Antitumor Efficacy, Pharmacokinetics, and Biodistribution of NX 211: A Low-Clearance Liposomal Formulation of Lurtotecan

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ABSTRACT

Lurtotecan is a clinically active water-soluble camptothecin analogue that has been formulated into a low-clearance unilamellar liposome, NX 211. Comparative studies between free drug and NX 211 have been performed assessing pharmacokinetics in nude mice, tissue distribution in tumor-bearing mice, and antitumor efficacy in xenografts. Compared with lurtotecan, NX 211 demonstrated a significant increase in plasma residence time and a subsequent 1500-fold increase in the plasma area under the drug concentration curve. The volume of distribution was also greatly restricted, suggesting altered tissue distribution. Evaluation of tissues 24 h after administration of either [^{14}C]NX 211 or [^{14}C]lurtotecan to ES-2 tumor-bearing mice demonstrated a 40-fold increase in radiolabeled compound in the tumors of NX 211-treated mice compared with mice treated with lurtotecan. In single-dose efficacy studies, NX 211 produced a consistent 3-fold or greater increase in therapeutic index compared with lurtotecan in both the KB and ES-2 xenograft models. When compared at equitoxic levels in repeat-dose efficacy studies, NX 211 generated durable cures lasting >60 days and a 2–8-fold increase in $\log_{10}$ cell kill, compared with lurtotecan and topotecan, respectively. Together, these data demonstrate that NX 211 has significant therapeutic advantage over lurtotecan and that the improved antitumor activity is consistent with increased exposure and enhanced drug delivery to tumor sites.

INTRODUCTION

The camptothecin-based topoisomerase I inhibitors are an important emerging class of oncology drugs that inhibit the ability of DNA topoisomerase I to relax torsionally strained DNA (1–5). The relaxation reaction involves single-strand cleavage and the formation of a short-lived catalytic intermediate referred to as a "cleavable complex." The intact DNA strand passes through the nicked strand, thereby relaxing torsion in the helix. The nick is then religated by the topoisomerase I enzyme, allowing completion of replication, transcription, and other DNA functions. Camptothecin and many of its analogues bind to and stabilize the cleavable complex, thereby preventing religation of the DNA strands and converting an essential nuclear enzyme into a cellular poison. This leads to inhibition of active replication forks and generates an accumulation of single- and double-strand breaks in the replicating DNA, resulting in cell cycle arrest and, in many cell types, apoptotic cell death (5–8).

Two camptothecins are clinically useful and commercially available [irinotecan (Camptosar) and topotecan (Hycamptin)], whereas many more camptothecin analogues are under intense study as oncolytic agents (1–4). Lurtotecan is a water-soluble analogue of camptothecin. It inhibits mammalian DNA topoisomerase I *in vivo* with greater potency than topotecan and demonstrates potent antitumor activity in multiple xenograft models (9–11). Phase I and II clinical development trails for lurtotecan were conducted in the United States and Europe by Glaxo-Wellcome Inc from 1994 to 1998 (12–25). Multicenter Phase II studies have shown that lurtotecan is active as a second-line agent in small cell lung cancer and ovarian cancer. Preclinical and clinical data suggest that prolonged exposure may enhance the cytotoxicity of camptothecins. We have formulated lurtotecan in low-clearance, small unilamellar liposomes (NX 211), with the expectation that liposomal encapsulation will prolong the plasma half-life of lurtotecan in human subjects, resulting in prolonged tumor exposure, enhanced efficacy, and an improved therapeutic index. The preclinical data indicate that: (*a*) liposomal encapsulation markedly increases the plasma residence time of lurtotecan in animals; (*b*) NX 211 is more potent than free lurtotecan *in vitro* and *in vivo*; and (*c*) NX 211 accumulates significantly better than free lurtotecan in xenografted tumors. Based on these data, it is believed that liposomal encapsulation may indeed improve the efficacy and therapeutic index of lurtotecan.

MATERIALS AND METHODS

Cells and Reagents. The ES-2 tumor cell line was obtained from the American Type Culture Collection (Manassas, VA) and cultured in McCoy's 5a media + 10% FCS. The U251 tumor cell line was obtained from the National Cancer Institute Tumor Repository (Frederick, MD) and cultured in RPMI 1640 + 10% FCS. The KB tumor cell line and the multidrug-resistant (MDR^+) variant KBV were obtained from the laboratory of Dr. Igor Roninson (University of Illinois, Chicago, IL) and main-

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tained in DMEM + 10% FCS ± 1 μg/ml vincristine (Sigma). Cells were subcultured in the usual fashion with mild trypsinization and maintained in the appropriate growth media at 37°C in a 5% $C0_2$ incubator. Lurtotecan, topotecan, and radiolabeled [^{14}C]lurtotecan were obtained from Glaxo-Wellcome (Research Triangle Park, NC) and used in the preparation of [^{14}C]NX 211. The specific activity of [^{14}C]NX 211 was 9.75 μCi [^{14}C]lurtotecan/ml, with a concentration of 0.4 mg lurtotecan/ml. Sterile D5W[3] (McGaw, Inc., Irvine, CA) was used as a diluent. A stable liposome formulation of lurtotecan (NX 211) was formulated with proprietary methodology similar to that of DaunoXome (26, 27). This methodology yields small unilamellar liposomes under 100 nm with a 20:1 lipid:drug ratio and a lipid composition of 2:1 fully hydrogenated soy phosphatidylcholine:cholesterol.

Animal Studies. Female athymic Nu-Nu mice (18–24 g) were obtained from Harlan Sprague Dawley (Indianapolis, IN), housed in microisolator filtration racks, and maintained on sterile water and sterile laboratory chow *ad libitum*. Animals were allowed to acclimate to their new environment for 1 week before tumor cell implantation. The institutional animal care and use committee approved all animal protocols. Xenografts were established by injecting harvested tumor cells in a single s.c. site on the flank of the mice in the axillary region. The tumors were allowed to grow until they were approximately 200 ± 50 mm^3 in size. The animals were then randomized into treatment groups and tattooed on the tail for permanent identification. Twice weekly tumor measurements were obtained with vernier calipers by taking two-dimensional measurements using the formula length × $width^2/2$. All efficacy studies contained at least eight animals per group, whereas the biodistribution and PK study groups contained three or four animals per time point.

Biodistribution Studies. Each mouse received 0.5 μCi of either [^{14}C]lurtotecan or [^{14}C]NX 211 (approximately 1.0 mg/kg GI147211) in a final total volume of 100 μl. The drugs were administered i.v. via the lateral tail vein. At 1, 3, 6, 24, or 48 h, the tumor, liver, kidney, spleen, intestine, and brain were removed and weighed. Tissue sections (approximately 1 g or less) or whole organs (if less than 1 g total weight) were burned in a Packard Tissue Oxidizer model 307 (Packard Instrument Co., Meriden, CT). The samples were counted on a Packard Tri-Carb 2100RT scintillation counter (Packard Instrument Co.). Efficiency of the tissue oxidizer was determined by comparing counts of standard solutions of [^{14}C]NX 211 or [^{14}C]-lurtotecan counted by liquid scintillation with counts of standard solutions of [^{14}C]NX 211 or [^{14}C]lurtotecan burned in the oxidizer and then counted by liquid scintillation. The ratio of the two counts was defined as the efficiency of the tissue oxidizer. This ratio was used as a correction factor to determine the true counts in the tissue. The data were reported as the true counts (cpm) divided by the weight of tissue in grams. The chemical identity of the radioactive material was not identified in these studies.

Statistical Analysis. All analysis was performed to compare for a drug effect for each of the 10 tissues measured. Wilcoxon rank-sum tests were performed to test for pairwise differences between drugs for each tumor type at each time point. The minimum achievable P was 0.05 for a one-sided test or 0.10 for a two-sided test. Tests that achieved this P were noted as significant on the summary tables. In addition, rank ANOVA was performed to test for differences between tumor types and drugs simultaneously. If the tumor types were able to be pooled, $P > 0.05$, and a significant difference between drugs was observed, $P > 0.05$, a significant difference was noted on the summary table for the pooled tumor types. However, if the tumor types were not able to be pooled, $P \leq 0.05$, no statistical test results for the comparison of drugs were reported for the pooled tumor types.

Xenograft Studies. All antitumor efficacy studies were performed with s.c. established xenograft tumors with initial tumor size of 200 ± 50 mg in all treatment groups. All drug substances were prepared fresh each day in either D5W or D5W containing empty liposomes just before administration. Body weight and tumor size were determined twice weekly. In repeat-dose studies, the drug was administered weekly for either 2 or 3 consecutive weeks. Tumor growth was monitored until a maximum size was achieved (10% of animal body weight) or, in the case of cures, 60 days. Both the percentage of tumor volume and the percentage of body weight change were plotted for each experiment. Tumor measurements were used for determining the percentage TGI [$\%TGI = 100\ (Wc - Wt)/Wc$ where Wc is the mean tumor weight of control group, and Wt is the mean tumor weight of treated group] and the LCK [$LCK = (T - C)/3.32 \times (Td)$ where T is the time in days for the treated group mean tumor volume to reach a final tumor volume, C is the time in days for the control treatment group to reach the defined final tumor volume, and Td is the tumor doubling time of control tumors]. Any cures were excluded from the LCK calculations.

PK Studies. Naïve, non-tumor-bearing female Nu/Nu mice (20–25 g) were divided into two treatment groups (28 mice/group) and given a 1 mg/kg i.v. bolus dose of lurtotecan (uncorrected for salt and water) or 1 mg/kg NX 211 (as free base GI147211) in D5W via the tail vein. Blood samples were obtained from four mice/group at predetermined time points by cardiac puncture using heparinized syringes. Samples were kept on wet ice until centrifuged to obtain plasma. Plasma samples were stored at −20°C until analyzed. The time points for blood collection after lurtotecan administration were 5, 15, and 30 min and 1, 2, 4, and 6 h. Blood was collected from NX 211-treated mice at 10 min and 2, 4, 8, 24, 32, and 48 h after the dose. Samples were prepared for HPLC analysis using a modification of a previously published method (28). Briefly, lurtotecan samples (50 μl) were precipitated and acidified with a 2:1 mixture of 10% perchloric acid:acetonitrile containing 6,7-dimethoxy-4-methyl-coumarin (Aldrich Chemical Co., St. Louis, MO) as an internal standard. After centrifugation, the supernatant was injected directly into the HPLC column. The mobile phase consisted of 84:15:1:0.1 sodium phosphate [0.1 M (pH 2.2)]:acetonitrile:tetrahydrofuran:acetic acid. The flow rate was 0.35 ml/min, and the column temperature was 45°C. The injection

[3] The abbreviations used are: D5W, 5% (w/v) dextrose in water; AUC, area under the drug concentration curve; LCK, $\log_{10}$ cell kill; TGI, tumor growth inhibition; PK, pharmacokinetic; HPLC, high-performance liquid chromatography; MTD, maximum tolerated dose.

volume was 25 μl. Detection was achieved using an excitation wavelength of 390 nm and emission at 425 nm. HPLC analyses were performed using a Model 600S Controller, a Model 717 Plus Autosampler, a Model 626 Pump, and a Model 474 Scanning Fluorescence Detector (Waters Corp., Milford, MA). A 3 mm × 25 cm Zorbax Rx C-18 HPLC column fitted with a 12.5 mm × 4.6 mm Zorbax Rx C-18 guard column was used. Chromatographic data were acquired and analyzed using a commercial computer system (Millennium 32; Waters Corp.). Samples containing less than 50 ng/ml drug were analyzed by preparation (as described above) using 100 μl of plasma, an increased injection volume of 70 μl, and incorporation of post-column photodegradation to enhance the fluorescence signal. A photodegradation cell (Beam Boost; Advanced Separation Technologies, Whippany, NJ) equipped with a 254 nm lamp and a 3 mm × 10 m reaction coil was used in this determination. Detection was achieved using an excitation wavelength of 378 nm and emission at 420 nm. The GI147211 calibration standards were prepared in control mouse plasma. Samples were quantitated against calibration curves obtained from the $1/x^2$-weighted linear regression of the peak height ratios of the drug to 6,7-dimethoxy-4-methyl-coumarin in the calibration standards. The precision and accuracy of the method were determined by the analysis of samples prepared by adding known amounts of NX 211 to control mouse plasma. Use of the liposomal material in this experiment assured that the method was capable of fully disrupting the liposomes. The method was demonstrated to give relative SDs of less than 15% and had a relative accuracy (percentage of deviation from nominal) of −2 to +16% over concentrations ranging from 0.5 to 200,000 ng/ml. The limit of quantitation was set at 1.4 ng/ml due to a potential interference observed in control mouse plasma. The mean plasma concentrations for each group at each time point were analyzed by noncompartmental analysis using commercial software (WinNonlin version 1.5; Scientific Consulting, Inc., Carey, NC).

RESULTS

Xenograft Studies. The therapeutic index of lurtotecan, topotecan, and NX 211 was determined in two separate xenograft models. This experiment was initiated to compare the antitumor efficacy of NX 211 with that of free drug and with topotecan, an approved topoisomerase I inhibitor with clinical activity against a variety of solid tumors and hematological malignances. A broad range of dosages was evaluated for each agent in an attempt to determine a therapeutic index as well as the antitumor efficacy at an optimum dosage for each compound. Two experimental controls were included, untreated and empty liposomes. All treatments were administered as single bolus i.v. injections, and each group contained 10 mice. Topotecan was administered at 6, 9, 12, 16, 20, 30, and 40 mg/kg; lurtotecan was administered at 6, 9, 12, 16, 20, and 30 mg/kg; and NX 211 was administered at 3, 6, 9, 12, 16, 20, 30, and 40 mg/kg. The therapeutic index for each experiment was determined by extrapolating the LD_{50}, ED_{60}, and ED_{80} values from the dose *versus* mortality curves and the dose *versus* %TGI graphs. The results of the KB tumor study demonstrated that the 3 mg/kg dose of NX 211 produced significantly greater tumor growth delay than that produced by either 6 mg/kg lurtotecan or 12 mg/kg topotecan ($P = 0.00004$). The growth delays produced by lurtotecan and topotecan were not significantly different from each other. In the ES-2 experiment, slightly different MTDs were determined. However, the overall pattern of differences remained the same. The growth delay produced by 9 mg/kg NX 211 was significantly greater than that produced by either 12 mg/kg lurtotecan ($P = 0.0006$) or 16 mg/kg topotecan ($P = 0.00004$). The overall results demonstrate that NX 211 is more potent and has a consistent increase in the therapeutic index ranging from 3–14-fold over that of lurtotecan (Table 1).

Table 1 Determination of the therapeutic index for topotecan, lurtotecan, and NX 211 in the KB and ES-2 xenograft models

Single bolus dose of drugs was administered i.v. on day 1, and antitumor activity and toxicity were determined on day 27 after dose. ED_{60} and ED_{80}, the effective dose at which 60% or 80% tumor growth inhibition occurred. LD_{50}, the lethal dose for 50% of animals.

Drug	KB tumor (LD_{50}/ED_{60})	ES-2 tumor (LD_{50}/ED_{80})
Topotecan	0.5	ND[a]
Lurtotecan	1	1.1
NX 211	2.9	14.4

[a] ND, not determined because an ED_{80} value was never reached.

Additional antitumor efficacy studies with repetitive administration of NX 211 and lurtotecan were performed in several other xenografts. These studies compared free drug and liposomal drug at equitoxic doses and schedules in the ES-2 ovarian tumor and the KB and KBV epidermoid tumors. Drugs were administered i.v. once weekly for 3 consecutive weeks for the KB and ES-2 xenografts and administered once every 2 weeks for the KBV xenograft. The MTD for each drug tested in these models was determined on the basis of body weight loss and toxic deaths. For NX 211, the MTD was determined to be 9 mg/kg/week, whereas the MTD for lurtotecan and topotecan were 14 and 16 mg/kg/week, respectively. The repeat-dose efficacy results for the ES-2 xenograft study are presented in Table 2 and Fig. 1. In this study, both lurtotecan and topotecan were compared with NX 211 at equitoxic doses, and all three drug groups had a significant effect on tumor growth compared with the vehicle control group. However, among the different treatment groups, there were significant differences in the magnitude of response and the duration of response, with NX 211 demonstrating a significant advantage. The amount of body weight loss and the number of toxic deaths that occurred in this experiment demonstrated that all three groups were dosed at their maximum tolerated levels. In the NX 211-treated group, three durable cures were generated with an overall LCK of 4.08, whereas no durable cure occurred in the free lurtotecan group, which had a LCK of 2.14. The topotecan group failed to generate any cures, and the LCK for this group was 0.58. The difference in TGI between the NX 211 and lurtotecan groups in the KB xenograft model was less dramatic, but NX 211 still showed greater activity. In this experiment, two different groups were tested at an identical dose of 14 mg/kg lurtotecan. In one group, D5W was used as the diluent, whereas in the second group, a suspension of empty liposomes was used as the diluent to control for any additional effects the lipid may have had on tumor growth. The results shown in Table 3 demonstrate that the empty liposomes had no effect on the observed efficacy because the two lurtotecan-treated groups

Table 2 Comparison of antitumor efficacy of NX 211, lurtotecan, and topotecan in the ES-2 tumor xenograft model.
Drugs were administered i.v. on days 1, 8, and 15. Durable cures were determined on day 60.

Drug (dose)	Maximum body weight loss	No. of toxic deaths	% TGI[a]	LCK[b]	T-C[c]	No. of durable cures
NX 211 (9 mg/kg)	19%	0/8	99.5%	4.08	51.4	3/8
Lurtotecan (14 mg/kg)	9%	3/8	95.0%	2.14	27.0	0/8
Topotecan (16 mg/kg)	16%	3/8	57.0%	0.58	7.4	0/8

[a] TGI = 100 (1 − *Wt*/*Wc*); *Wt*, mean tumor volume of treated group at day 15; *Wc*, mean tumor volume of control group at day 15.
[b] LCK = T-C/(3.32) (*Td*) where *Td* = tumor doubling time of control tumors (days).
[c] T-C, difference in days for treated and control groups to reach 400% tumor volume increase.

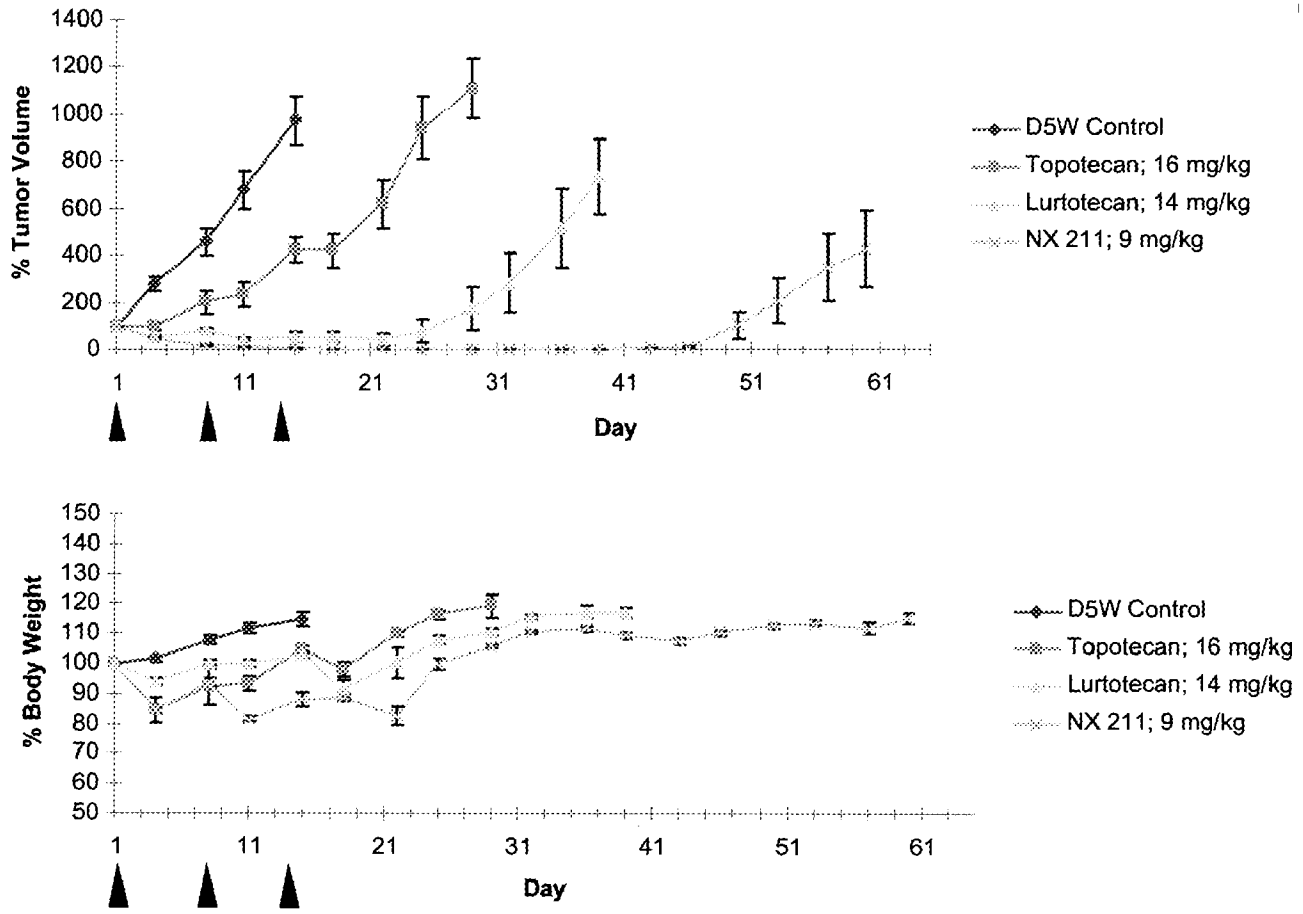

Fig. 1 Tumor growth curve and body weight graph of the ES-2 ovarian tumor xenograft experiment comparing lurtotecan, NX 211, and topotecan at an equitoxic dose. All three compounds were administered i.v. weekly via tail vein. Data shown are the mean ± SE from eight mice at each time point, except where noted. *Arrows* indicate dose days.

were essentially identical, with TGI values of 90% and 92% and a LCK of 1.66. The group treated with 9 mg/kg NX 211 had a 98% TGI and a LCK index of 2.7. Thus, the NX 211-treated group demonstrated a greater tumor cell kill effect than the lurtotecan-treated groups. In the multidrug-resistant tumor model, KBV, the NX 211-treated groups demonstrated a dose-response inhibition of tumor growth, whereas the lurtotecan-treated group did not (Fig. 2). In fact, the lowest dose of NX 211, 4 mg/kg, was as effective as the high dose of lurtotecan, 16 mg/kg. Overall, the effectiveness of lurtotecan in this tumor model was minimal compared with the control, with a LCK index of 0.59 for the group treated with 16 mg/kg lurtotecan (Table 4). In contrast, NX 211 produced a LCK index of 1.63 at the 9 mg/kg dose level, demonstrating that NX 211 is nearly three times as effective in cell kill. These results are consistent with those seen in previous studies of equal dose comparison and single-dose therapeutic index determinations where NX 211 consistently demonstrated superior efficacy compared with lurtotecan and topotecan.

PK Studies. In the PK studies, NX 211 and lurtotecan were administered at 1 mg/kg on an "as is" basis. This dose

Table 3 Comparison of antitumor efficacy of NX 211 and lurtotecan in the KB xenograft model

Empty liposomes were used as diluent for one lurtotecan group to control for nonspecific liposomal effects.

Drug (dose)	Maximum body weight loss	No. of toxic deaths	% TGI[a]	LCK[b]	T-C[c]	No. of durable cures
Lurtotecan (14 mg/kg/D5W)	14%	0/8	92%	1.66	27	1/8
Lurtotecan (14 mg/kg/empty liposome)	11%	0/8	90%	1.66	27	0/8
NX 211 (9 mg/kg)	17%	0/8	98%	2.74	44.5	1/8

[a] TGI = 100 (1 − *Wt*/*Wc*); *Wt* is mean tumor volume of the treated group at day 19; *Wc* is mean tumor volume of control group at day 19.

[b] LCK = T-C/ (3.32) (*Td*). T-C is the difference in days for treated and control groups to reach 400% tumor volume increase; *Td* is tumor doubling time of control tumor group (days).

[c] T-C, the difference in days for treated and control groups to reach 400% tumor volume increase.

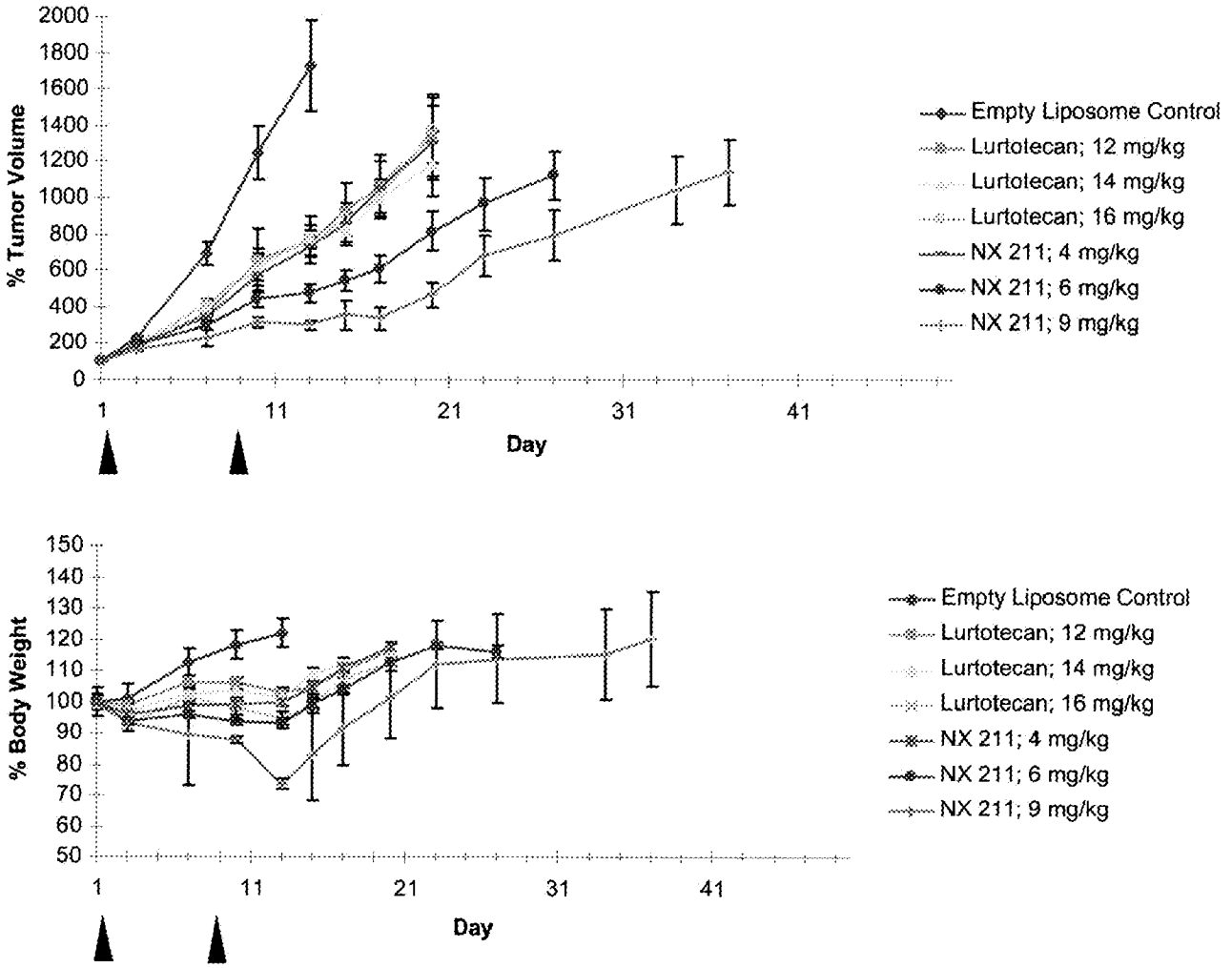

Fig. 2 Tumor growth and body weight graphs comparing the activity of NX 211 and lurtotecan in the MDR+ KBV tumor xenograft model. Data shown are the mean ± SE from eight mice at each time point, except where noted. *Arrows* indicate dose days.

translated to 1 mg/kg GI147211 as the free base for the NX 211-treated group and 0.78 mg/kg GI147211 for the lurtotecan-treated group (corrected for the dihydrochloride salt and water content). The results, shown in Fig. 3 and Table 5, demonstrated that NX 211 (liposomal encapsulated lurtotecan) has significantly increased plasma C_{max}, AUC, mean residence time (MRT), and half-life. Plasma clearance (Cl) for NX 211 was decreased almost 1500-fold, and the extensive volume of distribution (V_{ss}) observed for lurtotecan (9 liters/kg) was reduced essentially to that of the plasma compartment (35 ml/kg). These results are consistent with a model of a stable liposomal drug whose circulation is restricted primarily to the plasma compartment.

Biodistribution Studies. These studies were carried out in several different xenograft models and revealed that consistently greater amounts of radioactive material accumulated in

Table 4 Comparison of antitumor activity of NX 211 and lurtotecan in the KBV (MDR+) tumor xenograft model
Drugs were administered on days 1 and 8. Empty liposomes were used as diluent for all lurtotecan groups.

Drug (dose)	Maximum body weight loss	No. of toxic deaths	% TGI[a]	T-C[b]	LCK[c]	No. of durable cures
Lurtotecan (12 mg/kg)	10%	2/8	47%	7.6	0.53	0/8
Lurtotecan (14 mg/kg)	10%	3/8	49%	8.4	0.59	0/8
Lurtotecan (16 mg/kg)	15%	1/8	47%	8.4	0.59	0/8
NX 211 (4 mg/kg)	5%	2/8	48%	8.4	0.59	0/8
NX 211 (6 mg/kg)	7%	0/8	65%	15	1.06	0/8
NX 211 (9 mg/kg)	17%	4/8	80%	23	1.63	0/8

[a] TGI = 100(1 − *Wt*/*Wc*); *Wt* is mean tumor volume of the treated group at day 13; *Wc* is mean tumor volume of control group at Day 13.
[b] T-C, the difference in days for treated and control groups to reach 500% tumor volume increase.
[c] LCK = T-C/(3.32) (*Td*); *Td* is the tumor doubling time of control tumor group (days).

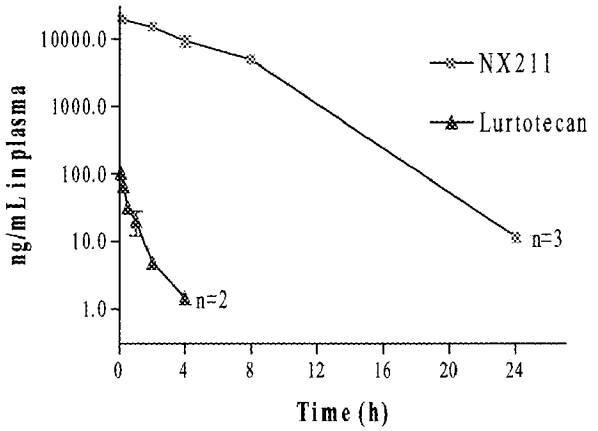

Fig. 3 Plasma concentration *versus* time profiles obtained after single-dose i.v. bolus administration of 1 mg/kg drug (free base) as either lurtotecan or NX 211. Data shown are the mean ± SD from four mice at each time point.

Table 5 Summary of noncompartmental pharmacokinetic parameters of NX 211 and lurtotecan as determined in nude mice

Parameter	Units	NX 211 Estimate	Lurtotecan Estimate	ΔNX 211/LRT[a]
C_{max}	μg/ml	20.1	0.128	122-fold ↑
$AUC_{(0\rightarrow t)}$	μg·h/ml	127	0.0672	1,474-fold ↑
$AUC_{(0\rightarrow\infty)}$	μg·h/ml	127	0.0690	1,435-fold ↑
Portion of AUC extrapolated	%	0.03	2.52	
Cl	ml/h/kg	7.89	11,309	1,433-fold ↓
$MRT_{(0\rightarrow\infty)}$	h	4.45	0.775	5.7-fold ↑
V_{ss}	ml/kg	35.1	8,902	254-fold ↓
$t_{1/2\lambda z}$	h	2.00	0.832	2.4-fold ↑

[a] Relative change in parameter estimate observed for NX 211 over that obtained for free drug. The lurtotecan (LRT) C_{max} and AUC values were corrected for the dose difference (1 mg/kg lurtotecan = 0.78 mg/kg GI147211 free base) for this comparison.

the tumors of the NX 211-treated mice than in the tumors of lurtotecan-treated mice. Fig. 4 compares the results of four different tumor xenografts and shows the cpm/g tumor tissue, 24 h after injection with either [^{14}C]NX 211 or [^{14}C]lurtotecan. Tumors from animals treated with [^{14}C]NX 211 contained 10,255 (ES-2), 7,817 (KB), 9,474 (KBV), and 1,981 (U251) cpm/g tumor tissue compared with 256 (ES-2), 118 (KB), 162 (KBV), and 221 (U251) cpm/g tumor tissue in mice treated with [^{14}C]lurtotecan. These data reflect a 9–67-fold increase in the amount of radioactive material in the tumors of [^{14}C]NX 211-treated animals. The amount of radioactive material of different tissues 24 h after injection with either [^{14}C]NX 211 or [^{14}C]-lurtotecan is shown in Fig. 5. At all selected times up to 24 h, the differences in accumulation of lurtotecan and NX 211 were statistically significant. Overall, the amount of radioactive material recovered was nearly 10-fold greater in [^{14}C]NX 211-treated animals than in [^{14}C]lurtotecan-treated animals. We also observed a different biodistribution profile for the NX 211 material compared with the free lurtotecan. The spleen was the major site of localization of radioactive material for [^{14}C]NX 211-treated animals, whereas the liver was the major site of localization of radioactive material for the [^{14}C]lurtotecan-treated animals. Tumor type differences did not influence the overall organ tissue distribution for each group.

The biodistribution of NX 211 over time is seen in Fig. 6, which plots the cpm/g tissue at selected times over a 48-h period from tumor-bearing mice treated with [^{14}C]NX 211. The spleen was the major site of localization at all time points examined, which is consistent with the known uptake of liposomes by the reticuloendothelial system. The KB tumor showed an apparent peak of radioactive material of over 2-fold by 6 h, compared with the amount at 1 h. The spleen and intestines also had a peak in radioactive material by 6 h. The other tissues examined had a peak in radioactive material at 1 h, followed by a gradual elimination of radioactive material until 48 h. Thus, NX 211 showed a greater propensity for tumor localization, compared to free lurtotecan, which varied with different tumor types.

DISCUSSION

Liposomal encapsulation can significantly prolong the plasma residence time of drugs that would otherwise be rapidly distributed or cleared (28–32). Altered plasma pharmacokinetics is one mechanism by which liposomal drugs may demonstrate substantial changes in tissue distribution, efficacy, and toxicity from their nonencapsulated counterparts. This may be especially important with cytotoxic drugs such as camptothecins, where the major cytotoxic effects are manifested in a cell cycle-specific fashion (5). Thus, the increase in LCK and the greater overall antitumor efficacy seen in the present experiments may be due in large part to prolonged exposure as

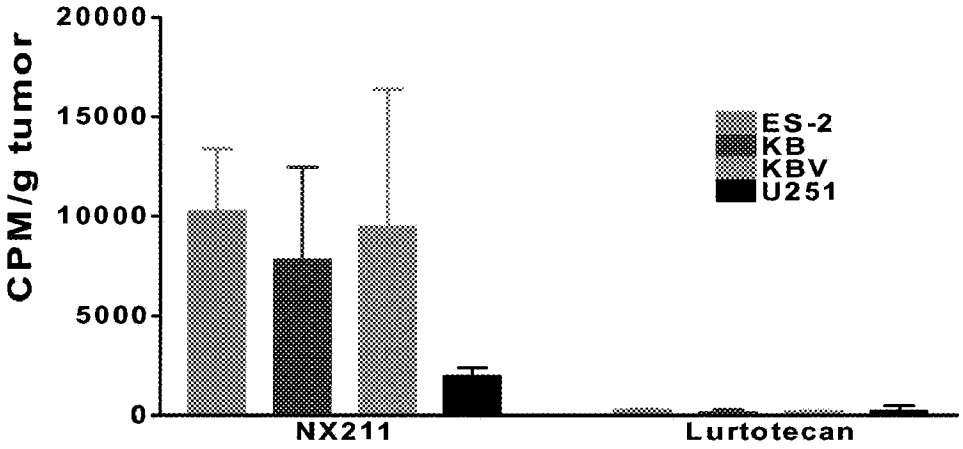

Fig. 4 Localization comparison of [^{14}C]NX 211 and [^{14}C]lurtotecan in tumors from four different tumor xenograft models 24 h after drug dose administration. Data shown are the mean ± SD from three mice at each time point.

determined by the increase in plasma AUC and to greater accumulation of the drug at the tumor site. The 1000–1500-fold increase in the AUC of NX 211 compared with that of the free drug was seen consistently in both rats and mice (33). Similar pharmacokinetics have been observed for both free and liposomal drug in these species and have been demonstrated in rats to be dose linear from at least 0.1 mg/kg to 9 mg/kg NX 211 (data not shown). The 2-log difference in apparent C_{max} between the liposomal and free drug is ascribed to the dramatic decrease in volume of distribution after liposomal encapsulation (over 250-fold). The rapid and extensive distribution of free drug from the bloodstream prohibits the accurate assessment of C_{max}, which is defined as the initial concentration after bolus i.v. administration in a well-stirred model.

Previous work performed in rats using pegylated liposomal GI147211 (SPI-355) also demonstrated a 1250-fold increase in AUC as compared with the free drug, although the absolute increase is difficult to determine because more than 60% of the AUC was determined by extrapolation past actual data points (34). In addition, there was a 4-fold increase in potency over the free drug and an increase in the therapeutic index and the number of durable cures in the HT29 xenograft model. However, no biodistribution studies were performed with this pegylated liposome. Other studies comparing pegylated and nonpegylated liposomal formulations of doxorubicin demonstrate a lack of beneficial effects by coating liposomes with polyethylene glycol (35). These conclusions were based on tumor localization studies in which pegylated liposomes containing doxorubicin accumulated less well than the nonpegylated version. These authors concluded that pegylation, which contributes to prolonged plasma circulation time, may be of little advantage in terms of maximizing liposomal drug accumulation at sites of tumor growth.

The increased local exposure of the tumor to the drug has been demonstrated by other liposomal drugs (26, 27, 36, 37) and is supported by the comparative differences in the present experiments, in which NX 211 was found to increase the overall exposure to lurtotecan by 9–67-fold over the free drug. However, the prolonged plasma concentrations of NX 211 were not solely responsible for the greater amount of radioactive material in both the tumor and spleen because the rate of elimination from these tissues was slower than the rate of elimination from plasma. This is consistent with other reports in which extravasation of small liposomes has been demonstrated to occur in tumor sites due to leaky vasculature (38–40). The variation we see within different xenografts would also argue that the increased exposure is due in part to liposomal extravasation, which varies between different tumors.

