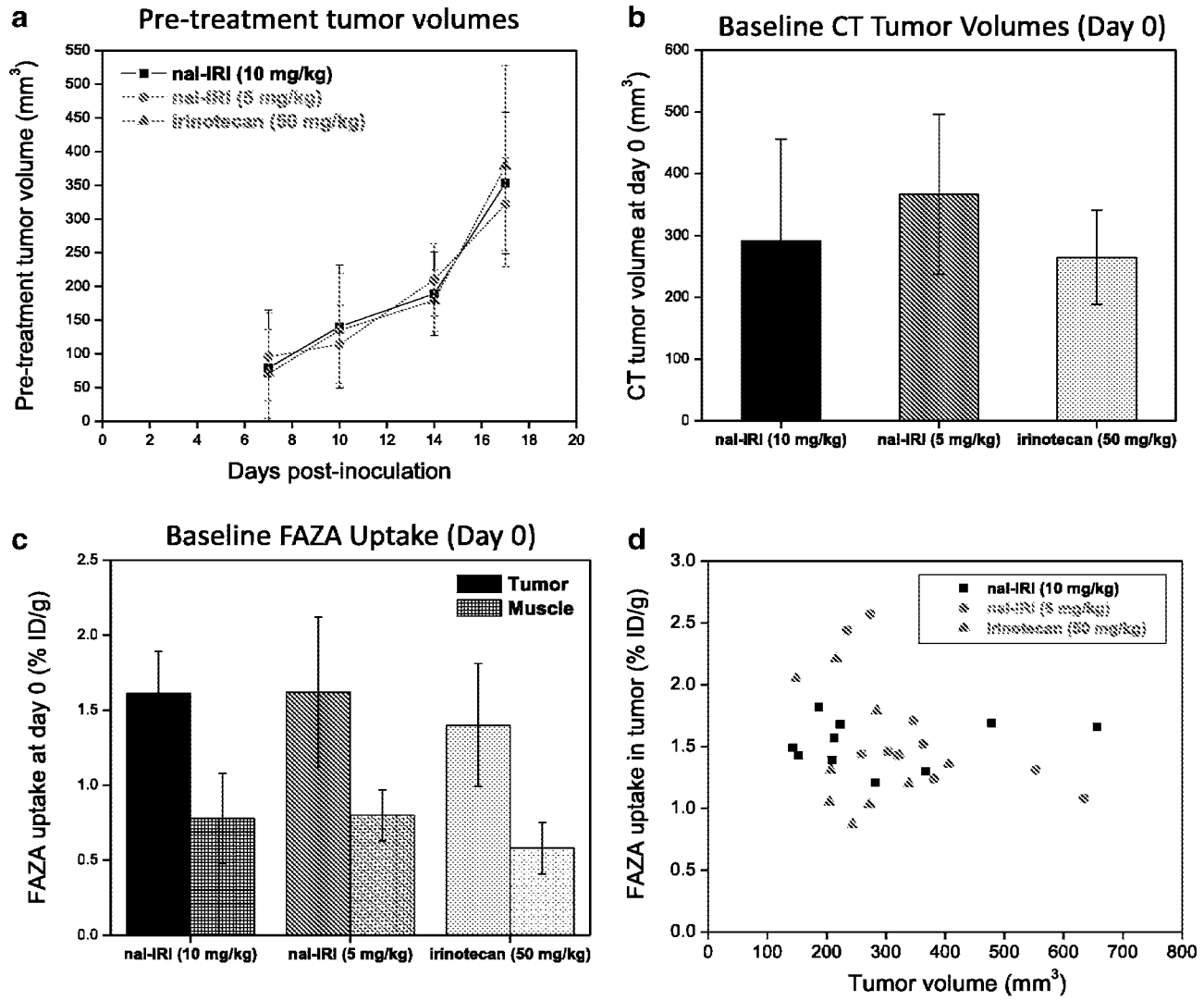

Fig. 2 Study baseline data including **a** pre-treatment mean tumor growth kinetics for each group (day 17 post-inoculation corresponds to day 0 of treatment initiation), **b** the baseline tumor volume obtained by contouring the volumetric CT data sets ($p = 0.202$), **c** the pre-treatment tumor ($p = 0.579$) and muscle ($p = 0.304$) FAZA uptake, and **d** the lack of correlation between tumor FAZA uptake and tumor volume. Each treatment group is composed of 5 mice, each bearing bilateral subcutaneously implanted HT-29 colon cancer xenograft, for a total of 10 tumors per treatment group

between the tumor volumes estimated based on digital caliper measurements and CT volume contours was within a 10 % tolerance (caliper volume = 0.938 × CT volume, 30 tumors over 7 measurement days).

On the day of treatment initiation, baseline FAZA uptake in mean %ID/g for both tumor and muscle (ROI drawn on the inner thigh muscle) was calculated. No statistically significant differences between the baseline tumor uptake for the three groups was found ($p = 0.579$, tumor uptake = 1.40 ± 0.41 to 1.62 ± 0.50 %ID/g, Fig. 2c). Most importantly, no correlation ($R^2 = 0.008$, Fig. 2d) was found between the mean FAZA tumor uptake and the CT tumor volume at study baseline (on the day of treatment initiation). Muscle FAZA uptake (Additional file 1: Figure S3) averaged ~50 % of the uptake measured in tumors at baseline consistent with previous publications [4, 8]. The differences measured in the baseline mean FAZA muscle uptake among the three treatment groups were relatively small (0.58 ± 0.18 %ID/g for the irinotecan group, 0.78 ± 0.32 %ID/g for the 5 mg/kg nal-IRI group, and 0.80 ± 0.18 %ID/g for the 10 mg/kg nal-IRI group) and were not statistically significant ($p = 0.304$). Muscle FAZA uptake was not used to normalize FAZA tumor uptake (i.e., to calculate tumor-to-muscle ratios) at the various imaging time points, since a reproducibility study previously conducted by our group [32]

had shown that there is higher day-to-day variation in the muscle FAZA uptake compared to tumor.

Treatment time course FAZA uptake

Response to treatment was evaluated over a 3-week period through CT-based tumor volume measurement (Fig. 3a). The seven imaging sessions and the three treatment doses were well-tolerated by all animals (Fig. 3b). The mean FAZA uptake measured in the 30 tumors (15 mice) ranged between 0.83 and 4.29 %ID/g over the course of the study (Fig. 3c). Treatment with 10 mg/kg nal-IRI maintained significantly lower levels of hypoxia and smaller hypoxic

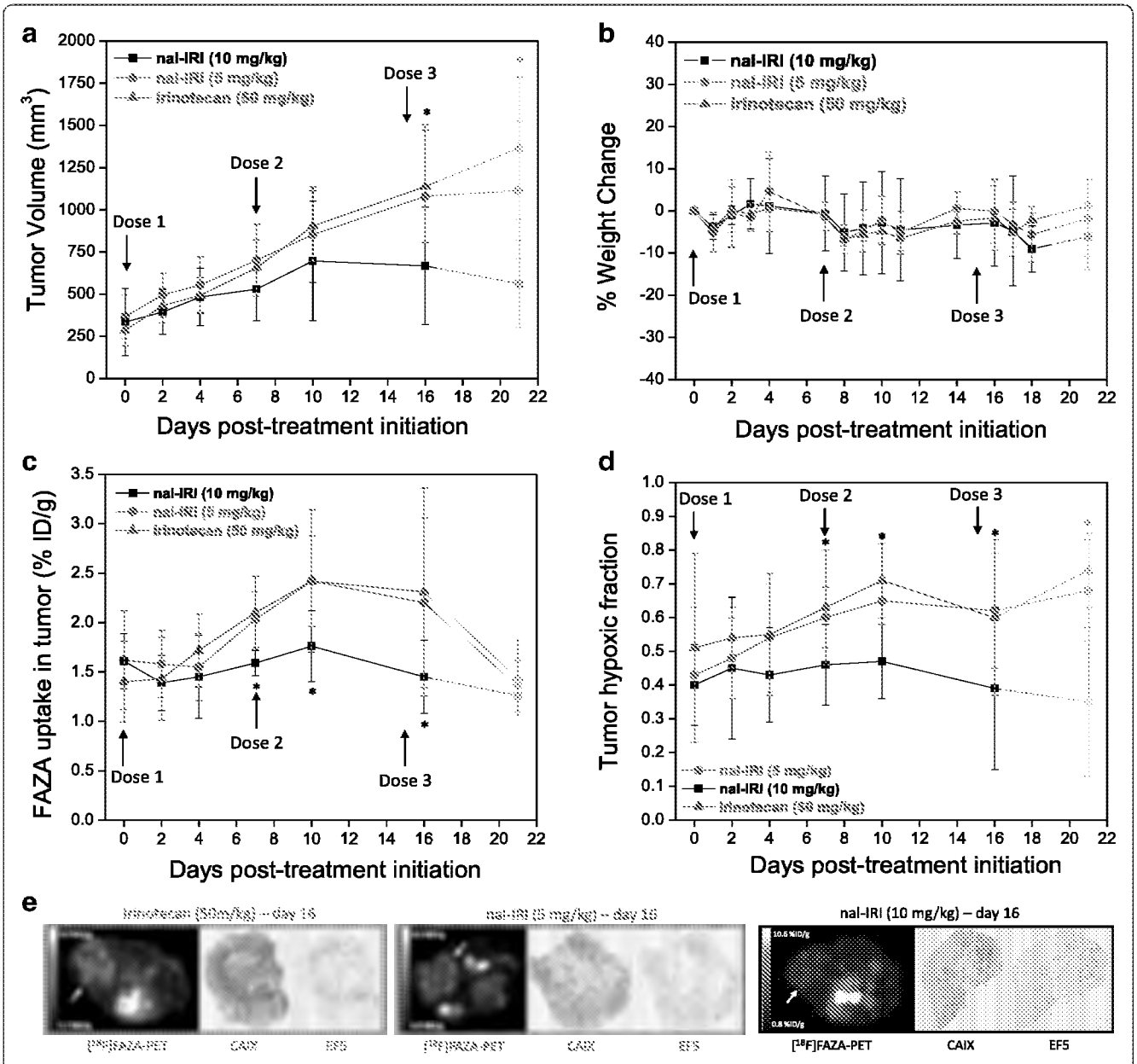

Fig. 3 **a** Tumor volume changes and **b** animal weight fluctuations over the study period of 21 days. **c** Longitudinal tumor FAZA uptake (%ID/g) and **d** tumor hypoxic fraction calculated from the PET data set. Each data point (except for day 21, transparently masked in *gray*) represents the mean ± standard deviation obtained from five animals, each bearing bilateral tumors, for a total of 10 tumors per treatment group. *Asterisk* denotes statistically significant difference ($p < 0.05$) between the nal-IRI (10 mg/kg) and either one or both the other two treatment groups. **e** Three sets of representative PET image (left) and CAIX (middle) and EF5 (right) stained tumor sections obtained from three animals, one belonging to each treatment group. Qualitative demonstration that the degree of FAZA-PET signal is confirmed by the level of CAIX and EF5 immunohistochemistry staining (i.e., high FAZA uptake correlates to high CAIX and high EF5 staining)

fractions compared to tumors that received irinotecan (Fig. 3c, d), and these differences between the treatment groups were significant as early as day 7 post-treatment initiation (Table 1). Tumor growth control was also apparent in the 10 mg/kg nal-IRI treatment group (Fig. 3a). However, differences in tumor volume only became statistically significant on day 10 (nal-IRI at 10 mg/kg vs. irinotecan at 50 mg/kg, $p = 0.038$) and day 16 (nal-IRI at 10 mg/kg vs. irinotecan at 50 mg/kg, $p = 0.006$; nal-IRI at 10 mg/kg vs. nal-IRI at 5 mg/kg, $p = 0.029$) (Table 1). This suggests that tumor hypoxia imaging using FAZA-PET has the potential to provide early prediction of treatment response. Five animals (one from each of the nal-IRI groups and three from the irinotecan treatment group) were sacrificed between day 16 and 21 for ethical reasons (tumor ≥ 1.5 cm in any direction). As a result, the data reported for day 21 only represents the surviving subgroup and is therefore skewed toward better response as shown by an overall decrease in tumor FAZA uptake (Fig. 3c). It is interesting to note that the tumor hypoxic fraction measure (Fig. 3d) better correlates with tumor response on day 21 (Fig. 3a) than the mean FAZA tumor uptake (Fig. 3c). This suggests that quantification of tumor hypoxia using the hypoxic fraction measure may be a more reliable evaluation metrics than the mean FAZA uptake value.

Initial histological validation of the FAZA-PET signal was done by staining with two distinct hypoxia markers, CAIX and EF5, on adjacent tumor tissue slides (Fig. 3e). The degree of FAZA-PET tumor uptake is proportional to the amount of CAIX and EF5 positive staining. Specifically, the percent CAIX and EF5 positive tumor areas for the 10 mg/kg nal-IRI group (17.6 ± 7.4 % and 1.9 ± 1.6 %, respectively) are significantly lower ($p < 0.05$) compared to those treated with 5 mg/kg of nal-IRI (28.8 ± 8.5 % and 5.8 ± 3.9 %, respectively) and 50 mg/kg of irinotecan (29.2 ± 5.5 % and 9.2 ± 4.7 %, respectively) This is in agreement with the significantly lower FAZA-positive tumor fraction quantified using the PET data (Table 1). A decrease in cellular density following treatment with nal-IRI at 10 mg/kg compared to the other treatments was also observed (Fig. 4).

Longitudinal changes in tumor FAZA uptake

Relative changes in tumor volume and hypoxia over time with respect to baseline values (day 0) were calculated for each tumor individually. The relative change in FAZA uptake in tumors over the 16-day period compared to baseline is shown in Fig. 5a. An increase in median FAZA uptake can be observed for the group that received 50 mg/kg of irinotecan starting as early as 4-day post-treatment initiation and for the group that received 5 mg/kg of nal-IRI 7-day post-treatment initiation. Only with the nal-IRI treatment at 10 mg/kg did the tumors maintain their pre-treatment hypoxia level on day 7 ($p = 0.264$ with respect to day 0 baseline values, paired-samples t test) and beyond following treatment initiation, despite significant increases in their tumor volumes ($p < 0.005$ for day 7, volumes with respect to day 0 baseline, paired-samples t test). Thus, the treatment with 10 mg/kg nal-IRI was able to stabilize the hypoxia levels in tumors. In addition, disease progression in terms of tumor volume increase was better controlled in this treatment group compared to the other two treatment groups (Fig. 3a).

Normalized time course trajectories for individual tumors of changes in FAZA uptake and tumor volume with respect to baseline values are shown in a three-dimensional plot (Fig. 5b). Ellipsoidal contours were generated for all trajectory data points from each treatment group (ellipsoid coverage = 70 % of data points).

Table 1 P values calculated from independent samples t tests (two-tailed, equal variance not assumed) for tumor volume, tumor FAZA uptake, and tumor hypoxic fraction parameters at various study time points.

***P* Value Table**

	p value (bold and yellow highlight indicate $p < 0.05$)														
	Day 4			Day 7			Day 10			Day 16			Day 21		
	Tumor volume (mm^3)	FAZA Uptake (%ID/g)	Tumor Hypoxic Fraction	Tumor volume (mm^3)	FAZA Uptake (%ID/g)	Tumor Hypoxic Fraction	Tumor volume (mm^3)	FAZA Uptake (%ID/g)	Tumor Hypoxic Fraction	Tumor volume (mm^3)	FAZA Uptake (%ID/g)	Tumor Hypoxic Fraction	Tumor volume (mm^3)	FAZA Uptake (%ID/g)	Tumor Hypoxic Fraction
nal-IRI (10 mg/kg) vs. nal-IRI (5 mg/kg)	0.364	0.554	0.104	0.070	**0.001**	**0.010**	0.070	**0.021**	**0.000**	**0.029**	**0.025**	**0.027**	**0.005**	0.366	**0.005**
nal-IRI (10 mg/kg) vs. irinotecan (50 mg/kg)	0.875	0.143	0.105	0.129	**0.002**	**0.018**	**0.038**	**0.003**	**0.000**	**0.006**	**0.033**	0.060	**0.022**	0.435	**0.003**
nal-IRI (5 mg/kg) vs. irinotecan (50 mg/kg)	0.352	0.305	0.886	0.592	0.669	0.564	0.938	0.980	0.172	0.735	0.809	0.881	0.361	0.745	0.465

Statistically significant difference in tumor FAZA uptake (expressed both in terms of %ID/g and tumor hypoxic fraction) is found on day 7 post-treatment initiation between the nal-IRI (10 mg/kg) and the other two treatment groups. Statistically significant difference in tumor volume, for the same groups, is seen on day 10 and 16. A total of 10 tumors were included in the analysis for each group on days 4 through 16. On day 21, the number of tumors per group was 4, 6, and 8 for irinotecan, nal-IRI (10 mg/kg) and nal-IRI (5 mg/kg), respectively

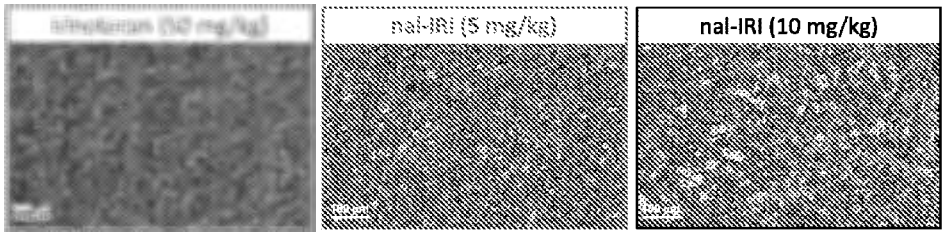

Fig. 4 Representative H&E stained tumor sections (20× magnification, day 16) showing a decrease in cellular density following treatment with nal-IRI at a dose of 10 mg/kg compared to treatment with nal-IRI at 5 mg/kg and treatment with irinotecan at 50 mg/kg

We observed treatment-specific divergence among all three treatment groups; data points from tumors treated with 10 mg/kg nal-IRI were clustered around the smallest changes in both tumor volume and FAZA uptake (blue ellipsoid), while data points from tumors treated with 50 mg/kg irinotecan were clustered around the largest changes (gray ellipsoid). The data points from tumors treated with 5 mg/kg nal-IRI (red ellipsoid) were shifted toward intermediate changes, particularly along the percent change in FAZA uptake axis. In addition, the trajectory path lengths of tumors treated with nal-IRI at either 5 or 10 mg/kg were significantly different ($p = 0.0012$, one-way ANOVA) from those treated with free irinotecan (data not shown). This time course assessment of individual tumor performance allowed for identification of more subtle differences that was not appreciated using group statistics alone.

Discussion

Longitudinal imaging studies are challenging to perform, even in well-controlled preclinical animal models. In hypoxia imaging, the baseline hypoxia status of the tumor can be significantly different even for tumors originating from the same cell line with similar volumes

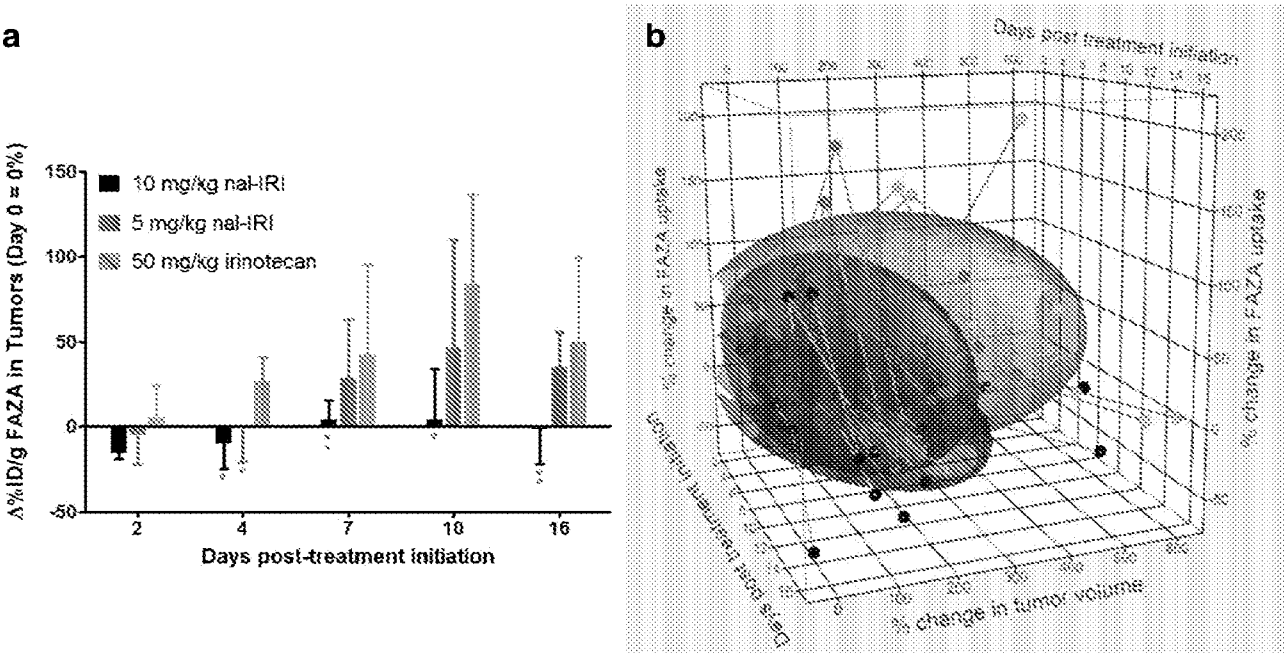

Fig. 5 **a** Longitudinal relative changes in tumor FAZA uptake with respect to pre-treatment values (%ID/g on day 0). Median values are plotted with the interquartile range indicated by bars. *Green asterisk* and *red asterisk* indicate statistically significant difference with respect to the 50 mg/kg irinotecan group and the 5 mg/kg nal-IRI group ($p < 0.05$), respectively. **b** Three-dimensional scatterplot of individual tumors at each treatment day with regard to % change in FAZA uptake from baseline (day 0) and % tumor volume change from day 0. For each treatment group, contour ellipsoids (ellipsoid coverage of data points = 70 %) are presented. Tumors are treated with 10 mg/kg nal-IRI (blue ellipsoid), 5 mg/kg nal-IRI (red ellipsoid), and 50 mg/kg irinotecan (gray ellipsoid)

and growth rates. Considerable effort was thus taken to minimize randomization bias as this could become more pronounced over the course of repeated measurements performed in the same animals and tumors. Our study showed the feasibility of performing FAZA-PET imaging for monitoring response to treatment, even in a relatively heterogeneous group of tumors with higher variability in pre-treatment tumor volume and hypoxia level, when appropriate randomization steps are employed. Using these methods, this study further demonstrated that FAZA-PET imaging-based tumor hypoxia assessment may be utilized as an early predictor of chemotherapy treatment response. Specifically, our findings illustrate that differences in tumor hypoxia can be detected significantly earlier (3 to 9 days) than tumor volume differences between treatment groups.

Our results show no immediate hypoxia reduction, both in terms of %ID/g of FAZA tumor uptake and tumor hypoxic fraction, on days 2 to 4 after treatment with free irinotecan or nal-IRI. This is consistent with the mechanism of action of camptothecins, which requires a prolonged exposure time to SN-38 for maximum cytotoxic effects [33]. Free irinotecan is rapidly cleared from plasma and tumor tissue, thereby not allowing sufficient time for tumor cells to be exposed to SN-38. Only with the nal-IRI treatment at 10 mg/kg did the tumors maintain their pre-treatment hypoxia level on day 7 and beyond following treatment initiation, despite significant initial increases in their tumor volumes, thus suggesting changes in the tumor microenvironment. Previous experiments in an HT-29 tumor model grown in NOD-SCID mice have shown that the 10 mg/kg nal-IRI dose achieves a prolonged SN-38 tumor duration of ~96 h, while the exposure with 50 mg/kg free irinotecan is ~36 h [24]. Similar to the published report on another liposomal irinotecan formulation, irinophore C [28], data from this study showed that tumors presented a less hypoxic profile following the 10 mg/kg nal-IRI treatment, and the prolonged SN-38 exposure resulted in decreased cellular density thus potentially alleviating solid stress and reducing blood vessel compression during tumor growth [34]. It has been shown that irinotecan and other camptothecins can inhibit the hypoxia-inducible factor-1α (HIF-1α) protein accumulation in vivo. This can interfere with the capacity of tumor cells to adapt to a hypoxic environment [10] and may increase their treatment sensitivity [1]. Furthermore, the modulation of HIF-1α protein levels has been reported to occur independently of significant changes in intratumoral hypoxia [10]. However, this study was aimed at quantifying longitudinal tumor hypoxia changes and could not address these mechanistic questions.

Results from our investigation confirmed that a dose of 10 mg/kg of nal-IRI was sufficient to provide growth control in the bilateral HT-29 subcutaneous xenograft model, and it demonstrated a significant benefit in therapeutic response compared to the other two treatment groups. The administration of a therapeutically more effective dose of nal-IRI (i.e., 20 mg/kg), while safe and efficacious [35], would have reduced sensitivity in the measurement of FAZA uptake due to potential disappearance of tumor hypoxia following partial or complete response and could also have introduced measurement bias due to rapid tumor volume shrinkage. Dosing in murine models for both nal-IRI and free irinotecan employed in this study scales appropriately to the clinical setting where nal-IRI is given at a dose density of 40 mg/m^2/week [36].

The apparent discordance between FAZA uptake (Fig. 3c) and hypoxic fraction (Fig. 3d) on day 21 post-treatment initiation for the nal-IRI treated groups is likely due to a decrease in focal areas of intense hypoxia which diminishes the mean tumor FAZA uptake value, while the overall volume fraction of hypoxia is maintained in the tumor compared to day 16. This observation motivates further studies aimed at investigating the intratumoral hypoxia heterogeneity. In fact, more sophisticated image analysis and acquisition strategies exist to further increase the sensitivity of FAZA-PET for hypoxia measurement. For example, quantification of intratumoral heterogeneity can be performed both in terms of subregional steady-state FAZA uptake and variations in distribution, as well as uptake and clearance kinetics through the identification of intratumoral multi-voxel clusters. In addition, kinetic modeling of dynamic FAZA uptake enables improved inter-tumor normalization achieved through the measurement of the perfusion characteristics of each tumor during the early imaging frames.

Conclusions

This study demonstrated the feasibility of performing longitudinal and repeated tumor hypoxia assessment using FAZA-PET imaging for early prediction of treatment response. Statistically significant differences in hypoxia within tumor-size matched groups in response to different treatments were successfully detected. Specifically, the liposomal irinotecan formulation nal-IRI showed enhanced ability to halt progression of tumor hypoxia compared to free irinotecan. Overall, hypoxia changes following anticancer therapy has the potential to provide an early assessment of treatment activity.

Additional file

Additional file 1: Figures S1–S3. Figure S1. Mean FAZA administration dose (MBq/g) calculated for animals belonging to each treatment group over 7 imaging sessions. Figure S2. Tumor volume correlation between measurements performed using caliper and CT. Figure S3. Longitudinal muscle FAZA uptake (%ID/g) measured from the CT data set over a 21-day

period following the first treatment dose administration (day 0). Each data point (except for day 21, transparently masked in gray) represents the mean ± standard deviation obtained from 5 animals. (PDF 86 kb)

Competing interests
JZ and DAJ are collaborators of Merrimack; a portion of the manuscript data was generated under a sponsored research agreement. SGK and JF are current employees of Merrimack Pharmaceuticals and have stock and/or stock options in Merrimack. RDS has no conflict of interest.

Authors' contributions
JZ and SGK designed the study, performed data analysis, and drafted the manuscript. JZ and RDS performed experimental work and image analysis. JF and DAJ participated in the study conception, provided intellectual input during results interpretation, and edited the manuscript. All authors read and approved the final manuscript.

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Author details
[1]TECHNA Institute for the Advancement of Technology for Health, University Health Network, 101 College Street, Rm 7-302, Toronto, Ontario M5G 1L7, Canada. [2]Department of Radiation Physics, Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada. [3]Institute of Biomaterials & Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada. [4]Merrimack Pharmaceuticals, Inc., Cambridge, MA, USA. [5]Radiation Oncology, University of Toronto, Toronto, Ontario, Canada.

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FULL PAPER

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Preferential potentiation of topoisomerase I poison cytotoxicity by PARP inhibition in S phase

P Znojek[1], E Willmore[1] and N J Curtin*[,1]

[1]Newcastle University, Northern Institute for Cancer Research, Paul O'Gorman Building, Medical School, Newcastle upon Tyne NE2 4HH, UK

Background: Topoisomerase I (Topo I) poisons (e.g., camptothecin (CPT)), used to treat cancer, cause DNA breaks that are most cytotoxic during S phase. PARP-1 promotes DNA repair and PARP inhibitors (PARPi) sensitise cells to Topo I poisons. We aimed to determine whether chemosensitisation is also S phase specific using rucaparib, a potent PARPi in advanced clinical evaluation.

Methods: The impact of rucaparib, on CPT-induced cytotoxicity was measured in human colon cancer (LoVo) and leukaemic (K562) cells in asynchronous and cell cycle phase-separated cultures. Topoisomerase I and PARP levels and activity and the effect of rucaparib on DNA single-strand breaks (SSBs), double-strand breaks (DSBs) and collapsed replication fork induction and repair were determined in cell cycle phase-separated cells.

Results: The cytotoxicity of CPT was greatest during S phase, partially attributable to high Topo I activity, and rucaparib preferentially sensitised S-phase cells. Rucaparib increased CPT-induced DNA SSBs in all phases of the cell cycle, and increased DSB and γH2AX foci in S and G2, with γH2AX foci being highest in S-phase cells. Repair of SSBs and DSBs was most rapid during S then G2 phases and was substantially hindered by rucaparib.

Conclusions: Rucaparib preferentially sensitises S-phase cells by increasing the frequency of collapsed replication forks.

Topoisomerase I (Topo I) forms a reversible complex with DNA catalysing the formation and re-annealing of single-strand breaks (SSBs) in DNA to relieve torsional stress associated with transcription, replication or repair. Topo I poisons such as camptothecin (CPT) stabilise the complex in the broken conformation leading to persistent SSB. Their cytotoxicity is thought to be primarily due to collision of the replication fork with the cleavable complex forming a stalled replication fork and single-ended DNA double-strand break (DSB; reviewed in Pommier, 2006; Gilbert *et al*, 2012) and is highest during S phase. The cytotoxicity of the Topo I poisons correlates with the level of Topo I-generated DNA breaks, which is dependent on Topo I activity (Pfister *et al*, 2009). Topo I activity is higher in malignant cells and correlates with disease progression in colorectal and ovarian cancers, making it an attractive target for anticancer chemotherapy (van der Zee *et al*, 1991; Tsavaris *et al*, 2009; Smith *et al*, 2013). Camptothecin derivatives with improved pharmacological properties, irinotecan (Camptosar) and topotecan (Hycamptin), are used in the treatment of colorectal and ovarian cancers, respectively (Douillard *et al*, 2000). Targeting the repair of Topo I poison-mediated DNA damage may improve the activity of Topo I poisons.

Camptothecin-induced DNA lesions are repaired by overlapping DNA repair pathways. Topo I-associated SSBs are repaired by the base excision repair (BER) pathway (Caldecott and Jeggo, 1991; Barrows *et al*, 1998) and stalled replication forks and DSBs are primarily repaired by homologous recombination repair (HRR), including excision of Topo I from the DNA by the MRN exonuclease complex that initiates HRR (reviewed in Pommier, 2006; Gilbert *et al*, 2012). Base excision repair and HRR defects confer a five-fold and 10-fold sensitivity to CPT, respectively (Smith *et al*, 2005). Poly(ADP-ribose) polymerase-1 (PARP-1)

*Correspondence: Professor NJ Curtin; E-mail: nicola.curtin@ncl.ac.uk

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plays a major role in the sensing and repair of DNA SSBs and DSBs. It is a key component of BER and contributes to the restart of stalled replication forks during HRR (Bryant *et al*, 2009). PARP-1 is activated by CPT-induced DNA breaks and, via recruitment of XRCC1 (El-Khamisy *et al*, 2003) it promotes the cleavage of Topo I from the DNA by tyrosyl-DNA phosphodiesterase 1 (TDP1) and subsequent DNA repair (Plo *et al*, 2003). PARP reduces CPT-induced replication fork reversal and limits DNA strand breakage (Ray Chaudhuri *et al*, 2012). Genetic inactivation of PARP-1 sensitises cells to Topo I poisons (Chatterjee *et al*, 1989) and PARP-1 null mice are hypersensitive to Topo I poison toxicity (Burkle *et al*, 2000). PARP inhibitors (PARPi) enhance the cytotoxicity of Topo I poisons *in vitro* (Delaney *et al*, 2000; Bowman *et al*, 2001; Smith *et al*, 2005; Miura *et al*, 2012) and *in vivo* (Miknyoczki *et al*, 2003; Calabrese *et al*, 2004; Tentori *et al*, 2006). Enhancement of Topo I activity by PARPi has been associated with inhibition of DNA repair (Bowman *et al*, 2001; Smith *et al*, 2005). Although Topo I poison-induced DNA breakage and cytotoxicity in replicating and non-replicating cells has been studied, little is known about the potentiation by PARPi during different phases of the cell cycle.

PARPi are undergoing clinical evaluation, including combinations with Topo I poisons and initial reports indicate that the PARPi, ABT-888 (Veliparib) increased topotecan-induced DNA breaks in circulating tumour cells (Kummar *et al*, 2011). A greater understanding of the interaction of PARPi with Topo I poisons is needed to optimise the combination clinically. Here we demonstrate that the clinically active PARPi, rucaparib (AG-014699) increases total CPT-induced DNA breaks and inhibits their repair in all phases of the cell cycle but increased DSB and γH2AX formation predominantly in S phase.

MATERIALS AND METHODS

Chemicals. All chemicals and reagents, including tissue culture media, were provided by Sigma (Poole, Dorset, UK) unless otherwise stated. The PARP-1 inhibitor rucaparib(AG0140699: 1-(4 dimethylaminomethylphenyl)-8-9-dihydro-7H-2,7,9a-benzo(cd) azulen-6-one) provided by Agouron/Pfizer Pharmaceuticals GRD, La Jolla, CA, USA) was stored at −20°C at a concentration of 5 mM in water and used at a final concentration of 0.4 μM. Camptothecin was stored at −20°C as 10 mM aliquots in anhydrous DMSO.

Cytotoxicity assays. Human colon carcinoma (LoVo) and chronic myelogenous leukaemia (K562) cells were obtained from the ATCC (Manassas VA, USA), maintained at low in RPMI 1640 culture medium supplemented with 10% fetal calf serum passage and authenticated by STR profiling (LGC standards, Teddington, UK). Cell survival was determined by clonogenic survival assay following exposure of exponentially growing cells to CPT ± rucaparib as indicated in the Results section, prior to re-seeding for colony formation either directly (LoVo) or in 0.15% low melting point agarose (SeaKem ME Cambrex, Berks, UK) in medium (K562). Colonies were stained with crystal violet (LoVo) or MTT (K562).

Centrifugal elutriation. The use of chemicals, serum starvation or double thymidine block to synchronise cells have been criticised, not only for the cytotoxicity of these methods, but also their inability to genuinely synchronise cells (Cooper, 2003; Cooper *et al*, 2006, 2008). We therefore used centrifugal elutriation to separate the cells into different phases of the cell cycle. Cells were separated into G1, S and G2/M fractions using a Beckman Avanti J-20 centrifuge equipped with JE-5.0 elutriation rotor and Sanderson elutriation chamber (BeckmanCoulter.com). The apparatus was pre-sterilised with 6% H_2O_2, rinsed with sterile PBS and filled with sterile culture medium (flow rate 15 ml min^{-1}) and a rotor speed of 2500 rpm prior to injecting 1.5–2.5 × 10^8 cells in 5 ml medium into the system to equilibrate. Cell fractions were collected into 50 ml tubes by increasing the flow rate by 5 ml min^{-1} under sterile conditions. An aliquot of each fraction was stained with propidium iodide and analysed by flow cytometry (FASCcan, with CellQuest software, BD Biosciences, Oxford, UK) and processed on ModFit LT software (Verity Software, Topsham, ME, USA). The purest fractions enriched with cells in G1, S and G2 cell cycle phase were selected for subsequent experiments.

PARP activity. PARP activity in digitonin-permeabilised cells was measured by immunological detection of the ADP-ribose polymer product with the 10H antibody (kind gift from Dr A Burkle, University of Konstanz, Konstanz, Baden-Württemberg, Germany) after maximal stimulation of PARP activity with exogenous oligonucleotide in the presence of an excess of NAD^+ as previously described (Plummer *et al*, 2008).

Topo I activity. Topo I activity was measured using the Topo I relaxation activity kit (www.Topogen.com). Nuclear lysates were prepared in ice-cold TEMP buffer (10 mM Tris-HCL, pH = 7.5, 1 mM EDTA, 4 mM $MgCl_2$, 0.5 mM PMSF) by centrifugation at 1500 **g** for 10 min at 4°C followed by suspension in ice-cold TEP buffer (10 mM Tris-HCL, pH = 7.5, 1 mM EDTA, 0.5 mM PMSF) and an equal volume of 1 M NaCl for 40 min followed by centrifugation at 15 000 **g** for 30 min (4°C) to remove final debris. The protein concentration of the supernatant was measured by BCA assay (Fisher Scientific, Loughborough, UK) and reactions were performed according to manufacturer's instructions using proprietary supercoiled DNA and 1 μl of test extract. Reactions were terminated with loading buffer and loaded on to a 1% agarose gel. Electrophoresis was run at 2.5 V cm^{-1} in TAE buffer (40 mM Tris, pH = 8, 20 mM acetic acid, 1 mM EDTA). The gel was than stained with 0.5 μg ml^{-1} ethidium bromide for 20 min, images were captured using the GelDoc system (Bio-Rad, Hemel Hempstead, UK) and a trans illuminator and analysed by ImageJ (http://imagej.nih.gov/ij/; gel analysing options).

DNA breakage and stalled replication forks. DNA breaks were determined by single cell gel electrophoresis using the Trevigen Comet Assay Kit (Trevigen, AMS Biotechnologies, Oxford, UK) according to the manufacturer's protocol. All preparation was done under dimmed light to avoid additional DNA damage. Briefly, 5 × 10^5 cells, suspended in low melting point agarose were spread on the pre-coated wells of the comet slide and allowed to gel prior to lysis. To measure SSB ('total' breaks as a fraction will also be DSB and alkali-labile sites) comets slides were incubated in chilled alkaline solution (300 mM NaOH, 40 mM EDTA, pH > 13) for 30 min at 4°C to denature the DNA strands prior to electrophoresis in alkaline solution at a current of 200–300 mA under applied voltage 0.75 V cm^{-1} for 30 min. To measure DSB electrophoresis was carried out with 1 × TBE, pH = 10 buffer, which does not permit DNA denaturation, at 1 V cm^{-1} for 30 min. Breakage was assessed by determining the Olive tail moment (OTM: expressed as (tail mean − head mean) × % of DNA in the tail/100) in >100 cells for each experimental condition.

Camptothecin-induced DSB and stalled replication forks were also measured by immunofluorescence microscopy of H2AX phosphorylation (γH2AX; Furuta *et al*, 2003) in K562 cells deposited onto microscope coverslips using a Thermo-Shandon cytospin centrifuge (Fisher Scientific) and fixed in cold methanol at −20°C. Coverslips were incubated with anti-phospho serine 139 H2AX (clone JBW301, mouse monoclonal antibody; Upstate, Millipore Corp, Watford, UK) diluted 1 : 1000 in blocking buffer (10% milk, 0.1% TritonX-100) for 1 h at 37°C, and secondary antibody (goat polyclonal to Mouse IgG antibody Chromeo 546) at a dilution of 1 : 1000 for 1 h at 37°C, mounted onto microscope slides using Vector-shield Mounting Medium

(Vector Laboratories, Peterborough, UK). For each sample, two images of DAPI and two corresponding images of γH2AX were recorded on a Leica Epi-fluorescence Microscope using Image Spot Advance software and analysed by ImageJ and a custom macro, PZFociEZ (http://www.pzfociez.com/), to count the number of foci/nucleus in at least 100 nuclei per assay.

RESULTS

We investigated the potentiation of CPT by the PARPi, rucaparib in human colon carcinoma, LoVo cells, because the Topo I poison, irinotecan, is commonly used to treat colorectal cancer. There was a marked time-dependency of the sensitivity of these cells, with 99% of cells killed by a 24-h exposure to 10 nM CPT (Figure 1A) but only 45% killed by a 1-h exposure to the same concentration of CPT (Figure 1B). Indeed, there was a substantial CPT-resistant population as increasing the CPT concentration to 300 nM only resulted in 63% cell kill after 1 h (Figure 1B). There was a 1.5- to two-fold enhancement of CPT cytotoxicity by the PARPi, rucaparib, during the 24 h exposure but only a 1.1 to 1.3-fold enhancement after a 1-h exposure. We postulated that the difference in sensitivity to CPT after a 1-h compared to a 24-h exposure may have been due to the fraction of cells passing through S phase during the exposure period. LoVo cells have a cell cycle time of ~24 h with ~40% of cells in S phase (Supplementary Figure 1), suggesting that the cells killed by a 1-h exposure to CPT were the 40–45% of cells that passed through S phase during the exposure period. To investigate the cell cycle-dependency further we examined the cytotoxicity of CPT at different phases of the cell cycle. Rather than using synchronisation of the cells with cytotoxic chemicals or nutrient deprivation we separated the cells into different phases by centrifugal elutriation. Since this requires the cells to be in suspension we determined the cytotoxicity of a 1-h exposure of LoVo cells in suspension to CPT, with and without rucaparib, and the data were not significantly different from those obtained after exposure of adherent LoVo cells (Figure 1B). It proved difficult to obtain pure populations of cells in the different phases due to the high degree of aneuploidy in these cells (Supplementary Figure 1; Drewinko *et al*, 1976). Nevertheless, using cell cycle phase-enriched populations it was apparent, not only that CPT-induced cytotoxicity was greatest during S phase, but also that PARP inhibition only sensitised S-phase cells to CPT (Table 1).

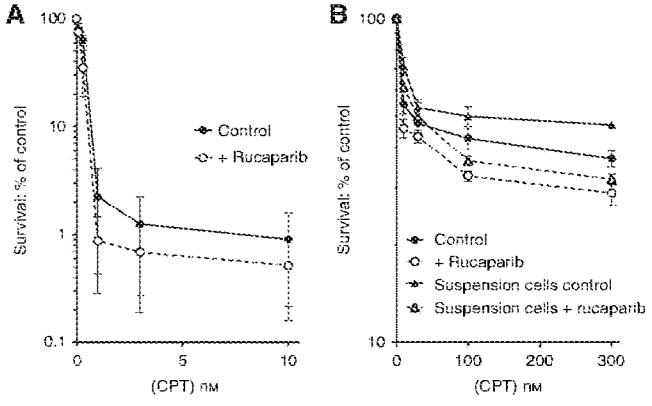

Figure 1. Effect of the PARP inhibitor, rucaparib, on clonogenic survival of LoVo cells. Exponentially growing LoVo cells were treated with increasing concentrations of CPT in the presence (○) or absence (●) of rucaparib for (A) 24 h or (B) 1 h. The comparison between incubating LoVo cells either as a monolayer or in suspension is also shown in (B), where cells in suspension were exposed to CPT in the presence (Δ) or absence (▲) of rucaparib.

Cells that have a stable modal chromosome number and are not highly aneuploid, such as the human myeloid leukaemia K562 cells, (Chen, 1985) make the best candidates for cell cycle phase separation by centrifugal elutriation and it was possible to obtain G1, S and G2 fractions that were 85% pure (Supplementary Figure 2). Following the cell cycle progression of the G1 fraction of K562 cells indicated that most cells remained in G1 at 2 h but 90% had progressed to S phase by 4 h (Supplementary Figure 3) and progression to G2 occurred between 8 and 10 h. Centrifugal elutriation did not affect the viability of these cells since the G1, S and G2 fractions grew at the same rate as the asynchronous, non-elutriated cells (Supplementary Figure 4) and it did not induce DNA strand breaks (Supplementary Figure 5). Previous studies have demonstrated that PARP inhibitors sensitise asynchronous K562 cells to CPT (Smith *et al*, 2005). We now show that, like the LoVo cells, not only are the S-phase K562 cells two-fold more sensitive to CPT alone, as expected, but also sensitisation by PARP inhibition was ⩾two-fold in the S-phase fraction but not significant in G1 or G2 (Figure 2).

A major determinant of sensitivity to CPT is Topo I activity, which we found to be greatest in S phase (Figure 3A and B), suggesting that this may contribute to the greater sensitivity of this fraction. Topo I activity appeared not to be related to Topo I protein levels as these showed a modest increase as the cells progressed from G1 to G2 (Supplementary Figure 6). Differences in Topo I activity did not seem to adequately explain the differential sensitivity of the different phases

Table 1. Effect of rucaparib on Topo I poison (CPT)-induced cytotoxicity in LoVo cells elutriated and separated into G1, S and G2 cell cycle phase-enriched fractions

	% Survival		
	G1	**S**	**G2**
30 nM CPT	53.2	36.1	57.4
30 nM + AG014699	58.3	21.9	57.5
Potentiation factor (at 50% survival)	**0.9**	**1.6***	**1**
100 nM CPT	45.3	26	58.5
100 nM + AG014699	59.3	13.6	53.6
Potentiation factor (at 50% survival)	**0.8**	**1.9***	**1.1**

Abbreviations: CPT = camptothecin; Topo I = topoisomerase I. Cells were separated by centrifugal elutriation into G1, S, and G2 phases prior to drug treatment. Data are means from 2 to 3 replicates in a single experiment where LoVo cells were elutriated and then treated for 1 h with CPT ± rucaparib before plating for colony formation. *$P<0.05$, other figures in bold are not significant.

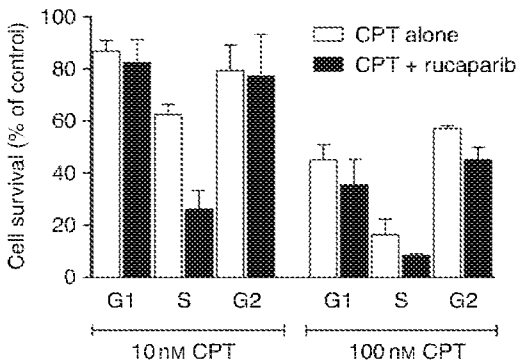

Figure 2. Cell survival elutriated K562 cells. Elutriated K562 cells in G1, S and G2 cell cycle phases were exposed to 10 or 100 nM CPT for 1 h in the presence (black bars) or absence (white bars) of 0.4 μM rucaparib. Cytotoxicity was measured by clonogenic assay and survival was expressed as percentage of untreated control. Data are mean of three independent experiments ± s.e.m.

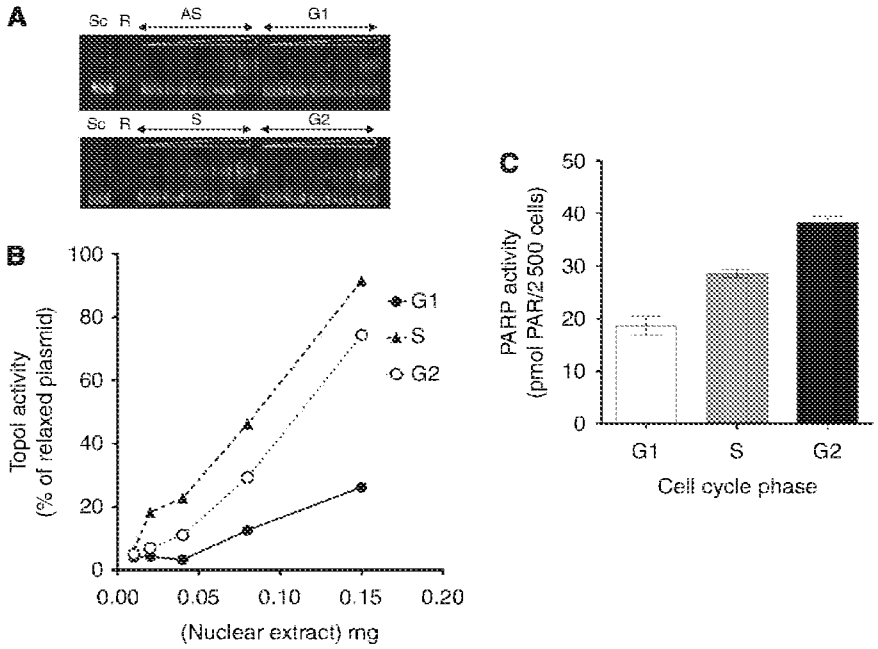

Figure 3. Topo I and PARP activity in G1, S and G2 elutriated K562 cells. (**A**) Nuclear extracts were prepared from asynchronous or elutriated cells. Approximately, 1 μl of the extracts (containing from 0.01 to 0.15 μg protein) was incubated with 1 μg of supercoiled plasmid and reaction products were separated on agarose gel. 'Sc' refers to a negative control supercoiled plasmid, and 'R' is a positive control of fully relaxed plasmid. Samples from asynchronous (AS), and G1, S and G2 are shown with increasing amount of protein extract, going from left to right. (**B**) the bands from A were quantified using ImageJ gel analysis, and Topo I activity is shown as the amount of relaxed DNA as a percentage of total DNA. (**C**) Exponentially growing K562 cells were elutriated and fractions containing >90% cells in G1, S and G2 cell cycle phases were used to measure PARP-1 activity (data are mean ± s.e.m. of three independent experiments).

as Topo I activity was around three-fold higher in G2 compared to G1 (Figure 3A and B), but the sensitivity of G1 and G2 fractions to CPT was similar (Figure 2). Also, changes in PARP activity in different phases of the cell cycle did not appear to be determined by PARP-1 protein levels (Supplementary Figure 6) and did not explain the greater sensitisation of S-phase cells: we found that PARP activity/cell increased throughout the cell cycle and was approximately twice as high in G2 compared to G1 (Figure 3C). This may reflect the increase in DNA content as PARP-1 is reported to be present at the concentration of 1 molecule per kB DNA (D'Amours *et al*, 1999).

Since the cytotoxicity of Topo I poisons is related to the number of DNA breaks induced, we determined whether CPT-induced DNA breakage in the presence and absence of rucaparib explained the cell cycle-dependent sensitivity of K562 cells. Measurement of DNA breakage by single cell gel electrophoresis (comet assay) under alkaline conditions measures DNA SSBs and DSBs and alkali-labile sites, however, the vast majority of the breaks will be single stranded. Camptothecin induced a concentration-dependent increase in breaks at all phases of the cell cycle (Figure 4A). DNA breakage was lowest in G1-phase cells with both S and G2-phase cells having substantially higher numbers of DNA breaks. PARP inhibition significantly increased DNA breakage by >two-fold ($P=0.08$ to 0.01) in all phases of the cell cycle (Figure 4A) such that DNA break levels induced by CPT+ rucaparib were similar in S and G2 and both were higher than in G1.

DNA DSBs are more profoundly cytotoxic than SSBs and the cytotoxicity of Topo I poisons is thought to be related to DSB formation at replication. Surprisingly, measurement of DSB formation by CPT indicated that they were only slightly higher in S-phase cells (Figure 4B). Rucaparib did not significantly increase the DSBs in G1-phase cells but increased the DSBs in S and G2-phase cells 1.5 to 1.8-fold ($P=0.03$). We also measured stalled replication forks together with DSBs by determining γH2AX foci formation following exposure to CPT and rucaparib. Not surprisingly, foci numbers per cell were lowest in G1-phase cells and highest in S-phase cells following exposure to CPT alone (Figure 4C). PARP inhibition barely altered the foci levels in G1-phase cells but caused a 1.7-fold and 2.4-fold increase in foci in S-phase cells exposed to 10 and 1000 nM CPT, respectively ($P<0.0001$ for both concentrations), with the corresponding increases in G2-phase cells being two-fold and 1.8-fold ($P=0.06$ and <0.0001, respectively). Cells exposed to the combination during S phase had the highest number of foci.

Although rucaparib increased DNA SSBs and DSBs to a similar extent in S- and G2-phase cells (Figure 4) it caused a much more profound cytotoxic sensitisation of S-phase cells (Figure 2) and our previous studies indicated that the effect of PARP inhibition on DNA repair was an important factor in CPT sensitisation (Smith *et al*, 2005). We therefore investigated whether DNA repair differed at different phases of the cell cycle and whether there was a cell cycle-dependent differential effect of rucaparib on repair. After a 2-h recovery period in drug-free medium there was substantial repair of the total breaks induced by a 30-min pulse of 100 nM CPT (Figure 5A). Repair was more rapid in S phase, with only 38% of breaks remaining at 2 h compared to 46 and 48% in G1 and G2, respectively. PARP inhibition retarded repair in all phases of the cell cycle but with a greater effect in S- and G2- phase cells. Investigation of DNA DSBs indicated that these too were rapidly repaired in S and G2 phases, such that only 25% of breaks remained after 2 h (Figure 5B). In G1-phase cells fewer DSBs were detected and they were repaired more slowly, with 55–60% of breaks remaining after 2 h incubation in fresh medium. Rucaparib inhibited repair at all phases of the cell cycle such that 75–80% of the breaks remained unrepaired after 2 h.

DISCUSSION

Topo I poisons have been in clinical use for several years, showing efficacy in colorectal tumours (Douillard *et al*, 2000). One

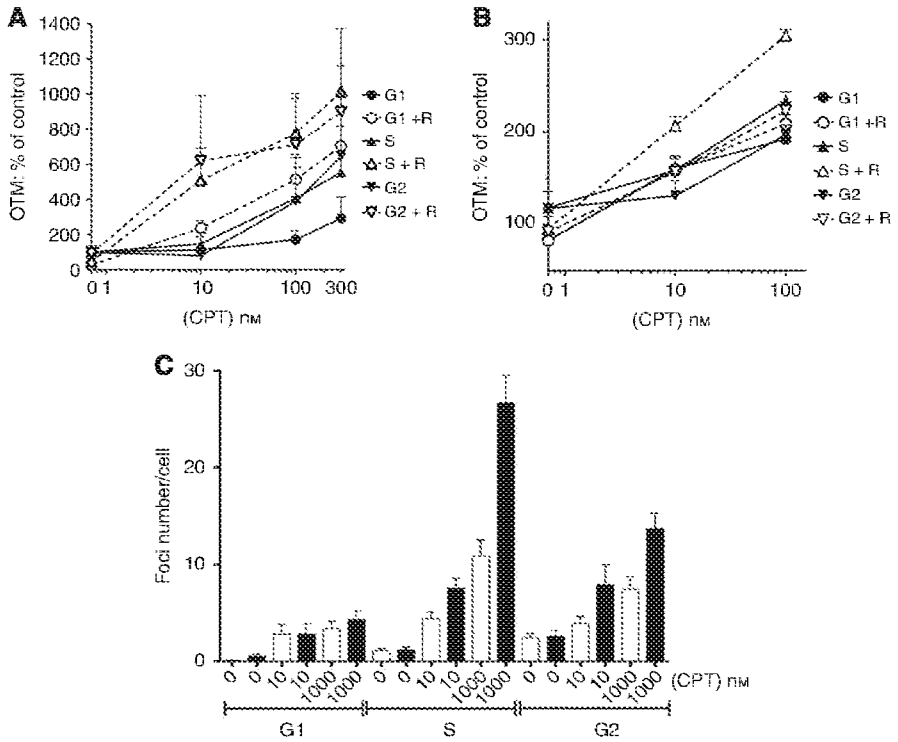

Figure 4. The effect of PARP inhibition on the level of DNA breaks in cell cycle phase-separated K562 cells. (**A**) Exponentially growing K562 cells were separated into cell cycle phases and exposed to increasing concentrations of CPT in the presence (open symbols) or absence (solid black symbols) of 0.4 μM rucaprib (R) for 30 min, prior to measurement of total DNA breaks by alkaline comet assay. Graphs showing mean Olive tail moment (OTM) ± s.e.m. from four independent experiments expressed as percentage of control untreated cells. (**B**) Similarly, elutriated (G1, S, and G2) K562 cells were exposed to increasing concentrations of CPT ± 0.4 μM rucaprib (R) for 30 min, and DNA DSB measured using the neutral comet assay. Data are mean + s.e.m. from single representative experiment expressed as a percentage of control. (**C**) K562 cells were treated with increasing concentrations of CPT in the presence (black bars) or absence (white bars) of 0.4 μM rucaparib for 30 min, and then separated into individual cell cycle phases and stained for the presence of γH2AX foci. Foci number per cell was measured using ImageJ and the macro PZFociEZ, as described in methods. Data show mean ± 95% confidence interval calculated from at least 100 cells per treatment, from one experiment (representative of three individual experiments).

promising new way to improve the effectiveness of Topo I poison therapy is the use of PARPi. PARPi are currently undergoing clinical trial but their use in combination with Topo I poisons has not been optimised (Gilbert *et al*, 2012). Veliparib has been investigated in phase I clinical trials with both topotecan and irinotecan, and olaparib with topotecan (Kummar *et al*, 2011; LoRusso *et al*, 2011; Samol *et al*, 2012). In the topotecan study, veliparib reduced PARP activity in both tumour and peripheral blood mononuclear cells (PBMC), increased DNA breaks in circulating tumour cells and PBMCs, and importantly resulted in some disease stabilisation. However, in all these studies the toxicities associated with the Topo I poison were exacerbated, indicating that improvements in either dosing or scheduling are needed.

Initially our studies focussed on the colorectal cell line, LoVo. Results revealed that CPT-induced cytotoxicity was both time- and concentration-dependent, with virtually all cells killed by low concentrations (10 nM) of CPT when cells were exposed for a complete cell cycle. However only ~50% of cells were killed by a short pulse with much higher concentrations of CPT, where the plateau in survival suggested a resistant subpopulation. On the basis of published literature, and our data showing that 40% of Lovo cells are in S phase, we assumed that the S-phase cells represented the sensitive fraction. We estimated that only 40–50% of cells would pass through S phase and be killed during the 1-h exposure period, compared to virtually all cells passing through S phase over 24 h. Inhibition of PARP-1 activity by rucaparib cells resulted in a two-fold sensitisation of CPT-induced cytotoxicity during 24-h exposure, consistent with previous reports using other PARP inhibitors and cell lines (Bowman *et al*, 2001; Calabrese *et al*, 2004; Bryant *et al*, 2009). Potentiation of CPT-induced cytotoxicity during a short pulse exposure appeared to be slightly greater than at 24 h, suggesting that potentiation may be greater for S-phase cells.

We used centrifugal elutriation, to separate the cells into different phases of the cell cycle. This technique did not cause DNA damage and, since the S-phase fraction did not have a higher alkaline comet OTM, Okazaki fragments formed during S phase did not contribute to comet tails. Neither did it affect cell viability. In both Lovo and K562 cells CPT was substantially more cytotoxic to S-phase cells than to G1- or G2-phase cells. Rucaparib did not significantly enhance CPT cytotoxicity to G1- and G2-phase cells in either of the cell lines but caused an approximately two-fold sensitisation of CPT in S-phase Lovo and K562 cells. The S-phase specificity of enhancement by PARP-1 inhibitors is not exclusive to Topo I poisons: veliparib potentiated the DNA methylating agent, temozolomide to a much greater extent in S phase compared to G1 (Liu *et al*, 2008).

Increased sensitivity to Topo I poisons has been related to elevated levels of Topo I (Pfister *et al*, 2009). We found that Topo I activity was highest in S-phase K562 cells, linking the increased sensitivity to CPT during this phase to increased Topo I activity. However, Topo I activity in G2 was greater than in G1 but the cytotoxicity was similar in both phases. The high Topo I activity in G2 was associated with a higher level of DNA SSB but not the much more cytotoxic DSB. Cytotoxicity may be related more to DSB and particularly collapsed replication forks that were highest

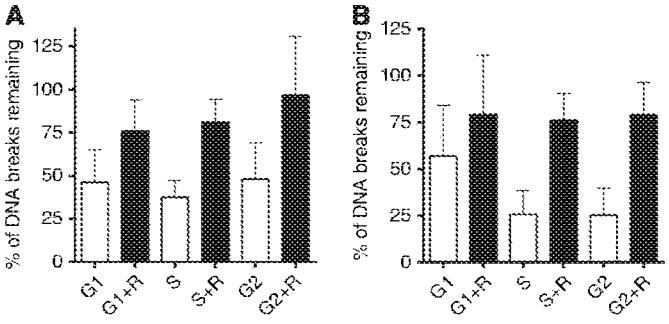

Figure 5. The effect of PARP-1 inhibition on DNA strand break repair in elutriated K562 cells. (**A**) K562 cells were separated into G1, S and G2 phases, and treated with 100 nM CPT for 30 min (black bars). After 2 h of incubation in drug-free medium (white bars) or rucaprib (R)-containing medium (grey bars) the break levels were measured by alkaline comet assay. The level of total DNA breaks at the end of 2-h recovery period was evaluated by expressing the breaks remaining as percentage of those at the end of the 30 min CPT exposure (time 0) using the formula: $100 \times T/C \times \sqrt{((t/T)^2 + (c/C)^2)}$ where c is s.d. of time 0 (C) and t is s.d. of post 2 h of recovery (T). Data showed mean + s.e.m. of three independent experiments. (**B**) Similarly, the effect of rucparib on the repair of double-strand breaks was measured using the neutral comet assay. K562 cells were separated into G1, S and G2 phases, and treated with 100 nM CPT for 30 min (black bars). After 2 h of incubation in drug-free medium (white bars) or rucaparib (R)-containing medium (grey bars) the percentage of DSBs remaining were assessed as described in A.

in S phase. In contrast, PARP activity increased throughout the cell cycle, and since the level in G2 was two-fold higher than in G1, this may be related to DNA content. Cell cycle phase changes in PARP-1 and Topo I activity were not related to the levels of PARP-1 or Topo I expression suggesting that both proteins are regulated by posttranslational modification. The highest activity of PARP occurred in G2 phase, but the effect of rucaparib on Topo I activity was no greater in G2 than in other phases, consistent with our previous observations that PARP inhibition did not increase Topo I activity (Smith *et al*, 2005).

Investigation of the mechanism underlying the greater potentiation of CPT cytotoxicity in S phase by PARP inhibition focussed on measuring the induction of DNA breaks and their repair. Alkaline comets measure total DNA breakage, but in the context of Topo I poisons, these will be largely SSB. The data indicated that although more SSBs were formed in S and G2 phases (possibly as a function of both the Topo I activity and DNA content), rucaparib increased these CPT-induced breaks to a similar degree in all phases of the cell cycle. Rucaparib also inhibited the repair of these lesions to a similar extent in all phases of the cell cycle. Therefore, it does not appear that the effect of rucaparib on SSB levels is responsible for the S-phase-specific sensitisation.

During S phase, the CPT-stabilised Topo I-DNA complexes can be converted into highly cytotoxic collapsed replication forks and DSBs. The hypothesis that these lesions were more closely associated with chemosensitisation was investigated using two methods; neutral comet assay and γH2AX focus formation. These complementary assays measure subtly different end points: neutral comet assays measure the migration of broken DNA into a gel under an electric current and are analogous to pulse-field gel electrophoresis (Olive, 2009) and the γH2AX focus assay measures the phosphorylation of H2AX by DNA damage-activated kinases (ATM, ATR and DNA-PK) and is therefore a sensitive measure of collapsed replication forks as well as DSBs (Darzynkiewicz *et al*, 2009). The advantage of both these methods is that analysis of individual cells is made allowing population dynamics to be discovered. Measurement of DSB induction by neutral comet assays revealed that they were indeed higher during S phase and that rucaparib significantly increased the level of DSBs in S phase. Similar studies showed that increased cytotoxicity of temozolomide by the PARP inhibitor ABT-888 in S phase was correlated with the level of DSB (Liu *et al*, 2008). We found that γH2AX foci were low in G1 and highest in S phase as expected, and very substantially increased by rucaparib in S phase. However, the increase in γH2AX foci in G2 cells was not accompanied by a corresponding increase in the induction of DSB as measured by neutral comets. Recent data implicate the XPF-ERCC1 endonuclease in an alternative pathway to PARP-TDP-1 for the removal of CPT-stabilised Topo I-DNA complexes and the induction of γH2AX foci (Zhang *et al*, 2011). XPF-ERCC1 is key to nucleotide excision repair of UV damage, which is known to induce γH2AX foci (Revet *et al*, 2011). It is possible that PARP inhibition shifts repair to this pathway leading to an increase in γH2AX foci and our data would suggest that this occurs in S and G2. Interestingly, the increase in γH2AX foci in G2 by rucaparib was not accompanied by a significant increase in cytotoxicity. However, the role of XPF-ERCC1-mediated repair of CPT-induced DNA damage and cytotoxicity is not entirely clear as although XPF knockdown was shown to reduce γH2AX foci it only caused a very marginal increase in CPT sensitivity, and ABT-888 sensitised cells irrespective of XPF levels (Zhang *et al*, 2011).