The increased efficacy seen with NX 211 compared with lurtotecan was evident in both single-dose and repeat-dose xenograft experiments, where a consistent increase in the therapeutic index, LCK, and the number of durable cures generated is consistent with an improved formulation of this active agent. This improvement in antitumor efficacy is significant and is consistent with the increased plasma AUC and tumor exposure. The increased efficacy of NX 211 in the KBV xenograft is especially intriguing because the free drug demonstrated only slight activity in this model, and active efflux of camptothecin molecules by the PGP-170 protein is believed to be less important as a multidrug resistance factor against this class of cytotoxic drugs.

In the clinic, continuous infusion schedules have been used with topotecan and lurtotecan in an attempt to prolong tumor exposure while reducing toxicity by attenuation of peak plasma drug levels. Phase I studies have evaluated lurtotecan in several i.v. dosing schedules, including daily i.v. doses for 5 consecutive days (12–14), continuous infusion for 3 days (14–19), and continuous infusion for 7, 14, or 21 days (19). Tumor responses in these Phase I studies were observed in the 3- and 21-day infusion schedules, but not in the consecutive 5-day i.v. schedule. Although the number of patients was limited, these results suggest enhancement of the antitumor activity of lurtotecan by continuous infusion. Prolongation of the infusion caused more pronounced thrombocytopenia but did not increase the severity of neutropenia, suggesting that the toxicity profile might also be influenced by the schedule of administration. Clinical findings suggest that prolonged i.v. infusion of topotecan may also increase tumor response (41). Collectively, the data are consistent with the hypothesis that a low-clearance liposomal lurtotecan formulation such as NX 211 will have a superior efficacy and therapeutic index in the treatment of solid tumors. Human clinical studies to determine the safety, tolerability, and phar-

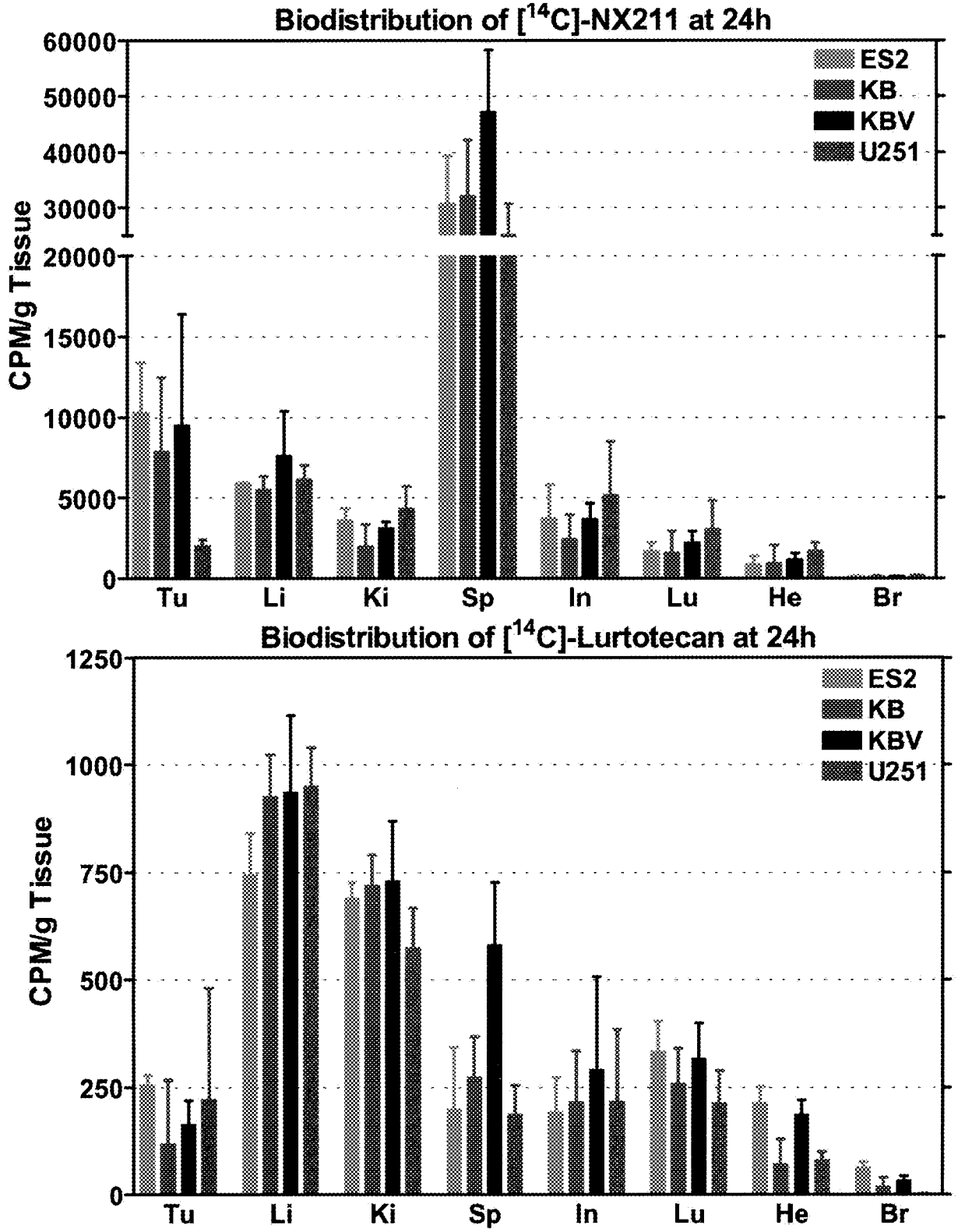

Fig. 5 Biodistribution of [^{14}C]NX 211 and [^{14}C]lurtotecan in eight different tissues from four different xenograft studies. Data shown are the mean ± SD from three mice at each time point. *Tu*, tumor; *Li*, liver; *Ki*, kidney; *Sp*, spleen; *In*, intestine; *Lu*, lung; *He*, heart; *Br*, brain.

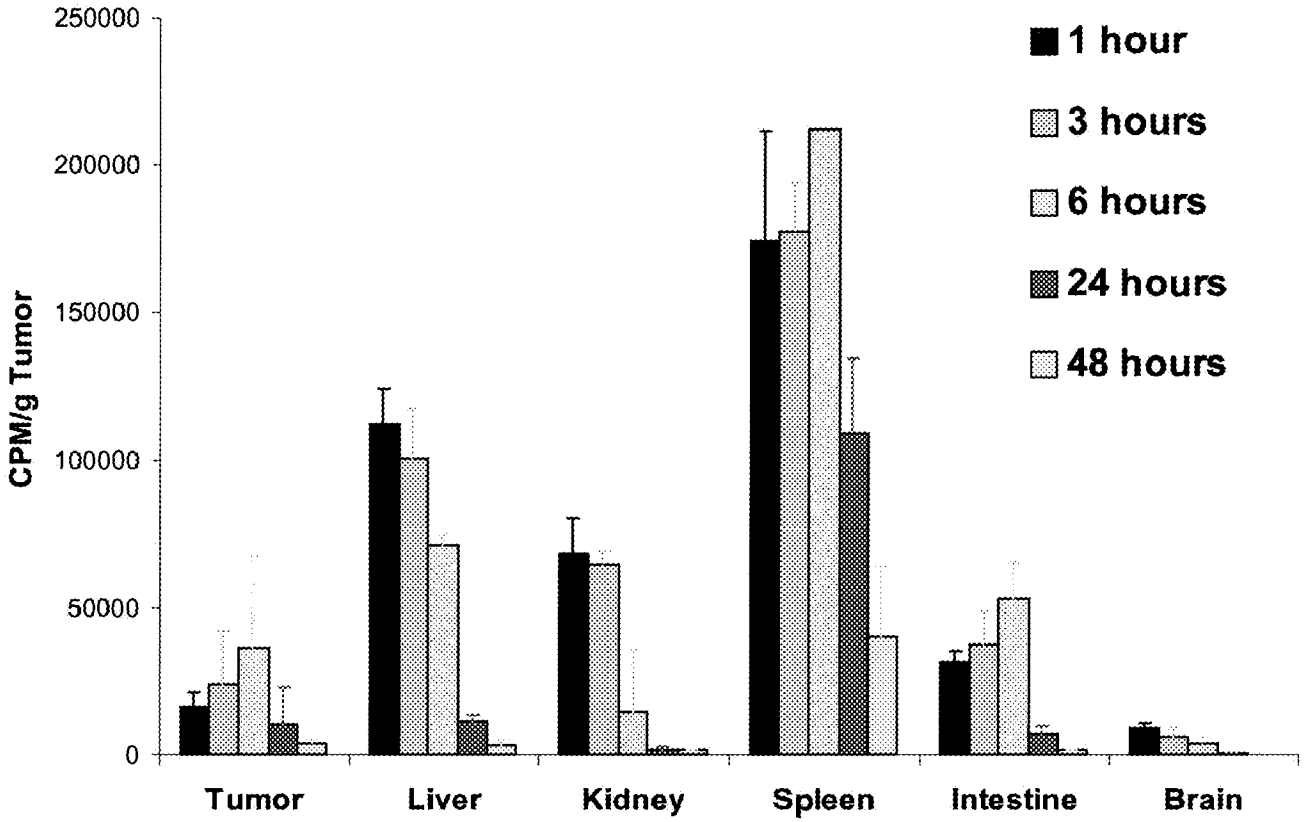

Fig. 6 Time course biodistribution of [^{14}C]NX 211 in six different tissues harvested from mice engrafted with the KB tumor. Data shown are the mean ± SD from three mice at each time point. Missing error bar from the 6 h spleen time point represents the mean of two mice.

macokinetics of NX 211 treatment in patients with advanced solid tumors are currently under way.

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Your Ref: 01208-0002-02JP
IPSEN Ref: 233IBL P2 PCT2 JP-B
Cited Reference: 4

(Translation)

Title: Biomarker as predictive safety testing in oncology

Abstract: Study on biomarkers is considered to be effective for individualization of therapy such as selection of an appropriate group to be treated with expensive molecular target drugs or prediction of life-threatening adverse events. Regarding biomarkers as predictive factors for side effects of cancer therapy, glucuronidase (UGT1A1) gene polymorphism and thiopurine methyltransferase (TPMT) gene polymorphism are clinically applied as predictive factors of side effects due to therapy with irinotecan and 6-mercaptopurine, respectively. Recently, cytidine deaminase (CDA) gene polymorphism has been reported to be a biomarker for predicting toxicity of antimetabolites such as gemcitabine. Development of biomarkers utilizing gene information as predictive factors for avoiding life-threatening adverse events is an important problem to be solved in the future in terms of development of safe pharmaceuticals. With the development of molecular target drugs, the importance of objective biological parameters for prediction of the agent effect, parameters of the therapeutic effect, and prediction of the safety/side effect of prognostic factors or agents has been recognized as biomarkers.

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バイオマーカー

薬剤副作用予測のバイオマーカー

Biomarker as predictive safety testing in oncology

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キーワード：グルクロン酸抱合酵素遺伝子，チオプリンメチル転移酵素遺伝子，シチジンデアミナーゼ遺伝子，遺伝子多型，バイオマーカー

分子標的薬の登場に伴い，適応症例の予測や治療効果の指標，さらには予後因子や薬剤の安全性・副作用予測のための客観的な生物学的指標の重要性がバイオマーカーとして認識されてきた．広義には，バイオマーカーは，①診断・staging，②治療予後因子，③治療標的（薬物の指標），④予後因子，⑤安全性・副作用予測因子，さらには，⑥薬物体内動態の予測，などが含まれる．これらは薬剤開発や臨床試験において科学的な効率化や安全性を担保する点で重要であり，また日常臨床では高価な分子標的薬の適切な治療群の選択や生命にかかわる有害事象の予測など，治療の個別化に力を発揮すると考えられている．本稿では，副作用予測因子としてのバイオマーカーについて述べるが，現時点では分子標的薬のひとつであるゲフィチニブの間質性・肝機能障害や，血管新生阻害薬による血管イベント・胃腸害などを予測するバイオマーカーは知られていないので，これまでの細胞傷害性薬剤に関する研究について紹介する．

細胞傷害性抗癌剤による化学療法では投与薬剤に関する安全域が狭く，また薬剤が最大耐量で投与されるため，薬剤に対する感受性が平均からはずれる患者にとっては著しく高い毒性を有する．臨床においてはその原因遺伝子の個体差があるが，ヒトゲノム情報の解析により遺伝子レベルで規定される個体の多様性の情報が蓄積されてきている．本稿では，塩酸イリノテカン，6-メルカプトプリンとアムルビシンを取り上げ，副作用予測因子のバイオマーカーとしての遺伝的多様性について概説する．

イリノテカンの感性予想に関するバイオマーカー

1．グルクロン酸抱合酵素遺伝子（UGT遺伝子）

イリノテカンはそれ自体はプロドラッグであり，抗腫瘍活性は低いが，カルボキシルエステラーゼにより加水分解されて活性体の数百倍高い抗腫瘍活性を発揮するSN-38に変換される．活性型に変換されたSN-38はさらに肝細胞内でグルクロン酸抱合酵素（uridine diphosphate glucuronosyltransferase：UGT）によりグルクロン酸抱合を受けて細胞毒性をもたないSN-38グルクロン酸抱合体（SN-38G）になり，胆汁を介して腸管に排泄

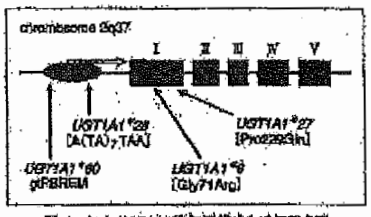

図1　ヒトUGT1A1遺伝子構成と遺伝子多型

される．この *UGT* 遺伝子には遺伝子多型が存在し，グルクロン酸抱合能に差があることが知られている．これまでに報告されたイリノテカンの毒性予測に関するお研究では，プロモーター領域の多型 *UGT1A1*28* と *UGT1A1*60*，エクソン1の多型 *UGT1A1*6* と *UGT1A1*27* が知られている．

*UGT1A1*28* 遺伝子多型はプロモーター領域に存在し，TATA ボックスとよばれる，転写因子が DNA に結合する短い繰り返し塩基配列部分の遺伝子多型である．通常（*UGT1A1*1*）は TA の2塩基配列の繰返しが6回であるのに対して，7回繰り返す配列を有する場合を *UGT1A1*28* とよぶ．その結果，*UGT1A1*28* では UGT の発現が低下することにより酵素活性が低下すると考えられている．同じくプロモーター領域で *UGT1A1*28* の3kb 上流でエンハンサー機能を有する *UGT1A1*60* が存在する．*UGT1A1*60* は1塩基置換による遺伝子多型（SNP）であり，転写因子の結合に影響を与えることにより転写活性を低下させることが，*in vitro* で示されている．*UGT1A1*6* と *UGT1A1*27* の遺伝子多型は1塩基置換による遺伝子多型（SNP）であり，いずれも蛋白質をコードするエクソン領域に存在し，アミノ酸配列の置換を伴うことにより酵素活性が低下することが報告されている（図1）．

2．プロモーター領域遺伝子多型（*UGT1A1*28* と *UGT1A1*60*）

*UGT1A1*28* の遺伝子多型とイリノテカンの毒性に関するこれまでの臨床研究から，*UGT1A1*28* を有する症例ではそうでない症例に比べ，多変量解析においてオッズ比 7～9 倍のリスクで重篤な毒性を発現することが示されている[1,5]．イリノテカン投与後の血漿中活性型イリノテカン（SN-38）からグルクロン酸抱合体（SN-38 G）への変換比（SN-38 G/SN-38）を *UGT1A1*28* の遺伝子多型で比較すると，*UGT1A1*28* の遺伝子多型をもたない患者，ヘテロ接合体（heterozygote）で *UGT1A1*28* の遺伝子多型を有する患者，ホモ接合体（homozygote）を有する患者の順に，SN-38 から SN-38 G への移行が悪くなっていくことが示されており，活性体である SN-38 が長く体内に留まり，生体に影響を及ぼすことがわかる．

これまでの研究結果に基づき，アメリカ食品医薬品局（FDA）は，*UGT1A1*28* 遺伝子多型のホモ型は 9倍のリスクでイリノテカンに対する毒性を示すと解析した．その結果，2005 年7月にアメリカにおいてはイリノテカンの使用説明書の改訂が実施され，*UGT1A1*28* 遺伝子多型のホモ型に対する治療では薬剤を減量して使用するように勧告が出された．また，*UGT1A1*28* をヘテロ接合体でもつ患者では好中球減少のリスクが高いとは予測されるものの，臨床研究の結果は一定せず，通常用量での治療開始が可能であるとしている[6]．

*UGT1A1*60* の遺伝子多型とイリノテカンの毒性に関する研究では，SN-38 から SN-38 G へのグルクロン酸抱合化をみると，*UGT1A1*60* 遺伝子多型を有する患者において低下しているが，とくに日本人においては *UGT1A1*28* との連鎖不平衡がみられ，多変量解析では *UGT1A1*28* のインパクトが強く残り，*UGT1A1*60* 遺伝子多型が統計学的に有意差を示さないことが示唆されている[6]．しかし，*in vitro* の解析から *UGT1A1*60* によるエンハンサー機能低下と *UGT1A1*28* によるプロモーター機能低下が異なり，生体における UGT1A1 酵素の機能低下をきたしていると推測される．

3．エクソン領域遺伝子多型（*UGT1A1*6* と *UGT1A1*27*）

遺伝子多型は人種による頻度やその存在に差があるが，*UGT1A1*6* は欧米人にはなく，アジア人にのみ報告されている遺伝子多型であり，その頻度はアジア人において *UGT1A1*28* と同程度存在することが報告されている．臨床研究から，*UGT1A1*6* のホモ接合体を有する患者では SN-

38 から SN-38 G への変換が *UGT1A1*28* と同程度低下することから，アジア人における *UGT1A1*6* の重要性が示された[5]．これまでの研究から，わが国を含むアジアでは *UGT1A1*28* に加えて *UGT1A1*6* がイリノテカンの毒性予想に重要であることが示唆される．

*UGT1A1*27* については，少数例であるため統計学的な解析はないが，著者らの症例ではヘテロ型の全 3 症例で重度の副作用が認められた．エクソン領域の遺伝子多型と *UGT1A1*28* あるいは *UGT1A1*60* 遺伝子多型を合わせもつ患者ではさらに重篤な副作用の発現が報告される．最近の解析では，*UGT1A1*27* を有する患者は *UGT1A1*28* を合わせもつ遺伝子型と同様の治療が予測されている．

このように，イリノテカンをはじめとしてグルクロン抱合酵素が薬剤代謝に関係する抗癌剤においては，毒性予測のバイオマーカーとして *UGT1A1*28*，*UGT1A1*6* ならびに *UGT1A1*27* が臨床的に有用であることが示されている[6]．

6-メルカプトプリンとチオプリンメチル転移酵素

6-メルカプトプリン(6-MP)は，それ自体は活性のないプロドラッグであり，上皮キサンチングアニンホスフォリボシル転移酵素(HGPRT)により活性化され，細胞内にチオグアニンヌクレオチド(TGNs)として蓄積される．さらに，TGNs がヌクレオチドアナログとして DNA に組み込まれることにより殺細胞効果が発揮される．6-MP はキサンチンオキシダーゼにより 6-チオ尿酸に代謝されるとともに，チオプリンメチル転移酵素(TPMT)によりメチル化され，6-メチルメルカプトプリン(6-MeMP)へと代謝される．これまでの研究から，TPMT 活性と細胞内の TGNs 濃度が関連し，TPMT 活性の低い患者では TGNs 濃度が高くなり，標準量の 6-MP 投与により重篤な骨髄抑制の副作用が出現することが報告されている．

白人では赤血球中の TPMT 活性の分布は三峰性を示し，89～94%の人は高い TPMT 活性をもち，6～11%の人が中間の TPMT 活性，0.3%の人が低い TPMT 活性群と報告されている．*TPMT* 遺伝子多型の研究から *TPMT*2*，*TPMT*3A*，*TPMT*3C* では TPMT 活性が低下していること，TPMT 低活性群の 95%以上では *TPMT*2*，*TPMT*3A*，*TPMT*3C* のうちの 2 つを有しているが，中間の TPMT 活性を示す群では 1 つを有することが報告されている(図 2)．

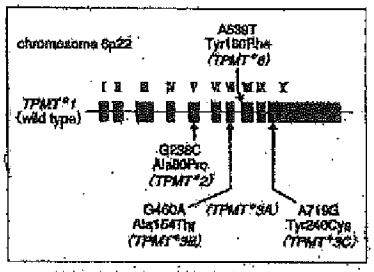

図 2　ヒト *TPMT* 遺伝子領域と遺伝子多型

小児急性白血病の治療で，2 つの無機能の *TPMT* 遺伝子をもつ症例では 6-MP の投与量を標準量の 6～10%とする一方，無機能の *TPMT* 遺伝子を 1 つ有する症例では，初期には標準の投与量で治療を行うが，治療経過中に副作用により投与量の減量が必要とされる．また，高い TPMT 活性を有する症例に比べ，中間の TPMT 活性あるいは TPMT 低活性群では無症状期間あるいは効果においてより成績が認められており，高い TPMT 活性を有する症例で 6-MP の用量を増量することにより，よい治療結果を得ることができる可能性が示唆されている．

このように，抗癌剤である 6-MP の毒性予測のバイオマーカーとしての *TPMT* 遺伝子多型の役割が明らかにされてきており，臨床的にも応用されている[7]．

ゲムシタビンとシチジンデアミナーゼ

ゲムシタビンは代謝拮抗薬に分類される抗癌剤として，膵癌をはじめ広い範囲の治療に用いられている．ゲムシタビンは代謝の過程でシチジンデアミナーゼ(CDA)により直接にゲムシタビンウラシル体へ不活性化され，体外へ排泄される．近年，*CDA* 遺伝子多型とゲムシタビンの薬物動態に関する研究が報告された(図 3)[8,9]．とくに，エクソ

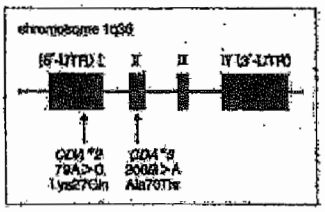

図3　ヒトCDA遺伝子構成と遺伝子多型

ンスの遺伝子多型(208G>A(Ala70Thr), *CDA*3*)のホモ接合体患者で重篤な骨髄抑制と非血液毒性を示した1症例が報告された。さらに，*CDA*3*を有する患者群ではゲムシタビン投与後の最高血中濃度(C_{max})もAUCも高い傾向にあり，とくに他剤と併用する場合に毒性が顕著であると報告されている。血漿中のCDA活性を測定することが可能であり，その活性とCDA遺伝子型がよく相関することから，臨床的に問題となる*CDA*3*ホモ接合体に対する薬作用予測として血漿中CDA活性測定がバイオマーカーとしての役割を果たす可能性が示唆された。しかし，*CDA*3*のアリル頻度は日本人では3.7%とされ，*CDA*2*ホモ接合体は1,000人に1人と予想されていることから，臨床応用とその必要性についてはさらなる検討が必要である。

おわりに

近年，分子標的薬の開発に関する臨床試験ではバイオマーカーを組み込んだ臨床試験が有効な結果を得るために必要であることが知られてきている。すでに乳癌領域ではHER2蛋白の発現やホルモンレセプターの発現により治療薬が選択され，バイオマーカーを基盤とした患者層別化戦略に基づく治療が実臨床で応用されている。このような効果予測因子としてのバイオマーカーの研究・応用は効率的な薬剤開発につながるとともに，一見効果がないと思われる疾患群のなかに顕著な効果を示す患者群を特定できる可能性を秘めている。一方で，生命を脅かす有害事象の回避を予測するバイオマーカーの開発は，安全な医薬品開発において重要な課題である。

FDAは検証されたバイオマーカーをvalid biomarkerと位置づけ，これまで承認した医薬品のなかで18のvalid biomarkerを示している[10]。18のvalid biomarkerはさらに，①レベル1(検査が必要)，②レベル2(検査が推奨)，③レベル3(情報のみ)と区分され，レベル1と評価されているのは，大腸癌に対するirinotecan使用時における上皮成長因子受容体(EGFR)発現の検査と，乳癌治療における trastuzumab(ハーセプチン®)使用時におけるHER2蛋白の過剰発現の検査である。レベル2に本稿で述べた*UGT1A1*遺伝子多型と*TPMT*遺伝子多型あるいは酵素測定，ならびにゼーファリン使用におけるプロテインC欠損検査の3種類があげられている。ヒト遺伝子多型情報が飛躍的に蓄積され，その機能解析と生体における役割について研究が進められている。今後，これらの研究に基づく有害事象を回避するためのバイオマーカーの研究・開発が進むことを期待したい。

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Date
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Reference	Application No./Patent No.
P41814EP-K/NJL	05745505.7 - 2112 / 1746976 PCT/US2005015349
Applicant/Proprietor	
Hermes Biosciences, Inc.	

Communication

The European Patent Office herewith transmits as an enclosure the supplementary European search report under Article 153(7) EPC for the above-mentioned European patent application.

If applicable, copies of the documents cited in the European search report are attached.

☑ 1 additional set(s) of copies of the documents cited in the European search report is (are) enclosed as well.

Refund of the search fee

If applicable under Article 9 Rules relating to fees, a separate communication from the Receiving Section on the refund of the search fee will be sent later.

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SUPPLEMENTARY EUROPEAN SEARCH REPORT

Application Number
EP 05 74 5505

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X,P	WO 2005/002546 A1 (SMITHKLINE BEECHAM CORP [US]; OH CHOON K [US]; IGNATIOUS FRANCIS [US]) 13 January 2005 (2005-01-13) * page 7, line 7 - page 8, line 22 *	1,2,12, 16, 18-20, 70-76, 78-80,82	INV. A61K9/127
X	WO 98/17256 A1 (KIRPOTIN DMITRI [US]) 30 April 1998 (1998-04-30)	1,11-21, 50,56, 70,71, 74,76-82	
Y	* page 3, line 4 - line 14 * * page 4, line 3 - page 5, line 31 * * page 9, line 1 - line 23 * * examples 1-9 *	1-27, 50-92, 94-97	
X	US 4 321 259 A (NICOLAU YVES-CLAUDE ET AL) 23 March 1982 (1982-03-23) * column 7, line 24 - line 67 * * claims 1-2 *	1-5,8-10	
Y	US 5 316 771 A (BARENHOLZ YECHEZKEL [IL] ET AL) 31 May 1994 (1994-05-31) * the whole document *	1-27, 50-92, 94-97	TECHNICAL FIELDS SEARCHED (IPC) A61K

The supplementary search report has been based on the last set of claims valid and available at the start of the search.

Place of search	Date of completion of the search	Examiner
Munich	25 August 2010	Sindel, Ulrike

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

3

EPO FORM 1503 03.82 (P04C04)

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing claims for which payment was due.

☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for those claims for which no payment was due and for those claims for which claims fees have been paid, namely claim(s):

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for those claims for which no payment was due.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:

☐ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

☒ The present supplementary European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims (Rule 164 (1) EPC).

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LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 05 74 5505

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. claims: 1-27, 70-92, 94, 95, 97(completely); 50-69, 96(partially)

   Liposomes in a medium said liposome comprising a polyanionized polyol or polyanionized sugar within the interior and having a transmembrane gradient effective for retention of an entity within said liposome

   ---

2. claims: 28-45(completely); 50-69, 96(partially)

   Liposome comprising one or more lipids and an antineoplastic therapeutic entity encapsulated therein at an entity-to-lipid molar ratio of at least 0.10, showing a four-fold higher activity and equal or less toxicity than the entity in a free non-liposomal form.

   ---

3. claims: 46(completely); 50-69, 96(partially)

   Liposomes in a medium said liposomes comprising one or more lipids and a vinca alkaloid drug encapsulated therein at a first drug/lipid ratio wherein 24 hours following administration into the bloodstream of a mammal the vinca alkaloid remains entrapped at a second drug/lipid ratio which is over 50% of the first one.

   ---

4. claims: 47-49(completely); 50-69, 96(partially)

   Liposomes in a medium said liposomes comprising lecithin and cholesterol in a molar ratio of 3:2 and a vinca alkaloid drug contained therein at a drug/lipid ratio of 0.15-0.55 mg/mmol lecithin wherein the vinca alkaloid drug is vinorelbine, vincristine or vinblastine. The interior of the liposomes contains also a biodegradable polyanionic polymer, a polyanionized polyol or a polyanionized sugar at a concentration of 0.5-1.0 gram-equivalent/L.

   ---

5. claims: 93(completely); 96(partially)

   Method for the preparation of liposomes containing an encapsulated entity which is an organic compound or a compound comprising a coordination complex of a metal of the platinum group; said method comprising the steps of providing a pre-entity, encapsulating it into a liposome and providing a condition inside the liposome to convert this encapsulated pre-entity into said entity within the interior space of said liposome.

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LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 05 74 5505

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

The common concept linking the different groups of invention is a liposome containing an entity. Since this concept is not new (see WO 98/17256, examples 1-9), the separate inventions are not so linked as to form a single general inventive concept and hence do not fulfill the requirements of unity.

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 05 74 5505

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
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25-08-2010

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		JP 2007522085 T	09-08-2007
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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

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Submission in opposition proceedings

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- representing the proprietor(s):	IPSEN BIOPHARM LTD
Proprietor/representative's reference	O008029EP

The information given below is pertaining to the following patent in opposition proceedings:

Patent No.	EP2861210
Application No.	EP13731230.2
Title of the invention	Methods for treating pancreatic cancer using combination therapies comprising liposomal irinotecan
Proprietor of the patent	IPSEN BIOPHARM LTD

Documents attached:

	Description of document	Original file name	Assigned file name
1	Main request in opposition	MR.pdf	MAINREQ-1.pdf
2	Auxiliary request in opposition	AR1.pdf	AUXREQ-1.pdf
3	Auxiliary request in opposition	AR2.pdf	AUXREQ-2.pdf
4	Auxiliary request in opposition	AR3.pdf	AUXREQ-3.pdf
5	Any annexes (other than citation) to an opposition letter - Statement of Grounds	O008029EP-statement of grounds_final.pdf	OTHER-1.pdf
6	Any annexes (other than citation) to an opposition letter - Covering letter	O008029EP-O-Letter-9837e305 (signed).pdf	OTHER-2.pdf

Evidence filed subsequently:

D23	Other evidence	D23 - Declaration of Amy McKee M.D. original file name: D23.pdf

		attached as: Other-evidence-1.pdf
D23A	Other evidence	D23A - Hoos, W.A. et al original file name: D23 A.pdf attached as: Other-evidence-2.pdf
D23B	Other evidence	D23B - Clinical Development Success Rates 2006-2015 original file name: D23B.PDF attached as: Other-evidence-3.PDF
D24	Other evidence	D24 - Belanger Declaration-signed original file name: D24 - Belanger_declaration_signed.pdf attached as: Other-evidence-4.pdf

Signatures

Place:	London
Date:	30 December 2019
Signed by:	/OATES, Edward Christopher/
Representative name:	Edward Christopher OATES
Capacity:	(Representative)

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Electronically submitted

Your Ref 13731230.2 / 2861210 - T2963/19 - 3.3.07
Our Ref O008029EP:ECO/SJD/FJT
Date 30th December 2019

Dear Sirs,

Re: European Patent No. 2861210
In the name of Ipsen Biopharm Ltd.
Opposed by Teva Pharmaceutical Industries Ltd

The appellant's statement of grounds of appeal is enclosed.

As mentioned in the enclosed, the appellant's Main Request is for the decision under appeal to be set aside, and for the patent to be maintained on the basis of the enclosed claim set entitled "Main Request -- December 2019". If the Main Request cannot be allowed, the appellant requests that the patent be maintained on the basis of one of the enclosed Auxiliary Requests 1-3, considered in that order.

Oral proceedings are requested should the Board of Appeal be considering anything other than the Main Request.

Yours faithfully,

// ELECTRONICALLY SIGNED AND SUBMITTED //

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

Encl. Statement of grounds of appeal
D23 and annexes

CARPMAELS & RANSFORD

D24
Main Request - December 2019
Auxiliary Requests 1-3 - December 2019

PROPRIETOR'S STATEMENT OF GROUNDS OF APPEAL

EUROPEAN PATENT 2 861 210 B1

IN THE NAME OF IPSEN BIOPHARM LTD

T2963/19 - 3.3.07 | 13731230.2 | O008029EP

1 INTRODUCTION

1.1 European patent EP2861210 ("the patent") was opposed by Teva Pharmaceutical Industries Ltd. and revoked by the Opposition Division in oral proceedings held on 10th July 2019. The Opposition Division's decision was dated 28th August 2019.

1.2 The appellant (patent proprietor) lodged an appeal against this decision on 7th November 2019 and paid the appeal fee on the same day. The appeal has been allocated number T2963/19. This is the appellant's statement of grounds of appeal.

1.3 In its decision, the Opposition Division found that the patent's first priority claim was invalid, meaning that several documents (most notably D15b) were citable as prior art, and that all claim requests did not comply with Article 56 EPC. Thus, this document will focus on the validity of the first priority claim, and inventive step. The Opposition Division correctly found that all claim requests satisfied Articles 83 and 123 EPC and Rule 80 EPC, and so these will not be addressed in this document.

2 REQUESTS

2.1 The appellant's Main Request is for the decision under appeal to be set aside, and for the patent be maintained on the basis of the enclosed claim set entitled "Main Request – December 2019". This claim set is identical to the claim set filed on 28th June 2019 labelled "Auxiliary Request 2 – June 2019". This request was the proprietor's Main Request in the oral proceedings held before the Opposition Division and was referred to as the Main Request in the decision of the Opposition Division. If the Main Request cannot be allowed, the appellant requests that the decision under appeal be set aside, and that the patent be maintained on the basis of one of Auxiliary Requests 1-3 – December 2019, considered in that order, which are enclosed. These auxiliary requests will be explained in more detail below. All of these claim requests were submitted at first instance and were the subject of the Opposition Division's Decision.

2.2 Should the Board be considering anything other than the Main Request, oral proceedings are requested.

2.3 The appellant requests permission to make further amendments to any of the requests, and/or to submit further auxiliary requests in response to points raised by the respondent or the Board.

2.4 For the avoidance of doubt, any deleted or unclaimed subject matter is not abandoned.

2.5 Should it prove necessary, the appellant requests that any amendment of the description be deferred until agreement has been reached on the claims.

3 DOCUMENTS

3.1 The appellant intends to rely on all of the documents which were on file during the first instance proceedings. In addition, the following documents are enclosed:

- D23 – Declaration of Amy McKee, M.D., including supporting annexes, and
- D24 – Declaration of Bruce Belanger, Ph.D.

3.2 D23 and D24 are declarations which are admissible because they are filed in response to the comments made in the Opposition Division's decision regarding document D15b, and which led to the Opposition Division's decision to revoke the patent. This statement of grounds is being filed prior to the revised version of the Rules of Procedure of the Boards of Appeal entering into force on 1 January 2020, and so Article 12, paragraphs 4 to 6, of the revised version shall not apply to this statement, in accordance with Article 25(2).

4 MAIN REQUEST - PRIORITY ENTITLEMENT

4.1 The Opposition Division found that the patent's first priority claim is invalid because "*the claimed subject matter which describes a dose of leucovorin in the l form* [sic] *cannot be derived directly and unambiguously from* [the first priority document]". In this section it will be explained that the subject matter of the Main Request can be directly and unambiguously derived from the first priority document (hereafter "PD1"), and that the Opposition Division was wrong to decide otherwise.

4.2 Claim 1 of the Main Request relates to liposomal irinotecan for use in a method of treating pancreatic cancer in a human patient. The method comprises:

"*co-administration of an effective amount each of liposomal irinotecan, 5-fluorouracil (5-FU) and leucovorin to the patient in at least one cycle wherein the cycle is a period of 2 weeks and, for each cycle:*

*(a) liposomal irinotecan is administered to patients not homozygous for the UGT1A 1 *28 allele on day 1 of each cycle at a dose of 80 mg/m^2 and to patients homozygous for the UGT1A1 *28 allele on day 1 of cycle 1 at a dose of 60 mg/m^2 and on day 1 of each subsequent cycle at a dose of 60 mg/m^2 or 80 mg/m^2;*

(b) 5-FU is administered at a dose of 2400 mg/m^2; and

(c) leucovorin is administered at a dose of 200 mg/m^2 (l form)".

This feature finds basis in claim 3 of PD1.

4.3 The final integer of claim 1:

"*and wherein in each cycle, the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU*"

finds basis in claim 4 of PD1.

4.4 The patient population specified in claim 1:

"*wherein the patient exhibits evidence of recurrent or persistent pancreatic cancer following primary chemotherapy and wherein the patient has failed prior treatment with gemcitabine or become resistant to gemcitabine*"

finds basis on page 11 (final sentence) and page 12 (second sentence) of PD1.

4.5 Claim 1 of the Main Request requires that the liposomal irinotecan:

"*is irinotecan sucrose octasulfate salt liposome injection*"

This feature finds basis on, for example, page 3 lines 3-4, page 4 lines 16-17, page 5 lines 4-5 and 27-28, and claim 11 of PD1.

4.6 Claim 1 of the Main Request specifies that the leucovorin is the "l-form", and PD1 refers to "leucovorin". Contrary to the Opposition Division's finding, the subject matter of claim 1 of the Main Request is directly and unambiguously derivable from PD1. As stated during the first instance proceedings, the skilled person would appreciate that the reference to "leucovorin" in PD1 is a direct and unambiguous reference to the "l-form" of leucovorin, meaning that the subject matter of claim 1 validly claims priority to PD1. Even if the Board disagrees with this interpretation, it remains the case that the first priority claim is valid, as explained below.

The requirement for leucovorin to be in the "l-form" does not result in a loss of priority

4.7 By way of background, the skilled person would have been aware that the leucovorin molecule is optically active, meaning that a given molecule of leucovorin can exist either as the l-form or as the d-form. The l-form and the d-form can be referred to as "optical isomers". At the filing date of PD1, leucovorin was commercially available as either the l-form (sometimes referred to as "levoleucovorin" or "l-leucovorin") or as a 50:50 isomeric mixture of the l- and d-forms (sometimes referred to as "l+d leucovorin" or "racemic leucovorin"). It was common general knowledge that, of the two optical isomers, the l-form of leucovorin is pharmaceutically active, and the d-form is not (see, for example, D1 at section "11 DESCRIPTION"). This is why the pure d-form of leucovorin was not approved as a drug at the priority date, nor has it been approved as a drug at the time of writing.

4.8 With this in mind, the skilled person considering PD1 would note from page 11, second complete paragraph (reproduced below) that, in the context of PD1, the term "leucovorin" was being used to refer to the "l-form" of leucovorin. This is because this passage on page 11 makes it clear that the term "leucovorin" is a reference to the precise species which acts as a biochemical cofactor – i.e. the species which actually has an effect *in vivo*.

> Leucovorin acts as a biochemical cofactor for 1-carbon transfer reactions in the synthesis of purines and pyrimidines. Leucovorin does not require the enzyme dihydrofolate reductase (DHFR) for conversion to tetrahydrofolic acid. The effects of methotrexate and other DHFR-antagonists are inhibited by leucovorin. Leucovorin can potentiate the cytotoxic effects of fluorinated pyrimidines (i.e., fluorouracil and floxuridine). After 5-FU is activated within the cell, it is accompanied by a folate cofactor, and inhibits the enzyme thymidylate synthetase, thus inhibiting pyrimidine synthesis. Leucovorin increases the folate pool, thereby increasing the binding of folate cofactor and active 5-FU with thymidylate synthetase.