Our data indicate that although PARP inhibition does not cause a greater induction of DSB in G2 it does impede DNA DSB resolution in G2 as well as S phase, but this is only critical to survival in S phase. Recent data suggest that CPT causes replication fork slowing (independently of DSB formation) and that PARP is needed to reverse arrested replication forks after exposure to Topo I poisons, such that PARP inhibition prevented fork reversal and restart leading to a greater accumulation of γH2AX foci (Ray Chaudhuri *et al*, 2012). Considering our data in the light of the model proposed by (Ray Chaudhuri *et al*, 2012) and the established role of PARP in the repair of Topo I poison-induced damage by BER, together with the mechanisms described by (Zhang *et al*, 2011), we propose a modified model (Figure 6). In this model Topo I-associated SSB are repaired by PARP and XRCC1-mediated sequential recruitment of TDP-1 then DNA Polβ and ligase I to execute repair. In the presence of rucaparib, SSB persist in G1 but in G2 XPF-ERCC1 may excise a portion of Topo I-bound DNA, creating NER intermediates that contribute to the measurement of SSB in alkaline comets and also activate H2AX phosphorylation. In S phase the DNA SSB stall replication forks and convert to DSB that activate H2AX phosphorylation. As the increase in γH2AX foci was the most remarkable effect of rucaparib during S phase, we suggest that inhibition of replication fork restart has the most profound implications for Topo I poison cytotoxicity.

The data presented here show that PARP inhibition causes S-phase-specific chemosensitisation of Topo I poisons that is related to the impact of PARP inhibition on stalled replication forks. This has clinical implications suggesting that rapidly growing tumours would be most sensitive to the combination, and that scheduling is critical to ensure both drugs are present for long enough for all tumour cells to enter S phase. Cancer cells generally have dysfunctional DNA cell cycle control and/or repair pathways, which underlie their differing vulnerabilities to a spectrum of cytotoxic agents (Curtin, 2012). We anticipate, but have not tested directly, that normal cells would also be most sensitive to Topo I poisons, alone and in combination with PARPi, during S phase. Most normal cells are in G1/G0 and replicating normal cells generally enter S phase in a synchronous fashion (Mormont and Levi, 2003) including cells in the gut mucosa, the site of dose-limiting toxicity by Topo I poisons. In mice (a nocturnal species) the peak in S phase occurs at 1.00 am and Topo I poisons are profoundly toxic when administered at 0200 hours (15% survival)

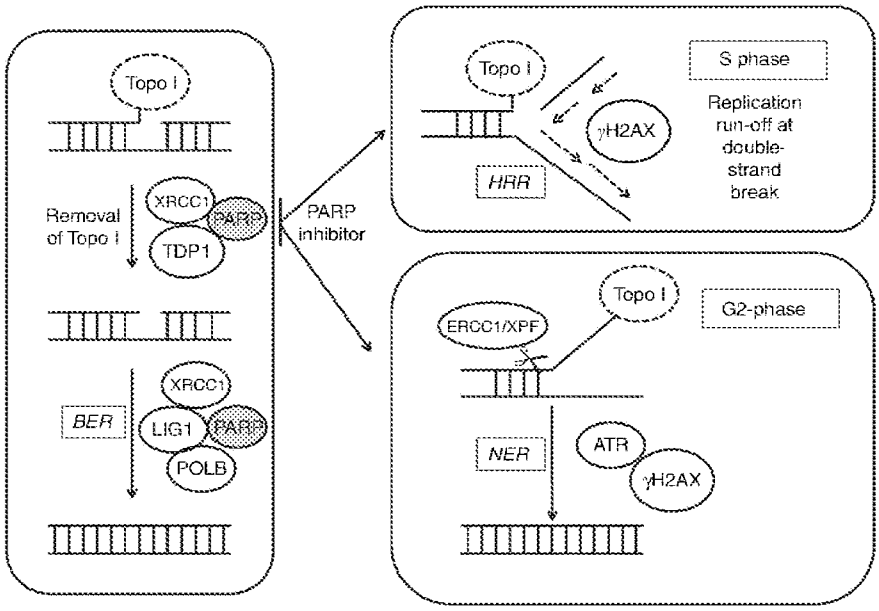

Figure 6. A model for repair of Topo I-induced lesions during the cell cycle. Topo I poisons (e.g., camptothecin) stabilise the covalent complex between DNA and Topo I protein. Recruitment of TDP1 (which hydrolyses the 3′-phosphotyrosyl bond that links Topo I to the DNA) is mediated via PARP and XRCC1. Base excision repair (BER) is completed by PARP and XRCC1 in concert with in-filling activity of POLB and ligase1. The presence of a PARP inhibitor results in the persistence of SSB in G1, and during S phase and homologous recombination repair (HRR), the SSB stall replication forks and create DSB that stimulate phosphorylation of H2AX. In G2, PARP inhibition may lead to activation of XPF/ERCC1-mediated nucleotide excision repair (NER) to remove the Topo I-bound DNA, which creates a NER intermediate that activates H2AX phosphorylation.

compared to 1400 hours (90% survival; reviewed in Rich *et al*, 2002). Similarly, in humans the peak in S phase is around midday (Smaaland *et al*, 2002) and in clinical trials irinotecan toxicity was less toxic if administered at 0500 hours. These data support the hypothesis that normal cells are also more sensitive to Topo I poisons during S phase and we would predict that the combination of a Topo I poison and a PARPi would also be more toxic in this phase. Using a chronotherapy approach it may therefore be possible to schedule the treatment to target the cancers, which are asynchronous but spare replicating normal cells, which generally enter S phase in a synchronous manner.

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First Named Inventor/Applicant Name:	Eliel Bayever
Customer Number:	153749
Filer:	Mary Rucker Henninger/Richard King
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APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $80 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $420 =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT	01/07/2020	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 18	Minus	** 20	= 0		x $100 =	0
	Independent (37 CFR 1.16(h))	* 2	Minus	*** 3	= 0		x $460 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	0

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.	SLIE
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".	/APRIL L. WALKER/
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".	
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.	

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/809,815	11/10/2017	Eliel Bayever	263266-421428	5137

153749 7590 02/27/2020

McNeill Baur PLLC/Ipsen
Ipsen Bioscience, Inc.
125 Cambridge Park Drive
Suite 301
Cambridge, MA 02140

EXAMINER	
RONEY, CELESTE A	

ART UNIT	PAPER NUMBER
1612	

NOTIFICATION DATE	DELIVERY MODE
02/27/2020	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docketing@mcneillbaur.com
eofficeaction@appcoll.com
patents.us@ipsen.com

Office Action Summary	Application No. 15/809,815	Applicant(s) Bayever et al.	
	Examiner CELESTE A RONEY	Art Unit 1612	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 07 January 2020.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☑ This action is **FINAL.** 2b) ☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 1,4-15,18-19 and 21-23 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 1,4-15,18-19 and 21-23 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date 1/7/2020(4); 1/9/2020 (4).

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

DETAILED ACTION

Previous Rejections

Applicant's arguments, filed 01/07/2020, have been fully considered. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn. The following rejections and/or objections are either reiterated or newly applied. They constitute the complete set presently being applied to the instant application.

Claim Rejections - 35 USC § 103 - Obviousness

The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent for a claimed invention may not be obtained, notwithstanding that the claimed invention is not identically disclosed as set forth in section 102, if the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 1, 5-8, 10 and 19 are rejected under 35 U.S.C. 103 as being unpatentable over Bayever et al (WO 2013/188586), in view of Conroy et al (NEJM, 34(19), 2011, 1817) and further in view of Melis et al (The Society for Surgery of the Alimentary Tract, 2011; http://meetings.ssat.com/abstracts/11ddw/P57.cgi).

Bayever et al disclosed a method for treatment of pancreatic cancer in a patient (e.g., a human, at page 3, 1st paragraph), comprising co-administering to the patient active agents, at a dose of 60 mg/m^2 (e.g., liposomal irinotecan). Bayever further disclosed 5-fluorouracil at a dose of 2400 mg/m^2 and leucovorin (*l* form administered at 200 mg/m^2 or the *l*+*d* racemic form administered at 400 mg/m^2). The method comprised

at least one cycle of administration, wherein the cycle was a period of two weeks (page 3, last full paragraph).

In one embodiment, Bayever's population was patients undergoing treatment for metastatic adenocarcinoma pancreatic cancer (e.g. a patient who has not previously received an antineoplastic agent) (page 12, section V, last embodiment, and claim 10).

Bayever did not disclose oxaliplatin, as recited in claim 9.

Conroy disclosed FOLFIRINOX (oxaliplatin; irinotecan; leucovorin and fluorouracil) treatment of patients having metastatic pancreatic cancer (title and the methods section of the abstract). Conroy disclosed that oxaliplatin has clinical activity against pancreatic cancer only when combined with fluorouracil, and that oxaliplatin and irinotecan have been shown to have synergistic activity *in vitro* (page 1818, left column, second paragraph).

Conroy did not disclose that the irinotecan was liposomal irinotecan.

Since Bayever disclosed treating metastatic pancreatic carcinoma with 5-fluorouracil and irinotecan, it would have been prima facie obvious to one of ordinary skill in the art to include oxaliplatin within Bayever's methods of treatment. An ordinarily skilled artisan would have been motivated because oxaliplatin has clinical activity against pancreatic cancer when combined with fluorouracil, and because oxaliplatin and irinotecan have synergistic activity *in vitro*, as taught by Conroy (Conroy, page 1818, left column, second paragraph).

Regarding the claims 1 and 19 limitation of 60 mg/m^2 oxaliplatin, the combination of Bayever (e.g., Bayever taught 85 mg/m^2 oxlaplatin at the abstract), though not silent the claimed amount of oxaliplatin, does not specifically teach 60 mg/m^2 oxaliplatin.

However, Melis taught [abstract] that a dosage of 60 mg/m^2 oxaliplatin was well tolerated in advanced pancreatic adenocarcinoma patients.

As such, oxlaplatin, and its amount, is recognized to have different effects (treatment of advanced pancreatic adenocarcinoma) with changing amounts used. Thus, the general condition (the dosage) is known and the amount of this ingredient is recognized to be result effective. Therefore, result effective variables can be optimized by routine experimentation, and it would have been prima facie obvious to optimize the dosage of the oxaliplatin present in the combined composition of Bayever and Conroy, as taught by Melis.

The combination of Bayever, Conroy and Melis reads on claims 1 and 19.

Claims 5-6 and 8 are rendered prima facie obvious because Bayever disclosed that 5-fluorouracil was administered intravenously over 46 hours, liposomal irinotecan was administered intravenously over 90 minutes, and that leucovorin was administered prior to 5-FU (page 12, section IV).

Claim 7 is rendered prima facie obvious because Bayever disclosed that active agents were administered on day one of a two-week cycle, where cycles comprised at least one administration. For example, Bayever's method overlaps that which is instantly recited (e.g. administration on days 1 and 15 of a 28-day cycle), because administration on day 1 of at least one 2-week cycle can also be administration on days 1 and 15 of a 28 day cycle (e.g. two 2-week cycles). In the case where the claimed ranges

"overlap or lie inside ranges disclosed by the prior art", a prima facie case of obviousness exists. MPEP 2144.05 A.

Claim 10 is rendered prima facie obvious because Bayever disclosed irinotecan sucrose octasulfate liposomal irinotecan, where the irinotecan was entrapped within the liposome, at page 4, and the last paragraph.

Response to Arguments

Applicant's arguments filed 01/07/2020 have been fully considered but they are not persuasive.

Applicants argued that neither Bayever, Conroy, nor Melis teach or suggests (solely or in combination) the claimed methods of treating a patient with metastatic adenocarcinoma of the pancreas who has not previously been treated with an antineoplastic agent (claim 1) or gemcitabine (claim 19), comprising co-administering to the patient 60 mg/m^2 liposomal irinotecan, 60 mg/m^2 oxaliplatin, leucovorin (200 mg/m^2 l-form or 400 mg/m^2 l+d form), and 2,400 mg/m^2 5-fluorouracil once every two weeks.

The Examiner disagrees that the combined prior art does not teach the claimed invention (claims 1 and 19). The combination of Bayever, Conroy and Melis reads on claims 1 and 19, the discussion of which was presented above.

Applicants argued that the Examiner has failed to establish a prima facie case of obviousness of the claimed methods, over the rejection of Melis.

The Examiner disagrees that a prima facie case of obviousness over Melis was not presented. Melis was relied upon to show that the dosage of oxaliplatin is a result effective variable. Result effective variables can be optimized by routine experimentation,

and it would have been prima facie obvious to optimize the dosage of the oxaliplatin present in the combined composition of Bayever and Conroy, as taught by Melis.

Regarding Melis, the Applicants argued that (1) the Melis Study involved patients with locally advanced pancreatic cancer and excluded patients with metastatic disease; (2) in contrast to the "once every two weeks" coadministration schedule recited in the pending claims, the Melis Study involved weekly administration of 60 mg/m^2 oxaliplatin; (3) the Melis study included continuous infusion of 200 mg/m^2 5-fluorouracil compared to the claimed coadministration of 2,400 mg/m2 5-fluorouracil once every two weeks; (4) the Melis treatment regime did not result in improved outcomes compared to other combination therapies for locally advanced pancreatic cancer and (5) "those who remained unresectable for cure but did not progress received a treatment regime involving a higher 85 mg/m^2 oxaliplatin dose every two weeks.

The Examiner responds that Melis was relied upon to show that the dosage of oxaliplatin is a result effective variable that can be optimized by routine experimentation (discussed above). Furthermore, cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986); & MPEP 2145(IV)].

In response to Applicant's argument that the Examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned

only from the applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971).

Applicants argued that the Examiner failed to account for the many factors, such as patient population, disease severity, drug combination, dose, dosing schedule, drug interactions and toxicities that affect tolerability and efficacy of a cancer treatment method.

The Examiner responds that the skilled artisan would be led by Bayever's guidance of study design and patient selection [pages 25-31], at the discretion of the investigator, to account for factors affecting tolerability and efficacy of the protocol.

Applicant reiterated that the skilled artisan would not be motivated to combine Melis with Bayever and Conroy, to which the Examiner disagrees. The combination of Bayever and Conroy taught oxaliplatin, though not specific to the instantly claimed amount. Melis was presented to show that the dosage of oxaliplatin could be optimized without undue experimentation, and with a reasonable expectation of success (discussed above).

Applicants argued that Melis excluded patients with metastatic disease, to which the Examiner responds that the rejection was based upon the combination of the prior art. Bayever taught patients undergoing treatment for metastatic cancer.

Claims 4, 9, 18 and 23 are rejected under 35 U.S.C. 103 as being unpatentable over Bayever et al (WO 2013/188586), in view of Conroy et al (NEJM, 34(19), 2011,1817) further in view of Melis et al (The Society for Surgery of the Alimentary Tract, 2011; http://meetings.ssat.com/abstracts/11ddw/P57.cgi) and further in view of Fleming et al

(http://www.oncologynurseadvisor.com/advisor-forum/importance-of-sequence-in-chemotherapy-administration/article/378072/).

The 35 U.S.C. 103 rejection over Bayever, in view of Conroy and Melis, has been discussed above.

Additionally, Bayever disclosed that prior to each administration of liposomal irinotecan, the patient was pre-medicated with dexamethasone (e.g. corticosteroid) and another anti-emetic (page 4, fourth embodiment from the top of the page).

Further, Conroy disclosed that a second active agent was given two hours after a first active agent (e.g., leucovorin was given two hours after oxaliplatin) (page 1819, 1st paragraph of the section entitled Treatment).

However, the combination of Bayever and Conroy did not specifically disclose oxaliplatin administration after liposomal irinotecan, as recited in claims 4, 18 and 23; liposomal irinotecan administration, followed by oxaliplatin administration, followed by leucovorin administration, followed by 5-fluorouracil administration, as recited in claim 9.

Fleming disclosed that the sequence of various chemotherapy drugs in general does not matter, as the half-life of each drug makes it impossible to determine what drug is at what level at any particular time, based on individual patient pharmacodynamics (last sentence of the first paragraph).

Since the combination of Bayever and Conroy disclosed administration of oxaliplatin, liposomal irinotecan, leucovorin and 5-fluorouracil, it would have been prima facie obvious to one of ordinary skill in the art to have varied the order of administration of the combined methods of Bayever and Conroy, such that the order of

administration was liposomal irinotecan, followed by oxaliplatin, followed by leucovorin, followed by 5-fluorouracil administration.

An ordinarily skilled artisan would have been motivated because the sequence of various chemotherapy drugs in general does not matter, as the half-life of each drug makes it impossible to determine what drug is at what level at any particular time, based on individual patient pharmacodynamics, as taught by Fleming (Fleming, last sentence of the first paragraph).

Response to Arguments

Applicant's arguments filed 01/07/2020 have been fully considered but they are not persuasive.

The Applicants reiterated the above arguments regarding a failing to show a prima facie case of obviousness and hindsight reasoning, to which the Examiner disagrees. A prima facie case of obviousness to combine each of the prior art was previously discussed. The Examiner disagrees that hindsight reasoning was used in the rejection of the claims, as discussed above. A motivation to combine the prior art (discussed above) was used in the rejection of the claims.

Claims 11-15 and 21-22 are rejected under 35 U.S.C. 103 as being unpatentable over Bayever et al (WO 2013/188586), in view of Conroy et al (NEJM, 34(19), 2011, 1817), further in view of Melis et al (The Society for Surgery of the Alimentary Tract, 2011; http://meetings.ssat.com/abstracts/11ddw/P57.cgi) and as evidenced by Bayever et al (WO 2016/094402).

The 35 U.S.C. 103 rejection over Bayever (2013), in view of Conroy and Melis, has been discussed above.

Although, Bayever (2013) disclosed MM-398 liposome (at page 4, last paragraph and as discussed above), Bayever was not specific as to the ingredients of the liposome, as recited in claims 11-12 and 21-22.

However, Bayever (2016) evidenced that MM-398 contained irinotecan sucrose octasulfate, DSPC, cholesterol and MPEG-2000-DSPE (page 30, section describing the drug product).

Thus, it is reasonable to assume that Bayever's (2013) MM-398 contained irinotecan, DSPC, cholesterol and MPEG-2000-DSPE, as evidenced by Bayever's (2016) disclosure of the liposomal constituents of MM-398.

Claims 13-15 and 21-22 are rendered prima facie obvious because Bayever disclosed that 5-fluorouracil was administered intravenously over 46 hours, liposomal irinotecan was administered intravenously over 90 minutes; liposomal irinotecan was administered prior to leucovorin; leucovorin was administered prior to 5-FU (page 12, section IV). Further, Bayever disclosed that active agents were administered on day one of a two-week cycle, where cycles comprised at least one administration.

For example, Bayever's method overlaps that which is instantly recited (e.g. administration on days 1 and 15 of a 28-day cycle) because administration on day 1 of at least one 2-week cycle can also be administration on days 1 and 15 of a 28-day cycle (e.g. two 2-week cycles). A prima facie case of obviousness exists because of overlap, as discussed above.

Response to Arguments

Applicant's arguments filed 01/07/2020 have been fully considered but they are not persuasive.

The Applicants reiterated the above arguments regarding a failing to show a prima facie case of obviousness and hindsight reasoning, to which the Examiner disagrees. A prima facie case of obviousness to combine each of the prior art was previously discussed. The Examiner disagrees that hindsight reasoning was used in the rejection of the claims, as discussed above. A motivation to combine the prior art (discussed above) was used in the rejection of the claims.

Nonstatutory Double Patenting

A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly

owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(l)(1) - 706.02(l)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Claims 1, 4-15, 18-19 and 21-23 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-18 of U.S. Patent No. 9,492,442, in view of Conroy et al (NEJM, 34(19), 2011, 1817) and further in view of Melis et al (The Society for Surgery of the Alimentary Tract, 2011; http://meetings.ssat.com/abstracts/11ddw/P57.cgi)

Although the claims at issue are not identical, they are not patentably distinct from each other. The issued claims recite all of the features instantly recited for the method of

treatment except for the administration of oxaliplatin. The instant claims require oxaliplatin, and such an ingredient is not recited by the issued claims.

Conroy disclosed FOLFIRINOX (oxaliplatin; irinotecan; leucovorin and fluorouracil) treatment of patients having metastatic pancreatic cancer (title and the methods section of the abstract). Conroy disclosed that oxaliplatin has clinical activity against pancreatic cancer only when combined with fluorouracil, and that oxaliplatin and irinotecan have been shown to have synergistic activity *in vitro* (page 1818, left column, second paragraph).

Melis taught [abstract] that a dosage of 60 mg/m^2 oxaliplatin was well tolerated in advanced pancreatic adenocarcinoma patients.

Thus, it would have been prima facie obvious to use oxaliplatin in the issued method, because oxaliplatin has clinical activity against pancreatic cancer only when combined with fluorouracil, and because oxaliplatin and irinotecan have been shown to have synergistic activity *in vitro*. It would have been prima facie obvious to use oxaliplatin at 60 mg/m^2 because the said dosage is well tolerated in advanced pancreatic adenocarcinoma patients.

Response to Arguments

Applicant's arguments filed 01/07/2020 have been fully considered but they are not persuasive.

The Applicants reiterated the above arguments regarding a failing to show a prima facie case of obviousness, to which the Examiner disagrees. A prima facie case of obviousness to combine each of the prior art was previously discussed.

Conclusion

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to CELESTE A RONEY whose telephone number is (571)272-5192. The examiner can normally be reached on Monday-Thursday; 7 AM-5 PM.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Frederick Krass can be reached on 571-272-0580. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see https://ppair-my.uspto.gov/pair/PrivatePair. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/CELESTE A RONEY/
Primary Examiner, Art Unit 1612

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/809,815	Bayever et al.
	Examiner	Art Unit
	CELESTE A RONEY	1612

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
west and palm searches	02/21/2018	CR
west and palm searches	09/02/2018	CR
west and palm searches	07/01/2019	CR
west and palm searches	02/20/2020	CR

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

/CELESTE A RONEY/ Primary Examiner, Art Unit 1612	

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	15809815
Filing Date	2017-11-10
First Named Inventor	Eliel Bayever
Art Unit	1612
Examiner Name	Celeste A. RONEY
Attorney Docket Number	01208-0007-01US

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Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	10350201	B2	2019-07-16	Hong et al.	
	2	10413510	B2	2019-09-17	Hong et al.	
	3	4604463	A	1986-08-05	Miyasaka et al.	
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	6	5192549	A	1993-03-09	Barenolz et al.	
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	9	5593622	A	1997-01-14	Yoshioka et al.	
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See attached certification statement.

The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Mary R. Henninger/	Date (YYYY-MM-DD)	2020-01-07
Name/Print	Mary R. Henninger	Registration Number	56992

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/CELESTE A RONEY/ 02/20/2020

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Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (02-18)
Approved for use through 11/30/2020. OMB 0651-0031
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	15809815
Filing Date	2017-11-10
First Named Inventor	Eliel Bayever
Art Unit	1612
Examiner Name	Celeste A. RONEY
Attorney Docket Number	01208-0007-01US

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Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

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	Examiner Name	Celeste A. RONEY
	Attorney Docket Number	01208-0007-01US

	1	ADIWIJAYA B, et al., "Population Pharmacokinetics of Liposomal Irinotecan in Patients With Cancer," Clin Pharmacol Ther. 102(6):997-1005 (2017).
	2	CHEN L, et al., "Effect of Baseline Carbohydrate Antigen 19-9 (CA19-9) Level on Overall Survival (OS) in NAPOLI-1 Trial: a Phase 3 Study of MM-398 (nal-IRI), with or without 5-Fluorouracil and Leucovorin (5-FU/LV), versus 5-FU/LV in Metastatic Pancreatic Cancer (mPAC) Previously Treated with Gemcitabine-based Therapy." Poster handout at the Gastrointestinal Cancers Symposium of the ASCO meeting of January 21-23, 2016, San Francisco, California, 2 pages.

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EXAMINER SIGNATURE

Examiner Signature	/CELESTE A RONEY/	Date Considered	02/20/2020

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

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CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

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See attached certification statement.

The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Mary R. Henninger/	Date (YYYY-MM-DD)	2020-01-09
Name/Print	Mary R. Henninger	Registration Number	56992

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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	1	VERREAULT M, et al., "Vascular Normalization in Orthotopic Glioblastoma Following Intravenous Treatment with Lipid-Based Nanoparticulate Formulations of Irinotecan (Irinophore C™), Doxorubicin (Caelyx®) or Vincristine," BMC Cancer. 11:124, pages 1-18 (2011).
	2	WATERHOUSE D, et al., "Lipid-Based Nanoformulation of Irinotecan: Dual Mechanism of Action Allows for Combination Chemo/Angiogenic Therapy," Nanomedicine 6(9):1645-54 (2011).
	3	WILSON W, et al., "Targeting Hypoxia in Cancer Therapy," Nat Rev Cancer. 11(6):393-410 (2011).
	4	YEH B, et al., "Structural Basis for Activation of Fibroblast Growth Factor Signaling by Sucrose Octasulfate," Mol Cell Biol. 22(20):7184-92 (2002).

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WEST Search History for Application 15809815

Creation Date: 2020022012:18

Prior Art Searches

Query	DB	Hits	Op.	Plur.	Thes.	Date
irinotecan with oxaliplatin	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	14417	ADJ	YES		02-21-2018
(irinotecan with oxaliplatin) and leucovorin	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	7488	ADJ	YES		02-21-2018
(irinotecan with oxaliplatin and leucovorin) and fluorouracil	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	7333	ADJ	YES		02-21-2018
(irinotecan with oxaliplatin and leucovorin and fluorouracil) and liposome	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	4086	ADJ	YES		02-21-2018
(irinotecan with oxaliplatin and leucovorin and fluorouracil and liposome) and pancreas or pancreatic	PGPB, USPT, USOC, EPAB, JPAB, DWPI,	194019	ADJ	YES		02-21-2018

	TDBD, FPRS					
irinotecan with oxaliplatin with leucovorin with fluorouracil	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	5104	ADJ	YES		02-21-2018
(irinotecan with oxaliplatin with leucovorin with fluorouracil) and pancreas	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	2596	ADJ	YES		02-21-2018
(irinotecan with oxaliplatin with leucovorin with fluorouracil and pancreas) and liposome	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	1571	ADJ	YES		02-21-2018
(irinotecan with oxaliplatin with leucovorin with fluorouracil and pancreas and liposome) and cycle	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	1312	ADJ	YES		02-21-2018
(irinotecan with oxaliplatin with leucovorin with fluorouracil and pancreas and liposome and cycle) and immunoliposome	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	71	ADJ	YES		02-21-2018
2013188586.pn.	PGPB, USPT, USOC, EPAB,	4	ADJ	YES		03-03-2018

	JPAB, DWPI, TDBD, FPRS					
2016094402.pn.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	5	ADJ	YES		03-03-2018
pancreatic adj2 cancer with (irinotecan and oxaliplatin and leucovorin and fluorouracil)	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	68	ADJ	YES		03-03-2018
(pancreatic adj2 cancer with (irinotecan and oxaliplatin and leucovorin and fluorouracil)) and liposome	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	51	ADJ	YES		03-03-2018
pancreas with irinotecan	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	128	ADJ	YES		09-02-2018
(pancreas with irinotecan) and oxaliplatin	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	103	ADJ	YES		09-02-2018
(pancreas with irinotecan and oxaliplatin) and leucovorin	PGPB, USPT,	85	ADJ	YES		09-02-2018

	USOC, EPAB, JPAB, DWPI, TDBD, FPRS					
(pancreas with irinotecan and oxaliplatin and leucovorin) and fluorouracil	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	82	ADJ	YES		09-02-2018
(pancreas with irinotecan and oxaliplatin and leucovorin and fluorouracil) and liposome	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	32	ADJ	YES		09-02-2018
("6545010").PN.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	2	ADJ	YES		07-01-2019
2003013536.pn.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	4	ADJ	YES		07-01-2019
2011153010.pn.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	6	ADJ	YES		07-01-2019

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	15809815
Filing Date	2017-11-10
First Named Inventor	Eliel Bayever
Art Unit	1612
Examiner Name	Celeste A. RONEY
Attorney Docket Number	01208-0007-01US

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Examiner Signature	/CELESTE A RONEY/	Date Considered	02/20/2020

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CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

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See attached certification statement.

The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Mary R. Henninger/	Date (YYYY-MM-DD)	2020-01-07
Name/Print	Mary R. Henninger	Registration Number	56992

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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	Art Unit	1612
	Examiner Name	Celeste A. RONEY
	Attorney Docket Number	01208-0007-01US

CERTIFICATION STATEMENT

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Name/Print	Mary R. Henninger	Registration Number	56992

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	Application Number	15809815
	Filing Date	2017-11-10
	First Named Inventor	Eliel Bayever
	Art Unit	1612
	Examiner Name	Celeste A. RONEY
	Attorney Docket Number	01208-0007-01US

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	8	2013158803	WO	A1	2013-10-24	Merrimack Pharmaceuticals, Inc.		
	9	2014113167	WO	A1	2014-07-24	Merrimack Pharmaceuticals, Inc.		
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	11	2017031445	WO	A1	2017-02-23	Merrimack Pharmaceuticals, Inc.		
	12	2017199093	WO	A1	2017-11-23	Ipsen Biopharm Ltd.		

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	First Named Inventor	Eliel Bayever
	Art Unit	1612
	Examiner Name	Celeste A. RONEY
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	Attorney Docket Number	01208-0007-01US

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Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

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In re Application of
Eliel BAYEVER et al.

Application Number	Filed
15/809,815	November 10, 2017

For: Methods for Treating Metastatic Pancreatic Cancer Using Combination Therapies Comprising Liposomal Irinotecan and Oxaliplatin

Art Unit	Examiner
1612	Celeste A. RONEY

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Electronic Patent Application Fee Transmittal

Application Number:	15809815
Filing Date:	10-Nov-2017
Title of Invention:	Methods for Treating Metastatic Pancreatic Cancer Using Combination Therapies Comprising Liposomal Irinotecan and Oxaliplatin
First Named Inventor/Applicant Name:	Eliel Bayever
Filer:	Mary Rucker Henninger/Richard King
Attorney Docket Number:	263266-421428

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
NOTICE OF APPEAL	1401	1	800	800
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Extension - 3 months with $0 paid	1253	1	1400	1400
Miscellaneous:				
			Total in USD ($)	**2200**

Electronic Acknowledgement Receipt

EFS ID:	40373141
Application Number:	15809815
International Application Number:	
Confirmation Number:	5137
Title of Invention:	Methods for Treating Metastatic Pancreatic Cancer Using Combination Therapies Comprising Liposomal Irinotecan and Oxaliplatin
First Named Inventor/Applicant Name:	Eliel Bayever
Customer Number:	153749
Filer:	Mary Rucker Henninger/Richard King
Filer Authorized By:	Mary Rucker Henninger
Attorney Docket Number:	263266-421428
Receipt Date:	25-AUG-2020
Filing Date:	10-NOV-2017
Time Stamp:	12:04:13
Application Type:	Utility under 35 USC 111(a)

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Warnings:					
Information:					
2	Notice of Appeal Filed	2020-08-25_01208-0007-01US_Notice_of_Appeal_as_filed.pdf	261074 173a1c925205cd6ffe6954f1c8872f5ecef92d6c	no	2
Warnings:					
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3	Fee Worksheet (SB06)	fee-info.pdf	32468 244a5c8f38b0be9c688f334213391edfd455ee4a	no	2
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PTO/AIA/22 (03-13)
Approved for use through 7/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)

Docket Number (Optional): 01208-0007-01US

Application Number: 15/809,815

Filed: November 10, 2017

For: Methods for Treating Metastatic Pancreatic Cancer Using Combination Therapies Comprising Liposomal Irinotecan and Oxaliplatin

Art Unit: 1612

Examiner: Celeste A. Roney

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	Micro Entity Fee	
☐ One month (37 CFR 1.17(a)(1))	$200	$100	$50	$
☐ Two months (37 CFR 1.17(a)(2))	$600	$300	$150	$
☑ Three months (37 CFR 1.17(a)(3))	$1,400	$700	$350	$ 1400
☐ Four months (37 CFR 1.17(a)(4))	$2,200	$1,100	$550	$
☐ Five months (37 CFR 1.17(a)(5))	$3,000	$1,500	$750	$

☐ Applicant asserts small entity status. See 37 CFR 1.27.

☐ Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

☐ A check in the amount of the fee is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☐ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number _____.

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I am the

☐ applicant.

☒ attorney or agent of record. Registration number 56,992.

☐ attorney or agent acting under 37 CFR 1.34. Registration number _____.

/Mary R. Henninger/ — Signature

August 25, 2020 — Date

Mary R. Henninger — Typed or printed name

404-891-1400 — Telephone Number

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

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PTO/SB/08a (02-18)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	15809815
Filing Date	2017-11-10
First Named Inventor	Eliel Bayever
Art Unit	1612
Examiner Name	Celeste A. RONEY
Attorney Docket Number	01208-0007-01US

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	1	COLUCCI G, et. al., "Randomized Phase III Trial of Gemcitabine Plus Cisplatin Compared With Single-Agent Gemcitabine as First-Line Treatment of Patients With Advanced Pancreatic Cancer: The GIP-1 Study," J Clin Oncol. 28(10):1645-51 (2010).
	2	CONROY T et al., Abstract 4010. "Randomized Phase III Trial Comparing FOLFIRINOX (F: 5FU/Leucovorin [LV], Irinotecan [I}, and Oxaliplatin [O]) Versus Gemcitibine (G) as First-Line Treatment for Metastatic Pancreatic Adenocarcinoma (MPA): Preplanned Interim Analysis Results of the PRODIGE 4/ACCORD 11 Trial" J Clin Oncol. 28 (15_Suppl):4010 (2010), 3 printed pages.
	3	CUSTODIO A, et. al., "Second-Line Therapy for Advanced Pancreatic Cancer: A Review of the Literature and Future Directions," Cancer Treat Rev. 35(8):676–84 (2009).
	4	DE JONG F, et al., "Effects of nal-IRI (MM-398; a Liposomal Formulation of Irinotecan) ± 5-Fluorouracil (5-FU) on Quality of Life (QoL) in NAPOLI-1: A Phase 3 Study in Patients With Metastatic Pancreatic Ductal Adenocarcinoma Previously Treated With Gemcitabine," Poster presented at the Australian Gastro-Intestinal Trials Group, 18th Annual Scientific Meeting, Melbourne, Australia, September 14-16, 2016, 10 pages.
	5	DE JONG F, et al., Abstract. "Effects of nal-IRI (MM-398; a Liposomal Formulation of Irinotecan) ± 5-Fluorouracil (5-FU) on Quality of Life (QoL) in NAPOLI-1: A Phase 3 Study in Patients With Metastatic Pancreatic Ductal Adenocarcinoma (mPDAC) Previously Treated With Gemcitabine," Australian Gastro-Intestinal Trials Group, 18th Annual Scientific Meeting, Melbourne, Australia, September 14-16, 2016, 2 pages.
	6	DEAN A, et al., "First-Line (1L) Liposomal Irinotecan + 5-Fluorouracil/Leucovorin (5-FU/LV) + Oxaliplatin (OX) in Patients With Locally Advanced or Metastatic Pancreatic Ductal Adenocarcinoma: Exploratory Subgroup Analyses of Survival by Changes in CA 19-9 Levels." Poster presented at the European Society for Medical Oncology (ESMO) Virtual Congress 2020, September 19-21, 2020, 7 pages.
	7	DEAN A, et al., "First-Line Liposomal Irinotecan + 5-Fluorouracil/Leucovorin + Oxaliplatin in Patients With Pancreatic Ductal Adenocarcinoma: Results From a Phase 1/2 Study." Presentation presented at the Clinical Oncology Society of Australia (COSA): Virtual meeting, November 11-13, 2020, 10 pages.
	8	DEAN A, et al., "Nanoliposomal Irinotecan (nal-IRI)-Containing Regimens Versus nab-paclitaxel Plus Gemcitabine as First-Line Therapy in Patients With Metastatic Pancreatic Adenocarcinoma (mPAC): A Randomized, Open-Label Phase 2 Study." Poster presented at the 18th European Society of Medical Oncology World Congress on Gastrointestinal Cancer; Barcelona, Spain; June 29 - July 2, 2016, 14 pages.
	9	DEAN A, et al., "NAPOLI-3: An Open-Label, Randomized, Phase III Study of First-Line Liposomal Irinotecan + 5-Fluorouracil/Leucovorin + Oxaliplatin Versus nab-Paclitaxel + Gemcitabine in Patients With Metastatic Pancreatic Ductal Adenocarcinoma." Presentation presented at the Clinical Oncology Society of Australia (COSA): Virtual meeting, November 11-13, 2020, 10 pages.
	10	DEAN A, et al., Abstract 1529P. "First-Line (1L) Liposomal Irinotecan + 5-Fluorouracil/Leucovorin (5-FU/LV) + Oxaliplatin (OX) in Patients With Locally Advanced or Metastatic Pancreatic Ductal Adenocarcinoma (mPDAC): Exploratory Subgroup Analyses of Survival by Changes in CA 19-9 Levels," Ann Oncol. 31(Suppl_4):S881-S897 10.1016/annonc/annonc285 (2020), 3 printed pages.
	11	DEAN A, et al., Abstract 222. "First-Line Liposomal Irinotecan + 5-Fluorouracil/Leucovorin + Oxaliplatin in Patients With Pancreatic Ductal Adenocarcinoma: Results From a Phase 1/2 Study," Asia-Pac J Clin Oncol. 16(Suppl. 3):118-119 (2020).

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	Attorney Docket Number	01208-0007-01US

	12	DEAN A, et al., Abstract 407. "NAPOLI-3: An Open-Label, Randomized, Phase III Study of First-Line Liposomal Irinotecan + 5-Fluorouracil/Leucovorin + Oxaliplatin Versus nab-Paclitaxel + Gemcitabine in Patients With Metastatic Pancreatic Ductal Adenocarcinoma," Asia-Pac J Clin Oncol. 16(Suppl. 8):202-3 (2020).
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	14	DEAN A, et al., Abstract P-287. "Nanoliposomal Irinotecan (nal-IRI)-Containing Regimens Versus nab-paclitaxel Plus Gemcitabine as First-Line Therapy in Patients With Metastatic Pancreatic Adenocarcinoma (mPAC): A Randomized, Open-Label Phase 2 Study." Annals of Oncology. 27(Suppl 2):ii1-i85 (2016), 1 page.
	15	DEAN A, et al., Abstract. "Expanded Analyses of NAPOLI-1: Phase 3 Study of MM-398 (nal-IRI), With or Without 5-Fluorouracil and Leucovorin (5-FU/LV), Versus 5-FU/LV, in Metastatic Pancreatic Cancer (mPAC) Previously Treated With Gemcitabine-Based Therapy," 18th Annual Scientific Meeting of the Australasian Gastro-Intestinal Trials Group (AGITG), Melbourne, Australia, September 14-16, 2016, 2 pages.
	16	DEAN A, et al., Abstract. "Liposomal Irinotecan (nal-IRI, MM-398)-Containing Regimens Versus nab-Paclitaxel Plus Gemcitabine as First-Line Therapy in Patients With Metastatic Pancreatic Adenocarcinoma (mPAC): A Randomized, Open-Label Phase 2 Study," 18th Annual Scientific Meeting of the Australasian Gastro-Intestinal Trials Group (AGITG), Melbourne, Australia, September 14-16, 2016, 2 pages.
	17	DIEGUEZ G, et al., "Real-World Rates of Hematologic Laboratory Abnormalities and Associated Cost Among Metastatic Pancreatic Cancer Therapeutic Regimens," Poster presented at the American Society of Clinical Oncology Gastrointestinal Cancers Symposium (ASCO GI), San Francisco, CA, January 23-25, 2020, 6 pages.
	18	DIEGUEZ G, et al., Abstract 670. "Real-World Rates of Hematology Lab Abnormalities and Associated Cost Among Metastatic Pancreatic Cancer (mPC) Therapeutic Regimens," J Clin Oncol. 38(4_Suppl):670 DOI: 10.1200/JCO.2020.38.4_suppl.670 (2020), 2 printed pages.
	19	DORIS J, et al., Abstract CT12. "The Cost of Adverse Events for FDA-Approved/NCCN Category 1 Treatments for Medicare Fee-For-Service (FFS) Patients With Metastatic Pancreatic Cancer: Focus on Liposomal Irinotecan-Based Regimens," Hematology Oncology Pharmacy Association (HOPA) Annual Conference, March 11-14, 2020, available at eventscribe.com/2020/posters/HOPAahead/SplitViewer.asp?PID=Njg0NzA2NjU1NjE, (2020), 2 pages.
	20	FIGER A, et. al., "A Comparison of Two Dose Regimens in Pancreatic Cancer," J Chemother. 12(5):442-5 (2000).
	21	GADDY D, et al., "A Systematic Literature Review to Identify and Compare Clinical Trials Evaluating Novel Therapeutic Agents in Post-Gemcitabine Advanced Pancreatic Cancer Patients." Poster presented at the International Society for Pharmacoeconomics and Outcomes Research (ISPOR) 18th Annual European Congress, Milan, Italy, November 7-11, 2015, 6 pages.
	22	GADDY D, et al., "Preclinical Anti-tumor Activity of Nanoliposomal Irinotecan (Nal-IRI, MM-398) Supports Utilization as a Foundation of Front-Line Pancreatic Cancer Regimens." Poster presented at the American Society of Clinical Oncology Gastrointestinal Cancers Symposium (ASCO GI), San Francisco, CA, January 19-21, 2017, 5 pages.

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	23	GADDY D, et al., Abstract 336. "Preclinical Antitumor Activity of Nanoliposomal Irinotecan (Nal-IRI, MM-398) and Utilization as a Foundation of Front-Line Pancreatic Cancer Regimens," J Clin Oncol. 35(4_Suppl):336 DOI: 10.1200/JCO.2017.35.4_suppl.336 (2017), 2 printed pages.
	24	GADDY D, et al., Abstract PCN29. "A Systematic Literature Review to Identify and Compare Clinical Trials Evaluating Novel Therapeutic Agents in Post-Gemcitabine Advanced Pancreatic Cancer," Value in Health. 18(7):A434 (2015).
	25	GEBBIA V, et al., "Second-Line Chemotherapy in Advanced Pancreatic Carcinoma: A Multicenter Survey of the Gruppo Oncologico Italia Meridionale on the Activity and Safety of the FOLFOX4 Regimen in Clinical Practice," Ann Oncol. 18(Suppl 6):vi124–7 (2007).
	26	GILL S, et al., "PANCREOX: A Randomized Phase III Study of Fluorouracil/Leucovorin With or Without Oxaliplatin for Second-Line Advanced Pancreatic Cancer in Patients Who Have Received Gemcitabine-Based Chemotherapy," J Clin Oncol. 34(32):3914-20 and Appendix (2016).
	27	GLASSMAN D, et al., "Nanoliposomal Irinotecan With Flurouracil for the Treatment of Advanced Pancreatic Cancer." Poster presented at the American Society of Clinical Oncology Gastrointestinal Cancers Symposium (ASCO GI), San Francisco, CA, January 18-20, 2018, 7 pages.
	28	GLASSMAN D, et al., Abstract 471. "Nano-Liposomal Irinotecan and 5-FU/LV (N+F) for the Treatment of Advanced PDAC: Memorial Sloan Kettering (MSK) Single Cancer Center Evaluation," J Clin Oncol. 36(4_Suppl):471 DOI: 10.1200/JCO.2018.36.4_suppl.471 (2018), 2 printed pages.
	29	GOUNARIS I, et. al., "Options for the Treatment of Gemcitabine-Resistant Advanced Pancreatic Cancer," JOP. J Pancreas (Online) 11(2):113-23 (2010).
	30	GOURZOULIDIS G, et al., "The Cost-Effectiveness of Nanoliposomal Irinotecan and 5-Fluorouracil (5-FU)/ Leucovorin (LV) for the Treatment of Patients With Metastatic Adenocarcinoma of Pancreas Who Have Progressed Following the Use of Gemcitabine-Related Therapies in Greece." Poster presented at the Virtual International Society for Pharmacoeconomics and Outcomes Research (ISPOR) European Congress, Milan, Italy, November 16-19, 2020, 9 pages.
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	32	HALLER D, "Chemotherapy for Advanced Pancreatic Cancer," Int J Radiat Oncol Biol Phys. 56(4 Suppl):16-23 (2003).
	33	HANN B, et. al., Abstract 5648. "Lipidic Nanoparticle CPT-11 in a Bioluminescent Orthotopic Pancreas Cancer Model," Cancer Res. 67(9 Suppl):5648 (2007), 4 printed pages.

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	34	HEINEMANN V, et. al., "Randomized Phase III Trial of Gemcitabine Plus Cisplatin Compared With Gemcitabine Alone in Advanced Pancreatic Cancer," J Clin Oncol. 24(24):3946-52 (2006).
	35	HERRERA-RESTREPO O, et al., "Budget Impact in the USA of Liposomal Irinotecan as a Post-Gemcitabine Treatment Option for Patients With Metastatic Pancreatic Adenocarcinoma (mPC)." Poster presented at the International Society for Pharmacoeconomics and Outcomes Research (ISPOR) Annual Meeting, New Orleans, LA, May 18-22, 2019, 12 pages.
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	Art Unit	1612
	Examiner Name	Celeste A. RONEY
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	47	HUBNER R, et al., "Effects of nal-IRI (MM-398) ± 5-Fluorouracil on Quality of Life (QoL) in NAPOLI-1: A Phase 3 Study in Patients With Metastatic Pancreatic Ductal Adenocarcinoma (mPDAC) Previously Treated With Gemcitabine," Presentation presented at the European Society for Medical Oncology (ESMO) World Congress on Gastrointestinal Cancer, Barcelona, Spain, June 29-July 2, 2016, 13 pages.
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	Examiner Name	Celeste A. RONEY
	Attorney Docket Number	01208-0007-01US

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ORIGINAL REPORT

Randomized Phase III Trial of Gemcitabine Plus Cisplatin Compared With Single-Agent Gemcitabine As First-Line Treatment of Patients With Advanced Pancreatic Cancer: The GIP-1 Study

Giuseppe Colucci, Roberto Labianca, Francesco Di Costanzo, Vittorio Gebbia, Giacomo Carteni, Bruno Massidda, Elisa Dapretto, Luigi Manzione, Elena Piazza, Mirella Sanmicolò, Marco Ciaparrone, Luigi Cavanna, Francesco Giuliani, Evaristo Maiello, Antonio Testa, Paolo Pederzoli, Massimo Falconi, Ciro Gallo, Massimo Di Maio, and Francesco Perrone

From the Medical and Experimental Oncology Unit, Oncology Institute "Giovanni Paolo II," Bari; Medical Oncology Unit, Ospedali Riuniti, Bergamo; Medical Oncology Unit, Azienda Ospedaliero-Universitaria Careggi, Firenze; Department of Experimental Oncology and Clinical Application, "La Maddalena" Hospital, University of Palermo; Oncology Unit, "Cardarelli" Hospital, Napoli; Medical Oncology Unit, University Hospital, Cagliari; Radiotherapy and Medical Oncology Unit, "S.Gerardo" Hospital, Monza; Medical Oncology Unit, "S.Carlo" Hospital, Potenza; Medical Oncology Unit, "L.Sacco" Hospital, Milano; Oncologic Day-Hospital, "S.Maria del Carmine" Hospital, Rovereto (Trento); Oncology Department, "S.Giovanni Calibita Fatebenefratelli" Hospital, Isola Tiberina, Roma; Department of Medical Oncology and Hematology, "Guglielmo da Saliceto" Hospital, Piacenza; Surgery B Unit, Department of Surgical and Gastroenterological Sciences, "G.B.Rossi" Hospital, University of Verona; Department of Medicine and Public Health, Second University of Napoli; and the Clinical Trials Unit, National Cancer Institute, Napoli, Italy.

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Written on behalf of the Gruppo Oncologico Italia Meridionale (GOIM), Gruppo Italiano per lo Studio dei Carcinomi dell'Apparato Digerente (GISCAD), Gruppo Oncologico Italiano di Ricerca Clinica (GOIRC).

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical Trials repository link available on JCO.org.

Corresponding author: Francesco Perrone, MD, PhD, Clinical Trials Unit, National Cancer Institute, via Mariano Semmola, 80131 Napoli, Italy; e-mail: francesco.perrone@uosc.fondazionepascale.it.

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ABSTRACT

Purpose
Single-agent gemcitabine became standard first-line treatment for advanced pancreatic cancer after demonstration of superiority compared with fluorouracil. The Gruppo Italiano Pancreas 1 randomized phase III trial aimed to compare gemcitabine plus cisplatin versus gemcitabine alone (ClinicalTrials.gov ID NCT00813696).

Patients and Methods
Patients with locally advanced or metastatic pancreatic cancer, age 18 to 75 years, and Karnofsky performance status (KPS) ≥ 50, were randomly assigned to receive gemcitabine (arm A) or gemcitabine plus cisplatin (arm B). Arm A: gemcitabine 1,000 mg/m^2 weekly for 7 weeks, and, after a 1-week rest, on days 1, 8, and 15 every 4 weeks. Arm B: cisplatin 25 mg/m^2 added weekly to gemcitabine, except cycle 1 day 22. Primary end point was overall survival. To have 80% power of detecting a 0.74 hazard ratio (HR) of death, with bilateral α .05, 355 events were needed and 400 patients planned.

Results
Four hundred patients were enrolled (arm A: 199; arm B: 201). Median age was 63, 59% were male, 84% had stage IV, and 83% had KPS ≥ 80. Median overall survival was 8.3 months versus 7.2 months in arm A and B, respectively (HR, 1.10; 95% CI, 0.89 to 1.35; $P = .38$). Median progression-free survival was 3.9 months versus 3.8 months in arm A and B, respectively (HR, 0.97; 95% CI, 0.80 to 1.19; $P = .80$). The objective response rate was 10.1% in A and 12.9% in B ($P = .37$). Clinical benefit was experienced by 23.0% in A and 15.1% in B ($P = .057$). Combination therapy produced more hematologic toxicity, without relevant differences in nonhematologic toxicity.

Conclusion
The addition of weekly cisplatin to gemcitabine failed to demonstrate any improvement as first-line treatment of advanced pancreatic cancer.

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INTRODUCTION

The majority of patients with pancreatic cancer are diagnosed in the advanced, unresectable stage, when the primary goals of treatment are survival prolongation and symptom palliation. The impact of systemic treatments in these patients is poor. Administration of gemcitabine is associated with some clinical benefit (CB) and a modest improvement in survival compared with fluorouracil.[1] Single-agent gemcitabine is currently recommended as standard first-line chemotherapy for patients with advanced disease.[2]

The combination of gemcitabine and cisplatin is supported by several preclinical data.[3-6] Gemcitabine increases cisplatin-induced DNA lesions and inhibits their repair, and cisplatin enhances the incorporation of gemcitabine triphosphate into DNA, inducing apoptosis of cancer cells. In a randomized trial published in 2002[7] by the Gruppo Oncologico Italia Meridionale (GOIM), 107 patients with locally advanced or metastatic pancreatic cancer were randomly assigned to single-agent gemcitabine or the combination of gemcitabine and cisplatin, in a weekly schedule. The combination significantly improved objective response rate (ORR) and time to

progression (TTP) compared with gemcitabine alone. CB rate was similar, and overall survival (OS) was longer with the combination, although the difference was not statistically significant.

These results were considered interesting but limited by the small number of patients, and three Italian cooperative groups (GOIM; Gruppo Italiano per lo Studio dei Carcinomi dell'Apparato Digerente [GISCAD]; Gruppo Oncologico Italiano di Ricerca Clinica [GOIRC]) decided to perform an Intergroup phase III trial, to compare the combination of gemcitabine and cisplatin, administered in the same schedule tested in the GOIM trial,[7] with single-agent gemcitabine. The aim of the Gruppo Italiano Pancreas (GIP) -1 study was to demonstrate a significant improvement in OS, chosen as primary end point.

Patients and Methods

The GIP-1 protocol was approved by ethical committees at each participating Institution. All patients gave written informed consent before starting study procedures.

Patient Selection

Patients age 18 to 75 years with histologic or cytologic diagnosis of pancreatic cancer, stage II (if unresectable) or III or IV according to International Union against Cancer 1997 staging system,[8] Karnofsky performance status (KPS) ≥ 50, and who had not received prior chemotherapy were eligible. Other eligibility criteria included: adequate hematology (absolute neutrophil count ≥ 2,000/μL, platelets ≥ 100,000/μL, hemoglobin ≥ 10 g/dL), and biochemistry (serum creatinine ≤ upper normal limit [UNL], AST and ALT ≤ 2.5 × UNL, and bilirubin ≤ 1.5 × UNL, unless due to liver metastases). Presence of brain metastases and history of other invasive malignancy in previous 5 years were exclusion criteria.

Before random assignment, complete history and physical examination, routine hematology and biochemistry, ECG, chest x-ray, abdominal computed tomography (CT) scans, assessment of CB measures, and compilation of quality of life (QoL) questionnaires were required.

Study Treatments

Eligible patients were randomly assigned to arm A (standard treatment) or arm B (experimental treatment).

In arm A, gemcitabine was administered as 30-minute intravenous infusion, 1,000 mg/m^2, weekly for 7 consecutive weeks (cycle 1), followed by 1 week of rest. Thereafter, gemcitabine was continued on days 1, 8, and 15 every 28 days.

In arm B, gemcitabine was administered as in arm A. Cisplatin was administered, 25 mg/m^2, 1 hour before gemcitabine, on days 1, 8, 15, 29, 36, and 42 of cycle 1. On day 22, only gemcitabine was administered. Thereafter, after 1 week of rest, treatment was continued with both drugs on days 1, 8, and 15 every 28 days.

Before each administration, the following criteria had to be met: absolute neutrophil count ≥ 1,000/μL, platelets ≥ 100,000/μL, and absence of grade ≥ 2 nonhematologic toxicity. Without these conditions, treatments were postponed by 1 week and eventually stopped if minimum treatment conditions were still not met after 2 consecutive delays. Dose reductions were planned according to severity of hematologic toxicity.

No maximum number of cycles was planned, and patients continued treatment until disease progression, refusal, or unacceptable toxicity. Patients with disease progression could also continue treatment if they were experiencing CB. Second-line treatment was not defined by protocol, and was at investigator's discretion.

Study Evaluations

During treatment, CBC, serum creatinine, AST, and ALT were repeated weekly in both arms. Complete biochemistry and ECG were repeated at the end of each cycle. Toxicity was coded according to National Cancer Institute Common Toxicity Criteria version 2.0.

ORR was categorized according to Response Evaluation Criteria in Solid Tumors.[9] ORR was assessed at the end of cycle 1 in both arms, repeating chest x-ray and abdominal CT scan. All instrumental exams were read by investigators at each center. Confirmation of response was not required.

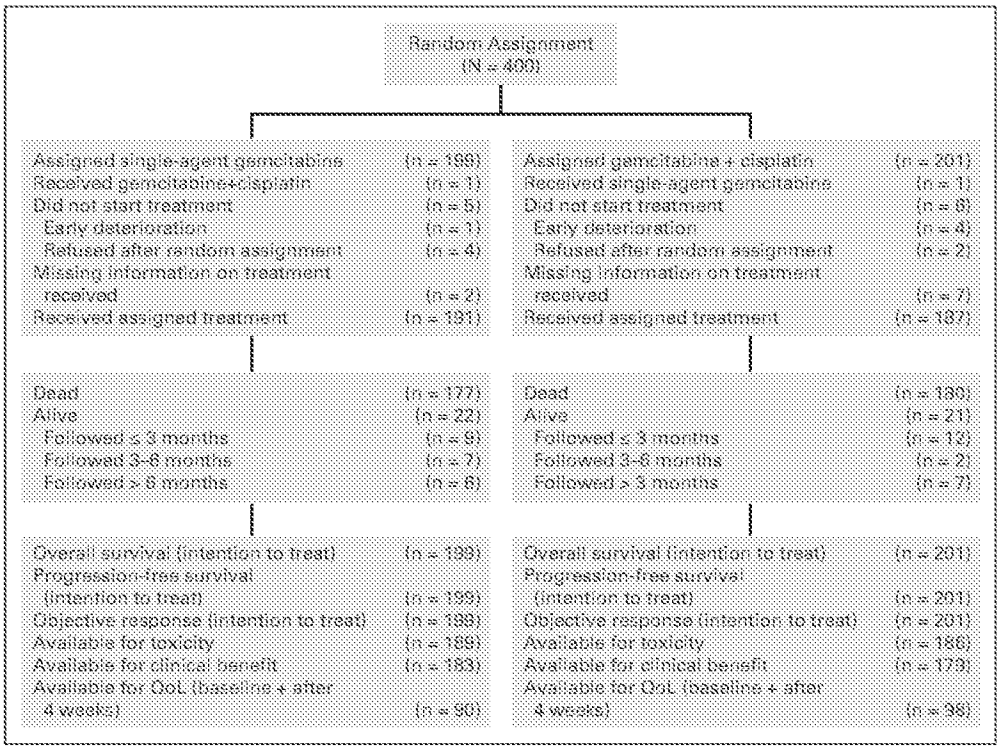

Fig 1. Flow of data collection according to CONSORT diagram.

CB rate was calculated measuring pain, functional impairment and weight loss, according to a previously described algorithm.[9] Pain (assessed by pain intensity and analgesic consumption) and functional impairment (assessed by KPS) represented primary measures. Weight change was a secondary measure. Pain intensity was recorded daily, the other parameters were assessed weekly. KPS was assessed by two independent observers. Each patient was classified as either positive, stable, or negative for each of the primary measures. Patients who were stable on both primary measures were classified as either responder or nonresponder based on weight. For patients to achieve an overall rating of posative CB, they had to be positive for at least one parameter without being negative for any of the others. This improvement had to last for at least 4 consecutive weeks.

The European Organisation for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire C30 (version 3.0)[10] and PAN26[11] questionnaires were used to evaluate QoL. PAN26 is specific for pancreatic cancer patients.[11] Most items of the EORTC questionnaires refer to the week preceding administration. Both questionnaires are designed to be completed by the patient. EORTC questionnaires were completed at baseline (before random assignment) and every 4 weeks, up to six questionnaires, in both arms.

Study Design

GIP-1 was a open-label, randomized phase III study. The primary end point was OS. Secondary end points included progression-free survival (PFS), ORR, treatment toxicity, CB, and QoL.

Overall, 400 patients were planned and 355 deaths were required to have 80% power of detecting a 0.74 hazard ratio (HR) of death, with two-tailed α .05 (EAST; Cytel Software, Cambridge, MA). This would represent an increase in proportion of patients alive at 1 year from 18% to 28%, corresponding to an increase in median OS from 4.8 to 6.5 months. One interim analysis was planned, to be performed 3 to 4 months after the accrual of 200 patients, using an α spending function,[12] based on an O'Brien Fleming[13] sequential group design. Interim analysis was performed by the study statistician (C.G.), with blinded treatment labels. Investigators were only informed that accrual remained open.

Patients were randomly assigned to standard arm or experimental arm in a 1:1 ratio. Telephone random assignment was performed centrally (Clinical Trials Unit, National Cancer Institute, Napoli, Italy), by a computer-driven minimization procedure. Stratification factors were center, KPS ($\geq$ 70 v $\leq$ 80), and stage (II-III v IV).

Data Analysis

Efficacy analyses were done on an intention-to-treat (ITT) basis. OS was defined as the interval between date of random assignment and date of death (or date of last follow-up for alive patients). PFS was defined as the interval between date of random assignment and date of progression or death whichever occurred first, or date of last follow-up for patients alive and without progression. Median follow-up was calculated according to the inverted Kaplan-Meier technique.[14] OS and PFS curves were estimated by Kaplan-Meier product limit method[15] and compared by log-rank test.[16] For OS, Cox proportional hazards model[17] was used to assess treatment effect after adjustment by baseline prognostic variables.

ORR was defined as the proportion of complete and partial responses on the total number of patients assigned to each arm. Patients who died or stopped treatment because of toxicity or refusal before restaging were conservatively defined as nonresponders. The statistical significance of the difference between ORRs in the two arms was assessed by χ^2 test.

All patients who received at least one chemotherapy administration were eligible for toxicity analysis. The worst grade of toxicity experienced throughout the treatment was computed for each patient. For each toxicity, two statistical tests were performed to assess the differences between arms: patterns of toxicity (considering all the possible grades) were compared by an exact linear rank test, while rates of severe toxicity (grade $\geq$ 3 v 0 to 2) were compared by χ^2 or Fisher's exact test as appropriate.

CB rate was defined as the proportion of responders on the number of patients with information available in each arm.

QoL analysis was performed according to EORTC manual.[18] Multi-item scales were computed by calculating the mean raw scores of single items and transforming them linearly so that all scales range from 0 to 100. For single items, only linear transformation was performed. For this article, only changes from baseline after 4 weeks were calculated for each domain and compared between arms, using baseline values as a covariate.

Statistical analyses were performed using S-Plus version 6.1 (Insightful Corp, Seattle, WA). Exact tests were performed using StatXact 7 (Cytel Software, Cambridge, MA).

RESULTS

Patient Characteristics

The CONSORT diagram of the trial is reported in Figure 1. Between April 2002 and April 2007, 400 patients were randomly assigned. Baseline characteristics were well balanced between arms (Table 1). Median age was 63 years, 59% of patients were male, 83% had KPS $\geq$ 80%, and 84% had stage IV disease. Most of the patients had adenocarcinoma, and 26% had received previous surgery.

Treatment Compliance

Information on treatment actually received was not available for nine patients (Fig 1). Of the remaining 391, 11 patients did not start treatment, five in arm A and 6 in arm B.

Table 1. Baseline Characteristics of Randomly Assigned Patients

Characteristic	Gemcitabine (n = 199) No.	%	Gemcitabine + Cisplatin (n = 201) No.	%	Overall (n = 400) No.	%
Sex						
Male	113	56.8	125	62.2	238	59.5
Female	86	43.2	76	37.8	162	40.5
Median age, years	63		63		63	
Range	37-75		35-75		35-75	
Karnofsky performance status						
$\leq$ 70	33	16.6	36	17.9	69	17.3
$\geq$ 80	166	83.4	165	82.1	331	82.7
Stage						
II	9	4.5	6	3.0	15	3.8
III	24	12.1	25	12.4	49	12.2
IV	165	82.9	170	84.6	335	83.8
Missing information	1	0.5	—	—	1	0.2
Location of pancreatic tumor						
Head	91	45.7	101	50.2	192	48.0
Body	52	26.1	34	16.9	86	21.5
Tail	26	13.1	20	9.9	46	11.5
Head + body	10	5.0	6	3.0	16	4.0
Body + tail	18	9.0	39	19.4	57	14.3
Head + body + tail	1	0.5	1	0.5	2	0.5
Missing information	1	0.5	—	—	1	0.2
Histology						
Undefined	31	15.6	27	13.4	58	14.5
Adenocarcinoma	161	80.9	170	84.6	331	82.7
Squamous	1	0.5	1	0.5	2	0.5
Cystoadenocarcinoma	5	2.5	2	1.0	7	1.8
Missing information	1	0.5	1	0.5	2	0.5
Previous surgery						
No	152	76.4	145	72.1	297	74.3
Yes	47	23.6	56	27.9	103	25.7

Table 2. Cox Proportional Hazards Model: Overall Survival

Parameter	Hazard Ratio	95% CI	P
Treatment (GemCis v gem)	1.10	0.89 to 1.35	.39
Sex (female v male)	1.02	0.82 to 1.28	.86
Age (≥ 65 v < 65 years)	0.89	0.72 to 1.12	.32
Karnofsky PS (≤ 80 v ≤ 70)	0.71	0.54 to 0.93	**.01**
Stage (IV v II-III)	1.82	1.34 to 2.47	**.0001**
Previous surgery (yes v no)	0.86	0.67 to 1.10	.22

NOTE. Bold font indicates statistical significance.
Abbreviations: GemCis, gemcitabine + cisplatin; Gem, gemcitabine; PS, performance status.

The median number of chemotherapy administrations was eight (range, one to 37) and seven (range, one to 31), in arms A and B, respectively. Median total dose of gemcitabine was 7,390 mg/m^2 in arm A (range, 920 to 31,000 mg/m^2) and 7,000 mg/m^2 in arm B (range, 980 to 28,000 mg/m^2; $P = .017$, Wilcoxon rank sum test). Median dose intensity of gemcitabine was 784 mg/m^2/week (range, 296 to 1,067 mg/m^2/week), corresponding to 95% of the planned dose intensity, and 712 mg/m^2/week (range, 333 to 1,000 mg/m^2/week), corresponding to 83% of the planned dose intensity, in arms A and B respectively ($P < .001$, Wilcoxon rank sum test). In arm B, median total dose of cisplatin was 150 mg/m^2 (range, 0 to 689 mg/m^2) and median dose intensity was 16.1 mg/m^2/week (range, 0 to 27.1 mg/m^2/week), corresponding to 83% of the planned dose intensity.