4.9 A similar definition is given on page 33 of the original application, under the heading "Description of 5-FU and Leucovorin", i.e. in the definitions section.

4.10 As stated at 4.7 above, l-leucovorin is the only form of the compound that is able to act as a biochemical cofactor in vivo. Therefore, within the context of PD1, the term "leucovorin" is used to refer to the active form of leucovorin, i.e. the l-form. Thus, the requirement in claim 1 that the leucovorin is in the "l-form" is implicitly, but directly and unambiguously, derivable from PD1.

4.11 If, unexpectedly, the Board finds that the skilled person would not have understood the reference to "leucovorin" in PD1 as a direct and unambiguous disclosure of the "l-form" of leucovorin, it remains the case that the first priority claim is valid. That is, if the Board is of the view that the skilled person would not have understood the "leucovorin" in PD1 to be a direct and unambiguous disclosure of the "l-form" of leucovorin, the only remaining sensible interpretation of the term "leucovorin" is as "l-leucovorin or racemic leucovorin". This is because, as stated above at 4.7, these were the only two forms of leucovorin which were available when PD1 was filed.

4.12 If the term "leucovorin" in PD1 were interpreted by the skilled person in PD1 as "l-leucovorin or racemic leucovorin", specifying "l-leucovorin" in claim 1 cannot generate new subject matter because it is, at worst for the appellant, a selection from two equally preferred alternatives. Thus, the subject matter of claim 1 of the Main Request is directly and unambiguously derivable from PD1, and thus the first priority claim is valid.

The case law on the novelty of enantiomers supports the first priority claim

4.13 The Opposition Division's reasoning for finding the first priority claim invalid was based on an analysis of several T-decisions which concern the question of novelty of specific enantiomers over racemic mixtures. As the Opposition Division stated, these decisions are relevant because "*the concept of disclosure is the same for both the assessment of novelty and the determination of the right to claim priority*". However, the Opposition Division incorrectly applied the cited case law to the facts of the present case, and this led to the Opposition Division's incorrect finding that the first priority claim is invalid.

4.14 The appellant appreciates that, following, for example, T296/87[1] and T1048/92, the disclosure of a racemic compound in a document will not always constitute a direct and unambiguous

[1] The Opposition Division's decision referred to "T269/87". However, based on the context, the appellant will proceed on the assumption that "T296/87" was intended.

disclosure of each optically active form of that compound. However, this only holds true when the document in question fails to provide any teaching which discusses the individual optical isomers or enantiomers. Where there is an individualised technical teaching of specific optical isomers in the document, the Boards have held that a claim to a specific optical isomer will lack novelty, as the specific optical isomer will be directly and unambiguously derivable from the document (see T600/95 and T658/91).

4.15 In view of the above, the question to be answered when determining whether a document (for example, a prior art document in a novelty analysis, or a priority document as is the case here) provides a direct and unambiguous disclosure of an individual optical isomer is: does the document provide an individualised technical teaching of the individual optical isomer to the skilled person explicitly or implicitly? When considering this question, it is essential to remember that the skilled person will read the document in light of the common general knowledge (T786/00 and T1117/14).

4.16 It is true that there is no explicit disclosure of individual optical isomers in PD1. However, the skilled person would know from his/her common general knowledge that leucovorin is an optically active molecule, with the l-form and the racemic form being available as drugs (see D1 and D1b, and 4.7 above). Even if the Board does not agree with the arguments made in 4.8 to 4.10**Error! Reference source not found.** above, the fact that PD1 provides no explicit disclosure of optical isomers does not prejudice the validity of the first priority claim, because the skilled person reading PD1 in light of his/her common general knowledge would appreciate that the "leucovorin" referred to in PD1 can only be a reference to either "l-leucovorin" or "racemic leucovorin". The Opposition Division failed to appreciate that PD1 directly and unambiguously discloses "l-leucovorin" or "racemic leucovorin" to the skilled person reading PD1 in light of the common general knowledge. The Opposition Division's failure to take the skilled person's common general knowledge into consideration meant that it came to an incorrect decision on priority. When the case law is applied correctly, and the disclosure of PD1 is considered in combination with the common general knowledge, it becomes clear that the first priority claim is valid.

The opponent's arguments on priority are wrong

4.17 During the first instance proceedings, the opponent argued that the skilled person would have understood the reference to "leucovorin" in PD1 to be a reference to the racemic form only (see, for example, paragraphs 4.5 and 4.6 of its submission of 10th May 2019). The Opposition Division's decision did not comment on this point. However, for the avoidance of doubt, the opponent's arguments on this point are wrong.

4.18 The opponent argued that an interpretation which allows the term "leucovorin" to be read as "l-leucovorin" or "racemic leucovorin" goes against "*well-established principles… from which the skilled person knows how to describe racemic compounds and enantiomers*". The appellant appreciates that, in the field of organic chemistry, there are indeed "well-established principles". However, it is important to bear in mind that, in this case, the skilled person, to whom PD1 is addressed, is not an organic chemist. The invention described in PD1 concerns "improvements in, and effective alternatives to, current therapies for pancreatic cancer" (page 2, penultimate sentence). It is not concerned with, for example, the provision of novel organic compounds, or novel processes for preparing organic compounds. Thus, it is clear that the

skilled person in this case is not an organic chemist. Rather, the skilled person is clearly an oncologist (i.e. a clinician specialising in the treatment of cancer).

4.19 An oncologist is concerned with the treatment of cancer patients. This is reflected in the disclosure of document D2 (a document written by and for clinicians) whose title refers to "leucovorin" and discloses that the l-form of leucovorin was being used (page 462, first paragraph under heading "Treatment Schedule"). D2 thus makes it clear that the term "leucovorin" was not used in the art to refer to the racemic form only.

4.20 The opponent's arguments on this point are thus based on a failure to consider the nature of the skilled person to whom PD1 is addressed and/or a failure to appreciate how the terminology was used and understood in the art. The skilled person in this case is an oncologist (i.e. a clinician). In spite of the fact that an oncologist would have been aware of the two forms of leucovorin which were available at the filing date of PD1 (see D1 and D1b, for example), s/he would not have stuck rigidly to strict chemical nomenclature when interpreting the disclosure of PD1. Rather, s/he would interpret the term "leucovorin" as "l-leucovorin" or "racemic leucovorin". As stated above, if one selects "l-leucovorin" from these two alternatives (which actually would have been the more natural thing to do given that l-leucovorin was the active form, as explained above), no new subject matter is generated, meaning that the first priority claim is valid.

Conclusion on Priority Entitlement of the Main Request

4.21 In this section it has been demonstrated that the term "leucovorin" in PD1 would be interpreted by the skilled person in possession of the common general knowledge as a direct and unambiguous reference to "l-leucovorin", meaning that claim 1 finds basis in PD1, and that the first priority claim is valid. Even if the Board disagrees with the appellant on this point, the first priority claim remains valid because, at worst for the appellant, the "l-leucovorin" feature in claim 1 arises as a result of a single selection from two equally preferred alternatives. This is in agreement with the case law cited in the decision under appeal, which the Opposition Division applied incorrectly to the facts of the case. It is wrong to argue that the reference to "leucovorin" is a reference to "racemic leucovorin" only.

4.22 Thus, when PD1 is properly interpreted and the nature of the skilled person reading PD1 is properly assessed, it becomes clear that the reference in claim 1 to the "l-form" of leucovorin is directly and unambiguously derivable from PD1. The remaining features of claim 1 are also directly and unambiguously disclosed in PD1 (see 4.2 to 4.5).

4.23 Claim 2 finds basis in claims 5, 7 and 8 of PD1. Claim 3 finds basis in claims 6, 9 and 10 of PD1. Claim 4 finds basis on page 12, fourth sentence of PD1.

4.24 Thus, the claims of the Main Request find direct and unambiguous support in PD1, meaning that the first priority claim is valid.

5 MAIN REQUEST - INVENTIVE STEP

5.1 The first priority claim is valid, meaning that documents D4, D8, D10, and D15b are not prior art. On this basis, it will be shown in this section that the claims of the Main Request involve an inventive step starting from D13 as the closest prior art.

5.2 If, unexpectedly, the Board finds that the first priority claim is invalid, it will also be shown in this section that the claims of the Main Request are nonetheless inventive.

The claims are inventive if the first priority claim is valid

Closest Prior Art

5.3 Documents D12 (Ko et al.) and D13 (Tsai et al.) are the only prior art documents on file which provide a disclosure of liposomal irinotecan being used to treat gemcitabine-resistant pancreatic cancer in a Phase II trial. The Phase II trials described in D12 and D13 had a primary endpoint of determining the patients' 3-month overall survival rate. That is, the trial was carried out with the aim of determining treatment efficacy. The results of the Phase II trial described in D12 and D13 are reported positively in both documents. For example, D12 states that liposomal irinotecan:

> "*appears to have both activity and tolerable side effects for* [patients] *with metastatic,* [gemcitabine]*-refractory PC, and represents a promising option for the* [patient] *population with few standard options"* (final sentence)

5.4 Both D12 and D13 report on the same Phase II trial. However, D13 is a more appropriate choice of closest prior art than D12. D12 is a conference abstract, whereas D13 is a full journal article whose disclosure is more comprehensive than that of D12 (for example, as well as the 3-month survival rate, D13 also reports the 6-month survival rate[2], whereas D12 does not). Thus, D13 would serve as a more promising springboard than D12. In addition, D13 was published closer to the priority date than D12, meaning that D13 is more reflective of the state of the art at the priority date. Indeed D13 references D12 (on page 189, bottom right hand corner; D12 is reference 30). Thus, D13 should be taken as the closest prior art.

5.5 For the avoidance of doubt, D7 is not an appropriate choice for the closest prior art. D7 describes a Phase I trial whose aims are to "define the dose-limiting toxicity (DLT), maximum tolerated dose (MTD), and pharmacokinetics (PK)" of liposomal irinotecan ("Background" section, second sentence). That is, unlike the Phase II trial described in D13, the primary purpose of the Phase I trial described in D7 was not to ascertain whether the liposomal irinotecan has therapeutic efficacy. A Phase I trial is carried out at an earlier stage of development than a Phase II trial of the type described in D13, and for this reason alone D7 cannot be considered closer to the claimed invention than D13, which describes Phase II data. Moreover, the trial described in D7 enrolled patients having "advanced refractory solid tumors" – there is no mention of the patients being gemcitabine resistant or having failed treatment with gemcitabine. It is true that one patient having pancreatic cancer being treated in the Phase I trial being reported in D7 did show a partial response. However, this does not take away from the fact that the purpose of the trial being reported in D7 was not to determine

[2] D13, page 191, third complete sentence.

the efficacy of liposomal irinotecan against pancreatic cancer, unlike D13. Thus, the partial response seen in a pancreatic cancer patient does not result in D7 being the closest prior art.

5.6 D15a gives some information regarding a Phase III clinical trial of liposomal irinotecan monotherapy, which uses the same regimen referred to in D12 and D13. D13 is a more appropriate choice of closest prior art at least because it discloses actual treatment of gemcitabine-resistant pancreatic cancer, whereas D15a does not. In addition, D13 is a more comprehensive disclosure than D15a, meaning that it would serve as a more promising springboard than D15a.

Closest prior art embodiment

5.7 The closest prior art document is D13. However, as well as the Phase II trial discussed above, D13 also contains information about other clinical trials involving liposomal irinotecan. Therefore, it is also important to identify the closest prior art embodiment within D13. This is consistent with the Board's guidance in T1287/14 (Reasons 5.2.1), where it is stated that:

> *"The specific starting point for assessing inventive step is … normally a set of features disclosed in combination in a document, e.g. an embodiment or example, the latter document being also referred to as "the closest prior art" in a more general sense … For assessing inventive step it is therefore necessary to establish the distinguishing features over that specific starting point and to assess whether it was obvious to arrive at the claimed subject-matter when starting from that specific point"* (emphasis added)

5.8 The relevant part of D13 to consider here is the paragraph which begins on page 189 (right-hand column) and runs over to page 191 (left-hand column). Briefly, this paragraph starts by describing a Phase I clinical trial using liposomal irinotecan monotherapy administered every three weeks in patients with standard therapy-failure solid tumors, with two patients showing a partial response. One of these patients was said to have pancreatic cancer. The paragraph then mentions a Phase I trial in which liposomal irinotecan was administered in combination with weekly 24-hour infusion of "high-dose 5-FU/LV" (this being a combination of 5-fluorouracil and leucovorin), although the term "high-dose" is not defined in D13. The results of both Phase I trials are then discussed together, and it is stated that, of the seven pancreatic cancer patients who received liposomal irinotecan (as a monotherapy or in combination with weekly 5-FU/LV) "the best response was partial response in one, stable disease in 4 and progressive disease in 2". As stated above, the partial response was seen in the pancreatic cancer patient who received liposomal irinotecan monotherapy, so the skilled person would have known from D13 that the monotherapy, not the combination with weekly 5-FU/LV, produced "the best response" in Phase I.

5.9 D13 then goes on to state that, based on the Phase I results, an international Phase II trial of liposomal irinotecan was pursued in patients with gemcitabine-resistant pancreatic cancer. This Phase II trial related to liposomal irinotecan monotherapy, and this can be confirmed by reference (30) of D13 (document D12). The results of this Phase II monotherapy trial are said to "*highlight the feasibility and activity of* [liposomal irinotecan monotherapy] *in previously heavily treated patients with gemcitabine-refractory advanced pancreatic cancer, which deserves further exploration*".

5.10 In view of this teaching, the skilled person would have noted that only the liposomal irinotecan monotherapy was taken forward from Phase I to a Phase II trial, where it was shown to be active in treating gemcitabine-resistant pancreatic cancer. The combination therapy of liposomal irinotecan with weekly 5-FU/LV was not taken forward to Phase II. The skilled person would have concluded from this that the combination regimen was deliberately not taken forward to Phase II because it was in some way inadequate relative to the liposomal irinotecan monotherapy, which showed a partial response in Phase I. Thus, the skilled person reading D13 would consider the liposomal irinotecan monotherapy which was taken into a Phase II trial to represent the closest prior art embodiment, as it clearly represents the most promising starting point within D13.

Distinguishing features

5.11 Claim 1 of the Main Request differs from the liposomal irinotecan monotherapy described in D13 at least because:

- Claim 1 requires co-administration of liposomal irinotecan, LV and 5-FU, whereas the monotherapy of D13 requires administration of liposomal irinotecan only;
- Claim 1 requests the three drugs to be administered "in at least one two-week cycle", whereas the monotherapy of D13 requires administration "once every three weeks";
- Claim 1 requires the liposomal irinotecan to be administered at a dose of 80 mg/m^2 on day 1 of each cycle to patients not homozygous for the UGT1A1*28 allele, and for liposomal irinotecan to be administered at a dose of 60 mg/m^2 on day 1 of each cycle for patients homozygous for the UGT1A1*28 allele and at a dose of 60 mg/m^2 or 80 mg/m^2 on day 1 of each subsequent cycle. The Phase II monotherapy trial reported in D12/D13 administered 120 mg/m^2 to all subjects irrespective of their UGT1A1*28 status;
- Claim 1 requires leucovorin (l-form) to be administered at a dose of 200 mg/m^2 and for the 5-FU to be administered at a dose of 2400 mg/m^2. The monotherapy described in D13 does not involve the administration of LV or 5-FU;
- Claim 1 requires the liposomal irinotecan to be administered prior to the leucovorin, and the leucovorin to be administered prior to the 5-FU; and
- Claim 1 requires that the liposomal irinotecan is "irinotecan sucrose octasulfate salt liposome injection", whereas D13 refers only to "nanoliposomal CPT-11"[3].

Technical Effects

5.12 Liposomal irinotecan for use according to claim 1 was approved by the FDA in the United States and the EMA in the EU following a pivotal Phase III trial, referred to as NAPOLI-1. The protocol for this trial is explained in detail in Example 7 of the patent. The results of this trial show that the claimed invention is associated with a number of beneficial technical effects.

5.13 In the NAPOLI-1 trial, and as explained in section B of Example 7, patients were separated into three arms. Patients in Arm A were administered 120 mg/m^2 of liposomal irinotecan

[3] Irinotecan is sometimes referred to as "CPT-11".

sucrose octasulfate salt (referred to as "MM-398" in Example 7) over 90 minutes every three weeks. The Arm A dosage regimen was the same regimen which was tested in the Phase II trial reported in D12 and D13. Arm B was referred to as the control arm, in which patients were treated with LV/5-FU. Patients in Arm C were administered 80 mg/m^2 of liposomal irinotecan sucrose octasulfate salt over 90 minutes every two weeks in combination with LV/5-FU according to claim 1. The results from each of Arms A and C were compared with those from Arm B.

The claimed dosage regimen shows improved efficacy

5.14 Several efficacy endpoints were considered in the NAPOLI-1 trial. The primary endpoint (i.e. the endpoint which was designed to ultimately show whether the therapy is efficacious) was overall survival (OS). OS is defined in paragraph 0164 of the patent as the time from the date of patient randomisation to the date of death or the date the patient was last known alive. The results of the trial showed a clinically relevant and statistically significant superiority in overall survival in patients receiving the liposomal irinotecan and LV/5-FU combination regimen according to claim 1 relative to patients receiving the LV/5-FU control arm (median OS was 6.1 months and 4.2 months, respectively – D19, page 1, "Findings"). By contrast, patients in the liposomal irinotecan monotherapy arm did not show superiority in overall survival relative to the LV/5-FU control (4.9 months versus 4.2 months D19, page 1, "Findings"). These data alone demonstrate the advantageous efficacy of the claimed combination regimen, and correspond to data cited by the FDA in its press release issued following the approval of Onivyde® (see D18).

5.15 In addition to the above, the claimed combination regimen also demonstrated its efficacy when the secondary endpoints were considered.

5.15.1 When progression free survival (PFS – defined in paragraph 0169 as "the number of months from the date of randomization to the date of death or progression, whichever occurred earlier") data are considered, patients treated with the combination regimen according to claim 1 achieved a median PFS of 3.1 months – approximately twice that of the patients in the LV/5-FU control arm who exhibited a median PFS of 1.5 months. By contrast, patients treated with liposomal irinotecan monotherapy showed a median PFS of 2.7 months compared to 1.6 months seen in the control arm (D19, paragraph spanning pages 6 and 7).

5.15.2 Patients in the liposomal irinotecan and LV/5-FU combination therapy arm achieved a median time to treatment failure (TTF – defined in paragraph 0171 as "the time from randomisation to either disease progression, death or study discontinuation due to toxicity") of 2.3 months compared to a median TTF of 1.4 months for patients in the LV/5-FU control arm. By contrast, patients in the liposomal irinotecan monotherapy arm had a TTF of 1.7 months which did not differ significantly from the LV/5-FU control, where the TTF was 1.4 months (D19, page 6, first complete paragraph).

5.15.3 Patients in the combination therapy arm showed an improved objective response rate (ORR – discussed in paragraphs 0172 and 0173) over the LV/5-FU control arm (16% vs. 1%, respectively). Patients in the monotherapy arm showed an ORR of 6% relative to the 1% seen in the control arm (D19, page 6, second complete paragraph).

5.15.4 Patients in the combination arm according to claim 1 showed lower levels of the pancreatic cancer tumour marker CA19-9 (discussed in paragraph 0174) than patients in the LV/5-FU control arm. Of the patients with elevated baseline CA19-9 who were treated in the study, 29% of patients treated with the liposomal irinotecan and LV/5-FU combination regimen of claim 1 showed reductions of ≥50% from baseline levels, compared to 9% of patients in the LV/5-FU control arm. Out of the patients with elevated baseline CA19-9 levels in the monotherapy arm, 24% of these patients showed reductions of ≥50% versus 11% seen in the control arm (D19, page 6, third paragraph).

5.15.5 In the patient reported outcome analysis (discussed in paragraph 0175), there were no substantial differences in patient quality of life between the three arms, indicating that the increased efficacy of the claimed combination regimen surprisingly does not have a detrimental effect on patients' quality of life (D19, paragraph spanning pages 7 and 8).

5.15.6 By way of a summary, the efficacy data endpoints from the NAPOLI-1 trial are reproduced in the table below. These data demonstrate that the claimed combination therapy regimen (right-hand column) is associated with therapeutic advantages without having a detrimental effect on patients' quality of life. The data reproduced below are also discussed in D19 (copy enclosed).

	Arm A (monotherapy)	**Arm C (combination)**
Median Overall survival / months (Arm B value)	4.9 (4.2)	6.1 (4.2)
Median progression-free survival / months (Arm B value)	2.7 (1.6)	3.1 (1.5)
Median time to treatment failure / months (Arm B value)	1.7 (1.4)	2.3 (1.4)
Overall response rate / % (Arm B value)	6 (1)	16 (1)
Patients showing ≥50% reduction in CA19-9 levels / % (Arm B value)	24 (11)	29 (9)

5.16 As explained before the Opposition Division, for the purposes of a problem-solution analysis, a comparison between the numbers given for Arm A and Arm C in the above table can be made, and from that comparison, the conclusion that Arm C (combination) was more efficacious than Arm A (monotherapy) can be drawn.

The increase in efficacy was not accompanied by an increase in treatment emergent adverse events (TEAEs)

5.17 Given the above improved overall survival data, the claimed combination regimen was also surprisingly associated with a lower frequency of serious treatment emergent adverse events

(TEAEs – discussed in paragraph 0176) than the liposomal irinotecan monotherapy regimen. In the NAPOLI-1 trial, 61% of patients in the liposomal irinotecan monotherapy arm experienced a serious TEAE, compared with the combination therapy arm, in which the percentage of patients experiencing a serious TEAE was similar to that of patients in the LV/5-FU control arm (48% for the combination therapy and 45% for the control – see D19, page 9, final sentence). This demonstrates that the efficacy of the claimed combination regimen did not come at the cost of additional TEAEs.

5.17.1 For example, patients treated with the combination regimen according to claim 1 also had less frequent severe diarrhoea than patients in the liposomal irinotecan monotherapy arm (13% of patients vs 21% of patients, respectively – see D19, Table 2, first row). As mentioned in paragraphs 0119 and 0120, irinotecan was known to induce both early and late forms of diarrhoea, the latter of which can be life-threatening. Thus, any regimen which is both therapeutically efficacious whilst causing less frequent severe diarrhoea would be greatly advantageous. The reduced frequency of severe diarrhoea in the combination arm coupled with the combination regimen's therapeutic efficacy would have come as a surprise to the skilled person, not least because diarrhoea is also a "frequently reported" side effect of 5-FU administration (D20).

5.17.2 Further, patients treated in the liposomal irinotecan/LV/5-FU combination arm showed a lower frequency of alopecia compared with patients in the liposomal monotherapy arm (14% vs. 22%, respectively – see D19, page 9, third complete paragraph, third sentence).

5.18 For ease of reference, the safety data discussed above are summarised in the below table. These data are also discussed in D19.

	Arm A (monotherapy)	Arm B (control)	Arm C (combination)
Frequency of serious TEAEs (%)	61.2	44.8	47.9
Frequency of patients experiencing severe diarrhoea (%)	21	5	13
Frequency of patients experiencing alopecia (%)	22	5	14

5.19 Thus, the improvement in efficacy seen for Arm C versus Arm A was not accompanied by an increase in TEAEs.

5.20 In summary, the technical effect achieved by claim 1 is that a safe and effective treatment for gemcitabine-resistant pancreatic cancer is provided that is improved relative to the monotherapy of D13.

Objective technical problem

5.21 In view of the above technical effects, the objective technical problem can be formulated as the provision of a safe and effective treatment for gemcitabine-resistant pancreatic cancer that is improved relative to the monotherapy of D13.

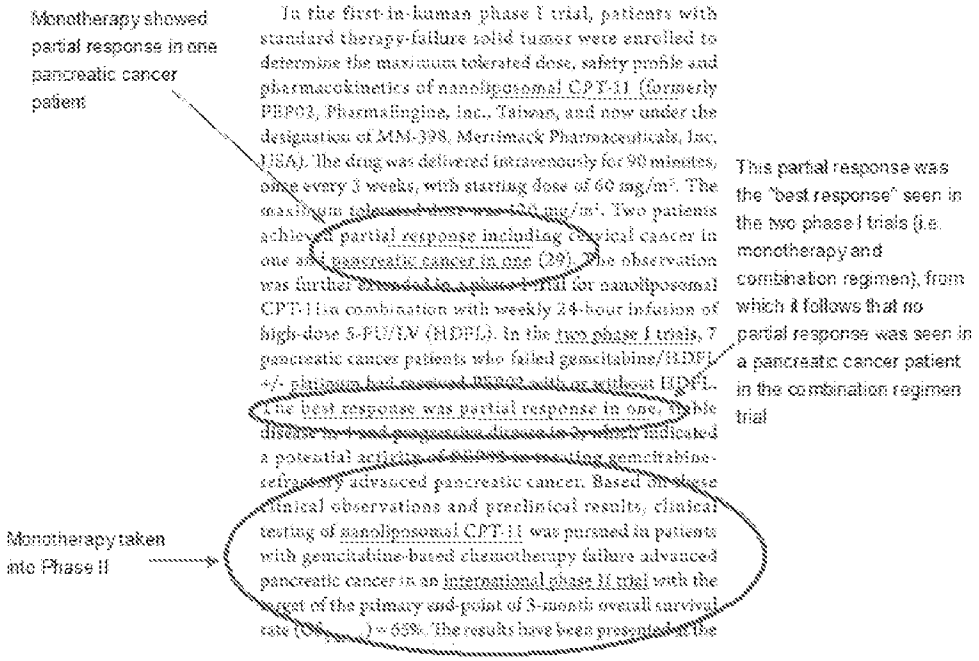
5.22 The data from the NAPOLI-1 trial demonstrate that the claimed subject matter solves this problem.

The claimed solution is not obvious

5.23 When faced with D13 and the above objective technical problem, the skilled person would not have solved the problem in the manner claimed with a reasonable expectation of success. D13 directs the skilled person towards a dosage regimen which is different from the one presently claimed (i.e. D13 teaches towards a monotherapy which administers a different dose with a different administration frequency), and any conclusion to the contrary necessarily involves the use of hindsight.

5.24 D13 does mention that liposomal irinotecan, both alone and in combination with LV/5-FU, had been studied in Phase I trials. The dosage regimen used in the Phase I combination trial is not fully disclosed. In the specific context of the Phase I trial, no explicit comparisons between the monotherapy and the combination regimen are made, and at no point is the combination regimen said to be in any way preferable over the monotherapy. In fact, due to its simplicity and the desire to avoid administering additional drugs unnecessarily, the skilled person would, all other things being equal, have wished to take forward for further development the monotherapy rather than the more complex combination regimen. Reasons why the skilled person would not have developed a combination regimen are elaborated on below, e.g. in paragraph 5.28 below.

5.25 After certain Phase I data are briefly mentioned in D12/D13, the discussion moves on to the Phase II monotherapy trial, and the combination regimen is not mentioned again. In clinical research, a Phase II trial is carried out after a Phase I trial, and aims to establish whether a drug or dosage regimen is sufficiently efficacious to be moved forward to a larger scale Phase III trial, the results of which may lead to an application for authorisation to market the drug or dosage regimen. In the present case, the monotherapy regimen was taken forward to Phase II, consistent with the partial response observed in a patient with refractory pancreatic cancer in the Phase I monotherapy trial but not the Phase I combination regimen trial; see D13, page 189, right hand column; emphasis added:



In fact, D13 does not present any efficacy data about the Phase I combination regimen.

5.26 The results of the Phase II trial of the monotherapy regimen are discussed in D13, which concludes that the liposomal irinotecan monotherapy trial demonstrated "the feasibility and activity of [liposomal irinotecan] in previously heavily treated patients with gemcitabine-refractory advanced pancreatic cancer, which deserves further exploration." (D13 – page 191, fifth complete sentence).

5.27 The skilled person seeking to provide an improved therapy for gemcitabine-resistant pancreatic cancer is thus taught by D13 that a liposomal irinotecan monotherapy should be developed. A move to instead develop a combination regimen, which was not taken into Phase II (unlike the monotherapy), would have been viewed as a backward step, obvious only with the benefit of hindsight. Thus, D13 teaches the skilled person away from the claimed combination dosage regimen, and claim 1 involves an inventive step for at least this reason.

5.28 Developing the liposomal irinotecan monotherapy regimen in an attempt to provide a safe and effective treatment for gemcitabine-resistant pancreatic cancer would have been consistent with the skilled person's common general knowledge. As mentioned above, the skilled person would have been aware that irinotecan is known to cause, amongst other things, both the early and late forms of diarrhoea, the latter of which can be life threatening. 5-FU was also known to cause diarrhoea (D20). Thus, the skilled person seeking an improved therapy would have been drawn to liposomal irinotecan monotherapy, as s/he would have had a strong motivation to avoid the combination regimen which combines two drugs which are both known to be associated with a potentially lethal adverse event (severe diarrhoea). This is particularly pertinent given that the monotherapy regimen, which did not require the use of multiple drugs, had shown safety and efficacy in both Phase I and Phase II trials, and was known to be the subject of a Phase III trial at the priority date (see paragraph 0007 of the patent, which refers to this trial, whose protocol is given in D15a).

5.29 The skilled person would also have been attracted to develop the liposomal irinotecan monotherapy because it is a simpler regimen, which requires less frequent (every three weeks) and relatively brief 90 minute administration of one drug only. In addition, the omission of 5-FU from the regimen would have meant that an infusion pump would not need to be used in the regimen.

5.30 Furthermore, D13 teaches the skilled person that the liposomal irinotecan should be administered once every three weeks at a dose of 120 mg/m^2 (see the "Methods" section of D12, for example). There is nothing in D13 which would have motivated the skilled person to administer liposomal irinotecan (as a monotherapy or in combination with other drugs) once every two weeks at a dose of 80 mg/m^2, as required by claim 1, with a reasonable expectation of providing a therapy which is both safe and efficacious.

5.31 Further, there is no teaching or suggestion in D13 that liposomal irinotecan in the form of "irinotecan sucrose octasulfate salt" should be used. Moreover, there is nothing in D13 which would have led the skilled person to administer "irinotecan sucrose octasulfate salt liposome injection" every two weeks at a dose of 80 mg/m^2 with a reasonable expectation of solving the objective technical problem.

5.32 Even if the skilled person had, for some reason, looked to the combination therapy disclosed in D13 when seeking to solve the problem, s/he would not have been any closer to the claimed subject matter. D13 states on page 189 (left-hand column) that irinotecan has a narrow therapeutic index, meaning that the margin for error when dosing any form of irinotecan is small. If the dose is too low, there will be insufficient efficacy. If the dose is too high, the side effects are likely to become dangerous. D13 teaches that 120 mg/m^2 was the maximum tolerated dose for liposomal irinotecan administration every three weeks in a Phase I trial. No dosages for any of the three drugs are given for the Phase I combination therapy trial. The skilled person would, in particular, have thus been at a loss as to the liposomal irinotecan dosage which should be used in a combination therapy. In particular, even if the skilled person had thought to use a dosage of 80 mg/m^2 (which is not conceded), s/he would have unable to make any predictions regarding the safety and efficacy of this dosage in a combination regimen. On the one hand, the skilled person may have expected reduced efficacy with 80 mg/m^2 as it is a lower dose than the 120 mg/m^2 dose reported as the MTD for the monotherapy. That said, s/he would have had no idea whether any loss of efficacy associated with the use of 80 mg/m^2 would be compensated for by the presence of LV/5-FU in the dosage regimen. In addition, the skilled person would have had no information on whether any combination regimen would be safe. On the one hand, the liposomal irinotecan dosage is being reduced from 120 mg/m^2 to 80 mg/m^2. On the other hand, the presence of two further agents in the dosage regimen (one of which is, like irinotecan, cytotoxic and known to cause diarrhoea) might be expected to be detrimental to the safety of any combination regimen. Thus, it cannot be said that the skilled person would have developed the combination regimen mentioned in D13, or indeed any combination regimen of liposomal irinotecan with LV/5-FU, with any expectation of success, and certainly not a reasonable expectation of success.

The claims remain inventive even if D13 is considered in combination with the other prior art documents

5.33 Even if D13 were considered by the skilled person in combination with any of the other prior art documents, the skilled person would not have been motivated to solve the objective technical problem in the manner claimed. In particular, none of the other prior art documents would have led the skilled person to administer liposomal irinotecan in a combination regimen every two weeks at a dose of 80 mg/m^2 according to claim 1 with a reasonable expectation of success.

5.34 For example, D7 is concerned with liposomal irinotecan monotherapy, and concludes that 120 mg/m^2 every three weeks should be administered in a future Phase II trial ("Conclusions" section, first sentence). D12 reports on the Phase II trial which is alluded to in D7, and it administered liposomal irinotecan monotherapy at a dose of 120 mg/m^2 every three weeks. Thus, neither D7 nor D12 adds anything to the disclosure of D13, and as such neither document would have directed the skilled person toward the claimed subject matter when read in combination with D13.

5.35 D22 makes no mention of pancreatic cancer, and certainly not any mention of gemcitabine-resistant pancreatic cancer. Thus, the skilled person would not have consulted D22 when seeking to solve the objective technical problem. If the skilled person did, for some reason, consult D22, s/he would note D22's conclusion that the MTD (maximum tolerated dose) of liposomal irinotecan, when administered every three weeks in combination with weekly 5-FU/LV, was 80 mg/m^2. With this in mind, the skilled person would have expected that administration of 80 mg/m^2 every two weeks - i.e. administration of the same dose more frequently - would have resulted in unacceptable side effects, because D22 teaches that 80 mg/m^2 is only safe when it is administered less frequently (once every three weeks). This certainly would not have directed the skilled person toward the claimed subject matter, and if anything, would have led the skilled person away from a regimen which requires 80 mg/m^2 once every two weeks.

The logic at paragraph 5.7.8 of the appealed decision is flawed

5.36 In paragraph 5.7.8 of the appealed decision, the Opposition Division acknowledged that "it is true that there are no indications on file that the compound MM-398 [which is the terminology used in D15b] was irinotecan sucrose octasulfate salt liposome injection", as required by the claims of the main request. Despite this, the Opposition Division erred and concludes that the claimed subject-matter contravenes Article 56. This cannot be right. The evidence fails to disclose one of the mandatory features of the claim, namely that the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome, and it is wrong for the Opposition Division to conclude, based on the patent, that this "would just represent an alternative liposomal irinotecan" which would, according to the Opposition Division, render the claimed subject-matter obvious. There is nothing on file to indicate that using the specific irinotecan sucrose octasulfate salt liposome in the claimed regimen would have been obvious. After all, none of the evidence before the Opposition Division even mentioned the irinotecan sucrose octasulfate salt liposome.

5.37 This point is expanded on in paragraphs 5.96 to 5.98 below.

The claimed subject matter satisfies a long-felt need

5.38 As alluded to above, in the prior art, pancreatic cancer patients who had failed gemcitabine-based first line therapy had no established options for further treatment. Whilst incidences of pancreatic cancer had markedly increased in the preceding several decades, efforts to evaluate other agents for use in second-line therapy had been disappointingly negative (see the patent, paragraphs 0002 and 0006, and D23, paragraphs 8-13). The state of the art had thus been inactive for a long period in spite of the fact that there was an urgent need in this period for a second-line therapy for pancreatic cancer. The claimed invention provides the first (and, thus far, only) approved treatment for patients who have progressed following gemcitabine-based therapy, and therefore the invention satisfies a long-felt need (Case Law of the Boards of Appeal, I.D.10.4).

Conclusion

5.39 When faced with D13 and the objective technical problem formulated at 5.21, the skilled person would not have been motivated to solve the problem in the manner required by claim 1. D13 directs the skilled person towards a monotherapy regimen which is different from the combination regimen of claim 1. Even if the skilled person had looked to the combination regimen that is discussed in, for example, D13 and D22, the skilled person could only have arrived at the claimed subject matter by going against the teaching in the prior art and by using inventive skill.

5.40 In the patent, the appellant has demonstrated for the first time that liposomal irinotecan, in the form of irinotecan sucrose octasulfate salt, is able to treat gemcitabine-resistant pancreatic cancer when administered at a dose of 80 mg/m^2 in combination with LV/5-FU in a particular dosage regimen. This dosage regimen is unexpectedly improved relative to the liposomal irinotecan monotherapy regimen described in the closest prior art, D13. Therefore, the claimed subject matter involves an inventive step.

The claims are inventive even if the first priority claim is invalid

Closest Prior Art

5.41 After finding that the first priority claim is invalid (which was incorrect), the Opposition Division found that D15b was the closest prior art. The Opposition Division was wrong to find that D15b is the closest prior art, at least because such a finding goes against the Boards' well-established case law on the selection of the closest prior art. When this case law is applied correctly to the present case, it becomes clear that D13, not D15b, is the closest prior art.

5.42 Within the problem-solution approach, the closest prior art should be a document which relates to the same purpose or effect as the claimed invention. In the case of a medical use claim, where achieving the therapeutic effect is a functional feature of the claim, a document relating to the same purpose or effect should be a document which discloses a treatment which achieves this therapeutic effect (see, for example, T2024/11 and T2571/12). This approach is logical because a skilled person wishing to treat a particular condition in a new way would naturally start with a known way of treating the same condition.

5.43 Where there is a choice between two prior art documents which both mention the condition of interest, with document (1) disclosing actual treatment of the condition and document (2)

merely providing an unsubstantiated verbal statement regarding the condition, the skilled person would always start from document (1). This is because a disclosure of actual treatment of the condition of interest would always be viewed as a more promising springboard than a mere verbal statement such as that found in document (2). This is consistent with the approach taken in T2154/14 (Reasons 32-39).

5.44 With this in mind, it becomes clear that D13, and not D15b, is the closest prior art. The claimed invention relates to the treatment of pancreatic cancer in patients who have failed first-line gemcitabine therapy. Verbal statements regarding the treatment of this condition in this particular patient population are given in both D13 (see, for example, page 189, right-hand column) and D15b ("Official title" and "Brief Summary"). However, D13, discloses actual treatment of pancreatic cancer in patients who have failed first-line gemcitabine therapy (see the passage spanning pages 189 and 191 which discuss the results of a Phase II trial), whereas D15b does not.

5.45 It follows that D13 constitutes a more promising springboard than D15b because, whilst both documents give verbal statements relating to pancreatic cancer in patients who have failed first-line gemcitabine therapy, D13 is the only document which provides an actual disclosure of treatment of pancreatic cancer in patients who have failed first-line gemcitabine therapy. Thus, D13 is the closest prior art.

5.46 The Opposition Division noted the total lack of any disclosure of treatment in D15b, but still erroneously held that D15b is the closest prior art. The Opposition Division's reasoning was that "*Boards of Appeal have on multiple occasions selected the disclosure of a clinical trial protocol with no results as the closest prior art*", citing T239/16 and T2506/12 (page 12 of the decision, fourth complete paragraph). It is true that, in T239/16 and T2506/12, the Boards did take a clinical trial protocol with no results as the closest prior art. However, in those two cases, it was common ground between the parties that there were no other prior art documents which could have been taken as the closest prior art[4]. For example, there were no other documents on file which disclosed actual treatment. Thus, the Boards had to take the clinical trial protocols as the closest prior art because no more suitable starting points were available. The situation in the present case differs from that of T239/16 and T2506/12 because, aside from the clinical trial protocol, there are other documents on file which do disclose treatment of the relevant disease in the patient population of interest (e.g. D13). As stated at 5.43, when faced with a document containing a verbal statement only (D15b) and a document containing a verbal statement supported by a disclosure of actual treatment (D13), the document disclosing actual treatment must be taken as the closest prior art. Thus, the Opposition Division failed to appreciate the factual background which led the Boards in T239/16 and T2506/12 to take a clinical trial protocol as the closest prior art, and this lead to the Opposition Division incorrectly finding that D15b is the closest prior art.

5.47 Of course, as well as considering the purpose of the prior art documents, consideration of the structural features of the prior art documents can also assist in the selection of the closest prior art (provided that care is taken to ensure that the closest prior art is not selected merely

[4] See T239/16, Summary of Facts and Submissions XI and X, and Reasons 6.2; and T2506/12, Reasons 6.2.

because it shows superficial structural similarities with the claim at issue – T506/95). If one considers structural features, it remains the case that D13 is the closest prior art.

5.48 Claim 1 of the Main Request relates to "liposomal irinotecan" which is "liposomal irinotecan sucrose octasulfate salt liposome injection" for use in a method of treatment. D13 is also concerned with "liposomal irinotecan" (see page 188, right-hand column to page 191, left-hand column). D15b on the other hand refers to "MM-398". D15b makes no mention of what "MM-398" is. The fact that D15b fails to give any description of what the drug being tested is did not deter the Opposition Division from selecting D15b as the closest prior art because "it was known from D13 that the MM-398 was nanoliposomal CPT-11" (page 12 of the decision, penultimate paragraph). Thus, it appears that the Opposition Division used a feature from D13 to compensate for a deficiency in the disclosure of D15b, and then held that D15b to be the closest prior art because Arm C of D15b uses the "same drugs" as claim 1 (page 12 of the decision).

5.49 The Opposition Division's approach here is totally at odds with a proper application of the problem-solution approach. It is of course true that the skilled person will consider the disclosure of a prior art document in light of his/her common general knowledge. Whilst there are prior disclosures which state that "MM-398" is a type of liposomal irinotecan, these disclosures would not have formed part of the skilled person's common general knowledge at the filing date, and thus D15b's failure to specify what MM-398 is cannot be remedied by common general knowledge. In any case, none of the documents on file specifies that MM-398 is "liposomal irinotecan sucrose octasulfate salt liposome injection".

5.50 The appellant is aware of T176/89 where the Board held that the closest prior art comprised two documents in combination with one another. However, in this case, the Board made clear that this was an exceptional case, and that the two documents could be considered together because they (1) had the same patentee, (2) had largely the same inventors, and (3) that they clearly related to the same set of tests. Clearly, D13 and D15b do not have the same "exceptional" relationship with one another within the meaning of T176/89, e.g., because they appear to emanate from different entities and relate to different studies. Therefore, the combination of D13 and D15b cannot be taken as the closest prior art. It therefore follows that the Opposition Division was totally wrong to fill the gaps in D15b using subject matter taken from D13, and then hold D15b to be the closest prior art.

5.51 Therefore, the Opposition was wrong to select D15b as the closest prior art over D13. Whilst both documents contain verbal statements regarding pancreatic cancer in patients who have failed prior gemcitabine therapy, only D13 discloses actual treatment. In addition, D13 is structurally closer to the claimed invention because it relates to therapy using "liposomal irinotecan", rather than an entity which is defined by a codename only.

Closest prior art embodiment

5.52 It has thus been established that the closest prior art document is D13. However, it is also important to identify the closest prior art embodiment within D13. As was explained above at 5.7 to 5.10, the skilled person reading D13 would consider the liposomal irinotecan monotherapy which was taken into a Phase II trial to represent the closest prior art

embodiment. Purely for the sake of brevity, these arguments 5.7 to 5.10 will not be repeated here.

Distinguishing features and technical effects

5.53 The differences between claim 1 of the Main Request and the liposomal irinotecan monotherapy described in D13 are given above at 5.11, and the technical effects associated with these differences are given at 5.12 to 5.20 above. Purely for the sake of brevity, the distinguishing features and technical effects will not be repeated here.

Objective technical problem

5.54 In view of the above technical effects, the objective technical problem can be formulated as the provision of a safe and effective treatment for gemcitabine-resistance pancreatic cancer. The data from the NAPOLI-1 trial discussed above demonstrate that the claimed subject matter solves this problem.

5.55 Alternatively, given the conclusion drawn in 5.16, it is also possible to formulate the objective technical problem more ambitiously as the provision of a safe and effective treatment for gemcitabine-resistant pancreatic cancer that is improved relative to the monotherapy of D13. This reformulation of the objective technical problem is allowable following the well-established jurisprudence which states that, where a specific problem is identified in the description (i.e. that formulated in 5.54), the appellant may put forward a modified version of the problem if new prior art comes to light (see "Case Law of the Board of Appeal", 9th Edition, EN version, I.D.4.4.1). This well-established jurisprudence is applicable here and confirms that the Opposition Division was wrong to state that this more ambitious problem cannot be considered (see 5.7.2 of the Opposition Division's decision). In particular, D15b as prior art has only come to light during the opposition proceedings. Therefore, following this established jurisprudence, it is allowable to reformulate the problem in a more ambitious way in response to D15b being cited as evidence and then becoming prior art by virtue of the erroneous decision on priority.

The claimed solution is not obvious

5.56 Even if the first priority claim is invalid, the skilled person faced with the problem formulated at 5.54 and starting from the liposomal irinotecan monotherapy results in D13 would not have arrived at the claimed subject matter without inventive skill.

5.57 It has already been explained above that D13 alone, and D13 in combination with the other relevant prior art, would not have led the skilled person towards the claimed dosage regimen. If the first priority claim is invalid, documents D4, D8, D10 and D15b become prior art. However, these documents would not have compensated for the lack of disclosure in D13, and thus they are not prejudicial to inventive step.

5.58 D4 and D10 are concerned with non-liposomal irinotecan only. Thus, the skilled person starting from D13 would not have looked to D4 or D10 when seeking to solve the objective technical problem.

5.59 D8 reports on a Phase I, first-in-human study of "IHL-305", which purports to be "a PEGylated liposome containing irinotecan" (sentence spanning pages 699 and 700). The trial aims to examine the safety profile and pharmacokinetics of IHL-305, and to establish the maximum

tolerated dose and RP2D ("recommended Phase II dose") in patients with advanced solid tumors. The study was not set up to test the efficacy of IHL-305. Three patients in the trial had pancreatic cancer (Table 1), although it is not clear from D8 what dose of IHL-305 these patients received, and nor is it stated in D8 whether these patients had been previously treated with gemcitabine. Most importantly, no information at all is given about whether IHL-305 is actually able to treat pancreatic cancer. Thus, the disclosure of D8 would not have supplemented the lack of teaching in D13, and would not have led the skilled person towards the claimed subject matter.

5.60 D15b also fails to direct the skilled person towards the claimed subject matter. D15b is a clinicaltrial.gov posting about a trial of MM-398 in metastatic pancreatic cancer in patients who have failed prior gemcitabine therapy. D13 states that MM-398 is a form of liposomal irinotecan. The trial in D15b has two experimental arms. Arm A is a liposomal irinotecan monotherapy which uses the same regimen which was used in the Phase II trial disclosed in D13. Arm C is a combination therapy. Whilst D13 does mention a combination therapy which was tested in Phase I only, the skilled person would note that the combination regimen being tested in Arm C is different from that mentioned in D13. For example, the combination regimen mentioned in D13 administered liposomal irinotecan once every three weeks and 5-FU/LV on a weekly basis, whereas the Arm C regimen administers all three drugs every two weeks. Thus, the skilled person looking at D15b in light of D13 would recognise the regimen being tested in Arm A, as it is the same regimen than was reported in D13 as being effective in Phase I and Phase II trials. By contrast, the fact that the regimen of Arm C was being tested would come as a complete surprise to the skilled person, who knows from D13 that a liposomal irinotecan/5-FU/LV combination therapy was not taken into Phase II. Moreover, the skilled person would note that the combination regimen being tested in D15b is not even the same regimen that was tested in Phase I.

5.61 Thus, the skilled person considering D15b in light of D13 would see two dosage regimens, one of which s/he knew from D13 to be a promising therapy for gemcitabine-resistant pancreatic cancer (Arm A), and another which seemingly comes from nowhere and which differs from the previously-trialled combination regimen (Arm C). It follows that the skilled person seeking to solve the objective technical problem considering D15b in view of D13 would have pursued the monotherapy regimen of Arm A which had come through Phase I and II trials successfully. Any suggestion that the skilled person would have ignored everything in D13 and pursued Arm C necessarily involves impermissible hindsight.

5.62 After taking Arm C of D15b as the closest prior art, the Opposition Division found that the skilled person would look to Arm C in D15b with a reasonable expectation of success. However, Arm C of D15b would, at best, have represented a mere "hope to succeed", meaning that it is not prejudicial to the inventive step of the claims. This is explained in more detail in D23 and below in 5.74 to 5.87. Purely in the interests of brevity, the arguments will not be repeated here.

5.63 Therefore, if the established case law on the selection of the closest prior art is correctly followed, D13 will be taken as the closest prior art. The skilled person starting from D13 and seeking to provide a safe and effective treatment for gemcitabine-resistant pancreatic cancer would not have been directed towards the claimed subject matter. The prior art leads the

skilled person towards a totally different dosage regimen (a monotherapy rather than a combination therapy), and even if the prior art disclosures of combination regimens were considered, they would not have led the skilled person towards the specific combination regimen that is claimed. Therefore the claims of the Main Request meet the requirements of Article 56 EPC.

5.64 Even if the Board disagrees with this conclusion, if the problem is formulated more ambitiously as it is at 5.55, the claims of the Main Request involve an inventive step because there is nothing in the prior art which would have suggested that Arm C in D15b would be improved relative to the liposomal irinotecan monotherapy of Arm A and D13. That is, Arm C of D15b considered in combination with D13 would not have given the skilled person a reasonable expectation that an improved therapy relative to Arm A could be provided by pursuing the combination regimen of Arm C. Thus, the claimed solution to the more ambitious technical problem involves an inventive step.

The claims are inventive even if D15b is taken as the closest prior art

5.65 It has been explained above that D15b is not an appropriate choice for the closest prior art. However, and purely for the sake of completeness, it will be shown in this section that the claims remain inventive if D15b is taken as the closest prior art.

5.66 If D15b is taken as the closest prior art document, it is important to consider what constitutes the closest prior art embodiment within D15b (see 5.7 above). Two options are possible here – Arm A (the monotherapy arm of the trial) and Arm C (the combination arm).

5.67 If Arm A is the closest prior art embodiment, the situation is essentially identical to that described at 5.56 to 5.62. The skilled person is starting from a liposomal irinotecan monotherapy which has shown to be safe and efficacious against gemcitabine-resistant pancreatic cancer. The problem-solution analysis given in the above section (5.52 to 5.62) applies equally if Arm A of D15b is taken as the closest prior art embodiment. Therefore, the claimed subject matter involves an inventive step if Arm A in D15b is taken as the closest prior art.

5.68 The remainder of this section will focus on Arm C of D15b being taken as the closest prior art. This was the approach taken by the Opposition Division in the decision under appeal.

5.69 Claim 1 of the Main Request differs from Arm C of D15b because it requires safe and effective treatment of gemcitabine-resistant pancreatic cancer. Claim 1 also differs from D15b because it requires that this safe and effective treatment is carried out using a combination regimen comprising the administration of "liposomal irinotecan" which is "irinotecan sucrose octasulfate liposome salt injection". D15b, by contrast, requires the administration of "MM-398", which is not defined in D15b. A further distinguishing feature is that claim 1 requires the liposomal irinotecan to be administered prior to LV, and for the LV to be administered prior to 5-FU. The order in which the drugs are administered is not specified in D15b. Claim 1 also requires that patients homozygous for UGT1A1*28 allele receive a lower starting dose of liposomal irinotecan compared to patients not homozygous for the UGT1A1*28 allele. D15b makes no mention of the UGT1A1*28 allele, and merely states that liposomal irinotecan is administered in an amount of 80 mg/m^2.

5.70 The technical effects of these distinguishing features are that a safe and effective treatment for gemcitabine-resistant pancreatic cancer is provided. The examples in the patent, particularly Example 6, show that this technical effect is present. The results given in the patent are backed-up by D19, which also demonstrates that the claimed combination regimen is improved relative to the monotherapy arm of the trial (Arm A).

5.71 In view of these technical effects, the objective technical problem can be formulated as the provision of a safe and effective treatment for gemcitabine-resistant pancreatic cancer.

5.72 In addition, a more ambitious technical problem – the provision of a safe and effective treatment for gemcitabine-resistant pancreatic cancer which is improved relative to the monotherapy arm of D15b – can also be formulated. The Opposition Division disagreed with this formulation of the objective technical problem in paragraph 5.7.2 of the decision (page 16), because an improvement relative to the monotherapy was allegedly not relevant because it is the combination arm of the trial that is the closest prior art. To the contrary, such an improvement vis-à-vis the monotherapy arm is relevant because, if the skilled person selects Arm C of D15b as the closest prior art and most promising starting point, s/he makes this selection because s/he hopes that it will result in the best dosage regimen in D15b, that is, a dosage regimen which is superior to Arm A. In addition, the Opposition Division was wrong to find that the objective technical problem may not be reformulated in this way (see 5.55 above).

5.73 When faced with the D15b and the problem formulated at 5.71, the skilled person would not have been motivated to solve the problem in the manner claimed with a reasonable expectation of success. In particular, when the Board's guidance in T239/16 is properly applied, it becomes clear than the claims involve an inventive step.

D15b would not have provided the skilled person with a reasonable expectation of success

5.74 The Opposition Division's finding that the claims lack inventive step in view of D15b are summarised in paragraph 5.7.3 of the decision. In this paragraph, the Opposition Division found that:

(i) A Phase III clinical trial such as that described in D15b would only be authorised "once it had been shown that the treatment under investigation has a high level of safety and efficacy in earlier preclinical and clinical studies";

(ii) The Phase III clinical trial mentioned in D15b would "require authority approval which takes ethical considerations into account"; and

(iii) In view of (i) and (ii), the mere fact that Arm C was being tested in a Phase III clinical trial would have led the skilled person to have a reasonable expectation of success for the combination regimen of Arm C.

5.75 The Opposition Division cited T239/16, Reasons 6.5, to support its position. The second paragraph of this extract is reproduced below, with emphasis added:

*"The board considers that the mere fact that an active agent selected from the group of bisphosphonates is being tested in a clinical study for the treatment of osteoporosis (as disclosed in document (55)) leads to an expectation of success, due to the fact that clinical studies are based on data obtained by pre-clinical testing both in vitro and in animals and require authority approval which takes ethical considerations into account. This means **in the present case** that the skilled person would expect all study arms to treat osteoporosis effectively, **unless he was dissuaded from this by the prior art**."*

5.76 When the above guidance is properly interpreted and applied to the facts of the present case, it becomes clear that the claims involve an inventive step, and that the Opposition Division was wrong to find otherwise. In particular, the Opposition Division ignored the factual background which led the Board in T239/16 to rule as it did, and also ignored the nuances in the decision. This led to the Opposition Division to incorrectly find that the claims lack inventive step.

5.77 As a starting point, the final sentence of the above extract, particularly the Board's use of the phrase "*in the present case*", makes it clear that the Board in T239/16 did not intend for its decision to be read as saying that any claim to a dosage regimen will lack an inventive step over the disclosure of a clinical trial protocol of a similar dosage regimen. Rather, the use of "*in the present case*" shows that the Board's decision regarding the reasonable expectation of success in T239/16 was **restricted to the facts of that particular case**. In T239/16, the Board noted that that:

a) the drug in question (zoledronic acid) was a member of the bisphosphonate class of drugs, whose use in the treatment of osteoporosis was well established;

b) there was nothing to suggest that zoledronic acid would behave differently to other bisphosphonates; and

c) there was speculation that bisphosphonates such as zoledronic acid would show persistent effects lasting for 12 months (the dosage interval recited in the claim in T239/16).

Additionally, the case concerned a specific citation (a "patient information and consent" form) and a specific disease (osteoporosis), both of which differ from the citation (a clinicaltrials.gov extract) and disease (gemcitabine-resistant pancreatic cancer) in the present case. These differences are crucial, as explained below.

In view of the factual background summarised in points a), b) and c) above, the Board found that the fact that administration of zoledronic acid every 12 months was being tested in a clinical trial for the treatment of osteoporosis would have given the skilled person a reasonable expectation that the trial would have been successful.

5.78 When the facts of the present case are taken into consideration, it becomes clear that D15b does not provide the skilled person with a reasonable expectation of success. This will be explained below, with reference to enclosed document D23. This document is a declaration from Amy McKee, M.D. As explained in D23, Dr McKee qualified as an oncologist before working at the United Stated Food and Drug Administration ("FDA") for eleven years. During her time at the FDA, Dr. McKee held several positions, such as the Deputy Center Director of

the Oncology Center of Excellence, Supervisory Associate Director of the Office of Hematology and Oncology Products (OHOP), and Deputy Office Director of the OHOP. At the FDA, Dr. McKee was involved in over 100 marketing application reviews, all relating to oncology treatments (see D23, paragraph 3). Dr. McKee is thus an expert in oncology clinical trials and the regulatory approval process for oncology drugs.

(i) **The presence of Arm C in D15b does not mean that the regimen has shown a "high level of safety and efficacy" in earlier studies**

5.79 The fact that a dosage regimen is being tested in a clinical trial, even a Phase III clinical trial, does not mean that the dosage regimen has shown a "high level of safety and efficacy" in earlier studies (as incorrectly asserted in the Opposition Division's decision at 5.7.3). This point is dealt with in paragraphs 22 et seq. of D23. Indeed, as explained in D24, at paragraph 7, the claimed therapy had never been trialled prior to Phase III. This explains why there is nothing in the prior art which mentions any Phase I or Phase II trials of the claimed dosage regimen in patients with gemcitabine-resistant pancreatic cancer until Arm C appears in the clinical trial protocol given in D15b.

5.80 Thus, the existence of Arm C in D15b, which appears in a Phase III trial despite never having been tested in Phase I or Phase II, itself demonstrates that the Opposition Division was wrong to find that Arm C would have shown "a high level of safety and efficacy" in earlier clinical studies.

(ii) **The Phase III clinical trial mentioned in D15b would not necessarily have required approval from a regulatory authority**

5.81 Document D15b is taken from the website clinicaltrials.gov. As stated by Dr McKee in D23 (paragraphs 14-20), this website is hosted by the United States National Library of Medicine ("NLM") and is intended to provide a central portal to provide information for patients and doctors on investigational therapies. Importantly, the fact that a clinical trial is listed on clinicaltrials.gov does not mean that the study has been evaluated or approved by the United States Federal government.

5.82 All of the information that appears on a clinicaltrials.gov website entry originates from the trial sponsor and investigators. No external regulatory body (e.g. the FDA, NLM or the United States National Institute of Health) verifies the scientific validity or technical relevance of the information that has been submitted or published on the website.

5.83 Turning to D15b, and as well as not necessarily being preceded by a "high level of safety and efficacy", and contrary to the Opposition Division's statement, it would not have been mandatory to obtain approval from the FDA, or indeed any government body, before adding Arm C to the Phase III clinical trial reported in D15b.

5.84 Before adding Arm C to the trial, it would have been necessary to obtain approval only from the relevant Institutional Review Board, or "IRB". The IRB would not have been part of the FDA, or any other government agency. When determining whether to approve the treatment arm, the IRB's primary consideration would have been patient safety and ensuring that any safety risks were acceptable, given the disease and patient population. The IRB would also

have considered whether the addition of the new arm may help the trial reach its objective. However, this consideration would always be secondary to safety considerations.

5.85 There are no general guidelines on the levels of risk that are considered to be acceptable by an IRB. Each new trial and trial amendment (e.g. the addition of a new treatment arm) is considered on a case-by-case basis, with the IRB taking into consideration the relevant risk-to-benefit ratio. For gemcitabine-resistant pancreatic cancer, the IRB would have been aware that there was no standard of care, and that the patients with gemcitabine-resistant pancreatic cancer would be terminally ill and likely to survive for less than one year. This would very much sway the balance in favour of running the trial even if the chances of any clinical benefit were extremely low. This is because, as the patients in question would have exhausted all established therapies and were terminally ill (the poor prognosis is described in D23, paragraph 8, with reference to D23A), the IRB would have appreciated that there is merit in allowing any new therapy to be tried even when the probability of success is extremely low. There would, of course, always have been a hope that the new arm (Arm C) would succeed in satisfying an unmet medical need, but the skilled person would not have had any reasonable expectation that it would succeed, particularly as no other approved second line therapies were available for advanced pancreatic cancer. This point is discussed in more detail in D23, paragraphs 22-30. It was known that pancreatic cancer presented "the toughest challenges in Phase III studies" – see D23, paragraph 22, with reference to D23B, page 15.

(iii) The fact that Arm C of D15b was being tested in a Phase III clinical trial does not lead to a reasonable expectation of success

5.86 It has been established above that the Opposition Division was wrong to find that the skilled person would have developed Arm C of D15b with a reasonable expectation of success.

- Contrary to the Opposition Division's ruling, it would not have been mandatory for the dosage regimen of Arm C to have shown "a high level of safety and efficacy" in earlier trials;
- The fact that Arm C was mentioned in D15b, a clinicaltrials.gov posting, does not mean that its addition to the trial was sanctioned or approved by any government agency;
- The body which allowed the addition of Arm C to the trial will have done so against a background of hope that the dosage regimen would succeed, rather than any expectation (let alone a reasonable expectation) of success.

5.87 Thus, contrary to the Opposition Division's finding, the mere presence of the D15b clinicaltrials.gov posting does not create a reasonable expectation of success.

The skilled person would have been dissuaded from Arm C of D15b by the other prior art documents

5.88 It has already been established that the presence of Arm C in D15b would not have led to a reasonable expectation of success. However, even if the skilled person did for some reason look to Arm C with a reasonable expectation of success (which is not conceded), the claimed subject matter would be inventive because the skilled person would have been dissuaded from the regimen of Arm C of D15b by the other prior art documents (see T239/16, Reasons 6.5, cited above in 5.75).

5.89 In particular, the prior art would have dissuaded the skilled person from believing that the claimed regimen was safe and efficacious. All of the prior art which reports on the successful treatment of gemcitabine-resistant pancreatic cancer with liposomal irinotecan uses a dosage of 120 mg/m^2, and there is nothing in the art to suggest that a dose of 80 mg/m^2 alone or in combination would be safe and effective.

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

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

5.92 D13 reports on liposomal irinotecan monotherapy and combination therapy regimens, although the only dosage of liposomal irinotecan it mentions is 120 mg/m^2. Very little information on the combination therapy is given, and it appears from D13 that the combination therapy was abandoned after Phase I in favour of the monotherapy. The only detail given for the combination therapy is the frequency of the administration of 5-FU/LV, which is administered weekly, in contrast to Arm C of D15b, which administers 5-FU/LV every two weeks. Thus, the skilled person consulting D13 would have had doubts about the efficacy of the combination therapy, which was seemingly abandoned after Phase I. In addition, there is still no pointer or motivation towards the use of a dose of 80 mg/m^2 liposomal irinotecan, and the skilled person is dissuaded from administration of 5-FU/LV once every two weeks.

5.93 D22 also dissuades the skilled person from expecting that Arm C in D15b would solve the objective technical problem. D22 teaches that the maximum tolerated dose of liposomal irinotecan when administered in combination with 5-FU/LV is 80 mg/m^2 once every three weeks. Thus, on the basis of D22, the skilled person would seriously doubt whether a dosage of 80 mg/m^2 once every two weeks (the dose described in D15b) would be safe, because this dose and administration frequency is more intense than that tolerated in D22. Thus, D22 would have dissuaded the skilled person from believing that Arm C of D15b was safe and effective. D22 also requires weekly administration of 5-FU/LV rather than every two weeks, which would further complicate matters and further reduce any expectation of success for Arm C of D15b. Thus, the skilled person considering D15b in combination with D22 would not have developed Arm C of D15b with a reasonable expectation of success.

5.94 In summary, the skilled person would not have had a reasonable expectation that the regimen of Arm C would have been able to provide a safe and effective treatment for gemcitabine-resistant pancreatic cancer. There is fundamental conflict between documents such as D7 which imply that a dose of 80 mg/m^2 might not be high enough to be effective and documents such as D22 which imply that 80 mg/m^2 every two weeks may be unsafe because that dose could only be tolerated if dosed every three weeks. This fundamental conflict in the prior art

would have dissuaded the skilled person from pursuing the regimen of Arm C of D15b with a reasonable expectation of success.

5.95 The fundamental conflict between the prior art documents was not appreciated by the Opposition Division, and this led the Opposition Division to fall into error when assessing obviousness. The Opposition Division's decision stated that "*it is common practice to lower the dosage of a drug when used in a combination treatment*", meaning that the dose reduction from 120 mg/m^2 in D7, D12 and D13 to 80 mg/m^2 in Arm C of D15b would not have taken away the skilled person's reasonable expectation of success. The Opposition Division did not cite any prior art documents to support its assertion that dosage reduction was standard in the art. In any case, in making this point, the Opposition Division ignores the fact that documents such as D22 teach the skilled person that a dosage of 80 mg/m^2 administered once every two weeks may actually be too high and thus unlikely to be tolerable, because D22 showed that dose was tolerated only when administered every three weeks. That is to say, the dosage used in Arm C actually represents an increase in the liposomal irinotecan dose intensity in going from D22 to D15b. The Opposition Division failed to appreciate the significance of this point, and this contributed to the Opposition Division's incorrect finding on inventive step.

5.96 The skilled person's common general knowledge would also have dissuaded him/her from a combination regimen. As mentioned above, the skilled person would have been aware that irinotecan is known to cause, amongst other things, both the early and late forms of diarrhoea, the latter of which can be life threatening. 5-FU was also known to cause diarrhoea (D20). Thus, all other things being equal, the skilled person would have been drawn to liposomal irinotecan monotherapy, as s/he would have been motivated to avoid the combination regimen which combines two drugs which are both known to be associated with a potentially lethal adverse event (severe diarrhoea). This is particularly pertinent given that the monotherapy regimen, which did not require the use of multiple drugs, had shown safety and efficacy in both Phase I and Phase II trials, whereas there was no such data for the combination regimen of Arm C. The Opposition Division failed to appreciate this in its decision, and presented an over-simplified argument on this point in paragraphs 5.7.4 and 5.7.5 of its decision. In particular, whilst it is of course true that regimens comprising the co-administration of (non-liposomal) irinotecan and 5-FU/LV had been used in the prior art, this does not mean that, all other things being equal, the skilled person would have been more inclined towards the combination regimen. Rather, the skilled person would have been more inclined towards the liposomal irinotecan monotherapy due to the lower risk of toxicity, simplicity, and the fact that it had been successfully tested in Phase I and Phase II trials.

The skilled person would not have been directed to the claimed liposomal irinotecan formulation

5.97 The skilled person seeking to solve the objective technical problem would not have been led to a dosage regimen which uses "irinotecan sucrose octasulfate salt liposome injection". None of the prior art documents discloses or points to such a formulation. Thus, in the absence of any document which refers to such a formulation, in particular, to the use of sucrose octasulfate salt, the claims cannot be obvious.

5.98 Even if the skilled person had been directed toward the use of "irinotecan sucrose octasulfate salt liposome injection", nothing in the prior art would have given the skilled person any

reasonable expectation that such a formulation would have been able to treat gemcitabine-resistant pancreatic cancer safely.

5.99 The Opposition Division's decision noted that there is nothing on file which discloses "irinotecan sucrose octasulfate salt liposome injection". However, the Opposition Division stated that "this feature does not appear to be associated with any technical effect" meaning that it allegedly represents merely "an alternative liposomal irinotecan" (see paragraph 5.7.8 of the decision, page 19). Once again, the Opposition Division's comments make it clear that it failed to appreciate the significance of the "irinotecan sucrose octasulfate salt liposome injection" feature. As stated above, D15b refers only to "MM-398" without giving any indication as to what "MM-398" is. It is wrong to suppose that, absent any evidence, a liposome with a specific chemical composition would have been considered an obvious alternative in the context a very specific and tailored dosage regimen. The fear would have been that another liposome, with a particular chemical composition, would have behaved differently so could not have been substituted into the dosing regimen in a non-obvious manner.

5.100 D13 does state that "MM-398" is a type of liposomal irinotecan (see Table 1 on page 193), although no further information on the formulation is given. D13, page 188, right-hand-column, half-way down, indicates that "MM-398" is "irinotecan hydrochloride". Thus, the skilled person seeking to provide a safe and efficacious treatment for gemcitabine-resistant pancreatic cancer starting from D15b would note that the trial uses a drug referred to merely as "MM-398". Even if s/he then looked to D13, s/he would only find out that MM-398 is a type of liposomal irinotecan, most likely irinotecan hydrochloride, and there would have been nothing to lead to skilled person toward the use of an "irinotecan sucrose ocatsulfate salt liposome injection", as required by claim 1. Even if the skilled person had had a reasonable expectation that Arm C of D15b would be able to safely and effectively treat gemcitabine-resistant pancreatic cancer (which is denied), s/he could not have arrived at the claimed subject matter because the use of "irinotecan sucrose octasulfate salt liposome injection" was not disclosed or suggested in any of the prior art documents. By contrast, the patent (particularly Example 6), demonstrates that irinotecan sucrose octasulfate salt liposome injection is able to treat gemcitabine-resistant pancreatic cancer. This is not disclosed or suggested in the prior art, and as such the presence of the "irinotecan sucrose octasulfate salt liposome injection" is a further reason why the claims are inventive.

The solution to the more ambitious technical problem is nonobvious

5.101 If the objective technical problem is formulated more ambitiously as the provision of a safe and effective treatment for gemcitabine-resistant pancreatic cancer which is improved relative to the monotherapy arm of D15b, the claims of the Main Request are inventive.

5.102 In particular, even if the Board finds the solution to the less ambitious technical problem formulated at 5.71 to be obvious, the solution to the more ambitious technical problem involves an inventive step at least because there is nothing in the prior art which would have taught or suggested to the skilled person that the combination regimen of Arm C would show an improvement relative to the monotherapy. Therefore the solution to the more ambitious technical problem involves an inventive step.

Inventive Step - conclusion

5.103 In this section it has been established that, regardless of whether or not the claims are entitled to priority, the requirements of Article 56 EPC are met by the enclosed claims. In particular, the prior art would have led the skilled person towards a liposomal irinotecan monotherapy, and any combination regimen would be obvious only with the use of hindsight.

5.104 Contrary to the arguments made in the Opposition Division's decision, it is not the case that the clinical trial protocol in D15b results in the skilled person having a reasonable expectation of success for Arm C of the trial. In addition, the other prior art documents (D7, D12, D13 and D22 in particular) would have dissuaded the skilled person from pursuing Arm C. Further, there are no documents on file that would have led the skilled person towards the specific liposomal irinotecan formulation that is recited in claim 1. The claimed subject matter is therefore inventive.

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6 AUXILIARY REQUESTS 1 AND 2

6.1 Enclosed Auxiliary Requests 1 and 2 (hereafter "AR1" and "AR2") are identical to Auxiliary Request 1 filed on 28th June 2019 and the Main Request filed on 24th August 2018, respectively. These requests are maintained. The basis and rationale for these claims is described in our submissions before the Opposition Division which will not be repeated here. They are being maintained in case they provide useful fall-backs in these appeal proceedings, in particular in response to points that the opponent might raise in reply to this appeal.

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7 AUXILIARY REQUEST 3

7.1 Enclosed Auxiliary Request 3 ("AR3") is identical to Auxiliary Request 3 filed on 28th June 2019 during first instance proceedings.

7.2 AR3 differs from the Main Request because it contains the additional feature that "*the patient achieves a response which is at least stable disease*". This amendment finds basis on pages 16 and 17 of the application as filed. Page 16, towards the bottom of the page, and continuing onto page 17, lists the various categories of treatment response. The middle of page 17, in the fourth complete paragraph on that page, specifically discloses an embodiment in which the patient exhibits stable disease, partial response, complete response, or pathologic complete response ("pCR, CR, PR, or SD"). Analogous passages can be found on pages 15 and 16 of PD1. Thus the amended claim finds basis in both PD1 and the application as filed.

7.3 Even if the first priority claim is invalid, the skilled person seeking to provide a safe and effective therapy for gemcitabine-resistant pancreatic cancer such that the patient achieves a response which is at least stable disease (SD) would not have been led to the claimed subject-matter by Arm C of D15b with a reasonable expectation of success. This is at least because D15b gives no information about clinical outcomes.

7.4 Even if the skilled person had a reasonable expectation of some success from Arm C of D15b (which is not conceded), the skilled person would not have had a reasonable expectation that Arm C would result in a patient exhibiting stable disease.

7.5 In its decision, the Opposition Division stated at paragraph 9.4 that it did not believe that "progressive disease" could constitute an effective treatment, meaning that if there is a reasonable expectation of success for Arm C of D15b, there will also be a reasonable expectation of success for achieving "stable disease". However, in coming to this decision, the Opposition Division failed to appreciate the proper meaning of the term "progressive disease", and this resulted in the Opposition Division coming to an incorrect decision on this claim request.

7.6 The definition for the term "progressive disease" is given in paragraph 0065 of the patent as "at least a 20% increase in the sum of dimensions of target lesions". However, the cancer in a patient who exhibits progressive disease following administration of a dosage regimen may still be being "treated" by the dosage regimen. For example, a patient's cancer may show, for example, a 40% increase in the sum of the dimensions of target lesions in the absence of treatment, but with treatment, the cancer may show, for example, only a 25% increase in the sum of the dimensions of target lesions. Thus, even though a patient's cancer falls within the definition of progressive disease, the patient has still responded to the dosage regimen and thus been treated because the rate of progression has been reduced by virtue of the claimed regimen. Even if this a reduction in the rate of progression would have been reasonably expected from D15b (which is not conceded), there is certainly no reasonable expectation that the therapy would have been even more efficacious, and resulted in patients exhibiting "stable disease", from D15b. Therefore, claim 1 of AR3, which raises the bar for what can be reasonably expected from D15b, involves an inventive step.

Main Request – December 2019

1. Liposomal irinotecan for use in a method of treating pancreatic cancer in a human patient, wherein the patient exhibits evidence of recurrent or persistent pancreatic cancer following primary chemotherapy and wherein the patient has failed prior treatment with gemcitabine or become resistant to gemcitabine, the method comprising co-administration of an effective amount each of liposomal irinotecan, 5-fluorouracil (5-FU) and leucovorin to the patient in at least one cycle wherein the cycle is a period of 2 weeks and, for each cycle:

(a) liposomal irinotecan is administered to patients not homozygous for the UGT1A 1 *28 allele on day 1 of each cycle at a dose of 80 mg/m^2 and to patients homozygous for the UGT1A1 *28 allele on day 1 of cycle 1 at a dose of 60 mg/m^2 and on day 1 of each subsequent cycle at a dose of 60 mg/m^2 or 80 mg/m^2;

(b) 5-FU is administered at a dose of 2400 mg/m^2; and

(c) leucovorin is administered at a dose of 200 mg/m^2 (l form);

and wherein in each cycle, the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU;

and wherein the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection.

2. The liposomal irinotecan for use according to claim 1 wherein:

(a) after cycle 1 the dose of liposomal irinotecan administered to the patient homozygous for the UGT1A1 *28 allele is increased to 80 mg/m^2; and/or

(b) the 5-FU is administered intravenously over 46 hours; and/or

(c) the leucovorin is administered intravenously over 30 minutes.

3. The liposomal irinotecan for use according to claim 1 or claim 2 wherein:

(a) the liposomal irinotecan is administered intravenously over 90 minutes; and/or

(b) prior to each administration of liposomal irinotecan, the patient is pre- medicated with dexamethasone and/or a 5-HT3 antagonist or another anti-emetic; and/or

(c) the pancreatic cancer is an exocrine pancreatic cancer selected from the group consisting of acinar cell carcinoma, adenocarcinoma, adenosquamous carcinoma, giant cell tumor, intraductal papillary-mucinous neoplasm (IPMN), mucinous cystadenocarcinoma, pancreatoblastoma, serous cystadenocarcinoma, and solid and pseudopapillary tumors.

4. The liposomal irinotecan for use according to any one of the preceding claims, wherein the cancer is advanced pancreatic cancer, which is a pancreatic tumor that exhibits:
(a) either of distant metastasis or peripancreatic extension of the tumor; or
(b) both distant metastasis and peripancreatic extension of the tumor.

Auxiliary Request 1 – December 2019

1. Liposomal irinotecan for use in a method of treating pancreatic cancer in a human patient such that the patient achieves a response which is at least stable disease, wherein the patient exhibits evidence of recurrent or persistent pancreatic cancer following primary chemotherapy and wherein the patient has failed prior treatment with gemcitabine or become resistant to gemcitabine, the method comprising co-administration of an effective amount each of liposomal irinotecan, 5-fluorouracil (5-FU) and leucovorin to the patient in at least one cycle wherein the cycle is a period of 2 weeks and, for each cycle:

(a) liposomal irinotecan is administered to patients not homozygous for the UGT1A 1 *28 allele on day 1 of each cycle at a dose of 80 mg/m^2 and to patients homozygous for the UGT1A1 *28 allele on day 1 of cycle 1 at a dose of 60 mg/m^2 and on day 1 of each subsequent cycle at a dose of 60 mg/m^2 or 80 mg/m^2;

(b) 5-FU is administered at a dose of 2400 mg/m^2; and

(c) leucovorin is administered at a dose of 200 mg/m^2 (l form);

and wherein in each cycle, the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU.

2. The liposomal irinotecan for use according to claim 1 wherein:

(a) after cycle 1 the dose of liposomal irinotecan administered to the patient homozygous for the UGT1A1 *28 allele is increased to 80 mg/m^2; and/or

(b) the 5-FU is administered intravenously over 46 hours; and/or

(c) the leucovorin is administered intravenously over 30 minutes.

3. The liposomal irinotecan for use according to claim 1 or claim 2 wherein:

(a) the liposomal irinotecan is administered intravenously over 90 minutes; and/or

(b) prior to each administration of liposomal irinotecan, the patient is pre- medicated with dexamethasone and/or a 5-HT3 antagonist or another anti-emetic; and/or

(c) the pancreatic cancer is an exocrine pancreatic cancer selected from the group consisting of acinar cell carcinoma, adenocarcinoma, adenosquamous carcinoma, giant cell tumor, intraductal papillary-mucinous neoplasm (IPMN), mucinous cystadenocarcinoma, pancreatoblastoma, serous cystadenocarcinoma, and solid and pseudopapillary tumors.

4. The liposomal irinotecan for use according to any one of the preceding claims, wherein the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection.

5. The liposomal irinotecan for use according to any one of the preceding claims, wherein the cancer is advanced pancreatic cancer, which is a pancreatic tumor that exhibits:

(a) either of distant metastasis or peripancreatic extension of the tumor; or

(b) both distant metastasis and peripancreatic extension of the tumor.