Treatment was stopped because of progression or death in 72% and 66%, because of toxicity or refusal in 12% and 20%, because of other or unspecified reason in 16% and 14% of the patients, in arms A and B, respectively.

Information about second-line treatment was available in 346 patients. Second-line treatment was received by 93 (53.1%) of 175 patients in arm A and 70 (40.9%) of 171 in arm B. Twenty patients (11.4%) in arm A received cisplatin-based second-line treatment; oxaliplatin alone or in combination was received by 29 patients (16.6%) and 21 patients (12.2%) in arms A and B, respectively. As expected, second-line treatment was received by patients who lived longer: median OS was 11.7 months for patients who received second line and 4.4 months for patients who did not.

Efficacy

All 400 patients were included in ITT analyses. At December 2008, with a median follow-up of 38.2 months, 357 deaths (89%) were recorded, 177 in arm A and 180 in arm B.

Median OS was 8.3 months for patients assigned to gemcitabine compared with 7.2 months for patients assigned to combination (HR, 1.10; 95% CI, 0.89 to 1.35, two-sided $P = .38$). At 1 year, 34.0% and 30.7% of patients were alive, in arms A and B respectively. At multivariate analysis, there are no significant differences between treatment arms (Table 2). OS curves are shown in Figure 2A. Exploratory survival analysis by subgroups according to sex, age, stage, KPS, and previous surgery is shown in Figure 3; no heterogeneity of treatment effect around the overall effect is apparent among subgroups.

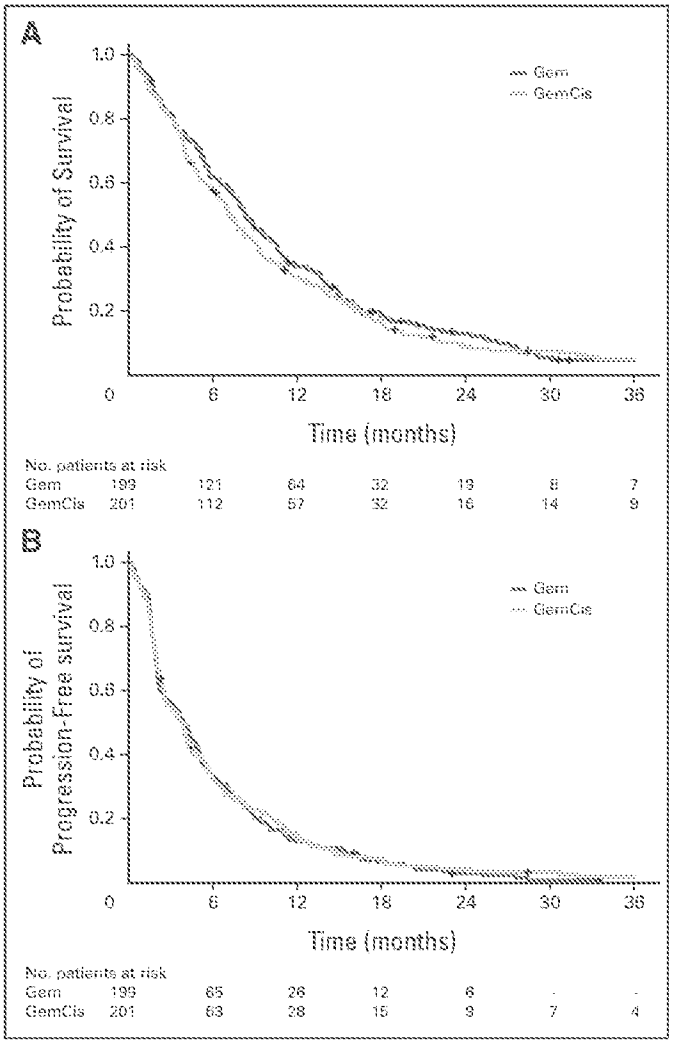

Fig 2. (A) Overall survival and (B) progression-free survival curves by treatment arm. Gem, gemcitabine; GemCis, gemcitabine + cisplatin.

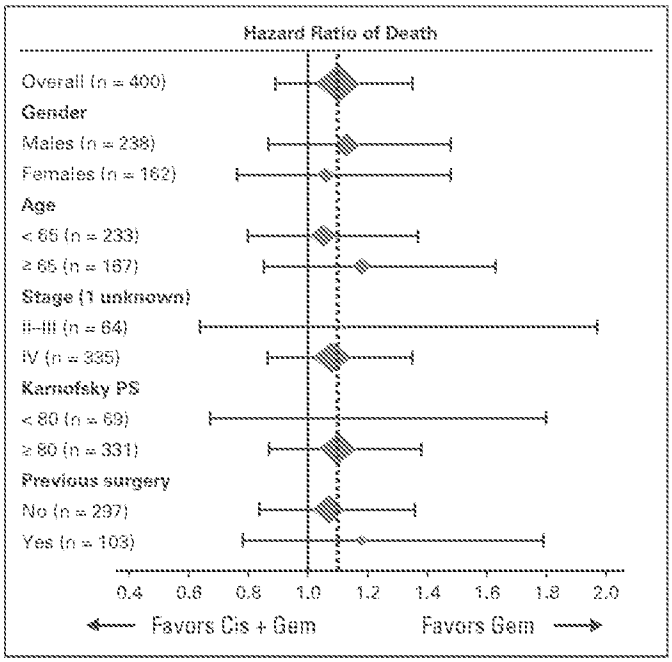

Fig 3. Treatment effect on overall survival within major patient subgroups. Vertical dotted line represents hazard ratio (gemcitabine [Gem] + cisplatin [Cis] v Gem) in the overall population.

With 382 progressions recorded (96%), median PFS was 3.9 and 3.8 months, in arms A and B, respectively (HR, 0.97; 95% CI, 0.80 to 1.19; two-sided $P = .80$). At 1 year, 12.8% and 14.5% of patients were progression free, in arms A and B, respectively. PFS curves are shown in Figure 2B.

Patients assigned to single-agent gemcitabine had complete response in 1.0% and partial response in 9.0%, for an ORR of 10.1% (95% CI, 6.6% to 15.0%). Patients assigned to combination had complete response in 1.5% and partial response in 11.4%, for an ORR of 12.9% (95% CI, 9.0% to 18.3%). ORR was not significantly different between arms ($P = .37$).

Toxicity

All patients with information on treatment who received at least one dose of chemotherapy were considered eligible for toxicity analysis (n = 380). Information about toxicity was missing for three patients (two in arm A, one in arm B). Two further patients were excluded (one in each arm) because they actually received the other treatment. The worst toxicity experienced by the remaining 375 patients is summarized in Table 3.

Hematologic toxicity was more frequent and severe with combination chemotherapy. Patients assigned to the experimental arm experienced more anemia (all grades: 51% *v* 39%, grade 3: 5% *v* 1%), more neutropenia (all grades: 45% *v* 36%, grade 3-4: 25% *v* 14%), and more thrombocytopenia (all grades: 58% *v* 30%, grade 3-4: 16% *v* 5%). No relevant differences were seen in nonhematologic toxicity.

There were five deaths potentially related to the treatment, two with gemcitabine (one stroke, one gastrointestinal bleeding) and three with combination chemotherapy (one deep venous thrombosis, one sudden death, one death for unknown reason in patient with severe thrombocytopenia).

CB

Information about CB was available for 362 patients (91%). Details of CB analysis are reported in Appendix Table A1 (online only). Overall CB responders were 23.0% of patients in arm A and 15.1% in arm B ($P = .057$).

QoL

Overall, 334 patients (161 arm A *v* 173 arm B) completed baseline QoL questionnaire. Of these, 188 completed the second questionnaire after 4 weeks (90 arm A *v* 98 arm B) and were eligible for this analysis. After 4 weeks, mean difference from baseline in global QoL (EORTC

Table 3. Worst Grade of Toxicity According to Treatment Arm

	NCI-CTC Grade																							
	Gemcitabine (n = 189)												Gemcitabine + Cisplatin (n = 186)											
	0		1		2		3		4		5		0		1		2		3		4		5			
Toxicity	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No	%	*P**	*P*†
Anemia	115	61	39	21	33	17	3	1	—	—	—	—	91	49	38	20	48	26	9	5	—	—	—	—	**.005**	**.03**
Leukopenia	136	72	19	10	26	14	6	3	2	1	—	—	127	68	18	10	27	15	13	7	1	< 1	—	—	35	.17
Neutropenia	121	64	20	11	22	12	22	12	4	2	—	—	102	55	16	9	22	12	35	19	11	6	—	—	**.02**	**.007**
Febrile neutropenia	188	99					1	< 1	—	—	—	—	186	100					—	—	—	—	—	—	1.00	1.00
Neutropenic infection	189	100											186	100											—	—
Non-neutropenic infection	186	98	1	< 1	2	1	—	—	—	—	—	—	186	100	—	—	—	—	—	—	—	—	—	—	0.25	—
Thrombocytopenia	133	70	33	17	13	7	10	5	—	—	—	—	78	42	38	20	41	22	22	12	7	4	—	—	**< .001**	**.001**
Allergy	188	99	1	1	—	—	—	—	—	—	—	—	186	100	—	—	—	—	—	—	—	—	—	—	1.00	—
Kidney	189	100	—	—	—	—	—	—	—	—	—	—	182	98	4	2	—	—	—	—	—	—	—	—	.06	—
Heart, rhythm	187	99	1	< 1	—	—	1	< 1	—	—	—	—	182	98	2	1	1	< 1	1	< 1	—	—	—	—	.15	1.00
Heart, general (CV)	189	100	—	—	—	—	—	—	—	—	—	—	183	98	1	< 1	1	< 1	—	—	—	—	1	< 1	.12	.50
Fatigue	112	59	39	21	32	17	6	3	—	—	—	—	111	60	23	12	42	23	10	5	—	—	—	—	.56	.29
Fever	161	85	20	11	8	4	—	—	—	—	—	—	163	88	15	8	7	4	1	< 1	—	—	—	—	.53	.50
Weight loss	172	91	11	6	5	3	1	< 1	—	—	—	—	164	88	15	8	6	3	1	< 1	—	—	—	—	.38	1.00
Hair loss	188	99	1	< 1	—	—							174	94	5	3	7	4							**.0008**	NA
Skin	187	99	1	< 1	1	< 1	—	—	—	—	—	—	185	99	1	< 1	—	—	—	—	—	—	—	—	.87	—
Anorexia	166	88	14	7	8	4	1	< 1	—	—	—	—	161	87	18	10	13	7	4	2	—	—	—	—	.07	.21
Constipation	157	83	19	10	10	5	2	1	1	< 1	—	—	157	84	16	9	9	5	4	2	—	—	—	—	.75	.72
Diarrhea	173	92	8	4	5	3	3	2	—	—	—	—	163	88	12	6	10	5	—	—	1	< 1	—	—	.23	.62
Nausea	120	63	45	24	22	12	2	1	—	—	—	—	115	62	39	21	27	15	5	3	—	—	—	—	.53	.28
Vomiting	154	81	13	10	15	8	1	< 1	—	—	—	—	144	77	22	12	15	8	5	3	—	—	—	—	.31	.12
Stomatitis	179	95	7	4	3	2	—	—	—	—	—	—	174	94	7	4	3	2	2	1	—	—	—	—	.55	.25
Liver	145	77	14	7	16	8	10	5	4	2	—	—	159	85	9	5	8	4	7	4	3	2	—	—	**.03**	.42
Neuropathy	183	99	1	< 1	—	—	—	—	—	—	—	—	181	97	1	< 1	2	1	2	1	—	—	—	—	.06	.25
Other	167	88	4	2	6	3	7	4	38	2	25	1	165	89	6	3	6	3	6	3	1‡	< 1	2¶	1	.87	.53

NOTE. Bold font indicates statistical significance.
Abbreviations: NCI-CTC, National Cancer Institute Common Toxicity Criteria; CV, cardiovascular; NA, not applicable.
*Any grade, tested for trend.
†Severe (grade 3 or higher).
‡Two grade 4 hyperglycemia; 1 grade 4 hypokalemia.
§One grade 5 stroke, 1 grade 5 GI bleeding.
‖One grade 4 hyper-gamma GT.
¶One death for unknown reason 7 days after G4 thrombocytopenia, one sudden death.

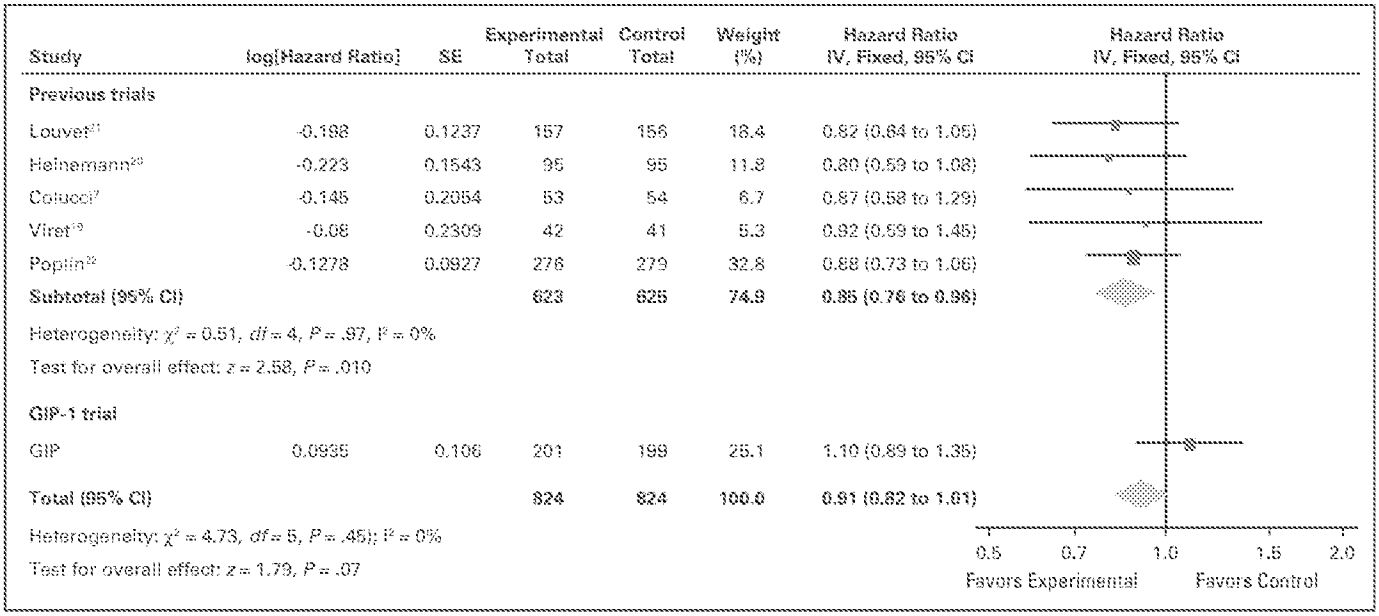

Fig 4. Updated meta-analysis of randomized trials comparing gemcitabine + platinum compound versus gemcitabine in advanced pancreatic cancer. IV, inverse variance; df, degrees of freedom.

C30, items 29-30) was 6.20 in arm A and 0.09 in arm B. This difference was not statistically significant ($P = .07$). Statistically significant differences were reported in social functioning and limitation in planning, both favoring single-agent gemcitabine, and in hepatic symptoms, favoring combination.

DISCUSSION

In this phase III trial, the addition of cisplatin to gemcitabine for patients with advanced pancreatic cancer failed to show any advantage. Improvement in OS, the primary end point, was not obtained, and there was no benefit in terms of PFS, ORR, CB, and QoL. Hematologic toxicity was higher with the addition of cisplatin. Nonhematologic toxicity, on the contrary, was similar between arms. The schedule adopted, characterized by weekly doses of cisplatin, was not associated with significant emesis or other typical effects of higher doses. The addition of cisplatin, however, was associated with a statistically significant reduction in the dose intensity of gemcitabine.

The cisplatin schedule adopted in this study was based on the previous GOIM trial.[7] In that small trial, the combination produced a significant increase in ORR and TTP, compared to single-agent gemcitabine, without significant prolongation of OS. After the start of GIP-1, two other trials testing the addition of cisplatin to gemcitabine in patients with advanced pancreatic cancer have been published, although with different cisplatin dose and schedule.[19,20] In a small French trial, cisplatin 75 mg/m^2 every 4 weeks added to gemcitabine did not demonstrate significant benefit.[19] In the larger German trial, enrolling 195 patients, subjects in experimental arm received gemcitabine 1,000 mg/m^2 and cisplatin 50 mg/m^2 on days 1 and 15 of a 4-week cycle.[20] Combination chemotherapy was associated with a statistically significant prolongation of PFS. Median OS was longer with combination (7.5 v 6.0 months), but the difference was not statistically significant. Furthermore, two randomized trials testing the addition of oxaliplatin to gemcitabine did not demonstrate a statistically significant prolongation in OS.[21,22]

All these trials had a sample size considered too small to demonstrate potentially relevant differences in survival. With this aim, several pooled analyses and meta-analyses have been performed.[23-26] In the meta-analysis by Heinemann,[25] pooling the above cited five trials that added oxaliplatin or cisplatin, the platin-based combination treatment was associated with a significant improvement in OS (HR, 0.85; 95% CI, 0.76 to 0.96; $P = .01$). We updated the meta-analysis including GIP-1 trial, adding 400 to the previous 1,248 patients (Fig 4). There was no statistical heterogeneity with the addition of GIP-1 to the five previous trials. Indeed, the addition of GIP-1 data produced a pooled HR of 0.91 (95% CI, 0.82 to 1.01). The pooled result is no longer statistically significant.

Other gemcitabine-based combinations have been tested. In a randomized phase III trial, the combination of gemcitabine and capecitabine produced a trend toward prolongation of OS,[27] but this advantage was not confirmed in another trial.[28] In recent years, phase III trials have tried to obtain a prolongation of survival using molecularly targeted agents.[29,30] A statistically significant OS benefit was obtained adding erlotinib to gemcitabine.[29] However, the small survival gain renders the clinical value of erlotinib debatable, and single-agent gemcitabine remains standard first-line treatment.

Subgroup analyses from several studies indicated that the benefit of gemcitabine-based combination chemotherapy in terms of OS is predominantly seen in patients with good KPS.[23,24,28] Although that finding should be considered only hypothesis generating, some guidelines consider combination chemotherapy a potential option for patients with good KPS.[2] Our data do not support this finding, and subgroup analysis shows no evidence of a differential effect of treatment in patients with KPS ≥ 80 versus KPS lower than 80. Also if patients, according to literature, are divided in good KPS (≥ 90) versus poor KPS (≤ 80), there is no evidence of interaction (data not shown).

In conclusion, the negative results of the GIP-1 trial add important evidence to the debate about the role of combination chemotherapy as first-line treatment of advanced pancreatic cancer. In this trial, the addition of weekly cisplatin to gemcitabine did not produce any benefit compared to single-agent gemcitabine. Prognosis of patients with advanced pancreatic cancer remains unacceptably poor. The best option for these patients remains enrollment in prospective clinical trials.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Giuseppe Colucci, Roberto Labianca, Francesco Di Costanzo, Paolo Pederzoli, Massimo Falconi, Ciro Gallo, Massimo Di Maio, Francesco Perrone

Provision of study materials or patients: Giuseppe Colucci, Roberto Labianca, Francesco Di Costanzo, Vittorio Gebbia, Giacomo Cartenì, Bruno Massidda, Elisa Dapretto, Luigi Manzione, Elena Piazza, Mirella Sannicolò, Marco Ciaparrone, Luigi Cavanna, Francesco Giuliani, Evaristo Maiello, Antonio Testa, Paolo Pederzoli, Massimo Falconi
Collection and assembly of data: Giuseppe Colucci, Roberto Labianca, Francesco Di Costanzo, Vittorio Gebbia, Giacomo Cartenì, Bruno Massidda, Elisa Dapretto, Luigi Manzione, Elena Piazza, Mirella Sannicolò, Marco Ciaparrone, Luigi Cavanna, Francesco Giuliani, Evaristo Maiello, Antonio Testa, Massimo Di Maio, Francesco Perrone
Data analysis and interpretation: Giuseppe Colucci, Roberto Labianca, Francesco Di Costanzo, Ciro Gallo, Massimo Di Maio, Francesco Perrone
Manuscript writing: Giuseppe Colucci, Roberto Labianca, Francesco Di Costanzo, Ciro Gallo, Massimo Di Maio, Francesco Perrone
Final approval of manuscript: Giuseppe Colucci, Roberto Labianca, Francesco Di Costanzo, Vittorio Gebbia, Giacomo Carteni, Bruno Massidda, Elisa Dapretto, Luigi Manzione, Elena Piazza, Mirella Sannicolò, Marco Ciaparrone, Luigi Cavanna, Francesco Giuliani, Evaristo Maiello, Antonio Testa, Paolo Pederzoli, Massimo Falconi, Ciro Gallo, Massimo Di Maio, Francesco Perrone

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GASTROINTESTINAL (NONCOLORECTAL) CANCER

Randomized phase III trial comparing FOLFIRINOX (F: 5FU/leucovorin [LV], irinotecan [I], and oxaliplatin [O]) versus gemcitabine (G) as first-line treatment for metastatic pancreatic adenocarcinoma (MPA): Preplanned interim analysis results of the PRODIGE 4/ACCORD 11 trial.

T. Conroy, F. Desseigne, M. Ychou, M. Ducreux, O. Bouche, R. Guimbaud, Y. Becouarn, C. Montoto-Grillot, S. Gourgou-Bourgade, A. Adenis, FNCLCC-FFCD PRODIGE Group

Centre Alexis Vautrin, Vandoeuvre les Nancy, France; Centre Léon Berard, Lyon, France; Centre Val d'Aurelle, Montpellier, France; Institut Gustave Roussy, Villejuif, France; University Hospital Robert Debre, Reims, France; University Hospital of Purpan, Toulouse, France; Institut Bergonié, Bordeaux, France; Fédération Nationale des Centres de Lutte Contre le Cancer, Paris, France; Centre Oscar Lambret, Lille, France

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Abstract

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Background: In a randomized phase II trial of F vs G in 88 MPA patients (pts), we reported that F induces a response rate > 30% (Ychou, ASCO 2007). Because the trial met its planned objectives, it has been pursued as a phase III to compare overall survival (OS).

Methods: Chemotherapy-naïve pts aged 18-75 years with histologically/cytologically-confirmed measurable MPA were randomized to receive F (O 85 mg/m² d1 + I 180 mg/m² d1 + LV 400 mg/m² d1 followed by 5FU 400 mg/m² bolus d1 and 2,400 mg/m² 46h continuous infusion biweekly) or G (1g/m² IV weekly x7, 1 w rest, then weekly × 3q4w). Eligibility included adequate organ function, performance status (PS) 0-1, no prior chemotherapy or radiotherapy. Pts were stratified by centre, PS, and primary tumor location (head vs. other). The primary endpoint was OS. With a planned sample size of 360 pts, the trial was designed to detect an improvement from 7 to 10 months (m) median survival (HR = 0.70) with 80% power and α = 0.05.

Results: 342 pts were enrolled between 01/2005 and 10/2009. Pts were well balanced for baseline characteristics: male 63%; median age 60 years; PS 0 38%. At the planned interim analysis, the Independent Data Monitoring Committee recommended to stop the study. Among 250 treated and monitored pts, median relative dose-intensities of 5FU, I, O and G were 0.81, 0.80, 0.78 and 1.01 respectively. Grade 3/4 toxicities per pts (%) in arms F/G were diarrhea 12.3/1.6, nausea 15.6/6.3, vomiting 17.2/6.3, fatigue 24/14.3, neutropenia 47.9/19.2 and febrile neutropenia 5.7/0. No toxic death occurred. Confirmed response rates (F/G) were 27.6% and 10.9% (p = 0.0008). Median follow-up was 19.5 m. Median PFS was 6.4/3.4 m (p < 0.0001). As of 09/2009, median OS was 10.5/6.9 m (HR = 0.61; 95%CI = 0.46-0.81; p < 0.001).

Conclusions: F is the first non containing G therapy that has shown a significantly longer OS, PFS and

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higher response rate than G alone. The toxicities of this regimen are manageable and F may emerge as a new standard for treatment of MPA in pts with good PS. Final analysis on the total population will be presented.

No significant financial relationships to disclose.

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ANTI-TUMOUR TREATMENT

Second-line therapy for advanced pancreatic cancer: A review of the literature and future directions ☆

Ana Custodio *, Javier Puente, Javier Sastre, Eduardo Díaz-Rubio

Department of Medical Oncology, Hospital Universitario Clínico San Carlos, Madrid, Spain

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SUMMARY

Whereas first-line chemotherapy (CT) with single-agent gemcitabine or gemcitabine-based combinations provides a proven benefit in patients with locally advanced or metastatic pancreatic cancer (PC), the role of salvage CT after gemcitabine-failure is not well-established and to date no regimen has emerged as preferred in this setting. Several clinical trials have investigated the efficacy and toxicity-profile of second-line CT and indicated that selected patients may obtain significant benefit from it, also with regard to survival. However, definitive results from large randomized phase III studies are still lacking, and the evidence for clinical benefit of salvage CT is based on small phase II trials that evaluated different treatment schedules in heterogeneous populations. The main goal of this paper is reviewing this topic.

Introduction

Pancreatic cancer (PC) is a devastating disease. As a whole, every patient diagnosed with a PC will die within 12 months, including the vast majority of those that underwent a potential curative surgery.[1,2] These dismal results are secondary to the fact that most of patients are diagnosed either with microscopic or macroscopic dissemination. Additionally, resistance to every current therapy is a feature inherent or acquired for PC cells during the evolution of the disease.

Gemcitabine-based combinations are the standard of care as first-line systemic chemotherapeutic treatment of patients with advanced PC.[3–6] These schedules showed a significant advantage with regard to clinical benefit response and prolongation of progression-free survival (PFS) and overall survival (OS). Thus there are an increasing number of patients with relatively low tumor burden metastatic disease and excellent performance status (PS) when progression on first-line therapy is diagnosed. Many oncologists are aware of this fact but hesitate about the best second-line therapy. In this regard and, although there is not an established salvage treatment for PC patients, we have summarized most relevant data in this setting to help physicians to make a choice.

On the other hand, due to the limited improvement derived from second-line chemotherapy (CT), identification of subgroups of patients with first-line refractory PC is needed in order to individualize treatments and properly treat them with either additional CT or best supportive care (BSC). Several studies have showed that the most important factors that predict survival time in patients with advanced PC after gemcitabine-failure are time to disease progression (TTP) following first-line treatment, PS, baseline serum carbohydrate antigen (CA 19.9) and serum albumin levels.[7–13]

Second-line treatment vs. supportive care

To date no data from a completed second-line trial that randomly compares CT plus BSC vs. BSC alone are available in this patient population. A survival benefit for salvage CT vs. BSC was suggested in a preliminary report from a small German phase III study in which patients failing first-line gemcitabine were randomly assigned to BSC alone vs. BSC with oxaliplatin/leucovorin (LV)/5-FU (OFF) CT.[14] OFF regimen consisted on oxaliplatin 85 mg/m^2 on days 8 and 22 and LV 200 mg/m^2 over 30 min followed by 5-FU 2000 mg/m^2 over 24 h, with both drugs given on days 1,8,15 and 22. Courses of CT were repeated every six weeks. After 46 patients included of the 165 planned, the study was closed prematurely due to the low accrual. Several centers participating in the trial refused to further accept a "standard" arm with BSC only after gemcitabine-failure. Both arms were well-balanced regarding age, tumor stage, sex and PS. Preliminary results showed that the experimental group had a significantly longer median survival

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* Corresponding author. Address: Medical Oncology Department, Hospital Clínico San Carlos, C/Profesor Martín Lagos, s/n. 28040, Madrid, Spain. Tel.: +34 680368448.
E-mail address: anabelencustodio@hotmail.com (A. Custodio).

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from the start of treatment (21 vs. 10 weeks; $p = 0.0077$) and longer median OS (40 vs. 34.4 weeks; $p = 0.0312$).

Choice of regimen

As we referred above, certain baseline patients characteristics (e.g. PS) have been shown to be strong and independent prognostic factors for survival.[7–13] Therefore, the study population can determine –in part– the survival results of a clinical trial. In this regard, published phase II studies of salvage CT for advanced PC included a wide range of different and noncomparable patient populations. For example, patients differed in terms of PS, tumor stage, prior CT lines or median PFS after previous treatments. Furthermore, the sample size of most series is limited to 20–30 patients per treatment arm, thus producing data with very large confidence intervals. Given these differences, the lack of information on important clinical factors, and other potential bias related to phase II trials design, results are difficult to compare across small phase II trials, especially in terms of survival. Only a well-designed, randomized phase III trial that stratifies patients according to prognostic factors will be able to determine a potential survival benefit of a second-line regimen in patients with advanced PC.

However, we consider that the second-line scenario is usually seen in clinical practice and, in spite of these limitations, it may be of interest to review this data to help oncologists to choose a non-phase III proven therapy for second-line patients.

Second-line single-agent chemotherapy

Results from prospective clinical trials that evaluated single-agent therapy in gemcitabine-pretreated patients with advanced PC are summarized in Table 1. Most of them were phase II studies[15,17,19–23] while two of these trials were two-arm randomized studies,[16,18] one comparing rubitecan with physicians best choice of treatment[16] and another comparing raltitrexed with the irinotecan plus raltitrexed combination.[18] Although few patients showed an objective response, the rate of disease-control ranged from 17% to 39% and the reported median OS from the beginning of salvage CT after gemcitabine-failure ranged from 3.1 to 7.6 months.

Rubitecan (Orathecin®), an orally active camprothecin derivative, is a topoisomerase I inhibitor which has demonstrated radiological and clinical responses in patients with advanced PC in early clinical trials.[15,16] Burris and colleagues developed a phase II study to asses the safety and efficacy of rubitecan in 58 patients with locally advanced or metastatic PC who had received at least one prior CT regimen.[15] They were treated with rubitecan by 1.5 mg/m^2 orally for five consecutive days per week, followed by 2 days off therapy, repeatedly. Among 43 patients with measurable disease, 7% achieved partial responses (PR) and 16% had stable disease (SD) for a clinical benefit of 23%. The median TTP and median OS were 59 (95% CI, 53–65) and 92 days (95% CI, 76–124), respectively. The median OS time was longer in patients with PR or SD than in the overall study cohort (10 months vs. 3 months, respectively). Treatment was generally well-tolerated. Gastrointestinal events were the most commonly reported toxicity (12–14% of the patients experienced grade 3–4 emesis and 9% grade 3–4 diarrhea) and the incidence of grade 3–4 neutropenia was 19%. Based on the outcome of this trial, a randomized phase III study was conducted to compare rubitecan vs. physicians best choice of treatment or care (BC) in patients with advanced PC who had failed or relapsed after ≥ 1 prior CT.[16] A total of 409 patients were randomized to receive either rubitecan 1.5 mg/m^2/day orally for 5 consecutive days each week for 8 or more weeks ($n = 198$) or BC ($n = 211$) until disease progression or toxicity. More than 70% were refractory to 2 or more prior regimens. BC was defined as the standard regimen of best alternative CT (89%) or supportive care (11%). A total of 49% of BC patients crossed over to rescue therapy with rubitecan at time of failure. No significant difference was noted in median survival time (108 vs. 94 days, respectively; p=0.626), whereas a significant survival advantage was observed for BC patients crossed over to rubitecan vs. BC non-rescue patients (147 vs. 60 days for median OS, respectively; $p < 0.0001$). In patients with measurable disease (59%), there were 12 responders for rubitecan (2 complete responses [CR] and 10 PR) for an overall response rate (RR) of 11%, compared to 1 responder (<1%; 1 CR) for BC ($p > 0.001$) and SD was also more common with this agent (44 vs. 27 patients). More than 2-fold more patients on rubitecan (28% vs. 13%) achieved tumor growth control (CR+PR+SD), a primary objective for palliative treatment of this disease. Median PFS was significantly longer for rubitecan (58 vs. 48 days; $p = 0.003$). Although grade 3–4 toxicity was more common for rubitecan (neutropenia 28% vs. 14%, anemia 21% vs. 9%, nausea/vomiting 14% vs. 9%; diarrhea 12% vs. 5%), it was generally well-tolerated and <5% patients discontinued treatment due to toxicity. In conclusion, rubitecan offers the convenience of oral dosing and can achieve control of tumor growth with an acceptable risk:benefit ratio in pretreated PC patients.

Second-line CT with single-agent pemetrexed after gemcitabine-failure has also been assessed. In patients with advanced PC, front line CT with pemetrexed has shown activity as a single-agent[24] as well as in combination with gemcitabine.[25] On the basis of these results, a multicenter phase II trial was conducted to evaluate the efficacy and safety of pemetrexed as a second-line

Table 1
Clinical trials investigating salvage single-agent chemotherapy in gemcitabine-pretreated patients with advanced pancreatic cancer.

Treatment regimen	No. of patients	Median age (years)	KPS 90–100%/ECOG 0–1 (%)	Metastatic disease (%)	RR (%)[a]	DCR (%)[a]	PFS/TTP (months)	OS (months)
Rubitecan (phase II)[15]	58	63	NA	90	5	17	2.0	3.1
Rubitecan (phase III)[b][16]	198	64	100	91	6	28	1.9	3.6
Pemetrexed[17]	52	63	94	89	4	23	1.6	4.7
Raltitrexed[18]	19	60	21	100	0	37	2.5	4.3
Paclitaxel[19]	18	59	NA	100	6	33	NA	4.1
Eribulin mesylate[20]	15	61	73	NA	0	42	NA	NA
Oxaliplatin[21]	18	61	72	72	0	17	NA	3.9
Capecitabine[22]	39	63	51	97	0	39	2.3	7.6
S-1[23]	40	62	90	100	15	58	2.0	4.5

KPS: Karnofski performance status; ECOG: Eastern Oncology Cooperative Group; NA: not available; RR: response rate; DCR: disease-control rate; PFS: progression-free survival; TTP: time to progression; OS: overall survival.

[a] Intention-to-treat analysis.

[b] Abstract publication only.

treatment in patients with unresectable or metastatic PC who had progressed on single-agent gemcitabine or gemcitabine-based first-line CT.[17] A total of 52 patients received pemetrexed 500 mg/m^2 as a 10-min infusion every 3 weeks until disease progression or occurrence of unacceptable toxicity. A dose-escalation by 100 mg/m^2 every other cycle was allowed- according to defined escalation criteria- at the investigator's discretion up to a maximum dose of 900 mg/m^2. The overall RR was 3.8% (2 PR); 10 patients (19.2%) experienced SD, 9 of them for >12 weeks. At least CA 19.9 reduction ⩾50% occurred in 12 patients (23.1%). The 3-months survival rate was 75% (95% CI, 63.2–86.8%), the median TTP was 7 weeks (range 1–62 weeks) and the median OS time was 20 weeks (range 1–84 weeks). Toxicity was manageable. Grade 3/4 hematological toxic effects were neutropenia (17.3%), thrombocytopenia (5.8%) and anemia (3.8%). Febrile neutropenia occurred in 2 patients (3.8%). The most frequent non-hematological toxic effects (any grade) were diarrhea, nausea and stomatitis (23.1% each). Authors concluded that pemetrexed is a safe treatment option with limited activity in the second-line setting after gemcitabine-failure.

Activity for raltitrexed, a pure thymidylate synthase inhibitor, has been shown in a randomized phase II trial that compared 21 days cycles of raltitrexed (3 mg/m^2 on day 1) to 21 days cycles of irinotecan (200 mg/m^2 day 1) plus raltitrexed (3 mg/m^2 day 2) in 38 patients with metastatic PC who progressed while receiving or within 6 months after discontinuation of palliative first-line CT with gemcitabine.[18] In the combination arm, the objective RR was 16% (95% CI, 3–40%), which was clearly superior to that observed in the control arm with raltitrexed alone, in which no response was evidenced. Therefore, the trial was stopped at the first stage of accrual. Also in terms of median PFS (2.5 vs. 4 months), OS (4.3 vs. 6.5 months) and clinical benefit response (8 vs. 29%), a clear benefit was noted in favour of the combination therapy. Overall, both chemotherapeutic drug regimens were fairly well-tolerated. The most frequently encountered toxicity was myelosuppresion, although grade 3/4 leukocytopenia/neutropenia occurred in only 4 and 5 patients in control and combined arms, respectively. Gastrointestinal symptoms (42% vs. 68%), partial alopecia (0% vs. 42%), and cholinergic syndrome (0% vs. 21%) were more commonly noted in irinotecan plus raltitrexed arm; however, grade 3 adverse events occurred in only three patients in both treatment groups.

Second-line weekly paclitaxel was also active in 18 patients who failed previous gemcitabine-containing schedule (gemcitabine as monotherapy and/or in combination with 5-FU and folinic acid).[19] At the time of analysis, 1 patient achieved a CR, while 5 others had SD and median survival time was 17.5 weeks. Regarding toxicity, grade 3–4 symptomatic toxicity was rare, except alopecia. Only one patient each presented with anemia and leukocytopenia grade III and hepatotoxicity with a temporary increase in aminotranferase grade II occurred in three patients. Eribulin mesylate, a halichondrin B analog that inhibits microtubule dynamics by a mechanism that is different from other tubulin-targeted agents, have also been evaluated as salvage therapy in advanced PC.[20] Fifteen patients were treated with this agent at a dose of 1.4 mg/m^2 on days 1 and 8 every 3 weeks. Grade 3–4 adverse events included neutropenia (29%), fatigue (14%), peripheral neuropathy (7%) and thrombosis (7%). There were no CR or PR, but 42% of patients had SD and for 3 patients this was maintained for more than 9 months. Oxaliplatin as second-line treatment for advanced PC has been studied in 18 patients previously treated with gemcitabine-based CT.[21] At a dose of 130 mg/m^2 iv every 21 days, it is well-tolerated and associated with improvement of tumor-related symptoms (3 patients had SD for >2 months and a clinical benefit response was observed in 5 patients) despite its failure to induce objective responses. On the other hand, single-agent capecitabine demonstrated to be an active and safe treatment option for patients with advanced PC who had already received at least one previous treatment regimen containing full-dose gemcitabine.[22] This agent was given orally to 39 patients at a dose of 1250 mg/m^2 twice daily for 14 days followed by 7 days of rest. After a median follow-up of 3 treatment cycles, 27 patients were evaluable for response: no CR or PR was observed, but 15 patients (39%) had SD. A CA 19.9 reduction of >20% after 2 cycles was documented in 6 patients (15%). Median TTP was 2.3 months and median OS was 7.6 months. Predominant grade 2 and 3 toxicities were hand-foot syndrome (28%, 13% grade 3), anemia (23%), leg edema (15%), diarrhea (13%), nausea/vomiting (10%) and leukocytopenia (10%). Finally, a japanese phase II study evaluated the efficacy and toxicity of single-agent S1 therapy in 40 patients with gemcitabine-refractory metastatic PC.[23] S1 is an oral drug consisting of the 5-FU prodrug tegafur combined with two modulators of the 5-FU activity. It was administered orally at 40 mg/m^2 twice daily for 28 days with a rest period of 14 days until disease progression or unacceptable toxicity. Although no CR was seen, PR was obtained in six patients (15%, 95% CI 3.9–26%) and SD in 17 patients (43%). The median PFS was 2 months, and the median survival time was 4.5 months with a 1-year survival rate of 14.1%. The most common adverse reactions were fatigue and anorexia, although most of those adverse events were tolerable and reversible. These preliminary efficacy data appears interesting an oral fluoropyrimidines may be a promising approach in second-line treatment regimens for advanced PC. However, further investigation of theses regimens is warranted in larger studies in order to confirm initial results.

In summary, single-agent CT may constitute a feasible treatment option with acceptable activity and favourable toxicity-profile in gemcitabine-resistant advanced PC patients.

Second-line combination chemotherapy

Several clinical trials have evaluated the activity and toxicity-profile of different combination CT regimens in gemcitabine-pretreated patients with locally advanced or metastatic PC. Most of the studies included a platinum compound (e.g., oxaliplatin or cisplatin), while other trials continued treatment with gemcitabine beyond progression and added one or even more cytotoxic agents in their second-lines schedules. Table 2 showed results of clinical trials investigating second-line CT in gemcitabine-pretreated patients with advanced PC.

Platinum-based combinations

Several studies have demonstrated benefit for oxaliplatin in combination with other chemotherapic agents in patients with advanced PC who have failed first-line gemcitabine-based treatments.[14,26–39] To date, five clinical trials have reported clinical outcomes from a FOLFOX or FOLFOX-like regimen after gemcitabine-failure.[14,26–29] As discussed above, oxaliplatin plus LV/5-FU as salvage therapy showed a survival benefit vs. BSC in gemcitabine-refractory disease.[14] The superiority of the OFF regimen compared with 5-FU and LV has been documented in the randomized phase II CONKO-003 trial.[26,27] Final results presented at 2008 American Society of Clinical Oncology (ASCO) Annual Meeting, showed that OFF schedule was associated with a significantly longer median PFS (13 vs. 9 weeks; $p = 0.012$) and OS (26 vs. 13 weeks; $p = 0.014$). Patients receiving oxaliplatin had significantly more neurotoxicity (mostly grade I or II) but otherwise, the regimen was well-tolerated.

A prospective phase II study also evaluated a similar second-line regimen in 30 patients with unresectable PC following relapse to gemcitabine.[28] Treatment consisted of oxaliplatin

Table 2
Clinical trials investigating second-line combination chemotherapy in gemcitabine-pretreated patients with advanced pancreatic cancer.

Treatment regimen	No. of patients	Median age (years)	KPS 90–100%/ECOG 0–1 (%)	Metastatic disease (%)	RR (%)[a]	DCR (%)[a]	PFS/TTP (months)	OS (months)
Oxa/5-FU Cl/LV vs. BSC[14]	46	NA	NA	NA	NA	NA	OFF:5.25 BSC:2.5	OFF:10 BSC:8.5
Oxa/5-FU Cl/LV vs. 5-FU Cl/LV[26,27]	168 (OFF:77; FF:91)	OFF: 61 FF:60	OFF:54 FF:50	OFF:85.5 FF:89.2	NA	NA	OFF:3.25 FF:2.25	OFF: 6.5 FF:3.35
Oxa/5-FU Cl/LV[28]	30	63	33[b]	97	23	53	5.1	5.8
FOLFOX-4[29]	42	64	62	83	14	52	4	6.7
Modified FOLFOX(a) vs. *modified* FOLFIRI.3(b)[30]	(a) 30 (b) 30	(a) 56 (b) 56	(a) 97 (b) 100	NA	NA	(a) 20 (b) 28	(a) 1.4 (b) 1.9	(a) 4 (b) 4
Oxa/5-FU CI[31]	18	57	22.2	94.5	0	17	0.9	1.3
Oxa + Gem[32]	33	57	88	64	21	58	4.2	6.0
Oxa + Cap[34]	39	62	80	NA	3	23	NA	5.8
Oxa + Cap[36]	15	64.1	NA	100	7	40	4.1	10
Oxa + irinotecan[37]	30	60	30	100	10	33	4.1	5.9
Oxa + pemetrexed[38]	16	NA	NA	NA	20	60	3.3	NA
Oxa + raltitrexed[39]	41	61	61	100	24	51	1.8	5.2
i-Cisplatin + Gem[40]	24	66	50	79	8	67	NA	4.0
Cisplatin + irinotecan + Gem + 5-FU + LV[41]	34	65	NA	100	24	44	3.9	10.3
Cisplatin + S-1[42]	17	60	NA	53	29	NA	NA	9.0
Cap + Gem + docetaxel[43]	35	59	52	100	29	60	NA	11.2
Mitomycin+docetaxel + irinotecan[44]	15	61	27	100	0	20	1.7	6.1
Irinotecan + raltitrexed[18]	19	63	21	100	16	47	4.0	6.5

KPS: Karnofsky performance status; ECOG: Eastern Cooperative Oncology Group; NA: not available; RR: response rate; DCR: disease-control rate; PFS: progression-free survival; TTP: time to progression; OS: overall survival; CI: continuous infusion; OFF: oxaliplatin+5-FU CI/LV; FF: 5-FU CI/LV; Oxa: oxaliplatin; 5-FU: 5-fluorouracil; LV: leucovorin; Gem: gemcitabine; Cap: capecitabine.

[a] Intention-to-treat analysis.

[b] KPS 80–100%.

50 mg/m^2 as a 2-h intravenous infusion, followed by LV 50 mg/m^2 delivered as an intravenous bolus and 5-FU 500 mg/m^2 as a 1-h infusion. This combination revealed an encouraging RR of 23.3% with a corresponding disease-control rate of 53%. Patients that had responded to first-line gemcitabine treatment were found more likely to respond or stabilize their disease with a second-line treatment. The median duration of response was 22 weeks and median OS was 25 weeks. Grade 3/4 toxicity included leukopenia (16%), anemia (3.2%), thrombocytopenia (3.2%), diarrhea (14.2%), fatigue (16.1%) and neurotoxicity (4.2%). Eight patients (27%) suffered a febrile neutropenic event, while 17 patients required G-CSF support. There were no treatment-related deaths. These data correlate well with a retrospective Italian analysis including 42 gemcitabine-pretreated patients who received FOLFOX-4 regimen.[29] Six PR (14%) and 16 stabilizations (38%) were recorded for a tumor growth control rate of 57%. The median TTP was 4 months (range 1–7) and median OS was 6.7 months (range 2–9). Twenty-seven patients (64%) had stabilization of PS or subjective improvement of cancer-related symptoms. Overall, side effects were moderate and easily manageable. Finally, a randomized phase II trial of modified FOLFOX vs. modified FOLFIRI.3 as second-line regimen has been conducted in 60 patients with gemcitabine-refractory PC.[30] With a median follow-up period of 6 months, the median OS was 4 months in both groups (HR = 0.95; 95% CI, 0.52–1.75) with 6-months survival rates of 25% and 20%, respectively. The median PFS was 1.4 months for FOLFOX and 1.9 months for FOLFIRI.3 (HR = 1.11; 95% CI, 0.64–1.92). Disease-control was achieved in 20% (FOLFOX) and 28% (FOLFIRI.3) of patients with measurable disease. The incidence of grade 3–4 toxicity was similar in both groups.

Other oxaliplatin-5-FU-based combinations have also been evaluated in different studies.[31,32] Within a French multicenter trial,[31] 18 patients received second-line treatment with the OXFU regimen (oxaliplatin 130 mg/m^2 as 2-h infusion combined with 5-FU 1000 mg/m^2/day continuous, days 1–4, every 3 weeks) after failure of single-agent first-line therapy.[31] However, all included patients in this trial received a gemcitabine-free front-line CT within a randomized phase II study. There was no objective response and 3 patients (17%) had SD. Median TTP from the start of second-line treatment was 0.9 months. Median OS was 4.9 months from the start of front-line therapy and 1.3 months from the start of second-line therapy.

Six phase II trials have investigated oxaliplatin-based second-line CT in combination with gemcitabine,[33] capecitabine,[34–36] irinotecan,[37] pemetrexed[38] or raltitrexed,[39] respectively in advanced PC. These regimens demonstrated to be active and well-tolerated in selected patients who have failed single-agent gemcitabine. The achieved clinical benefit rates and PFS ranged from 20% to 60% and 1.8 to 4.2 months, respectively and the OS from the start of salvage therapy were estimated at between 5.2 and 6 months. In the series of 30 patients treated with irinotecan and oxaliplatin,[37] 2 of the 3 patients who had a PR were down-staged and subsequently underwent surgery. In the phase II trial conducting by Xiong and colleagues[34] the dose of both oxaliplatin and capecitabine was modulated according to patients' age and PS. Thus, patients aged ≤65 years who had an ECOG PS of 0–1 received oxaliplatin at a dose of 130 mg/m^2 given on day 1 and capecitabine at a dose of 1000 mg/m^2 twice daily for 14 days. For patients aged >65 years or with an ECOG PS of 2, the oxaliplatin dose was 110 mg/m^2 on day 1 and the capecitabine dose was 750 mg/m2 twice daily for 14 days. The treatment was repeated every 3 weeks. Of the 39 evaluable patients, only 1 patients had a PR, but another 10 patients showed SD, conducting to a median PFS and OS of 9.9 weeks (95% CI, 9.6–14.5 weeks) and 23 weeks (95% CI, 17–31 weeks), respectively. Two Spanish phase II studies have also examined the efficacy and tolerability of oxaliplatin and capecitabine as second-line therapy after gemcitabine-failure.[35,36] In one of them[35] 18 patients with advanced pancreatic, biliary and gallbladder adenocarcinomas (50% with PC) received oxaliplatin 130 mg/m^2 day 1 and capecitabine 1000 mg/m^2 twice daily days 2–14, every 3 weeks until progression or [illegible] PR was

documented in 1 patient (5.6%) and 8 patients had SD (44.4%). Median PFS was 16.71 weeks and median OS was 24.71 weeks, significantly longer for patients with PS 0–1 than in those with PS 2 ($p = 0.001$). In the trial conducting by Gasent Blesa and coileagues,[36] 15 patients with metastatic PC were treated with oxaliplatin 100 mg/m^2 day 1 and capecitabine 1000 mg/m^2 twice daily days 1–14, every 21 days (XELOX regimen). Best radiological response was: 1 patient CR, 5 patients SD and 9 patients PD. Median PFS was 124 days (21–480, censored data) and median survival since the beginning of second-line CT was 163 days (censored data). Toxicity was manageable in both studies. Finally, Reni et al. performed univariate and multivariate analyses focused on the relationship between OS and patient-, treatment-, and tumor-related variables in 41 patients with metastatic PC treated with raltitrexed–oxaliplatin salvage therapy.[39] In total, 10 patients (24%) yielded a PR and 11 a SD. PFS at 6 months was 14.6% and median OS was 5.2 months. Treatment was well-tolerated. OS was significantly longer in patients with previous PFS ranging between 6.1 and 12 months relative to those with shorter PFS and in patients without pancreatic localization.

Cisplatin-based regimens were investigated in several studies as salvage CT after gemcitabine-failure.[40–42] Stathopoulos and coileagues performed a phase I–II trial that evaluated escalating dose of a liposomal cisplatin (lipoplatin) formulation in combination with gemcitabine in 24 advanced pretreated PC patients.[40] The gemcitabine dose was maintained at 1000 mg/m^2 and the lipoplatin dose was escalated from 25 mg/m^2 to 125 mg/m^2 by 25 mg/m^2 per dose level. Lipoplatin at 125 mg/m^2 was defined as dose-limiting toxicity (DLT) and 100 mg/m^2 as the maximum tolerated dose (MTD). Both agents were given on days 1 and 15 every 28 days. Preliminary objective RR data showed a PR in 2 patients (8.3%) and SD in 14 patients (58.3%) for a median duration of 3 months (range 2–7 months) and clinical benefit in 8 patients (33.3%). Median survival for the beginning of second-line treatment was 4 months. A second study was carried out as a retrospective analysis of 34 consecutive metastatic PC patients that received a four-drug combination of cisplatin, gemcitabine, irinotecan, 5-FU and LV (G-FLIP regimen).[41] G-FLIP was administered over 48 h and repeated every 2 weeks. Day 1 treatment consisted of sequentially administered gemcitabine 500 mg/m^2, irinotecan 80 mg/m^2, LV 300 mg/m^2, 5-FU 400 mg/m^2 bolus followed by infusional 5-FU 600 mg/m^2 over 8 h. Day 2 treatment consisted of LV 300 m/m^2 and 5-FU 400 mg/m^2 bolus, followed by cisplatin 50 to 75 mg/m^2, and then infusional 5-FU 600 mg/m^2 over 8 h. Efficacy results showed PR and SD in 8 (24%) and 7 (20.5%) patients, respectively. Median TTP was 3.9 months and median OS 10.3 months. The toxicity-profile of the regimen was moderated. Grade 3–4 hematological toxicities included anemia (23%), thrombocytopenia (53%) and neutropenia (38%). There were no grade 3–4 neutropenic fevers or treatment-related mortalities. Non-hematological grade 3–4 toxicities were rare: nausea/vomiting (3%), neurotoxicity (3%), nephrotoxicity (6%) and diarrhea (3%). The positive results reported in this publication should be regarded carefully due its retrospective design. Finally, a Japanese group recently published the data from 17 patients that showed progressive disease during adjuvant therapy with gemcitabine and were subsequently treated with S1 and cisplatin.[42] S1 was administered at a dose of 80 mg/m^2 per day for 21 consecutive days, followed by a 14-day rest period. Cisplatin (40 mg/m^2) was administered on day 8. These schedule was repeated every 5 weeks until disease progression or unacceptable toxicities. Five (29.4% patients) achieved a PR and 2 (11.8%) had SD, conducting to a median survival time of 10 months, with 63.7% and 31.9% of patients alive at 6 and 12 months, respectively. Major adverse reactions included gastrointestinal toxicities of grade 1 or 2 and only one patient (5.9%) developed grade 3 leucopenia.

Other regimens

A retrospective study analyzed the GTX regimen in a group of 35 patients, 66% untreated and 34% failed prior therapies.[43] GTX consisted of capecitabine 750 mg/m^2 twice daily on days 1–14, gemcitabine 750 mg/m^2 and docetaxel 30 mg/m^2 on days 4 and 11. The overall RR for all 35 patients was 29% and 31% had a minor response or SD. In the 12 patients who had progressed on previous CT, the RR was 25% at metastatic sites and 33% at the primary site. Median PFS and OS for responders were 6.3 (95% CI, 4.4–10.4 months) and 11.2 months (95% CI, 8.1–15.1 months), respectively. Grade 3/4 leukopenia and thrombocytopenia each occurred in 14% of patients and grade 3/4 anemia in 9%. The most frequent grade 3/4 non-hematologic toxicities were diarrhea and hand-foot syndrome. Although these data suggest that GTX has a potential as a regimen for untreated and treated metastatic PC, prospective data for its use are still lacking.

Reni and coworkers conducted a phase I/II trial with 15 patients evaluating the MTD and activity of mitomycin C, docetaxel and irinotecan (MDI regimen) on metastatic PC patients previously treated with gemcitabine-containing CT.[44] No objectives response was observed among patients with MTD or higher doses. Three patients had SD and all other patients had PD. The median TTP and median OS was 1.7 and 6.1 months, respectively. Based on this efficacy data, authors concluded that the regimen is ineffective in this population.

The combination of 5-FU, adriamycin and mitomycin-C (FAM) have also demonstrated to be a safe and feasible salvage therapy in patients with advanced pancreatic or biliary tract cancer previously treated with gemcitabine-based CT.[45] A total of 31 patients (15 with pancreatic cancer) received 5-FU 800 mg/m^2 on days 1–5, mitomycin-C 8 mg/m^2 on day 1 and doxorubicin 30 mg/m^2 on day 1 every 4 weeks. Four (12.9%) patients evidenced PR and eight (25.8%) patients showed SD. The median TTP and OS times were 2.3 (95% CI, 1–3.6) months and 6.7 (95% CI, 4.4–9) months, respectively. Major hematologic toxicities included grade 1–2 anemia (43.2%), thrombocytopenia (20%) and grade 3–4 neutropenia (13.7%). The most frequently detected non-hematological toxicities were grade 2 and 3 nausea/vomiting (31.3%).

Finally, an antihormonal therapeutic approach in the second-line setting was studied with the antiandrogen flutamide (250 mg orally three times per day) in 14 patients with advanced PC who had developed PD following therapy with one 5-FU-based regimen.[46] Authors concluded that flutamide treatment is ineffective with no objective tumor responses, no improvement in tumor-related symptoms and a median survival of 4.7 months.

Systemic therapy with targeted agents

Given the lack of any effective therapies in gemcitabine-refractory advanced PC, a strong need exists to investigate novel therapeutics that exploit the molecular basis of this neoplasm. Table 3 summarizes the most important targeted-agent-based studies in advanced pancreatic cancer after gemcitabine-failure.

Milella and colleagues were among the first authors to investigate the use of a targeted agent- celecoxib- in patients with gemcitabine-pretreated advanced PC.[47] Several lines of evidence indicate that selective cyclooxigenase-2 (COX-2) inhibition may be of therapeutic benefit in patients with advanced PC. First, COX-2 is up-regulated strongly in PC compared with normal pancreatic tissue or benign pancreatic lesions.[48–50] Second, it has been shown that COX-2 blockade by selective pharmacologic inhibitors reduces in vitro cell growth in preclinical models of PC.[51,52] Third, COX-2 inhibitors exert synergistic proapoptotic and antitumor effects when combined with either gemcitabine or 5-FU, the two drugs that currently constitute the mainstay of medical PC

treatment.[53,54] In the pilot study, 17 patients were treated with oral celecoxib (400 mg twice daily) and protracted intravenous infusion 5-FU 200 mg/m^2 per day, both given continuously, without scheduled interruptions, from day 1 until disease progression or unacceptable toxicity. Encouraging preliminary activity was observed: 2 confirmed PR and 2 patients with SD, for an overall RR of 12% (95% CI, 0–27%) in the intent-to-treat population. A significant decrease (≥50%) in serum CA 19.9 levels was observed in 3 of 9 evaluable patients. The median TTP was 8 weeks, and the median OS was 15 weeks. Treatment-related toxicity was minimal and manageable. Asymptomatic transaminase elevation was the most common adverse event and reached grade 3–4 in 4 of the 133 treatment weeks. No other hematologic or nonhematologic toxicity > grade 2 was observed. However, celecoxib administration was discontinued in 3 patients due to upper gastrointestinal tract toxicity.

On the other hand, oral EGF receptor (EGFR) tyrosine-kinase inhibitors, erlotinib and gefitinib, has been studied as salvage therapy after gemcitabine-failure.[55–57] Erlotinib in combination with gemcitabine have shown a significant, though modest, improvement in survival as first-line treatment for advanced PC when compared with gemcitabine alone (1-year survival: 23% vs. 17%, respectively).[6] Given the activity of this agent, Kulke et al performed a phase II multicenter study evaluating the safety and efficacy of capecitabine in combination with erlotinib in patients with advanced PC who had experienced treatment failure with standard gemcitabine first-line therapy.[55] Thirty patients were treated with capecitabine administered at a dose of 1000 mg/m^2 twice daily for 2 weeks, followed by 1-week break and erlotinib 150 mg daily. Three patients (10%) experienced a PR to therapy and five additional patients experienced biochemical responses, as defined by decreases in serum tumor marker CA 19.9 of more than 50%. The median PFS and OS time were 3.4 and 6.5 months, respectively and one-year OS was 26%. Grade 3 or 4 toxicities included diarrhea (17%), skin rash (13%), and hand–foot syndrome (13%). No correlation was found between the development of skin rash and either response or survival. The efficacy of erlotinib, dosed to achieve a rash, has also been investigated in 50 patients with locally advanced or metastatic PC who had progressed or were unable to tolerate gemcitabine-based CT.[56] Erlotinib was given at an initial dose or 150 mg/day and then it was increased by 50 mg every 2 weeks (maximum 300 mg/day) until more than grade 1 rash or other dose-limiting toxicities occurred. Dose-escalation to 200–300 mg of erlotinib was possible in 9 patients. Best response was SD in 14 of the 40 evaluable patients (0.35; 95% CI, 0.2–0.5%) and prolonged disease-control (SD > 8 weeks) was observed in 10 patients (0.25; 95% CI, 0.12–0.38). Median TTP was 1.6 months (95% CI, 1.6–2.1), median OS was 4.1 months (95% CI, 3.2–7.3), and 6 months OS rate was 39% (95% CI, 24–61%). Most common treatment adverse events of any grade were rash (74.5%), diarrhea (38.3%) and fatigue (17%). In contrast with previous results, docetaxel (75 mg/m^2 every 3 weeks) plus gefitinib (250 mg/day continuously) combination resulted in no significant clinical activity as salvage treatment for advanced PC in a recently published phase II study.[57]

An antiangiogenic approach has also been evaluated as second-line treatment in advanced PC. As the overexpression of vascular endothelial growth factor (VEGF) and its receptors VEGFR-1, VEGFR-2, and VEGFR-3 promote tumor growth via paracrine angiogenic and autocrine mitogenic pathways, targeting the VEGF pathway holds promise in this setting.[58] Thus, a phase II study was performed to study the safety profile and efficacy of bevacizumab 15 mg/kg every 21 days plus erlotinib 150 mg/day orally in 26 patients with gemcitabine-refractory metastatic PC.[59] Authors demonstrated that this combination can be safely administered to this patient population, but efficacy appears modest. Of the 25 evaluable patients, 1 had a PR and 7 had SD for ≥2 cycles, for an overall disease-control rate of 32%. Median TTP and median OS were 40 (95% CI, 37–59 days) and 103 (95% CI, 66–132) days, respectively, with 6-months survival rate of 16.7%.

Multitarget tyrosine-kinase (TK) inhibitors are currently being studied in previously treated advanced PC patients. Data from a phase II trial have recently been reported with sunitinib in 77 patients.[60] Sunitinib at a dose of 50 mg daily for 28 days followed by 14 days of rest (1 cycle) demonstrated to have modest activity as single-agent: CR and SD occurred in 1 and 14 patients (20%), respectively. Median PFS was 1.35 months (95% CI, 1.28–2.07) and median OS was 3.2 months (95% CI, 2.8–4.17). Grade 3–5 toxicities included: hematologic 13% (grade 3), fatigue 12%, bleeding 6% (grade 3), nausea 4% (grade 3), thrombosis/embolism 2% (grade 3), renal failure (2%) and gastrointestinal perforation (grade 5). Vatalinib, another VEGFR and platelet-derived growth factor receptor (PDGFR)-targeted small molecule TK inhibitor, was also examined in 65 patients with unresectable PC whose tumors progressed on or were unable to tolerate gemcitabine-based CT.[61] The treatment consisted of oral administration of vatalinib, starting at 250 mg on a twice daily basis, up to 500 mg BID in the second week, and up to 750 mg BID in the third week in patients who tolerated dose-escalation. This schedule was well-tolerated and resulted in promising 6-months RFS (14%) and OS (27%) rate among the 57 evaluable patients. Hypertension and fatigue were the most common grade 3/4 toxicities attributable to study treatment, occurring in 17% and 13%, respectively, of treated patients. Only 4% of patients experienced grade 3 dizziness. Given this preliminary results, lack of any effective therapies in this patient population, further studies with vatalinib may be of interest.

Flavopiridol, a cyclin-dependent kinase inhibitor (CDKI) that potentiates chemo-induced apoptosis in PC cell lines, has also been evaluated in combination with docetaxel in both phase I[62] and II studies.[63] In the phase I study,[62] 10 patients with PC were treated with docetaxel followed by flavopiridol, achieving 1 CR lasting >2 years, 1 PR and 4 SD. In the phase II trial,[63] 10 patients with stage IV PC who progressed on one prior gemcitabine-based therapy in the adjuvant, locally advanced or metastatic setting, received docetaxel 35 mg/m^2 followed 4–6 h later by flavopiridol 80 mg/m^2 on days 1,8 and 15 every 28 days (1 cycle). This regimen showed minimal activity and significant toxicity in patients previously treated for PC. The different outcomes in the phase I and II studies may be due to patients selection: the phase I patients were younger (56.5 vs. 64 years) with a higher PS (90 vs. 80). Those who achieved CR/PR/SD on the phase I study were less heavily treated.

Finally, the vast majority of PC harbor activating mutations in K-RAS, which promote cellular proliferation and survival through engagement of several downstream effector pathways, including PI3K/Akt/mTOR pathway.[64] Increased activation of the PI3K/Akt/mTOR pathway has been noted in approximately half of PC[65–67] and has been associated with a poorer prognosis.[65,66] In preclinical models of PC, inhibition of this pathway has demonstrated antitumor activity.[68–70] A phase II study of RAD001 (everolimus), an oral small-molecule inhibitor of mTOR (mammalian target of rapamycin) was recently performed to evaluate whether downstream inhibition of the PI3 K/Akt/mTOR pathway is safe and effective in patients with gemcitabine-refractory metastatic PC.[71] Thirty-three patients were treated continuously with RAD 001 at 10 mg daily until disease progression or unacceptable toxicity. Overall, treatment with RAD001 was well-tolerated and the most common adverse events were mild hyperglycemia and thrombocytopenia. Nevertheless, it had minimal clinical activity as a single-agent: no CR or PR were noted, and only seven patients (21%) had SD. Median PFS and OS were 1.8 months and 4.5 months, respectively. The results of two prospective studies using mTOR inhibitors in patients with gemcitabine-refractory PC have been presented at 2009 ASCO Annual Meeting.[72] Five patients [illegible] treated with

Table 3
Clinical trials investigating second-line systemic therapy with targeted agents in gemcitabine-pretreated patients with advanced pancreatic cancer.