Auxiliary Request 2 – December 2019

1. Liposomal irinotecan for use in a method of treating pancreatic cancer in a human patient, wherein the patient exhibits evidence of recurrent or persistent pancreatic cancer following primary chemotherapy and wherein the patient has failed prior treatment with gemcitabine or become resistant to gemcitabine, the method comprising co-administration of an effective amount each of liposomal irinotecan, 5-fluorouracil (5-FU) and leucovorin to the patient in at least one cycle wherein the cycle is a period of 2 weeks and, for each cycle:

(a) liposomal irinotecan is administered to patients not homozygous for the UGT1A 1 *28 allele on day 1 of each cycle at a dose of 80 mg/m^2 and to patients homozygous for the UGT1A1 *28 allele on day 1 of cycle 1 at a dose of 60 mg/m^2 and on day 1 of each subsequent cycle at a dose of 60 mg/m^2 or 80 mg/m^2;

(b) 5-FU is administered at a dose of 2400 mg/m^2; and

(c) leucovorin is administered at a dose of 200 mg/m^2 (l form);

and wherein in each cycle, the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU.

2. The liposomal irinotecan for use according to claim 1 wherein:

(a) after cycle 1 the dose of liposomal irinotecan administered to the patient homozygous for the UGT1A1 *28 allele is increased to 80 mg/m^2; and/or

(b) the 5-FU is administered intravenously over 46 hours; and/or

(c) the leucovorin is administered intravenously over 30 minutes.

3. The liposomal irinotecan for use according to claim 1 or claim 2 wherein:

(a) the liposomal irinotecan is administered intravenously over 90 minutes; and/or

(b) prior to each administration of liposomal irinotecan, the patient is pre- medicated with dexamethasone and/or a 5-HT3 antagonist or another anti-emetic; and/or

(c) the pancreatic cancer is an exocrine pancreatic cancer selected from the group consisting of acinar cell carcinoma, adenocarcinoma, adenosquamous carcinoma, giant cell tumor, intraductal papillary-mucinous neoplasm (IPMN), mucinous cystadenocarcinoma, pancreatoblastoma, serous cystadenocarcinoma, and solid and pseudopapillary tumors.

4. The liposomal irinotecan for use according to any one of the preceding claims, wherein the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection.

5. The liposomal irinotecan for use according to any one of the preceding claims, wherein the cancer is advanced pancreatic cancer, which is a pancreatic tumor that exhibits:

(a) either of distant metastasis or peripancreatic extension of the tumor; or

(b) both distant metastasis and peripancreatic extension of the tumor.

Auxiliary Request 3 – December 2019

1. Liposomal irinotecan for use in a method of treating pancreatic cancer in a human patient such that the patient achieves a response which is at least stable disease, wherein the patient exhibits evidence of recurrent or persistent pancreatic cancer following primary chemotherapy and wherein the patient has failed prior treatment with gemcitabine or become resistant to gemcitabine, the method comprising co-administration of an effective amount each of liposomal irinotecan, 5-fluorouracil (5-FU) and leucovorin to the patient in at least one cycle wherein the cycle is a period of 2 weeks and, for each cycle:

(a) liposomal irinotecan is administered to patients not homozygous for the UGT1A 1 *28 allele on day 1 of each cycle at a dose of 80 mg/m^2 and to patients homozygous for the UGT1A1 *28 allele on day 1 of cycle 1 at a dose of 60 mg/m^2 and on day 1 of each subsequent cycle at a dose of 60 mg/m^2 or 80 mg/m^2;

(b) 5-FU is administered at a dose of 2400 mg/m^2; and

(c) leucovorin is administered at a dose of 200 mg/m^2 (l form);

and wherein in each cycle, the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU;

and wherein the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection.

2. The liposomal irinotecan for use according to claim 1 wherein:

(a) after cycle 1 the dose of liposomal irinotecan administered to the patient homozygous for the UGT1A1 *28 allele is increased to 80 mg/m^2; and/or

(b) the 5-FU is administered intravenously over 46 hours; and/or

(c) the leucovorin is administered intravenously over 30 minutes.

3. The liposomal irinotecan for use according to claim 1 or claim 2 wherein:

(a) the liposomal irinotecan is administered intravenously over 90 minutes; and/or

(b) prior to each administration of liposomal irinotecan, the patient is pre- medicated with dexamethasone and/or a 5-HT3 antagonist or another anti-emetic; and/or

(c) the pancreatic cancer is an exocrine pancreatic cancer selected from the group consisting of acinar cell carcinoma, adenocarcinoma, adenosquamous carcinoma, giant cell tumor, intraductal papillary-mucinous neoplasm (IPMN), mucinous cystadenocarcinoma, pancreatoblastoma, serous cystadenocarcinoma, and solid and pseudopapillary tumors.

4. The liposomal irinotecan for use according to any one of the preceding claims, wherein the cancer is advanced pancreatic cancer, which is a pancreatic tumor that exhibits:

(a) either of distant metastasis or peripancreatic extension of the tumor; or

(b) both distant metastasis and peripancreatic extension of the tumor.

DECLARATION OF AMY MCKEE, M.D.

I, Amy McKee, declare and state as follows.

Qualifications

1. I, Amy McKee, M.D., am Vice President of PAREXEL Regulatory Consulting and have held this position for ten months.

2. Prior to my position at PAREXEL, I worked for eleven years in the U.S. Food and Drug Administration (FDA). During my time at the FDA, I held several positions including:
    - Deputy Center Director of the Oncology Center of Excellence,
    - Supervisory Associate Director of the Office of Hematology and Oncology Products (OHOP), and
    - Deputy Office Director of the OHOP.

3. I have experience in managing, developing and implementing the multi-disciplinary reviews for marketing applications within the OHOP. I have also managed an international regulatory program with outreach and program development with the European Medicines Agency (EMA) and other regulatory bodies. Overall, while I was at FDA, I was involved in over 100 marketing application reviews, all relating to oncology treatments.

Scope of declaration

4. I have been informed that European Patent EP 2 861 210 81 has been opposed at the European Patent Office. I understand that the claims of this patent relate to a particular combination of **liposomal irinotecan, 5-fluorouracil (5-FU)** and **leucovorin (LV)** for the treatment of pancreatic cancer in patients who exhibit evidence of recurrent or persistent pancreatic cancer following primary chemotherapy and who have either failed prior treatment with gemcitabine or become resistant to gemcitabine treatment.

5. I have been asked to provide an overview of the pancreatic cancer treatment field in 2012/2013 and the prognosis for such patients at that time.

6. I have been shown copies of the following two entries posted to the website clinicaltrials.gov and have been asked to comment on the content of these:

    - NCT01494506 on 2011_12_16 ("D15a")
    - NCT01494506 on 2013_01_25 ("D15b")

7. I have also been asked to provide information on the steps that would have been taken in order for these entries to be published on the website, the level of analysis that would have been undertaken before publication on the website, and how those entries would have been perceived by clinicians.

Treatment of pancreatic cancer

8. As of 2012/2013, pancreatic cancer was incredibly difficult to treat, and, in most cases, patients with this disease had a dismal prognosis. A 2013 paper reports that "the 5-year relative survival rate for pancreatic ductal adenocarcinoma (PDAC) is 6%, the only major cancer with a survival rate in the single digits".[3]

9. In spite of the fact that the incidence of pancreatic cancer has markedly increased in the past several decades, treatment options for pancreatic cancer were very limited, and there had been very little change in the field of pancreatic cancer treatment over the decade preceding 2012/2013.

10. Most commonly, treatment involved chemotherapy, with gemcitabine as the first line treatment. Surgery was used for the small proportion of patients who were deemed eligible for resection.

11. In patients whose tumors progressed on or after gemcitabine treatment or that had become resistant to such treatment, the options were very limited. They could try to enroll in a clinical trial. They could try another type of chemotherapy (depending on performance status), although there was no approved second line treatment.

12. In 2012/2013, there were no approved therapies for patients' whose cancer had progressed following gemcitabine therapy, thus any second-line treatment given would be at the discretion of the oncologist and would be associated with a very low chance of success. Aside from this, the only other option available was to discontinue active treatment and settle for palliative care (with the understanding that the disease, if left untreated, will continue to progress and lead to death).

13. There was therefore a desperate need as of 2012/2013 for new treatment options for gemcitabine-resistant pancreatic cancer patients and patients in whom gemcitabine treatment had failed.

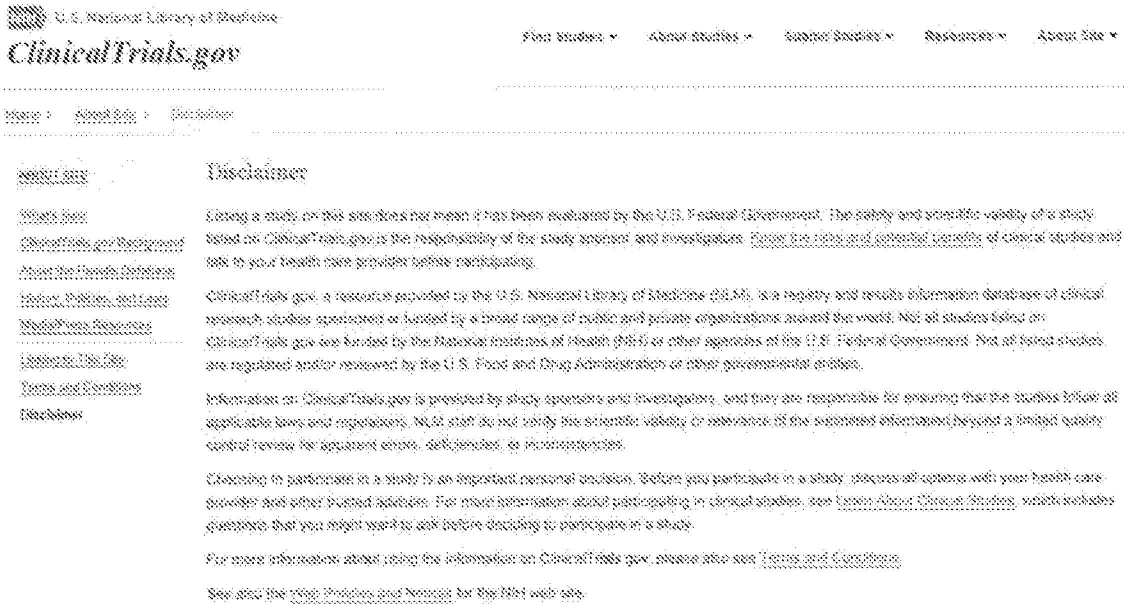
Clinicaltrials.gov website

14. According to the website clinicaltrials.gov, the purpose of the website is to provide a database of privately and publicly funded clinical studies conducted around the world.

15. The website is run by the U.S. National Library of Medicine (NLM) as a central portal on which information about certain clinical trials is posted to provide a single source of information for doctors, patients, and others on new therapeutic treatments being investigated. In practice, the website is helpful for doctors and patients interested in partaking in clinical trials. However, generally speaking, an oncologist would not consider clinicaltrials.gov to be a reliable source of information as to which experimental drugs and dosage regimens are promising therapies. Rather, an oncologist would reserve judgment until, for example, the results of the trial are published in a peer-reviewed journal. This is especially so given the high failure rate of pancreatic cancer trials, mentioned below.

[3] Hoos et al., J Clin Oncol 31:3432-3438. Annex A.

16. As the website makes clear in its disclaimer (below), the fact that a study has been listed on this site does not mean it has been evaluated by the U.S. Federal Government. It is the responsibility of the study sponsor and investigators to ensure that the trial is safe and scientifically valid. Not all studies listed on the website are regulated and/or reviewed by the FDA or other governmental entities:



(Screenshot taken from the clinicaltrials.gov)

17. As mentioned in the disclaimer, the NLM, which is responsible for the website, does not verify the scientific validity or relevance of the information submitted by the trial sponsors and investigators beyond a limited quality control review for obvious errors, deficiencies, or inconsistencies.

18. For all of the time that I worked at the FDA (since September 2008), it was always understood that the information on clinicaltrials.gov originates from the trial sponsor(s) and investigators and that no external regulatory body verifies the scientific validity or relevance of the submitted information that is published on the website.

19. Each clinical trial published on the website is assigned a different trial number which begins with "NCT". The website page for a given trial can be updated throughout the course of the trial, if needed, as and when more information on the trial and its results become available.

20. Even if/when the results of the clinical trial are published on the clinicaltrials.gov website, these also originate from the trial sponsor and investigators. The results are not reviewed or sanctioned by the FDA, NLM or any other regulatory body before they are published online.

Clinical trial NCT01494506

21. Having reviewed both D15a and D15b, I note that these are both entries taken from the clinicaltrials.gov website page for the same Phase III clinical trial (clinical trial no. NCT01494506). The two entries are dated around one year apart (16th December 2011

(D15a) and 25th January 2013 (D15b), respectively).

22. In the field of oncology, it is not necessarily true that a drug or dosage regimen being tested in a Phase III trial will have shown a high level of efficacy and safety in, for example, Phase I and/or Phase II trials. Sometimes the regimen/indication being studied in phase III is not preceded by studies of that regimen/indication in phase I or II. A clinical trial sponsor may choose to take a treatment into Phase III even if the chances of success are low, objectively speaking. A review of clinical development success rates indicates that, "Solid tumor drugs for pancreatic cancer seemed to have the toughest challenges in Phase III studies (13.3%, n=15)".[2]

23. According to the December 2011 entry, the trial NCT01494506 consisted of two treatment arms:

    - ***Arm A***: MM-398 (120 mg/m^2) administered once every three weeks
    - ***Arm B (comparator)***: 5-FU (2000 mg/m^2) and LV (200 mg/m^2) both for 4 weeks followed by 2 weeks of rest every 6 weeks

24. On 25th January 2013, just over a year later, the trial was modified to introduce a new treatment arm (Arm C):

    - ***Arm C***: MM-398 (80 mg/m^2) administered once every two weeks in combination with 5-Fluorouracil (2400 mg/m^2) every 2 weeks and leucovorin (400 mg/m^2) every 2 weeks

25. Although the trial sponsor and investigators would have had to gain approval from the relevant Institutional Review Board (IRB)(s) before adding this new arm to the trial, it would not have been mandatory for the sponsor/investigators to liaise with the FDA or any other regulatory body regarding this new treatment arm[3]. While the IRB would consider the likelihood of the new arm contributing to the trial's overall scientific objective, the IRB's primary consideration would be patient safety and whether the new arm risks safety in any way.

26. When deciding whether to consent to the addition of the new arm, the IRB would have taken into consideration the seriousness of the disease under investigation in the trial and the risk/benefit ratio of adding the new arm. The IRB would be aware that there was no standard of care for gemcitabine-resistant pancreatic cancer patients, and that these patients would be terminally ill and expected to survive for less than a year. This likely would sway the balance in favor of allowing the addition of another treatment arm. Provided that the risk to the patients would not be excessively high when weighed against the patient's dismal prognosis in the event of no treatment, the IRB would have been inclined to approve

[2] Clinical Development Success Rates 2006-2015, published by Biotechnology Innovation Organization (BIO) et al. Annex B, page 15.

[3] An amendment to the protocol would have to be submitted to any regulatory agency where the trial was being conducted before testing Arm C in patients, but the trial could have been started without any affirmative response from the agency.

the addition of Arm C. This is in spite of the extremely low probability of success.

27. In other words, given that the patients in question are terminally ill and would have already exhausted all approved therapies available, the IRB would have appreciated that there was merit in trying any new therapy options available in the context of a controlled clinical trial even when the probability of the trial being successful was extremely low. That is to say, whilst it may have been **hoped** that Arm C would be successful, there would certainly have been no **reasonable expectation** that Arm C would be successful, especially against the backdrop of the very limited options available to pancreatic cancer patients at that time and the lack of success for other investigational therapies in pancreatic cancer clinical trials in the preceding years.

28. The level of risk that was tolerated with a given clinical trial was very disease- and context-dependent, and each trial was analysed individually on a case-by-case basis. Consideration was given to a number of factors, including the relative risks that the trial would have placed on the patient and their safety, the therapies available to the patient (and in the case of pancreatic cancer, the well recognized unmet medical need), and the treatment setting (adjuvant versus relapsed/refractory). In view of the dismal prognosis of the patient population in clinical trial NCT01494506 (i.e. patients whose tumors have become resistant to or have failed with the only approved treatment option), the risk/benefit considerations that would have been weighed by the IRB for clinical trial NCT01494506 would have been very different to those weighed by an IRB for a different clinical trial relating to another disease and another patient population. Take, for example, the medical condition osteoporosis, which I have been asked about as at 2000/2001. While osteoporosis is an unpleasant condition, it is not terminal, and there were therapies already available on the market to treat and manage this condition. Accordingly, in assessing a clinical trial protocol for an osteoporosis drug, an IRB would have been more conservative than with respect to a protocol for a proposed treatment for gemcitabine-resistant pancreatic cancer and would have placed greater emphasis on the safety and avoidance of risk to patients who are not terminally ill coupled with a need to justify a reasonable expectation of efficacy, in order to justify the trial proceeding. One reason for this is that there were already a number of treatment options for osteoporosis on the market, so the overall risk/benefit analysis for a therapy that does not address unmet medical need would have required a showing of a reasonable expectation of success in order to justify the clinical trial.

29. As mentioned above, the situation for the gemcitabine-resistant pancreatic cancer patients in clinical trial NCT01494506 would have been completely different. Given the dismal prognosis for gemcitabine-resistant pancreatic cancer, IRBs most likely would have allowed any trials to take place in the circumstances above. This applies even though there was a mere hope, as opposed to an actual expectation, that the treatment would succeed. The IRBs would have been willing to tolerate a different level of risk (compared to, say, osteoporosis treatments in 2000/2001) in terms of the potential exposure of the patients to adverse events for even a slim chance of identifying a new treatment for patients who have run out of options to fight the cancer.

30. Although at the end of both D15a and D15b, the US FDA is identified as the health authority under whose purview the trial is intended to be run, this does not mean that the trial has been sanctioned or approved by the FDA.

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

Declared in Washington D.C., United States on 12/23/19

(AMY MCKEE)

Annex A

Annex B

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SPECIAL ARTICLE

Pancreatic Cancer Clinical Trials and Accrual in the United States

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See accompanying editorial on page 3312

All authors: Pancreatic Cancer Action Network, Manhattan Beach, CA.

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Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/13/3127w-0432w/$20.00

DOI: 10.1200/JCO.2013.49.4823

ABSTRACT

Purpose
Pancreatic cancer clinical trials open in the United States and their accrual were examined to identify opportunities to accelerate progress in the treatment of pancreatic cancer.

Methods
Pancreatic cancer–specific clinical trials open in the United States in the years 2011 and 2012 were obtained from the Pancreatic Cancer Action Network database. Accrual information was obtained from trial sponsors.

Results
The portfolio of pancreatic cancer clinical trials identified by type (adenocarcinoma or neuroendocrine), phase, disease stage, and treatment approach is reported. More than half of trials for patients with pancreatic ductal adenocarcinoma applied biologic insights to new therapeutic approaches, and 38% focused on optimization of radiation or chemotherapy delivery or regimens. In 2011, pancreatic cancer trials required total enrollment of 11,786 patients. Actual accrual to 93.2% of trials was 1,804 patients, an estimated 4.57% of the patients with pancreatic cancer alive in that year. The greatest need was for patients with resectable cancer. Trials open in 2011 enrolled an average of 15% of their total target accrual. Physician recommendations greatly influenced patients' decision to enroll or not enroll onto a clinical trial. Matching to a clinical trial within a 50-mile radius and identifying trials for recurrent/refractory disease were documented as challenges for patient accrual.

Conclusion
Overall trial enrollment indicates that pancreatic cancer trials open in 2011 would require 6.7 years on average to complete accrual. These results suggest that harmonizing patient supply and demand for clinical trials is required to accelerate progress toward improving survival in pancreatic cancer.

J Clin Oncol 31:3432-3438. © 2013 by American Society of Clinical Oncology

INTRODUCTION

Pancreatic cancer is the fourth-leading cause of cancer deaths in the United States.[1] In 2012, an estimated 44,000 Americans were diagnosed with pancreatic cancer, and 37,000 died as a result of the disease. It is the only one of the most commonly diagnosed cancers for which both the incidence and death rates are increasing.[2] As a result, by 2020, pancreatic cancer is projected to become second only to lung cancer as the leading cause of cancer death.[3]

The overall 5-year relative survival rate for all cancer has increased from 49% to 67% since the 1972 declaration of the war on cancer, and several major cancer types have 5-year relative survival rates that exceed 90%.[1] In contrast, the 5-year relative survival rate for pancreatic ductal adenocarcinoma (PDAC) is 6%, the only major cancer with a survival rate in the single digits. There are anatomic and biologic factors that are likely contributors to the striking lack of progress in PDAC survival.[4,5] Clinical progress in PDAC has been slow. In 1997, investigators showed gemcitabine improved median overall survival (OS) to 5.6 months compared with 4.4 months with bolus fluorouracil (FU).[6] Approximately 12 novel chemical or biologic agents have been tested in combination with gemcitabine with no significant benefit.[7] The addition of erlotinib was shown to improve OS from 5.9 to 6.2 months in 2005,[8] but this regimen has not become the standard of care, because the benefit is not deemed to outweigh the additional toxicities.[9] FOLFIRINOX, a combination of FU, oxaliplatin, leucovorin, and irinotecan, increased OS to 11.1 months compared

with 6.8 months for gemcitabine in 2011, but toxicities limit its use to patients with an excellent performance status.[7,10]

The need for accelerated clinical progress against pancreatic cancer, with an organizational goal of doubling the survival rate in pancreatic cancer by 2020, led the Pancreatic Cancer Action Network to examine the characteristics of ongoing clinical trials during the years 2011 and 2012. All clinical trials for pancreatic cancer are entered into a proprietary database at the Pancreatic Cancer Action Network and updated on a monthly basis. The database permits customized clinical trial searches to assist patients and their physicians in identifying trials and sites with appropriate eligibility requirements. Our results suggest that a critical look at harmonizing clinical trial opportunities and patient availability could more effectively leverage a limited patient resource to speed progress in identifying new therapeutic approaches for pancreatic cancer.

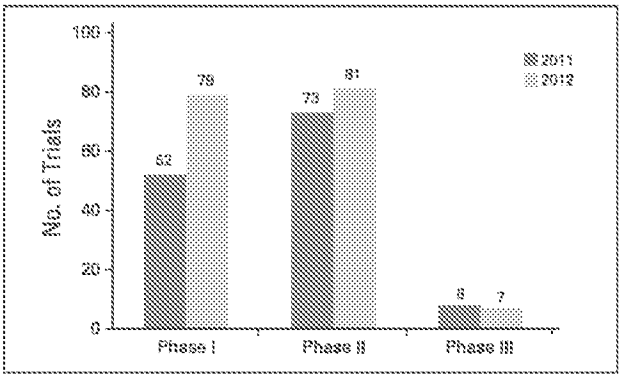

Fig 1. Pancreatic cancer clinical trials open in United States in 2011 and 2012 by phase.

METHODS

The Pancreatic Cancer Action Network developed and maintains a proprietary database of phase I, II, and III pancreatic cancer–specific clinical trials in the United States. Only trials for which pancreatic cancer is identified as a specific inclusion criterion are included in the database; general solid tumor trials are not included. Trial information is obtained through multiple channels, including the membership of the organization in various national committees, direct interaction with trial sponsors, Web sites such as www.clinicaltrials.gov, and institutional Web sites. Information is verified before it is entered into the database and updated on an ongoing basis to ensure accuracy. Trial sponsors provided information on 2011 US accrual only; a small number of trials included overseas accrual sites, which were not considered in the analysis.

All trials active at any time in 2011 and 2012 were included in the analysis. Trials listed as phase Ib/II, Ib, and I/II were categorized as phase I, and phase II/III as either phase II or phase III depending on current status. Trials were categorized by disease stage based on reviewing the title and inclusion/exclusion criteria for each trial and determining a best fit based on common definition of each category. If eligibility included more than one stage of disease, the more advanced stage was assigned. Trials were categorized by treatment type by reviewing the available literature on each compound and author judgment. Trials indicated as recurrent included studies where patients were identified as having disease refractory to a treatment or second-line therapy. Clinical trial searches were performed the first week of February 2013 using hypothetic patients to identify trial opportunities by geographic location. Surveys were sent to individuals who received a clinical trial search; 51% (761 of 1,495) returned the survey, and 253 respondents who did not enroll onto a clinical trial provided reasons for their decision that were identified as barriers to clinical trial accrual.

RESULTS

2011 and 2012 Pancreatic Cancer Clinical Trials

A total of 133 and 167 pancreatic cancer clinical trials were open in the United States at some time during the years 2011 and 2012, respectively. Of these trials, 103 were open both years, with 30 trials closing in 2011 and 64 new trials opening in 2012. In both years, the greatest number of open trials were phase II and the least number phase III studies (Fig 1). Phase I trials showed a 52% percent increase in 2012 relative to 2011. Trial sponsors for 2011 trials were listed as government (4.5%), industry (25.6%), or institutions (63.9%), with 6.0% sponsored by foundations or other organizations (Appendix Fig A1, online only). The oldest trial in either year opened in 2005, and the majority of trials opened in the same year (2011 or 2012) or in the prior 2 years (data not shown). Of the 30 trials that closed in 2011, results were reported for 27%; an additional 7% were closed for toxicity, and 7% were reported closed for slow accrual (Appendix Table A1, online only).

More than 90% of studies were designed for patients with PDAC at all stages of disease progression, with 8.7% of trials designed for patients with pancreatic neuroendocrine tumors (PNETs; Fig 2). The majority of the 120 PDAC trials in 2011 and 154 trials in 2012 focused on patients with metastatic disease, although trials for advanced cancer that included both nonresectable locally advanced and metastatic disease were characterized as metastatic. Thirteen percent of PDAC trials focused only on patients with locally advanced, nonresectable, nonmetastatic disease. Approximately 12% of PDAC trials required patients with resectable disease for neoadjuvant studies, and another 9% required the same population for adjuvant studies. Trials for

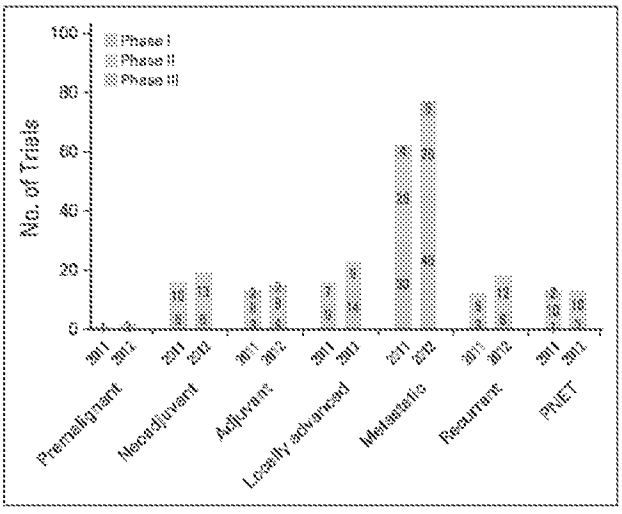

Fig 2. Pancreatic cancer clinical trials open in United States in 2011 and 2012 by disease stage. Premalignant indicates trials in patients with pancreatic cysts. All other stages refer to trials for pancreatic ductal adenocarcinoma. Trials indicated as recurrent include studies in which patients were identified as having disease refractory to treatment or second-line therapy. No. of phase I (blue), II (gold), and III (gray) trials is indicated within each category. PNET, pancreatic neuroendocrine tumor.

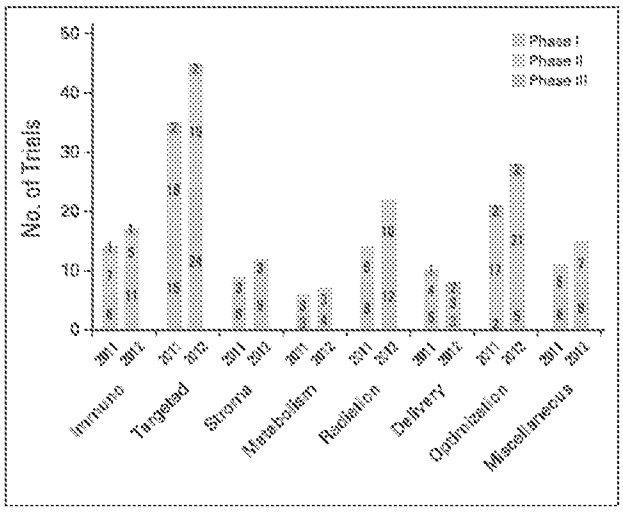

Fig 3. Pancreatic ductal adenocarcinoma clinical trials open in United States in 2011 and 2012 by treatment type. Targeted indicates those targeted to signal transduction pathways within tumor cell; stroma, targeted to pathways involved in tumor-stromal interactions; metabolism, targeted to alterations in tumor metabolism; radiation, involving radiotherapy; delivery, chemical modifications to improve delivery of chemotherapeutic drugs; optimization, optimization of approved cancer chemotherapy agents to test or improve their efficacy in pancreatic cancer; miscellaneous therapies include hyperthermia, vitamins, and natural products. No. of phase I (blue), II (gold), and III (gray) trials is indicated within each category. Immuno, immunotherapy.

patients with recurrent/refractory disease constituted 10% of PDAC trials. Few studies focused on patients with premalignant cysts.

The majority of PDAC trials tested novel small-molecule inhibitors or biologics directed at specific molecules or pathways (Fig 3). Therapies for PDAC targeted to tumor cell–specific intracellular signal transduction pathways made up the largest group (29%) and included studies of novel inhibitors of a number of tyrosine kinases, serine/threonine kinases, growth factors, and DNA repair and apoptosis cascades. Eight percent of PDAC trials addressed molecular targets that reside in the dense stroma in PDAC tumors, the majority with the intent to improve drug delivery to the tumor cells. A small number of trials (5%) were designed to capitalize on findings related to metabolic imbalance in pancreatic cancer. An additional 11% of trials were immunotherapeutic approaches to activate the natural immunologic defense system. Ten percent of PDAC trials represented a miscellaneous assortment of novel therapeutic approaches, including hyperthermia, vitamin treatment, and natural products.

More than one third of PDAC clinical trials (38%) were designed to optimize existing chemotherapies or radiation therapies. Thirteen percent of trials focused on radiation therapy and included proton-beam, stereotactic, intensity-modulated, and radiation-tagged biologic approaches. Seven percent of PDAC trials focused on chemical modifications of chemotherapeutic agents with nanoparticle or lipophilic tags to enhance drug delivery. Eighteen percent of studies were modifications to regimens currently in use for PDAC or other cancer types with the intent of testing or optimizing efficacy in patients with PDAC. PNET studies included a number of novel targeted therapies, targeted radiation, and optimization of existing chemotherapies or radiation therapies (data not shown).

Patient Supply and Demand

No organization or research entity tracks US trial participation rate by disease site, although the National Patient Advocate Foundation sponsored a baseline study of patient accrual to publically sponsored trials that included disease-site information.[11] To determine actual patient accrual to pancreatic cancer–specific clinical trials, we contacted sponsors of trials open in 2011 to obtain the total number of patients accrued to phase I to III pancreatic cancer–specific clinical trials during that year. A total of 1,804 patients in the United States were accrued to 93.2% of the pancreatic cancer clinical trials open during the year 2011.

To obtain an estimate of the percentage of all patients with pancreatic cancer who were enrolled onto a clinical trial in 2011, the number of patients with pancreatic cancer alive on January 1, 2011, was obtained as the SEER January 1, 2009, prevalence figure and corrected for increases of 1.4% and 1.7% per year in delay-adjusted incidence rates for women and men, respectively.[12] It is estimated that 4.57% of patients with pancreatic cancer were enrolled onto a clinical trial in 2011. This compares favorably to the overall estimate that less than 3% of adult patients with cancer in the United States are enrolled onto clinical trials.[13]

To determine the total number of patients required for clinical trials, studies open in 2011 were assigned to one of five categories: PDAC trials focused on localized (neoadjuvant and adjuvant trials), regional (locally advanced), distant (metastatic), or recurrent disease, and PNET trials. The total enrollment required for 29 (neo)adjuvant trials, 16 for patients with locally advanced disease, 62 for metastatic disease, and 12 for recurrent disease is summarized in Table 1. The estimated total number of patients anticipated to be diagnosed with localized, regional, or distant disease based on SEER stage distribution data was calculated and is listed in Table 1. If the entire trial portfolio were accrued in 2011 with US patients, 57% of the total number of patients with PNETs and 83%, 6%, and 26% of total patients with resectable, locally advanced, and metastatic PDAC, respectively, would be required to complete the trial. This far exceeds the overall average of 4.57% of patients with pancreatic cancer who enroll onto clinical studies.

It is generally estimated that 20% of patients are eligible for clinical trials,[14] and many are excluded because of poor performance status or inability to meet specific eligibility requirements. We calculated the enrollment capacity of trials open in 2011 relative to the number of patients eligible for trials using the 20% estimate (Table 1). Accruing the entire trial portfolio in each of the categories would require 4.2×, 1.3×, and 2.9× more patients with resectable, metastatic, and neuroendocrine disease, respectively, than estimated to be available on a yearly basis. Trials that only enroll patients with locally advanced disease would have the potential of completing accrual in less than a year. These results indicate that on average, the demand for patients exceeds the estimated supply of patients by two-fold, even if all eligible patients are recruited to clinical trials.

The percentage of patients who were accrued to (neo)adjuvant trials and trials for advanced, metastatic, and recurrent disease in 2011 was calculated by dividing the reported 2011 accrual for those trials for which accrual data were available by the total anticipated enrollment for the same trials (Table 1). The pancreatic cancer clinical trials open in 2011 at all disease stages achieved between 12.8% and 17.8% of total potential enrollment, with an average of 15%. On average, it would require 6.7 years to complete accrual for the pancreatic cancer studies

Table 1. Anticipated Total and Actual Accrual for Pancreatic Ductal Adenocarcinoma Clinical Trials in United States in 2011

Stage at Diagnosis	Distribution (%)*	Estimated No. of Patients†	No. of Trials	Total Enrollment Required	Enrollment Capacity (%)‡	Estimated No. of Patients at 20% Enrollment	Trial Capacity at 20% Enrollment§	Patients Enrolled (%)‖
Localized (neo]adjuvant)	8	3,451	29	2,874	83	690	4.17	14.9
Regional (locally advanced)	29	12,511	16	756	6	2,502	0.30	12.8
Distant (metastatic)	58	25,021	62	8,394	28	5,004	1.26	17.8
Recurrent	NA	NA	12	524	NA	NA	NA	14.1
PNET	5	2,157	13	1,238	57	431	2.87	15.5

Abbreviations: NA, not available; PNET, pancreatic neuroendocrine tumor.
*Data adapted.[14,15] Unstaged patients with pancreatic cancer (12%) were distributed as: PNET, 5%; locally advanced, 2%; metastatic, 5%.
†No. of new patient cases of pancreatic cancer in 2011 was estimated to be 43,140.[16]
‡Enrollment capacity is total potential enrollment for trials open in 2011 divided by estimated No. of available patients.
§Trial capacity at 20% enrollment is potential enrollment divided by estimated No. of patients available per stage if 20% of patients enrolled onto clinical trials.
‖Percent enrolled is No. of patients enrolled in 2011 divided by total potential enrollment for trials within that subgroup for which accrual numbers were available.

open in the year 2011. No clear patterns of accrual based on sponsor or length of time the trial was open could be discerned from the available data.

The majority of pancreatic cancer clinical trials required treatment-naive patients; in 2011, 4.5% of patients were required for 9.1% of trials for recurrent/refractory disease (Table 1). To estimate the treatment status of patients seeking information on clinical trials from the Pancreatic Cancer Action Network, we examined the records from the Patient and Liaison Services (PALS) program. The call center received 5,091 contacts from patients with pancreatic cancer or caregivers in 2011 and 7,771 contacts in 2012 (Table 2). This is an overestimate of the number of individual patients represented, because the same patient or caregiver may have called more than once with distinct questions. Of the patients who provided information on whether they had been treated for pancreatic cancer, 33% had not received prior treatment, indicating that approximately two thirds of contacts were ineligible for trials that required treatment-naive patients.

The PALS associates encourage contacts to consider clinical trials when making their treatment decisions and use the clinical trials database to provide up-to-date information on trials for which patients are eligible. Trial search criteria include type and stage of pancreatic cancer, treatment history, location, and ability to travel. A total of 874 clinical trial searches were performed in 2011 (Table 2). As the result of a focused awareness campaign, this number more than doubled in 2012, for a total of 2,183 searches in the year. Using a follow-up questionnaire, 78 of 761 respondents indicated they enrolled onto a clinical trial. The most frequent reason given for choosing to enroll onto a trial was physician recommendation (Table 2). Of the 253 contacts who indicated barriers to enrolling onto a clinical trial, 37% were not willing or able to travel to a trial location, 32% were too ill to participate, and 3% indicated financial or insurance reasons. Interestingly, 19% of respondents indicated their physician was opposed to their enrollment onto either a specific clinical trial (11%) or any clinical trial (8%).

Table 2. Patient Contact Information From PALS Program of Pancreatic Cancer Action Network

Characteristic	2011	2012
Total No. of PALS contacts	5,091	7,771
Total No. of contacts with known treatment status	2,687	4,684
Patients who have not received treatment, %	33.7	33.1
No. of clinical trial searches	874	2,183
Respondents enrolled onto clinical trial, %		10.2
Reasons for enrolling onto clinical trial (n = 78), %		
Physician recommendation		48.7
Better treatment option		21.8
Communication with PALS associate		9.0
Other/unknown		20.5
Barriers to enrolling onto clinical trial (n = 253), %		
Not willing/able to travel to trial location		37.2
Too sick to participate		31.8
Physician opposition		19.4
Financial/insurance issues		2.8
Other		9.1

Abbreviation: PALS, Patient and Liaison Services.

To examine the likelihood that a patient would be able to identify a clinical trial within a 50-mile radius from his or her home, clinical trial searches were performed for hypothetic patients who qualified for either adjuvant or neoadjuvant trials or for those with locally advanced, untreated metastatic, or previously treated metastatic disease. The search was performed using zip codes representing small, mid-sized, and large cities in the northeastern, southeastern, central, and western United States. Patients with resected or stage I pancreatic cancer in all regions and all city sizes could obtain information for at least one clinical trial within a 50-mile radius (Fig 4). In contrast, patients with more advanced disease received much less consistent clinical trial results. Residing in a large city increased the likelihood of identifying an appropriate clinical trial, as did living in the northeastern United States. The results of an expanded search using distances of 25, 50, 100, and 200 miles are presented in Appendix Table A2 (online only).

DISCUSSION

The analysis of pancreatic cancer clinical trials over the past 2 years reveals several features reflective of the state of the field. The number of studies focusing on PNETs compared with PDAC roughly reflects the 5% incidence of PNETs. The increase in phase I trials between 2011 and 2012 indicates there are many new approaches being considered

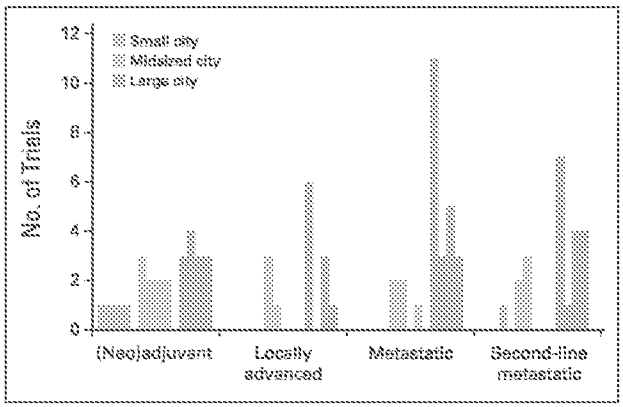

Fig 4. Pancreatic ductal adenocarcinoma cancer clinical trials available in select cities in United States. The Patient and Liaison Services database was queried for clinical trials within a 50-mile radius appropriate for hypothetic patients with resected or resectable disease (neo)adjuvant), locally advanced disease, and metastatic disease and for patients with metastatic disease who had been previously treated with gemcitabine (second-line metastatic). Zip codes from small (blue), midsized (gold), and large (gray) cities were used representing areas in northeastern (first bar), southeastern (second bar), central (third bar), and western (fourth bar) regions of United States.

for pancreatic cancer treatment. This is also supported by the observation that more than half of trials focus on approaches derived from advances in the biology of pancreatic cancer (immunotherapies or therapies targeted to signaling pathways, stroma, or metabolism). The small number of phase III trials reflects difficulty in identifying approaches with sufficient benefit for large-scale trials. Of the seven phase III trials open in 2012, five were open at the beginning of 2013; the study of ganitumab, a monoclonal antibody targeting the insulin-like growth factor 1 receptor, was closed for futility,[17] and the study of albumin-bound paclitaxel showed statistically significant benefit for patients with PDAC with metastatic disease.[18]

The most striking result of evaluating the US portfolio of pancreatic cancer clinical trials is the large excess of patients required to complete ongoing trials relative to the number of patients with pancreatic cancer who enroll onto trials. We determined that 1,804 patients with pancreatic cancer enrolled onto clinical trials in the year 2011; this is a slight underestimate because data were unavailable for 6.8% of open trials, and the number of patients with pancreatic cancer enrolled onto solid-tumor phase I trials is unknown. The number of patients needed to completely accrue the clinical trials that were open in 2011, however, was more than 6× greater than this number. In the event that clinical trial accrual would increase to 20% to include all patients estimated to be eligible for clinical trials, the average trial would accrue 75% of its enrollment within a year. However, there was considerable heterogeneity in the ratio of the estimated supply and demand for patients with different disease stages. In particular, the number of patients with resectable disease required for (neo)adjuvant trials was almost 4× greater than the number of patients estimated to be available for these studies, indicating it would take 3 to 4 years to complete accrual even at a robust 20% accrual rate. Pancreatic cancer trials open in 2011 overall accrued an average of 15% of their target enrollment (Table 1), indicating it would require 6.7 years on average to complete accrual. For a disease with survival times measured in months, the need to accelerate this process is evident.

Patients face many barriers to enrolling onto a clinical trial if offered one, including inability to meet eligibility criteria, desire for another treatment, distance from cancer center, and insurance denial.[19] Our survey confirmed that patients with pancreatic cancer who call the PALS program are unable to travel long distances, and clinical trial searches were unable to identify an appropriate trial for many hypothetic individuals residing in small and midsized cities. The excess capacity for patients with resectable disease was evident in that this was the only disease stage for which a trial was available within a 50-mile radius irrespective of city size or geographic region. In addition, a diagnosis of pancreatic cancer often results in immediate treatment without full consideration of all the options. Data from the PALS program suggest that two thirds of patients seeking clinical trial information from the Pancreatic Cancer Action Network have already received treatment and would therefore be ineligible for trials for untreated patients. Physician attitudes are well known to influence patient enrollment onto clinical trials,[19] an observation further documented by our observation that 49% of patients who enrolled onto a trial did so because their physician recommended it, and 19% of those who did not enroll attributed this to physician opposition.

The concept of supply and demand for pancreatic cancer clinical trials can be viewed from two perspectives. The perspective of the clinical trialists is they are seeking a supply of patients to fill the demand of accrual to clinical trials. The clinical trials community is actively producing a large number of scientifically driven trials designed to improve survival in pancreatic cancer, but progress is slow at least in part because the supply of eligible patients is inadequate to fulfill the demand. From the patient perspective, however, the patient has a demand for a study that meets his or her needs, and the supply of clinical trials that meet requirements for location and eligibility can be inadequate unless the patient lives in a large metropolitan area in the northeastern United States. From a patient/consumer perspective, it is not the number of clinical trials open that is important; rather, it is the ability to identify a clinical trial that provides the best opportunity for success in individual treatment and for advancement of pancreatic cancer treatment regimens in general.

Narrowing the disconnect between the supply and demand of patients for pancreatic cancer clinical trials can be approached from both sides: one, increasing the number of patients who enroll onto clinical trials, and two, modifying trial design and characteristics to better match the needs of patients. Increasing patient accrual to clinical trials has been the topic of national and international discussion for many years. The National Comprehensive Cancer Network states that the best management for any patient with cancer occurs in a clinical trial.[9] Studies have identified factors relevant to the patient, physician, and clinical research associate that influence a patient's decision to enroll onto a clinical trial, and these factors can be general, specific to the encounter, or specific to the trial under consideration.[20] A recent report from the National Cancer Clinical Trials Pilot Breakthrough Collaborative project addressed challenges associated with ineffective operational procedures, community relationships, and physician-patient communication and identified guiding principles to increase trial enrollment in the community setting.[21] Accreditation from the American College of Surgeons Commission on Cancer includes clinical trial accrual percentages that vary with the type of institution, suggesting that at least some of the incentives for increasing clinical trial enrollment are in place.[22] The National Cancer Institute (NCI) implemented the AccrualNet program in 2010 to provide strategies,

tools, and resources to support accrual to clinical trials.[23,24] The Pancreatic Cancer Action Network performed more than 2,000 clinical trial searches for patients diagnosed with pancreatic cancer in 2012. Despite these efforts, adult accrual to cancer clinical trials in the United States remains under 3%, with lower participation rates among ethnic and racial minorities and those age > 65 years.[23] Interestingly, the National Institute for Health Research Cancer Research Network in the United Kingdom initiated a program that successfully increased the recruitment of patients with cancer to National Institute for Health Research Cancer Research Network portfolio studies by five-fold over a 10-year period to 19.8% of patients recruited to trials in 2011.[25]

Modifying pancreatic cancer clinical trial demand has not been explored as thoroughly as attempts to understand how to increase the supply of patients to clinical trials. The NCI Clinical Trials Working Group proposed 22 initiatives to transform the NCI clinical trials enterprise, including establishing a network of steering committees to address the design and prioritization of phase III trials that leverages current intergroup, cooperative group, SPORE (specialized programs of research excellence), and cancer center structures.[26] The Gastrointestinal Steering Committee was established in 2006 and functions to harmonize an efficient, cost-effective, science-driven, and transparent process to identify and promote the best science by addressing the design and prioritization of large phase II and III trials.[27] The Pancreas Task Force of the Gastrointestinal Steering Committee meets on a monthly basis and makes recommendations for the improvement and suitability of trials that open within the cooperative group structure. However, of the 133 pancreatic cancer trials open in 2011, only three fell under the purview of the Pancreas Task Force, representing 2.3% of the open trials, 9.5% of the patients accrued, and 10.9% of the total anticipated accrual for these trials. This significantly limits the impact of the task force prioritization process relative to the existing landscape of competing trials.

The overarching need is to enroll patients with pancreatic cancer onto trials faster to shorten the cycle and increase the number of novel approaches that can be tested. An intriguing model for a clinical trials system for a cancer with low incidence is the Children's Oncology Group (COG). COG research has helped in moving childhood cancers from being virtually incurable 50 years ago to having a combined 5-year survival rate of 83%.[15] Part of the COG model involves uniting and coordinating efforts at more than 200 leading hospitals, universities, and cancer centers across North America, Australia, New Zealand, and Europe.[28] Survival rates from multiple myeloma have also increased in recent years to 41% in 2001 to 2007, from an average of 31% in the 1990s.[1,15] Much of this success can be attributed to the Multiple Myeloma Research Foundation, which initiated a clinical network dedicated to phase I and II myeloma clinical trials. The Multiple Myeloma Research Consortium, consisting of 16 integrated member institutions, has facilitated more than 40 clinical trials and was instrumental in the application of five new treatments, with more under investigation.[29] On the basis of these precedents, an integrated approach involving the NCI, the pharmaceutical industry, and patient advocacy groups is likely to be required to make significant changes in outcomes from pancreatic cancer within the foreseeable future. In the case of pancreatic cancer clinical trials, more is not necessarily better. Attention should be paid to more closely matching the supply of clinical trials to the demand of the patients with pancreatic cancer, resulting in more options for current patients and acceleration in the development of new and better treatments for future patients with this disease.

Author's Disclosures of Potential Conflicts of Interest

The author(s) indicated no potential conflicts of interest.

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Appendix

Table A1. Pancreatic Cancer–Specific Clinical Trials Closed in 2011 (N = 30)

Result	No.	%
No information on completion status (five open ≥ 3 years)*	8	30
Closed for slow accrual*	2	7
Closed for toxicity*	2	7
Results published or reported (two open ≥ 3 years)	8	27
Indicated as completed (one open ≥ 3 years)*	8	30

NOTE. Status of each trial was queried on clinicaltrials.gov and PubMed.
*Data collected from clinicaltrials.gov.

Table A2. Geographic Trial Landscape, February 2013

Location	Radius (miles)	Tumor Removed, No Additional Treatment and Stage I Untreated (adjuvant and neoadjuvant)	Locally Advanced Untreated (including trials for this and other stages)	Metastatic Untreated	Metastatic (current and at diagnosis) Second Line (one previous regimen, gemcitabine)
Large					
Los Angeles, CA (90071)	25	3	1	3	4
	50	3	1	3	4
	100	3	2	5	5
	200	3	3	6	6
New York, NY (10007)	25	3	5	10	6
	50	3	6	11	7
	100	4	9	16	10
	200	7	14	21	13
Chicago, IL (60604)	25	3	3	5	4
	50	3	3	5	4
	100	3	3	5	5
	200	4	5	8	5
Atlanta, GA (30334)	25	4	0	3	1
	50	4	0	3	1
	100	4	0	3	2
	200	5	0	7	5
Medium					
Portland, OR (97209)	25	2	0	1	0
	50	2	0	1	0
	100	2	0	1	0
	200	2	2	6	4
Pittsburgh, PA (15219)	25	2	3	2	2
	50	3	3	2	2
	100	3	3	3	3
	200	6	10	14	16
Milwaukee, WI (53202)	25	2	0	0	0
	50	2	0	0	0
	100	4	4	6	4
	200	4	5	7	5
Tampa, FL (33602)	25	2	1	2	3
	50	2	1	2	3
	100	3	1	2	3
	200	5	4	5	5
Small					
Boise, ID (83702)	25	1	0	0	1
	50	1	0	0	1
	100	1	0	0	1
	200	1	0	0	1
Concord, NH (03301)	25	1	0	0	0
	50	1	0	0	0
	100	3	5	7	4
	200	3	6	9	6
Des Moines, IA (50316)	25	1	0	0	0
	50	1	0	0	0
	100	1	0	0	0
	200	2	6	7	6
Savannah, GA (31401)	25	1	0	0	0
	50	1	0	0	0
	100	1	1	4	1
	200	4	4	6	3

NOTE. Clinical trial searches were performed during first week of February 2013 using hypothetic patients with resected or stage I pancreatic cancer with no chemotherapy treatment, untreated locally advanced, untreated metastatic, or recurrent metastatic disease after gemcitabine treatment. No. of trials for hypothetic patients is given without consideration of performance status.

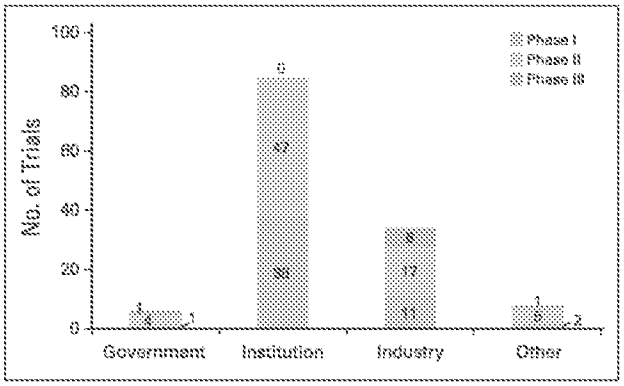
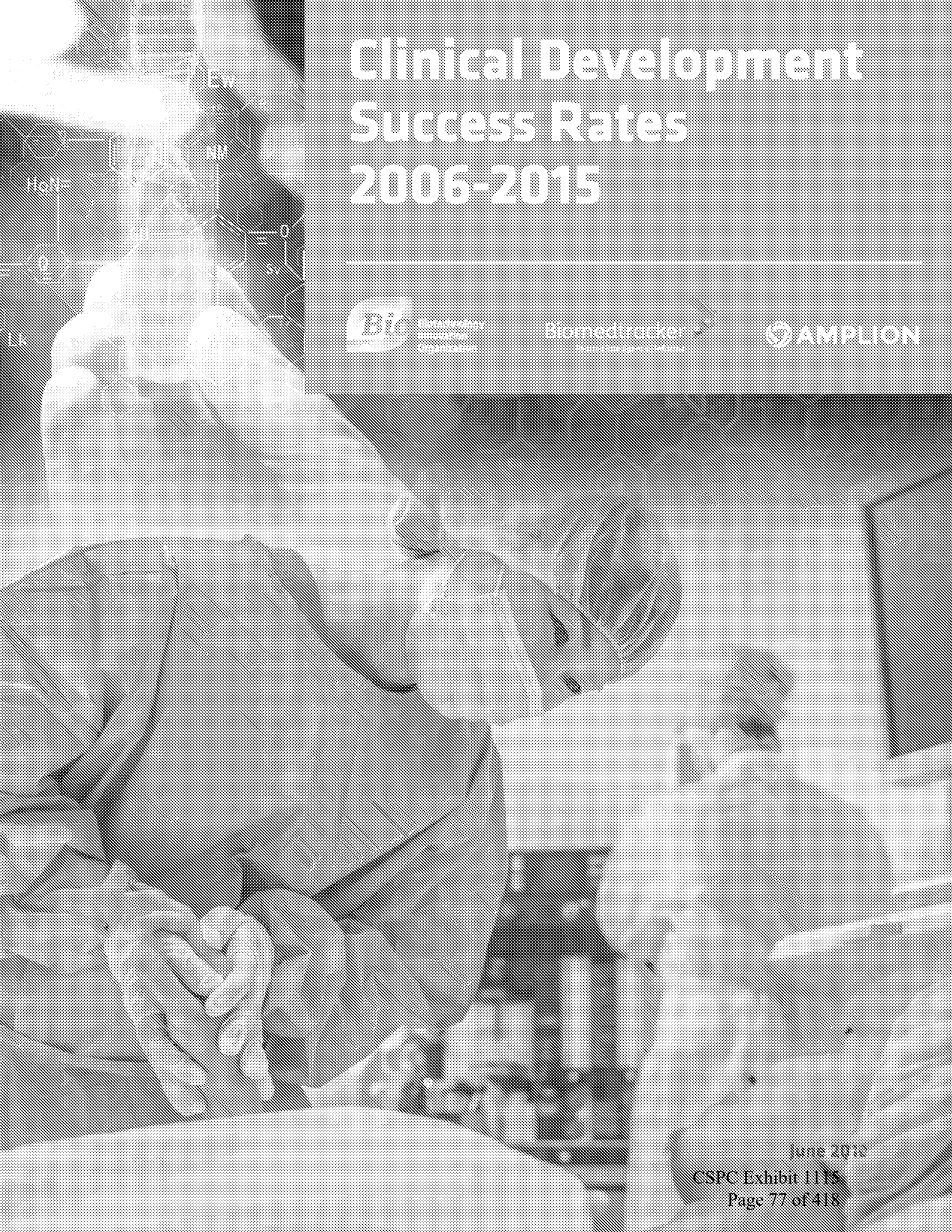


Fig A1. The 133 pancreatic cancer–specific clinical trials open in United States in 2011 were identified by sponsor indicated in clinicaltrials.gov. No. of phase I (blue), II (gold), and III (gray) trials is indicated within each category.

Clinical Development Success Rates 2006-2015



June 2016

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BioMedTracker, a subscription-based product of Informa, tracks the clinical development and regulatory history of investigational drugs to assess its Likelihood of Approval (LOA) by the FDA. BioMedTracker is populated in near real-time with updated information from press releases, corporate earnings calls, investor and medical meetings and numerous other sources.

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Executive Summary

This is the largest study of clinical drug development success rates to date. Over the last decade, 2006-2015, a total of 9,985 clinical and regulatory phase transitions were recorded and analyzed from 7,455 development programs, across 1,103 companies in the Biomedtracker database. Phase transitions occur when a drug candidate advances into the next phase of development or is suspended by the sponsor. By calculating the number of programs progressing to the next phase vs. the total number progressing and suspended, we assessed the success rate at each of the four phases of development: Phase I, II, III, and regulatory filing. Having phase-by-phase data in hand, we then compared groups of diseases, drug modalities and other attributes to generate the most comprehensive analysis yet of biopharmaceutical R&D success.

This work was made possible due to the years of clinical program monitoring and data entry by Informa's Biomedtracker service. BIO has long partnered with Biomedtracker to calculate success rates based on this data. More recently, BIO and Biomedtracker partnered with Amplion, the inventors of BiomarkerBase, to analyze the effects of biomarkers in clinical trial success.

Key takeaways:

- The overall likelihood of approval (LOA) from Phase I for all developmental candidates was 9.6%, and 11.9% for all indications outside of Oncology.
- Rare disease programs and programs that utilized selection biomarkers had higher success rates at each phase of development vs. the overall dataset.
- Chronic diseases with high populations had lower LOA from Phase I vs. the overall dataset.
- Of the 14 major disease areas, Hematology had the highest LOA from Phase I (26.1%) and Oncology had the lowest (5.1%).
- Sub-indication analysis within Oncology revealed hematological cancers had 2x higher LOA from Phase I than solid tumors.
- Oncology drugs had a 2x higher rate of first cycle approval than Psychiatric drugs, which had the lowest percent of first-cycle review approvals. Oncology drugs were also approved the fastest of all 14 disease areas.
- Phase II clinical programs continue to experience the lowest success rate of the four development phases, with only 30.7% of developmental candidates advancing to Phase III.

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Disease areas covered in this report:

- Allergy
- Autoimmune
- Cardiovascular
- Chronic High Prevalence Diseases
- Endocrine
- Gastroenterology
- Hematology
- Infectious Disease
- Metabolic
- Neurology
- Oncology
- Ophthalmology
- Psychiatry
- Rare Diseases
- Respiratory
- Urology

Table of Contents

Introduction

This study aimed to measure clinical development success rates to strengthen benchmarking metrics for drug development. To measure success rates for investigational drugs, we analyzed individual drug program phase transitions from January 1, 2006 to December 31, 2015. For the ten years studied, 9,985 transitions in the Biomedtracker database were analyzed. A phase transition is the movement out of a clinical phase – for example, advancing from Phase I to Phase II development, or being suspended after completion of Phase I development.

These transitions occurred in 7,455 clinical drug development programs, across 1,103 companies (both large and small), making this the largest study of its kind. With this broad set of data, we aimed to capture the diversity in drug development across levels of novelty, molecular modalities, and disease indications.

Only company-sponsored, FDA registration-enabling development programs were considered; investigator-sponsored studies were excluded from this analysis. A more detailed description of the data collection, composition, and analysis methodology are described at the end of this report under "Methods."

Individual Phase transition success rates were determined by dividing the number that advanced to the next phase by the total number advanced and suspended. This "advanced and suspended" number is often referred to as "n" in this report, and should be taken into account when drawing conclusions from the success rate results.

One of the key measures of success used in this report is the Likelihood of Approval (LOA) from Phase I. This LOA success rate is simply a multiplication of all four Phases success rates, a compounded probability calculation. For example, if each phase had a 50% chance of success, then the LOA from Phase I would be 0.5 x 0.5 x 0.5 x 0.5 = 6.25%.

Phase Transition Success and Likelihood of Approval (LOA) - Overall

Consistent with previous studies of drug development phase transition success rates, we found Phase II success rates to be far lower than any other phase.[1] Phase I and III rates were substantially higher than Phase II, with Phase I slightly higher than Phase III. The highest success rate of the four development phases was the NDA/BLA filing phase.

The Phase I transition success rate was 63.2% (n=3,582). As this Phase is typically conducted for safety testing and is not dependent on efficacy results for candidates to advance, it is common for this phase to have the highest success rate among the clinical phases across most categories analyzed in this report. Phase I success rates may also benefit from delayed reporting bias, as some larger companies may not deem failed Phase I programs as material and thereby not report them in the public domain. The Phase II transition success rate (30.7%, n=3,862) was substantially lower than Phase I, and the lowest of the four phases studied. As this is generally the first stage where proof-of-concept is deliberately tested in human subjects, Phase II consistently had the lowest success rate of all phases. This is also the point in development where industry must decide whether to pursue the large, expensive Phase III studies and may decide to terminate development for multiple reasons including commercial viability. The second-lowest phase transition success rate was found in Phase III (58.1%, n=1,491). This is significant as most company-sponsored Phase III trials are the longest and most expensive trials to conduct.

The probability of FDA approval after submitting a New Drug Application (NDA) or Biologic License Application (BLA), taking into account re-submissions, was 85.3% (n=1,050). Multiplying these individual phase components to obtain the compound probability of progressing from Phase I to U.S. FDA approval (LOA) reveals that only 9.6% (n=9,985) of drug development programs successfully make it to market (**Figure 1**).

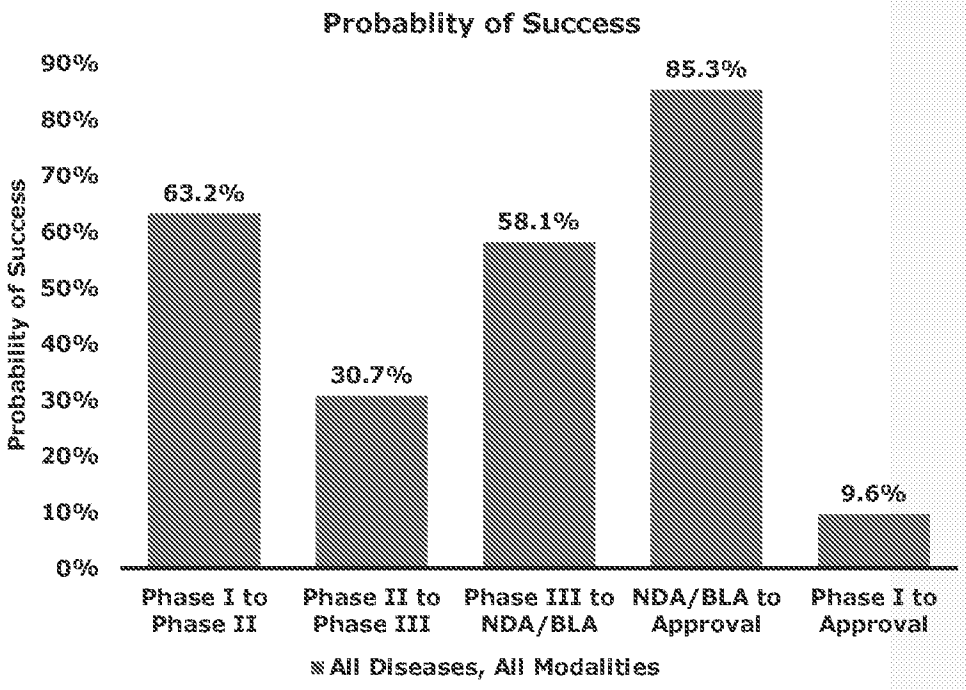


Figure 1. Phase transition success rates and LOA from Phase I for all diseases, all modalities.

Phase Transition Success and Likelihood of Approval (LOA) - by Disease

We segmented major disease areas according to the convention used by Biomedtracker, and categorized 21 major diseases and 558 indications for the 2006-2015 timeframe. For reporting at the disease area level, we analyzed only major diseases with more than 100 total transitions from Phase I to NDA/BLA approval. This resulted in 14 categorized disease areas: Allergy, Autoimmune, Cardiovascular, Endocrine, Hematology, Infectious disease, Gastroenterology (non-IBD), Metabolic, Neurology, Oncology, Ophthalmology, Psychiatry, Respiratory, and Urology. Disease areas with n <100 were placed into the "Other" category. This includes Dermatology, Renal, Obstetrics, Rheumatology (for non-autoimmune indications), Dental, and Orthopedics.

As can be seen in **Figures 2a**, there is a wide range of Likelihood of Approval (LOA) from Phase I. At the high end, Hematology towers over the other groups at 26.1% (n=283). A large portion of Hematology transitions came from Hemophilia, Anemia, and Blood Protein Deficiencies, Thrombocytopenia, and Hemostasis. Some of these Hemophilia indications had overall LOA that reached above 50%. This more than offset some of the weaker Hematology success rates that were observed in Venous Thromboembolism and Neutropenia. Hematology's LOA from Phase I was 5x the success rate for Oncology, which at 5.1% (n=3,163) had the lowest of all the major disease areas.

The next-highest LOA from Phase I under Hematology's 26.1% was Infectious Disease with an impressive 19.1% (n=916). Five disease areas follow closely in the 14-17% range: Ophthalmology > Other > Metabolic > Gastroenterology > Allergy. Below 14% there is a third group of diseases that was slightly above the overall average of 9.6%: Endocrine > Respiratory > Urology > Autoimmune. Falling under the overall LOA of 9.6% was a fourth group made up of four disease areas: Neurology > Cardiovascular > Psychiatry > Oncology. The fact that Oncology and Neurology had the two highest n values while also having low LOA values suggests that these two disease categories are a significant factor in bringing down the overall industry LOA.

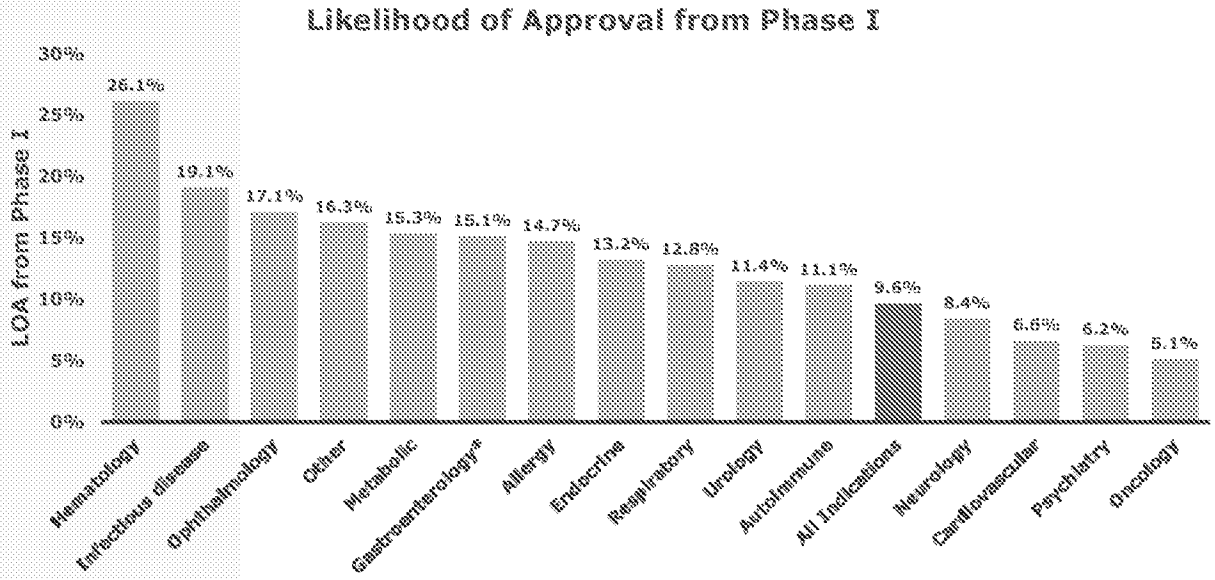


Figure 2a. Chart of LOA from Phase I, displayed highest to lowest by disease area.

Phase Success	Phase I to Phase II		Phase II to Phase III		Phase III to NDA/BLA		NDA/BLA to Approval	
	Advanced or Suspended	Phase Success	Advanced or Suspended	Phase Success	Advanced or Suspended	Phase Success	Advanced or Suspended	Phase Success
Hematology	86	73.3%	83	56.6%	64	75.0%	50	84.0%
Infectious disease	347	69.5%	286	42.7%	150	72.7%	133	88.7%
Ophthalmology	66	84.8%	101	44.6%	60	58.3%	40	77.5%
Other	96	66.7%	116	39.7%	46	69.6%	43	88.4%
Metabolic	95	61.1%	84	45.2%	35	71.4%	27	77.8%
Gastroenterology*	41	75.6%	56	35.7%	33	60.6%	26	92.3%
Allergy	37	67.6%	40	32.5%	14	71.4%	16	93.8%
Endocrine	299	58.9%	242	40.1%	143	65.0%	107	86.0%
Respiratory	150	65.3%	196	29.1%	45	71.1%	37	94.6%
Urology	21	57.1%	52	32.7%	21	71.4%	14	85.7%
Autoimmune	297	65.7%	319	31.7%	135	62.2%	86	86.0%
All Indications	3882	62.2%	3862	38.7%	1493	68.1%	1058	85.3%
Neurology	462	59.1%	465	29.7%	216	57.4%	161	83.2%
Cardiovascular	209	58.9%	237	24.1%	110	55.5%	76	84.2%
Psychiatry	154	53.9%	169	23.7%	70	55.7%	58	87.9%
Oncology	1222	62.8%	1416	24.6%	349	40.1%	176	82.4%

Likelihood of Approval	Phase I to Approval		Phase II to Approval		Phase III to Approval		NDA/BLA to Approval	
	LOA n	Phase LOA	LOA n	Phase LOA	LOA n	Phase LOA	LOA n	Phase LOA
Hematology	283	26.1%	197	35.7%	114	63.0%	50	84.0%
Infectious disease	916	19.1%	569	27.5%	283	64.5%	133	88.7%
Ophthalmology	267	17.1%	201	20.1%	100	45.2%	40	77.5%
Other	301	16.3%	205	24.4%	89	61.5%	43	88.4%
Metabolic	241	15.3%	146	25.1%	62	55.6%	27	77.8%
Gastroenterology*	156	15.1%	115	20.0%	59	55.9%	26	92.3%
Allergy	107	14.7%	70	21.8%	30	67.0%	16	93.8%
Endocrine	791	13.2%	492	22.4%	250	55.9%	107	86.0%
Respiratory	428	12.8%	278	19.6%	82	67.3%	37	94.6%
Urology	108	11.4%	87	20.0%	35	61.2%	14	85.7%
Autoimmune	837	11.1%	540	17.0%	221	53.5%	86	86.0%
All Indications	9885	9.6%	6403	15.3%	2641	40.6%	1058	85.3%
Neurology	1304	8.4%	842	14.2%	377	47.8%	161	83.2%
Cardiovascular	632	6.6%	423	11.2%	186	46.7%	76	84.2%
Psychiatry	451	6.2%	297	11.6%	128	49.0%	58	87.9%
Oncology	3163	5.1%	1941	8.1%	525	33.0%	176	82.4%

Figure 2b. Phase transition success and LOA by disease. Table of phase transition success and LOA by disease with corresponding n values. 'Advanced or Suspended' refers to the total number of transitions used to calculate each success rate, with the n value noted in the text. The LOA n value is the total 'Advanced or Suspended' transitions of all phases used to calculate LOA. 'Phase Success' is the probability of successfully advancing to the next phase, whereas 'Phase LOA' is the probability of FDA approval for drugs from this phase of development. *Gastroenterology does not include IBD.

Phase I Transition Success Rates by Disease

Success rates for Phase I ranged from 63.9% to 84.8%, with the average for all disease indications coming in at 63.2%. Looking at the distribution, we find that most disease area Phase I success rates cluster within +/-10% of the overall Phase I success rate. Two disease areas were outliers, and they are both to the upside. Ophthalmology registered an 84.8% (n=66) success rate, which was substantially higher (by >20%) than the overall Phase I success rate. Gastroenterology programs also exhibited an above average rate of successfully overcoming initial clinical safety hurdles with a 75.6% (n=41) Phase I success rate.

Phase II Transition Success Rates by Disease

In every disease area, Phase II had the lowest transition success rate of the four phases. As shown in **Figure 3**, Phase II success rates ranged from a high of 56.6% (Hematology, n=83) to a low of 23.7% (Psychiatry, n=169). Although it is widely known that drug program attrition is high in Phase II, it is interesting to find that the rate of success can vary by 33% among disease groups. The only Phase II success rate above 50% was seen in Hematology, which largely explains how that indication attained the highest LOA from Phase I.

Excluding Hematology, we can group the Phase II success rates into three clusters: success rates below the 30% overall success rate, those 31-36%, and those in the 40-45% range. Unlike what is observed in the LOA from Phase I, Oncology does not have the lowest success rate for Phase II. Cardiovascular and Psychiatry both registered slightly below the 25% success rate seen for Oncology.

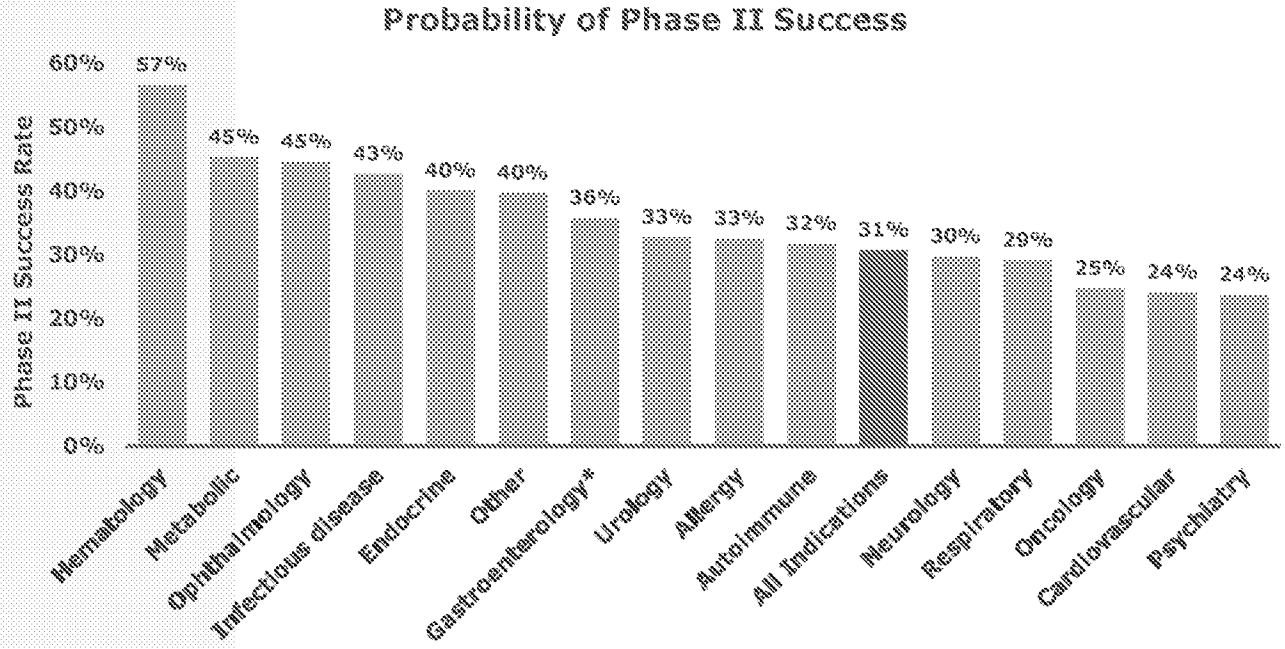


Figure 3. Phase II transition success rates by disease area. Categories are listed from highest to lowest based on the probability of transitioning from Phase II to Phase III. *Gastroenterology does not include IBD.

Phase III Transition Success Rates by Disease

For Phase III transition success rates, Oncology was the outlier with the lowest transition success rate. As seen in **Figure 4**, the Phase III success rates for 14 specific disease areas clustered into two ranges: near 70% and 55-65%. This places Oncology into a group of its own at just 40.1% (n=349).

In addition to Oncology, Neurology, Psychiatry and Cardiovascular were also below the overall Phase III success rate of 58.1% (n=1,491) at 57.4%, 55.7%, and 55.5%, respectively. Each of these areas included disease indications with large patient populations. Later in this report, we break down these high prevalence diseases and compare them with low prevalence disease areas.

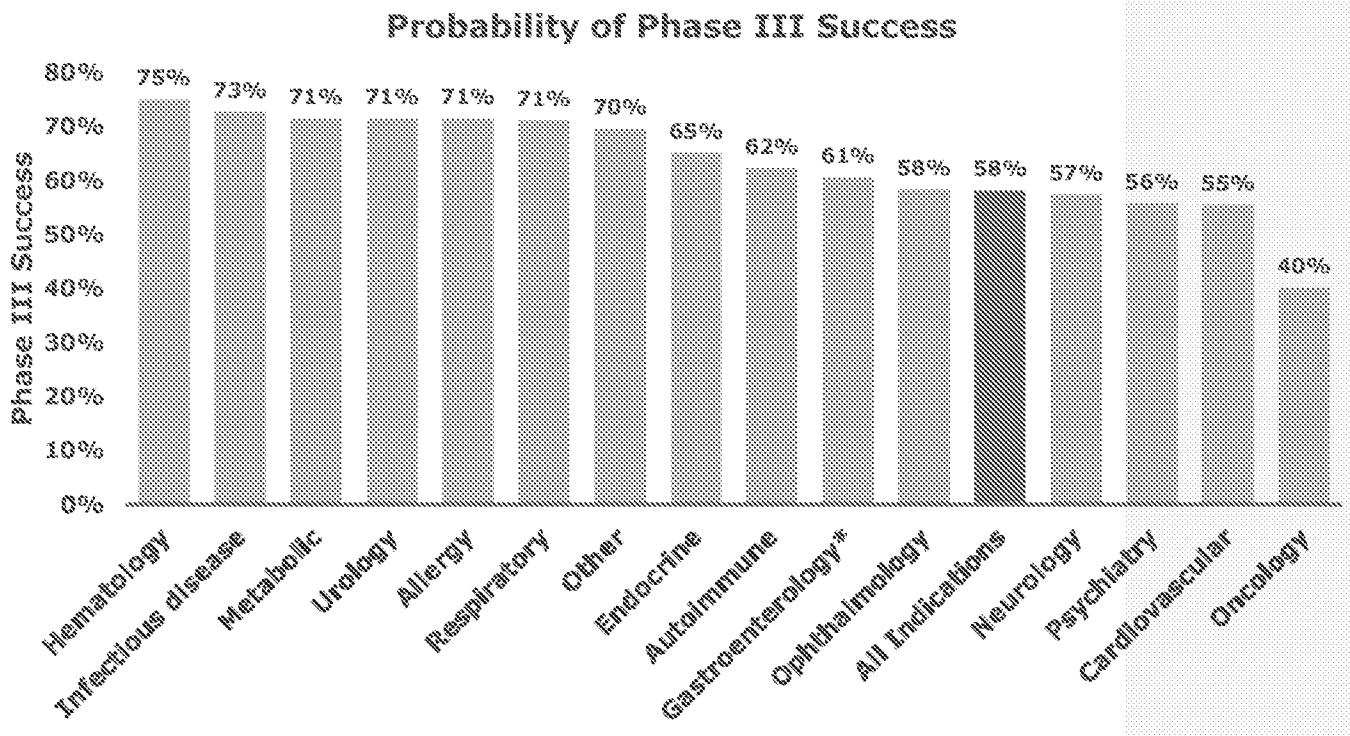


Figure 4. Phase III transition success rates by disease area. Categories are listed from highest to lowest based on the probability of transitioning from Phase II to NDA/BLA filing. *Gastroenterology does not include IBD.