Treatment regimen	No. of patients	Median age (years)	KPS 90–100%/ECOG 0–1%	Metastatic disease (%)	RR (%)[a]	DCR (%)[a]	PFS/TTP (months)	OS (months)
Celecoxib/5-FU[57]	17	60	94	82	12	24	1.9	3.5
Capecitabine/erlotinib[58]	30	60	100	100	10	NA	3.4	6.5
Erlotinib[59]	50	61	92	90	0	28	1.6	4.1
Docetaxel/gefitinib[57]	26	65	92	NA	0	19	2.1	2.9
Bevacizumab/erlotinib[58]	26	60	100	100	4	32	1.3	3.4
Sunitinib[60]	77	65	93	100	1	21	1.35	3.2
Vatalanib[61]	65	64	90	91	3	31	2	NA
Docetaxel/flavopiridol[62]	10	64	NA	100	0	33	NA	NA
Everolimus[71]	33	61	100	100	0	21	1.8	4.5

KPS: Karnofsky performance status; ECOG: Eastern Cooperative Oncology Group; NA: not available; RR: response rate; DCR: disease-control rate; PFS: progression-free survival; TTP: time to progression; OS: overall survival; 5-FU: 5-fluorouracil.
[a] Intention-to-treat analysis.

Table 4
Ongoing second-line clinical trials in patients with advanced pancreatic cancer.

Clinical trial identifier	Study design	Agents	Number of patients	Primary endpoint	Previous treatment
NCT 00813163	Phase II	Liposomal irinotecan	39	PFS	Gemcitabine-based therapy
NCT 00690300	Phase II	Docetaxel + oxaliplatin	44	RR	First-line CT
NCT 00786006	Randomized phase II	FOLFOX vs. FOLFIRI-3	60	OS	Gemcitabine-based therapy
EFC6596	Randomized phase II	Larotaxel vs. capecitabine(5-FU)	400	OS	Gemcitabine-based therapy
NCT 00397787	Phase II	Sunitinib	64	RR	Gemcitabine-based therapy
NCT 00365144	Phase II	Bevacizumab + erlotinib	40	6-months survival, toxicity	Gemcitabine-based therapy
NCT 00703625	Phase I	Docetaxel + temsirolimus	25	MTD	Gemcitabine-based therapy
NCT 00703170	Phase I	Doxil + temsirolimus	24	MTD	Gemcitabine-based therapy

5-FU: 5-fluorouracil; PFS: progression-free survival; RR: response rate; OS: overall survival; MTD: maximum tolerated dose; CT: chemotherapy.

temsirolimus 25 mg iv weekly and 16 patients in study B with everolimus 30 mg weekly plus erlotinib 150 mg daily. Neither study demonstrated objective responses or SD. Sirolimus have also been evaluated as second-line therapy in 30 patients with advanced PC.[73] It was administered at a dose of 5 mg/day for 28 days. Seven (23%) patients achieved SD and 6-months survival rate was 20%. Treatment was well-tolerated. Despite this modest results and given substantial preclinical data implicating activation of the PI3K/Akt/mTOR pathway in PC, this pathway remains an interesting target in the treatment of patients with this disease. To realize the potential of this strategy, future studies of mTOR inhibitors will likely need to assess the combination of these agents with drugs that inhibit upstream components of the PI3K/Akt/mTOR pathway. Concurrent work will be necessary to verify target inhibition and investigate potential mechanisms of resistance in patients with this difficult to treat disease.

Conclusion

There is increasing evidence that selected patients with locally advanced or metastatic PC may obtain significant clinical benefit from salvage CT after failure to first-line gemcitabine-based regimens. A limited amount of data suggest that median survival in gemcitabine-resistant advanced PC patients receiving BSC is approximately 2 months, whereas survival times (calculated from the start of second-line CT after gemcitabine-failure) of between 3 and 9 months have been reported from prospective clinical trials in patients receiving salvage CT. However, final results from large randomized studies confirming a survival advantage for second-line therapy compared to BSC only in this patient population are still lacking. Interpretation of data from phase II studies are often limited by the fact that the patients populations recruited in these studies are heterogeneous and noncomparable. Thus, randomized phase III trials that stratifies patients according to prognostic factors are warranted to determine the real survival benefit of a second-line regimen in advanced PC patients.

After failure to first-line therapy, all patients with advanced PC and adequate PS should be enrolled on clinical trials testing new strategies. Table 4 summarizes some of the ongoing studies in this setting. When investigational therapy is not available, no evidence-based treatment recommendation can be given and each therapeutic decision is based on individual patient. The clinical factors that may help to select patients with advanced PC who will benefit most from second-line therapy are good PS, previous response to first-line CT and late recurrence after primary pancreatectomy. Furthermore, better understanding of validated predictions markers will provide new opportunities to optimize the management of patients with advanced PC.

With regard to the optimal treatment, to date a standard second-line CT regimen has yet to be defined after gemcitabine-failure. In patients with PS 0–1, we recommend oxaliplatin-based schedules or a combination of capecitabine with erlotinib as the standard second-line therapy. It is supported by encouraging disease-control rates over 50% and median survival times of approximately 6 months in phase II studies using both regimens. Oxaliplatin, fluoropyrimidines or paclitaxel monotherapy are proposed as our treatment of choice for patients with PS 2 who are considered adequate candidates for further therapy. These agents are well-tolerated and associated with improvement of tumor-related symptoms. Although few patients showed objective responses, the rates of disease-control ranged from 23% to 39% and the reported median OS times from the start of salvage CT after gemcitabine-failure ranged from [illegible] Finally,

targeted therapies as single-agent offer relatively modest results for the majority of patients and they do not appear to be as efficacious as regimens that include traditional cytotoxic agents as a component. For this reason and, at least for now, we do not support them outside clinical trials.

Conflict of interest statement

The authors declare that they have no conflict of interest relating to the publication of this manuscript.

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Effects of nal-IRI (MM-398, a liposomal formulation of irinotecan) ± 5 fluorouracil/leucovorin (5-FU/LV) on Quality of Life in NAPOLI-1: A Phase 3 study in patients with metastatic pancreatic ductal adenocarcinoma previously treated with gemcitabine

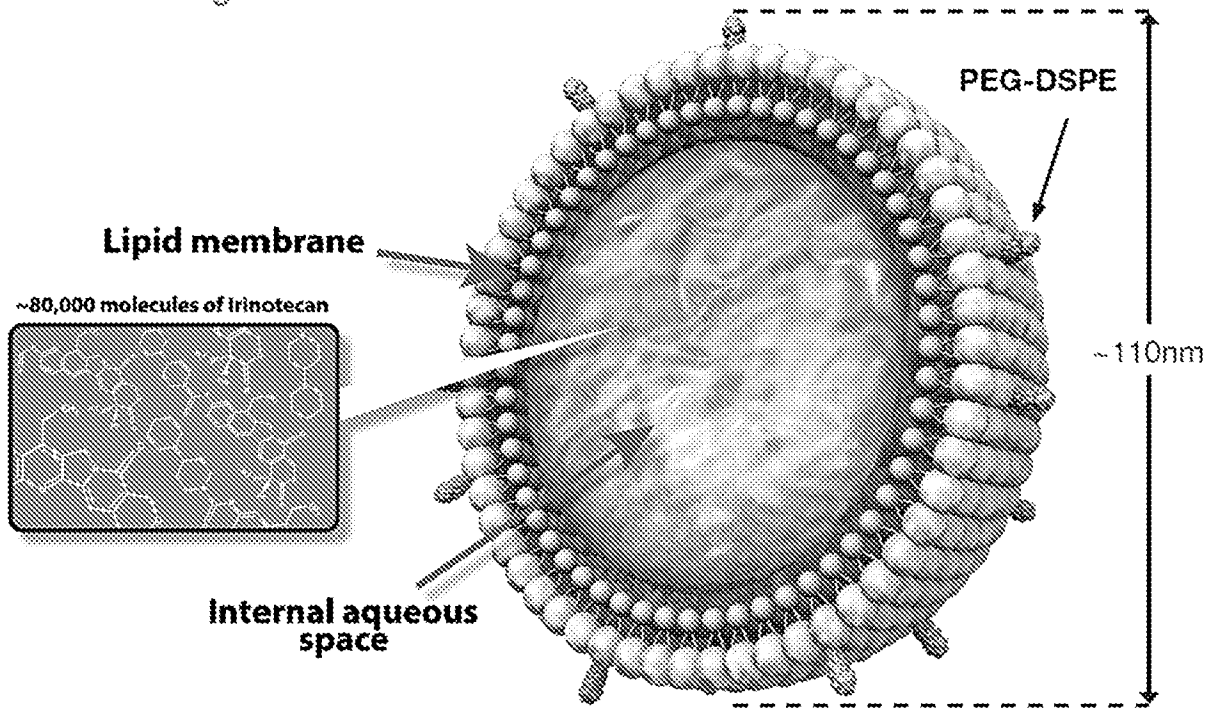
Floris de Jong,[1] Richard Hubner,[2] Antonio Cubillo,[3] Jean-Frédéric Blanc,[4] Davide Melisi,[5] Daniel D. Von Hoff,[6] Andrea Wang-Gillam,[7] Li-Tzong Chen,[8] Claus Becker,[9] Khalid Mamlouk,[9] Bruce Betanger,[9] Yoojung Yang,[10] Jens T. Siveke[11]

[1]Shire GmbH, Glattpark, Zürich, Switzerland; [2]Christie Hospital NHS Foundation Trust, Manchester, UK; [3]Centro Integral Oncológico Clara Campal, Madrid, Spain; [4]Hôpital Saint-André, Bordeaux, France; [5]Digestive Molecular Oncology Unit, University of Verona, Verona, Italy; [6]TGen and Honor Health, Phoenix/Scottsdale, AZ, USA; [7]Washington University in St. Louis, St. Louis, MO, USA; [8]National Health Research Institutes (NHRI) – National Institute of Cancer Research, Tainan, Taiwan; [9]Merrimack Pharmaceuticals, Inc., Cambridge, MA, USA; [10]Shire Inc., Cambridge, MA, USA; [11]West German Cancer Center, University Hospital Essen, Essen, Germany

BACKGROUND

- Pancreatic cancer is the fourth leading cause of cancer-related death in Europe, and the seventh leading cause of cancer-related death worldwide[1,2]
- Survival rates for pancreatic cancer are poor because of rapid disease progression and difficulties in diagnosis[3]
  - 1-year survival, 15%
  - 5-year survival, 4%
- Gemcitabine-based therapies and FOLFIRINOX are the standard first-line treatments for pancreatic cancer; however, there is no standard treatment for patients with metastatic disease who have progressed on first-line therapy[4]
- nal-IRI (MM-398) is a novel liposomal formulation of irinotecan that exhibits extended circulation and enhanced intratumoral drug deposition when compared with nonliposomal (conventional) irinotecan (Figure 1)[5,6]
- nal-IRI is approved by the US Food and Drug Administration, in combination with 5-fluorouracil (5-FU) and leucovorin (LV), for use following disease progression in patients with metastatic pancreatic ductal adenocarcinoma (mPDAC) previously treated with gemcitabine-based therapy, and in Europe it has recently received positive opinion from the European Medicines Agency Committee for Medicinal Products for Human Use[7,8]

Figure 1. nal-IRI design



nal-IRI, liposomal irinotecan; PEG-DSPE, poly(ethylene glycol)-distearoylphosphatidylethanolamine

- NAPOLI-1 was a phase 3 trial evaluating the efficacy and safety of nal-IRI, as monotherapy and in combination with 5-FU/LV, compared with 5-FU/LV alone, in patients with mPDAC previously treated with gemcitabine-based therapy[9]
  - At the primary analysis data cut-off (February 14, 2014), median overall survival (OS) was significantly increased with nal-IRI+5-FU/LV relative to 5-FU/LV (6.1 vs. 4.2 months; unstratified hazard ratio [HR], 0.67 [95% confidence interval (CI), 0.49-0.92]; P = 0.012; stratified HR, 0.57 (0.41–0.80), P = 0.0009),[9,10] but did not differ significantly between nal-IRI monotherapy and 5-FU/LV (4.9 vs 4.2 months; unstratified HR, 0.99 [95% CI, 0.77–1.28]; P = 0.94)
  - Median progression-free survival was significantly longer with nal-IRI+5 FU/LV compared with 5-FU/LV (3.1 vs 1.5 months; unstratified HR, 0.56; 95% CI, 0.41-0.75; P = 0.0001)
  - Median ORR was significantly higher with nal-IRI+5-FU/LV compared with 5-FU/LV (16% vs 1%; P < 0.0001)
  - nal-IRI+5-FU/LV exhibited a manageable safety profile; Grade 3/4 adverse events (AEs) occurring more frequently with nal-IRI+5-FU/LV vs. 5-FU/LV included neutropenia (27% vs. 1%), fatigue (14% vs. 4%), diarrhea (13% vs. 4%), and vomiting (11% vs. 3%)

OBJECTIVES

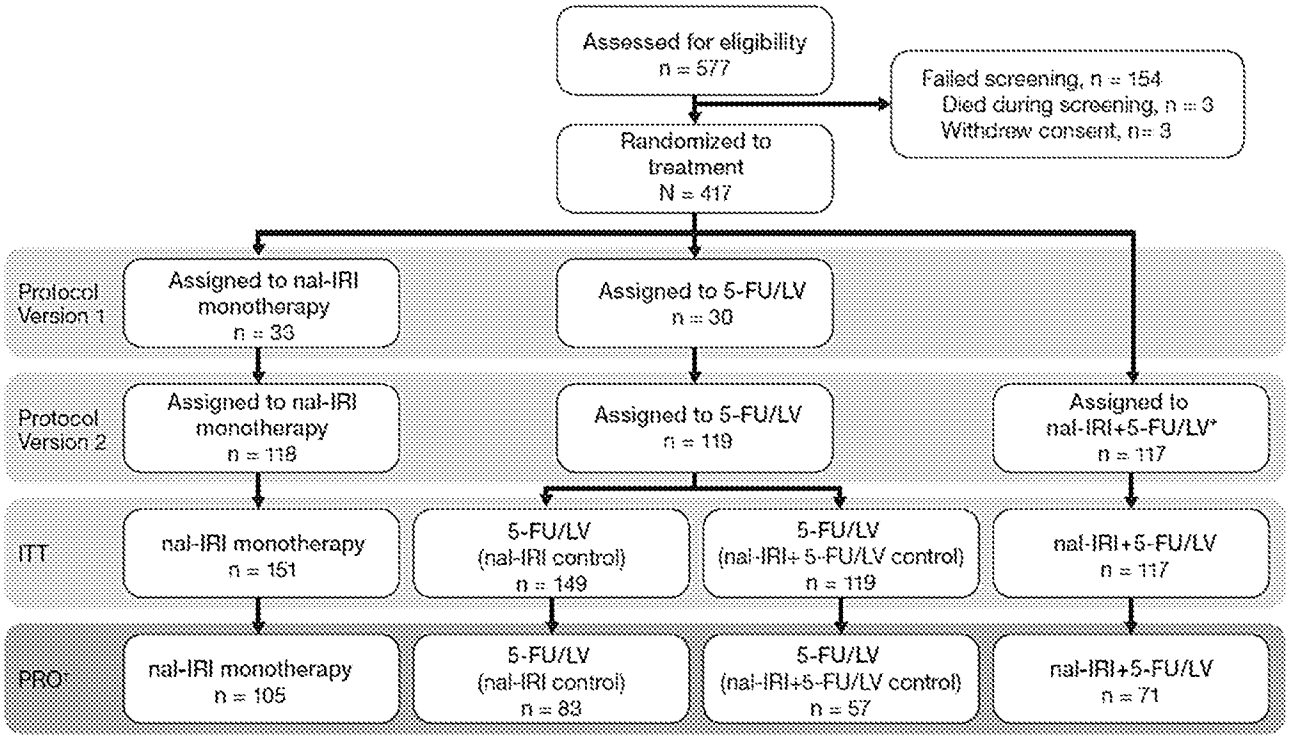
- To assess the quality of life (QoL) of patients receiving nal-IRI+5-FU/LV and 5-FU/LV in the NAPOLI-1 study. QoL was a secondary endpoint in NAPOLI-1

METHODS

Study design

- NAPOLI-1 was an international, open-label, randomized, Phase 3 trial (Figure 2)
  - Patients were initially randomized to nal-IRI monotherapy (120 mg/m^2 irinotecan hydrochloride trihydrate salt equivalent to 100 mg/m^2 irinotecan free base every 3 weeks) or 5-FU/LV (200 mg/m^2 LV and 2000 mg/m^2 5-FU, every week for the first 4 weeks of each 6-week cycle; protocol version 1)
  - Once safety data for the combination treatment became available from a concurrent study in metastatic colorectal cancer, the protocol was amended to include a third arm: nal-IRI+5-FU/LV (80 mg/m^2 irinotecan hydrochloride trihydrate salt equivalent to 70 mg/m^2 irinotecan free base every 2 weeks; 400 mg/m^2 LV and 2400 mg/m^2 5-FU every 2 weeks; protocol version 2)
- QoL was assessed at baseline, every 6 weeks, and at the 30-day post–follow-up visit, using the European Organization for Research and Treatment of Cancer QoL core questionnaire (EORTC-QLQ-C30) version 3.0[11]
  - QoL was assessed in all patients in the intention-to-treat (ITT) population who provided baseline and ≥1 subsequent QoL assessment (patient-reported outcome [PRO] population); patients were classified by the treatment arm to which they were randomized (Figure 2)

Figure 2. Trial profile



*Study was amended to add the nal-IRI+5-FU/LV arm once safety data on the combination became available; 63 patients had already been enrolled in the original 2-arm study at the time of amendment.

†Used for QoL analyses; included all patients in the ITT population who provided a baseline and ≥1 subsequent QoL assessment. 5-FU, fluorouracil; ITT, intention-to-treat; LV, leucovorin; nal-IRI, liposomal irinotecan; PRO, patient-reported outcome population; QoL, quality of life.

- QoL was assessed in 3 independent domains (global health status, functionality, and symptomatology) across 15 scales
- Linear transformations were applied to raw scores so that the reported score ranged from 0–100 for each scale
- Patients were classified into 1 of 3 categories:
  - Improved: Patient had scores ≥10% above baseline and remained above baseline value for ≥6 weeks
  - Stable: Patient did not meet criteria for improved or worsened
  - Worsened: Patient did not meet improvement criteria and died, or had scores that decreased by 10%
- Pairwise treatment group comparisons were performed using Cochran-Mantel-Haenszel testing adjusted for multiplicity with a Benjamini-Hochberg correction to control false discovery rate at 0.05 level for the 15 comparisons

Eligibility criteria

Key inclusion criteria

- Adults ≥18 years of age
- Histologically or cytologically confirmed PDAC
- Documented measurable or non-measurable distant metastatic disease (as defined by Response Evaluation Criteria in Solid Tumors, version 1.1)
- Disease progression after prior gemcitabine or gemcitabine-containing therapy in a neoadjuvant, adjuvant (only if distant metastases occurred within 6 months of completing adjuvant therapy), locally advanced, or metastatic setting
- Karnofsky performance status (KPS) score ≥70
- Adequate hematologic (including absolute neutrophil count >1.5 × 10^9 cells per L), hepatic (including normal serum total bilirubin and albumin levels ≥30 g/L), and renal function

Key exclusion criteria

- Active central nervous system metastasis
- Clinically significant gastrointestinal disorders
- Severe arterial thromboembolic events <6 months before inclusion
- New York Heart Association Class III or IV congestive heart failure, ventricular arrhythmias, or uncontrolled blood pressure
- Active infection or uncontrolled fever

RESULTS

Patient characteristics

- 71 patients (61% of the ITT population randomized under protocol version 2) in the nal-IRI+5-FU/LV arm and 57 patients (48% of the ITT population randomized under protocol version 2) in the 5-FU/LV arm provided baseline and ≥1 subsequent EORTC assessment (PRO population)
- Patient demographics and baseline characteristics were similar between treatment arms (Table 1)

Table 1. Demographics and baseline characteristics (PRO population)

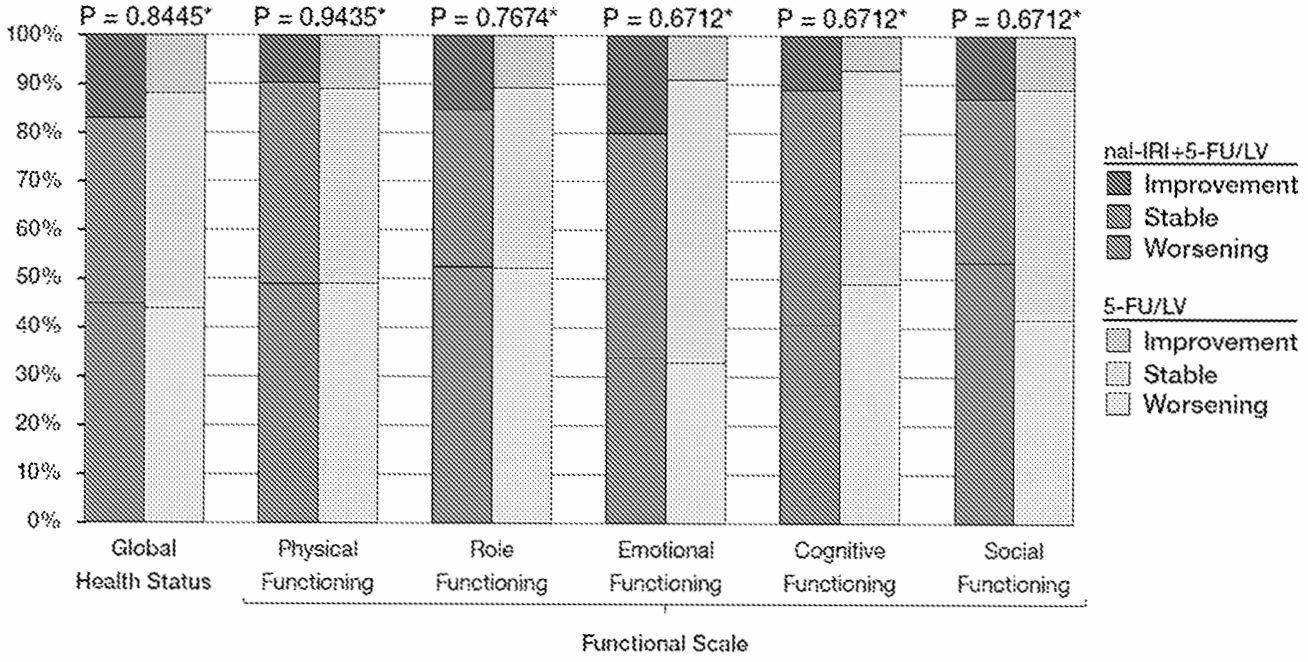
Parameter	nal-IRI+5-FU/LV n = 71	5-FU/LV n = 57
Sex, n (%)		
Male	43 (61)	31 (54)
Female	28 (39)	26 (46)
Age, median (range), years	63.0 (41-81)	63.0 (41-80)
Ethnicity, n (%)		
White	42 (59)	39 (68)
East Asian	22 (31)	16 (28)
Other	7 (10)	2 (4)
KPS score, n (%)		
100	12 (17)	8 (14)
90	31 (44)	23 (40)
80	24 (34)	22 (39)
70	3 (4)	4 (7)
60	1 (1)	0

5-FU, 5-fluorouracil; KPS, Karnofsky performance status; LV, leucovorin; nal-IRI, liposomal irinotecan.

Quality of life

- No substantial differences were identified in the proportion of patients exhibiting improved, stable, or worsening QoL in the domains of global health status or functional scale scores between the nal-IRI+5-FU/LV and 5-FU/LV arms (Figure 3)

Figure 3. Proportion of patients demonstrating improvement, stability, or worsening in global health status and functional scale scores (nal-IRI+5-FU/LV, n = 71; 5-FU/LV, n = 57)

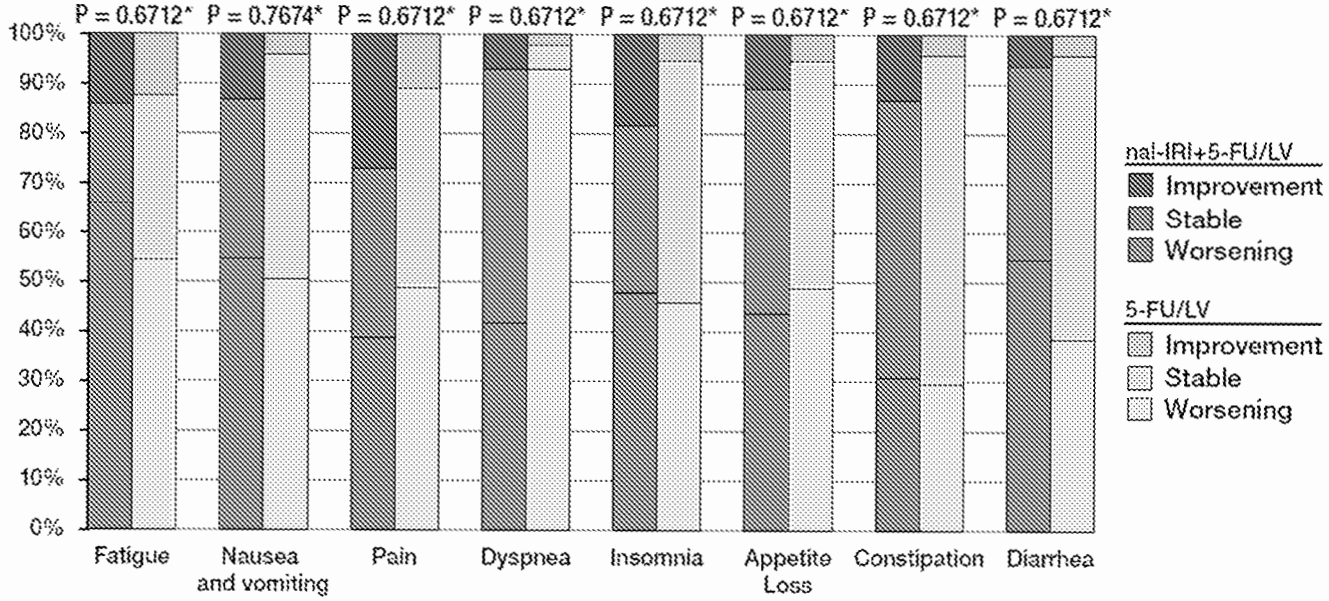


*Benjamini-Hochberg-adjusted P-values. The adjustment was conducted across the 15 domains of the QoL questionnaire to control for the overall false discovery rate (FDR).

nal-IRI+5-FU/LV values are shown to the left for each scale item.

- No substantial differences were identified in the proportion of patients exhibiting improved, stable, or worsening QoL in symptom scale scores between the nal-IRI+5-FU/LV and 5-FU/LV arms (Figure 4)

Figure 4. Proportion of patients demonstrating improvement, stability, or worsening in symptom scale scores (nal-IRI+5-FU/LV, n = 71; 5-FU/LV, n = 57)

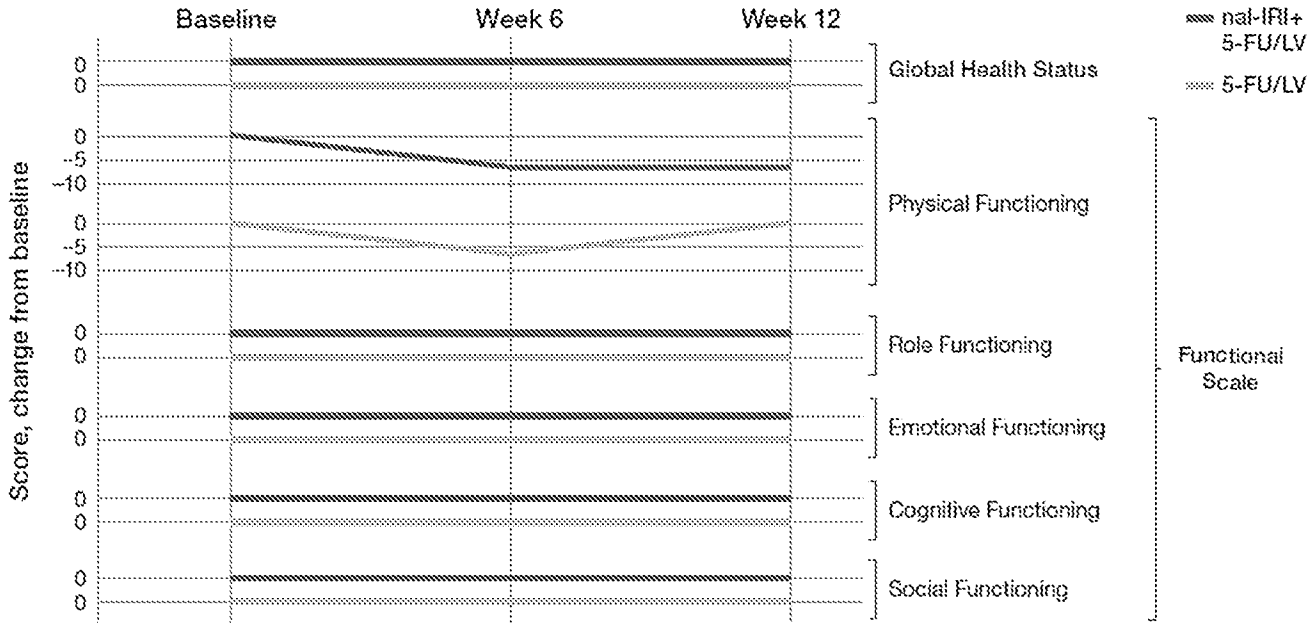


*Benjamini-Hochberg-adjusted P-values. The adjustment was conducted across the 15 domains of the QoL questionnaire to control for the overall FDR.

nal-IRI+5-FU/LV values are shown to the left for each scale item.

- Baseline global health status and functional scale scores ranged from 58-83 and were similar between the treatment arms
- Overall, there were no appreciable changes from baseline to week 12 in global health status and functional scale scores between the nal-IRI+5-FU/LV and 5-FU/LV arms (Figure 5)
  - The observed median change from baseline to week 6 in physical functioning score was 6.7 points in both arms; which corresponds to "a little" decrease[12]

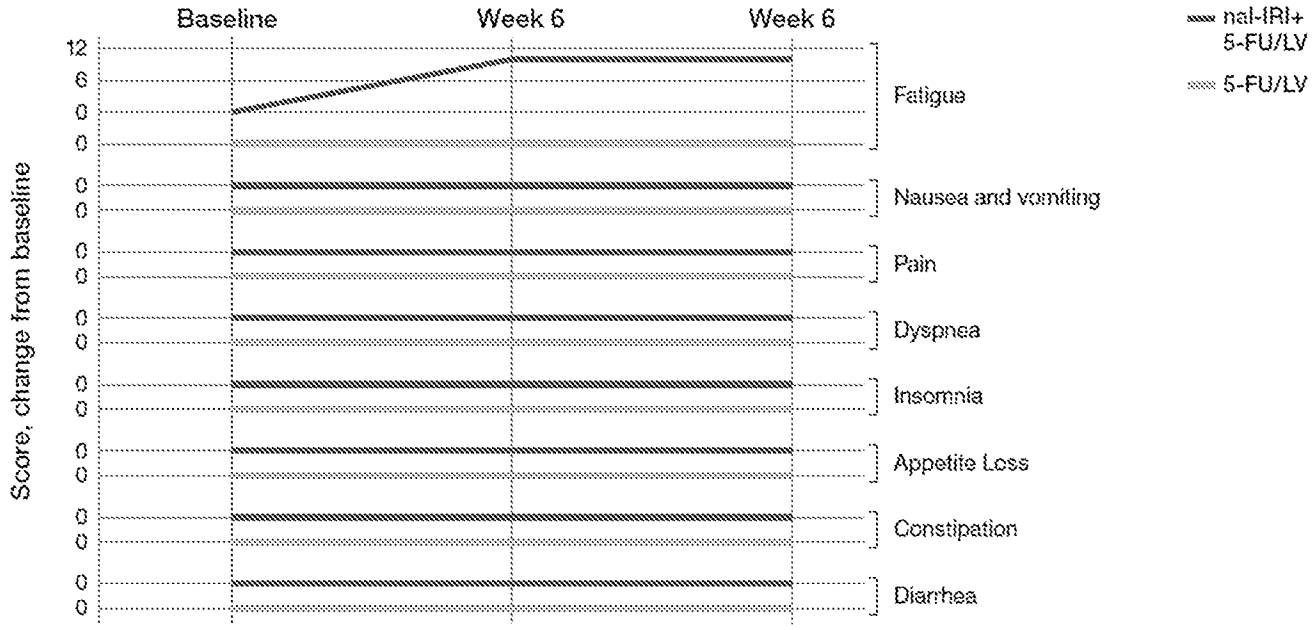
Figure 5. Median change from baseline to week 12 in global health status and functional scale scores (nal-IRI+5-FU/LV, n = 71 at baseline; 5-FU/LV, n = 57 at baseline)



nal-IRI+5-FU/LV values are shown on the top for each scale item.

- Baseline symptom scale scores ranged from 0-33 and were similar between the treatment arms
- Overall, there were no appreciable changes from baseline to week 12 in symptom scale scores between the nal-IRI+5-FU/LV and 5-FU/LV arms (Figure 6)
  - The observed median change from baseline to week 6 in fatigue score was approximately 11 points in the nal-IRI+5-FU/LV arm, which corresponds to a "moderate" increase[12]

Figure 6. Median change from baseline to week 12 in symptom scale scores (nal IRI+5 FU/LV, n = 71 at baseline; 5-FU/LV, n = 57 at baseline)



nal-IRI+5-FU/LV values are shown on the top for each scale item.

CONCLUSIONS

- nal-IRI+5-FU/LV significantly improves OS in patients with mPDAC previously treated with gemcitabine-based therapy compared with 5-FU/LV
- Overall, patients treated with nal-IRI+5-FU/LV had no deterioration in QoL over 12 weeks, despite the addition of a second chemotherapeutic agent
  - Global health status and functional scale scores were not significantly different between treatment arms at baseline, and showed no appreciable change over 12 weeks
  - Median symptom scale scores at baseline ranged from 0-33 (low levels of symptomatology), and showed no appreciable change over 12 weeks
- Study limitations included small patient numbers with QoL data, open-label design, and inherent variability in PROs
- nal-IRI+5-FU/LV provides a new treatment option that does not compromise QoL in patients with mPDAC previously treated with gemcitabine-based therapy

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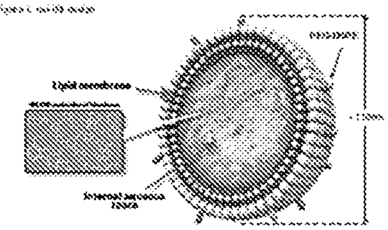
Disclosures

FdJ: Shire employee; owns Shire stock. RH: Celgene advisory board honoraria, conference attendance, educational grant. JFB: Baxalta, now part of Shire, speaker activity, advisory board. DVH: Alpha Med consulting. AWG: Served on Merrimack, Pfizer, Newlink advisory boards. CB, KM and BB: Merrimack employees. YY: Shire employee. JTS: Advisory board honoraria from Celgene and Baxalta, now part of Shire; Celgene grant.

Australian Gastro-Intestinal Trials Group, 18th Annual Scientific Meeting, Melbourne, 14–16 September, 2016

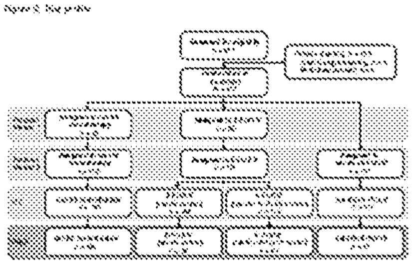
Effects of nal-IRI (MM-398, a liposomal formulation of irinotecan) ± 5 fluorouracil/leucovorin (5-FU/LV) on Quality of Life in NAPOLI-1: A Phase 3 study in patients with metastatic pancreatic ductal adenocarcinoma previously treated with gemcitabine

BACKGROUND

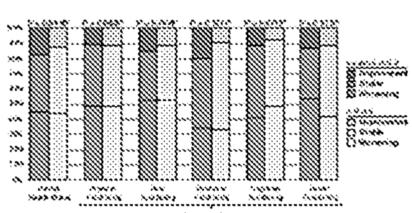


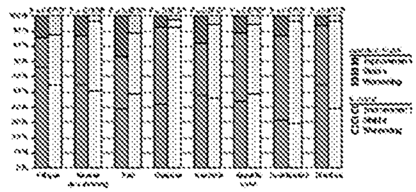
OBJECTIVES

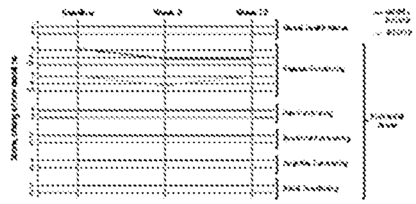
METHODS

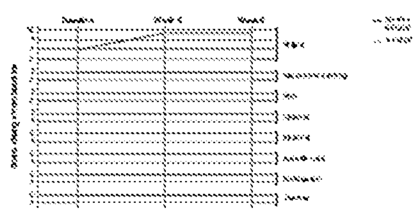


RESULTS









CONCLUSIONS

References

Acknowledgments

Disclosures

CSPC Exhibit 1097

Effects of nal-IRI (MM-398; a liposomal formulation of irinotecan) ± 5-fluorouracil (5-FU) on quality of life (QoL) in NAPOLI-1: a phase 3 study in patients with metastatic pancreatic ductal adenocarcinoma (mPDAC) previously treated with gemcitabine

Floris de Jong,[1] Richard Hubner,[2] Antonio Cubillo,[3] Jean-Frédéric Blanc,[4] Davide Melisi,[5] Daniel D. Von Hoff,[6] Andrea Wang-Gillam,[7] Li-Tzong Chen,[8] Claus Becker,[9] Khalid Mamlouk,[9] Bruce Belanger,[9] Yoojung Yang,[10] Jens T. Siveke[11]

[1]Shire GmbH, Glattpark (Opfikon), Zürich, Switzerland; [2]Christie Hospital NHS Foundation Trust, Manchester, UK; [3]Centro Integral Oncologico Clara Campal, Madrid; [4]Hôpital Saint-André, Bordeaux, France; [5]Digestive Molecular Oncology Unit, University of Verona, Italy; [6]TGen and Honor Health, Phoenix/Scottsdale, AZ, USA; [7]Washington University in St. Louis, St. Louis, MO, USA; [8]National Health Research Institutes (NHRI) - National Institute of Cancer Research, Tainan, Taiwan; [9]Merrimack Pharmaceuticals, Inc., Cambridge, MA, USA; [10]Baxalta Inc., Cambridge, MA, USA; [11]West German Cancer Center, University Hospital Essen, Essen, Germany

Target Congress: 18th AGITG, September 14 – 16, 2016, Melbourne, Victoria, Australia

Abstract Category: Clinical Pancreatic Cancer

Abstract Subcategory: Clinical

Word count: 300 (limit, 300)

Suggested keywords: mPDAC, nal-IRI, nal-IRI+5-FU/LV, NAPOLI-1, QoL, EORTC-QLQ-C30, pancreatic, MM-398

Background: Patients with mPDAC frequently experience significant symptom burden, negatively impacting QoL. QoL was a secondary endpoint in NAPOLI-1 (ClinicalTrials.gov, NCT01494506), in which nal-IRI±5-FU/LV vs. 5-FU/LV were evaluated. nal-IRI+5-FU/LV significantly improved OS vs. 5-FU/LV (6.1 vs. 4.2 months; unstratified HR 0.67; *P*=0.012) (Wang-Gillam, Lancet 2016).

Methods: QoL was assessed using the European Organization for Research and Treatment of Cancer QoL core questionnaire (EORTC-QLQ-C30), including functional, symptom, Global Health and QoL scales, at treatment start, every 6 weeks and 30 days post-follow-up visit. Patients with a baseline and ≥1 subsequent EORTC-QLQ-C30 assessment were analyzed. Patients were classified as improved, worsened, or stable for each subscale. Pairwise treatment group comparisons were performed using Cochran-Mantel-Haenszel

testing, adjusted for multiplicity, with a Benjamini-Hochberg correction to control false discovery rate at 0.05 level.

Results: Of analyzed patients (n=154), 69% (49/71) in the nal-IRI+5-FU group vs. 53% (44/83) in the 5-FU/LV group had evaluable data at 12 weeks. Baseline median Global Health Status scores approached the scoring range midpoint, median Functional Scale scores were high, and Symptom Scale scores were low, with similar values between treatment groups. There was no median score change at 12 weeks (both treatment groups) for Global Health Status and these subscale scores: role, emotional functioning, cognitive, social functioning, nausea and vomiting, pain, dyspnea, insomnia, appetite loss, constipation, diarrhea, financial difficulties. Where the median change was not 0 (nal-IRI+5-FU/LV: physical functioning, fatigue), there were no substantial between-group differences. The proportion of improved, worsened or stable patients did not differ significantly between groups. Adjusted comparison *P* values were >0.05 across subscales.

Conclusion: NAPOLI-1 reported no significant QoL response differences in nal-IRI+5-FU/LV vs. 5-FU/LV-treated patients, despite addition of a cytotoxic agent. Evaluable nal-IRI+5-FU/LV-treated patients tended to maintain baseline QoL over 12 weeks. Results are limited by small patient numbers and QoL subscale score variability.

1529P

First-line liposomal irinotecan + 5-fluorouracil/leucovorin + oxaliplatin in patients with locally advanced or metastatic pancreatic ductal adenocarcinoma: exploratory subgroup analyses of survival by changes in CA 19-9 levels

Andrew Dean,[1] Tanios Bekaii-Saab,[2] Patrick M Boland,[3] Farshid Dayyani,[4] Teresa Macarulla,[5] Kabir Mody,[6] Bruce Belanger,[7] Fiona Maxwell,[8] Yan Moore,[7] Tiffany Wang,[7] Bin Zhang,[7] Zev A Wainberg[9]

[1]St John of God Subiaco Hospital, Perth, WA, Australia; [2]Mayo Clinic (ACCRU), Phoenix, AZ, USA; [3]Rutgers Cancer Institute of New Jersey, New Brunswick, NJ, USA; [4]University of California, Irvine, CA, USA; [5]Vall d'Hebron University Hospital and Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain; [6]Mayo Clinic, Jacksonville, FL, USA; [7]Ipsen, Cambridge, MA, USA; [8]Ipsen, Abingdon, UK; [9]University of California, Los Angeles, CA, USA

BACKGROUND

- FOLFIRINOX (non-liposomal irinotecan + 5-fluorouracil/leucovorin [5-FU/LV] + oxaliplatin)[1] is an established first-line treatment for metastatic pancreatic ductal adenocarcinoma (PDAC).[2]
  - However, non-liposomal irinotecan has a short half-life,[3] and toxicities can be dose limiting.[4]
- Liposomal irinotecan (ONIVYDE, ONIVYDE pegylated liposomal) is a formulation that encapsulates irinotecan in a lipid bilayer, such that 95% of irinotecan is retained in the liposome during circulation.[5]
- Liposomal irinotecan is indicated, in combination with 5-FU/LV, for the treatment of adults with metastatic PDAC that has progressed following gemcitabine-based therapy.[5]
- In a phase 1/2 study of NALIRIFOX (liposomal irinotecan + 5-FU/LV + oxaliplatin) as a first-line treatment for patients with locally advanced or metastatic PDAC (EudraCT number, 2015-003086-28; ClinicalTrials.gov identifier, NCT02551991), for the 32 patients receiving the selected dose (data cut-off: 26 February 2020):
  - no new safety signals were observed[6]
  - median progression-free survival (PFS) was 9.2 (95% confidence interval [CI]: 7.69, 11.96) months, and median overall survival (OS) was 12.6 (95% CI: 8.74, 18.69) months (secondary study outcomes).[6]

OBJECTIVES

- The aim of these *post hoc* analyses of this phase 1/2 study was to evaluate survival in subgroups characterized by post-treatment changes in levels of carbohydrate antigen 19-9 (CA 19-9) for patients receiving the selected dose of NALIRIFOX (exploratory endpoint).
  - Serum CA 19-9 levels are typically elevated in patients with locally advanced or metastatic PDAC and, separately, post-treatment decreases have been associated with prolonged survival.[7]

METHODS

Study design

- This was a two-part, open-label, phase 1/2 study comprising a dose-exploration part (using a traditional 3 + 3 design) and a dose-expansion part.

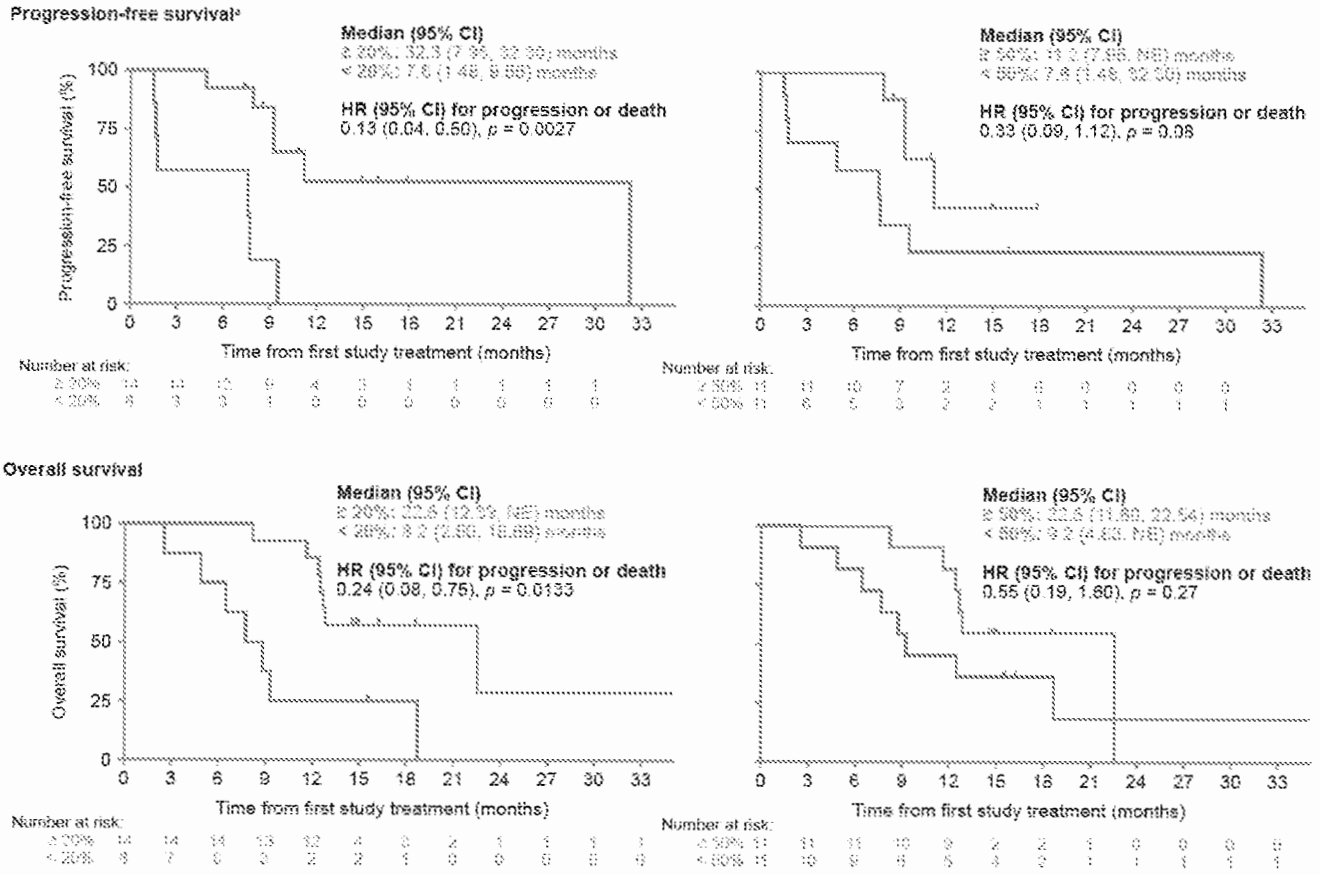
Study population

- Patients were adults, aged 18 years or older, with:
  - unresectable locally advanced or metastatic PDAC
  - a diagnosis that was received no more than 6 weeks before screening
  - no previous treatment in the metastatic setting
  - an Eastern Cooperative Oncology Group Performance Status score of 0 or 1 (dose-exploration part only)
  - a Karnofsky Performance Status score of at least 70 (dose-expansion part only)
  - adequate organ function.

Treatment regimen

- Based on the results of the dose-exploration part of the study, the selected (maximum tolerable) dose consisted of liposomal irinotecan 50 mg/m^2 free base, 5-FU 2400 mg/m^2 (continuous infusion), LV 400 mg/m^2 and oxaliplatin 60 mg/m^2, administered on days 1 and 15 of each 28-day cycle.

Figure 1. Survival in *post hoc* subgroups according to best reduction in CA 19-9 levels up to 16 weeks after baseline (n = 22)



[a]Patients who progressed/died after receiving new therapy or who progressed/died more than 16 weeks after last non-PD assessment were censored for PFS.
CA 19-9, carbohydrate antigen 19-9; CI, confidence interval; HR, hazard ratio; NE, not estimable; OS, overall survival; PD, progressive disease; PFS, progression-free survival.

Table 1. CA 19-9 levels at baseline and percentage changes by week 16

	Pooled population (n = 34)	Patients with elevated baseline CA 19-9 (> 37 U/mL) (n = 23)
Patients with CA 19-9 data at baseline, n (%)	30 (94)	23 (100)
Baseline levels, median (range), U/mL	315.5 (2 to 127,115)	1265.0 (38 to 127,115)
Patients with baseline CA 19-9 data and another observation by week 16, n (%[a])	22 (73)	17 (74)
Best change by week 16, median (range), %	−49.4 (−100 to +376)	−35.9 (−100 to +278)

[a]Percentages based on number of patients with CA 19-9 data at baseline.
CA 19-9, carbohydrate antigen 19-9

- All patients in the dose-expansion part received the selected dose until radiologically determined progressive disease (as per Response Evaluation Criteria In Solid Tumours version 1.1 [RECIST v1.1]), unacceptable toxicity or consent withdrawal.

Assessments

- Efficacy measures included PFS and OS.
  - Disease was assessed using RECIST v1.1 at screening, every 8 weeks thereafter and at the end of study treatment.
  - Patients were followed for survival every 2 months from the end-of-treatment visit until death or study closure, whichever occurred first.
- Serum CA 19-9 levels were measured at screening (day of first dose) and every 8 weeks during the treatment period.

Exploratory survival analyses by change in CA 19-9 levels

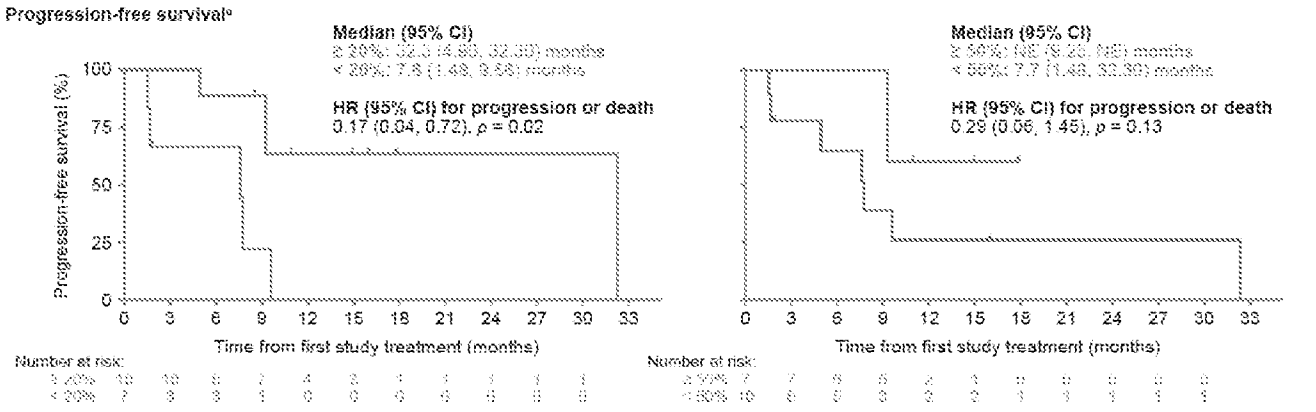
- In these *post hoc* analyses, patients were classified according to their best change in CA 19-9 during the first 16 weeks of treatment.
  - The four subgroups included patients with and without decreases in CA 19-9 levels from baseline of at least 20% and of at least 50%.
- Kaplan–Meier methods were used to estimate PFS and OS.
- Hazard ratios and 95% CIs were based on Cox regression analysis.
- Analyses were repeated for patients who had elevated CA 19-9 levels (> 37 U/mL) at baseline.

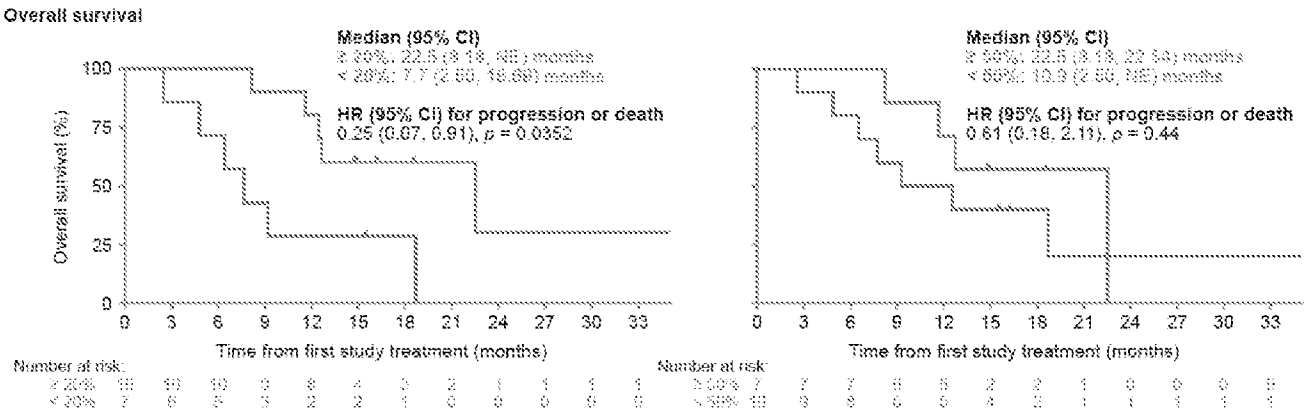
RESULTS

Patient disposition and CA 19-9 levels through week 16

- In total, 32 patients received the selected dose (pooled population), seven during dose exploration and 25 during dose expansion.[6]

Figure 2. Survival in *post hoc* subgroups according to best reduction in CA 19-9 levels up to 16 weeks after baseline among patients with elevated baseline CA 19-9 levels (> 37 U/mL, n = 17)





*Patients who progressed/died after receiving new therapy or who progressed/died more than 16 weeks after last non-PD assessment were censored for PFS.
CA 19-9, carbohydrate antigen 19-9; CI, confidence interval; HR, hazard ratio; NE, not estimable; OS, overall survival; PD, progressive disease; PFS, progression-free survival.

- Baseline CA 19-9 measurements were available for 30/32 patients (94%) (Table 1); of these, 22 (73%) had post-baseline measurements by week 16.
  - Median PFS for these patients was 9.6 (95% CI: 7.59, 32.30) months (12 events), and median OS was 12.7 (95% CI: 8.74, 22.54) months (14 events).
- Of 23 patients with an elevated CA 19-9 level (> 37 U/mL) at baseline, 17 (74%) had measurements by week 16 (Table 1).
  - Median PFS for these patients was 9.2 (95% CI: 7.59, 32.30) months (9 events), and median OS was 12.6 (95% CI: 7.69, 22.54) months (11 events).
- Baseline CA 19-9 levels and best changes by week 16 in both groups are summarized in Table 1.

Survival by change in CA 19-9 levels

- Median PFS and OS were longer in patients who achieved post-treatment reductions in CA 19-9 levels of at least 20% (vs those who did not) during the first 16 weeks of treatment (Figure 1).
- Results were similar when PFS and OS were assessed in patients who had elevated CA 19-9 levels (> 37 U/mL) at baseline (Figure 2).

CONCLUSIONS

- First-line NALIRIFOX reduced CA 19-9 levels in patients with locally advanced or metastatic PDAC.
- Post-treatment reductions in CA 19-9 of at least 20% during the first 16 weeks may be associated with better survival outcomes.
- Thus, change in CA 19-9 is a potential predictor of NALIRIFOX treatment response and is under investigation in a large, ongoing, phase 3 study, in which NALIRIFOX will be compared with gemcitabine plus nab-paclitaxel.

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For further information, please send your question(s) to Dr Andrew Dean (adean747@mac.com)

To download the poster or for audio commentary, please scan the Quick Response (QR) code.

Author contributions All authors have contributed to study conception/design, drafting the publication or revising it critically for scientific accuracy and important intellectual content, and final approval of the publication.

Conflicts of interest AD – non-remunerated advisory/consultancy activities: Shire, Specialised Therapeutics, Ipsen/accommodation/expenses: Amgen. PB-S – 1Globe Health Institute, AbCellomics, Amgen, Array BioPharma, AstraZeneca, Bayer, Boehringer Ingelheim, Boston Biomedical, Bristol Myers Squibb, Celgene, Clovis Oncology, Eli Lilly, Exelixis, Genentech, Immunomedics, Imugene, Incyte, Ipsen, Merck, Pancreatic Cancer Action Network (PanCAN), Seattle Genetics, Taiho, Sun BioPharma. Travis Eli: PMB – research grant/funding: Advaxis, Bayer, Boehringer Ingelheim, Boston Biomedical, Celgene, Clovis Oncology, Genentech, Merck, advisory/consultancy: Bayer, Merrimack Pharmaceuticals; honoraria: Eisai, Merck, FG – research grant/funding (institution): Amgen, AstraZeneca, Bristol Myers Squibb, Exelixis, Ipsen, Taiho Pharmaceutical, advisory/consultancy: Eisai, Exelixis, Foundation Medicine, Genentech, Ipsen, Natera (Signatera), QED Therapeutics; speaker bureau/expert testimony: Amgen, Decipher Pharmaceuticals, Eisai, Exelixis, Ipsen, Natera (Signatera), Sirtex Medical; spouse/financial dependent and full/part-time employment: Foundation Medicine. TM – research grant/funding: Agios, ASLAN Pharmaceuticals, AstraZeneca, Bayer, Biogen, Celgene, Eli Lilly, Genentech, Halozyme Therapeutics, Immunomedics, Merrimack Pharmaceuticals, Millennium Pharmaceuticals, Novartis, Novocure, OncoMed Pharmaceuticals, Pfizer Pharmacyclics, Roche; honoraria: Eli Lilly, Ipsen, Roche, Sanofi, Sanofi Genzyme, Shire, Tesaro; advisory/consultancy: Baxalta, Celgene, H3 Biomedicine, Incyte, QED Therapeutics, Sanofi Genzyme, Servier, Shire; speaker bureau/expert testimony: Celgene, Sanofi, Shire; travel/accommodation/expenses: Bayer, H3 Biomedicine, Merck, Sanofi. KM – research grant/funding: Agios, ArQule, AstraZeneca, Genentech, Incyte, National Cancer Institute of the National Institutes of Health award # NCI/NIH P50 CA210964, Purple Biotechnology, Sentrera Biosciences, Taiho Pharmaceutical; advisory/consultancy: AstraZeneca, Bayer, Celgene, Eisai, Exelixis, Ipsen, Merrimack Pharmaceuticals, Virus Therapeutics. BB – full/part-time employment: Ipsen. PM – full/part-time employment and shareholder/stockholder/stock options: Ipsen. YM – full/part-time employment, leadership role and shareholder/stockholder/stock options: Ipsen. TW – full/part-time employment and shareholder/stockholder/stock options: Ipsen. RZ – full/part-time employment, shareholder/stockholder/stock options and licensing/royalties: Ipsen. ZAW – research grant/funding (institution): Five Prime Therapeutics, Ipsen, Novartis, Pfizer/Arc; advisory/consultancy: AstraZeneca, Bayer, Daiichi Sankyo, Eli Lilly, Five Prime Therapeutics, Ipsen, Merck, QED Therapeutics.

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First-line liposomal irinotecan + 5-fluorouracil/leucovorin + oxaliplatin in patients with locally advanced or metastatic pancreatic ductal adenocarcinoma: exploratory subgroup analyses of survival by changes in CA 19-9 levels

1529P

BACKGROUND

OBJECTIVES

METHODS

RESULTS

CONCLUSIONS

References

First-line liposomal irinotecan + 5-fluorouracil/ leucovorin + oxaliplatin in patients with pancreatic ductal adenocarcinoma: results from a phase 1/2 study

Andrew DEAN,[1] Tanios S. BEKAII-SAAB,[2] Patrick M BOLAND,[3] Farshid DAYYANI,[4] Teresa MACARULLA,[5] Kabir MODY,[6] Bruce BELANGER,[7] Fiona MAXWELL,[8] Yan MOORE,[7] Arunthathi THIAGALINGAM,[7] Haofei Tiffany WANG,[7] Bin ZHANG,[7] Zev A. WAINBERG[9]

[1]St John of God Subiaco Hospital, Subiaco, WA, Australia; [2]Mayo Clinic (ACCRU), Phoenix, AZ, USA; [3]Rutgers Cancer Institute of New Jersey, New Brunswick, NJ, USA; [4]University of California, Irvine, CA, USA; [5]Vall d´Hebron University Hospital and Vall d´Hebron Institute of Oncology (VHIO), Barcelona, Spain; [6]Mayo Clinic, Jacksonville, FL, USA; [7]Ipsen, Cambridge, MA, USA; [8]Ipsen, Abingdon, UK; [9]University of California Los Angeles, Los Angeles, CA, USA

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Disclosures

Author	Disclosure
Andrew Dean	Amgen, Shire, Specialised Therapeutics
Tanios Bekaii-Saab	1Globe Health Institute, AbGenomics, Amgen, Array BioPharma, AstraZeneca, Bayer, Boehringer Ingelheim, Boston Biomedical, Bristol Myers Squibb, Celgene, Clovis Oncology, Eli Lilly, Exelixis, Genentech, Immuneering, Imugene, Incyte, Ipsen, Merck, Pancreatic Cancer Action Network (PanCAN), Seattle Genetics, Sobi, Sun BioPharma, Treos Bio
Patrick M Boland	Advaxis, Bayer, Boehringer Ingelheim, Boston Biomedical, Cascadian Therapeutics, Genentech, Merck, Merrimack Pharmaceuticals, Sirtex Medical
Farshid Dayyani	Amgen, AstraZeneca, Bristol Myers Squibb, Deciphera Pharmaceuticals, Eisai, Exelixis, Foundation Medicine, Genentech, Ipsen, Natera, QED Therapeutics, Sirtex Medical, Taiho Pharmaceutical; spouse is an employee of Roche Diagnostics
Teresa Macarulla	Agios, ASLAN Pharmaceuticals, AstraZeneca, Baxalta, Bayer, BeiGene, Biogen, Celgene, Eli Lilly, Genentech, Genzyme, H3 Biomedicine, Halozyme Therapeutics, Immunomedics, Incyte, Ipsen, Merck, Merrimack Pharmaceuticals, Millennium Pharmaceuticals, Novartis, Novocure, OncoMed Pharmaceuticals, Pfizer, Pharmacyclics, QED Therapeutics, Roche, Sanofi, Servier, Shire, Tesaro
Kabir Mody	Agios, ArQule, AstraZeneca, Bayer, Celgene, Eisai, Exelixis, Genentech, Incyte, Ipsen, Merrimack Pharmaceuticals, Puma Biotechnology, Senwa Biosciences, Taiho Pharmaceutical, Vicus Therapeutics, NCI of the NIH award # NCI/NIH P50 CA210964
Bruce Belanger	Ipsen
Fiona Maxwell	Ipsen
Yan Moore	Ipsen
Arunthathi Thiagalingam	Ipsen
Tiffany Wang	Ipsen
Bin Zhang	Ipsen
Zev A Wainberg	AstraZeneca, Bayer, Daiichi Sankyo, Eli Lilly, Five Prime Therapeutics, Ipsen, Merck, Novartis, QED Therapeutics, Plexxikon

Objectives

- To assess the safety, tolerability and efficacy of the **NALIRIFOX regimen** as a first-line treatment for patients with locally advanced or metastatic PDAC

Primary objectives

- Safety and tolerability of NALIRIFOX
- Characterize dose-limiting toxicities (DLTs) associated with NALIRIFOX and determine the recommended dose for future development

Secondary objectives

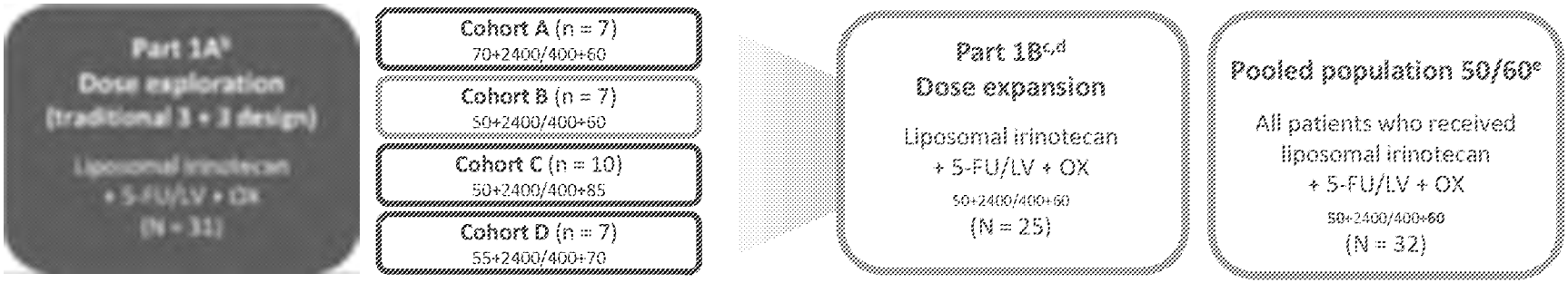
- Clinical response
  - Progression-free survival (PFS)
  - Overall survival (OS)
  - Other: Best overall response (BOR); Overall response rate (ORR); Disease control rate at week 16 (DCR16); Duration of response (DOR)

Antitumour activity was assessed using RECIST v1.1 at screening (baseline), every 8 weeks until PD and at EOT

5-FU/LV, 5-fluorouracil/leucovorin; EOT, end of treatment; PD, progressive disease; PDAC, pancreatic ductal adenocarcinoma; RECIST v1.1, Response Evaluation Criteria in Solid Tumours version 1.1

Methods

- An open-label phase 1/2 study in treatment-naïve[a] patients with locally advanced or metastatic PDAC
- Patients were aged ≥ 18 years with an ECOG PS ≤ 1 and adequate organ function
- The study comprised two parts: dose exploration and dose expansion
- Patients received NALIRIFOX on days 1 and 15 of each 28-day cycle, at different doses
- The dose selected for expansion was based on DLTs and safety data from dose exploration



[a]Not previously treated in the metastatic setting. [b]Enrolled between 26 Oct 2015 and 28 Mar 2018. [c]Enrolled between 11 Jun 2018 and 29 Oct 2018. [d]Patients were required to have a Karnofsky PS score ≥ 70. [e]Dose of liposomal irinotecan (free base)/dose of oxaliplatin expressed in mg/m² (administered in combination with 5-FU 2400 mg/m² and LV 400 mg/m² every 2 weeks).
DLT, dose-limiting toxicity; ECOG, Eastern Cooperative Oncology Group; PS, Performance Status

Pooled population: patient demographics

	Pooled population (50/60[a,b]) N = 32
Age (years)	
Median (range)	58 (39–76)
Age group, n (%)	
< 65 Years	23 (71.9)
Sex, n (%)	
Men	14 (43.8)
Race, n (%)	
White	28 (87.5)
Tumour stage at diagnosis	
IIA[c]	1 (3.1)
III	3 (9.4)
IV	28 (87.5)
Baseline ECOG Performance Status score	
Fully active (ECOG 0)	14 (43.8)
Restricted activity (ECOG 1)	18 (56.3)
Disposition	
Discontinued treatment,[d] n (%)	31 (96.9)

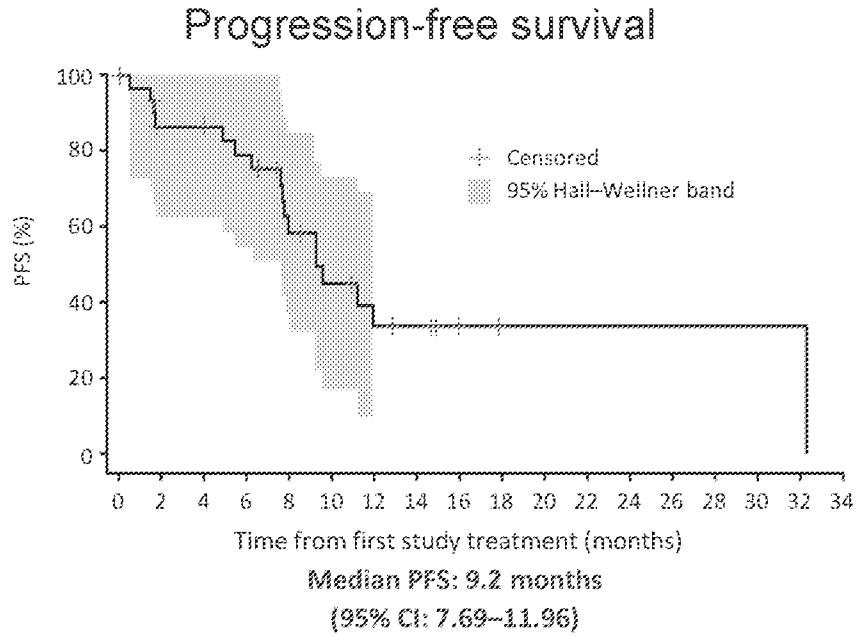
[a]Dose of liposomal irinotecan (free base)/dose of oxaliplatin expressed in mg/m² (administered in combination with 5-FU 2400 mg/m² and LV 400 mg/m² every 2 weeks). [b]Comprised cohort 8 and the dose-expansion cohort. [c]One patient in the dose-expansion cohort was diagnosed as stage IIA, but entered the treatment phase as stage IV. [d]At time of data cut-off (26 Feb 2020).
5-FU, 5-fluorouracil; ECOG, Eastern Cooperative Oncology Group; LV, leucovorin.

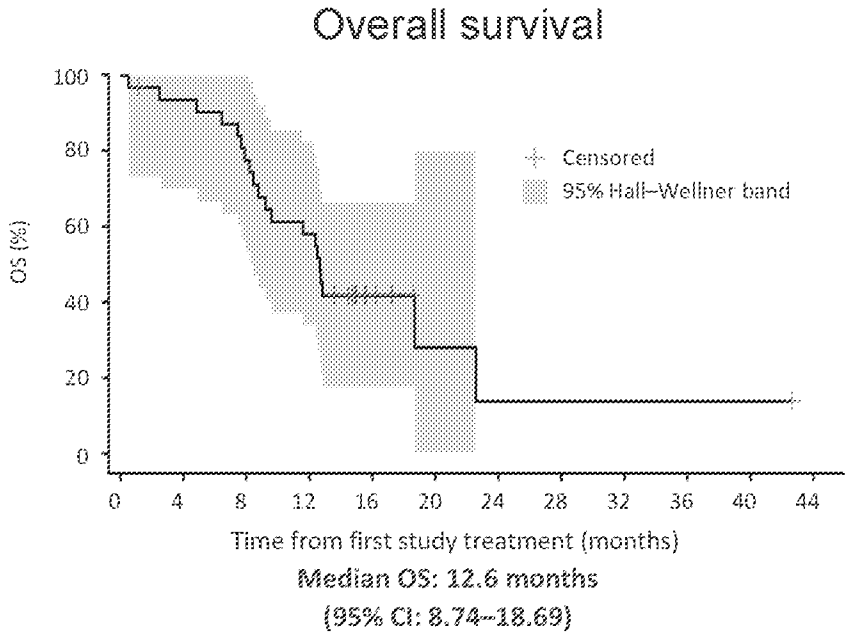
Pooled population: safety overview

	Pooled population (50/60[a,b]) (N = 32)
Any TEAE, n (%)	32 (100)
Leading to dose discontinuation[c]	8 (25.0)
Leading to dose adjustment[c]	26 (81.3)
Any serious TEAE, n (%)	17 (53.1)
Leading to death	3 (9.4)[d]
Treatment-related[e]	10 (31.3)[f]
Treatment-related TEAE, n (%)	32 (100)
Treatment-related and grade ≥ 3	22 (68.8)
Treatment-related TEAE of grade ≥ 3 in at least 5% of the pooled population, n (%)	
Neutropaenia	10 (31.3)
Febrile neutropaenia	4 (12.5)
Neutrophil count decreased	3 (9.4)
Anaemia	2 (6.3)
Diarrhoea	3 (9.4)
Nausea	3 (9.4)
Vomiting	2 (6.3)
Hypokalaemia	4 (12.5)
Hyponatraemia	2 (6.3)
Alanine aminotransferase increased	2 (6.3)
GGT increased	2 (6.3)
Lymphocyte count decreased	2 (6.3)
White blood cell count decreased	2 (6.3)

[a]Dose of liposomal irinotecan (free base)/dose of oxaliplatin expressed in mg/m^2 (administered in combination with 5-fluorouracil 2400 mg/m^2 and leucovorin 400 mg/m^2 every 2 weeks). [b]Comprises cohort B and the dose-expansion cohort. [c]Refers to any of the four treatments administered. [d]Malignant gastrointestinal obstruction, upper gastrointestinal haemorrhage and disease progression, none were considered related to treatment. [e]TEAEs considered by the investigator to be related to any of the four treatments administered or for which the relationship was missing. [f]Most common were febrile neutropaenia and nausea, each reported in 3 patients (9.4%). GGT, gamma-glutamyltransferase; NA, not applicable; TEAE, treatment-emergent adverse event

Pooled population: clinical response (1)





Data are from the safety pooled population. PFS and OS were analysed using the kaplan–Meier method. Median (95% CI) values were calculated using the Brookmeyer–Crowley method.
One patient with minimal progressive disease per RECIST v1.1 was approved for treatment continuation as the investigator believed there was a benefit from treatment. Data from this patient were censored at PD data.
CI, confidence interval; OS, overall survival; PD, progressive disease; PFS, progression-free survival; RECIST v1.1, Response Evaluation Criteria in Solid Tumors version 1.1

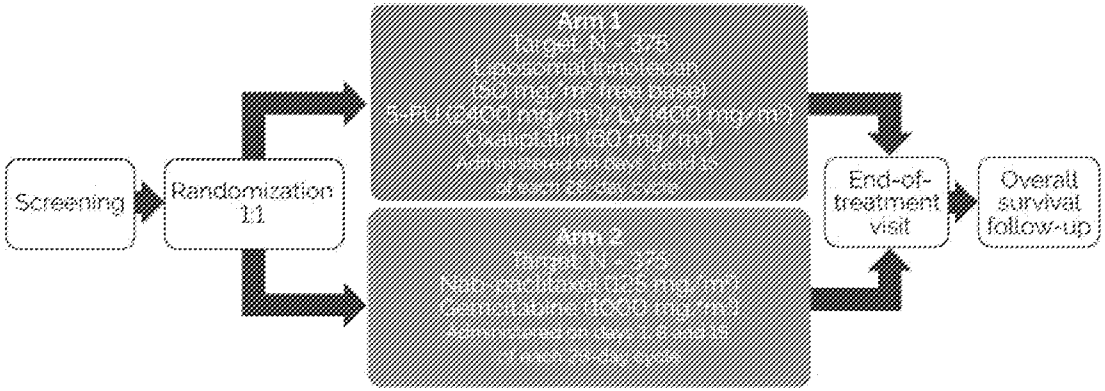
Pooled population: clinical response (2)

	Pooled population (50/60) (N = 32)
Best overall response[a]	
CR	1 (3.1)[b]
PR	10 (31.3)
SD	15 (46.9)
PD	3 (9.4)
Non-PD/non-CR	0
NE	3 (9.4)
Overall response rate	
(CR + PR), rate [95% CI][c]	34.4 [18.6–53.2]
Disease control rate at 16 weeks	
(CR + PR + SD), rate [95% CI][d]	71.9 [53.3–86.3]
Duration of response[e]	(n = 11)
Median, months [95% CI]	9.4 [3.52–NE]
Rate, % [95% CI], at:	
6 months	63.6 [30.8–89.1]
12 months	27.3 [6.0–61.0]
24 months	9.1 [0.2–41.3]

Data are from the safety population and with responses determined using RECIST v1.1. [a]Recorded from start of study treatment until disease progression or start of new anticancer therapy. [b]Patient received a diagnosis of locally advanced stage III disease. [c]95% CIs were calculated using the Clopper–Pearson method. [d]Patients who died, whose tumours were no longer assessed or who started new anticancer treatment before the week-16 assessment were not considered to have achieved disease control at week 16. [e]Time from the first date of response (CR or PR) to date of first documented radiologically determined PD per RECIST v1.1; duration of response was not calculated for patients who started a new anticancer treatment before the first response.
CI, confidence interval; CR, complete response; NE, not evaluable; PD, progressive disease; PR, partial response; RECIST v1.1, Response Evaluation Criteria in Solid Tumors version 1.1; SD, stable disease

Conclusions

- First-line NALIRIFOX raised no new safety signals in patients with locally advanced or metastatic PDAC
- Anti-tumour activity was promising at the identified recommended dose
- The ongoing randomized phase-3 NAPOLI-3 study (NCT04083235) will compare NALIRIFOX with gemcitabine + nab-paclitaxel



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Thank you for listening!

P-287

Nanoliposomal Irinotecan (nal-IRI)-Containing Regimens Versus nab-Paclitaxel Plus Gemcitabine as First-Line Therapy in Patients With Metastatic Pancreatic Adenocarcinoma: A Randomized, Open-Label Phase 2 Study

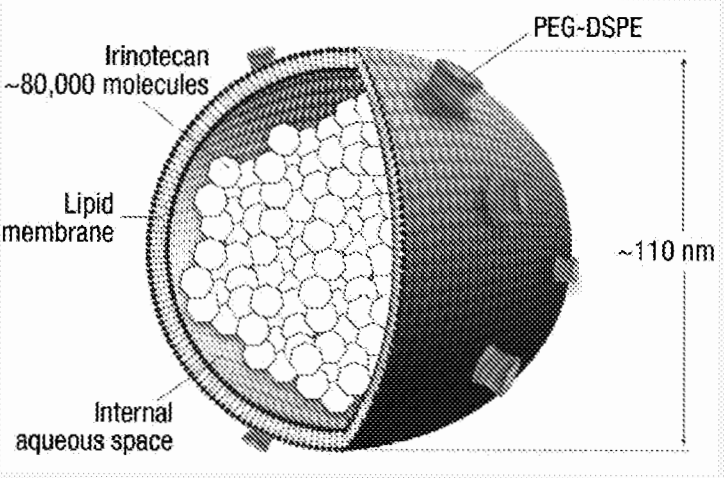
Andrew Dean,[1] Ramesh K Ramanathan,[2] Brooke Belota,[3] Bruce Belanger,[3] Deyaa Adib,[4] Eliel Bayever,[3] Li-Tzong Chen[5]

[1]St John of God Hospital, Subiaco, Australia; [2]Mayo Clinic Cancer Center, Scottsdale, Scottsdale, AZ, USA; [3]Merrimack Pharmaceuticals, Inc., Cambridge, MA, USA; [4]Shire, Cambridge, MA, USA; [5]National Health Research Institutes; National Institute of Cancer Research, Tainan, Taiwan

BACKGROUND

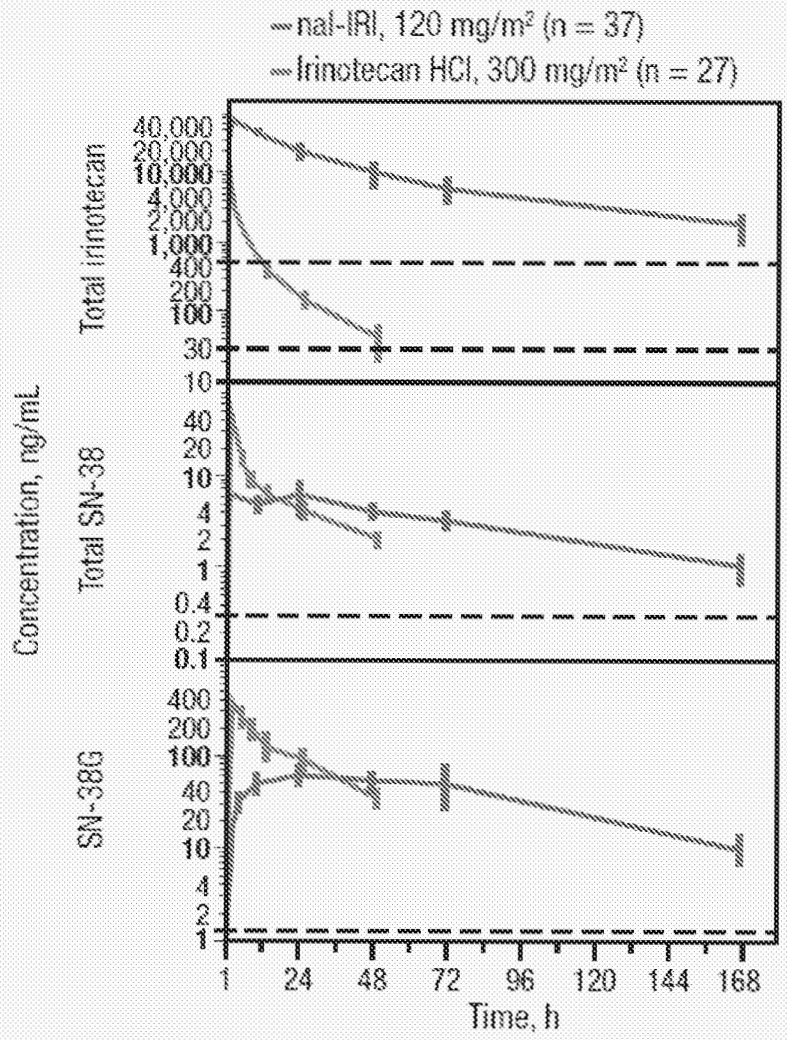
- Two combination chemotherapy regimens have emerged as the standard of care for first-line treatment of metastatic pancreatic adenocarcinoma (mPAC):
  - FOLFIRINOX (5-fluorouracil [5-FU] + leucovorin [LV] + irinotecan + oxaliplatin)
  - nab-paclitaxel + gemcitabine
- These regimens are associated with a median overall survival (OS) of less than 1 year in phase 3 studies (11.1 and 8.5 months, respectively)[1,2]
- nal-IRI (liposomal irinotecan; MM-398) is a nanoliposomal formulation of irinotecan, a topoisomerase inhibitor, for intravenous use (**Figure 1**)[3]
  - Pharmacokinetic analyses in patients with gastric cancer treated with either nal-IRI 120 mg/m^2 or irinotecan HCL 300 mg/m^2 showed extended circulation of irinotecan within the liposome in patients treated with nal-IRI (**Figure 2**)[4,5]
  - The liposome facilitates intratumoral drug deposition through the enhanced permeability and retention effect[6]
  - Preliminary data from a pilot study across different cancer types showed higher levels of SN-38 (the active metabolite of irinotecan) in tumor biopsies compared with plasma at 72 hours, suggesting local metabolic activation of irinotecan to SN-38 (**Figure 3**)[3]

Figure 1. nal-IRI design.[3]



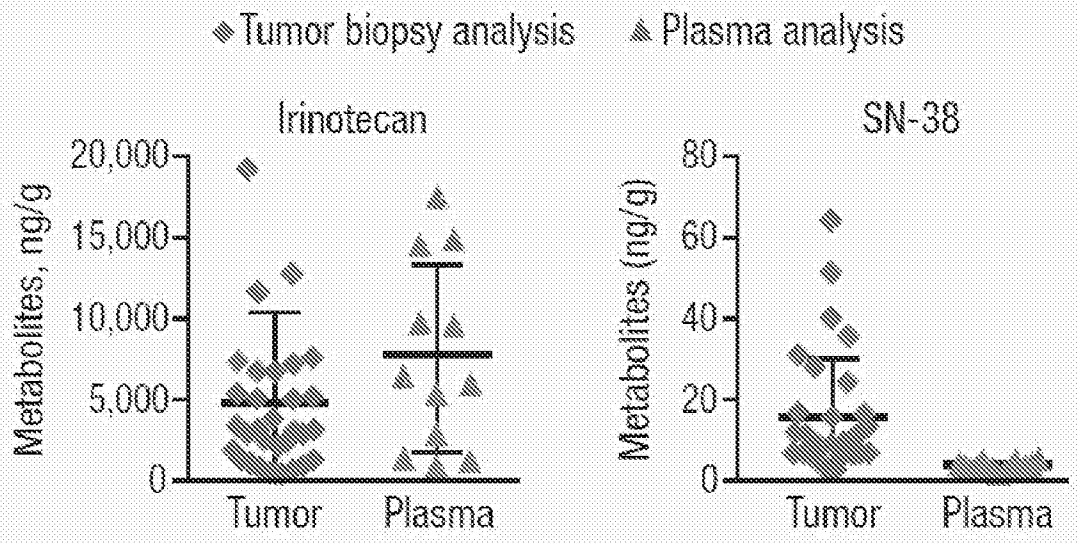
nal-IRI, liposomal irinotecan.

Figure 2. Sustained circulation of nal-IRI.[4,5]



AUC, area under the curve; C_{max}, maximal concentration; nal-IRI, liposomal irinotecan.
Comparing nal-IRI with irinotecan HCl, the total irinotecan AUC was 46 times greater and the total irinotecan C_{max} was 13.4 times greater; the SN-38 AUC was 1.4 times greater and the SN-38 C_{max} was 0.19 times greater. The peak of SN-38 metabolite was lower with nal-IRI versus irinotecan HCl, without an increase in SN-38 plasma AUC.

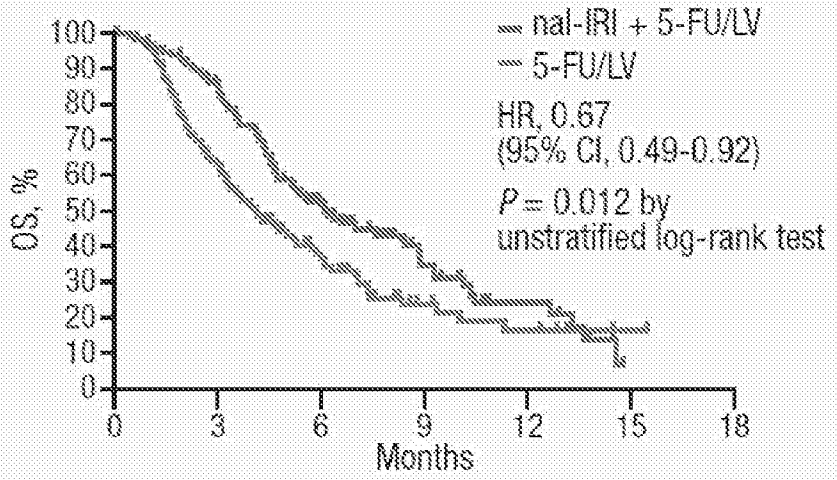
Figure 3. Irinotecan and SN-38 levels 72 hours after nal-IRI treatment.[3]



LLoQ, lower limit of quantification; nal-IRI, liposomal irinotecan.
Drug metabolite quantification in tumor biopsies and plasma analyses from a study of patients (N = 14) with advanced solid tumors. Biopsy material averaged 10.5 mg (range, 3.3–21.9 mg); metabolite detection was in an LC/MS/MS TSQ Vantage instrument, with LLoQ of 50 pg/mL for irinotecan and 100 pg/mL for SN-38.
Plasma analysis was performed at GPS according to validated procedures, with LLoQ of 140 ng/mL for irinotecan and 800 pg/mL for SN-38.

- nal-IRI is approved by the US Food and Drug Administration (FDA) for use in combination with 5-FU/LV for the treatment of patients with mPAC after disease progression following gemcitabine-based therapy, based on results from the primary analysis of the large (N = 417), international, randomized, phase 3 NAPOLI-1 study[7]
  - Median OS increased significantly with nal-IRI + 5-FU/LV relative to 5-FU/LV (6.1 vs 4.2 months; unstratified hazard ratio [HR], 0.67 [95% confidence interval (CI), 0.49-0.92]; $P = 0.012$; **Figure 4**)
  - Median OS did not differ between patients assigned nal-IRI monotherapy and those allocated to 5-FU/LV (4.9 vs 4.2 months; unstratified HR, 0.99 [95% CI, 0.77-1.28]; $P = 0.94$)
  - Median progression-free survival (PFS; 3.1 vs 1.5 months; unstratified HR, 0.56 [95% CI, 0.41-0.75]; $P = 0.0001$) and objective response rate (ORR; 16% vs 1%; $P < 0.0001$) were also improved with nal-IRI + 5-FU/LV compared with 5-FU/LV
  - nal-IRI + 5-FU/LV demonstrated a predictable and manageable safety profile; the most frequently reported grade ≥3 treatment-emergent adverse events (AEs) were neutropenia, fatigue, diarrhea, and vomiting

Figure 4. Kaplan-Meier OS in the NAPOLI-1 trial.[7]



No. at risk:

nal-IRI + 5-FU/LV:	117	97	51	20	8	0	0
5-FU/LV:	119	68	34	11	6	1	0

5-FU, 5-fluorouracil; CI, confidence interval; HR, hazard ratio; LV, leucovorin; nal-IRI, liposomal irinotecan; OS, overall survival. OS analysis includes all patients randomized after implementation of a protocol amendment that added the nal-IRI + 5-FU/LV combination arm.

STUDY OBJECTIVES

- The overall goal of the current study is to determine the preliminary safety and efficacy of nal-IRI + 5-FU/LV with or without oxaliplatin, as compared with nab-paclitaxel + gemcitabine in previously untreated patients with mPAC (ClinicalTrials.gov Identifier: NCT02551991)

Part 1

- The primary objectives of Part 1 are to
  - Evaluate the safety and tolerability of nal-IRI + 5-FU/LV + oxaliplatin
  - Characterize dose-limiting toxicities (DLTs) associated with nal-IRI + 5-FU/LV + oxaliplatin and determine the Part 2 triplet combination dose
- The secondary objective of Part 1 is to characterize the pharmacokinetics of nal-IRI + 5-FU/LV + oxaliplatin

Part 2

- The primary objective of Part 2 is to compare the PFS in patients in each nal-IRI–containing arm versus nab-paclitaxel + gemcitabine
- The secondary objectives of Part 2 are to

- Compare the OS and ORR in patients in each nal-IRI–containing arm relative to nab-paclitaxel + gemcitabine
- Assess tumor marker carbohydrate antigen 19-9 (CA19-9) response in patients in each nal-IRI–containing arm relative to nab-paclitaxel + gemcitabine
- Evaluate health-related quality of life (HRQoL) of patients in each treatment arm
- Compare the safety and AE profile between treatment arms
- Assess the potential for QTcF prolongation with nal-IRI treatment

- Additional exploratory objectives are to
  - Evaluate the relationship between plasma pharmacokinetics of nal-IRI (total irinotecan, SN-38), oxaliplatin, and efficacy and safety endpoints
  - Evaluate patient blood samples and archived tumor tissue for potential biomarkers that may correlate with nal-IRI pharmacokinetics, toxicity, and/or response

Key Eligibility Criteria

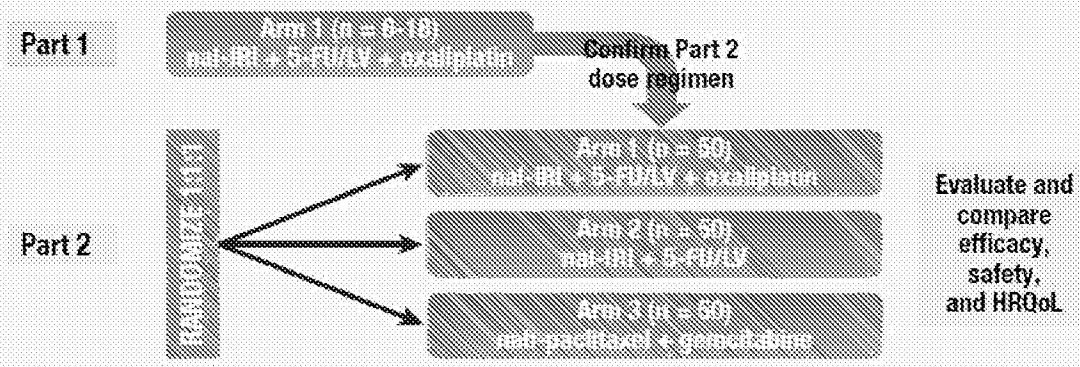
- Adults aged ≥18 years
- Pathologically confirmed, measurable or nonmeasurable mPAC, as defined by Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1, that has not been previously treated in the metastatic setting
  - Part 1: unresectable, locally advanced or metastatic disease is allowed, diagnosed within 6 weeks before enrollment
  - Part 2: must have metastatic disease diagnosed within 6 weeks before randomization; locally advanced disease is not allowed
- Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1
- No uncontrolled metastases to the central nervous system
- No prior treatment of pancreatic cancer with cyctotoxic doses of chemotherapy
- No history of any second malignancy in the last 3 years
- Adequate hematologic parameters, and hepatic and renal function
- Normal electrocardiogram or electrocardiogram without clinically significant findings
- No use of strong CYP3A4 inhibitors or inducers

- No use of strong CYP2C8 inhibitors or inducers (Part 2 only)
- No known contraindications or hypersensitivity to any study drugs (Part 2 only for nab-paclitaxel and gemcitabine)
- No clinically significant gastrointestinal disorder, concurrent illnesses, active infection or unexplained fever >38.5°C at screening/baseline, or any other condition deemed likely to interfere with the study
- Not pregnant or breast feeding; both males and females must agree to highly effective methods of birth control during the study and for 4 months following the last dose of study drug

STUDY DESIGN

- This phase 2, open-label, comparative study is being conducted in 2 parts (**Figure 5**)

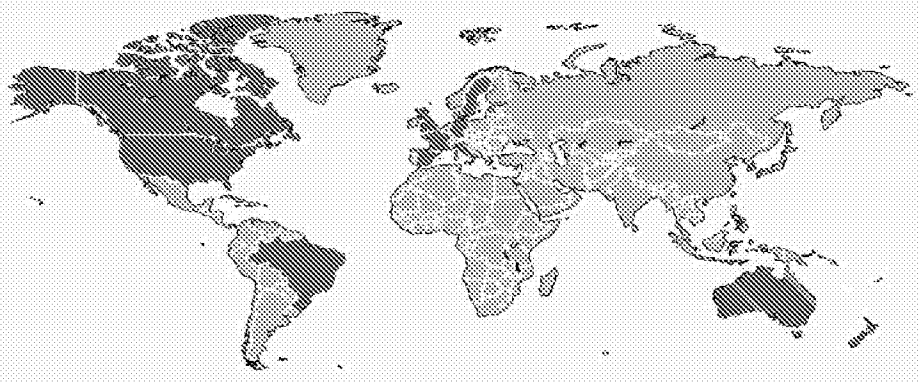
Figure 5. Study design.



5-FU, 5-fluorouracil; HRQoL, health-related quality of life; LV, leucovorin; nal-IRI, liposomal irinotecan.

- Participating sites are located in 14 countries in North America, Europe, Asia-Pacific, and South America (**Figure 6**)

Figure 6. Countries with participating sites.



Part 1

- A dose-escalation safety run-in (traditional 3+3 design) is being performed to confirm an appropriate Part 2 dose regimen for the nal-IRI + 5-FU/LV + oxaliplatin triple combination arm (**Table 1**)
  - If no DLTs are reported for a dose cohort within the first treatment cycle, the subsequent cohort will be initiated
  - If 1 patient in a dose cohort experiences a DLT within the first treatment cycle, the cohort will be expanded from 3 to 6 patients
  - If ≥2 patients within a dose cohort experience a DLT, that dose will be considered to exceed the safety criteria and the dose will not be escalated further. The Part 2 dose will then be defined as the next lower dose level in which 6 patients were treated and ≤1 patient experienced a DLT

Table 1. Dose-Escalation Scheme

	Oxaliplatin		5-FU/LV		nal-IRI	
Level	Dose (mg/m^2)	Dose day	Dose (mg/m^2)	Dose day	Dose (mg/m^2)[a]	Dose day
1	60	1, 15	2400/400	1, 15	80	1, 15
–1	60	1, 15	2400/400	1, 15	60	1, 15
2	85	1, 15	2400/400	1, 15	80	1, 15
–2A	75	1, 15	2400/400	1, 15	80	1, 15
–2B	85	1, 15	2400/400	1, 15	60	1, 15

5-FU, 5-fluorouracil; LV, leucovorin; nal-IRI, liposomal irinotecan.

Shaded dose levels are for de-escalation only. Enrollment in these dose levels will only be initiated upon agreement of the investigators, study sponsor, and medical monitor.

[a]The above nal-IRI doses are expressed as the irinotecan HCl trihydrate salt. Converting the dose from irinotecan HCl trihydrate salt to irinotecan free base is accomplished by substituting the molecular weight of irinotecan HCl trihydrate salt (677.19 g/mol) with that of irinotecan free base (586.68 g/mol), which results in a conversion factor of 0.866. The above nal-IRI doses of 80 and 60 mg/m^2 approximate to 70 and 52 mg/m^2 irinotecan free base.

Part 2

- Patients will be randomized (1:1:1) in the active-comparator phase to evaluate and compare efficacy, safety, and HRQoL between treatment arms

- Randomization will be stratified based on region (East Asia vs rest of the world) and ECOG PS (0 vs 1)
- Part 2 will include the following 3 treatment arms (28-day cycles):
  - Arm 1: nal-IRI + 5-FU/LV + oxaliplatin at dosages determined in Part 1, on days 1 and 15 of each cycle
  - Arm 2: nal-IRI (80 mg/m^2 over 90 minutes) + 5-FU (2400 mg/m^2 over 46 hours)/LV (400 mg/m^2) on days 1 and 15 of each cycle
  - Arm 3: nab-paclitaxel (125 mg/m^2 over 35 minutes) + gemcitabine (1000 mg/m^2 over 30 minutes) on days 1, 8, and 15 of each cycle

- Patients will be treated until disease progression, intolerable toxicity, withdrawal of consent, or at the discretion of the treating physician
- Primary efficacy endpoint: PFS
  - Each nal-IRI–containing arm will be assessed for an increased PFS relative to the control arm using a 1-sided stratified log-rank test; hazard ratios for PFS will be estimated using stratified Cox models
  - If the true hazard ratio for PFS for an experimental arm compared with the control arm is 0.60, occurrence of at least 70 PFS events would provide 80% power to detect an improvement with a pairwise 1-sided 0.10 level test

STUDY POPULATIONS

- Several study populations will be used in the analysis of data (**Table 2**)

Table 2. Study Populations

Population	Description
Safety population	Part 1: Patients receiving any part of ≥1 dose of study drug. This will be the only study population for assessing Part 1 Part 2: Patients receiving any part of ≥1 dose of study drug; this population will be summarized according to treatment actually received
Intent-to-treat population	Part 2 only: All randomized patients; this population will be analyzed according to the randomized treatment arm. This is the primary population for efficacy evaluations
EQ-5D-5L population	Part 2 only: Treated patients who have provided baseline and ≥1 postbaseline assessment for EQ-5D-5L
EORTC-QLQ-C30 population	Part 2 only: Treated patients who have provided baseline and ≥1 postbaseline assessment for EORTC-QLQ-C30
Pharmacokinetic population	nal-IRI–treated patients with ≥1 post–study drug pharmacokinetic assessment

STUDY EVALUATIONS

Efficacy

- Tumor response will be evaluated at least every 8 weeks according to RECIST, version 1.1, based on computed tomographic or magnetic resonance imaging, until determination of progressive disease
- CA19-9 levels will be evaluated at least every 8 weeks during treatment and at the end-of-treatment visit. The maximum reduction (percentage change from baseline) in CA19-9 will be computed, including analyses by time period
- Follow-up for survival status will continue at least once every 2 months after treatment discontinuation until death, lost to follow-up, withdrawal of consent, or study closure. OS will be descriptively summarized for each treatment arm using Kaplan-Meier methodology

Safety and Tolerability

- Safety evaluations, including AE and hospitalization reporting, vital signs, complete blood count, and serum chemistry, will be collected throughout the study
- AEs will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 4.03, and will be summarized according to severity and relatedness to treatment

Quality of Life

- HRQoL will be assessed via the EORTC-QLQ-C30 and EQ-5D-5L instruments at baseline, Day 1 of each subsequent cycle, and at the end-of-treatment visit

Pharmacokinetics

- Arm 1: Plasma sampling for pharmacokinetic analyses of nal-IRI, SN-38, 5-FU, and oxaliplatin will occur on days 1, 3, 8, and 15 of Cycle 1 and at end-of-treatment during Parts 1 and 2
- Arm 2: Plasma sampling for pharmacokinetic analyses between nal-IRI, SN-38, and 5-FU, and ECG findings, will occur on days 1, 3, 15, and 17 of Cycle 1

Biomarkers

- Analyses will be performed to assess the associations between potential biomarkers (from plasma and archived tissue) and efficacy parameters
- Plasma sampling for biomarker analyses will be collected on Day 1 of each cycle and at the end-of-treatment visit
- Whole blood and serum sampling for biomarker analyses will be collected on Cycle 1 Day 1
- Examples of potential analyses include cytokine levels, growth factors, and enzyme levels

Management of Toxicities

- Guidelines for specific dose modifications are provided in **Table 3** and **Table 4**
  - Any patient who requires >2 dose reductions or a dose interruption of >2 weeks due to toxicity should be discontinued from study treatment
  - If oxaliplatin is not well tolerated in arm 1, the patient may discontinue oxaliplatin and continue to receive nal-IRI + 5-FU/LV

Table 3. Dose[a] Modification Guidelines for Arms 1 and 2

Grade 3/4 Toxicity[b]	nal-IRI	5-FU	Oxaliplatin (arm 1 only)
Neutropenia or febrile neutropenia	1st occurrence: Reduce to 60 mg/m^2 2nd occurrence: Reduce to 50 mg/m^2	1st occurrence: Reduce dose by 25% 2nd occurrence: Reduce dose by another 25%	*Grade ≥2 events:* 1st occurrence: None 2nd occurrence: Reduce to 52 mg/m^2
Other hematologic toxicity	1st occurrence: Reduce to 60 mg/m^2 2nd occurrence: Reduce to 50 mg/m^2	1st occurrence: Reduce dose by 25% 2nd occurrence: Reduce dose by another 25%	1st occurrence: Reduce to 52 mg/m^2 2nd occurrence: Maintenance of reduced dose at 52 mg/m^2
Hand-foot syndrome	1st occurrence: Reduce to 60 mg/m^2 2nd occurrence: Reduce to 50 mg/m^2	*Grade 2:* 1st occurrence: Reduce dose by 25% 2nd occurrence: Reduce dose by another 25% *Grade 3/4:* Discontinue therapy	None
Neuro-cerebellar toxicity	None	*Any grade:* Discontinue therapy	None
Cardiac toxicity	None	*Grade ≥2 events:* Discontinue therapy	None
Sensory neuropathy	None	None	Persistent grade 2, or grade 3 that recovers before next cycle: Reduce to 52 mg/m^2 Persistent grade 3, or grade 4: Discontinue therapy
Other non-hematologic toxicity[c]	1st occurrence: Reduce to 60 mg/m^2 2nd occurrence: Reduce to 50 mg/m^2	1st occurrence: Reduce dose by 25% 2nd occurrence: Reduce dose by another 25%	1st occurrence: None 2nd occurrence: Reduce to 52 mg/m^2

5-FU, 5-fluorouracil; nal-IRI, liposomal irinotecan.

[a] Following dose reduction, the patient's dose should remain reduced for the duration of the study.

[b] The above nal-IRI doses are expressed as the HCl trihydrate salt.

[c] Grade 3/4 toxicity unless otherwise noted.

[d] For grade 3/4 nausea and vomiting, patients should also receive optimized antiemetic therapy.

Table 4. Dose Modification Guidelines for Arm 3

Grade 3/4 Toxicity[a]	nab-Paclitaxel	Gemcitabine
Hematologic or nonhematologic toxicity	1st occurrence: Reduce to 100 mg/m^2 2nd occurrence: Reduce to 75 mg/m^2	1st occurrence: Reduce to 800 mg/m^2 2nd occurrence: Reduce to 600 mg/m^2
Mucositis or diarrhea	Withhold until improves to grade ≤1; reduce dose per row 1	
Cutaneous toxicity	**Grade ≥2 events:** Reduce dose per row 1; discontinue therapy if toxicity persists	
Peripheral neuropathy	Withhold until improves to grade ≤1; reduce dose per row 1	None
Febrile neutropenia	Withhold until fever resolves and ANC ≥1500/mm^3; reduce dose per row 1	
Neutropenia or thrombocytopenia	Detailed dose reduction and delay guidelines per administration day and severity of decreased ANC and/or platelet levels	

ANC, absolute neutrophil count.
Following dose reduction, the patient's dose should remain reduced for the duration of the study.
[a]Grade 3/4 toxicity unless otherwise noted.

SUMMARY

- **nal-IRI has demonstrated clinical activity and predictable and manageable toxicity in combination with 5-FU/LV in patients with mPAC following gemcitabine-based therapy,[7] leading to its FDA approval in this setting**
- **This phase 2 study will evaluate the safety and efficacy of nal-IRI + 5-FU/LV with or without oxaliplatin versus nab-paclitaxel + gemcitabine in patients with previously untreated mPAC**
- **The study may also provide important information on the impact of nal-IRI combination treatment on patient HRQoL and identify potential biomarkers of response**

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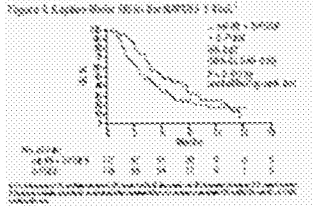
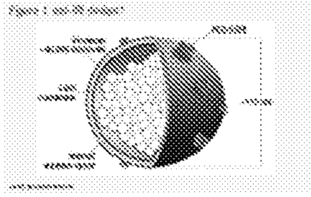
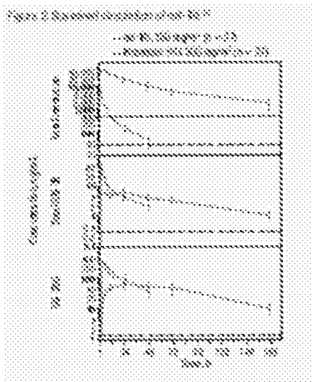
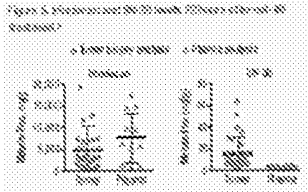
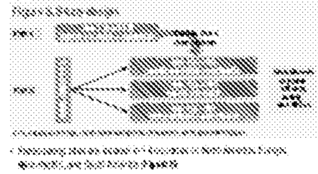
Nanoliposomal Irinotecan (nal-IRI)-Containing Regimens Versus nab-Paclitaxel Plus Gemcitabine as First-Line Therapy in Patients With Metastatic Pancreatic Adenocarcinoma: A Randomized, Open-Label Phase 2 Study











POSTER PRESENTED AT THE 18TH ESMO WORLD CONGRESS ON GASTROINTESTINAL CANCER; 29 JUNE–2 JULY, 2016; BARCELONA, SPAIN

NAPOLI-3: an open-label, randomized, phase III study of first-line liposomal irinotecan + 5-fluorouracil/leucovorin + oxaliplatin versus nab-paclitaxel + gemcitabine in patients with metastatic pancreatic ductal adenocarcinoma

Andrew DEAN,[1] Zev A. WAINBERG,[2] Tanios S. BEKAII-SAAB,[3] Richard HUBNER,[4] Teresa MACARULLA,[5] Andrew Scott PAULSON,[6] Eric Van CUTSEM,[7] Fiona MAXWELL,[8] Yan MOORE,[9] Haofei Tiffany WANG,[9] Bin ZHANG,[9] Eileen Mary O'REILLY[10]

[1]St John of God Subiaco Hospital, Subiaco, WA, Australia; [2]University of California Los Angeles Medical Center, Los Angeles, CA, USA; [3]Mayo Clinic, Scottsdale, AZ, USA; [4]Christie NHS Foundation Trust, Manchester, UK; [5]Vall d'Hebrón University Hospital and Vall d'Hebrón Institute of Oncology, Barcelona, Spain; [6]Texas Oncology/The US Oncology Network, Dallas, TX, USA; [7]University of Leuven, Leuven, Belgium; [8]Ipsen, Abingdon, UK; [9]Ipsen Bioscience, Boston, MA, USA; [10]Memorial Sloan Kettering Cancer Center, New York, NY, USA

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Author	Disclosure
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Richard Hubner	Bayer, Bristol Myers Squibb, BTG, Eisai, Ipsen, prIME Oncology, Roche
Teresa Macarulla	Agios, ASLAN Pharmaceuticals, AstraZeneca, Baxalta, Bayer, BeiGene, Biogen, Celgene, Eli Lilly, Genentech, Genzyme, H3 Biomedicine, Halozyme Therapeutics, Immunomedics, Incyte, Ipsen, Lilly, Merck, Merrimack Pharmaceuticals, Millennium Pharmaceuticals, Novartis, Novocure, OncoMed Pharmaceuticals, Pfizer, Pharmacyclics, QED Therapeutics, Roche, Sanofi, Servier, Shire, Tesaro
Andrew Scott Paulson	Actinium, Advanced Accelerator Applications, Amgen, Aptose Biosciences, AstraZeneca, Bristol Myers Squibb, Cardinal Health, Eisai, Exelixis, Hutchinson, Immunomedics, Incyte, Ipsen, Lilly, Seattle Genetics, Taiho Pharmaceutical
Eric Van Cutsem	Array BioPharma, AstraZeneca, Bayer, Biocartis, Bristol Myers Squibb, Boehringer Ingelheim, Celgene, Daiichi Sankyo, Halozyme, GlaxoSmithKline, Incyte, Ipsen, Lilly, Merck Sharp & Dohme, Merck KGaA, Novartis, Pierre Fabre, Roche, Servier, Sirtex, Taiho Pharmaceutical
Fiona Maxwell	Ipsen
Yan Moore	Ipsen
Haofei Tiffany Wang	Ipsen
Bin Zhang	Ipsen
Eileen Mary O'Reilly	Acta Biologica, AstraZeneca, Bayer, BioLineRx, Bristol Myers Squibb, CytomX, Genentech, Ipsen, Loxo Oncology, MabVax Therapeutics, Merck, Polaris Pharma, Roche, Silenseed, Targovax

Background

- Liposomal irinotecan (ONIVYDE® pegylated liposomal) is indicated with 5-fluorouracil/leucovorin (5-FU/LV), for the treatment of adults with metastatic pancreatic ductal adenocarcinoma (mPDAC) after disease progression following gemcitabine-based therapy[1]

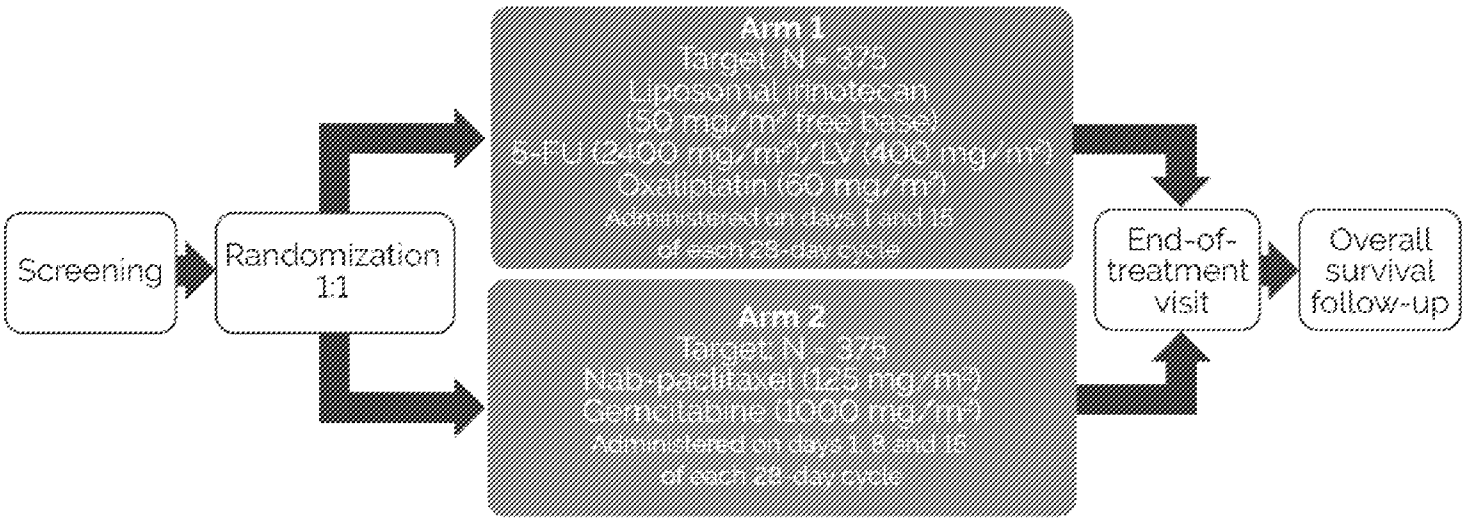
- A phase 1/2 study (NCT02551991) in previously untreated locally advanced/metastatic PDAC showed promising anti-tumor activity with liposomal irinotecan + 5-FU/LV + oxaliplatin (also known as NALIRIFOX) on days 1 and 15 of a 28-day cycle[2]

- NAPOLI-3 (NCT04083235) is an ongoing phase 3 study investigating the efficacy and safety of the NALIRIFOX regimen as first-line (1L) therapy in patients with mPDAC

1. Ipsen Biopharmaceuticals, Inc. Prescribing Information, ONIVYDE (irinotecan liposome injection). US Food and Drug Administration. 2017; 2. Wainberg *et al. Ann Oncol* 2019;30 Suppl 4: 5O-005
FDA, Food and Drug Administration

NAPOLI-3 study design

- An open-label, randomized, multicenter, phase 3 study comparing 1L NALIRIFOX (arm 1) with 1L nab-paclitaxel + gemcitabine (arm 2) in adult patients with mPDAC



1L, first-line; 5-FU, 5-fluorouracil; LV, leucovorin; mPDAC, metastatic pancreatic ductal adenocarcinoma

Study objectives

Primary objective	
Overall survival	Time from the date of randomization to the date of death by any cause
Secondary objectives	
Progression-free survival	Time from randomization to the first documented objective disease progression as per RECIST v1.1 or death by any cause, whichever comes first
Objective response rate	The proportion of patients with a BOR of complete or partial response as per RECIST v1.1; BOR is defined as the best response from treatment initiation to disease progression
Safety	Severity of AEs and SAEs graded according to NCI-CTCAE v5.0, AEs leading to treatment discontinuation and/or death, AEs related to study treatment, laboratory abnormalities, incidence of patients experiencing dose modifications (including infusion interruptions, dose omissions and dose delays) and/or premature treatment discontinuation (including reason for discontinuation)

AE, adverse event; BOR, best overall response; NCI-CTCAE v5.0, National Cancer Institute – Common Terminology Criteria for Adverse Events version 5.0; RECIST v1.1, Response Evaluation Criteria in Solid Tumors version 1.1; SAE, serious adverse event

Study population

- Eligible patients are those aged ≥ 18 years with:
  - histologically or cytologically confirmed adenocarcinoma of the pancreas that has not been previously treated in the metastatic setting
  - ≥ 1 metastatic tumor measurable with CT or MRI
  - an ECOG PS score of 0 or 1
  - adequate hematologic, hepatic and renal functions
  - an ECG without clinically significant findings

- Patients will be stratified according to ECOG PS (0 or 1), region (North America, East Asia, other) and liver metastases (yes or no)

CT, computed tomography; ECG, electrocardiogram; ECOG PS, Eastern Cooperative Oncology Group Performance Status; MRI, magnetic resonance imaging

Data collection and analyses

- All patients will receive 28-day cycles of treatment until disease progression, treatment toxicity or withdrawal from the study
  - Thirty days after permanent discontinuation of study treatment, patients will undergo a follow-up assessment and will be observed for survival status every 2 months until death or study end (when all patients have died or withdrawn consent, or are lost to follow-up)

- Tumor assessments will be performed every 8 weeks using RECIST v1.1 criteria

- OS will be assessed using Kaplan–Meier methodology, and differences between treatment arms will be assessed using a stratified log-rank test
  - PFS and ORR (secondary endpoints) will only be compared between treatment arms if NALIRIFOX shows superiority in OS compared with nab-paclitaxel + gemcitabine

ORR, objective response rate; OS, overall survival; PFS, progression-free survival; RECIST v1.1, Response Evaluation Criteria in Solid Tumours version 1.1

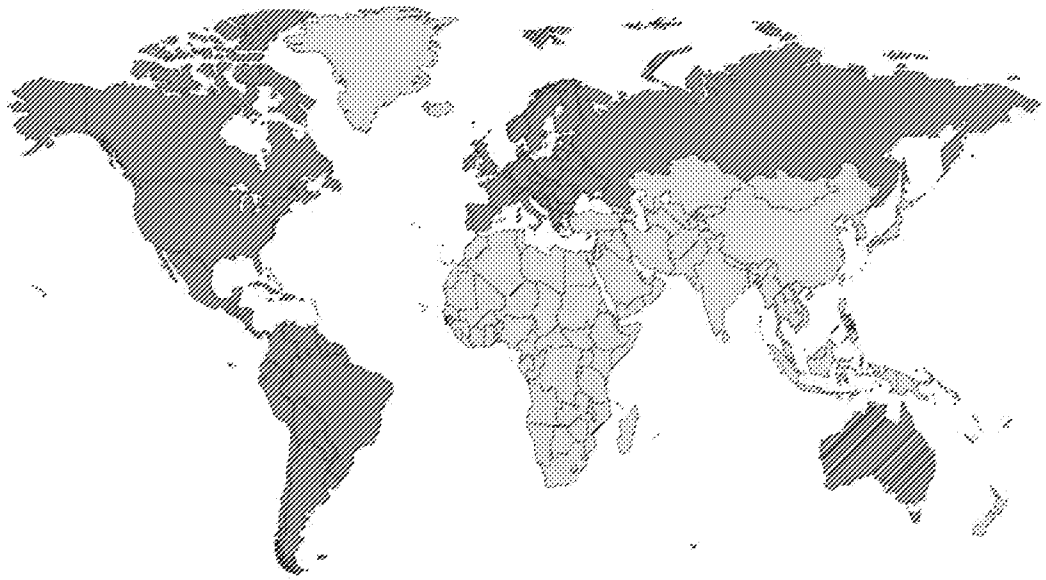
Dose modifications

- Treatment dose may be reduced owing to toxicity, and re-escalation will generally not be permitted
- To allow time for recovery from toxicity, the treatment dose may also be delayed
- At the investigator's discretion, oxaliplatin may be discontinued if not well tolerated, and patients can continue treatment with liposomal irinotecan + 5-FU/LV
  - Discontinuation of any other treatment will result in discontinuation from the study

5-FU/LV, 5-fluorouracil/leucovorin

Recruitment update

- Site activation began in December 2019
- Recruitment is ongoing or planned in:
  - North America
  - South America
  - Europe
  - Australia



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Thank you for listening!

FIRST-LINE (1L) LIPOSOMAL IRINOTECAN + 5 FLUOROURACIL/LEUCOVORIN (5-FU/LV) + OXALIPLATIN (OX) IN PATIENTS WITH LOCALLY ADVANCED OR METASTATIC PANCREATIC DUCTAL ADENOCARCINOMA (MPDAC): EXPLORATORY SUBGROUP ANALYSES OF SURVIVAL BY CHANGES IN CA 19-9 LEVELS

Date
17 Sep 2020

Session
E-Poster Display

Presenters
Andrew Dean

Citation
Annals of Oncology (2020) 31 (suppl_4): S881-S897. 10.1016/annonc/annonc285

Authors
A. Dean[1], T. Bekaii-Saab[2], P.M. Boland[3], F. Dayyani[4], E. Macarulla[5], K. Mody[6], B Belanger[7], F. Maxwell[8], Y. Moore[7], T. Wang[7], B. Zhang[9], Z.A. Wainberg[10]

Author affiliations
More

Resources

Abstract 1529P

Background

Liposomal irinotecan + 5-FU/LV is approved for treating patients with mPDAC following progression with gemcitabine-based therapy. Liposomal irinotecan + 5-FU/LV + OX (NALIRIFOX) is being investigated 1L in a phase I/II study of patients with locally advanced/mPDAC. Serum CA 19-9 levels are typically elevated in such patients and post-treatment decreases are associated with prolonged survival. We report exploratory survival analyses from the phase I/II study for subgroups defined by post-treatment changes in CA 19-9 levels.

Methods

Following dose exploration, the regimen of liposomal irinotecan 50 mg/m^2 (free base), OX 60 mg/m^2, 5-FU 2400 mg/m^2 and LV 400 mg/m^2, on days 1 and 15 of 28-day cycles, was selected for dose expansion and 25 more patients were enrolled. In total, 32 patients received the selected (maximum tolerable) dose. Tumors (RECIST v1.1) and serum CA 19-9 were assessed at screening, every 8 weeks and at end of treatment (EoT); tumor assessments continued after EoT. Progression-free and overall survival (PFS and OS) were compared across subgroups defined by best change within the first 16 weeks (data cut-off 26 Feb 2020).

Results

In total, 30/32 patients had a baseline CA 19-9 measurement (median 315.5 U/mL, range 2–127115) of whom 22 had measurements by week 16 (analysis set) with median best change −49.4% (range −100%, +376%). Survival data (Table) were similar for patients with above-normal (≥ 37 U/mL) baseline CA 19-9 levels (n = 17; median best change −35.9% [range −100%, +278%]). Table: 1529P

PFS	PP 30/60 N = 32	All n = 22	Analysis set, best CA 19-9 change
≥ 20% decrease	≥ 50% decrease		
Yes n = 14	No n = 8	Yes n = 11	No n = 11
Progressed/died,[a] n (%)	17 (53.1)	12 (54.5)	6 (42.9)	6 (75.0)	4 (36.4)	8 (72.7)

PFS	PP 50/60 N = 32	All n = 22	Analysis set, best CA 19-9 change			
≥ 20% decrease	≥ 50% decrease					
Yes n = 14	No n = 8	Yes n = 11	No n = 11			
HR (95% CI)	-	-	0.13 (0.04, 0.50)	0.33 (0.09, 1.12)		
OS						
Died, n (%)	20 (62.5)	14 (63.6)	7 (50.0)	7 (87.5)	6 (54.6)	8 (72.7)
Median, mo (95% CI)	12.6 (8.74, 18.69)	12.7 (8.74, 22.54)	22.5 (12.39, NE)	8.2 (2.50, 18.69)	22.5 (11.60, 22.54)	9.2 (4.83, NE)
HR (95% CI)	-		0.24 (0.08, 0.75)	0.55 (0.19, 1.60)		

[a] Patients who progressed/died after new therapy or >16 wks after last non-PD assessment were censored.

Conclusions

1L NALIRIFOX reduced CA 19-9 levels in patients with locally advanced/mPDAC. Median OS and PFS were numerically higher in patients with a ≥20% decrease within the first 16 weeks. CA 19-9 is a potential biomarker of post-NALIRIFOX outcomes.

Clinical trial identification

NCT02551991.

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Legal entity responsible for the study

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Health. In The Health Effects of Cannabis and Cannabinoids: The Current State of Evidence and Recommendations for Research, National Academies Press (US) 2017 by the National Academy of Sciences. All rights reserved.: Washington (DC).

220 | Exploration of current dietetic practices for patients with gynaecological cancers undergoing radiotherapy in Australia: A cross sectional survey

Emilie Croisier[1,2], Kelly D'cunha[1], Teresa Brown[1,2], Judy Bauer[1]

[1]*School of Human Movement Nutrition Sciences, University of Queensland, Brisbane, Queensland, Australia*

[2]*Nutrition and Dietetics, Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia*

Radiotherapy for gynaecological cancers often causes Gastro Intestinal (GI) toxicities, presenting as diarrhoea. However, there is an absence of clear evidence-based guidelines to inform clinical practice for GI symptom management in this patient cohort. This study aims to provide an overview of current opinions and clinical practice of dietitians treating this patient cohort and to examine current models of nutrition care in Australian cancer clinics. A mixed-method, exploratory cross-sectional survey using semi-structured interviews was used. The survey utilised purposive sampling and comprised: demographic characteristics, referral protocol and follow-up pathways, management strategies and interventions prescribed, and attitudes and confidence in service provided. Descriptive analysis was performed on quantitative data and thematic analysis was performed on qualitative data once data saturation was achieved. In total, 17 dietitians participated in the study, from seven of the eight states and territories of Australia. Most centres did not have an automatic referral pathway (94%) or post-treatment pathway (88%). The results demonstrate heterogeneity in dietitians' opinions and prescription of dietary interventions for symptom management secondary to radiotherapy. The majority of respondents (71%) had either prescribed and/or seen patients who had been prescribed a low fibre diet, however contextualized this as a 'trial and error' or 'last resort' strategy. Dietitians acknowledge there is a knowledge gap in effective symptom management and that the current dietetic services provided may not be adequately meeting the needs of this patient cohort, and is not standardized within Australia (82%). There is no clear consensus on best practice for dietary management of GI symptoms in patients undergoing radiotherapy for gynaecological cancers. This variation in practice warrants more robust studies to investigate the efficacy of dietary interventions in symptom management to inform the development of a suitable model of nutrition care, in addition to collaborating with patients to effectively determine perceived needs.

221 | Metabolic tumour volume (MTV) on 18-fluorodeoxyglucose positron emission tomography (FDGPET) as a prognostic marker of survival in patients with metastatic neuroendocrine neoplasms (mNENs) receiving ^{177}Lutetium-DOTA-octreotate (Lutate)

Madhawa De Silva[1], David Chan[1], Elizabeth Bernard[2], Alice Connor[1], Sophie Mascall[1], Dale Bailey[2], Paul Roach[2], Nick Pavlakis[1], Geoffrey Schembri[2]

[1]*Department of Medical Oncology, Royal North Shore Hospital, St Leonards, NSW, Australia*

[2]*Department of Nuclear Medicine, Royal North Shore Hospital, St Leonards, NSW, Australia*

Introduction/Aims: Prior work has demonstrated quantitative analysis of FDGPETs in mNENs is feasible. FDG avidity correlates with high-grade disease, and high MTV and total lesion glycolysis (TLG) portend poor prognosis. We aimed to investigate MTV and TLG on baseline FDGPET as prognostic markers for survival in patients with mNENs receiving Lutate.

Methods: A retrospective review of mNENs undergoing baseline FDGPET and Lutate at Royal North Shore Hospital (December 2010 to November 2019). Images were analysed with automated segmentation (SUV cut-off 4.0) followed by contour verification by a nuclear medicine physician and manual segmentation where required. Variables collected included MTV and TLG (dichotomised by median into high vs low), and SUV_{max}/SUV_{mean}. Primary outcome was overall survival (OS) by MTV (high vs low). Secondary outcome was progression-free survival (PFS) by MTV (high vs low). Survival data were compared using the log-rank test.

Results: One hundred and five patients were included (median age 64 years, 50% male). Primary sites of mNENs were small bowel (44%), pancreas (40%), and lung (8%). Median MTV was 3.8 ml (IQR 0 - 58.7) and median TLG was 19.3 (IQR 0 - 310.3). Overall median OS was 72 months; OS was not different based on MTV-high vs MTV-low (47.4 months vs not reached, HR 0.43, CI 0.18-1.04, $P = .0594$). Overall median PFS was 30.4 months; PFS was different based on MTV-high vs MTV-low (21.6 months vs 45.7 months, HR 0.35, CI 0.19-0.64, $P = .007$).

Conclusions: Low MTV on baseline FDGPET was associated with a statistically significant PFS benefit in mNEN patients receiving Lutate. Low MTV also showed a trend toward OS benefit, though this was not statistically significant. Quantitative analysis of FDGPET in mNENs is feasible, and may assist in treatment decisions, in particular determining the urgency.

222 | First-line liposomal irinotecan + 5-fluorouracil/leucovorin + oxaliplatin in patients with pancreatic ductal adenocarcinoma: results from a phase 1/2 study

Andrew Dean[1], Tanios Bekaii-Saab[2], Patrick M Boland[3], Farshid Dayyani[4], Teresa Macarulla[5], Kabir Mody[6], Bruce Belanger[7],

Fiona Maxwell[8], Yan Moore[7], Arunthathi Thiagalingam[7], Tiffany Wang[7], Bin Zhang[7], Zev A Wainberg[9]

[1]St John of God Subiaco, Subiaco, WA, Australia
[2]Mayo Clinic Cancer Center (MCCC), Scottsdale, Scottsdale, AZ, USA
[3]Rutgers Cancer Institute of New Jersey, New Brunswick, NJ, USA
[4]University of California, Irvine, CA, USA
[5]Vall d'Hebron University Hospital, Barcelona, Spain
[6]Mayo Clinic, Jacksonville, FL, USA
[7]Ipsen Bioscience, Inc., Cambridge, MA, USA
[8]Ipsen, Abingdon, UK
[9]University of California, Los Angeles, Medical Center, Los Angeles, CA, USA

Background: Liposomal irinotecan + 5-fluorouracil/leucovorin (5-FU/LV) is approved for adults with metastatic pancreatic ductal adenocarcinoma following progression with gemcitabine-based therapy. We report results from an open-label phase 1/2 study (NCT02551991) of adults with untreated, unresectable, locally advanced/metastatic-PDAC receiving liposomal irinotecan + 5-FU/LV + oxaliplatin (NALIRIFOX).