NDA/BLA Submission Success Rates

NDA/BLA transition success rates (approval rates) for the disease areas listed in **Figure 2b** ranged from the low end of 77.5% (Ophthalmology) to a high of 94.6% (Respiratory). The distribution of rates (17.1%) were within the tightest range among the four phases analyzed in this report. These rates are the result of eventual success, not success on first review, meaning some programs may have as many as four Complete Response Letters (CRLs) and attempts at approval. This unrestricted time-frame and number of re-submissions pushes the overall success above 85% across all diseases.

When looking at how many original NDA/BLA filings were approved on the first review by FDA, the rates are far from concentrated (**Figure 6**). In fact, Psychiatry had only a 37% chance of first-cycle approval vs. Oncology at nearly 80%. Although this is an extreme range, upon subsequent submissions and reviews, both of these disease areas ended up with 91% of original drug indication applications being approved. There was a large increase in cumulative success rates after the second submission, but only marginal increases after the third review.

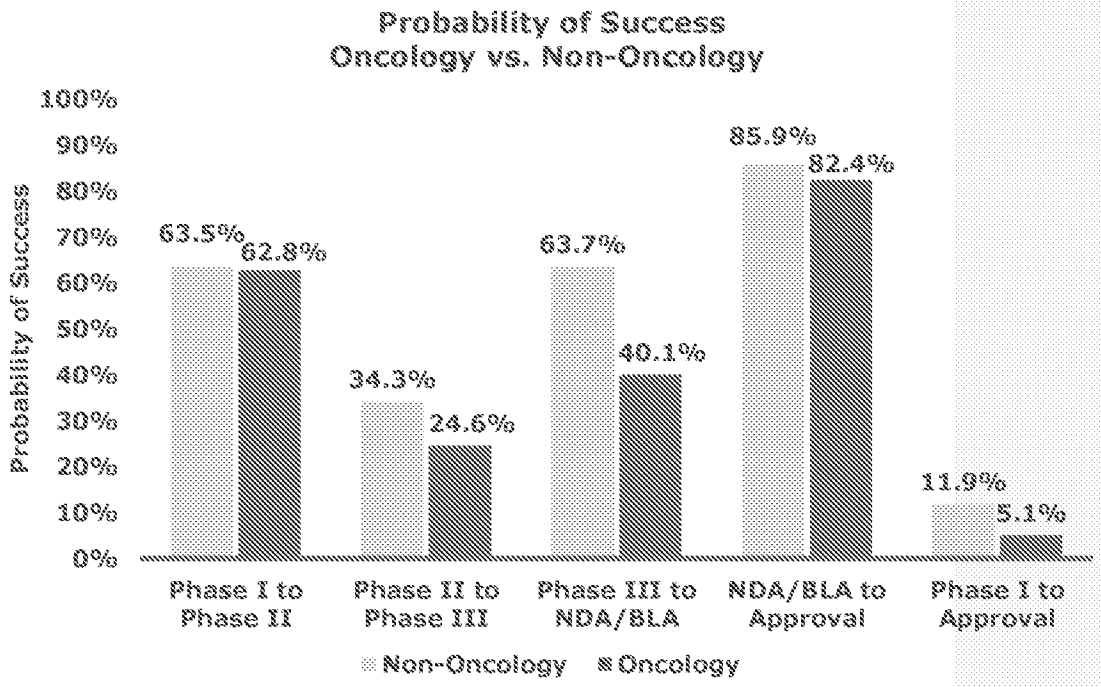
Time from filing to approval also varied by disease area. Neurology drugs took the longest to approve on average, at 2 years, while Oncology drugs were approved almost twice as fast at 1.1 years. Many Oncology drugs for unmet medical need may have benefited from expedited approval pathways and associated increased interactions with FDA such as Breakthrough Therapy and Accelerated Approval, contributing to the faster overall time to approval. As might be expected, calculating time to approval for all disease areas put the time to approval in the middle of these extremes, at 1.6 years.

Disease Area	% Approved on 1st Review	% Approved by 2nd Review	% Ultimately Approved	Filing to Approval Time (Years)
Oncology	79%	89%	89%	1.1
Allergy	71%	93%	93%	1.3
Respiratory	71%	94%	94%	1.6
Cardiovascular	69%	83%	85%	1.4
Infectious disease	69%	86%	92%	1.4
Urology	64%	73%	82%	1.7
Autoimmune	63%	82%	86%	1.6
Metabolic	63%	83%	83%	1.5
Ophthalmology	62%	69%	73%	1.3
All Diseases	61%	80%	86%	1.6
Hematology	60%	76%	90%	1.6
Gastroenterology	56%	84%	92%	1.8
Endocrine	56%	77%	83%	1.8
Neurology	45%	70%	81%	2.0
Psychiatry	37%	70%	91%	1.6

Figure 6. Time to FDA approval and percent approved by FDA for original NDA/BLA filings only. Data shown does not include supplemental applications.

Oncology and Non-Oncology Diseases

Oncology drug development program transitions in the 2006-2015 period accounted for 31% of the 9,985 total transitions. With the lowest LOA from Phase I (5.1%, n=3,163), Oncology had an outsized effect on the overall industry success rate. To further understand this contribution, we compared phase transition success rates and LOA for non-oncology development programs against oncology development programs alone. **Figure 6** shows Phase success rates and LOA from Phase I for oncology and non-oncology development programs. The LOA from Phase I across non-oncology indications was twice that for oncology alone, at 11.9% (n=6,822). Looking at individual phase transition success rates, it is clear that Phase III transition success rates were the reason Oncology ended up with the lowest overall success across our 14 disease categories. Oncology Phase III success was 23% lower than Non-Oncology disease areas.

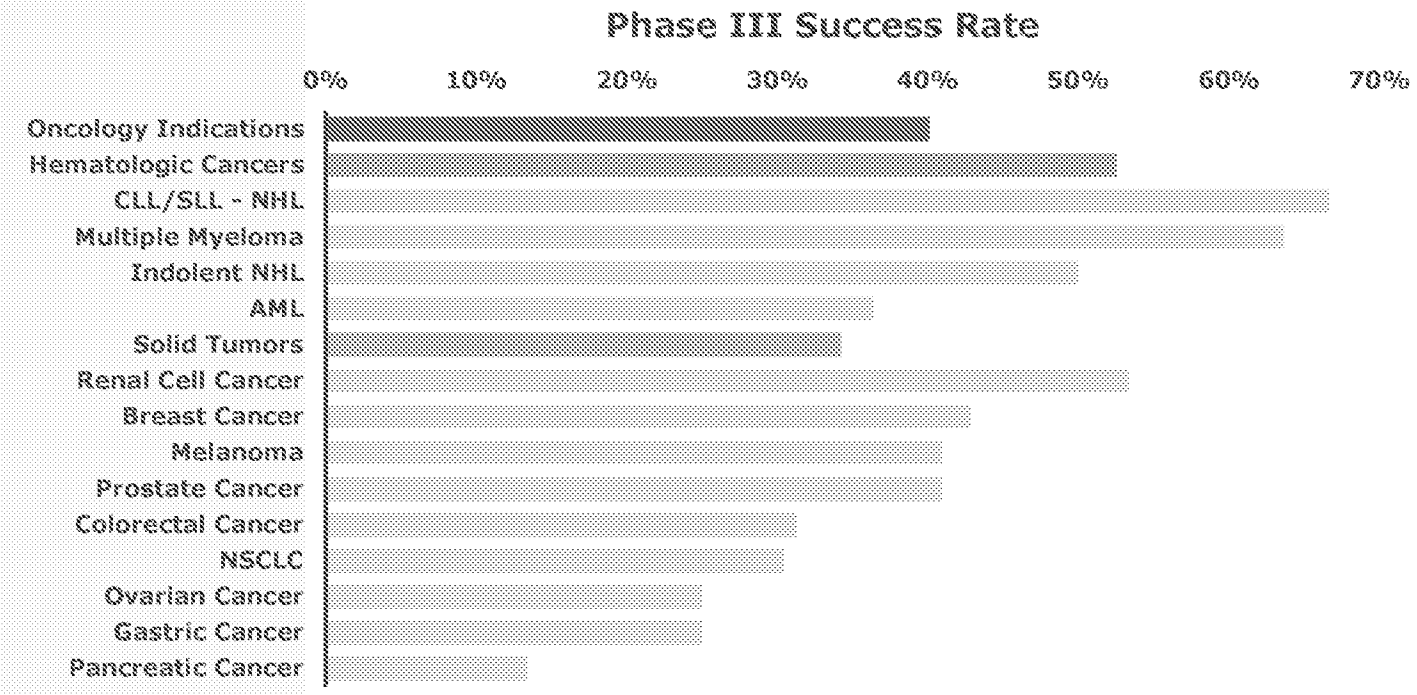


Phase Success	Phase I to Phase II		Phase II to Phase III		Phase III to NDA/BLA		NDA/BLA to Approval	
	Advanced or Suspended	Phase Success	Advanced or Suspended	Phase Success	Advanced or Suspended	Phase Success	Advanced or Suspended	Phase Success
Oncology	1222	62.8%	1416	24.6%	349	40.1%	176	82.4%
Non-Oncology	2360	63.5%	2446	34.3%	1142	63.7%	874	85.9%
Likelihood of Approval	**Phase I to Approval**		**Phase II to Approval**		**Phase III to Approval**		**NDA/BLA to Approval**	
	LOA n	Phase LOA	LOA n	Phase LOA	LOA n	Phase LOA	LOA n	Phase LOA
Oncology	3163	5.1%	1941	8.1%	525	33.0%	176	82.4%
Non-Oncology	6822	11.9%	4462	18.7%	2016	54.7%	874	85.9%

Figure 6. Oncology vs. Non-Oncology phase transition success rates and LOA. Top: Chart of LOA from Phase I. Bottom: Table of phase transition success rates and LOA for Oncology vs. Non-Oncology indications, with corresponding n values. 'Advanced or Suspended' refers to the total number of transitions used to calculate each success rate, with the n value noted in the text. The LOA n value is the total 'Advanced or Suspended' transitions of all phases used to calculate LOA. 'Phase Success' is the probability of successfully advancing to the next phase, whereas 'Phase LOA' is the probability of FDA approval for drugs in this phase of development.

Oncology Sub-Indication Phase Transition Success Rates and LOA

Oncology drugs were further categorized into two main types of cancer: solid tumors and hematological cancers. Solid tumors had twice as many transitions in the data set (2,283 vs. 805), but only half the LOA from Phase I vs. hematological cancers (4.0% vs. 8.1%). These are shown in **Figure 7** in more detail.



Phase Success	Phase I to Phase II		Phase II to Phase III		Phase III to NDA/BLA		NDA/BLA to Approval	
	Advanced or Suspended	Phase Success	Advanced or Suspended	Phase Success	Advanced or Suspended	Phase Success	Advanced or Suspended	Phase Success
Oncology	1222	62.8%	1416	24.6%	349	40.1%	176	82.4%
Solid	860	64.1%	1055	23.0%	260	34.2%	108	79.6%
Hematologic	327	61.8%	341	28.7%	78	52.6%	59	86.4%

Likelihood of Approval	Phase I to Approval		Phase II to Approval		Phase III to Approval		NDA/BLA to Approval	
	LOA n	Phase LOA	LOA n	Phase LOA	LOA n	Phase LOA	LOA n	Phase LOA
Oncology	3163	5.1%	1941	8.1%	525	33.0%	176	82.4%
Solid	2283	4.0%	1423	6.3%	368	27.3%	108	79.6%
Hematologic	805	8.1%	478	13.1%	137	45.4%	59	86.4%

Figure 7. Phase transition success rates and LOA for Oncology indications with corresponding n values. 'Advanced or Suspended' refers to the total number of transitions used to calculate each success rate, with the n value noted in the text. The LOA n value is the total 'Advanced or Suspended' transitions of all phases used to calculate LOA. 'Phase Success' is the probability of successfully advancing to the next phase, whereas 'Phase LOA' is the probability of FDA approval for drugs in this phase of development.

Phase II transition success rates by sub-indication tended to range close to the overall 25% Oncology calculation for Phase II, +/-10%.

Narrowing in on Phase III transition success rates, only 34.2% of the 260 drug programs in solid tumor cancers were deemed sufficiently successful to file an NDA/BLA with the FDA. This was the underlying cause for the 2x difference we see in overall LOA, as the hematological cancer programs recorded a 52.6% success rate in Phase III. Since Phase III was identified as the weakest phase for Oncology, Phase III transition success rates for a number of major oncology sub-indications were included in **Figure 7**.

The solid tumor Phase III transition success rate (34.2%, n=260) ended up as the lowest for any of the major disease categories studied. Solid tumor drugs for pancreatic cancer seemed to have the toughest challenges in Phase III studies (13.3%, n=15). However, Phase III success rates for Ovarian and Gastric cancers also fell below 30%.

Hematological cancer Phase III transition success rates benefited from transition successes in CLL/SLL (66.7%, n=12) and MM (63.7%, n=11). ALL, Hodgkin's and CML had Phase III success rates of 100% but only had fewer than five completed clinical development programs each (data not shown). Only AML (36.4%, n=11) came in below 40% for Phase III, which helped the overall hematologic cancer Phase III success rate remain above 50%.

The NDA/BLA to approval success rate for all hematological cancers (86.4%, n=59) was impacted positively by Multiple Myeloma, ALL, and CML success, as each had more than five completed filings and 100% approval rates. The NDA/BLA success rate for all solid tumors was lower at 79.6% (n=108).

Abbreviated cancer indications:

ALL - Acute Lymphocytic Leukemia

AML - Acute Myelogenous Leukemia

CLL - Chronic Lymphocytic Leukemia

CML - Chronic myelogenous Leukemia

MM - Multiple Myeloma

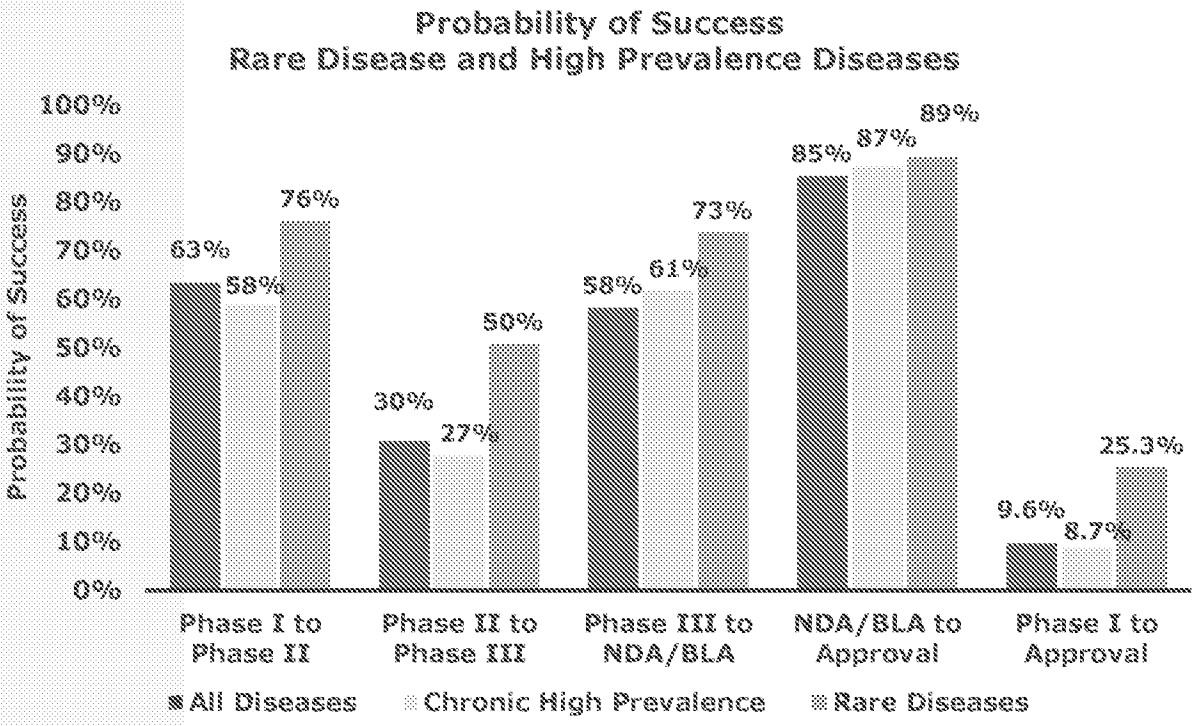
NHL - Non-Hodgkin's Lymphoma

NSCLC - Non-Small Cell Lung Cancer

SLL - Small Lymphocytic Lymphoma

Rare Diseases and Chronic High Prevalence Diseases

In recent years, there has been an increase in funding for companies focused on rare diseases.[2] This is welcome news as there are reportedly 7,000 rare diseases and most do not have an approved therapeutic treatment.[4] One question that is often asked is if the probabilities of success are any better for rare diseases, especially for those in which a particular defective gene has been confirmed as the sole contributor. On the other extreme, we have observed less venture funding for high prevalence, chronic diseases.[2] The question we wanted to explore is whether investors may have scaled back funding because there is a higher hurdle to developing and gaining approval for medicines that treat highly prevalent conditions.



Phase Success	Phase I to Phase II		Phase II to Phase III		Phase III to NDA/BLA		NDA/BLA to Approval	
	Advanced or Suspended	Phase Success	Advanced or Suspended	Phase Success	Advanced or Suspended	Phase Success	Advanced or Suspended	Phase Success
All Diseases	3582	63.2%	3862	30.7%	1491	58.1%	1050	85.3%
Chronic High Prevalence	732	58.7%	726	27.7%	268	61.6%	196	87.2%
Rare Diseases	150	76.0%	168	50.6%	110	73.6%	93	89.2%
Likelihood of Approval	Phase I to Approval		Phase II to Approval		Phase III to Approval		NDA/BLA to Approval	
	LOA n	Phase LOA	LOA n	Phase LOA	LOA n	Phase LOA	LOA n	Phase LOA
All Diseases	9985	9.6%	6403	15.3%	2541	49.6%	1050	85.3%
Chronic High Prevalence	1922	8.7%	1190	14.9%	464	53.7%	196	87.2%
Rare Diseases	521	25.3%	371	33.3%	203	65.7%	93	89.2%

Figure 8. Non-Oncology Rare disease and high prevalence, chronic disease. Top: Chart of phase transition success rates and LOA from Phase I, for rare and chronic, high prevalence. Bottom: Table of phase transition success and LOA by disease with corresponding n values. 'Advanced or Suspended' refers to the total number of transitions used to calculate each success rate, with the n value noted in the text. The LOA n value is the total 'Advanced or Suspended' transitions of all phases used to calculate LOA. 'Phase Success' is the probability of successfully advancing to the next phase, whereas 'Phase LOA' is the probability of FDA approval for drugs in this phase of development.

We isolated rare disease programs in the Biomedtracker database by first identifying all prior FDA Orphan-designated indications, then pooling all drug programs in these indications, regardless of whether they obtained FDA's Orphan designation. All Oncology indications were removed to make this rare disease analysis more concentrated on inborn genetic disorders. For chronic diseases, we first obtained a list of conditions from the Center for Medicare & Medicaid Services (CMS) Chronic Conditions Data Warehouse (CCW). We removed any cancer indications, then identified those disease with > 1 million patients afflicted with the disease in the United States.

With programs from both groups identified, we compared phase transition success rates and LOA as shown in **Figure 8**. At 25.3%, the overall LOA from Phase I for Non-Oncology rare diseases was 2.6x higher than the LOA for all diseases and 3x higher than the 8.7% LOA for chronic, high prevalence diseases. Chronic, high prevalence diseases accounted for almost 20% of the transitions, and Oncology, 31% of the transitions. The combined weighting of 50% of the dataset for these two categories with low LOAs (Chronic, high prevalence disease, 8.7% and Oncology, 5.7%) contributed significantly to the low overall industry LOA of 9.6%.

All four transition success rates were higher for the rare disease group than the overall dataset. The largest difference between was found in Phase II transition success rates (50.6% for rare disease vs 30.7% overall). Phase III and Phase I each had higher transition success rates by at least 10%.

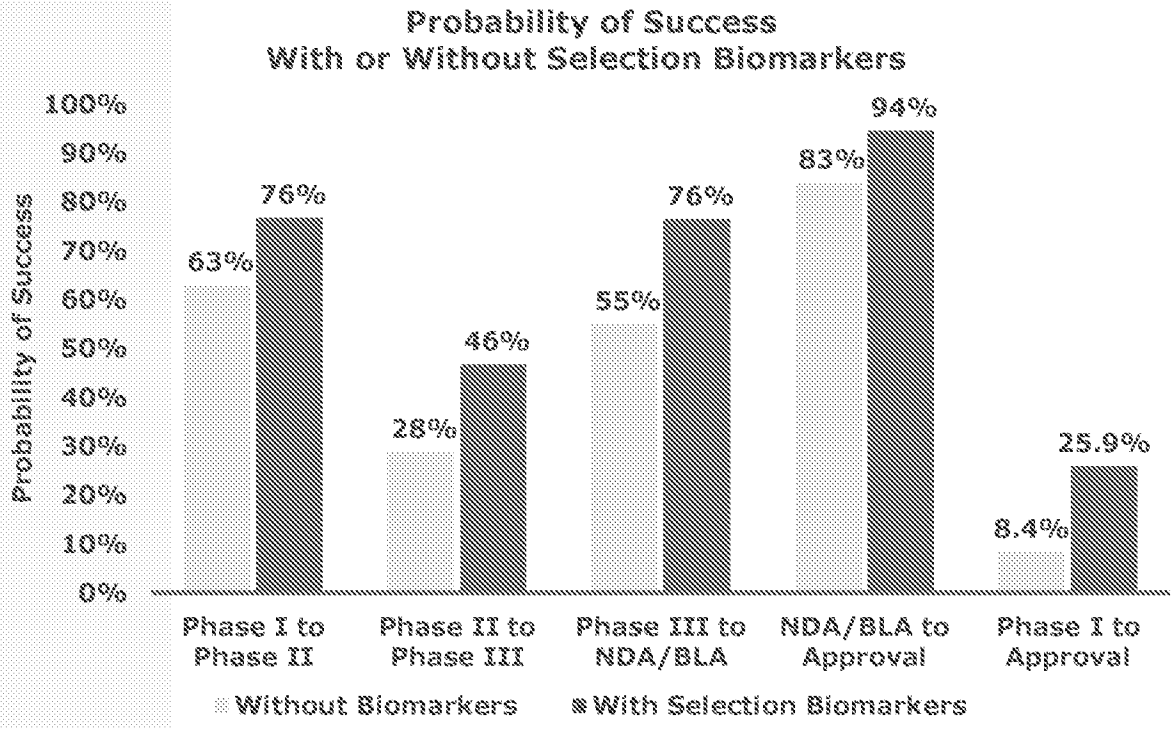
Chronic, high prevalence diseases transition success rates were lower in Phase I (58.7% vs 63.2%) and Phase II (27.7% vs. 30.7%) vs. the overall dataset. The opposite was seen in Phase III and NDA/BLA, where slightly higher rates were observed: 61.6% vs. 58.1% for Phase III and 87.2% vs. 85.3% for NDA/BLA.

As the chronic, high prevalence group in this study does not include Oncology, we compared these results with the Non-Oncology rates found in **Figure 6**. Although the chronic, high prevalence Phase III transition success rate is higher than that seen with all diseases, it is lower than the Non-Oncology transition success rates (61.6% vs. 63.7%). Furthermore, the LOA of 11.9% (n=6,822) for all Non-Oncology indications is 3% higher than for chronic, high prevalence diseases (8.7%), suggesting that these chronic indications are negatively impacting overall success rates outside Oncology.

Patient Selection Biomarker Programs

The use of biomarkers as inclusion or exclusion criteria, or 'selection biomarkers', for enrolling patients into clinical studies has increased dramatically since the sequencing of the human genome. We identified 512 phase transitions out of 9,985 (5%) that incorporated a selection biomarker for patient stratification. This was accomplished by mapping the NCT numbers (clinicaltrial.gov identifier) from Amplion's BiomarkerBase to programs in the Biomedtracker transition database.[6] For programs that filed an NDA/BLA, we only included filings that used selection biomarkers in the Phase III study design.

The LOA from Phase I can be found in **Figure 9**. The benefit from selection biomarker use raises the LOA from Phase I to one in four compared to less than one in 10 when no selection biomarker was used.



Phase Success	Phase I to Phase II		Phase II to Phase III		Phase III to NDA/BLA		NDA/BLA to Approval	
	Advanced or Suspended	Phase Success	Advanced or Suspended	Phase Success	Advanced or Suspended	Phase Success	Advanced or Suspended	Phase Success
No Biomarkers	3480	63.0%	3396	28.8%	1254	55.0%	882	83.9%
Selection Biomarkers	43	76.7%	246	46.7%	132	76.5%	91	94.5%
Likelihood of Approval	Phase I to Approval		Phase II to Approval		Phase III to Approval		NDA/BLA to Approval	
	LOA n	Phase LOA	LOA n	Phase LOA	LOA n	Phase LOA	LOA n	Phase LOA
No Biomarkers	9012	8.4%	5532	13.3%	2136	46.2%	882	83.9%
Selection Biomarkers	512	25.9%	469	33.8%	223	72.3%	91	94.5%

Figure 9. Selection Biomarker phase transition success rates and LOA. Top: Chart of individual phase transition success rates and LOA from Phase I for all indications without and with selection biomarkers. Bottom: Table of Phase transition success and LOA by disease with corresponding n values. 'Advanced or Suspended' refers to the total number of transitions used to calculate each success rate, with the n value noted in the text. The LOA n value is the total 'Advanced or Suspended' transitions of all phases used to calculate LOA. 'Phase Success' is the probability of successfully advancing to the next phase, whereas 'Phase LOA' is the probability of FDA approval for drugs in this phase of development.

As shown in the table of **Figure 8**, a three-fold higher LOA from Phase 1 was calculated for programs that utilized selection biomarkers (25.9%, n=512) vs. programs that did not (8.4%, n=9,012).

Individual phase transition success rates can be found in **Figure 8**. All four phase transition success rates were much higher for programs incorporating selection biomarkers vs. those not incorporating selection biomarkers. Phase III transition success rates for selection biomarker programs were 76.5% (n=132) vs. only 55.0% (n=1,254) for non-selection biomarker programs - the largest percentage difference among the four phases of development.

There were fewer completed Phase 1 trials that utilized a selection biomarker (43) than those observed in NDA/BLA filings (91). This is likely due to the fact that biomarkers are more commonly used in Phase II and III.

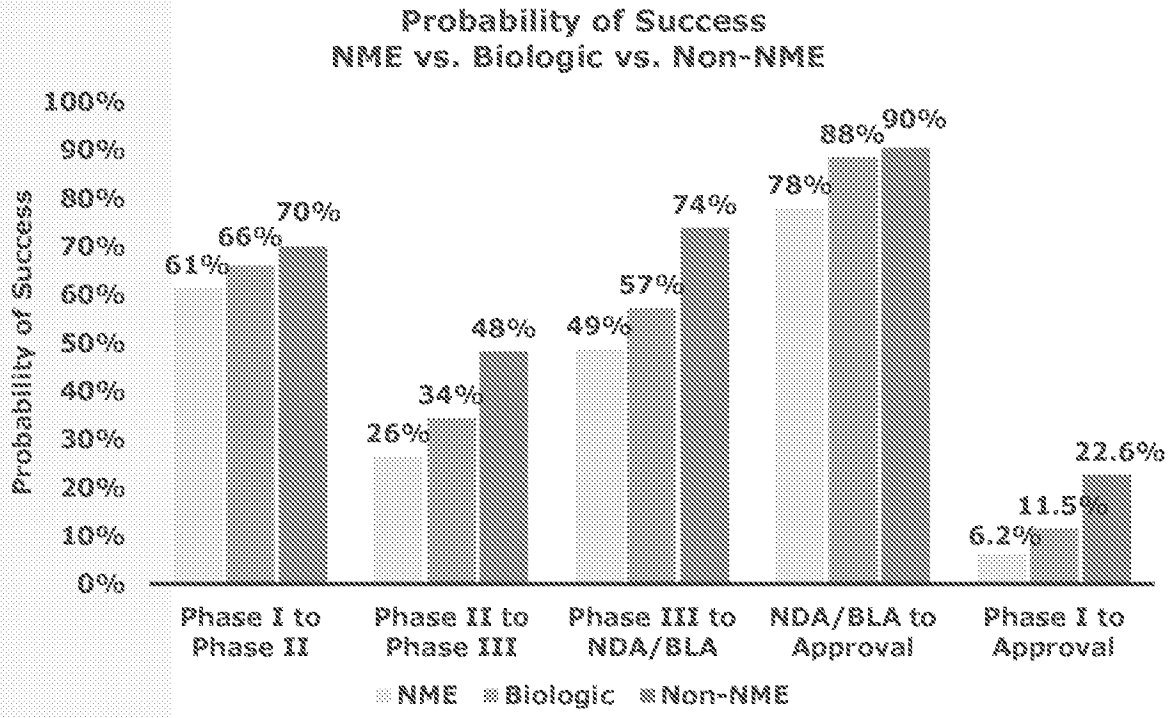
The large differences in Phase II and III transition success rates are quite convincing, quantitatively, of what many drug developers have long argued anecdotally – enrichment of patient enrollment at the molecular level is a more successful strategy than heterogeneous enrollment.

With respect to statistical rigor, while having a smaller number of transitions for programs that utilized a selection biomarker (512) vs. for non-biomarker phase transitions (9,012), when analyzed for statistical significance, all phases, save for Phase I, were found to be statistically significant. This is in large part due to the low n value for Phase I, a clinical phase in which few selection biomarkers are actually used.

Drug Classification and Modality

Drugs in the dataset were annotated by: new molecular entity (NME), non-NME, biologic and vaccine.

Most NMEs are small molecules, although in some cases FDA will designate larger molecules, such as proteins and peptides, as NMEs. When comparing small-molecule NMEs to all NMEs we found very little difference in success rates. The Non-NME classification often includes drugs with the same molecular properties as NMEs, but are frequently reformulations or combinations of approved products. Non-NMEs often use the 505(b)(2) pathway to gain FDA approval. Monoclonal antibodies made up the majority of biologics in the dataset. Other biologics in the dataset included gene therapy, cell therapy, RNAi, and other large molecules. Generic and over-the-counter (OTC) drugs were not included.



Phase Success	Phase I to Phase II		Phase II to Phase III		Phase III to NDA/BLA		NDA/BLA to Approval	
	Advanced or Suspended	Phase Success	Advanced or Suspended	Phase Success	Advanced or Suspended	Phase Success	Advanced or Suspended	Phase Success
NME	2236	61.3%	2482	26.5%	731	48.7%	409	78.0%
Biologic	882	66.0%	883	34.4%	313	57.2%	199	88.4%
Non-NME	314	70.1%	406	48.3%	398	73.9%	406	90.4%
Vaccine	95	66.3%	76	32.9%	35	74.3%	32	100.0%
Likelihood of Approval	Phase I to Approval		Phase II to Approval		Phase III to Approval		NDA/BLA to Approval	
	LOA n	Phase LOA	LOA n	Phase LOA	LOA n	Phase LOA	LOA n	Phase LOA
NME	5858	6.2%	3622	10.1%	1140	38.0%	409	78.0%
Biologic	2277	11.5%	1395	17.4%	512	50.6%	199	88.4%
Non-NME	1524	22.6%	1210	32.2%	804	66.8%	406	90.4%
Vaccine	238	16.2%	143	24.4%	67	74.3%	32	100.0%

Figure 10. Phase transition success rates and LOA by drug type and class. Top: Chart of phase transition success rates and LOA from Phase I by FDA classification for NME, Biologic, Non-NME. Bottom: Table of phase transition success rates and LOAs for FDA's classifications and vaccines. 'Advanced or Suspended' refers to the total number of transitions used to calculate each success rate, with the n value noted in the text. The LOA n value is the total 'Advanced or Suspended' transitions of all phases used to calculate LOA. 'Phase Success' is the probability of successfully advancing to the next phase, whereas 'Phase LOA' is the probability of FDA approval for drugs in this phase of development.

NMEs were found to have the lowest transition success rates in every phase of development and an LOA from Phase I of just 6.2% (n=5,858). Biologics had almost 2x this LOA (11.6%, n=2,277), as shown in **Figure 10**. The breakout of Monoclonal Antibodies yielded the exact same 11.6% LOA from Phase I (n= 1,258).

Non-NMEs had the highest LOA from Phase I of 22.6% (n=1,524), with transition success rates well above the NME and biologic classifications in every phase. However, many non-NMEs begin development in Phase II or Phase III, so the actual approval rate is likely higher (assuming that successful Phase I outcomes would contribute positively to the LOA from Phase I).

Although a much smaller subset of the modality breakout, vaccines generated a healthy 16.1% (n=238) LOA from Phase I.

Discussion

During the time frame of this study (2006-2015), programs entering clinical development in Phase I were found to have only a one in ten (9.6%) chance of advancing all the way to FDA approval. Although this is an overall probability calculation and does not follow each program from its first trial to final outcome (which is often be outside of a 10-year time-frame), we believe that these low success rates for individual drug programs accurately reflect overall industry realities. This is particularly important in the context of cost and time of unsuccessful clinical trials.

Potential factors that may have impacted success rates in this report include:

Clinical and regulatory related:

- Patient population and selection strategy. The impact that a targeted, well-defined patient population can have was observed in the success rates for both selection biomarker usage and rare disease studies.
- Clinical validation of a target, drug class, or mechanism of action. Note that non-NME drugs presented in this report are drugs developed for already approved classes and targets and have 3x higher LOA from Phase I vs. novel NMEs.
- Clinical trial complexity. In chronic, high prevalence diseases, where large heterogeneous patient populations will be treated, additional safety and efficacy studies may be required. These areas have experienced below average LOA from Phase I.

Corporate related:

- Lack of funding. This is a common issue for emerging companies, especially during times of economic stress such as in the years following the global financial of 2008. Programs terminated due to lack of resources or funding would be counted as "suspended" in this study and not differentiated from programs terminated based on disappointing clinical data.
- Commercial decisions and portfolio prioritization. Competition, IP litigation, and other market factors could result in the termination of a program. Internal programs at companies may also compete during pipeline review and prioritization, potentially leading to program termination.

The overall low rates for Phase III programs to successfully transition to an NDA/BLA filing is concerning, as 35% of all R&D spending is now spent on Phase III development, and Phase III trials account for 60% of all clinical trial costs.[5] A main contributor to the overall low Phase III transition success rate was found to be Oncology clinical programs. While Oncology remains a very challenging area in which to develop drugs, some more recent successes in immunotherapies give hope that there will be highly successful drug programs in Oncology in coming years.

Outside of Oncology, clinical programs targeting heterogeneous patient populations in chronic, high prevalence indications also contributed to lower overall success rates. For this group, all three clinical-stage transition success rates were lower than the Non-Oncology disease group.

As selection biomarkers are used more frequently in clinical development (presently, they are only being used in a small proportion of studies), phase transition success rates in high prevalence diseases should improve as patient selection improves. The higher success rates for trials run with biomarker-selected patients suggests that the broader industry is already on the right path. For example, Phase III transition success rates in programs utilizing selection biomarkers in the last decade were 76.5% (n=132) compared to only 55.0% (n=1,254) for non-biomarker trials. Experiencing one in four failures (with selection biomarkers) vs. two in four Phase III failures (without biomarkers) could have significant cost implications.

As many rare diseases are identified by specific genetic mutations, it is not surprising that success rates in rare disease indications closely match the success rates observed in clinical trials that utilized selection biomarkers. Phase transition success rates for rare disease candidates and candidates utilizing selection biomarkers were very similar for every stage of development. Transition success rates for each classification, rare disease programs and programs with selection biomarkers, respectively, were; Phase I: 76% and 76.7%; Phase II: 50.6% and 46.7%; Phase III: 73.6% and 76.5%; and NDA/BLA: 89.2% and 94.6%. Both of these specific classifications significantly outpaced the success rates seen for chronic, high prevalence disease drug development at 58.7% in Phase I, 27.7% in Phase II, 61.6% in Phase III, and 87.2% in the NDA/BLA stage.

Greater flexibility with alternative and novel surrogate endpoints, the utilization of adaptive clinical trial design, improved methodologies for assessing patient benefit-risk, and improvements in communication between sponsors and regulators could help improve the success rates reported in this study. Simultaneously, improvements in basic science can enable better success rates. For example, more predictive animal models, earlier toxicology evaluation, biomarker identification and new targeted delivery technologies may increase future success in the clinic.

Advancing industry-wide understanding of the evidentiary standards or considerations governing regulatory acceptance of drug development tools including biomarkers would increase adoption and use of innovative drug development tools, and serve to expedite development and review timelines and improve chances of success.

The ability to utilize these modern approaches to drug development and modern regulatory review processes combined with healthy capital markets supporting private-sector investment will enable biopharmaceutical companies to develop the next generation of innovative medicines.

Methods

Drug Development Programs analyzed in this report track a specific indication for each drug. For example, Rituxan in non-Hodgkin's lymphoma (NHL) qualifies as a different development path than Rituxan in multiple sclerosis (MS). Biomedtracker assigns a unique internal identifier which can be used to isolate all development paths.

In addition to tracking the phase of development, Biomedtracker also assigns "lead" status to certain development paths. This is used to denote the most advanced indication in clinical development for a specific drug. Drugs can only have one lead development program (with some rare exceptions). For example, cancer drugs developed in multiple indications will have the most advanced program assigned as the lead, and the rest as "non-lead". However, in this report we do not differentiate the most advanced programs, and analyze the data on a program/development path level, which more accurately reflects company resource utilization.

Individual Phase Transition Success Rates were calculated as the number of drugs that moved from one phase to the next phase divided by the sum of the number of drugs that progressed to the next phase and the number of drugs that were suspended. The n value associated with the phase transition success rates represent the number of drugs that have advanced plus the number of drugs that have been suspended, which we label as phase transitions. Phase transition Success rates reported in this study were based on transition rates, not necessarily resulting from safety or efficacy data. Transition rates are negatively impacted by early development termination due to commercial and regulatory uncertainty as well as economic and portfolio management decisions.

Biomedtracker further classifies events by phase of development, summarized in the table below:

Biomedtracker phase	Description for purposes of the study
I	Drug is currently in Phase I
I/II, II, IIb	Drug is currently in Phase II
II/III, III	Drug is currently in Phase III
NDA/BLA	Application for approval has been submitted to the FDA and is currently under review
Approved, Withdrawn From Market, Approved (Generic Competition)	Drug has been approved for marketing in the United States
Suspended	Drug is no longer in development
Approved in Europe, Approved in other than U.S./E.U., Development, Development Outside U.S.	The company developing this drug does not plan to market it in the United States

Generic products were not included, but generic manufacturers developing novel investigational drugs were represented.

Likelihood of Approval (LOA) denotes the probability of reaching FDA approval from the current phase, and is also expressed as a percentage. LOA is calculated as the product of each Phase Success probability leading to FDA approval. The n value associated with LOA is the sum of the n values for each Phase Success included in the LOA calculation.

For example, if a drug is currently in Phase II, and the phase success for Phase II is 50% (n=10), Phase III is 50% (n=10), and FDA Approval is 50% (n=10), then the LOA for the Phase II drug would be 12.5% (50%×50%×50%=12.5%, n=30).