Methods: Eligible patients were adults with ECOG performance status (PS) ≤ 1 and adequate organ function who received NALIRIFOX (liposomal irinotecan 50 mg/m^2 (free-base), 5-FU 2400 mg/m^2, LV 400 mg/m^2, oxaliplatin 60 mg/m^2) on days 1 and 15 of each 28-day cycle. The primary endpoint was safety; secondary endpoints included progression-free-survival (PFS), overall survival (OS), best overall response, overall response rate (ORR), 16-week disease control rate (DCR_{16}) and duration of response (DoR). RECIST v1.1 was assessed at 8-week screenings and treatment end.

Results: Thirty-two patients were included (median [range] age 58.0 [39–76] years; 43.8% men; 87.5% metastatic disease; 56.3% ECOG PS 1). Twenty-two patients experienced grade ≥3 treatment-emergent-adverse-events (TEAEs): neutropenia (31.3%), febrile neutropenia (12.5%), hypokalaemia (12.5%), diarrhea (9.4%), nausea (9.4%) and decreased neutrophil count (9.4%). 17 patients had serious TEAEs: nausea (9.4%) and febrile neutropenia (9.4%). TEAEs led to 3 deaths (none treatment-related), dose-adjustment in 26 and discontinuation in eight patients. Median (95% CI) PFS and OS were 9.2 (7.69–11.96) months and 12.6 (8.74–18.69) months, respectively. One patient, with locally advanced disease, had complete response, 10 partial response, and 15 stable disease. ORR (95% CI) was 34.4 (18.6–53.2)%, DCR_{16} was 71.9 (53.3–86.3)% and median (95% CI) DoR was 9.4 (3.52, NE) months.

Conclusions: First-line NALIRIFOX raised no new safety signals in patients with locally advanced/metastatic PDAC; anti-tumour activity was promising. The randomized phase-3 NAPOLI-3 study (NCT04083235) will compare NALIRIFOX with gemcitabine + nab-paclitaxel.

This study is funded by Ipsen.

This abstract was originally presented virtually at the 2020 ESMO World Congress on Gastrointestinal Cancer.

223 | The utility of screening tools for the initial screening for chemotherapy induced peripheral neuropathy

Pavandeep Dhaliwal[1], Bogda Koczwara[1], Timothy Tune[1], Vanessa Chong[1], Leila Mohammadi[1]

[1]Flinders University, Bedford Park, SA, Australia

Aims: Chemotherapy induced peripheral neuropathy (CIPN) is a disabling condition associated with a poor quality of life. Given that assessment is lengthy and not routinely done for all patients, screening tools could provide an efficient and cost-effective method for early detection of CIPN. The aim of this review was to examine the utility of screening tools for CIPN and to assess their psychometric properties.

Methods: A search was conducted on Medline, ProQuest, Scopus and Cochrane for papers written in the last 15 years, examining screening tools for CIPN in adult patients undergoing neurotoxic chemotherapy. Psychometric properties (discrimination, sensitivity, specificity and reliability) were the outcomes of interest. Randomized controlled trials, case-series and cross-sectional studies written in the English language were included. Two reviewers screened studies based on title and abstract before full text screening and data extraction. Any discrepancies were resolved by consensus.

Results: After removing duplicates, 2649 studies were identified with 6 cross sectional studies meeting the eligibility criteria. Adult patients undergoing neurotoxic chemotherapies with colorectal cancer, testicular cancer, multiple myeloma and others were included. Screening tools included: sEMG, mTNS, DN 4, SCIN, ICPNQ, peripheral sensory neuropathy item from NCI-CTCAE v4.03, symptom severity item from numbness and tingling section of PRO-CTCAE and a pilot screening tool. Good discrimination was reported with SCIN, PSRI-NCI-CTCAE, SSINT-PRO-CTCAE, mTNS and ICPNQ. SCIN was also found to have good reliability. Studies found high sensitivity in DN4, mTNS and sEMG with DN4 showing high specificity as well. Moderate to high correlation between screening tools (PSRI - NCI-CTCAE, SSINT-PRO-CTCAE, PST) and assessment tools (TNSr, FACT-GOG/Ntx) was reported.

Conclusion: All screening tools have shown good psychometric properties. Further research regarding their acceptability in the clinical context is required. Comparison studies to choose the ideal screening tool and discussion regarding severity cut-off scores for further evaluation are also needed.

224 | Changes in body composition and sarcopenia status with four weeks of HIIT in breast and prostate and colorectal cancer survivors

Morgan J Farley[1], Tina L Skinner[1], Mia A Schaumberg[2], David G Jenkins[2]

[1]University of Queensland, Brisbane, Qld, Australia
[2]The University of Sunshine Coast, Sippy Downs, Qld, Australia

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observed for the low function compared to high function group (31% vs 14%, respectively; $P < .01$).

Conclusion: Consistent with benefits reported in clinical trials, an exercise program embedded in routine care was effective for improving fatigue and physical function post-HSCT. These findings highlight, irrespective of baseline physical function, exercise is possible and beneficial. However, people with higher function likely require a higher exercise stimulus to obtain the same benefit as those with lower function. Tailored, progressive aerobic and resistance training programs may reduce acute and long-term treatment side effects and should be offered to HSCT recipients to support recovery.

406 | Interventions for cancer-related fatigue: A review of systematic reviews

Jasmine Yee[1,2], Corrinne Renton[3,4], Stephanie Konings[4], Stefanie Baios[4,5], Renée Bultijnck[1,6,7], Haryana M Dhillon[1,4], Meera R Agar[8], Joanne M Shaw[4]

[1]*CeMPED, School of Psychology, The University of Sydney, Campderdown, NSW, Australia*

[2]*Sydney School of Health Sciences, The University of Sydney, Lidcombe, NSW, Australia*

[3]*CeMPED, Concord Clinical School, The University of Sydney, Concord, NSW, Australia*

[4]*Psycho-Oncology Cooperative Research Group (PoCoG), School of Psychology, The University of Sydney, Campderdown, NSW, Australia*

[5]*Department of Psychology, Queen's University, Kingston, ON, Canada*

[6]*Department of Human Structure and Repair, Ghent University, Ghent, East Flanders, Belgium*

[7]*Research Foundation-Flanders (FWO), Brussels, Belgium*

[8]*Cancer Symptoms Trial Group, University of Technology Sydney, Ultimo, NSW, Australia*

Introduction: Cancer-related fatigue (CRF) is the most common symptom associated with cancer and significantly impacts daily activities and quality of life. Mechanisms of CRF are multi-factorial, influenced by biological and psychosocial factors. A variety of interventions to address CRF have been explored, including exercise, psychosocial and pharmacological treatments. Existing systematic reviews of interventions, typically focused on one intervention type, report inconsistent findings. We aimed to compile evidence from systematic reviews to describe all interventions addressing CRF.

Method: We searched Medline, EMBASE, CINAHL, Cochrane and PsycInfo to June 2019. Systematic reviews of interventions where CRF was a specified outcome of the review were included. All titles and abstracts were independently assessed for inclusion. Relevant data related to study characteristics and intervention content, timelines and delivery mode were extracted. Risk of bias was evaluated using AMSTAR 2.

Results: We identified 35 reviews that met criteria, these predominantly included: adults (80%) of both sexes (63%) with mixed cancer types (63%) and stages (75%). Eight (23%) reviews focused on interventions during active treatment, four (11%) following treatment and 20 (57%) both phases. In most reviews (77%), exercise was part of the intervention: 15 (43%) exercise only and 6 (17%) combined exercise + psychosocial. Four (11%) reported pharmacological and three (9%) psychosocial only interventions. Assessment of fatigue was highly variable, measured using 59 different tools. Review quality varied, with 14% of high and 6% moderate quality.

Conclusion: Impact of exercise alone or in combination with other treatments has been most explored for addressing CRF. Reviews include mixed cancer types and stages, with a wide variety of tools to measure fatigue. This variability may distort intervention effects and contribute to inconsistent findings. Further research is needed to determine the most effective intervention for an individual with consideration of medical, treatment and psychosocial factors.

407 | NAPOLI-3: An open-label, randomized, phase III study of first-line liposomal irinotecan + 5-fluorouracil/leucovorin + oxaliplatin versus nab-paclitaxel + gemcitabine in patients with metastatic pancreatic ductal adenocarcinoma

Andrew Dean[1], Zev A Wainberg[2], Tanios S Bekaii-Saab[3], Richard Hubner[4], Teresa Macarulla[5], Andrew Scott Paulson[6], Eric V Cutsem[7], Fiona Maxwell[8], Yan Moore[9], Haofei Tiffany Wang[9], Bin Zhang[9], Eileen O'Reilly[10]

[1]*St John of God Subiaco, Subiaco, WA, Australia*

[2]*University of California, Los Angeles, Medical Center,, Los Angeles, CA, USA*

[3]*Mayo Clinic Cancer Center (MCCC), Scottsdale, Scottsdale, AZ, USA*

[4]*Christie Hospital NHS Foundation Trust, Manchester, UK*

[5]*Vall d'Hebron University Hospital, Barcelona, Spain*

[6]*Texas Oncology/The US Oncology Network,, Dallas, TX, USA*

[7]*University of Leuven, Leuven, Belgium*

[8]*Ipsen, Abingdon, UK*

[9]*Ipsen Bioscience, Inc., Cambridge, MA, USA*

[10]*Memorial Sloan Kettering Cancer Center, New York, NY, USA*

Background: Liposomal irinotecan administered with 5-fluorouracil/leucovorin (5-FU/LV) is FDA-approved in USA for metastatic pancreatic ductal adenocarcinoma (mPDAC) following progression with gemcitabine-based therapy. A phase 1/2 study in previously untreated locally advanced/metastatic PDAC showed promising anti-tumour activity with liposomal irinotecan 50 mg/m free-base + 5-FU 2400 mg/m + LV 400 mg/m + oxaliplatin (OX) 60 mg/m on days 1 and 15 of a 28-day cycle (Wainberg et al. Ann Oncol 2019;30 Suppl 4: SO-005). We present the phase-3 NAPOLI-3 study

design, investigating the efficacy and safety of this regimen as first-line therapy in patients with mPDAC.

Methods: NAPOLI-3 (NCT04083235) is a phase-3, open-label, randomized, global study in adults with histologically/cytologically confirmed pancreatic adenocarcinoma, previously untreated in the metastatic setting. Patients must have ≥1 metastatic tumour measurable with computed tomography/magnetic resonance imaging and an Eastern Cooperative Oncology Group performance status score of 0–1. Site activation began in Dec-2019 and enrolment is ongoing. Random allocation (1:1) of 750 patients is planned to liposomal irinotecan + 5-FU/LV + OX (same regimen as phase 1/2 study) or nab-paclitaxel 125 mg/m + gemcitabine 1000 mg/m on days 1, 8 and 15 in a 28-day cycle. The primary endpoint is overall survival. Secondary endpoints (progression-free survival and overall response rate assessed with Response Evaluation Criteria in Solid Tumors v1.1) will be compared if primary endpoint shows superiority for liposomal irinotecan + 5-FU/LV + OX over nab-paclitaxel + gemcitabine. Safety assessments include adverse-event monitoring. Patients will continue treatment until disease progression, unacceptable toxicity or study withdrawal, and will then be followed for survival every 2-months until death or study-end (when all patients have died, withdrawn consent or lost to follow-up).

This study is funded by Ipsen.

408 | The interplay between inflammatory markers and body composition with 6 months of HIIT in breast, colorectal and prostate cancer survivors

Morgan J Farley[1], Tina L Skinner[1], Mia A Schaumberg[2], David G Jenkins[2]

[1]*University of Queensland, Brisbane, QLD, Australia*

[2]*The University of Sunshine Coast, Sippy Downs, QLD, Australia*

Breast, colorectal and prostate cancer have among some of the highest cancer survival rates in Australia, yet treatments for these cancers have been associated with increased fat mass, reduced skeletal muscle mass and increased systemic inflammation. Collectively, these treatment-related changes not only reduce quality of life but also increases the risk of both disease recurrence and comorbidities. Exercise following cancer diagnosis and treatment can reduce the risk of disease recurrence. However, the physiological mechanisms that explain this relationship are not clear. One potential mechanism relates to the acute release of myokines in response to exercise. The aim of this randomised control trial is to compare changes in the acute response of myokines (IL-15, IL-10, TNF-α and IL-6) to a single bout of high intensity interval training (HIIT) (4 × 4 min) session and changes in cardiorespiratory fitness and body composition in breast, prostate and colorectal cancer survivors following before and after six months of HIIT. At baseline, participants (n = 50 for each group) will complete a $\dot{V}O_2$peak test and have their body composition assessed (using dual-energy X-ray absorptiometry). Before and after a single HIIT session, venous blood will be sampled for the analysis of IL-6, IL-10, IL-15 and TNF-α. Participants will then complete three HIIT sessions per week for six months before all testing is repeated. It is hypothesised that the acute changes in the myokine response to a single HIIT session will be greater following training but that there will be no change in baseline (resting) levels. Training-induced changes in the myokine response to HIIT will be related to changes in body composition and cardiovascular fitness that occur over the six months.

409 | Evaluation of the YMCA Cancer Survivors' Program: A study protocol

Eliza Keating[1], Tina Skinner[1], Phillip Lynem[2], Tsiamis Michael [2], Sjaan Gomersall[1, 3]

[1]*School of Human Movement and Nutrition Sciences, The University of Queensland, St Lucia, Queensland, Australia*

[2]*YMCA of Brisbane, Brisbane, Queensland, Australia*

[3]*School of Health and Rehabilitation Sciences, The University of Queensland, St Lucia, Queensland, Australia*

Provision of high-quality supportive care services for cancer survivors is becoming increasingly challenging as available resources fail to match the growth of this population. Community-based exercise programs are a critical component of oncological treatment for the longer-term health and wellbeing of cancer survivors. The YMCA Cancer Survivors Program is a free exercise program offered at several YMCA's across Brisbane, Australia. The program offers two, 1-hour supervised aerobic- and resistance-based sessions per week for 12 weeks. The YMCA Cancer Survivors Program has been running since 2016 and to date, over 440 participants have completed the program. The aim of this study is to conduct a process and effectiveness evaluation of the YMCA Cancer Survivors Program. Cancer survivors participating in the YMCA Cancer Survivors Program during 2021 and 2022 will be invited to participate in this pragmatic trial. The process evaluation will involve measures of safety, attendance, adherence and uptake. Evaluation of the effectiveness of the program will include measures of physical activity, quality of life, fatigue, exercise capacity, strength, sleep, cognition, emotional distress, flexibility, balance and body composition of participants measured before and after the 12-week exercise program. The findings from the evaluation will be used to iteratively refine and develop the program, with plans for broader dissemination and uptake in other YMCA sites.

410 | ZENERGISE trial and COVID-19 tribulation: A snapshot of progress

Ashanya Malalasekera[2, 1], Jane Turner[2], Sue Butler[2], Eileen Hozack[2], Kim Kerin-Ayres[2], Janette Vardy[2, 1]

[1]*University of Sydney, Sydney, NSW*

[2]*Concord Cancer Centre, Concord West, NSW, Australia*

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A phase 1/2, open-label dose-escalation study of liposomal irinotecan (nal-IRI) plus 5- fluorouracil/leucovorin (5-FU/LV) and oxaliplatin (OX) in patients with previously untreated metastatic pancreatic cancer (mPAC).

Andrew Peter Dean, Zev A. Wainberg, Ramesh K. Ramanathan, Patrick McKay Boland, Kabir Mody, Bin Zhang, Bruce Belanger, Floris A de Jong, Stephan Braun

St. John of God Hospital, Subiaco, Australia; Department of Medicine, University of California Los Angeles School of Medicine, Los Angeles, CA; Mayo Clinic, Phoenix, AZ; Roswell Park Cancer Institute, Buffalo, NY; Mayo Clinic, Jacksonville, FL; Ipsen Bioscience, Paris, France; Ipsen Bioscience, Inc., Cambridge, MA; Shire International GmbH, Zug, Switzerland; Ipsen Biopharmaceuticals, Basking Ridge, NJ

Show Less

Abstract Disclosures

Abstract

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Background: nal-IRI+5-FU/LV is effective for patients with mPAC after disease progression following gemcitabine-based therapy. The current study (NCT02551991) is a phase 1/2, open-label trial to assess the safety, tolerability, and dose-limiting toxicities (DLT) of nal-IRI+5-FU/LV+OX (NAPOX) for the first-line treatment of patients with mPAC and to determine Phase 3 dosing. **Methods:** NAPOX is being evaluated in patients ≥18 yrs with previously untreated mPAC, with an ECOG performance status ≤1 and adequate organ function. Three of 4 dose-escalation cohorts of NAPOX, dosed on day 1 and 15, have been initiated. Safety and tolerability are the primary endpoints of this study, with assessment of exploratory efficacy signals. **Results:** As of 10 Nov 2017, 24 patients (Cohort A: n = 7; Cohort B: n = 7; Cohort C: n = 10) have received ≥1 dose of NAPOX (median age: 66.0 yrs, range: 44–78 yrs). Five patients reported ≥1 DLT (Cohort A: n = 2/7; Cohort B: n = 1/7; Cohort C: n = 2/10). The most frequent treatment-emergent adverse events (TEAEs) were gastrointestinal (GI) disorders (Cohort A: 71%; Cohort B: 71%; Cohort C: 60%). Grade 3 or 4 TEAEs were GI disorders (Cohort A: 43%; Cohort B: 14%; Cohort C: 50%) and neutropenia (Cohort A: 43%; Cohort B: 29%; Cohort C: 40%). The best overall response was partial response (PR) in 6/24 patients (Cohort B: n = 3/7; Cohort C: n = 3/10). In Cohort B (the lowest and most tolerable cohort), n = 5/7 patients reached disease control (PR or stable disease > 16 weeks), with n = 4/7 patients were treated for ≥24 weeks. **Conclusions:** Initial analysis suggests a well-tolerated dose and promising antitumor clinical activity of NAPOX. Dose escalation and expansion is ongoing. Clinical trial information: NCT02551991.

	Dose			Current Patients		Grade 3/4 TEAEs			
Cohort	nal-IRI (mg/m²)	5-FU/LV (mg/m²)	Oxaliplatin (mg/m²)	Dosed (n)	Ongoing (n)	Neutropenia (n)	Diarrhea (n)	Vomiting (n)	Nausea (n)
A	80	2,400 / 400	60	7	0	3	3	1	0
B	60	2,400 / 400	60	7	2	2	1	0	0
C	60	2,400 / 400	85	10	3	4	3	3	2
D*	65	2,400 / 400	70	-	-	-	-	-	-

* Cohort not yet initiated

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posters

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Nanoliposomal irinotecan (nal-IRI)-containing regimens versus nab-paclitaxel plus gemcitabine as first-line therapy in patients with metastatic pancreatic adenocarcinoma (mPAC): a randomized, open-label phase 2 study

Dean Andrew[1], Ramanathan Ramesh[2], Belota Brooke[3], Belanger Bruce[4], Adib Deyaa[5], Bayever Eliel[6], Chen Li-Tzong[7]
[1]*St John of God Hospital Subiaco, Subiaco, Australia*
[2]*Mayo Clinic, Scottsdale, Arizona, USA*
[3]*Merrimack Pharmaceuticals, Cambridge, Massachusetts, USA*
[4]*Merrimack Pharmaceuticals, Inc., Cambridge, Massachusetts, USA*
[5]*Baxalta, Cambridge, Massachusetts, USA*
[6]*Merrimack, Cambridge, Massachusetts, USA*
[7]*National Health Research Institutes (NHRI) – National Institute of Cancer Research, Tainan, Taiwan*

Introduction: Patients with mPAC have a poor prognosis, with median survival of <1 year. Current standard first-line treatment options include: 5-fluorouracil (5-FU)/leucovorin (LV) + irinotecan + oxaliplatin (FOLFIRINOX) and nab-paclitaxel + gemcitabine. nal-IRI (MM-398) is a nanoliposomal formulation of irinotecan. In the randomized phase 3 NAPOLI-1 study, nal-IRI + 5-FU/LV significantly improved overall survival (OS) compared with 5-FU/LV (6.1 vs 4.2 months; $P = 0.012$), and was generally well tolerated in patients with mPAC previously treated with gemcitabine-based therapy (Wang-Gillam et al, *Lancet.* 2016). Based on these results, nal-IRI + 5-FU/LV has been incorporated into ESMO Clinical Practice Guidelines as recommended second-line therapy for gemcitabine-refractory mPAC (Ducreux et al., *Ann Oncol.* 2015). The current study (ClinicalTrials.gov, NCT02551991) was designed to determine the preliminary safety and efficacy of nal-IRI + 5-FU/LV with or without oxaliplatin, compared with nab-paclitaxel + gemcitabine, in previously untreated patients with mPAC.

Methods: This open-label, phase 2 comparative study will be conducted in 2 parts: A safety run-in (part 1), and a randomized, open-label study (part 2). Key eligibility criteria include: age ≥18 years; pathologically confirmed pancreatic cancer; unresectable locally advanced or metastatic disease (part 1) or metastatic disease (part 2); no prior treatment for metastatic disease; Eastern Cooperative Oncology Group performance status 0-1; no known metastases to the central nervous system; and adequate hematologic, hepatic, and renal function. In part 1, small cohorts of patients will be enrolled following a traditional 3 + 3 dose escalation design (n = ~6-18). The primary objectives of part 1 are to evaluate the safety and tolerability of nal-IRI + 5-FU/LV + oxaliplatin, to characterize dose-limiting toxicities, and to determine the target dose of oxaliplatin in combination with nal-IRI + 5-FU/LV for part 2. The secondary objective of part 1 is to characterize the pharmacokinetics of nal-IRI + 5FU/LV + oxaliplatin. In part 2, an additional 150 patients will be randomized 1:1:1 to a nal-IRI + 5-FU/LV + oxaliplatin regimen (arm 1), the nal-IRI + 5-FU/LV combination that previously demonstrated efficacy in the NAPOLI-1 trial (arm 2), and a nab-paclitaxel + gemcitabine control arm (arm 3). The primary objective of part 2 is to assess the efficacy of the nal-IRI–containing regimens (arms 1 and 2) compared with nab-paclitaxel + gemcitabine (arm 3) with progression-free survival as the primary end point. Secondary objectives of part 2 include: OS, objective response rate according to Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1, CA19-9 tumor marker response, health-related quality of life according to the European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire core module (EORTC-QLQ-C30) and the European Quality of Life Questionnaire (EQ-5D-5L), and safety. This study is currently recruiting patients.

Results:

Conclusion:

Expanded analyses of NAPOLI-1: phase 3 study of MM-398 (nal-IRI), with or without 5-fluorouracil and leucovorin (5-FU/LV), versus 5-FU/LV, in metastatic pancreatic cancer (mPAC) previously treated with gemcitabine-based therapy.

Andrew Dean,[1] Li-Tzong Chen,[2] Daniel D. Von Hoff,[3] Chung-Pin Li,[4] Andrea Wang-Gillam,[5] Jens T. Siveke,[6] György Bodoky,[7] Yan-Shen Shan,[8] Gayle S. Jameson,[3] Teresa Macarulla,[9] Kyung-Hun Lee,[10] David Cunningham,[11] Jean-Frédéric Blanc,[12] Chang-Fang Chiu,[13] Gilberto Schwartsmann,[14] Fadi S. Braiteh,[15] Eliel Bayever,[16] Bruce Belanger,[16] Floris de Jong,[17] Richard Hubner[18]

[1]St. John of God Hospital, Subiaco, Western Australia, Australia; [2]National Health Research Institutes (NHRI) - National Institute of Cancer Research, Tainan, Taiwan; [3]TGen and Honor Health, Phoenix/Scottsdale, AZ, USA; [4]Division of Gastroenterology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan; [5]Washington University in St. Louis, St. Louis, MO, USA; [6]West German Cancer Center, University Hospital Essen, Essen, Germany ; [7]Szent László Hospital, Budapest, Hungary; [8]National Cheng Kung University Hospital, Tainan City, Taiwan; [9]Vall d'Hebron University Hospital, Barcelona, Spain; [10]Department of Internal Medicine and Cancer Research Institute, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, South Korea; [11] Royal Marsden NHS Foundation Trust, London and Surrey, United Kingdom; [12]Hôpital Saint-André, Bordeaux, France; [13]China Medical University Hospital, Taichung, Taiwan; [14]Federal University of Rio Grande do Sul, Porto Alegre, Brazil; [15]Comprehensive Cancer Centers of Nevada, Las Vegas, NV; [16]Merrimack Pharmaceuticals Inc., Cambridge, MA; [17]Shire GmbH, Zürich, Switzerland; [18]Christie Hospital NHS Foundation Trust, Manchester, UK

Target Congress: 18th AGITG, September 14 – 16, 2016, Melbourne, Victoria, Australia

Abstract Category: Clinical Pancreatic Cancer

Abstract Subcategory: Clinical

Word count: 300 (limit, 300)

Keywords: nal-IRI, mPAC, nal-IRI+5-FU/LV, pancreatic, NAPOLI-1, Per-Protocol, MM-398

Background: nal-IRI is a liposomal encapsulation of irinotecan. Median overall survival (OS) in the NAPOLI-1 Intent to Treat (ITT) population was significantly longer with nal-IRI+5-FU/LV (n=117) vs. 5-FU/LV alone (n=119; 4.2 vs. 6.1 months, unstratified

HR=0.67; *P*=0.012; NCT01494506). The most frequent grade 3+ adverse events included neutropenia, fatigue and Gastro-intestinal effects (diarrhea and vomiting). Expanded, pre-specified analyses of the phase 3 study are presented.

Methods: Patients (n=417) with mPAC previously treated with gemcitabine-based therapy were randomized 1:1:1 in an open-label study to receive either: (A) nal-IRI (120 mg/m^2 IV over 90 min) Q3W; (B) 5-FU (2,000 mg/m^2 over 24 h) with LV (200 mg/m^2 over 30 min) x 4 weeks followed by a 2-week rest; or (C) a combination of nal-IRI (80 mg/m^2 IV over 90 min) prior to 5-FU (2,400 mg/m^2 over 46 h) and LV (400 mg/m^2 over 30 min) Q2W. The primary endpoint was OS. The ITT population included all randomized patients; the Per-Protocol (PP) population included patients who received at least 80% of the target treatment dose in the first 6 weeks and who did not violate any inclusion/exclusion criteria.

Results: Analysis of the PP population (8.9 vs. 5.1 months, HR=0.57 [0.37-0.88]; *P*=0.011 for nal-IRI+5-FU/LV [n=66] vs. 5-FU/LV [n=71]) confirmed the favorable median OS of the combination nal-IRI+5-FU/LV arm vs. the control 5-FU/LV arm in the ITT population, which was also reflected by the progression-free survival, overall response rate and CA19-9 results. No statistically significant improvement in OS was demonstrated for nal-IRI monotherapy vs. 5-FU/LV. Subgroup analysis, based on pretreatment characteristics including stage at diagnosis, prior lines of therapy and CA 19-9 levels, favored OS for the nal-IRI+5-FU/LV arm vs. the 5-FU/LV arm.

Conclusions: Expanded analysis of the PP population and sensitivity analyses support the benefit of nal-IRI+5-FU/LV compared with 5-FU/LV.

Liposomal irinotecan (nal-IRI, MM-398)-containing regimens versus nab-paclitaxel plus gemcitabine as first-line therapy in patients with metastatic pancreatic adenocarcinoma (mPAC): a randomized, open-label phase 2 study

Andrew Dean,[1] Ramesh K Ramanathan,[2] Brooke Belota,[3] Bruce Belanger,[3] Qi Zhu,[4] Marc Pipas,[3] Li-Tzong Chen,[5]

[1]St John of God Hospital, Subiaco, Australia; [2]Mayo Clinic Cancer Center (MCCC), Scottsdale, Scottsdale, AZ, USA; [3]Merrimack Pharmaceuticals, Inc., Cambridge, MA, USA; [4]Shire, Cambridge, MA, USA; [5]National Health Research Institutes (NHRI) – National Institute of Cancer Research, Tainan, Taiwan

Target congress: Australian Gastro-intestinal trials group 18th Annual Scientific Meeting September 14th – September 16th, Melbourne, Australia

Word count: 300 (limit 300)

Suggested key words: mPAC, pancreatic, nal-IRI, nal-IRI+5-FU/LV, nab-paclitaxel, phase 2, first line, gemcitabine, MM-398

Background: mPAC results in a poor prognosis, with median overall survival (OS) <1 year. Current first-line treatments include 5-fluorouracil (5-FU)/leucovorin (LV) + irinotecan + oxaliplatin and nab-paclitaxel+gemcitabine. nal-IRI+5-FU/LV significantly improved OS compared with 5-FU/LV (6.1 vs. 4.2 months; HR=0.67; *P*=0.012) in the randomized, phase 3 NAPOLI-1 study and was generally well-tolerated in patients previously treated with gemcitabine-based therapy (Wang-Gillam et al., Lancet 2016). nal-IRI+5-FU/LV is therefore recommended as second-line therapy for gemcitabine-refractory mPAC (Ducreux et al., Ann Oncol 2015). This trial (NCT0251991) was designed to determine the preliminary safety and efficacy of nal-IRI+5-FU/LV±oxaliplatin compared with nab-paclitaxel+gemcitabine.

Trial design: This phase 2 comparative study comprises parts 1 (safety run in) and 2 (randomized, open-label study). Eligibility criteria include age ≥18 years; pathologically confirmed PAC; unresectable locally advanced (part 1) or metastatic disease absent from the central nervous system (parts 1 and 2); no prior treatment of metastases; Eastern Cooperative Oncology Group performance status ≤1; and adequate hematologic, hepatic, and renal function. In part 1, patient cohorts will be enrolled using a 3+3 dose escalation design (n=6-18). Primary objectives are to evaluate safety and tolerability of nal-IRI+5-FU/LV+oxaliplatin, identify dose limiting toxicities, and determine the target dose of the triplet combination for part 2. The secondary objective is to characterize nal-IRI+5-FU/LV+oxaliplatin pharmacokinetics. In part 2, 150 additional patients will be

randomized 1:1:1 to nal-IRI+5-FU/LV+oxaliplatin (arm 1), nal-IRI+5-FU/LV (arm 2) and nab-paclitaxel+gemcitabine control (arm 3). The primary objective is to assess efficacy, comparing arms 1 and 2 with arm 3. Progression-free survival is the primary endpoint. Part 2 secondary objectives include: safety, OS, objective response rate, CA19-9 response, and health-related quality of life (assessed via European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire core module [EORTC-QLQ-C30] and European Quality of Life Questionnaire [EQ-5D-5L]). This study is currently recruiting patients.

J1

Real-world rates of hematologic laboratory abnormalities and associated cost among metastatic pancreatic cancer therapeutic regimens

Gabriela Dieguez,[1] Andy Surinach,[2] Daniel Mercer,[2] Paul Cockrum,[3] George P Kim,[4] Pamela Pelizzari[1]

[1]Milliman, New York, NY, USA; [2]Genesis Research, Hoboken, NJ, USA; [3]Ipsen Biopharmaceuticals, Inc., Cambridge, MA, USA; [4]George Washington University, Division of Hematology & Oncology, Washington, DC, USA

BACKGROUND

- Pancreatic cancer is the thirteenth most commonly diagnosed cancer globally, accounting for 2.5% of all new cancer cases.[1]
  - In the USA, in 2019, pancreatic cancer comprised an estimated 3.2% of total new cancer diagnoses and is estimated to be the third leading cause of cancer-related death. The overall 5-year survival for all stages of pancreatic cancer combined is 9%.[2]
- At diagnosis, the majority of pancreatic cancer cases (53%) are metastatic owing to the lack of specific, early symptoms and the aggressive nature of the disease.[3]
- Liposomal irinotecan can be used to treat metastatic pancreatic ductal adenocarcinoma in combination with other chemotherapeutic agents, including fluorouracil, leucovorin and oxaliplatin.[4]
- Low hematologic counts are a common and costly side effect of chemotherapy, and can include anemia, neutropenia, lymphocytopenia and thrombocytopenia.[4–7]

OBJECTIVE

- The aim of this observational study was to examine the rates of hematologic laboratory abnormalities and associated costs in patients with metastatic pancreatic cancer (mPANC).

METHODS

Data sources

- Medicare 100% limited data set (LDS) claims files were used to investigate healthcare costs.
  - The files contain all Medicare fee-for-service (FFS) Part A and Part B claims, except those for professional services and durable medical equipment, for 45 million beneficiaries.
  - The files include diagnosis, procedure and diagnosis-related group codes, site-of-service information and beneficiary information (e.g. age, eligibility status and health-maintenance organization (Medicare Advantage) enrollment).
- Flatiron Health clinical database files were used to investigate laboratory data.
  - The Flatiron Health database is a US-nationwide longitudinal, demographically and geographically diverse database derived from electronic-health-record data.

Study population

- Deidentified patient-level data were extracted from the US-nationwide Flatiron Health database on adverse events (AEs) reported between January 1, 2014, and January 31, 2019.
- From the 100% LDS claims files, included patients were 18 years of age or older and received a diagnosis of mPANC according to diagnostic codes from the International Classification of Diseases and Related Health Problems, 9th and 10th revisions, Clinical Modification (ICD-9/10-CM), based on:
  - two or more claims with a pancreatic cancer diagnosis more than 30 days apart
    AND
  - one or more claim(s) with a secondary malignancy (metastasis) diagnosis on or after the date of the first pancreatic cancer diagnosis.

- In addition, patients were required to have received at least one of the following treatments in any line of therapy (e.g. first- or second-line treatment).
  - Folinic acid + 5-fluorouracil + oxaliplatin (FOLFOX).
  - Folinic acid + 5-fluorouracil + irinotecan (FOLFIRI).
  - Folinic acid + 5-fluorouracil + irinotecan + oxaliplatin (FOLFIRINOX).
  - Gemcitabine + nab-paclitaxel (gem/nab).
  - Any liposomal-irinotecan-based therapy.
  - Other gemcitabine-based therapy.
- The index date was identified as the earliest metastasis diagnosis date.
- For the healthcare costs analysis using Medicare FFS, patients were excluded if they had pre-index non-pancreatic malignancies or if they were not enrolled at least 6 months before, and 3 months after, the index date or until the date of death (i.e. the earlier of the two).

Safety outcomes

- AEs and laboratory abnormalities of interest included anemia, neutropenia, lymphocytopenia and thrombocytopenia, and were identified directly from the database using ICD-9/10-CM diagnosis codes and/or structured laboratory data.
- Laboratory abnormalities were stratified by grade based on the grading system of the National Cancer Institute Common Terminology Criteria for Adverse Events, and findings are reported for all patients with any grade event together and for those with a grade 3+ event.
- Control patients from the Medicare 100% LDS without an AE, who were matched to the same treatment line and regimen, were randomly sampled and assigned shadow AE dates.

Associated costs of treating adverse events

- Costs associated with treating AEs of interest from January 1, 2013, to December 31, 2017, in patients with mPANC were assessed using the Medicare 100% LDS.
- The 30-day costs were calculated after generation of the AE onset data or the shadow AE date (for control patients).
  - For each regimen–treatment-line combination with at least 80 patients (cases and controls), 30-day AE incremental costs were estimated using log-link generalized linear models and gamma-distributed errors.
  - Mean 30-day AE incremental costs relative to controls were estimated using recycled projections and bootstrapped 95% confidence intervals to determine statistical significance relative to zero.

RESULTS

Patient characteristics and treatment

- In total, 4,592 patients with mPANC who had received at least one of the prespecified treatment regimens were identified from the Medicare 100% LDS.
  - The median age at diagnosis was 68 years (interquartile range, 61–75 years).
- All therapies and treatment lines are provided in Table 1.
  - FOLFIRINOX and gem/nab were the most commonly prescribed first-line treatments, and were less frequently prescribed as second- or third-line therapies.
  - FOLFOX, FOLFIRI and liposomal irinotecan were prescribed as second-line therapies more frequently than as first- or third-line therapies.

Adverse events/laboratory abnormalities of interest

- Rates of anemia, neutropenia, lymphocytopenia and thrombocytopenia AEs calculated using the Flatiron Health database are shown in Figure 1.

Associated costs of treating adverse events

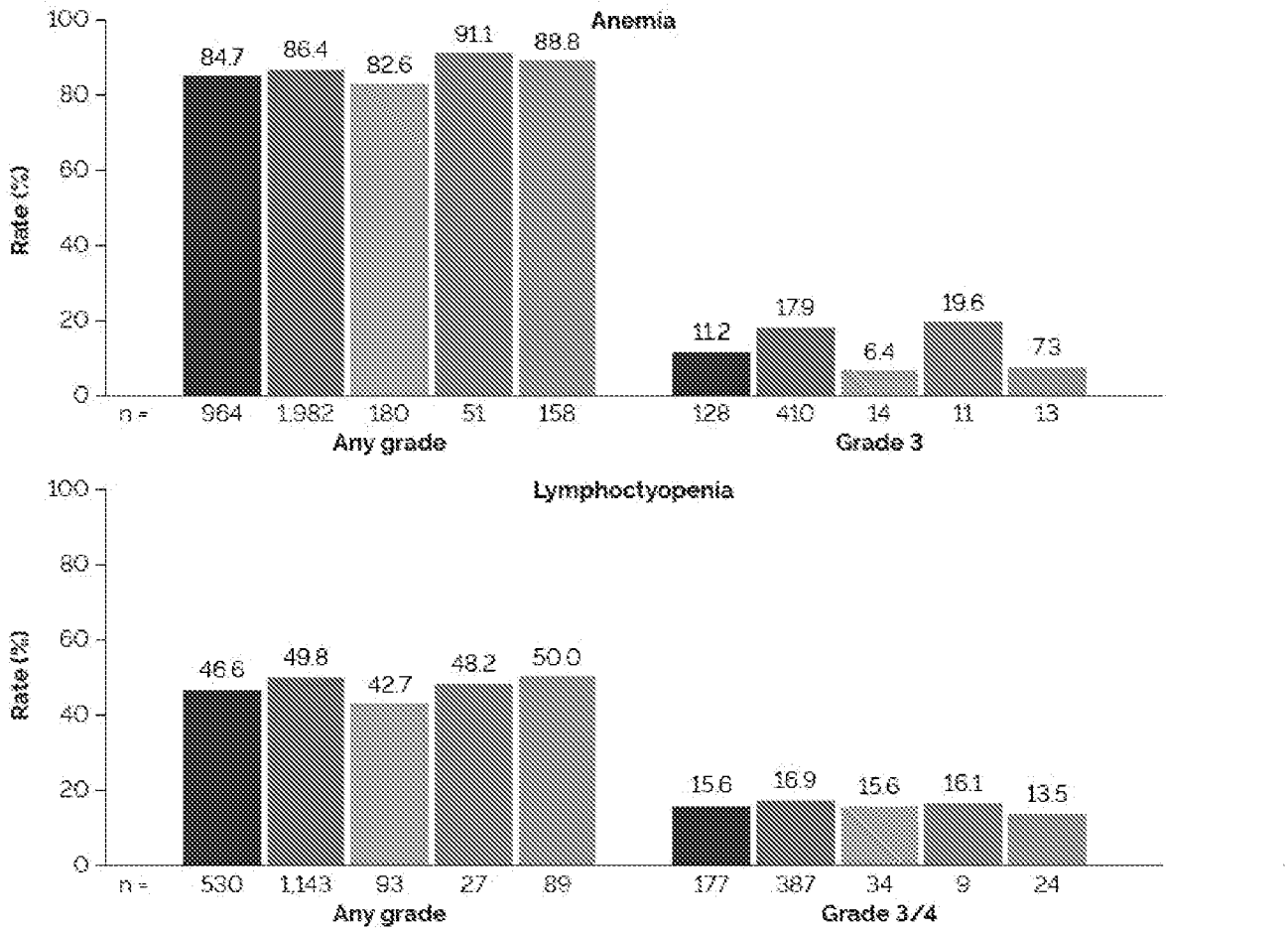
- Mean adjusted incremental costs for first-line FOLFIRINOX and gem/nab and second-line FOLFOX, FOLFIRI and liposomal-irinotecan-based treatments in patients with 'any grade' anemia and 'any grade' neutropenia are shown in Table 2.
  - For both anemia and neutropenia AEs, the lowest associated costs of treatment were observed in patients receiving liposomal-irinotecan-based therapy, and the highest associated costs of treatment were observed in those receiving FOLFIRI.
- Not enough data were available to enable comparisons to be made between treatment costs associated with lymphocytopenia or thrombocytopenia.

Table 1. Numbers of patients per line of therapy based on the Medicare 100% LDS

Treatment line	Patients per therapy					
	FOLFIRINOX	Gem/nab	FOLFOX	FOLFIRI	Liposomal irinotecan*	Other
All lines	1,435	2,965	491	135	406	707
First line	1,138	2,295	189	35	57	–
Second line	231	576	218	56	178	–
Third line	54	75	66	35	130	–

*Liposomal-irinotecan-based therapy
FOLFIRI, folinic acid + 5-fluorouracil + irinotecan; FOLFIRINOX, folinic acid + 5-fluorouracil + irinotecan + oxaliplatin; FOLFOX, folinic acid + 5-fluorouracil + oxaliplatin; gem/nab, gemcitabine + nab-paclitaxel; LDS, limited data set.

Figure 1. Rates of hematologic AEs in prespecified treatment regimens based on the Flatiron Health database



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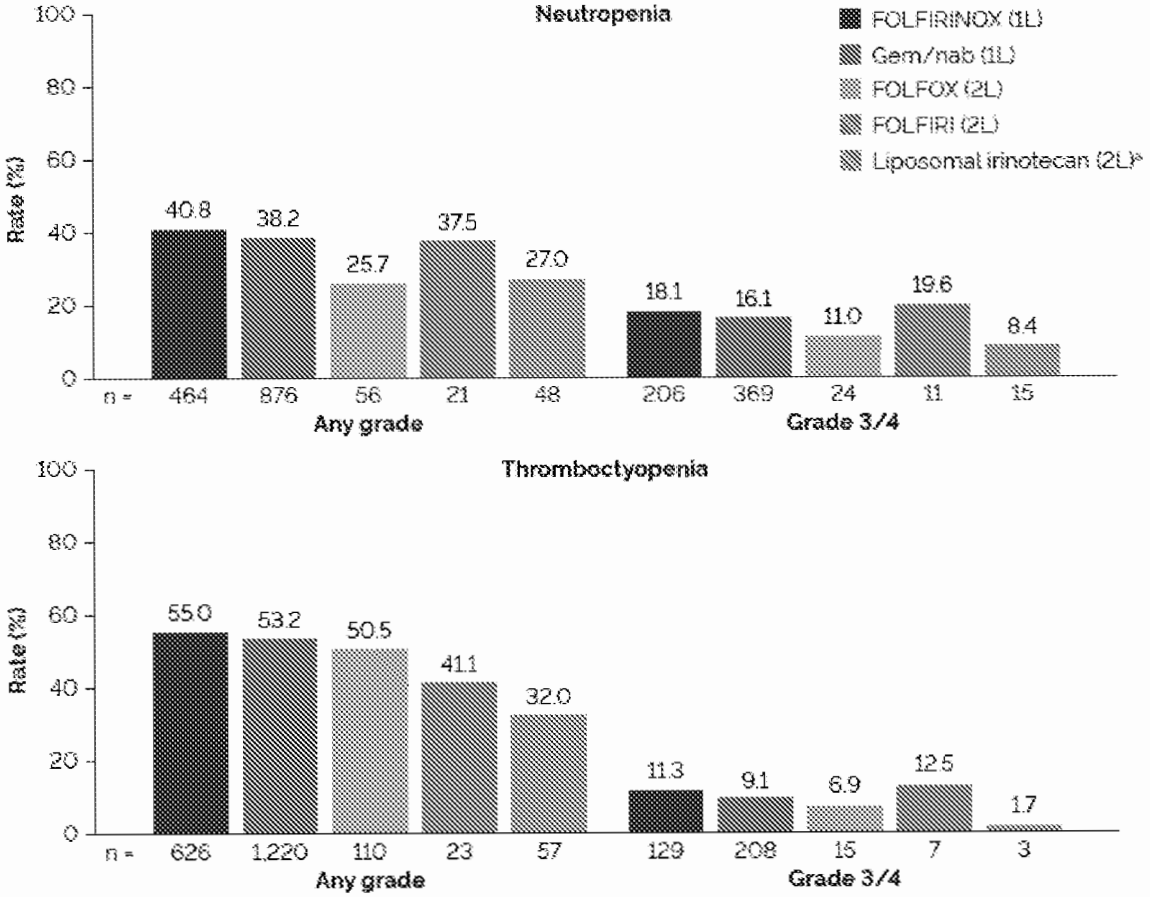

[a]Liposomal-irinotecan-based therapy.
Grades were based on the grading system of the National Cancer Institute Common Terminology Criteria for Adverse Events.
1L, first line; 2L, second line; AE, adverse event; FOLFIRI, folinic acid + 5-fluorouracil + irinotecan; FOLFIRINOX, folinic acid + 5-fluorouracil + irinotecan + oxaliplatin; FOLFOX, folinic acid + 5-fluorouracil + oxaliplatin; gem/nab, gemcitabine + nab-paclitaxel.

Table 2 Associated per-patient healthcare costs for 'any grade' hematologic AEs based on the Medicare 100% LDS

Treatment regimen	Anemia	
	Mean adjusted incremental costs, USD ($)	Confidence intervals, USD ($)
FOLFIRINOX (1L)	3,864	2,931–4,662
Gem/nab (1L)	3,818	3,409–4,247
FOLFOX (2L)	3,536	2,438–4,677
FOLFIRI (2L)	3,976	2,241–5,817
Liposomal irinotecan (2L)[a]	2,963	1,544–4,400

Neutropenia	
Mean adjusted incremental costs, USD ($)	Confidence intervals, USD ($)
2,352	1,616–3,211
2,440	1,969–2,945
2,688	1,473–4,228
3,561	1,227–6,039
2,307	703–4,313

[a]Liposomal-irinotecan-based therapy.
1L, first line; 2L, second line; AE, adverse event; FOLFIRI, folinic acid + 5-fluorouracil + irinotecan; FOLFIRINOX, folinic acid + 5-fluorouracil + irinotecan + oxaliplatin; FOLFOX, folinic acid + 5-fluorouracil + oxaliplatin; gem/nab, gemcitabine + nab-paclitaxel; LDS, limited data set; USD, US dollars.

CONCLUSIONS

- The present real-world analyses of patient-level data indicated that 'any grade' anemia was common across chemotherapy regimens and treatment lines.
- 'Any grade' neutropenia was less common than anemia across regimens and had lower overall incremental costs.
  - Based on the Medicare data, associated costs of treating 'any grade' neutropenia and anemia were lower with liposomal-irinotecan-based therapy than with other regimens, which may suggest that AEs in patients receiving liposomal irinotecan were of lower severity than those in patients receiving other regimens.
- The lower costs found in the Medicare data for liposomal-irinotecan-based therapy are consistent with Flatiron Health's relatively low rates of grade 3+ neutropenia and anemia among patients treated with second-line liposomal irinotecan.

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Conflicts of interest

GD and PP are employed by Milliman, Inc., and received consulting fees from Ipsen Biopharmaceuticals, Inc.
AS and DM are employees of Genesis Research LLC, which received consulting funding from Ipsen Biopharmaceuticals, Inc.
PC is an employee of Ipsen Biopharmaceuticals, Inc., and holds stock or stock options.
GPK received funding for consulting from Ipsen Biopharmaceuticals, Inc.

Corresponding author: gabriela.dieguez@milliman.com

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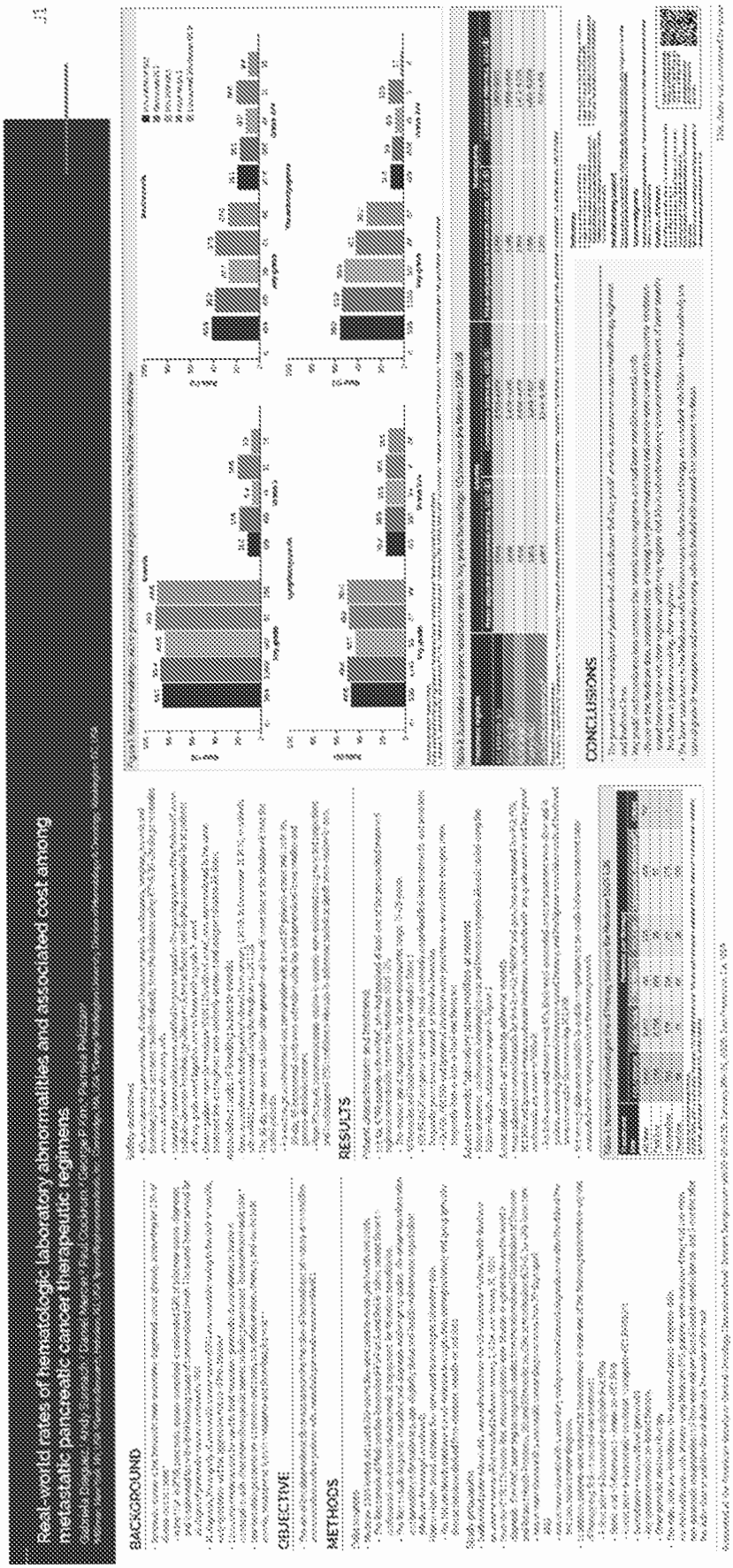
Real-world rates of hematologic laboratory abnormalities and associated costs among metastatic pancreatic cancer therapeutic regimens
BACKGROUND
OBJECTIVE
METHODS
RESULTS
CONCLUSIONS

PANCREATIC CANCER

Real-world rates of hematology lab abnormalities and associated cost among metastatic pancreatic cancer (mPC) therapeutic regimens.

Gabriela Dieguez, Andy Surinach, Daniel Mercer, Paul Cockrum, George P. Kim, Pamela Pelizzari

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Milliman, Inc., New York, NY; Genesis Research, Hoboken, NJ; Ipsen Biopharmaceuticals, Cambridge, MA; 22nd and I Street, NW, Washington, DC; Milliman, New York, NY

Abstract Disclosures

Abstract

670

Background: Low hematologic counts are a common, costly side effect of chemotherapy. This observational study examines rates and associated cost to treat. **Methods:** Data on adverse events (AEs) were extracted from the clinical Flatiron Health database for mPC patients (pts) from 01/2014-01/2019. Anemia, neutropenia, and lymphopenia occurrence was assessed via diagnosis codes and structured lab data. Costs due to AEs were derived from a claims analysis of mPC pts from 2013-2017 in Medicare Limited Data Set claims. Mean adjusted incremental costs (ICs) were estimated by comparing 30-day costs of pts with and without AEs, with controls selected among pts without AEs with the same regimen and line of therapy, when at least 80 cases/controls were identified. **Results:** 4592 treated mPC pts were identified (median age at diagnosis: 68y, IQR: 61 – 75). 1138 pts were treated with FOLFIRINOX (FFX) in first-line (1L), 2295 pts with 1L gemcitabine plus nab-paclitaxel (gem-nab), 218 pts with second-line (2L) FOLFOX, 56 pts with 2L FOLFIRI, and 178 pts with 2L liposomal irinotecan (nal-IRI) based therapy. Observed rates of anemia and neutropenia are shown in the table below. Lymphopenia rates were similar across regimens and ICs were not statistically significant. ICs for patients with any grade anemia were $3864, $3818, $3536, $3978, and $2963 for FFX, gem-nab, FOLFOX, FOLFIRI, and nal-IRI treated pts, respectively. ICs for pts with any grade neutropenia were $2382 for FFX, $2440 for gem-nab, $2688 for FOLFOX, $3551 for FOLFIRI and $2307 for nal-IRI. **Conclusions:** Any grade anemia ICs ranged from $2963 [$1544, $4400] (nal-IRI) to $3978

[$2241, $5817] (FOLFIRI), and any grade neutropenia ICs ranged from $2307 [$703, $4313] (nal-IRI) to $3551 [$1227, $6039] (FOLFIRI). Pts treated with nal-IRI had similar any grade AE rates but lower ICs, which suggest lower severity of AEs. These results are consistent with Flatiron Health's lower rates of grades 3+ neutropenia and anemia.

	Any Grade Anemia	Grades 3+ Anemia	Any Grade Neutropenia	Grades 3+ Neutropenia
1L FFX	84.7%	11.2%	40.8%	18.1%
1L GEM-NAB	86.4%	17.9%	38.2%	16.1%
2L FOLFOX	82.6%	6.4%	25.7%	11.0%
2L FOLFIRI	91.1%	19.6%	37.5%	19.6%
2L NAL-IRI	88.8%	7.3%	27.0%	8.4%

Research Sponsor:

Ipsen Biopharmaceutical Inc

Completed Research Clinical/Translational

Poster Reception

CT12 | The Cost of Adverse Events for FDA-Approved/NCCN Category 1 Treatments for Medicare Fee-for-Service (FFS) Patients with Metastatic Pancreatic Cancer: Focus on Liposomal Irinotecan-Based Regimens (Screen 8)

Thu, Mar 12 6:40 PM – 7:25 PM

BACKGROUND/RATIONALE: Adverse events (AEs) related to cancer therapy reduce patients' quality of life and generate substantial healthcare costs. There is limited real-world evidence regarding AE costs for patients with metastatic pancreatic cancer (m-PANC) who receive FDA-Approved/NCCN Category 1 treatments.

OBJECTIVES: We analyzed the costs of three common AEs (neutropenia, anemia, and thrombocytopenia) for patients with m-PANC receiving FDA-Approved/NCCN Category 1 Lines of Therapies (LOTs): first line (1L) gemcitabine/nab-paclitaxel, gemcitabine monotherapy, and FOLFIRINOX, and second line (2L) liposomal irinotecan.

METHODS: We identified m-PANC patients and AEs using ICD-9/10 diagnosis codes in 2013-2017 Medicare claims. Patients in our study had both multiple claims with a pancreatic cancer (PANC) diagnosis 30+ days apart and one+ claim(s) with a secondary malignancy diagnosis on/after the first PANC diagnosis date. LOTs were assigned based on the order of therapies used. LOTs ended when a new regimen began, 28 days after the last chemotherapy, or upon death.
We randomly sampled control patients in the same LOT and regimen without an AE and assigned them shadow AE dates. We calculated 30-day costs after AE or shadow AE onset, trended to 2017. For LOTs and regimens with sufficient volume, we estimated 30-day AE incremental costs using log-link generalized linear models and gamma-distributed errors. We predicted mean 30-day AE incremental costs relative to controls using recycled projections and bootstrapped 95% confidence intervals to determine statistical significance.

RESULTS: Anemia was the most common AE, ranging from 41% of patients receiving 1L gemcitabine/nab-paclitaxel to 32% of patients receiving 2L liposomal irinotecan. Mean 30-day incremental anemia costs ranged from $3,080 for 1L gemcitabine monotherapy to $3,924 for 1L gemcitabine/nap-paclitaxel, and was $3,257 for 2L liposomal irinotecan.

Neutropenia ranged from 32% of those receiving 1L FOLFIRINOX to 16% of those receiving 1L gemcitabine monotherapy. Mean 30-day neutropenia incremental costs ranged from $1,284 for 2L liposomal irinotecan to $2,503 for 1L gemcitabine/nap-paclitaxel.

Thrombocytopenia ranged from 18% of patients receiving 1L FOLFIRINOX to 8% of those receiving 2L liposomal irinotecan. Mean 30-day incremental thrombocytopenia costs ranged from $2,678 for 1L gemcitabine/nap-paclitaxel to $3,721 for 1L FOLFIRINOX.

CONCLUSIONS/

Discussion: AEs impose substantial costs for Medicare fee-for-service patients with m-PANC receiving FDA-Approved/NCCN Category 1 treatments. For 1L regimens, we observed statistically significant incremental costs associated with anemia, neutropenia, and thrombocytopenia. Among 2L liposomal irinotecan patients, only anemia incremental costs were statistically significant; neutropenia incremental costs were not statistically different from zero, and there were insufficient thrombocytopenia cases to estimate incremental costs.

Poster Presenter(s)

JD

Jeff Doris

Email: jeff.doris@ipsen.com

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A Comparison of Two Dose Regimens in Pancreatic Cancer

A. FIGER [1] - E. SADIKOV [2] - M. MISHAELI [2] - R. KOREN [3]
R. GAL [3] - I. LEVIN [2] - B. KLEIN [2]

From the Rabin Medical Center [1] Institute of Oncology, Campus Beilinson, [2] Oncology Unit, Campus Golda, [3] Department of Pathology, Campus Golda, Petach-Tikva, Israel.

Correspondence: B. Klein, Oncology Unit, Rabin Medical Center – Golda Campus, 7 Kakal St., Petach-Tikva 49372 Israel. Tel: +972-3-9372551, 590, 477; Fax: +972-3-9372590. E-mail: bklein@post.tau.ac.il

Summary

Forty-seven patients with pancreatic cancer were treated with two different schedules of 5-fluorouracil (5FU) and leucovorin (LCV): standard and dose-intense schedules. The standard regimen was monthly and the dose intense was biweekly. Partial response was observed in one patient (4%), no change in 12 (48%) and progression of disease in another 12 patients.

Clinical benefit, measured by symptomatic improvement, was observed in 19% of all the patients, in 12% of those treated with the standard regimen and in 27% of the intense group. Median survival was 8 months for all the patients. The 1-year survival rate was 32%. Toxicity was mild. There was no survival benefit for the dose intense regimen. These results indicate that clinical benefit can be obtained with 5FU and LCV regimens despite the lack of objective response and that a dose-intense schedule is of little benefit in treating pancreatic cancer.

Key words: **Pancreatic cancer, 5-fluorouracil, 5FU, leucovorin.**

INTRODUCTION

Pancreatic cancer is a highly malignant disease with approximately 27,000 deaths per year in the USA [1]. Only a minority of the patients have resectable tumor and most of them present with advanced disease. The 5-year survival rate is 1-4% [2]. Glimelius *et al.* [3] compared chemotherapy to supportive care in 90 patients with pancreatic cancer and found a significant survival advantage in the chemotherapy arm. Many chemotherapeutic agents have been studied in patients with pancreatic cancer, but 5-fluorouracil (5FU) has been the most widely used single agent. The response rate to 5FU was reported to be 28% [4], but recent studies have shown objective response to be only 15% [5]. Various combination chemother-

apy regimens, adding doxorubicin or mitomycin, have not resulted in improvement either in response rate or survival [6,7]. Since modulation of 5FU by leucovorin (LCV) resulted in improved response rates in colon cancer patients, several studies have been published studying 5FU with LCV in the treatment of pancreatic cancer [8-12], most of them with small numbers of patients.

In the present article, we summarize our experience treating patients with pancreatic cancer with 5FU and LCV in a standard and in a dose-intense regimen, with special attention to symptom relief in addition to the usual parameters of response rate and survival.

PATIENTS AND METHODS

Forty-seven consecutive patients with pancreatic cancer were studied. All patients had histologically confirmed pancreatic cancer. Patients' characteristics are shown in *Table 1*. The median age was 65 years (range 41-82). Twenty-three patients had stage III disease and 24 patients had stage IV disease. Twenty-five patients had measurable disease on CT and all patients were evaluable for clinical benefit. Standard response criteria were used.

TABLE 1 - *Patients' characteristics.*

Number of patients	47	
Median age	65 (41 - 77)	
Stage of disease	III	23
	IV	24
Chemotherapy	Dose-intense	22
	Standard	25

Statistics

Comparison between the groups was done using the Chi square test.

Treatment

5FU and LCV were given in two different schedules. Patients treated at the Beilinson Campus received a dose-intense regimen of 5FU 900 mg/m^2 intravenously preceded by LCV 200 mg/m^2, both as rapid intravenous infusion every 2 weeks (dose intense schedule). Patients treated at the Golda Campus received a standard schedule of LCV 20 mg/m^2 followed by 5FU 370 mg/m^2 i.v. bolus for 5 consecutive days every 28 days (standard schedule). Subjective measures of response were also recorded and included reduction in analgesic consumption, weight gain and improvement in performance status.

RESULTS

The median duration of treatment was 4.3 months (5.1 for the dose intense and 3.6 for the standard schedule). Of the 47 patients treated, 25 were evaluable by CT. *Table 2* outlines the response rate according to the two different dose regimens. One patient obtained a partial response, 12 patients had no change and 12 patients had disease progression while on treatment. Median survival for all patients was 8 months, 15 months for those with stable disease, and 5 months for those who progressed. The one-year survival rate was 32% (15 patients).

Clinical benefit was evaluated in all patients (*Table 3*). Nine patients experienced symptomatic improvement (19%), 8 patients had no

TABLE 2 - *Response rate and survival.*

Response	Standard		Dose-intense		All patients	
	N.	%	N.	%	N.	%
Partial response	1	10	-	-	1	4
No change	4	40	8	53	12	48
Disease progression	5	50	7	47	12	48
Median survival (mo.)	5		9		8	
1-year survival	-		-		15	32

change in symptoms, and in 30 patients symptoms worsened during treatment. Clinical benefit was noted more frequently with the dose-intense regimen. There was no significant difference between regimens with regard to response and survival.

Toxicity (Table 4)

Toxicity was mild in both regimens and the treatment was generally well tolerated. Only one patient had grade 4 diarrhea. There were no toxicity-related deaths.

DISCUSSION

In the present study treatment with 5FU and LCV resulted in only one objective response, which was consistent with other studies [9-11]. We also measured clinical benefit and observed that about 20% of the patients improved symptomatically. In addition, one-third of all patients lived longer than 1 year. Although the overall median survival was 8 months, for patients with stable disease it was 15 months. Moreover, the objective response rate by standard criteria was very low, but survival was longer than that reported for supportive care only.

Only 5 studies have evaluated the use of 5FU and LCV in pancreatic cancer [8-12] (*Table 5*), most of them including a small number of patients. In all of these other studies, the response rate was very low and survival was up to 6 months. The longer survival in our study may be due to the fact that we included patients with stage III disease. Since chemotherapy in pancreatic cancer is essentially palliative in nature, clinical benefit parameters have been studied and formally accepted as valid response criteria. Indeed the prospective studies with gemcitabine provided evidence that symptomatic improvement occurs in patients regardless of concomitant objective response [13]. In the present study symptomatic improvement was observed despite the fact that only one patient obtained an objective partial response. The dose-intense regimen was of less clinical benefit to the patients than the standard one.

TABLE 3 - *Clinical benefit.*

	Standard		Dose-intense		Total	
	N.	%	N.	%	N.	%
Improvement	3	12	6	27	9	19
No change	5	20	4	18	9	19
Worsened	17	68	12	54	29	62

TABLE 4 - *Toxicity.*

Grade	1		2		3		4	
	N.	%	N.	%	N.	%	N.	%
Standard schedule								
Leukopenia	1	4	1	4	–	–	–	–
Platelets	3	12	4	16	–	–	–	–
Diarrhea	4	16	4	16	1	4	1	4
Stomatitis	–	–	1	4	–	–	–	–
Dose-intense schedule								
Leukopenia	3	13.5	1	4.5	–	–	–	–
Platelets	3	13.5	–	–	–	–	–	–
Diarrhea	6	27	4	18	–	–	–	–
Stomatitis	2	9	1	4.5	–	–	–	–

TABLE 5 - *Summary of studies of 5FU and LCV in patients with pancreatic cancer.*

Studies	Dose and schedule	Response	
		Objective	Subjective
Bruckner et al [8]	LCV 100-20mg 5FU 30 mg/kg q 2 w	4/8	NA
De Caprio et al [9]	5FU 500 mg/m^2 LCV 500 weekly x 6	2/27	NA
Crown et al [10]	5FU 370 mg/m^2 d 1-5 LCV 500 mg/m^2 C.I. d 1-6 q 28 d	0/19	NA
Rubin et al [11]	5FU 425 mg/m^2 LCV 20 mg/m^2 d 1-5, q 28 d	0/31	NA
Weinerman et al [12]	5FU 370 mg/m^2 LCV 200 mg/m^2 d 1-5 q 28 d	3/23	NA
Present study	5FU 370 mg/m^2 LCV 200 mg/m^2 d 1-5 q 28 d Or: 5FU 900 mg/m^2 LCV 500 mg/m^2 d 1 q 14 d	1/47	20%

NA = not available
C.I. = continuous infusion

The fact that clinical benefit was observed in patients with no objective response further supports the notion that dose intensity is less important in pancreatic cancer. Clinical benefit parameters should be measured in patients with pancreatic cancer rather than response rates *per se*.

In conclusion our study regimens give similar outcome results, with some improvement in quality of life for a small percentage of patients.

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PCN29

A SYSTEMATIC LITERATURE REVIEW TO IDENTIFY AND COMPARE CLINICAL TRIALS EVALUATING NOVEL THERAPEUTIC AGENTS IN POST-GEMCITABINE ADVANCED PANCREATIC CANCER PATIENTS

Daniel F. Gaddy[1], Claus C. Becker[1], Haihong Li[1], Robert Bennett[1], Yoojung Yang[2], Jonathan B. Fitzgerald[1], Eliel Bayever[1]

[1]Merrimack, Cambridge, MA, USA

[2]Baxalta US Inc., Cambridge, MA, USA

INTRODUCTION

- Pancreatic cancer is the eleventh most common and sixth most lethal malignancy world-wide, resulting in more than 330,000 deaths per year [1]. In 2015, greater than 85,000 deaths are expected in the European Union [2], and nearly 41,000 deaths are expected in the United States [3], where pancreatic cancer is predicted to be the second-leading cause of cancer-related mortality by 2030 [4].
- Early detection of pancreatic cancer is hindered by the non-specific symptoms of early-stage disease and a lack of effective screening methods. Most patients present with advanced disease, including approximately 53% with distant metastases, and another 28% with regional metastases at the time of initial diagnosis [3].
- While surgery can be curative for local disease, treatment options for metastatic pancreatic cancer are limited to chemotherapeutic regimens. Gemcitabine-based regimens are the most commonly prescribed, and represent the standard of care, for first-line metastatic pancreatic cancer.
- In the two decades since the approval of gemcitabine, only two new agents have been approved for treatment of metastatic pancreatic cancer (Erlotinib and nab-Paclitaxel), both in the front-line setting in combination with gemcitabine.
- There is no standard of care, and no agents are approved specifically for treatment of patients with metastatic pancreatic cancer who progress following gemcitabine-based therapy. Available treatment options have been limited by a lack of therapeutic breakthroughs, and primarily utilize different combinations and dosing schedules of established chemotherapeutic agents.
- The current review assesses the relative efficacy of new therapeutic agents tested, alone or in combination, since 2003 in patients with pancreatic cancer who progressed following gemcitabine-based therapy.

METHODOLOGY

- A systematic literature review was performed in PubMed/MEDLINE, EMBASE and ASCO meeting abstracts between January 2003* and June 2015. This review identified randomized controlled trials (RCTs) and single-arm trials evaluating new post-gemcitabine regimens in patients with advanced pancreatic cancer.
- Inclusion criteria:

- Histologically proven metastatic pancreatic cancer. Studies enrolling patients with metastatic and locally advanced disease were allowed, and the reported percentage of metastatic disease was documented.
- Prior treatment with a gemcitabine-based regimen.
- Regimen must contain at least one novel agent (indicating agents other than established chemotherapeutics that were evaluated in pancreatic cancer prior to 2003), including targeted small molecule inhibitors, antibodies, nanotherapeutics and immunotherapies.
- Regimens combining novel agents and established chemotherapeutic agents were allowed.

- Relevant reported clinical outcomes for data extraction included objective response rate (ORR), disease control rate (DCR), progression-free survival (PFS) and overall survival (OS).

**Between 1996 (year of gemcitabine approval) and 2003, only standard chemotherapeutic agents were evaluated in the post-gemcitabine setting.*

FIGURE 1: PRISMA FLOW DIAGRAM FOR TRIAL SELECTION

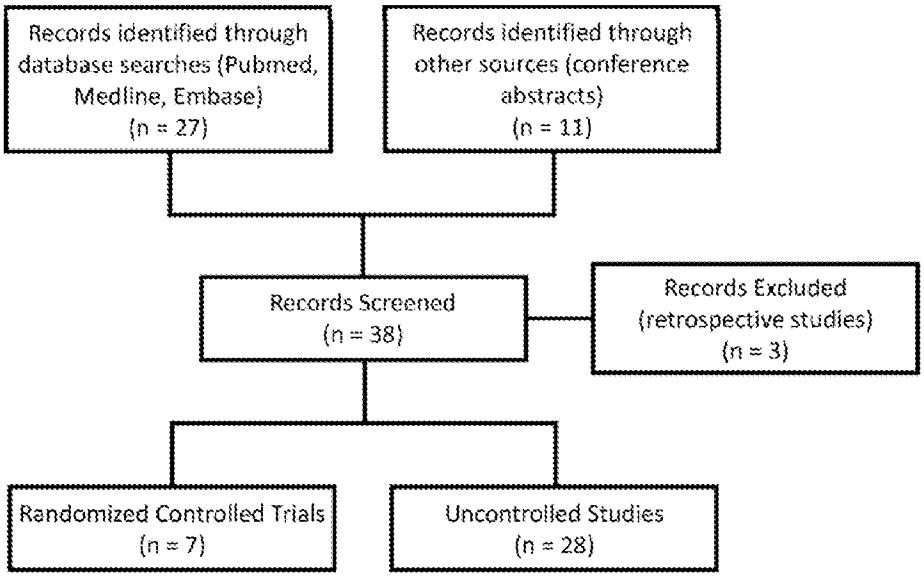

FIGURE 2: NOVEL AGENTS INCLUDED IN ANALYSIS

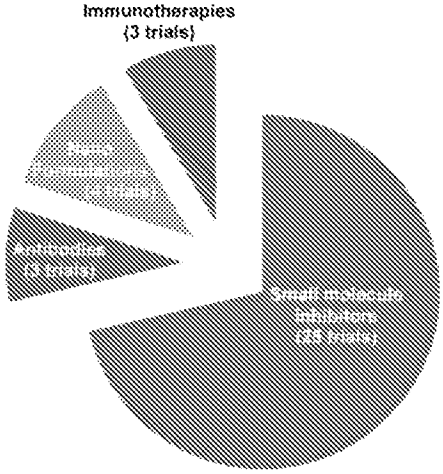

Figure 2. Thirty-five trials have reported outcomes for 26 distinct novel agents in the post-gemcitabine metastatic pancreatic cancer setting since January 2003. A total of 1385 patients were enrolled.

FIGURE 3: THE LANDSCAPE OF NOVEL AGENTS

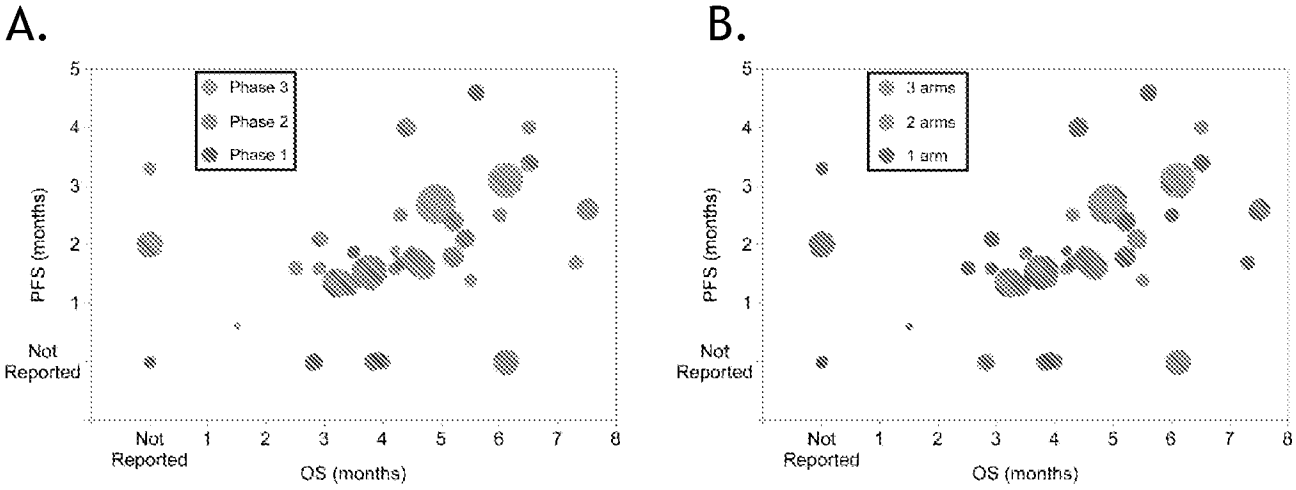

Figure 3. Thirty-five trials evaluated novel agents in post-gemcitabine metastatic pancreatic cancer, exhibiting a broad range of therapeutic outcomes. A) Trials included 1 Phase 3 study (red), 29 Phase 2 studies (green), and 5 Phase 1 studies (blue). B) The majority of studies were small, uncontrolled trials (n=28, blue). Seven studies were randomized controlled trials (RCTs), including predominantly 2-arm studies (n=6, green) and a 3-arm Phase 3 (red). Each circle represents a trial arm containing a novel agent. Circle size indicates the number of patients enrolled .

FIGURE 4: RELATIVE EFFICACY OF NOVEL AGENTS IN RCTs

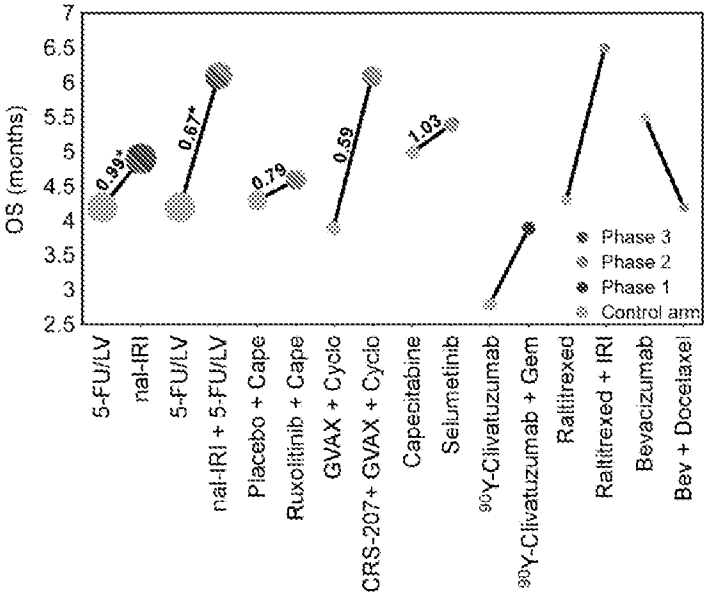

Figure 4. The NAPOLI-1 trial is the first Phase 3 study to demonstrate statistically significant improvement in OS for a novel agent in post-gemcitabine pancreatic cancer. Reported hazard ratios (HR) are indicated on the lines connecting the treatment and control arms. Circle size indicates numbers of patients enrolled per arm, sorted left-to-right in descending order.

**Unstratified HR; stratified HR: 0.93 for nal-IRI (Arm A) and 0.57 for nal-IRI + 5FU/LV (Arm C)*

TABLE 1: KEY CHARACTERISTICS OF RCTs TESTING NOVEL

Reference	Regimen	Phase	Location	Number of Sites	Patients (#)	Performance Score KPS ≥90 or ECOG ≤1)	Metastatic Disease (%)	Primary Localization (%, Head/Other)	ORR (%)	DCR (%)	Median PFS (months)	Median OS (months)	HR (OS)
[5]	Raltitrexed + Irinotecan	2	Austria	4	19	21	100	NR	16	47	4	6.5	NR
[6, 7]	**nal-IRI + 5FU/LV (NAPOLI Arm C)**	**3**	**14 Countries**	**105**	**117**	**59**	**100**	**64/36**	**16**	**58.1**	**3.1**	**6.1**	**0.67**
[8]*	CRS207 + GVAX + Cyclophosphamide	2	USA	10	62	100	100	NR	0	31	NR	6.1	0.59
[9]	Bevicizumab	2	USA	1	16	100	100	NR	0	12.5	1.4	5.5	NR
[10]	Selumetinib	2	USA, EU	15	38	NR	92	NR	5.3	36.8	2.1	5.4	1.03
[6, 7]	**nal-IRI (NAPOLI Arm A)**	**3**	**14 Countries**	**105**	**151**	**56**	**100**	**65/35**	**3.3**	**55.6**	**2.7**	**4.9**	**0.99**
[11]	Ruxolitinib + Capecitabine	2	USA	60	64	56.8	100	NR	7.8	40.6	1.7	4.6	0.79
[5]	Raltitrexed	2	Austria	4	19	21	100	NR	0	37	2.5	4.3	NR
[9]	Bevacizumab + Docetaxel	2	USA	1	16	100	100	NR	0	18.8	1.6	4.2	NR
[12]	^{90}Y-Clivatuzumab + Gemcitabine	1b	USA	24	29	NR	100	NR	41	75	NR	3.9	NR
[8]*	CRS207 + GVAX	2	USA	10	31	100	100	NR	0	24	NR	3.9	1.69
[12]	^{90}Y-Clivatuzumab	1b	USA	24	29	NR	100	NR	NR	NR	NR	2.8	NR

**The CRS207+GVAX trial included an unspecified percentage of patients who received treatment other than Gem-based regimens in first-line.*
NR, not reported.

SUMMARY

- The present review highlights the limited number of RCTs evaluating new therapeutic agents in patients with metastatic pancreatic cancer who progressed following gemcitabine-based therapy.
- Most new agents have only been evaluated in small, uncontrolled trials.
- Despite much research in this difficult-to-treat patient population with high unmet medical need, only one novel agent has been evaluated in a randomized Phase 3 study.
- The Phase 3 NAPOLI-1 trial is the only global trial evaluating a novel therapeutic agent (nal-IRI, in combination with 5FU/LV) to demonstrate significant improvement in overall survival in patients with metastatic pancreatic cancer who had progressed following gemcitabine-based therapy.

REFERENCES

- The present review highlights the limited number of RCTs evaluating new therapeutic agents in patients with metastatic pancreatic cancer who progressed following gemcitabine-based therapy.
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- The Phase 3 NAPOLI-1 trial is the only global trial evaluating a novel therapeutic agent (nal-IRI, in combination with 5FU/LV) to demonstrate significant improvement in overall survival in patients with metastatic pancreatic cancer who had progressed following gemcitabine-based therapy.

A SYSTEMATIC LITERATURE REVIEW TO IDENTIFY AND COMPARE CLINICAL TRIALS EVALUATING NOVEL THERAPEUTIC AGENTS IN POST-GEMCITABINE ADVANCED PANCREATIC CANCER PATIENTS

Daniel F. Gaddy[1], Claus C. Becker[1], Haihong Li[2], Robert Bennett[2], Yanjing Yang[2], Jonathan B. Fitzgerald[1], Eliel Bayever[1]

[1]Merrimack, Cambridge, MA, USA
[2]Baxalta US Inc., Cambridge, MA, USA

MERRIMACK Baxalta

PCN29

INTRODUCTION

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METHODOLOGY

[illegible]

FIGURE 1: PRISMA FLOW DIAGRAM FOR TRIAL SELECTION

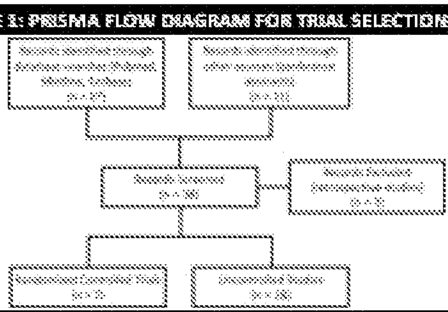

FIGURE 2: NOVEL AGENTS INCLUDED IN ANALYSIS

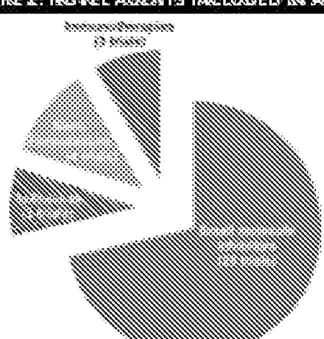

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FIGURE 3: THE LANDSCAPE OF NOVEL AGENTS EVALUATED IN POST-GEMCITABINE PANCREATIC CANCER

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FIGURE 4: RELATIVE EFFICACY OF NOVEL AGENTS IN RCTs

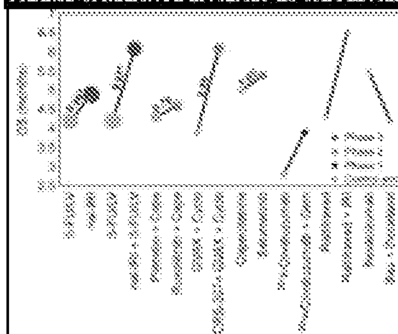

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TABLE 1: KEY CHARACTERISTICS OF RCTs TESTING NOVEL AGENTS

[illegible]

SUMMARY

[illegible]

REFERENCES

[illegible]

Abstract #336

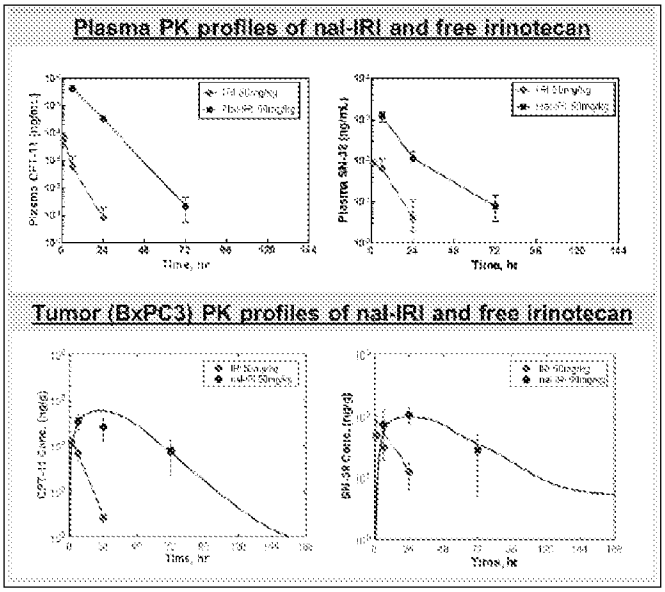
Preclinical Anti-tumor Activity of Nanoliposomalirinotecan(nal-IRI, MM-398) Supports Utilization as a Foundation of Front-Line Pancreatic Cancer Regimens

Daniel F. Gaddy[1], Helen Lee[1], Nancy Paz[1], Shannon C. Leonard[1], Ashish Kalra[1], Ninfa L. Straubinger[2], Charlene M. Thomas[2], Robert M. Straubinger[2], Bryan M. Gillard[3], Michael T. Moser[3], Barbara A. Foster[3], Daryl C. Drummond[1], Stephan G. Klinz[1], Bart S. Hendriks[1], Jonathan B. Fitzgerald[1]

[1]Merrimack Pharmaceuticals, Inc., Cambridge, MA, USA; [2]State University of New York at Buffalo, Buffalo, NY, USA; [3]Roswell Park Cancer Institute, Buffalo, NY, USA

INTRODUCTION

- Liposomal irinotecan (nal-IRI) is designed to extend circulation, resulting in improved pharmacokinetics and enhanced drug exposure in tumors, when compared with free (nonliposomal) irinotecan (see figure below, illustrating improved plasma and tumor PK of nal-IRI in a BxPC3 pancreatic cancer xenograft model).
- Nal-IRI is approved in combination with 5-fluorouracil and leucovorin (5-FU/LV) in patients with metastatic pancreatic ductal adenocarcinoma (PDAC) following progression on a gemcitabine-based regimen.
- Nal-IRI + 5-FU/LV significantly improved overall survival, with a well-characterized toxicity profile, in pretreated patients [1].
- We have previously demonstrated that nal-IRI combined with front-line PDAC standard of care (SOC) agents, 5-FU and oxaliplatin, is tolerable and has anti-tumor activity in mouse models of PDAC ([2], summarized in Figure 3).
- Gemcitabine (Gem) and nab-paclitaxel (nab-P) are additional SOC agents that are highly active in first-line metastatic PDAC, and the combination of Gem with nab-P is a commonly used regimen in this setting [3].



Note: Nal-IRI and nonliposomal irinotecan (IRI) concentrations in this poster are specified in HCl salt concentrations. Nal-IRI 10 mg/kg (salt) is approximate to 9 mg/kg (free base).

- **Here, we further evaluate nal-IRI as a potential backbone of first-line metastatic PDAC by assessing the preclinical anti-tumor activity of nal-IRI relative to, and in combination with, Gem and nab-P.**

EFFECT OF SOC DRUG PRETREATMENT ON NAL-IRI DEPOSITION N TUMORS

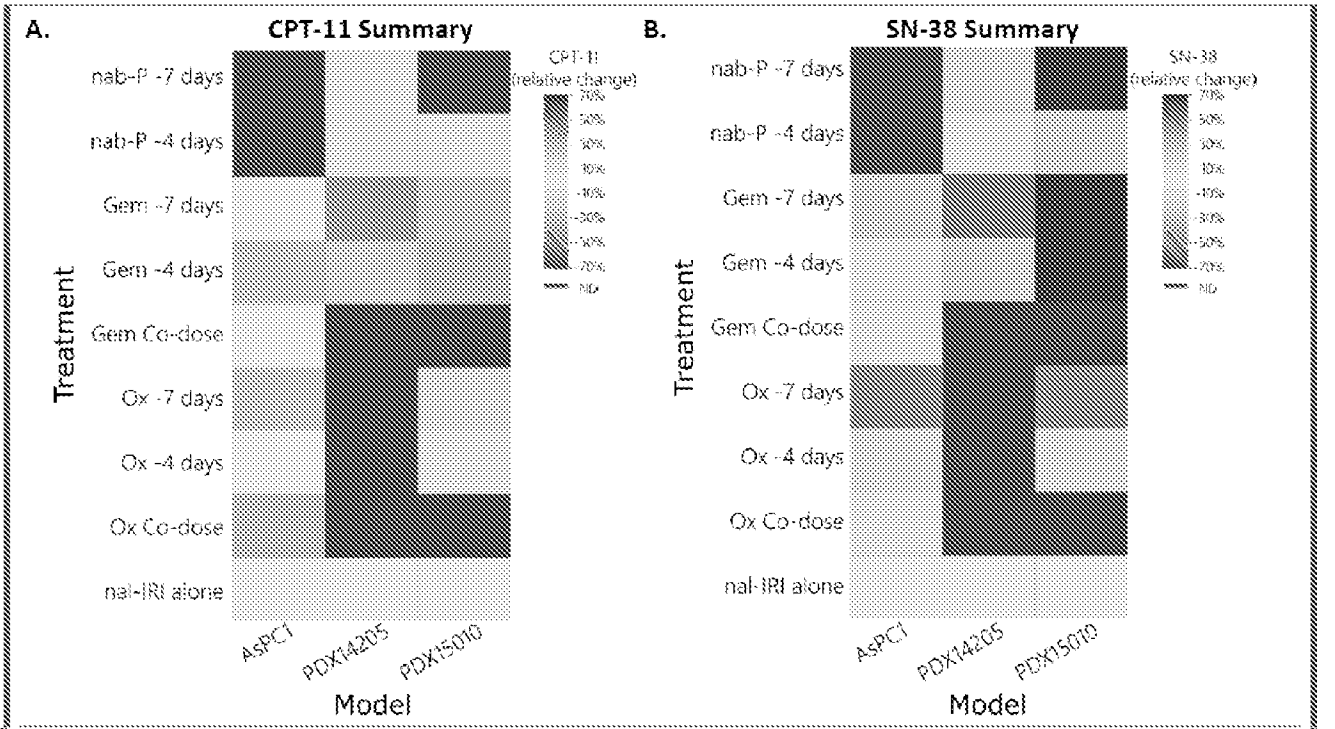


Figure 1. Pretreatment with PDAC SOC agents, including oxaliplatin, Gem and nab-P, improves nal-IRI tumor deposition in pre-clinical PDAC models. Gem (100mg/kg), nab-P (30mg/kg), or oxaliplatin (Ox, 5mg/kg) were co-dosed with nal-IRI, or dosed 4- or 7-days prior to administration of nal-IRI (10mg/kg). Nal-IRI deposition was assessed by HPLC measurement of irinotecan (CPT-11) and its active metabolite, SN-38, in cell line-derived xenograft (CDX; AsPC1) and patient derived xenograft (PDX) models of PDAC. CPT-11 (A) and SN-38 (B) levels are summarized for all models tested based on changes relative to nal-IRI alone. Dark gray boxes indicate the result has not been determined (ND).

NAL-IRI TUMPR DEPOSITION IN A PDX MODEL OF PDAC

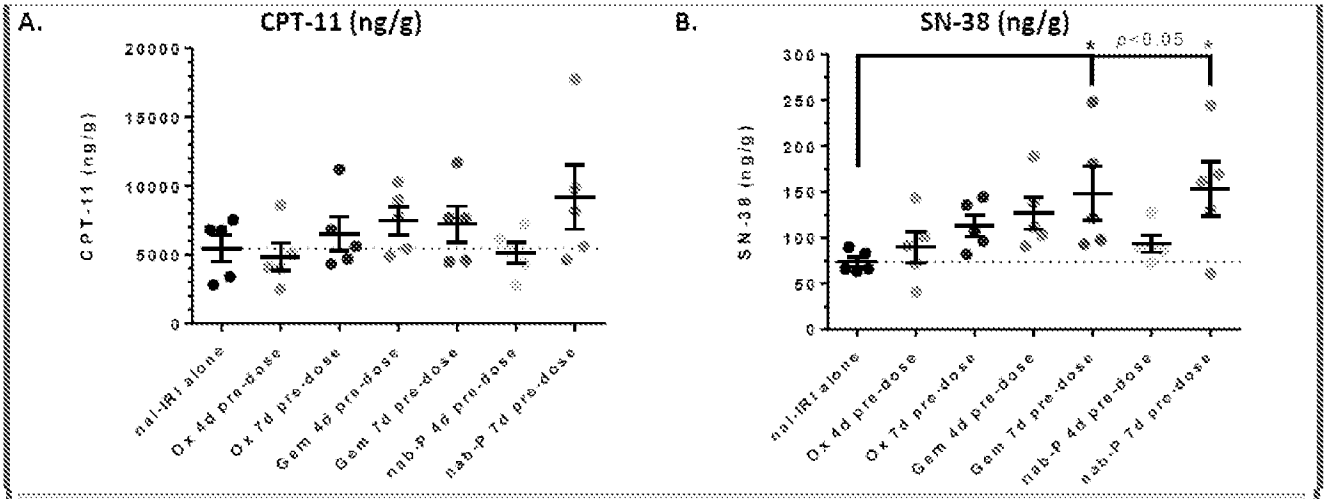


Figure 2. CPT-11 and SN-38 levels in PDX15010 tumors when nal-IRI is administered after Ox, Gem or nab-P.

SUSTAINED RESPONSES TO NAL-IRI IN COMBINATION WITH PDAC SOC AGENTS

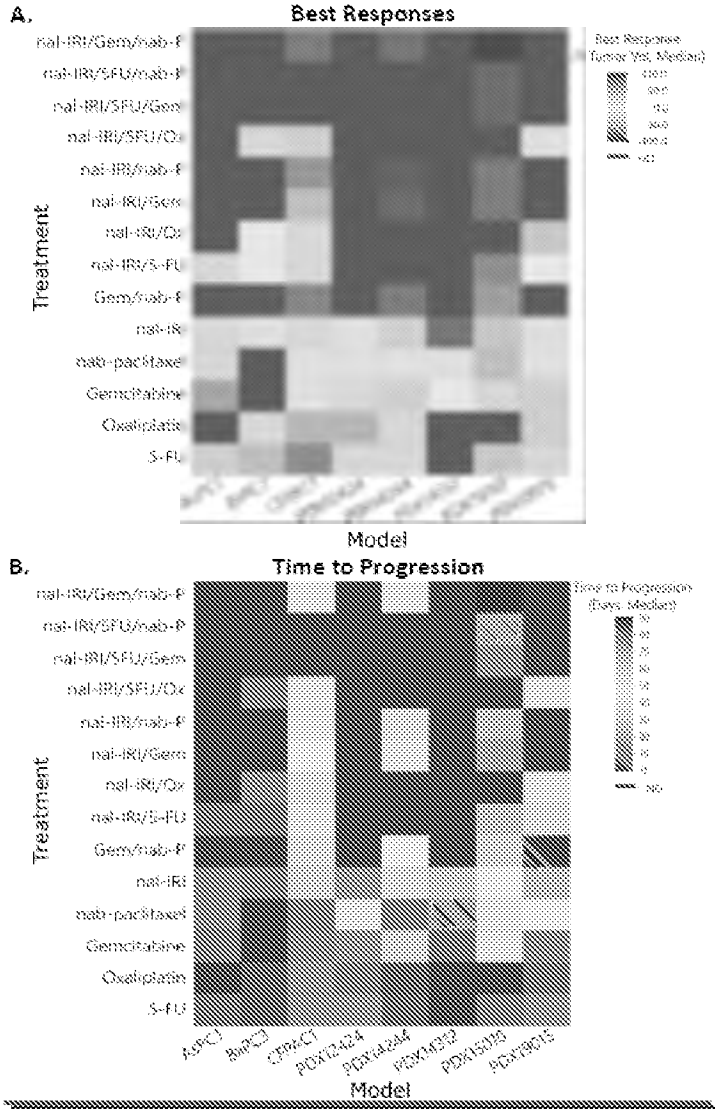


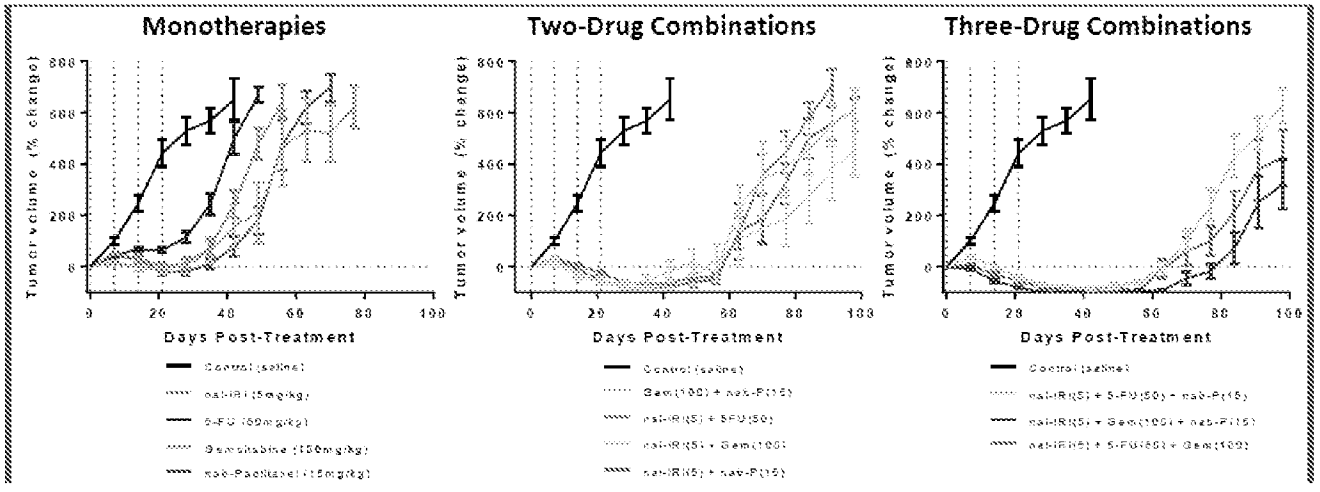
Figure 3. Nal-IRI combinations with 5-FU, Ox, Gem and/or nab-P result in significant and sustained tumor responses across a panel of CDX and PDX models.

A) Monotherapies (5-FU, 50mg/kg; Ox, 5mg/kg; Gem, 100mg/kg; nab-P, 30mg/kg, nal-IRI, 10mg/kg) were evaluated in a panel of 3 CDX and 5 PDX models. Combinations were tested in a subset of models. Combination regimens improved response rates in all models tested, including significant increases in complete responses (CR). Responses to nal-IRI combinations were comparable or improved relative to the combination of Gem + nab-P.

B) The addition of 5-FU, Ox, Gem and/or nab-P to nal-IRI significantly improved duration of tumor growth control and time to progression relative to the respective monotherapies. The durations of response to nal-IRI combinations were consistent or improved relative to the combination of Gem + nab-P.

Dark gray boxes indicate the result has not been determined (ND).

NAL-ITI PRECLINICAL ACTIVITY WITH 5-FU, GEM AND NAB-P



	Control	nal-IRI	5-FU	Gem	nab-P	Gem + nab-P	nal-IRI + 5-FU	nal-IRI + Gem	nal-IRI + nab-P	nal-IRI + 5-FU + nab-P	nal-IRI + Gem + nab-P	nal-IRI + 5-FU + Gem
Best Response = CR (≥95% reduction)	0	0	0	10%	0	50%	22%	67%	63%	67%	100%	67%
Median Time to Progression (days)	7	35	7	43.5	42	63	63	77	70	73	91	77
Median Overall Survival (days)	28	56	45	75	63	88	77	98	84	88	115.5	91

Figure 4. Combinations of 5-FU, Gem and/or nab-P with nal-IRI improve response in PDX15010.

- All two-drug combinations improved response rate and tumor growth control relative to each monotherapy.
- The addition of nal-IRI to Gem + nab-P significantly (p<0.0001) improved tumor growth control and survival relative to Gem + nab-P (n = 8-10 animals per group).
- Nal-IRI + Gem provided comparable tumor growth control as Gem + nab-P, and markedly improved survival.

ALL DRUG COMBINATION WERE WELL-TOLERATED IN MICE

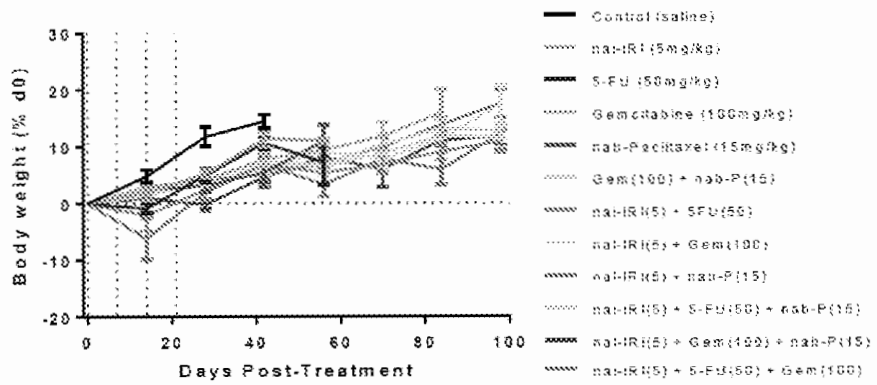


Figure 5. All drug combinations were well-tolerated as determined by body weight change. There was no significant difference in changes in body weight between monotherapies, two-drug combinations or three-drug combinations.

SUMMARY

- **Combinations of nal-IRI with front-line PDAC standard of care agents, including Ox, Gem and nab-P, may enhance nal-IRI tumor deposition in preclinical models.**
- **Nal-IRI combinations with 5-FU, Ox, Gem and/or nab-P result in significant and sustained tumor responses in preclinical models of pancreatic cancer.**
- **Responses to nal-IRI combinations were comparable or improved relative to the combination of Gem + nab-P.**
- **Nal-IRI combinations with 5-FU, Gem and/or nab-P were well-tolerated in mouse models.**
- **Based on these preclinical data, clinical investigation is warranted.**

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2. Gaddy D, et al. 2016. American Association for Cancer Research, Abstract #4830.
3. Von Hoff D, et al. 2013. *NEJM*. 369(18):1691-1703.

Preclinical Anti-tumor Activity of Nanoliposomal Irinotecan (nal-IRI, MM-398) Supports Utilization as a Foundation of Front-Line Pancreatic Cancer Regimens

Abstract #336

Daniel F. Gabby[1], Helen Lee[1], Nancy Paz[1], Shannon E. Leonard[1], Ashish Kalra[1], Nikita L. Straubinger[1], Charlene M. Thomas[1], Robert M. Straubinger[2], Bryan M. Gillard[1], Michael I. Moyer[1], Barbara A. Foster[1], Eleryl C. Drummond[1], Stephen G. Klein[1], Bart S. Hendrikx[1], Johnathan B. Fitzgerald[1]

[1]Merrimack Pharmaceuticals, Inc., Cambridge, MA USA; [2]State University of New York at Buffalo, Buffalo, NY USA; [3]Roswell Park Cancer Institute, Buffalo, NY, USA

CANCERS OF THE PANCREAS, SMALL BOWEL, AND HEPATOBILIARY TRACT

Preclinical antitumor activity of nanoliposomal irinotecan (Nal-IRI, MM-398) and utilization as a foundation of front-line pancreatic cancer regimens.

Daniel F. Gaddy, Helen Lee, Nancy Paz, Shannon C. Leonard, Ashish Kalra, Ninfa L. Straubinger, Robert M Straubinger, Bryan M. Gillard, Michael T. Moser, Barbara A. Foster, Daryl C. Drummond, Stephan G. Klinz, Bart Hendriks, Jonathan B. Fitzgerald

Merrimack Pharmaceuticals, Inc., Cambridge, MA; State University of New York at Buffalo, Buffalo, NY; Department of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, State University of New York at Buffalo, Buffalo, NY; Roswell Park Cancer Institute, Buffalo, NY

Show Less

Abstract Disclosures

Abstract

336

Background: Nanoliposomal irinotecan (nal-IRI, MM-398) recently gained approval in combination with 5-fluorouracil/leucovorin (5-FU/LV) in post-gemcitabine metastatic pancreatic ductal adenocarcinoma (PDAC) based on the extended survival and manageable safety profile observed in the Phase 3 NAPOLI-1 trial. Preclinically, we have previously demonstrated the anti-tumor activity of nal-IRI with 5-FU and oxaliplatin, standard of care agents in first-line PDAC, and are currently investigating this combination in patients with previously untreated metastatic PDAC in a Phase 2 clinical trial (NCT02551991). Herein, we further evaluate nal-IRI as a potential backbone of first-line metastatic PDAC by assessing the preclinical anti-tumor activity of nal-IRI relative to, and in combination with, gemcitabine and nanoparticle albumin-bound-paclitaxel (nab-P). **Methods:** Nal-IRI tumor metabolite (CPT-11 and SN-38) levels were measured in mice treated with nal-IRI in combination with gemcitabine or nab-P. Anti-tumor activity and tolerability of nal-IRI, 5-FU, gemcitabine and nab-P monotherapies and combinations were evaluated using pancreatic cancer cell line (ASPC-1 and CFPAC-1)-derived xenograft models, as well as a panel of five patient-derived xenograft models. **Results:**Administration of gemcitabine or nab-P prior to or simultaneously with nal-IRI resulted in unchanged or increased nal-IRI deposition, as measured by tumor CPT-11 and SN-38 levels at 24 hours post-injection. Moreover, in both cell

line-derived and patient-derived xenograft models of PDAC, nal-IRI monotherapy demonstrated comparable or improved anti-tumor activity relative to gemcitabine or nab-P monotherapies. Further, nal-IRI consistently improved tumor growth inhibition and survival when used in combination with either 5-FU, gemcitabine and/or nab-P, relative to the combination of gemcitabine plus nab-P. All treatments were well-tolerated in these preclinical models. **Conclusions:** These findings illustrate the compatibility and therapeutic potential of nal-IRI as a foundation of first-line PDAC combination regimens, and warrant clinical evaluation.

Limited information was available linking disease severity to QoL. **CONCLUSIONS:** In studies of patients receiving treatment for recurrent or metastatic SCCHN, median OS did not differ systematically among populations receiving regimens containing cetuximab, docetaxel, methotrexate, or paclitaxel. Among platinum-refractory patients, no treatment was identified as having demonstrated significant improvements in QoL.

PCN26

LIPEGFILGRASTIM FOR REDUCTION OF CHEMOTHERAPY-INDUCED NEUTROPENIA RELATED EVENTS: A META-ANALYSIS

Bond TC[1], Mueller U[2], Barnes G[3], Gennero R[4], Tang B[5], Schwartzberg L[5]
[1]Covance Market Access, Gaithersburg, MD, USA, [2]Teva Pharmaceutical, Ulm, Germany, [3]Teva Pharmaceutical, Frazer, PA, USA, [4]Global Health Economics and Outcomes Research - Growth Markets, Miami, FL, USA, [5]The West Clinic, Memphis, TN, USA

OBJECTIVES: The purpose of the current meta-analysis was to compare the efficacy of lipegfilgrastim (LIP) to pegfilgrastim (PEG) and filgrastim (FIL). **METHODS:** EMBASE was searched for head-to-head trials examining the efficacy of LIP, PEG, or FIL. Outcomes included incidence of febrile neutropenia (FN), incidence of severe neutropenia (SN), duration of SN (DSN), and time to recovery of absolute neutrophil count (ANC). Direct comparisons of SN/FN between LIP and PEG were made using random-effects models estimating relative risk (RR). No trials directly compared LIP and FIL; indirect comparisons were made with PEG or placebo/no treatment (PLA) as the common comparator. For DSN/ANC recovery, generic inverse variance methods were employed. **RESULTS:** Sixty-five studies were identified and 24 were included after full-text review and quality assessment via PRISMA criteria. Over all treatment cycles, LIP was non-inferior to PEG for risk of FN (RR 0.34, 95% CI: 0.05, 2.14). The indirect estimate of FN for LIP versus FIL was also non-significant (RR 0.22, 95% CI: 0.03, 1.51). For SN during cycle 1, LIP had a RR of 0.80 (95% CI: 0.63, 1.03) versus PEG and 0.79 (95% CI: 0.61, 1.03) versus FIL. For subsequent cycles, the RR was 0.53 (95% CI: 0.35, 0.79) LIP versus PEG and 0.45 (95% CI: 0.27, 0.75) versus FIL. Time to ANC recovery was significant: -1.75 days (95% CI: -2.61, -0.90) for LIP versus PEG and -1.88 days (95% CI: -2.82, -0.95) for LIP versus FIL. No comparions were significant for DSN. **CONCLUSIONS:** LIP showed non-inferiority to PEG for risk of at least one FN episode and SN in cycle 1. LIP was more effective than both PEG and FIL for prevention of SN in cycles 2-4 and reduced ANC recovery time. However for DSN differences were not significant. These results suggest that LIP is a possibly more effective treatment.

PCN27

COMPARATIVE EFFECTIVENESS OF GRANULOCYTE COLONY-STIMULATING FACTORS (G-CSF) FOR REDUCING INCIDENCE OF FEBRILE NEUTROPENIA (FN) -RELATED HOSPITALIZATION: A RETROSPECTIVE COHORT STUDY USING GERMAN CLAIMS DATA

Wetten S[1], Li X[2], Haas J[3], Worth C[3], Jacob C[3], Braun S[3], Taivelekis S[3]
[1]Amgen Ltd, Uxbridge, UK, [2]Amgen Inc, Thousand Oaks, CA, USA, [3]Xcenda GmbH, Hannover, Germany, [4]Amgen Ltd, Zug, Switzerland

OBJECTIVES: Effectiveness of daily G-CSF prophylaxis can be decreased when given in short courses. The objective was to determine the difference in odds of FN-related hospitalizations with once per cycle G-CSF (pegfilgrastim) prophylaxis compared to daily G-CSF (filgrastim/lenograstim) prophylaxis for patients receiving high/intermediate FN-risk chemotherapy for breast cancer or Non-Hodgkin lymphoma (NHL). **METHODS:** This retrospective cohort study used claims data from the Health Research Institute research database with <4 million insured individuals in Germany. Patients receiving first-line, high/intermediate FN-risk chemotherapy for breast cancer or NHL from January 1, 2009 to December 31, 2013 were included and those cycles with G-CSF administration initiated ≤5 days following chemotherapy were assessed. G-CSF types were identified by ATC codes and FN-related hospitalizations within each cycle were identified by ICD-10-GM codes with a primary/secondary diagnosis of neutropenia (D70.1*, D70.7). Odds ratios (OR) for FN-related hospitalization and 95% confidence intervals (CI) were estimated with generalized estimating equation models and adjusted for age, gender, tumour type, metastatic status, cycle number, chemotherapy FN-risk and history of anaemia and surgery. **RESULTS:** In total, 2,278 patients representing 7,918 cycles (6316 pegfilgrastim, 1602 daily G-CSF) were included in the analysis; 2,037 (89%) patients had breast cancer and 241 (11%) had NHL. More than half of patients receiving pegfilgrastim prophylaxis initiated it in cycle 1, primary prophylaxis, (56%) whereas 37% of patients receiving daily G-CSF prophylaxis initiated it in cycle 1. Three-quarters of patients receiving daily G-CSF were prescribed 5 or less doses in at least one cycle. Cycles with prophylactic daily G-CSF were associated with an increased risk of FN-related hospitalisations (adjusted OR=2.19, 95% CI: 1.41-3.39; p-value < .001) in comparison to cycles with prophylactic pegfilgrastim. **CONCLUSIONS:** This comparative effectiveness analysis showed a significantly higher likelihood of FN-related hospitalizations in cycles with daily G-CSF prophylaxis versus those with pegfilgrastim prophylaxis.

PCN28

ANALYSIS OF ERIBULIN MESYLATE DOSING MODIFICATIONS IMPACT ON ADMINISTRATION PERSISTENCE IN PATIENTS WITH METASTATIC BREAST CANCER (MBC)

Feinberg BA[1], Dreming J[2], Garofalo DF[1], Lal LS[3], Montgomery J[2]
[1]Cardinal Health, Dublin, OH, USA, [2]Cardinal Health, Dallas, TX, USA, [3]Cardinal Health, Missouri City, TX, USA

OBJECTIVES: Eribulin mesylate is a microtubule inhibitor FDA approved for patients with MBC after treatment with at least two prior chemotherapeutic regimens. The recommended dose of eribulin is 1.4 mg/m2 administered on Days 1 and 8 of a 21-day cycle with options for dose modification (dose reduction/dose delay) based on severity and duration of specific toxicities. Recent studies, limited to the clinical trial setting, have shown dose modifications lead to greater treatment persistence and improved patient outcomes. This study utilized real-world claims data to evaluate the relationship between dose modifications and persistence among patients that receive 5 or more administrations. **METHODS:** Using data from the Cardinal Health Specialty Solutions Revenue Cycle Management medical claims database, 267 patients who received 5 or more eribulin administrations and completed therapy between May 2014 and April 2015 were included in the analyses. The Relative Dose Intensity (RDI) methodology compared the intensity of dose received per day of treatment against expected dose (recommended dose) intensity. RDI values and total number of eribulin administrations were calculated for each patient based on the presence or absence of either dose reduction and/or dose delay. Data was analyzed using an independent samples t-test. **RESULTS:** An analysis of patient distribution revealed the mean number of eribulin administrations was 13.4 with a mean RDI of 85%. Persistence was statistically higher in patients that had eribulin therapy managed through dose delay and dose reduction strategies. Patients with no modification (100% RDI) received an average of 8.1 eribulin administrations. Patients with dose modification (81% RDI) received an average of 14.5 eribulin administrations ($p < 0.001$). **CONCLUSIONS:** Management of eribulin therapy in patients with MBC via dose delay and/or reduction resulted in a statistically significant increase in persistence among responding patients.

PCN29

A SYSTEMATIC LITERATURE REVIEW TO IDENTIFY AND COMPARE CLINICAL TRIALS EVALUATING NOVEL THERAPEUTIC AGENTS IN POST-GEMCITABINE ADVANCED PANCREATIC CANCER

Gaddy DF[1], Becker C[1], Li H[2], Bennett R[1], Yang Y[2], Fitzgerald JB[1], Bayever E[2]
[1]Merrimack Pharmaceuticals, Inc., Cambridge, MA, USA, [2]Baxalta, Inc., Cambridge, MA, USA

OBJECTIVES: There is currently no standard of care for patients with advanced pancreatic cancer (APC), including locally advanced and metastatic disease, who progressed following first-line therapy. Available treatment options have been limited by a lack of therapeutic breakthroughs, and primarily utilize different combinations and dosing schedules of established chemotherapeutic agents. The current review assesses the relative efficacy of new therapeutic agents tested, alone or in combination, since 2003 in patients with APC who progressed following gemcitabine-based therapy. **METHODS:** A systematic literature review was performed in PubMed/MEDLINE, EMBASE and ASCO meeting abstracts between January 2003 and June 2015. This review identified randomized controlled trials (RCTs) and single-arm trials evaluating new post-gemcitabine regimens in patients with APC. **RESULTS:** A total of 34 trials, evaluating 1263 patients, were identified. New agents that have been tested include small molecules (24 trials), antibodies (3 trials), nanotherapeutics (4 trials), and immunotherapies (3 trials). The majority of studies were small, single-arm trials (n=27). RCTs (n=7, enrolling 835 patients) were further investigated as they represent the standard for demonstrating therapeutic efficacy. At the time of analysis, the only Phase 3 RCT to evaluate a new therapeutic agent in post-gemcitabine APC was the NAPOLI-1 trial (nanoliposomal irinorecan (MM-398, nal-IRI) + 5-fluorouracil and leucovorin (5FU/LV) versus 5FU/LV), which was a large, global study that demonstrated a statistically significant improvement in overall survival in patients with metastatic disease, including heavily-pretreated patients. **CONCLUSIONS:** The present review highlights the limited number of RCTs evaluating new therapeutic agents in patients with APC who previously received gemcitabine. Most new agents fail to be evaluated beyond small, uncontrolled trials of APC. Despite much research in this difficult-to-treat patient population with high unmet medical need, only one Phase 3 RCT of a new agent (nal-IRI + 5FU/LV demonstrated significant improvement in overall survival in patients with APC who had progressed following gemcitabine-based therapy.

PCN30

A REAL-WORLD ANALYSIS OF KOREAN NATION-WIDE DATABASE: PATTERN, ADHERENCE, AND ASSOCIATED HEATLHCARE COSTS OF IMATINIB AMONG PATIENTS WITH CHRONIC MYELOID LEUKEMIA

Shin S[1], Lee J[2], Kim J[1], Shin M[1], Kwon H[1]
[1]National Evidence based Health-care Collaborating Agency, Seoul, South Korea, [2]National Evidence-based Healthcare Collaborating Agency (NECA), Seoul, South Korea

OBJECTIVES: This study aimed to determine the demographic features, treatment pattern, medication adherence, survival rates and associated healthcare costs in patients with newly diagnosed Ph+ CML from Korean National health insurance (NHI) claims database. **METHODS:** We conducted a longitudinal analysis of patients with newly diagnosed Ph+ CML (ICD-10: C92.1) and started treatment with imatinib in 2005 enrolled in the Korean NHI program. Patients were excluded if they had ≥ 1 claim with a diagnosis of other cancer within one year before diagnosis of CML. All data were retrieved from the NHI Database provided by National Health Insurance Corporation in Korea. **RESULTS:** In the study, a total of 8,986 patients with a diagnosis of Ph+ CML between January 1, 2004 and December 31, 2013 were identified. Among them, our study population consisted 268 patients (mean age: 46.4±14.7 years, male: 57.4%) with the diagnosis of CML in 2005. The majority of patients (75.9%) initiated imatinib therapy at a starting dose was 400mg/day. With over 7 years of follow-up data, based on the 180-day gap definition of discontinuation, 33 (11.7%) patient was discontinued and discontinuation period was 395.4±137.2 days (range: 189-1,023). Overall, 44.3% (n=125) of patients were defined as Good Medication Possession Ratio (MPR) (≥ 90%) and 19.2% (n=54) were as Poor MPR (<70%). During follow-up period, 69 patients (24.5%) were deceased and the time to death for them was 3.18 years (1,159.5 ± 845.1 days) after initiation of imatinib. Patients with Good MPR had significantly higher survival compared to patients with Poor MPR ($p<0.001$). **CONCLUSIONS:** In a retrospective assessment of a large cohort of patients with CP-CML treated with imatinib, we have shown that nonadherence to therapy is important factor for survival. Adherence to therapy must be included as an important evaluation parameter in all future studies of CML.

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Second-line chemotherapy in advanced pancreatic carcinoma: a multicenter survey of the Gruppo Oncologico Italia Meridionale on the activity and safety of the FOLFOX4 regimen in clinical practice

V. Gebbia[1]*, E. Maiello[2], F. Giuliani[3], N. Borsellino[4], M. Caruso[4], G. Di Maggio[2], F. Ferraù[5], R. Bordonaro[6], F. Verderame[7], P. Tralongo[8], L. Di Cristina[9], R. Agueli[1], P. Russo[1] & G. Colucci[3]

[1]*Department of Experimental Oncology and Clinical Applications, University of Palermo, La Maddalena Clinic for Cancer, Palermo;* [2]*IRCCS, Casa Sollievo della Sofferenza, San Giovanni Rotondo;* [3]*Istituto Oncologico, Bari;* [4]*Centro Catanese di Oncologia---Humanitas, Catania;* [5]*Ospedale San Vincenzo, Taormina;* [6]*Ospedale Garibaldi, Catania;* [7]*Ospedale Civile, Sciacca;* [8]*Ospedale De Maria, Avola;* [9]*Ospedale di Castelvetrano, AULS 9, Trapani, Italy*

Background: In daily clinical practice second-line chemotherapy (SLCT) is frequently given to patients with advanced pancreatic cancer failing gemcitabine-based first-line chemotherapy without solid scientific support.
Patients and methods: A retrospective survey was carried out including 42 patients. Patients received standard FOLFOX4 regimen biweekly until progression or unacceptable toxicity.
Results: Six partial responses (14%) and 16 stabilizations (38%) were recorded for a tumor growth control rate of 57%. The median time to progression (TTP) was 4 months (range 1–7 months), and median overall survival (OS) was 6.7 months (range 2–9 months). A stabilization of performance status (PS) and a subjective improvement of cancer-related symptoms were recorded in 27 patients.
Conclusions: Data presented in this paper support the use of FOLFOX4 regimen in the second-line treatment of adenocarcinoma of the pancreas patients. The use of SLCT, however, should be carefully proposed to patients with good PS or those who had a good response to first-line therapy.
Key words: FOLFOX4 regimen, pancreatic carcinoma, second-line chemotherapy

introduction

Although survival in patients with locally advanced adenocarcinoma of the pancreas (APC) is still largely unsatisfactory, however, the availability of gemcitabine (GEM) has represented a major progress in the medical management of APC. In fact, GEM-based chemotherapy (CT) represents the gold standard for systemic treatment of advanced pancreatic cancer for the majority of patients, being able to improve cancer-related symptoms and patient's PS and to confer a modest survival advantage [1]. Combination regimens incorporating GEM, cisplatin (CDDP), 5-fluorouracil (5-FU), oxaliplatin (OXP), or irinotecan (CPT11) have generally shown to improve outcomes in objective response rates but with little or no improvement in survival parameters in phase III trials [1, 2]. Today a significant percentage of APC patients progressing after GEM-based CT are still in relatively good clinical conditions and may require a second- and even a third-line therapy. 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.

Phase II trials evaluating second-line chemotherapy (SLCT) in patients failing GEM-based CT are relatively scarce in medical literature. Some agents, such as paclitaxel, OXP, CPT11, capecitabine (CAP), rubitecan, pemetrexed, and flutamide, have been tested as single agents [1, 2]. Other drugs shown to be active in first-line have been tested in combination regimens as SLCT. At present, there is no standard SLCT for patients who have become refractory to GEM, although a recently reported study has indicated that the OXP/5-FU/leucovorin regimen is superior to best supportive care (BSC) in these patients [1, 2].

In this paper we report a retrospective survey of the efficacy and toxicity of SLCT employed in daily clinical practice in a series of unselected patients affected by APC progressing after GEM-based first-line treatment.

patients and methods

patient population

Enrolled patients had to shown APC progressing after GEM-based first-line treatment. Patients were enrolled into the study if they satisfied the

*Correspondence to: Dr V. Gebbia, Department of Experimental Oncology and Clinical Applications, University of Palermo, La Maddalena Clinic for Cancer, via san Lorenzo Colli n. 312d, Palermo, Italy. Tel: +3909168806710; Fax: +3909168806806; E-mail: vittorio.gebbia@tin.it

following inclusion criteria: (i) histological diagnosis of APC; (ii) bidimensionally measurable disease [World Health Organization (WHO) criteria]; (iii) age between 18 and 75 years; (iv) Eastern Cooperative Oncology Group (ECOG) PS of two or more; (v) absence of severe uncontrolled cardiovascular, metabolic, infectious, or neurological diseases; (vi) informed written consent; and (vii) adequate bone marrow, hepatic, and renal function as previously described [3].

pretreatment evaluation

Staging procedures consisted of medical history, physical examination, Electrocardiogram (EKG), peripheral blood cell counts, serum chemistry panel, CEA 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 WHO criteria [3]. The sum of PR and SD was reported as tumor growth control rate (TGCR). TtP was estimated from the date of first treatment to the first evidence of PD. OS was estimated from the date of first treatment 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.

treatment schedule

The CT schedules were as follows: intravenous (i.v.) OXP 85 mg/m^2 on day 1, i.v. levo-leucovorin at a dose of 100 mg/m^2, and i.v. bolus 5-FU at a dose of 400 mg/m^2 and continuous intravenous infusion (c.v.i.) 5-FU at a dose of 600 mg/m^2 on day 1 and day 2 every 2 weeks. Treatment was biweekly administered 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. 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 previously described [3]. OXP was reduced in the event of persistent paresthesia/dysesthesia between cycles or with pain lasting for >7 days according to cancer center guidelines and staff physician's decision. When paresthesia/dysesthesia with either pain or functional impairment persisted between cycles, OXP was discontinued.

statistical analysis

Objective responses were reported as their relative rates adjusted to the nearest unit with 95% confidence interval. TtP and OS were calculated as previously described [3].

results

patient characteristics

Forty-two patients were collected from nine Gruppo Oncologico Italia Meridionale centers (Table 1). There were 26 males and 16 females with a median age of 63 years and a median PS of one according to the ECOG scale. Five patients (12%) had a PR to front-line treatment, and 16 patients (38%) had SD. All patients except seven had stage IV APC, and 62% had multiple sites of disease. Liver metastases were present in 67% of patients, locoregional lymph nodes in 57%, lung metastases in 14%, and peritoneal carcinomatosis in 33% of patients.

Table 1. Patients' clinical and demographical characteristics

No. of patients	42 (100%)
Median age (range)	64 (40–76)
Sex	
Male	26 (62%)
Female	16 (38%)
PS (ECOG)	
Median	1
PS 0	2 (05%)
PS 1	24 (57%)
PS 2	16 (38%)
Stage	
III	7 (17%)
IV	35 (83%)
Previous surgery	4 (9%)
Previous RT	None
Previous CT	
GEM	23 (55%)
GEMOX	1 (02%)
GEM/CDDP	18 (43%)
Response to previous CT	
PR	5 (12%)
SD	16 (38%)
PD	21 (50%)
Clinical benefit response to previous chemotherapy	
Yes	15 (36%)
No	27 (64%)
Site of disease	
Pancreas	40 (95%)
Lymph node	24 (57%)
Liver	28 (67%)
Peritoneum	14 (33%)
Lung	6 (14%)
Other	4 (9%)

CDDP, cisplatin; CT, chemotherapy; ECOG, Eastern Cooperative Oncology Group; GEM, gemcitabine; GEMOX, gemcitabine and oxaliplatin; PD, progressive disease; PR, partial response; PS, performance status; RT, radiotherapy; SD, stable disease.

antitumor activity and survival

As shown in Table 2 there were six objective PR (14%), and 16 patients (38%) had an SD for a TGCR of 62%. No complete response was recorded. Median duration of PR was 5.4 months (range 2–8). Median TtP from the start of second-line treatment was 4 months (range 1–7 months). No correlation has been found between length of TtP during first-line CT and length of TtP in SLCT or objective response. It had been proposed that the activity of an SLCT could be documented by showing that the TtP following SLCT is longer than the TtP following front-line therapy in each single patient. The ratio of TtPs has been defined as the growth modulation index (GMI) with each patient being his own control [4]. A GMI >1 means that TtP was longer with the SLCT and treatment that produces a GMI ≥1.33 (33%

Table 2. Activity and survival parameters

No. of patients	42 (100%)
CR	0
PR	6 (14%)
SD	16 (38%)
PD	20 (47%)
TGCR	24 (57%)
Clinical benefit	27
Median TtP (months)	4 (range 1–7)
GMI (≥1.33)	11 (26%)
Median OS (months)	6.7 (range 2–9)

CR, complete response; GMI, growth modulation index; OS, overall survival; PD, progressive disease; PR, partial response; SD, stable disease; TGCR, tumor growth control rate; TtP, time to progression.

improvement) should be considered to have excellent activity [5]. In this series 11 patients (26%) had a GMI >1.33, and two further patients had a GMI = 1. Median OS from the start of front-line therapy was 6.7 months (2 to 9+ months). Twenty-seven patients (64%) had a stabilization of PS or a subjective improvement of cancer-related symptoms.

tolerability and safety

Patients had received between 2 and 12 cycles. Median delivered dose intensity was higher than 90% in the whole series. Most patients received full treatment. Twenty patients had some treatment delay, but only three patients experienced more than two cycle delay. Reasons for delay were not treatment related in six cases. Reasons for treatment discontinuation were PD in all patients but three cases. Two patients had grade 3–4 toxicity which precluded continuation of CT and one patient refused to continue for psychological distress.

Overall, side-effects were moderate and easily manageable. Grade 3 anemia was recorded in 14% of patients, grade 3–4 neutropenia occurred in 17% of patients (two cases of febrile neutropenia). Thrombocytopenia occurred in seven patients and was severe in three cases (7%) with massive liver disease. Most of the non-hematological symptoms were mild being less than grade 3. Mild OXP-related peripheral sensory neurotoxicity occurred only in five patients (12%) most probably due to the low median number of CT cycles administered. Mild hand-foot syndrome was observed in two cases.

discussion

Several GEM-based regimens have been recently tested where GEM is given in association with other agents [1–3]. This paper reports the results of a retrospective survey on the efficacy and safety of the FOLFOX4 regimen as SLCT in a series of 42 nonconsecutive patients progressing after GEM-based first-line therapy. In this series of unselected patients the FOLFOX4 regimen yielded a 14% PR rate with 38% of patients showing SD for a TGCR of 57%. Median duration of PR was 5.2 months, while median TtP and OS were 4 and 6.7 months, respectively. The activity in terms of GMI (GMI ≥ 1.33 in 26% of cases) in the evaluation of palliative treatment of APC is interesting, but it should be interpreted with caution.

Overall, these data support the use of SLCT in patients with good PS progressing after a front-line CT. These data should, however, be interpreted with caution because SLCT has been evaluated in a few trials. Various OXP-based regimens have been tested in seven phase II trials involving relatively small numbers of patients. The impact of the addition of OXP to GEM was tested in 31 patients failing single-agent GEM who achieved a PR in 23% of cases and an SD >2 months in 35% of cases [6]. Median TtP and OS were 4.2 and 6.0 months, respectively. Other investigators carried out a phase II trial involving 18 patients treated with OXP and c.v.i. 5-FU [7]. There were no PR and three patients (17%) had an SD with poor survival. In a series of 41 patients treated with raltitrexed/OXP, 10 patients (24%) yielded a PR and 11 had SD with a progression-free survival (PFS) at 6 months of 14.6%, and a median OS of 5.2 months [8]. A clinically relevant improvement of quality of life was observed in numerous domains. OS was significantly longer in patients with previous PFS >6 months and in patients without pancreatic localization, raising the issue of selecting patients who may be more likely to benefit from salvage treatment. A weekly combination of OXP, leucovorin, and 5-FU has been tested in a phase II study in a series of 30 patients achieving a PR in 23.3% of cases, and SD in 30.0% of patients with a median OS of 25 weeks and an improvement in PS in 43% of patients [9]. Patients who had responded to first-line GEM were found more likely to respond or stabilize their disease with SLCT. A phase II trial of OXP plus CAP in a series of 41 patients reported a PR in one case and SD in eight patients with a median OS of 5.8 months, and a 6-month and 1-year survival rate of 48% and 22%, respectively [10]. Toxicity was, however, significant. Preliminary results of another trial of OXP/5-FU in a series of 23 patients have shown an OS of 4 months [11]. Finally, it is worth mentioning the phase III trial by Oettle et al. [12], comparing OXP/5-FU/folinic acid with BSC in GEM-refractory APC. Median OS of SLCT was 21 weeks in the treatment arm compared with 10 weeks in the BSC arm ($P = 0.007$). By demand from participating centers the BSC arm was thus closed early.

Because of the good activity shown by camphotecins, CPT11-containing regimens have also received significant attention. In a trial including 25 patients treated with an OXP/CPT11 combination, one patient had a PR and six patients (24%) attained a CBR [13]. Median survival from the start of this regimen was 5.6 months. A series of 38 patients were treated with raltitrexed/CPT11, reporting a 16% ORR and 32% SD rate with a median PFS of 4.0 months and a median OS of 6.5 months [14]. A retrospective evaluation of the four-drug G-FLIP (gemcitabine, 5-fluorouracil, leucovorin, and cisplatin) regimen reported a TGCR of 47% with 24% of PR among 34 patients [4]. Median TtP was of 3.9 months and the OS of 10.3 months. A retrospective analysis of 20 consecutive patients treated with GEM/5-FU/leucovorin/CDDP every 2 weeks reported two patients with a PR and two with an SD despite PD with prior GEM-based therapy [15]. These data support the hypothesis that adding a single new drug

such as CPT11 to the same first-line CT upon disease progression may be an important alternative for the treatment of relapsed/resistant cancer.

In conclusion, data presented in this paper support the use of FOLFOX4 regimen in the second-line treatment of APC patients. Data from medical literature support the use of SLCT, although its use should be carefully proposed to patients with good PS or those who had a good response to first-line therapy. A prospective randomized trial using a controlled arm without treatment would be needed to definitely demonstrate and validate the place of second-line treatment in progressing APC.

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ORIGINAL REPORT

PANCREOX: A Randomized Phase III Study of Fluorouracil/Leucovorin With or Without Oxaliplatin for Second-Line Advanced Pancreatic Cancer in Patients Who Have Received Gemcitabine-Based Chemotherapy

Sharlene Gill, Yoo-Joung Ko, Christine Cripps, Annie Beaudoin, Sukhbinder Dhesy-Thind, Muhammad Zulfiqar, Pawel Zalewski, Thuan Do, Pablo Cano, Wendy Yin Han Lam, Scot Dowden, Helene Grassin, John Stewart, and Malcolm Moore

Sharlene Gill and Malcolm Moore, BC Cancer Agency, Vancouver; Muhammad Zulfiqar, BC Cancer Agency, Abbotsford; Thuan Do, BC Cancer Agency, Surrey; Wendy Yin Han Lam, Burnaby Hospital Cancer Centre, Burnaby, British Columbia; Yoo-Joung Ko, Sunnybrook Health Sciences Centre; Malcolm Moore, Princess Margaret Hospital, Toronto; Christine Cripps, Ottawa Hospital Cancer Centre, Ottawa; Sukhbinder Dhesy-Thind, Juravinski Cancer Centre, Hamilton; Pawel Zalewski, RSM Durham Regional Cancer Centre, Oshawa; Pablo Cano, Sudbury Regional Hospital, Sudbury, Ontario; Annie Beaudoin, Centre Hospitalier Universitaire de Sherbrooke, Sherbrooke; Helene Grassin and John Stewart, Sanofi Canada, Laval, Quebec; and Scot Dowden, Alberta Health Service, Calgary, Alberta, Canada.

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Corresponding author: Sharlene Gill, MD, MPH, British Columbia Cancer Agency, 600, 10th Ave West, Vancouver, British Columbia, Canada V5Z 4E6; e-mail: sgill@bccancer.bc.ca.

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ABSTRACT

Purpose

The standard of care for second-line therapy in patients with advanced pancreatic cancer after gemcitabine-based therapy is not clearly defined. The CONKO-003 phase III study reported a survival benefit with second-line fluorouracil (FU) and oxaliplatin using the oxaliplatin, folinic acid, and FU (OFF) regimen.[1] PANCREOX was a phase III multicenter trial to evaluate the benefit of FU and oxaliplatin administered as modified FOLFOX6 (mFOLFOX6, infusional fluorouracil, leucovorin, and oxaliplatin) versus infusional FU/leucovorin (LV) in this setting.

Patients and Methods

Patients with confirmed advanced pancreatic cancer who were previously treated with gemcitabine therapy and with an Eastern Cooperative Oncology Group performance status of 0-2 were eligible. A total of 108 patients were randomly assigned to receive biweekly mFOLFOX6 or infusional FU/LV until progression. Progression-free survival (PFS) was the primary end point.

Results

Baseline patient characteristics were similar in both arms. No difference was observed in PFS (median, 3.1 months *v* 2.9 months; $P = .99$). Overall survival (OS) was inferior in patients assigned to mFOLFOX6 (median, 6.1 months *v* 9.3 months; $P = .02$). Increased toxicity was observed with the addition of oxaliplatin, with grade 3/4 adverse events occurring in 63% of patients who received mFOLFOX6 and 11% of those who received FU/LV. More patients in the mFOLFOX6 arm withdrew from study due to adverse events than in the FU/LV arm (20% *v* 2%), whereas the use of post-progression therapy was significantly higher in the FU/LV arm (25% *v* 7%, $P = .015$). No significant differences were observed in time to deterioration on the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 global health scale.

Conclusion

No benefit was observed with the addition of oxaliplatin, administered as mFOLFOX6, versus infusional FU/LV in patients with advanced pancreatic cancer previously treated with first-line gemcitabine.

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INTRODUCTION

An estimated 48,960 Americans and 4,800 Canadians were diagnosed with pancreatic cancer in 2015. Because of its high lethality, an almost equal number (40,560 Americans and 4,600 Canadians) will have died of pancreatic cancer in the same year.[1,2] The 5-year survival for all stages is 7.2%, with less than 10% of patients presenting with localized disease.[3]

Gemcitabine has been considered in the first-line management of advanced pancreatic cancer since 1997, based on clinical benefit and a modestly improved survival when compared with fluorouracil (FU).[4] Recent phase III trials have demonstrated the superiority of first-line combinations of FOLFIRINOX (FU, leucovorin [LV], irinotecan, and oxaliplatin), and gemcitabine plus nab-paclitaxel over gemcitabine alone in appropriately selected patients with metastatic disease.[5,6] Given that patients with advanced

pancreatic cancer often present with a significant burden of symptoms and compromised performance status, a combination approach may not be suitable for many patients. As such, there remains an unmet need for therapeutic strategies that can extend survival while minimizing treatment-related toxicities. In particular, for those patients who experience first-line therapy failure, there is increasing interest in understanding the optimal second-line management.

In 2008, the results of the CONKO-003 trial were presented, and these demonstrated an improvement in time to progression (TTP) and overall survival (OS) in gemcitabine-refractory pancreatic cancer with the addition of oxaliplatin to FU.[7] As reported in its subsequent publication in 2014,[8] treatment with FU and LV administered by a 24-hour continuous intravenous (IV) infusion on days 1, 8, 15, and 22 every 6 weeks (FF; n = 76) was compared with FF plus oxaliplatin, 85 mg/m^2 IV on days 8 and 22 (OFF; n = 81). The median OS was improved with the addition of oxaliplatin in the OFF arm (5.9 months *v* 3.3 months; $P = .01$).

Because CONKO-003 represents the only phase III trial to demonstrate a benefit with postprogression oxaliplatin in advanced pancreatic cancer, and because oxaliplatin is more commonly combined with FU and LV using the biweekly infusional fluorouracil, LV, and oxaliplatin (FOLFOX) schedule, a multicenter, randomized phase III trial was conducted to confirm the efficacy and safety of oxaliplatin administered in the modified FOLFOX6 (mFOLFOX6) schedule compared with biweekly infusional FU and LV in patients with advanced pancreatic cancer previously treated with gemcitabine-based therapy.

PATIENTS AND METHODS

Patients

Eligible adult patients had a histologically or cytologically confirmed diagnosis of advanced, unresectable pancreatic cancer with an Eastern Cooperative Oncology Group (ECOG) performance status of 0-2, measurable disease, a life expectancy of longer than 3 months, and adequate hepatic function (defined as total bilirubin < 1.5× the upper limit of normal [ULN], AST lower than 3× the ULN or 5× the ULN in the presence of liver metastases, and ALT lower than 3× the ULN or 5× the ULN in the presence of liver metastases); adequate renal function (defined as a creatinine clearance > 50 mL/min) and adequate hematologic function (defined as neutrophils ≥ 1.5× 10^9/L and platelets ≥ 100× 10^9/L). Patients must have received prior first-line treatment with gemcitabine and confirmed radiographic evidence of disease progression within 4 weeks prior to randomization. Exclusion criteria were prior treatment with oxaliplatin or FU (except as a radiation sensitizer), the presence of peripheral sensory or motor neuropathy greater than National Cancer Institute Common Toxicity Criteria (NCIC-CTC) grade 1; serious cardiac arrhythmia, diabetes, or serious active infection or other illness that would preclude study participation; and prior or current other malignancy within 5 years. The trial was approved by the research ethics boards of each participating center, and all patients provided written informed consent.

Study Design and Treatment

Patients enrolled in this open-label, randomized, two-arm, multicenter phase III trial were randomly assigned (in a 1:1 fashion) to receive infusional FU and LV (FU/LV) or mFOLFOX6. Patients were stratified according to age (< 70 years, ≥ 70 years), sex, ECOG (0, 1, or 2), and presence of liver metastases. The standard arm of infusional FU/LV consisted of a dose of LV 400 mg/m^2 administered as a 2-hour IV infusion on day 1 and FU administered as a bolus IV dose of 400 mg/m^2 on day 1 followed by a 2,400 mg/m^2 continuous infusion for 46 hours, administered every 14 days. mFOLFOX6 consisted of the same plus an oxaliplatin dose of 85 mg/m^2 given as a 2-hour IV infusion on day 1, administered every 14 days. Patients were treated until disease progression, unacceptable toxicity, or patient request.

Assessments

Within 14 days before enrollment, a complete history was taken and a physical examination performed, including neurologic evaluation and assessment of ECOG performance status, in addition to hematology and biochemistry blood work. Neurologic examination and toxicity assessments (in accordance with NCIC-CTC adverse event grading) were completed after each cycle, along with quality-of-life questionnaires composed of the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30 (EORTC-QLQ-C30), to collect overall cancer-specific quality-of-life data. Radiographic tumor assessments by computed tomography or magnetic resonance imaging were performed at baseline, 6 weeks, and 12 weeks, and in the presence of clinical signs of disease progression.

Statistical Methods

The primary end point was progression-free survival (PFS) defined as the time from the start of treatment to disease progression (based on Response Evaluation Criteria in Solid Tumors [RECIST]) or death from any cause. A planned sample size of 64 patients per arm was determined to allow for an 80% chance of detecting a 15% improvement in the rate of PFS at 16 weeks, based on a log-rank statistic and two-sided type I error rate of 0.05. Secondary efficacy end points were OS, overall response rate (ORR) per RECIST criteria, duration of response, and disease control rate. The efficacy analysis was conducted in the intent-to-treat (ITT) population using the log-rank statistic to determine if the Kaplan-Meier curves for PFS were significantly different between the two arms. The relative risk reduction for the experimental arm of the study was reported by hazard ratios (HRs) and 95% CIs determined using the Cox proportional hazards models. Two-sided *P* values were reported. Consistency of effect across predefined patient and disease characteristics was performed by Cox regression analysis. Safety and quality of life were reported using descriptive statistics.

RESULTS

Patients

During the study period from May 2010 to December 2012, 108 patients from 12 centers across Canada were randomly assigned as follows: mFOLFOX6, n = 54; and infusional FU/LV, n = 54 (Fig 1). The study was closed before its target enrollment of 128 patients because of slow accrual in the latter half of the study period. All randomly assigned patients were included in the ITT analysis; five patients in the mFOLFOX6 arm did not receive treatment, due to medical event (n = 3), death before study start (n = 1), and progression (n = 1); one patient in the infusional FU/LV arm did not receive treatment because of death before study start. The safety analysis population consisted of 102 patients: mFOLFOX6 (n = 49) versus infusional FU/LV (n = 53) and the quality-of-life analysis population consisted of 83 patients: mFOLFOX6 (n = 39) versus infusional FU/LV (n = 44).

Baseline characteristics by treatment arm are listed in Table 1. The majority of patients had metastatic disease (mFOLFOX6, 93%; infusional FU/LV, 94%), were previously treated with first-line gemcitabine monotherapy rather than combination therapy

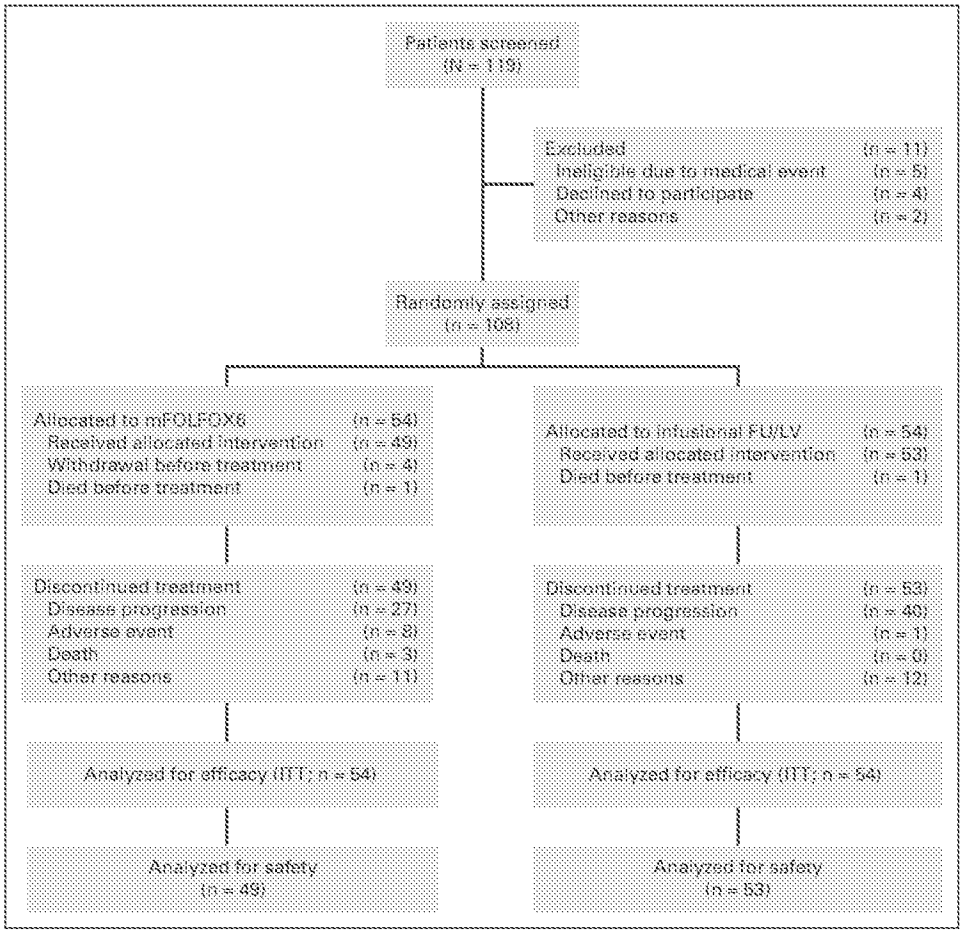

Fig 1. CONSORT diagram. FU/LV, fluorouracil/leucovorin; ITT, intent to treat; mFOLFOX6, modified infusional fluorouracil, leucovorin, and oxaliplatin.

(mFOLFOX6, 74%; infusional FU/LV, 78%), and were ECOG PS 0–1 (mFOLFOX6, 89%; infusional FU/LV, 94%). Characteristics were generally well balanced with the exceptions of patients assigned to the mFOLFOX6 arm having a longer duration of advanced disease and patients assigned to the infusional FU/LV arm having a slightly greater frequency of ECOG PS 0 and presence of liver metastases.

Table 1. Baseline Characteristics

Characteristic	mFOLFOX6 (n = 54) No.	mFOLFOX6 (n = 54) %	Infusional FU/LV (n = 54) No.	Infusional FU/LV (n = 54) %
Median age (range), years	66 (38-82)		67 (48-78)	
< 70	34	63.0	36	66.7
≥ 70	20	37.0	18	33.3
Sex				
Male	31	57.4	30	55.6
Female	23	42.6	24	44.4
Body mass index (kg/m^2)	23.7		24.3	
Stage				
Locally advanced disease	4	7.4	3	5.6
Metastatic disease	50	92.6	51	94.4
Presence of liver metastases	31	57.4	37	68.5
ECOG performance status				
0	7	13.0	10	18.9
1	41	75.9	41	75.5
2	6	11.1	3	5.7
Prior gemcitabine therapy				
Monotherapy	40	74.1	42	77.8
Combination	14	25.9	12	22.2
Median time since diagnosis of advanced disease (months)	7.9		5.7	

Abbreviations: FU/LV, fluorouracil/leucovorin; ECOG, Eastern Cooperative Oncology Group; mFOLFOX6, modified infusional fluorouracil, leucovorin, and oxaliplatin.

However, these differences were not significantly different (all comparative P values > .05).

Efficacy

After a median follow-up of 8.8 months, the median PFS in the ITT population was 3.1 months in the mFOLFOX6 arm and 2.9 months in the infusional FU/LV arm (HR, 1.00; 95% CI, 0.66 to 1.53; log-rank P = .989; Fig 2) The median OS was lower in the mFOLFOX6 arm at 6.1 months versus 9.9 months in the infusional FU/LV arm (HR, 1.78; 95% CI, 1.08 to 2.93); log-rank P = .024; Fig 3). ORRs in the evaluable population were not significantly different between arms, with a partial response rate of 13.2% in the mFOLFOX6 arm versus 8.5% in the FU/LV arm (P = .361). No complete responses were observed. The stable disease rate was 44.7% in mFOLFOX6 versus 55.3% in the FU/LV arm.

In the prespecified subgroup analysis for PFS (Fig 4), the HR point estimates for PFS seemed to favor FU/LV for female sex, whereas mFOLFOX6 was favored for ECOG PS 0; however, the 95% CIs for these variables crossed unity. For the subgroup analysis by age, FU/LV was favored among patients younger than 70 years (n = 70) versus those older than 70 years (n = 70; P = .015). For OS, a significant interaction was again observed for age, with FU/LV favored in patients younger than 70 years old and mFOLFOX6 favored in patients older than 70 years (P = .005). In a post hoc exploratory analysis by age (Table 2), patients younger than 70 years who were randomly assigned to the mFOLFOX6 arm tended to have a more adverse prognostic profile than those randomly assigned to FU/LV, with a trend for a longer time since diagnosis of advanced disease (8.2 months *v* 5.6 months; P = .058) and a greater proportion of patients with PS 2 (12% *v* 3%; P = .053). No such imbalances were observed among patients older than 70 years.

Adverse Events

The overall incidence of NCIC-CTC grade 3/4 adverse events (defined as possibly related treatment-emergent adverse events) was 63% in the mFOLFOX6 arm versus 11% in the FU/LV arm. Incidence of adverse events leading to permanent treatment discontinuation was higher in the mFOLFOX6 arm (10% *v* 0%). Frequencies of grade 3/4 adverse events in the safety-evaluable population are listed in Table 3. There were no treatment-related deaths. A dose reduction of oxaliplatin was required in 45% of patients assigned to mFOLFOX6, most commonly because of hematologic toxicity (77%) and neuropathy (9%). FU dose reductions were more frequent in the mFOLFOX6 arm (45%), with hematologic toxicity as the most commonly cited reason (59%) versus a 15% rate of dose reductions of infusional FU/LV (P = .007). Dose delays were also significantly more common with mFOLFOX6 (77% *v* 47%; P = .003).

Quality of Life

Deterioration of quality of life was defined as a decrease of 10 points or more on the EORTC-QLQ-C30 cancer-specific global health status score. As evaluated in the population assessable for quality of life (n = 74), there were no significant differences observed in the time to deterioration across both treatment arms: 2.2 months for mFOLFOX6 versus 3.8 months for FU/LV (HR, 1.37; 95% CI, 0.73 to 2.57; P = .328) as shown in Appendix Figure A1 (online only).

Study Withdrawal and Postprogression Therapy

Study withdrawal due to disease progression was observed more frequently in the FU/LV arm (75% *v* 55%), whereas

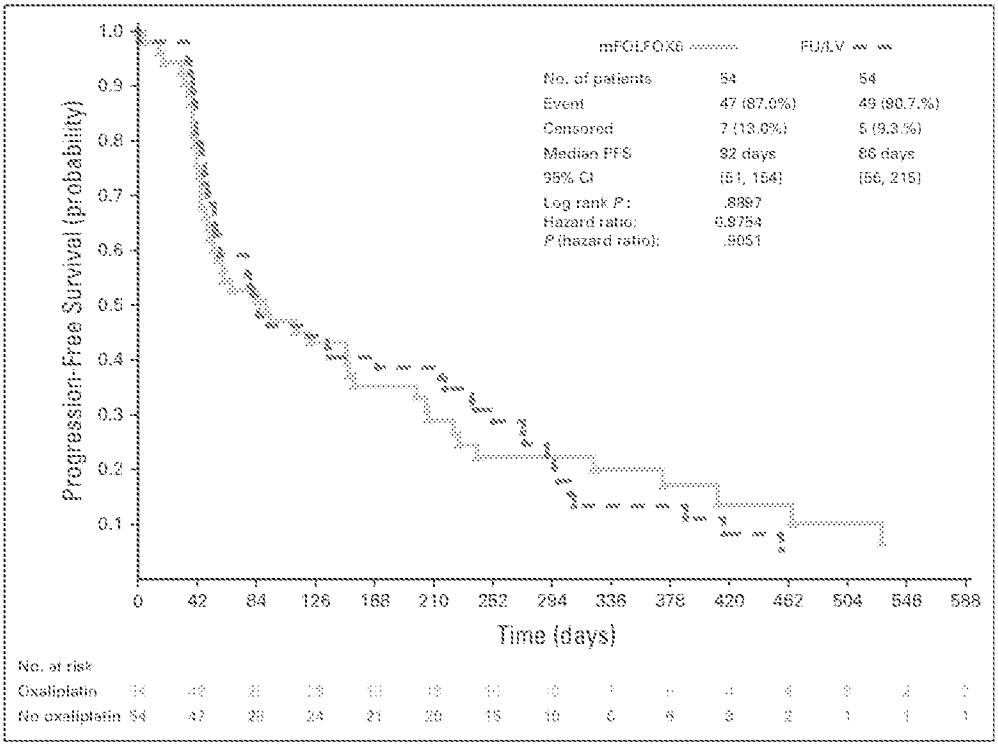

Fig 2. Progression-free survival. FU/LV, fluorouracil/leucovorin; mFOLFOX6, modified infusional fluorouracil, leucovorin, and oxaliplatin; PFS, progression-free survival.

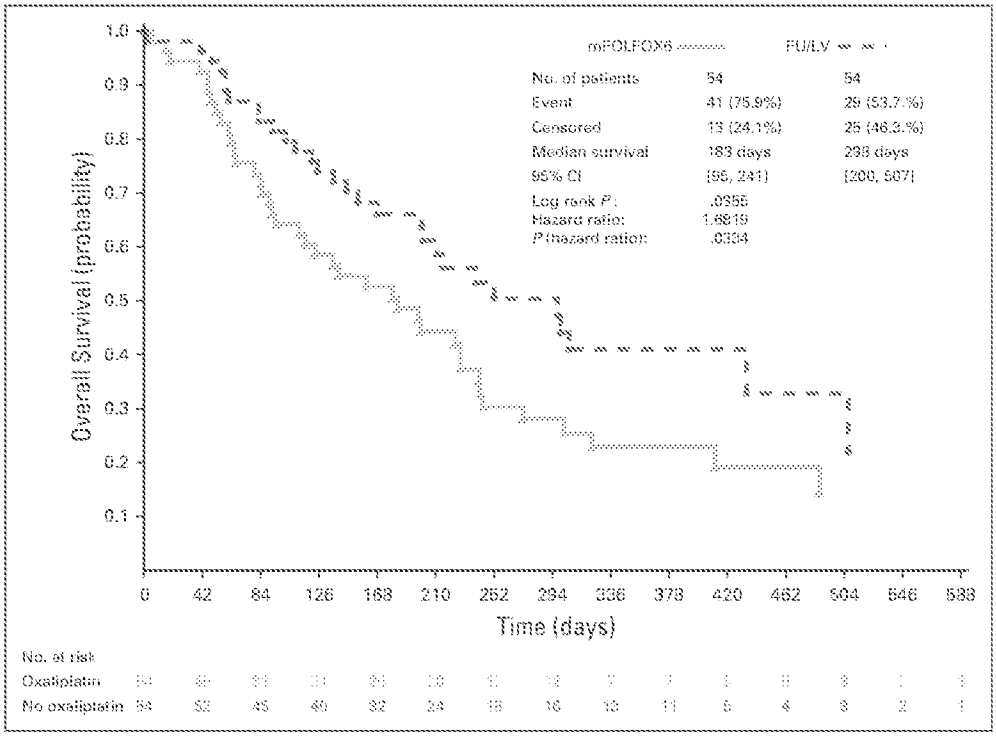

Fig 3. Overall survival. FU/LV, fluorouracil/leucovorin; mFOLFOX6, modified infusional fluorouracil, leucovorin, and oxaliplatin.

withdrawal due to adverse events was greater in the mFOLFOX6 arm (20% *v* 2%). Significantly more patients assigned to FU/LV went on to receive postprogression therapy (23% *v* 7%; $P = .015$). Among patients assigned to the FU/LV arm, the most frequent poststudy treatments included retreatment with FU/LV (33%), FOLFOX (17%), nab-paclitaxel (17%), FOLFIRINOX (8%), and paclitaxel (8%). Among patients assigned to mFOLFOX6, poststudy treatments included FU/LV (33%), capecitabine (33%), and paclitaxel (33%).

DISCUSSION

In this multicenter, open-label, randomized phase III trial in patients with advanced pancreatic cancer previously treated with first-line gemcitabine, no efficacy benefit was observed with the addition of oxaliplatin, administered as mFOLFOX6, versus infusional FU/LV alone. Given the demonstrated benefit of oxaliplatin in the ACCORD trial,[5] when administered as the FOLFIRINOX regimen, our PANCREOX results imply that the indication for using oxaliplatin-based chemotherapy in advanced pancreatic cancer would primarily be in the first-line setting.

The tolerability of the infusional FU/LV arm was remarkably better than the mFOLFOX6 arm, with a nearly six-fold lower incidence of grade 3/4 adverse events (11% *v* 63%). As such, the proportion of study withdrawals due to adverse events in the absence of progression was 10-fold higher in the mFOLFOX6 arm (20% *v* 2%); this suggests that second-line treatment tolerance is of considerable importance in patients with advanced pancreatic cancer. It is noted that the time to deterioration of quality of life, as measured by EORTC-QLQ-C30, was longer at 3.8 months for FU/LV versus 2.2 months for mFOLFOX6; however, this difference was not statistically significant.

Interestingly, for the secondary end point of OS, a more favorable OS was observed in patients randomly assigned to infusional FU/LV. Although treatment characteristics were generally well balanced, the time from diagnosis of advanced disease was shorter in the infusional FU/LV (5.7 months *v* 7.9 months; $P = .199$). More importantly, the frequency of postprogression therapy was significantly greater among patients assigned to infusional FU/LV ($P = .015$). The reason for the lesser delivery of postprogression treatment among patients assigned to mFOLFOX6 may have been related to its poorer tolerability. Because the PFS was similar across arms, it can be postulated that the differences observed in OS are most likely attributable to the differences in use of postprogression therapy.

In the preplanned subgroup analysis, a significant treatment interaction for age was observed with evidence of a differential treatment benefit favoring the FU/LV arm in younger patients (younger than 70 years old). In an exploratory analysis of baseline characteristics by age, it seems that there were some prognostic imbalances in the randomization of younger patients, favoring the FU/LV arm. Given the post hoc nature of this analysis and the smaller numbers within the age subgroups, the reasons for the treatment interaction by age are difficult to confirm but may be worthy of further investigation.

Why do our results differ from that of the previously reported CONKO-003 trial?[8] Although cross-trial comparisons are difficult, certain differences may be considered. The investigational arm of the German CONKO-003 trial used the OFF regimen, which has a lower dose intensity of oxaliplatin administered on days 8 and 22 of a 42-day cycle than the every 14-day administration of oxaliplatin per the mFOLFOX6 regimen. Thus, the reduced oxaliplatin

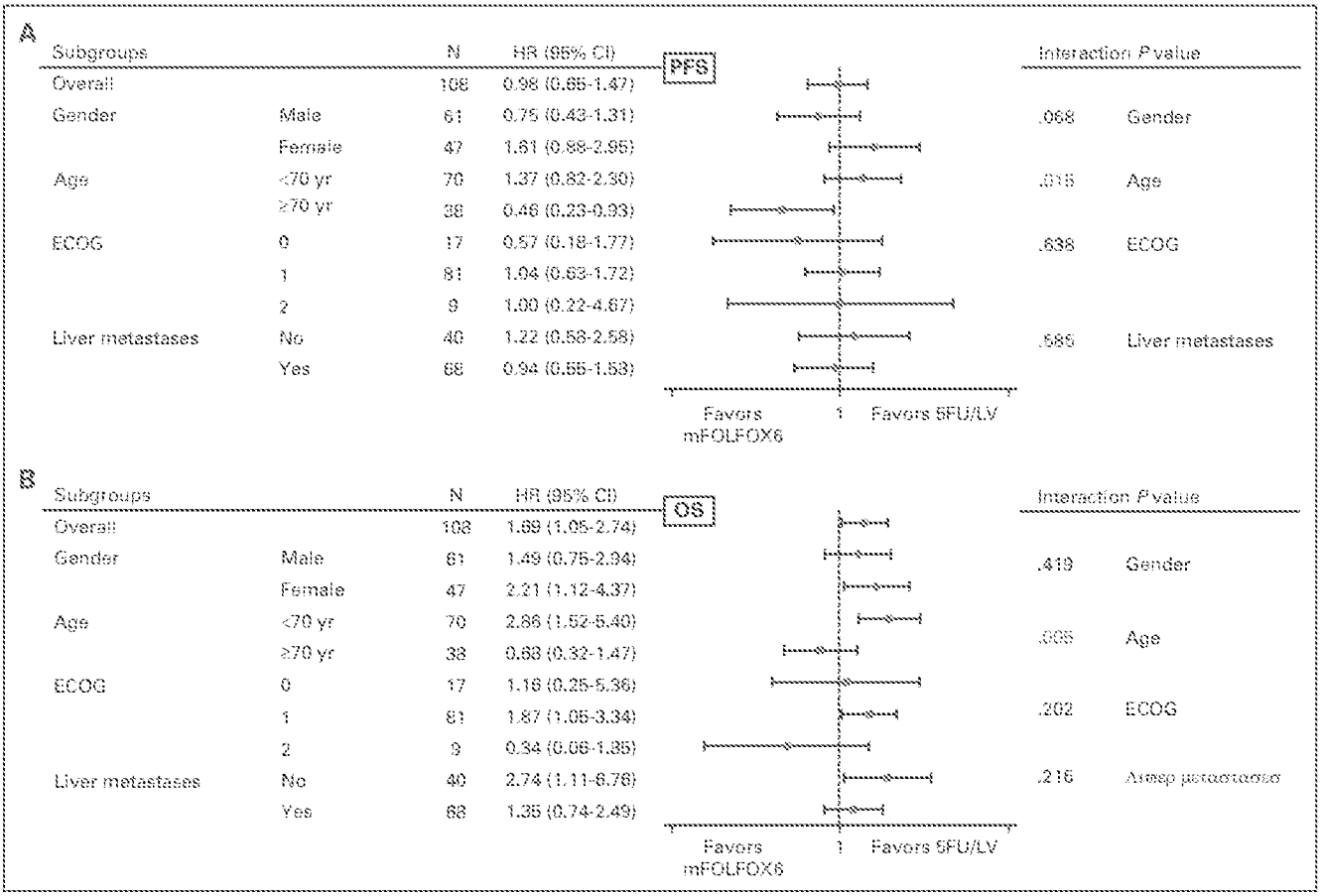

Fig 4. Prespecified subgroup analysis by forest plot, with HRs for (A) PFS and (B) OS. FU/LV, fluorouracil/leucovorin; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; mFOLFOX6, modified infusional fluorouracil, leucovorin, and oxaliplatin; OS, overall survival; PFS, progression-free survival.

intensity may have resulted in a better tolerability of the combination regimen. Eligibility in CONKO-003 required evidence of disease progression while on gemcitabine therapy, whereas PANCREOX eligibility required evidence of disease progression within 4 weeks of randomization either during or following prior gemcitabine therapy. Finally, a similar proportion of patients in CONKO-003 went on to receive postprogression therapy in both arms of CONKO-003 (OFF, n = 22; FF, n = 18), in contrast to the experience observed in PANCREOX. Although noting these differences may aid in the clinical interpretation of the PANCREOX results, it is recognized that the value of such cross-trial comparisons is inconclusive and ultimately limited to hypothesis generation. Given the established benefit of FOLFIRINOX in the untreated setting,[5] the PANCREOX data suggest that the opportunity for benefit from oxaliplatin-based chemotherapy would be in the first-line setting.

An important limitation of the PANCREOX trial was that it was closed prematurely because of slow accrual after randomly assigning 108 of the planned 128 patients. First-line FOLFIRINOX became available during this trial and, consequently, the availability of patients with an acceptable performance status who were previously treated with gemcitabine therapy was significantly curtailed during the latter study period. However, given the observed nonsignificant *P* value of .989 for PFS, it is highly unlikely that the recruitment of 20 additional patients would have changed the direction of the primary end point. Another limitation is that

Table 2. Exploratory Analysis of Baseline Characteristics by Age

Characteristic	Age < 70 years			Age ≥ 70 years		
	mFOLFOX6 (n = 34)	FU/LV (n = 36)	*P*	mFOLFOX6 (n = 20)	FU/LV (n = 18)	*P*
Median age (range), years	61 (38-69)	62 (48-69)	.154	73 (70-82)	73 (70-78)	.893
ECOG PS 0-1, %	88.1	97.1	.053	90.0	88.9	.914
ECOG PS 2, %	11.8	2.1		10.0	11.1	
Median time since diagnosis of advanced disease (months)	8.2	5.6	.058	7.0	6.0	.914

Abbreviations: FU/LV, fluorouracil/leucovorin; ECOG PS, Eastern Cooperative Oncology Group performance status; mFOLFOX6, modified infusional fluorouracil, leucovorin, and oxaliplatin.

Table 3. Frequency of Grade 3/4 Adverse Events in the Safety Population

	mFOLFOX6 (n = 49)		FU/LV (n = 53)	
Adverse Events*	No.	%	No	%
Neutropenia	16	32.7	2	3.8
Febrile neutropenia	2	4.1	0	0
Fatigue	7	14.2	1	1.9
Thrombocytopenia	4	8.2	1	1.9
Dehydration	4	8.2	0	0
Pulmonary embolism	2	4.1	0	0
Vomiting	2	4.1	0	0
Hypokalemia	2	4.1	0	0
Peripheral neuropathy	2	4.1	0	0
Anemia	1	2.0	0	0
Diarrhea	1	2.0	0	0
Cholangitis	1	2.0	0	0
Muscular weakness	1	2.0	0	0

Abbreviations: FU/LV, fluorouracil/leucovorin; mFOLFOX6, modified infusional fluorouracil, leucovorin, and oxaliplatin.
*Possibly related treatment-emergent adverse events.

PANCREOX was conducted before the availability of nab-paclitaxel,[6] thus the impact of treatment sequencing with FU/LV versus mFOLFOX6 after first-line therapy with gemcitabine and nab-paclitaxel is not known. In practice, the risk of neuropathy with nab-paclitaxel, albeit potentially reversible, may limit the suitability for considering subsequent second-line oxaliplatin therapy.

The treatment landscape of advanced pancreatic cancer has changed appreciably over the last 5 years. With the availability of more efficacious first-line combination treatment options, it is now of increasing importance to understand the optimal strategy for sequencing available therapies. Although first-line FOLFIRINOX is a valuable option in patients with good performance status, it is recognized that many patients with advanced pancreatic cancer will not be eligible for this approach and will likely receive first-line gemcitabine-based therapy.[9] There has been heightened interest in investigating post-gemcitabine progression therapies, with FU recognized as the standard treatment comparator after gemcitabine progression. NAPOLI-1, a randomized phase III trial in patients with metastatic pancreatic cancer previously treated with gemcitabine therapy, recently reported a survival benefit with the addition of nano-liposomal irinotecan to FU/LV versus FU/LV.[10] Promising results were observed in the randomized phase II RECAP trial of capecitabine with or without ruxolitinib (a JAK1/JAK2 inhibitor) in patients with metastatic pancreatic cancer previously treated with gemcitabine, with a survival benefit suggested in patients' elevated C-reactive protein levels.[11] However, the preliminary findings of the phase III JANUS-2 trial of capecitabine with or without ruxolitinib failed to confirm this signal. Nonetheless, such trials advance the hope of more efficacious, tolerable, and novel therapeutic options for patients with advanced pancreatic cancer.

In summary, the findings of the Canadian phase III PANCREOX study confirm that infusional 5FU/LV is a reasonable and well-tolerated second-line option for patients with advanced pancreatic cancer with an ECOG performance status of 2 or better, previously treated with first-line gemcitabine-based therapy.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: Sharlene Gill, Helene Grassin, John Stewart, Malcolm Moore
Financial support: Helene Grassin
Administrative support: Helene Grassin
Provision of study materials or patients: Sharlene Gill, Yoo-Joung Ko, Christine Cripps, Annie Beaudoin, Sukhbinder Dhesy-Thind, Muhammad Zulfiqar, Pawel Zalewski, Thuan Do, Pablo Cano, Wendy Yin Han Lam, Scot Dowden, Helene Grassin, Malcolm Moore
Collection and assembly of data: Sharlene Gill, Yoo-Joung Ko, Christine Cripps, Annie Beaudoin, Sukhbinder Dhesy-Thind, Muhammad Zulfiqar, Pawel Zalewski, Thuan Do, Pablo Cano, Wendy Yin Han Lam, Scot Dowden, Malcolm Moore
Data analysis and interpretation: Sharlene Gill, Helene Grassin, John Stewart, Malcolm Moore
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

PANCREOX: A Randomized Phase III Study of Fluorouracil/Leucovorin With or Without Oxaliplatin for Second-Line Advanced Pancreatic Cancer in Patients Who Have Received Gemcitabine-Based Chemotherapy

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Sharlene Gill
Honoraria: Celgene, Eli Lilly and Company
Consulting or Advisory Role: Celgene, Baxalta, Eli Lilly and Company
Research Funding: Celgene

Yoo-Joung Ko
Honoraria: Celgene, Eli Lilly and Company
Consulting or Advisory Role: Celgene, Eli Lilly and Company
Research Funding: Celgene
Travel, Accommodations, Expenses: Celgene

Christine Cripps
No relationship to disclose

Annie Beaudoin
Consulting or Advisory Role: Novartis
Research Funding: Sanofi Canada (Inst)
Travel, Accommodations, Expenses: Roche Canada

Sukhbinder Dhesy-Thind
No relationship to disclose

Muhammad Zulfiqar
No relationship to disclose

Pawel Zalewski
Stock or Other Ownership: Merck
Consulting or Advisory Role: Bristol-Myers Squibb, Roche Canada, AstraZeneca, Sanofi

Thuan Do
Stock or Other Ownership: Johnson & Johnson, AbbVie
Honoraria: Boehringer Ingelheim, Eli Lilly and Company

Pablo Cano
No relationship to disclose

Wendy Yin Han Lam
No relationship to disclose

Scot Dowden
Honoraria: Roche Canada, Celgene, Sanofi, Eli Lilly and Company
Consulting or Advisory Role: Roche Canada, Sanofi, Celgene, Eli Lilly and Company
Speakers' Bureau: Roche Canada, Celgene, Amgen

Helene Grassin
Employment: Sanofi Canada
Stock or Other Ownership: Sanofi Canada

John Stewart
Employment: Sanofi Canada
Stock or Other Ownership: Sanofi

Malcolm Moore
No relationship to disclose

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Appendix

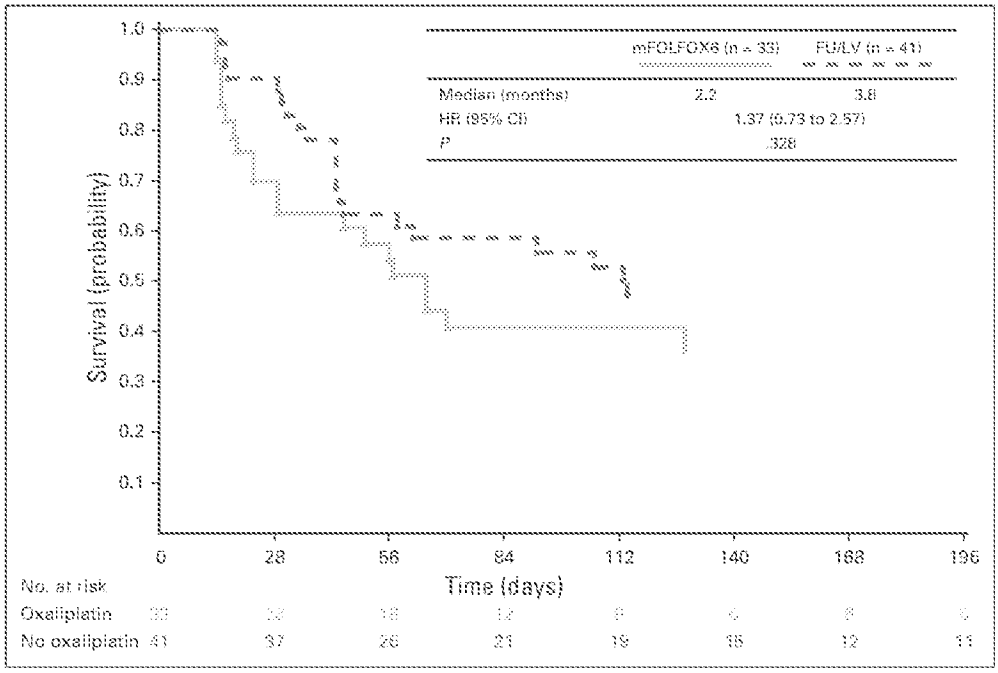
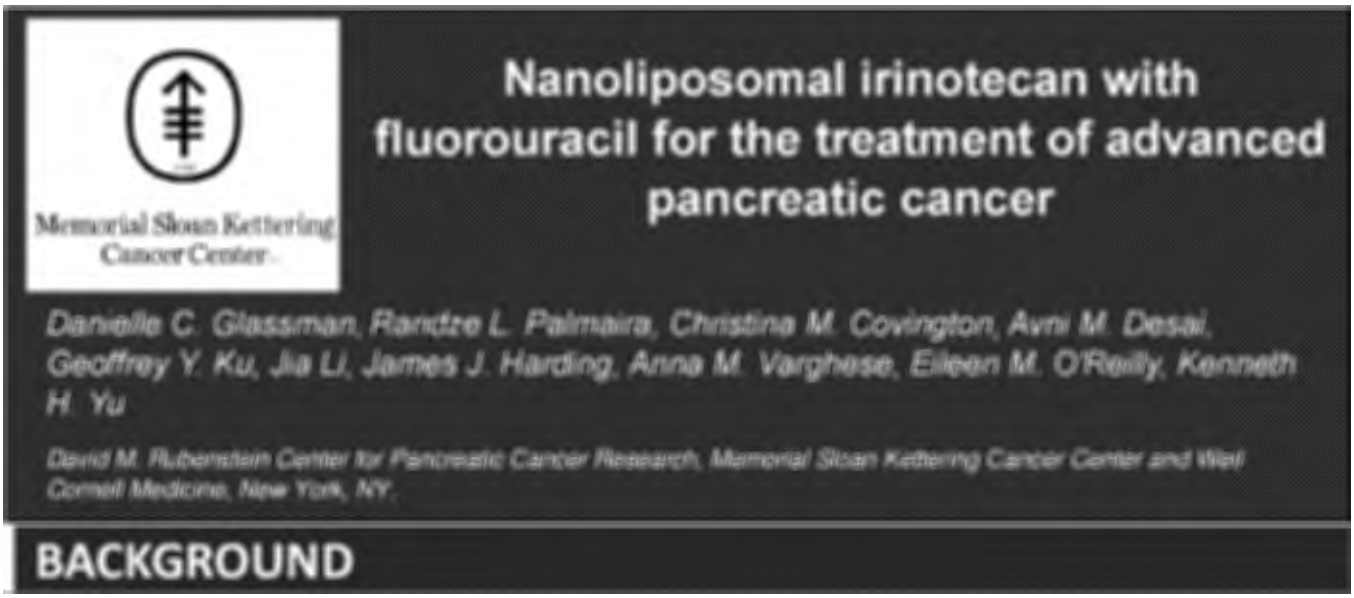


Fig A1. European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30 Time to Deterioration (decrease of 10 points). FU/LV, fluorouracil/leucovorin; HR, hazard ratio; mFOLFOX6, modified infusional fluorouracil, leucovorin, and oxaliplatin.



BACKGROUND

Therapy options for advanced pancreatic adenocarcinoma (PDAC) are finite. NAPOLI-1, a phase III randomized trial, demonstrated the efficacy of nano-liposomal irinotecan and 5-FU/LV (nal-IRI+5-FU/LV) following progression on gemcitabine-based therapy (mPFS 3.1 mo, mOS 6.1 mo).[1] There are limited additional data on the safety and efficacy of nal-IRI+5-FU/LV following FDA approval in October 2015. We examined the post approval safety, tolerability and effectiveness of nal-IRI+5-FU/LV in advanced PDAC patients at Memorial Sloan Kettering Cancer Center.

METHODS

A retrospective chart review was conducted of all patients who began treatment with nal-IRI+5-FU/LV from October 2015 through June 2017. A total of 56 patients were identified using the electronic medical record. Information was extracted regarding demographics, prior and nal-IRI+5-FU/LV therapy, adverse events, treatment response and survival.

All treatment related adverse events (AEs) that occurred while patients were treated with nal-IRI+5-FU/LV were collected. All AEs and SAEs were graded per National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE V4.0). Patients were assessed every 8-12 weeks by computed tomography (CT). Disease response was assessed using RECIST version 1.1 criteria. Date of disease progression on nal-IRI+5-FU/LV treatment and date of death were recorded. Patients without progression or death were censored at the last follow-up date as of November 2nd, 2017.

RESULTS

Table 1. Characteristics of patients and tumors

	N = 56 (%)			*N = 56 (%)*	
Median Age (years, range)	68 (42-88)		**Metastatic sites**		
Gender			Liver	41	(73)
Male	29	(52)	Peritoneum	16	(29)
Female	27	(48)	Lung	15	(27)
ECOG Performance Status			Distant lymph nodes	18	(32)
0	3	(5)	Other	10	(18)
1	41	(73)	**Number of metastatic sites**		
2	11	(20)	1	29	(52)
Primary tumor location			2	8	(14)
Head	28	(50)	3 or more	16	(29)
Body	11	(20)	**Prior lines of advanced disease therapy**		
Tail	12	(21)	0	4	(7)
Body and tail	5	(9)	1	20	(36)
Stage at start of treatment			2	21	(38)
III	2	(4)	3 or more	11	(20)
IV	54	(96)			

Table 2. Dosing, dose reductions and sequencing

	N = 56 (%)
Starting nal-IRI dose (mg/m^2)	
≤ 50	23 (41)
55	9 (16)
60	7 (13)
70	17 (30)
Dose reductions (#)	
0	38 (68)
1	15 (27)
2	3 (5)
Treatment sequencing	
FOLF(IRIN)OX ←→ (nab-P)+Gem → nal-IRI+5-FU/LV	26 (46)
nab-P+Gem → nal-IRI+5-FU/LV	25 (45)
nab-P+Gem → Gem/Cap → nal-IRI+5-FU/LV	3 (5)
other	2 (4)

Figure 1. Irinotecan (A) and schematic of nal-IRI (B). Each ~110 nm nanoliposome contains ~80,000 irinotecan salt molecules.[2]

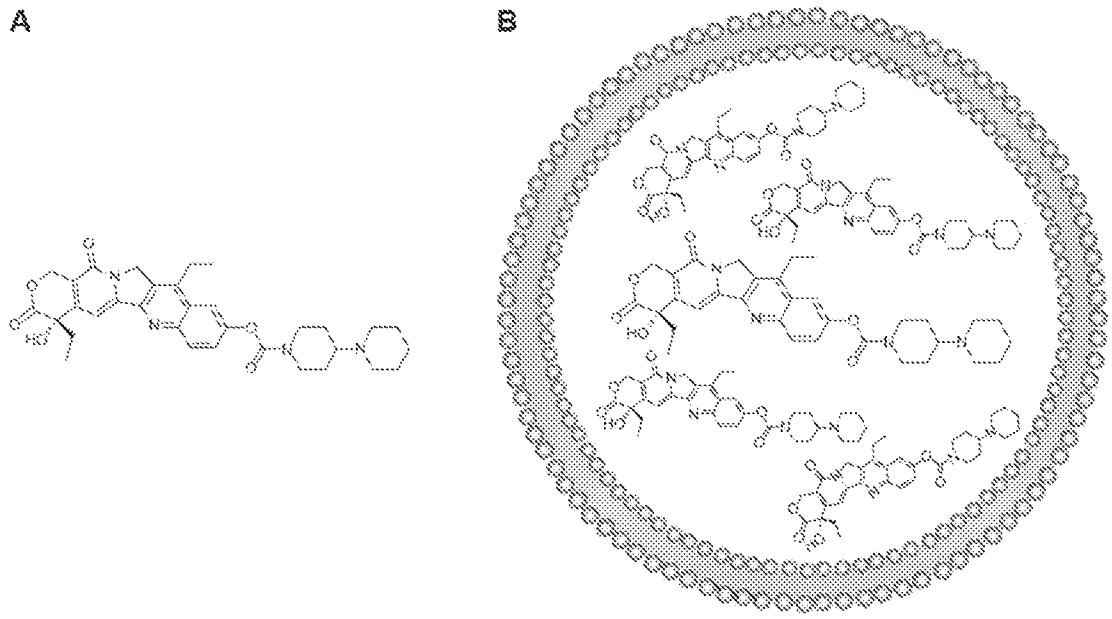


Table 3. Dose reductions		
	N = 18 (%)	
1	15	(83)
2	3	(17)
Reason attributed for dose reduction		
Fatigue	8	(44)
Diarrhea	8	(44)
Nausea	2	(11)
Neutropenia	2	(11)
Anorexia	2	(11)
Abdominal cramping	1	(6)
Ageusia	1	(6)
Not defined	1	(6)

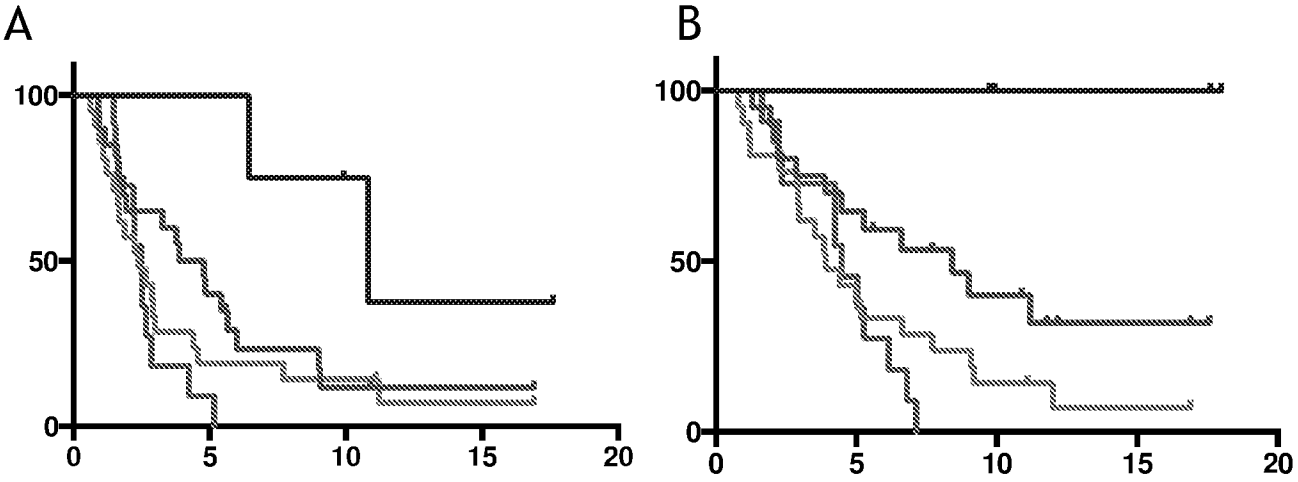
Table 4. Overall efficacy and response

	N = 56 (%)	
PFS (median, mo)	2.9	
OS (median, mo)	5.3	
Response rate		
Partial response	3	(5)
Stable disease	23	(41)
Progressive disease	23	(41)
Not evaluabe	7	(13)
CA 19-9 response (maximal response/baseline)		
≥ 1	19	(34)
0.5 to 1	15	(27)
< 0.5	10	(18)
not evaluable	10	(18)
not measurable	2	(4)
Advanced disease, OS (median, mo)		
All	24.2	
Sequence 1	25.5	
Sequence 2	23.0	
nab-P+Gem è Gem/Cap è nal-IRI+5-FU/LV	28.6	
other	23.0	

Table 5. Adverse events

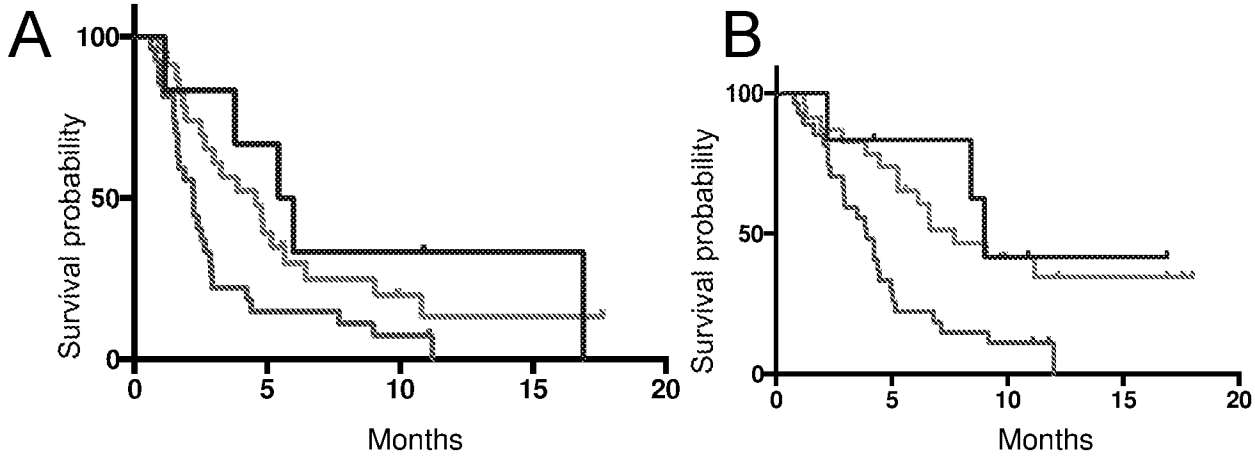
	MSKCC		NAPOLI-1[1]	
Treatment	nal-IRI+5-FU/LV		nal-IRI+5-FU/LV	
Patients	56		117	
toxicities	any grade (%)	grade 3/4 (%)	any grade (%)	grade 3/4 (%)
Nausea	33 (59)	2 (4)	60 (51)	9 (8)
Vomiting	18 (32)	2 (4)	61 (52)	13 (11)
Diarrhea	35 (63)	1 (2)	69 (59)	15 (13)
Fatigue	45 (80)	1 (2)	47 (40)	16 (14)
Anorexia	32 (57)	0 (0)	52 (44)	5 (4)
Neutropenia	16 (29)	1 (2)	46 (39)	32 (27)
Anemia	50 (89)	10 (18)	44 (38)	11 (9)

Figure 2. PFS (A) and OS (B) of nal-IRI+5-FU/LV, stratified by line of therapy.



	mPFS (mo)	mOS (mo)
1st line (n = 4):	10.8	not reached
2nd line (n = 20):	4.3	8.4
3rd line (n = 21):	2.4	3.9
> 3rd line (n = 11):	2.5	4.5
	PFS	**OS**
Log-rank test for trend	p = **0.0031**	p = **0.0002**

Figure 3. PFS (A) and OS (B) of nal-IRI+5-FU/LV, stratified by prior irinotecan treatment.



	mPFS (mo)	mOS (mo)
Prior IRI without progression (n = 6)	5.71	9.01
Prior IRI with progression (n = 27)	2.24	3.91
No prior IRI (n = 23)	4.57	7.7

	PFS		OS	
	Log-rank test (p)	HR (logrank)	Log-rank test (p)	HR (logrank)
IRI, no progression v IRI, progression	0.041	0.41 (0.19 to 0.86)	0.035	0.31 (0.13 to 0.70)
no prior IRI v IRI, progression	0.022	0.51 (0.28 to 0.93)	0.0021	0.38 (0.20 to 0.72)

CONCLUSIONS

- These data support the safety and efficacy of nal-IRI+5-FU/LV, reinforcing results of NAPOLI-1.
- Patients without disease progression on prior irinotecan-based therapy fared significantly better than patients with progression, when treated with nal-IRI+5-FU/LV.
- Studies to identify predictive markers of treatment response are ongoing.
- Sequential therapy with nab-P+Gem followed by nal-IRI+5-FU/LV demonstrates encouraging median overall disease survival. Collectively these findings underscore the utility of nal-IRI+5-FU/LV in the therapy of advanced PDAC.

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[1] Lancet. 2016 Feb 6;387(10018):545-57.

[2] Onco Targets Ther. 2016 May 20;9:3001-7.

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Nanoliposomal irinotecan with fluorouracil for the treatment of advanced pancreatic cancer

Danielle C Glassman, Randae L Palmaira, Christina M Covington, Avni M Desai, Geoffrey Y Ko, Jia Li, James J Harding, Anna M Varghese, Eileen M O'Reilly, Kenneth H Yu

Memorial Sloan Kettering Cancer Center

David M Rubenstein Center for Pancreatic Cancer Research, Memorial Sloan Kettering Cancer Center and Weill Cornell Medicine, New York, NY

BACKGROUND

Therapy options for advanced pancreatic adenocarcinoma (PDAC) are finite. NAPOLI-1, a phase III randomized trial, demonstrated the efficacy of nanoliposomal irinotecan and 5-FU/LV (nal-IRI+5-FU/LV) following progression on gemcitabine-based therapy (mPFS 3.1 mo, mOS 6.1 mo)[1]. There are limited additional data on the safety and efficacy of nal-IRI+5-FU/LV following FDA approval in October 2015. We examined the post approval safety, tolerability and effectiveness of nal-IRI+5-FU/LV in advanced PDAC patients at Memorial Sloan Kettering Cancer Center.

METHODS

A retrospective chart review was conducted of all patients who began treatment with nal-IRI+5-FU/LV from October 2015 through June 2017. A total of 66 patients were identified using the electronic medical record. Information was extracted regarding demographics, prior and nal-IRI+5-FU/LV therapy, adverse events, treatment responses and survival.

All treatment related adverse events (AEs) that occurred while patients were treated with nal-IRI+5-FU/LV were collected. All AEs and SAEs were graded per National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE V4.0). Patients were assessed every 8-12 weeks by computed tomography (CT). Disease response was assessed using RECIST version 1.1 criteria. Date of disease progression on nal-IRI+5-FU/LV treatment and date of death were recorded. Patients without progression or death were censored at the last follow-up date as of November 2nd, 2017.

RESULTS

RESULTS

CONCLUSIONS

- These data support the safety and efficacy of nal-IRI+5-FU/LV, reinforcing results of NAPOLI-1.
- Patients without disease progression on prior irinotecan-based therapy fared significantly better than patients with progression, when treated with nal-IRI+5-FU/LV.
- Studies to identify predictive markers of treatment response are ongoing.
- Sequential therapy with nab-P-Gem, followed by nal-IRI+5-FU/LV demonstrates encouraging median overall disease survival. Collectively, these findings underscore the utility of nal-IRI+5-FU/LV in the therapy of advanced PDAC.

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Lancet. 2016 Feb 6;387(10018):545-57.
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CANCERS OF THE PANCREAS, SMALL BOWEL, AND HEPATOBILIARY TRACT

Nano-liposomal irinotecan and 5-FU/LV (N+F) for the treatment of advanced PDAC: Memorial Sloan Kettering (MSK) Single Cancer Center Evaluation.

Danielle C. Glassman, Avni Mukund Desai, Geoffrey Yuyat Ku, Jia Li, James J. Harding, Anna M. Varghese, Eileen Mary O'Reilly, Kenneth H. Yu

Memorial Sloan Kettering Cancer Center, New York, NY; Yale School of Medicine, Yale University, New Haven, CT; Memorial Sloan Kettering Cancer Center and Weill Cornell Medical College, New York, NY; Memorial Sloan Kettering Cancer Center/ Weill Cornell Medical College, New York, NY;

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Abstract Disclosures

Abstract

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Background: Therapy options for advanced pancreatic adenocarcinoma (PDAC) are finite. NAPOLI-1, a phase III randomized trial, demonstrated the efficacy of N+F following progression on gemcitabine (G) based therapy (mPFS 3.1 mo, mOS 6.1 mo). There are limited additional data on the safety and efficacy of N+F following FDA approval in October 2015. We examined the post approval safety, tolerability and effectiveness of N+F in advanced PDAC patients at MSK. **Methods:** A retrospective chart review was conducted of all patients treated with N+F from Feb 2016 and ending in Aug 2017. Using the EMR and institutional database, information was extracted pertaining to demographics, performance status (ECOG), prior therapies, dose, duration of N+F, adverse events, PFS, OS and treatment response per RECIST. **Results:** N = 56 identified. Demographics: M/F 29/27, age 68 (range 42-88), prior lines of palliative chemotherapy (0/1/2/3/ > 3, 4/20/21/11). Median PFS was 2.9 months and median OS was 5.3 months. There was a significant difference in PFS, OS and prior lines of therapy between patients who previously progressed on irinotecan (N = 27) versus not (N = 29); PFS = 2.4 v 4.8 mo, *p = 0.0154*; OS = 3.9 v 8.4 mo, *p = 0.0021*; 2 v 1 line. ECOG score was not predictive of PFS or OS. There were 19 dose reductions (DR), most frequent reasons: fatigue (42%) and diarrhea (37%). Regarding RECIST: PR = 2 (4%), SD = 19 (34%). 10/43 (23%) experienced > 50% CA 19-9 reduction. Dose reductions were not associated with worse outcomes, in fact, patients with 1 or

more DR experienced significantly longer PFS v none (DR 2, not reached; DR 1, 5.2 mo; DR 0, 2.5 mo, *p = 0.0185*). For the subset who received sequential therapy with G+nab-paclitaxel (P) followed by N+F (N = 25) mOS of 25.4 mo. **Conclusions:** These data support the safety and efficacy of N+F, re-inforcing results of NAPOLI-1. Patients whose disease previously progressed on irinotecan fared significantly worse than patients who did not, when treated with N+F. N+F appears active even in patients requiring DR. Sequential therapy with G+P followed by N+F demonstrates encouraging mOS. Collectively these findings underscore the utility of N+F in the therapy of advanced PDAC.