Data Source for Drug Program Transitions. Data used for this study were extracted from Biomedtracker using a Probability of Technical Success (PTS) tool, which identified all 'Advanced' and 'Suspended' drugs by development phase from January 1, 2006 to December 31, 2015. Biomedtracker, a subscription-based product of Informa, tracks the clinical development and regulatory history of investigational drugs to assess their Likelihood of Approval (LOA) by the FDA. Biomedtracker is populated in near real-time with updated information from press releases, corporate earnings calls, investor and medical meetings and numerous other sources. These data are recorded in BioMedTracker and tagged with a date. BioMedTracker also uses other sources, including regular communication with companies conducting clinical trials, to ensure accuracy and timeliness of the data.

Drug Classification Methods. Biomedtracker records FDA classification (i.e. new molecular entity (NME), non-NME, biologic, or vaccine) as well as the biochemical profile (e.g. small molecule, monoclonal antibody, peptides, natural proteins, antisense, vaccine, etc.). Biologics, as defined by FDA, can be sugars, proteins, or nucleic acids or complex combinations of these substances, or may be living entities such as cells and tissues.

Sub-Indications. Drilling down into sub-indications within each major disease area has limitations due to lower n values for each phase. The statistical significance of the results must be taken into account when comparing data from low sample sizes. Although this study is over a 10-year period, some sub-indications had a limited number of Phase III programs completed or NDA/BLA filings submitted. For example, because AML had four drugs submitted for FDA approval, and no wins, the success rate for AML at the NDA/BLA stage is 0% (n=4). That also means that the LOA calculation for AML yields 0% (n=103), due to the compounded approach of multiplying individual phase probabilities to arrive at the overall LOA. With that caveat in mind, it is best to approach sub-indications starting with the NDA/BLA filing phase to assess n values and % success.

Data Source for Selection Biomarkers. Selection biomarkers are gene products used as inclusion or exclusion criteria for enrolling patients into clinical studies. The biomarker subject selection data used for this study were extracted from BiomarkerBase, which identifies selection biomarkers described in clinical trials posted at ClinicalTrials.gov. BiomarkerBase is a subscription-based service from Amplion that tracks selection biomarker usage in clinical trials, drug labels, and tests (including laboratory-developed, FDA-cleared, and FDA-approved tests). BiomarkerBase is updated weekly with information from these sources and publications, using supervised machine learning algorithms for natural language processing (Amplion BiomarkerEngine) to identify selection biomarkers. BiomarkerBase includes primarily human genes and proteins. Other diagnostic measurements, including clinical blood chemistry, liver enzymes, white blood cell count, heart rate, blood oxygenation, blood glucose, clotting times, etc. are not included in BiomarkerBase.

Comparison with Previous BIO/Biomedtracker Study. The Biomedtracker database has expanded since our original publication in Nature Biotechnology, January 2014.[1] The first study contained 5,820 phase transitions from 2003-2011. The current study contains 9,985 transitions from 2006-2015 (10 years). This more recent finding for overall LOA success rates falls within the same statistical range as our previous report (near 10.4%, n=5,820 for 2003-2011).[1,2] While the more recent study captures four additional years (2012-2015), it begins in 2006 and thus cuts 2003-2005 data, leaving a 10-year window for evaluation. Importantly, there are 4,165 more transitions in the current dataset. Such broad sample size comparisons makes it very difficult for significant changes to be found in overall rates. To see significant change would require more than just a few disease areas to experience profound improvements in productivity. For example, the impressive success rates found in Hematology might need to be seen in the larger groups such as Oncology or Neurology to move the needle. Narrowing the time-frame to detect improvements, can lead to program biases as R&D timelines are typically closer to a 10-year time-frame and not less.

Lead Drug vs. Non-Lead. In our previous report, we had included "Lead" drug success rates.[1] That allows us to answer the question: "What is the probability that a drug will reach approval for *any* indication?" However, the Lead program analysis will not represent the true costs, both monetary and time, that is required for approval. Furthermore, it can be a source of confusion in that it refers not to a company's lead program, but rather the most advanced indication for a given drug. Lead indication status is constantly changing within the database through time as programs fail and secondary indication then become the Lead indication. For this reason, the current study focuses on each development path to answer the question: "What is the probability that a drug developed for a *specific* indication will reach approval?"

For those interested in the Lead indication success rates, the overall Lead success rate was 16.8% (n = 5,511), 1.7x higher than the 9.6% reported for individual development paths. This is a slightly wider difference than our previous finding (for 2003-2011) of 15.3% for the Lead indication. As the spread between Lead and subsequent individual indication programs widens, it could be argued that more R&D is required (across multiple indications) to reach final approval of a chemical entity in at least one of its indications.

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6. BiomarkerBase: https://biomarkerbase.com/

Acknowledgements

This research was supported by Informa, Amplion, and the Biotechnology Innovation Organization (BIO). The authors are indebted to the analysts at Biomedtracker and Amplion who have collected these data. We would also like to thank our colleagues at Informa (special thanks to Amanda Micklus), Amplion, and BIO (special thanks to Cartier Esham and Scott McGoohan) and for their input, advice, and review of this study.

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European Patent No. 2 861 210

Methods of Treating Pancreatic Cancer Using Combination Therapies Comprising Liposomal Irinotecan

Declaration of Bruce Belanger, Ph.D.

I, Bruce Belanger, Ph.D., declare and state as follows:

1. I, Bruce Belanger Ph.D., am employed by Ipsen as a Senior Director, Biostatistics, Due Diligence. I have held this position since 2017.

2. In 1994, I obtained a Ph.D. in Statistics from North Carolina State University. Since then I have worked as a statistician in the pharmaceutical industry. As well as Ipsen, I have worked as a statistician at Schering-Plough, Praecis Pharmaceuticals, Genzyme Corporation, Idenix Pharmaceuticals, Tolerx, Inc., and Merrimack Pharmaceuticals.

3. My experience covers a range of areas in the drug development process including clinical trials, pharmacokinetics, drug discovery, and scale-up manufacturing. I am experienced with FDA and EMA submissions and interactions with regulatory agencies.

4. During my time at Merrimack, I was the lead statistician on the MM-398 (nanoliposomal irinotecan) development program for pancreatic cancer after disease progression following gemcitabine-based therapy. I was responsible for writing the statistical analysis plan for the pivotal Phase III study ("NAPOLI-1"), and managed all biometrics activities for our NDA submission. The NDA submission, on which I worked, included data related to all of the clinical development studies for MM-398. In 2015, MM-398 was approved by the FDA for the treatment of pancreatic cancer after disease progression following gemcitabine-based therapy under the trade name Onivyde®. In 2017, the rights to Onivyde® were acquired from Merrimack by Ipsen.

5. I am aware that European Patent No. 2861210 was revoked by the Opposition Division during an oral hearing which I attended. I am also aware that the case is now before the Board of Appeal. I have also been informed that claim 1 of the Main Request current on file at the EPO is as follows:

   "Liposomal irinotecan for use in a method of treating pancreatic cancer in a human patient, wherein the patient exhibits evidence of recurrent or persistent pancreatic cancer following primary chemotherapy and wherein the patient has failed prior treatment with gemcitabine or become resistant to gemcitabine, the method comprising co-administration of an effective amount each of liposomal irinotecan, 5-fluorouracil (5-FU) and leucovorin to the patient in at least one cycle wherein the cycle is a period of 2 weeks and, for each cycle:

   *(a) liposomal irinotecan is administered to patients not homozygous for the UGT1A 1 *28 allele on day 1 of each cycle at a dose of 80 mg/m² and to patients homozygous for the*

*UGT1A1 *28 allele on day 1 of cycle 1 at a dose of 60 mg/m² and on day 1 of each subsequent cycle at a dose of 60 mg/m² or 80 mg/m²;*

(b) 5-FU is administered at a dose of 2400 mg/m²; and

(c) leucovorin is administered at a dose of 200 mg/m² (l form);

and wherein in each cycle, the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU;

and wherein the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection."

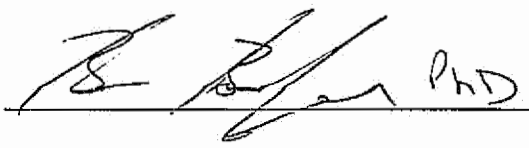
6. The dosage regimen recited in the above claim corresponds to the dosage regimen that is set out in the Onivyde® regulatory documents, e.g. the European summary of product characteristics. This regimen was approved after being tested in the Napoli-1 Phase III trial.

7. Prior to being tested in the NAPOLI-1 Phase III trial, the dosage regimen recited in the above claim had not been tested in any human subjects having pancreatic cancer whose disease had progressed following gemcitabine-based therapy.

8. A different dosage regimen involving the co-administration of liposomal irinotecan, 5-FU and leucovorin had been tested in a Phase I trial in patients having advanced solid tumors. This Phase I trial took place before the NAPOLI-1 trial referred to above. The dosage regimen that was tested in this Phase I trial was different from that recited in claim 1 of the Main Request reproduced above. For example, the liposomal irinotecan was administered every three weeks, rather than every two weeks. In addition, 5-FU and LV were administered weekly, rather than every two weeks. That regimen never progressed beyond Phase I.

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

Declared on December 30, 2019



(Bruce Belanger, Ph.D.)

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Germany

Our Ref: X112963EP KJG VMM

Via Online Filing

27 July 2020

Appeal No. T2963/19-3.3.07 following Opposition against EP2861210 (13731230.2)
in the name of
Ipsen Biopharm Ltd.
by Teva Pharmaceutical Industries Limited

Dear Sir or Madam

Enclosed is our reply to the Grounds of Appeal filed in connection with the above mentioned case.

Yours faithfully
for D Young & Co LLP

Signed and Filed Online

Kirk Gallagher
Partner, Patent Attorney
kjg@dyoung.com

European Patent No. 2861210

Ipsen Biopharm Ltd.

T2963/19-3.3.07

Reply to Proprietor's Appeal

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1. REQUESTS

1.1. This is a reply to the Statement of Grounds of Appeal of the Patent Proprietor ("PP") dated 30 December 2019 and the Communication from the Boards of Appeal dated 21 January 2020. An extension to the period for response was granted on 28 May 2020.

1.2. We request that the appeal is dismissed and that European Patent No. 2861210 ("the Patent") is revoked in its entirety. Oral Proceedings are requested in the event that the Patent is not to be revoked on the basis of the written submissions alone.

1.3. We also request that new documents D23 (together with its Annexes) and D24 are not admitted into these appeal proceedings.

2. EVIDENCE

2.1. We rely on all of the documents that were filed during the first instance proceedings (D1 to D22, including D1b and D15b). In addition, the following new documents are enclosed:

D15c	EU clinical trial database for NAPOLI-1 study from 12 October 2012, corresponds to D15b
D25	Pin-Yuan Chen et al, Neuro-Oncology 15(2):189–197 (December 2012)
D26	Drummond DC et al, Cancer Res 2006; 66: 3271–3277 (2006)
D27	Roy AC et al, Annals of Oncology 24(6): 1567-1573 (February 2013)
D28	Svenson S, Current Opinion in Solid State and Materials Science, 16(6) pp 287-294 (October 2012)
D29	Makrilia N et al, JOP. Journal of the Pancreas, 12(2) pp 110-113 (2011)
D30	Chen LT et al, Journal of Clinical Oncology, 30(4 Suppl) pp 613-613 (February 2012)
D31	Cunningham D et al, Journal of Clinical Oncology 29 (4 Suppl): 6-6 (2011)
D32	Gerber DE, Journal of Thoracic Oncology 7(12) Supplement 5_ S387-S389 (December 2012)
D33	Noble et al, Cancer Res 2006; 66: (5). March 1, 2006
D34	Krauze MT et al, Neuro-Oncology 9(4): 393-403 (2007).
D35	Mullard A, Nature Reviews Drug Discovery, vol. 17, page 777 (2018)
D36	The Medicines for Human Use (Clinical Trials) Regulations (MHCTR) 2004.

2.2. Document D15c is the corresponding entry for the NAPOLI-1 trial obtained from the EU clinical trials database on 12 October 2012, specifically for the trials conducted in the UK. Note that the sponsor's code (organization study ID) is identical ("MM-398-07-03-01") in both D15b and D15c. D15c and D36 primarily addresses issues raised in D23.

2.3. Documents D26, D29, D30, D31, D33 and D34 were published before the earliest claimed priority date (13 June 2012); whereas, documents D25, D27, D28 and D32 were published after the earliest priority date but before the filing date of the second priority document (14 March 2013) and are therefore prior art if the claim to the earliest priority date is not valid.

2.4. These documents are filed in response to the PP's allegation that D15b discloses only MM-398 and not liposomal irinotecan or that the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection. These allegations were raised for the first time during oral proceedings before the OD and so this represents the first opportunity to file evidence in relation to this issue.

3. FORMAL ISSUES

3.1. The PP has filed four requests and two new documents (D23 and D24) with its Grounds of Appeal. The requests correspond to those considered by the opposition Division (OD) in its Decision as follows:

First Instance	Appeal
MR (formerly (AR2)	MR
AR1	AR1
AR2 (formerly MR)	AR2
AR3	AR3

3.2. Document D23 is a post published declaration from Dr Amy McKee. This document addresses a number of issues concerning the status and relevance of D15b as well as providing further background on the treatment of pancreatic cancer ("PC").

3.3. This document should not be admitted on appeal because it constitutes new evidence that could (and should, if felt relevant) have been filed during first instance proceedings.

3.4. For instance, D15b (then simply D15) was filed with the grounds of opposition. Should the PP have had issues with the status or relevance of this disclosure it had ample opportunity to raise them during the opposition procedure.

3.5. Similarly, the fact that the Patent concerned the treatment of PC was obvious from the outset. Should the PP have felt it necessary to provide additional information about the treatment of PC in 2012/13 there was ample opportunity to do so during the opposition phase of this case.

3.6. The primary purpose of D23 appears to be an attempt to undermine the OD's finding that the publication of Arm C of D15b, as part of a Phase III study, would have given the skilled person a reasonable expectation that the regimen would be successful, see the Grounds of Appeal at pars. 5.85-5.86.

3.7. However, D15b was identified as a closest prior art document in the Annex to the Notice of Opposition and basis for the OD's finding that there was a reasonable expectation of success is supported by established case law (T0239/16 and T2506/12) which was detailed by the opponent in writing during first instance proceedings, see, for example, pars. 5.14-5.22 of the opponent's letter dated 9 May 2019. As such, should the PP have felt it necessary to

address this issue it could have done so during the first instance proceedings and not waited until the appeal phase.

3.8. Overall, there is no justification for the PP to file this new evidence only on appeal and as such, this new evidence should not be admitted.

3.9. Document D24 is a post published declaration from Dr Bruce Belanger, an employee of the PP. Primarily, this document should not be admitted because it is not relevant. The document has been submitted on appeal as part of the PP's arguments on inventive step and particularly to show that, prior to the Phase III NAPOLI-1 study, the claimed regimen had not been tested in human subjects having PC, whose disease had progressed following gemcitabine-based therapy.

3.10. However, the question of inventive step is addressed by the skilled person based on the state of the art. The information contained in D24 is not part of the state of the art in this case, it is secret information known only to the PP, and is therefore simply not relevant to the skilled person's assessment of inventive step.

3.11. Moreover, should the evidence in D24 be considered relevant it should not be admitted on appeal because it could have been filed during the first instance proceedings.

3.12. D24 allegedly addresses the OD's point that publication of the Phase III study in D15b and particularly the regimen in Arm C indicates the existence of earlier successful human studies with that regimen, see pars. 5.79-5.80 of the PP's Grounds of Appeal.

3.13. However, this point was raised in writing during first instance proceedings, see, for example, pars. 5.14-5.22 of the opponent's letter dated 9 May 2019. As such, should the PP have felt it necessary to address this issue it could and should have done so during those first instance proceedings and not waited until the appeal phase.

4. THE PATENT

4.1. European Patent no. 2861210 ("the Patent") was granted on 3 May 2017, based on application no. 13731230.2. The Patent has a filing date of 12 June 2013 and claims priority from two earlier US applications: US 201261659211 P, filed on 13 June 2012; and US 201361784382 P, filed on 14 March 2013.

4.2. Independent claim 1 of the Main Request, is directed to:

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

mg/m^2 and on day 1 of each subsequent cycle at a dose of 60 mg/m^2 or 80 mg/m^2;

(b) 5-FU is administered at a dose of 2400 mg/m^2; and

(c) leucovorin is administered at a dose of 200 mg/m^2 (l form);

and wherein in each cycle, the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU;

and wherein the liposomal irinotecan is irinotecan sucrose octasulfate liposome injection."

4.3. Dependent claims 2-4 correspond to granted claims 2, 3 and 5.

4.4. Claim 1 has been amended from granted claim 1 to remove reference to administering racemic leucovorin and to specify that the liposomal irinotecan is irinotecan sucrose octasulfate liposome injection. This form of liposomal irinotecan is also referred to as MM-398 or PEP02, see paragraph [0032] of the Patent.

4.5. The Patent contains seven examples.

4.6. Examples 1-4 describe tests with MM-398 alone in animal cancer models. Example 5 describes a human pharmacokinetic study with MM-398 alone. As such, Examples 1 to 5 do not concern the claimed clinical dosing regimen.

4.7. Example 6 describes a Phase I dose escalation study combining MM-398 (80 mg/m^2), 5-FU and leucovorin in 16 patients with solid tumours. The study was prior published as D22. Whilst the level of detail is different, the study described in Example 6 of the Patent is the same study reported in D22 - same number of patients enrolled (16), same number of evaluable patients (15), same number of patients receiving 80 mg/m^2 of MM-398 (6), same number of responses (PR-2 (in gastric cancer and breast cancer), SD-9 and PD-4).[1]

4.8. Of the 16 patients in the study it can be determined that two must have had a positive result in the treatment of PC, see OD's Decision at par. 3.3.2. However, Example 6 does not provide details of the dose of 5-FU, the dose of leucovorin, the order of administration of the drugs, the two weekly dosing cycle for all drugs or the treatment status of the patients[2] all of which are functional features of the claims.

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

[1] A comparison of the content of D22 and Example 6 is set out in the Annex to this document.
[2] Recurrent or persistent pancreatic cancer following primary chemotherapy and wherein the patient has failed prior treatment with gemcitabine or become resistant to gemcitabine.

5. SUFFICIENCY OF DISCLOSURE

Introduction

5.1. In summary, it is the Opponent's position that the claimed subject matter cannot meet the requirements of both Article 83 EPC and Article 56 EPC. In its argumentation the PP relies heavily on many aspects of the dosing regimen to distinguish the claimed subject matter from the prior art, but the Patent does not provide any evidence of the suitability of these features.

5.2. Example 6 of the Patent appears to describe the suitability of MM-398, at a dose of 80 mg/m^2, in combination with 5-FU and leucovorin for treating PC, although this information was already known from the prior art. However, the Patent fails to describe the suitability of the other features of the claimed dosing regimen including the two weekly dosing cycle for all drugs (including MM-398), the dose of 5-FU, the dose of leucovorin and the order of administration of the drugs. Example 6 does not describe that the initial dose of MM-398 is dependent on UGT1A1*28 allele status and that for homozygous patients an initial dose of 60 mg/m^2 is effective. Similarly, Example 6 is silent on the treatment of PC that is recurrent or persistent following primary chemotherapy and resistant to gemcitabine.

5.3. Therefore, in order to meet the requirements of Article 83 EPC, it is necessary to rely on the common general knowledge to teach the suitability of the claimed dosing regimen for treating the recited form of PC. However, in its Decision the OD failed to point to the necessary disclosures in the common general knowledge that would fill the whole of this information gap. Instead, the OD relied on mere verbal statements in the Patent, at least for the doses of leucovorin and 5-FU and the 2-weekly dosing cycle, thereby confusing the concepts of a literal disclosure under Article 123 EPC and support under Article 83 EPC.

5.4. Either the Patent is insufficiently disclosed because the claimed dosing regimen is not fully supported by the information content of the Patent together with the common general knowledge, or the missing features of the claimed dosing regimen are to be found in the common general knowledge, which has implications for the assessment of inventive step.

The OD erred in finding that the claimed dosing regimen was sufficiently disclosed

5.5. We disagree with the OD that the Patent meets the requirements of Article 83 EPC, especially that the claimed therapeutic regimen was at least plausibly disclosed at the relevant date. Aside from the arguments presented below, we also rely on paragraphs 25-30 of the Annex attached to the Notice of Opposition and Section 3, paragraphs 3.1-3.23 of our letter dated 9 May 2019.

5.6. The established case law of the Boards of Appeal requires that in the case of a medical use claim the patent / application must disclose the suitability of the product for use in the claimed therapeutic application.[3] The relevant disclosure need not be in the form of clinical studies but it is clear that a simple verbal statement is not enough (T0609/02, reasons 9).

[3] Although the originally decided cases concerned claims in the Swiss-style the law has since been applied to EPC 2000-stype medical use claims (T0895/13, reasons 5).

5.7. Only if this suitability hurdle has been overcome may post-published evidence be taken into account to back-up the findings in the patent in relation to the claimed medical use.

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

5.9. The prior art, e.g. D12 and D13, already describes the suitability of a combination of MM-398, 5-FU and leucovorin for use in a method of treating gemcitabine-refractory PC. Therefore, the alleged technical contribution in this case relates not to the therapeutic application *per se* but to the dosing regimen. Therefore, according to the case law (e.g. T1592/12) the Patent must demonstrate the suitability of the claimed dosing regimen for treating the form of PC mentioned in the claims.

5.10. In finding that the requirements of Article 83 EPC are met, the OD relies, initially, on Example 6 and its description of a Phase I study with promising efficacy and safety results. Analysing Example 6 the OD concludes that it provides evidence of a partial response (PR) or stable disease (SD) in two out of three patients with PC treated with 80 mg/m^2 of MM-398 in combination with 5-FU and leucovorin, see par. 3.3.2 of its Decision.

5.11. However, Example 6 fails to describe any of the other features that make up the claimed dosing regimen. Example 6 does not disclose the two weekly dosing cycle for any of the drugs (including MM-398), Example 6 does not describe the dose of 5-FU, Example 6 does not describe the dose of leucovorin and Example 6 does not disclose the order of drug administration which is specified in the claims. Moreover, Example 6 does not describe that the PC is recurrent or persistent following primary chemotherapy, or that the PC is gemcitabine refractory, nor does Example 6 describe that the initial dose of MM-398 is dependent on UGT1A1*28 allele status and that for homozygous patients an initial dose of 60 mg/m^2 is effective.

5.12. In resolving the issue of the claim features missing from Example 6, the OD erroneously considers it adequate that at least some of the claim features are simply stated in the Patent even though no evidence of their actual suitability is provided. In other words it appears that the OD has confused the disclosure requirements of Article 123 EPC and the support requirements of Article 83 EPC.

5.13. Specifically, as regards the doses of 5-FU and leucovorin recited in the claims, the OD notes that these doses are mentioned consistently in the Patent (Decision, par. 3.3.2, last 5 lines). However, this finding goes against the established case law in this area, see the Case Law of the Boards of Appeal of the European Patent Office, 9th edition ("CLBA"), II.C.7.2. In particular, it is clearly incorrect to rely merely on a verbal (albeit consistent) statement as support for the suitability of these specific doses for treating a particular form of PC. The case law makes clear that evidence of suitability is required, either from the Patent or from the common general knowledge.

5.14. In a similar manner, the OD relies on mere statements in the Patent concerning the 2-weekly dosing cycle for all three drugs and the order of addition of the drugs (Decision, par. 3.3.4),

even though there is no evidence in the Patent that a 2-weekly cycle or that the recited order of addition is effective for combination therapy in treating PC.[4]

5.15. In sum, the OD erred in simply believing what was written in the Patent without any evidence to support its conclusion. This contravenes the established case law in the field of medical use claims that requires some evidence, either in the Patent or from the common general knowledge, showing that the claimed treatment is effective.

5.16. In this regard, D22 is a prior art disclosure of the Phase I study involving MM-398, 5-FU and leucovorin presented in Example 6 of the Patent. In its Decision, the OD disregarded the content of D22 in determining sufficiency of disclosure because as a single article it does not represent common general knowledge. However, what D22 does demonstrate is the danger of relying on mere verbal statements in a patent as evidence of the suitability of a dosing regimen in treating a particular disease. Specifically, it is clear from D22 that despite verbal statements to the contrary neither the dose of 5-FU nor the 2-weekly dosing cycle were used in the regimen of Example 6 which underlies the suitability of the claimed dosing regimen.

5.17. In paragraph 3.3.7 of its Decision the OD attempts to distinguish the present case from T1592/12 concerning a dosing regimen for Herceptin. In particular, the OD appears to argue that it is acceptable to rely on the statements in the Patent concerning the features of the dosing regimen not evidenced by Example 6 because in the present case (unlike in the case of Herceptin) there is no evidence that these features are not suited to the therapeutic use.

5.18. However, the ODs interpretation of the law in this area is wrong. Firstly, as noted above, it is established case law that verbal statements, such as relied on by the OD in this case, are not sufficient in connection with medical use claims. Secondly, whilst it is true that the Board in T 1592/12 was able to point to serious doubts about the claimed dosing regimen to support its decision, that is not the legal standard for a finding of insufficiency in the case of a medical use claim.

5.19. Rather, in the case of a medical use claim it is necessary to positively demonstrate suitability. In other words, the case law concerning the requirement for "serious doubts" to substantiate insufficiency is not relevant to medical use claims. This point is made most clearly in T1045/13 where in relation to a medical use claim the appellant argued that a sufficiency objection could only be raised on the basis of "serious doubts substantiated by verifiable facts" (T 19/90). In reply the Board stated that:

> "The board does not consider T 19/90 to be relevant for the present decision. T 19/90 does not deal with a situation where a therapeutic effect which is a functional technical feature of the claim under consideration has to be established for the first time. This requires (see above) the present application to disclose the suitability of NGF for the claimed therapeutic application. Such suitability has not been convincingly demonstrated." (emphasis added)

[4] In the discussion of inventive step the OD do state that the same order of drug administration as recited in the claims is consistently mentioned in the prior art concerning combination therapy using nonliposomal irinotecan (e.g. D2-D6), see Decision par. 5.7.7.

5.20. Thus, in respect of a proprietor's obligation to show the suitability of a claimed dosing regimen for a therapeutic use, the established law relating to the demonstration of serious doubts does not apply until suitability has been convincingly demonstrated. In the present case, repeated reliance on mere verbal statements means that suitability of the dosing regimen has not been demonstrated and therefore there is no obligation on any third party to verify serious doubts about the claimed subject matter.

5.21. To summarise: the established case law concerning medical uses requires a technical not simply a verbal teaching as to the suitability of the claimed use. The case law applies to the functional technical features of medical use claims, especially dosing regimens, which are relied on as the technical contribution to the art. In the present case no technical teaching is provided as to the suitability of the claimed dosing regimen for treating recurrent or persistent PC which is also gemcitabine refractory. These deficiencies have not been completely addressed with references to the common general knowledge.

5.22. As such, in the present case the claimed therapeutic application has not been plausibly disclosed and the post-published evidence may not be relied on to back-up the findings that are in the Patent. Accordingly, the requirements of Article 83 EPC are not fulfilled.

6. PRIORITY

6.1. We agree with the OD that the claim to the first priority date, 13 June 2012, is not valid because there is no direct and unambiguous disclosure in PD1 that "*leucovorin is administered at a dose of 200 mg/m^2 (l form)*".

Claim 1 impermissibly combines two separate embodiments

6.2. The PP argues that basis for claim 1 of the MR can be found in PD1 by combining the disclosures of claims 3 and 4, the final sentence on page 11, the second sentence on page 12 and, for example, claim 11, and that reference to "leucovorin" in PD1 is a direct and unambiguous reference to the "l-form" of leucovorin.

6.3. However, the final sentence on page 11 and the second sentence on page 12 of PD1 relate to separate embodiments as follows:

> "In one embodiment, a patient treated using the methods and compositions disclosed herein exhibits evidence of recurrent or persistent PC following primary chemotherapy."
>
> "In an additional embodiment, the patient has failed prior treatment with gemcitabine or become resistant to gemcitabine."

6.4. The patient group in claim 1 of the MR has the features of both of these embodiments, i.e. the patient group in claim 1 of the MR "*exhibits evidence of recurrent or persistent PC following primary chemotherapy **and** ... has failed prior treatment with gemcitabine or become resistant to gemcitabine.*"

6.5. There is no basis in PD1 to combine these two separate embodiments to create a new patient group. In other words, the technical information that patients can be treated with the

described drug regimen when suffering from recurrent or persistent PC following primary chemotherapy and wherein the PC is refractory to gemcitabine is not directly and unambiguously disclosed in the priority document. As such, for this reason alone, the claim to the first priority date is not valid.

Leucovorin in the l-form is not directly and unambiguously disclosed in PD1

6.6. In paragraph 4.7 of its Grounds of Appeal, the PP indicates that at the filing date of PD1, leucovorin was commercially available in its pure l-form (sometimes referred to as "levoleucovorin" or "l-leucovorin")[5] or as a 50:50 isomeric mixture of the l- and d-forms (**sometimes referred to as "l+d leucovorin" or "racemic leucovorin"**).

6.7. However this information is, at least, incomplete because at the filing date of PD1 the usual term for racemic leucovorin was simply "leucovorin". This is apparent from D1b which is the FDA approved product label for "leucovorin calcium" and which states that "*Leucovorin is a mixture of the diastereoisomers of the 5-formyl derivative of tetrahydrofolic acid (THF).*"

6.8. It is well-established that the application of EPO law must be consistent and that the concept of disclosure is the same for the purposes of Articles 54, 87 and 123 EPC (G2/03, reasons 2.2.2, par. 4).

6.9. In the context of Article 123(2) EPC, the standard of proof that must be met for an amendment to be allowed is a high one, namely "beyond a reasonable doubt" (CLBA-II.E.5). The same standard therefore applies when considering whether the subject matter of a claim is directly and unambiguously disclosed in a priority document.

6.10. In the present case, PD1 does not directly and unambiguously disclose leucovorin in its l-form ("levo-leucovorin") and therefore the claim to the first priority date is not valid.

6.11. Throughout PD1, reference is made simply to "leucovorin", which is typically used to refer to the racemic compound, see paragraph 6.7 above. Moreover, there is no disclosure in PD1 that leucovorin is a chiral molecule and no disclosure of any enantiomers.

6.12. However, the PP argues that the skilled person would have been aware that leucovorin is an optically active molecule and that it was common general knowledge that, of the two optical isomers, the l-form of leucovorin is pharmaceutically active. In this regard, the PP references D1, which is the product label for FUSILEV (levo-leucovorin).

6.13. However, whilst it is correct that, when assessing the right to claim priority, PD1 should be read through the eyes of the skilled person, it is not the case that the common general knowledge can make up for the lack of a direct and unambiguous disclosure in PD1.

6.14. In this regard, the dangers of relying on the common general knowledge when assessing compliance with Article 123(2) EPC, and hence Article 87 EPC, are well documented, CLBA-II.E.5. Particularly, the Boards note the notorious difficulty of objectively determining the extent of common general knowledge and the dangers of undetected abuse by allowing amendments on the basis of ostensibly proven common general knowledge. In other words it

[5] We will use the term "levo-leucovorin".

seems highly unlikely that the requisite standard of proof, i.e. beyond a reasonable doubt, can be met when relying on common general knowledge as the basis for an amendment.

6.15. In paragraphs 4.8-4.10 of its Grounds of Appeal, the PP refers to a passage on page 11 of PD1 which describes a biological function for leucovorin as a biochemical cofactor for 1-carbon transfer reactions in the synthesis of purines and pyrimidines. The PP argues that only levo-leucovorin is able to act as a biochemical cofactor and that therefore any disclosure of leucovorin in PD1 must be interpreted by the skilled person as a reference to levo-leucovorin.

6.16. This is not correct. Even if the PP is right about the extent of common general knowledge then the skilled person might equate the teaching on page 11 of PD1 with levo-leucovorin as the biologically active enantiomer present in leucovorin. However, this does not mean that the skilled person would unambiguously understand that the drug administered in the claimed dosing regimen is levo-leucovorin, rather than the disclosed racemate, leucovorin. In this regard, many drugs are administered as racemates when it is well-known that only one of the enantiomers is active. Moreover, very similar language to that relied on by the PP from page 11 of PD1 can be found in D1b, the FDA product label for (racemic) leucovorin, see page 1, col. 1 under the heading 'clinical pharmacology'. In other words, the biological functions described on page 11 of PD1 would occur regardless of whether racemic leucovorin or the pure l-isomer was administered to a patient.

6.17. The PP continues in paragraphs 4.11-4.12 of its Grounds of Appeal, to assert that if the Board are not convinced that the term "leucovorin" is in fact a direct and unambiguous disclosure of levo-leucovorin, it is nevertheless the case that the only remaining sensible interpretation of the term "leucovorin" is as "levo-leucovorin or racemic leucovorin". In which case, the PP argues, selecting one item "leucovorin" from the list of two equally preferred alternatives is permissible and that the right to claim priority is therefore maintained. The PP is wrong on both issues.

6.18. Firstly, in its analysis of how the skilled person would interpret the term "leucovorin" in PD1, the PP seems to ignore the most straightforward option, i.e. that the skilled person would simply take the disclosure at face value, that "leucovorin" means "leucovorin" and that the disclosure is that of the racemic compound. This is a technically and linguistically sensible interpretation of the relevant disclosure and in line with usual practice, as mentioned above.

6.19. Secondly, the PP is wrong to associate the term "leucovorin" with a list containing two members (levo-leucovorin and racemic leucovorin). Whilst we maintain that the term "leucovorin" is a reference to the racemic compound in line with common practice, at best, it is a generic term encompassing at least two members, but not disclosing any of these members in individualised form.

6.20. This assessment is consistent with established EPO case law, see, for example, T1046/97 (reasons 2.1.1.6) which decided that the term "optically-active forms" could not be equated to an individualised disclosure of any specific enantiomer, and T0833/11 (reasons 5.4) which decided that the term "furoate" is a generic term encompassing two compounds, namely 2-furoate and 3-furoate, but not disclosing either of them in individualised form, see also CLBA-I.C.6.2.3. Similarly, in the well-known case T0181/82 (reasons 8) it was decided that the

generic term "C1-C4 alkyl bromides" does not individualise any of the compounds conceptually covered by the term, other than methyl (C1) bromide, see also CLBA-I.C.6.2.1(c).

6.21. The PP's arguments in paragraphs 4.13-4.16 of its Grounds of Appeal fail for the same reasons. PD1 discloses "leucovorin" - either this is (as we believe) a disclosure of the racemate, in line with conventional naming as evidenced by D1b, or it is a generic term encompassing, but not individualising, more than one compound. In either case there is no direct and unambiguous disclosure in PD1 of "levo-leucovorin", certainly not up to the standard of proof required.

6.22. Further, the PP comments on a point raised during first instance proceedings and not discussed by the OD; namely that if the skilled person had intended to disclose both the racemic compound (leucovorin) and the l-enantiomer (levo-leucovorin) s/he would have done so according to well-established principles from which the skilled person knows how to describe both a racemic compound and its enantiomers.

6.23. The PP acknowledges that these well-established principles exist but argues that in the present case the skilled person is an oncologist, rather than an organic chemist, and that an oncologist "*would not have stuck rigidly to strict chemical nomenclature when interpreting the disclosure of PD1.*" However, in our view, both for safety and efficacy reasons, an oncologist would pay close attention to whether a disclosure of an optically active compound was that of the racemate or of a single enantiomer.

6.24. As noted by the PP in its submission, an oncologist would be aware that leucovorin (along with many other chemotherapeutic agents) is available both in racemic form and as an individual enantiomer. The oncologist would also be aware from common general knowledge that different dosages are given depending on whether the drug is in racemic form or is a single enantiomer. Accordingly, a skilled oncologist is likely to pay close attention when an optically active compound is disclosed for administration to a patient to avoid administering the wrong dose.

6.25. In the present case, PD1 simply discloses "leucovorin". The skilled oncologist would realise that if the single enantiomer were intended to form part of the dosing regimen this would be clearly specified, according to the well-established principles, by an unambiguous reference to "levo-leucovorin" or the "l-form" etc., to avoid giving the wrong dose. Similarly the skilled oncologist would be aware that the usual means of referring to the racemic product is simply to use the term "leucovorin" as evidenced by the FDA label for the racemic product D1b.

6.26. Finally, the PP points to D2, which in one place uses the term folinic acid (leucovorin) where in fact the drug actually administered during the study was levo-folinic acid.[6] However, an isolated disclosure in an academic paper, such as D2, is not evidence of how the skilled person would behave in general any more than a single academic paper is evidence of the skilled person's common general knowledge.

[6] In its Decision (Section 4.2, par. 3), the OD refers to a similar inconsistency in D3. However, this is not correct (see our letter of 9 May 2019, par. 4.9) and the allegation is not repeated by the PP in its Grounds of Appeal.

6.27. In summary, there is no direct and unambiguous disclosure of "levo-leucovorin" in PD1, certainly not to the standard of beyond a reasonable doubt. It is more than conceivable that the term "leucovorin" in PD1 refers to the racemic compound alone. As such, the claim to the first priority date is not valid.

7. INVENTIVE STEP

Selection of the closest prior art

7.1. We agree with the OD that D15b is a suitable closest prior art document and in particular that Arm C of the Phase III study disclosed therein represents the closest prior art. However, we also believe that D12, D13 and D5 are suitable starting points for the inventive step analysis.[7] Therefore, to meet the requirements of Article 56 EPC the claimed subject matter should be inventive over all of these disclosures.

7.2. In particular, D12 and D13 both describe positive results in treating gemcitabine-refractory PC with the combination of MM-398, leucovorin and 5-FU. Thus, D12 and D13 describe the treatment of the same patient group as in the Patent with the same combination of drugs.

7.3. D5 also describes a clinical study in patients with PC who had failed first line gemcitabine based chemotherapy. The patients were treated according to a modified FOLFIRI.3 (m FOLFIRI.3) regimen which consisted of (non-liposomal) irinotecan, leucovorin (400 mg/m^2) and 5-FU (2000 mg/m^2) every two weeks. Thus, D5 describes treatment of the same patient group as in the Patent with the same active ingredients, albeit irinotecan is formulated differently, according to a similar dosing regimen.

D15b as Closest Prior Art

7.4. The PP argues that D15b is not the closest prior art. Mainly because it believes D13 is "closer" to the claimed subject matter. However, this is not the correct approach.

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

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

7.7. We maintain that D15b (along with D12, D13 and D5) are all feasible closest prior art documents over which the claimed subject matter is required to demonstrate an inventive step. In particular, D15b is clearly directed to the same purpose as the Patent, i.e. the

[7] Decision of OD, par. 5.1.

treatment of PC in patients who have failed prior gemcitabine-based therapy, and also requires a minimum of structural and functional modifications to arrive at the claimed subject matter, i.e. Arm C of D15b teaches a substantially identical dosing regimen to that claimed.

7.8. Whilst it is the case that D15b does not disclose the actual results of the Phase III trial this is an issue related to other aspects of the problem-solution approach (see below), not whether the disclosure is a feasible starting point for further consideration by the skilled person.

7.9. In paragraphs 5.43 and 5.44 of its Grounds of Appeal, the PP argues that in the case of a medical use claim, the closest prior art should be a document that discloses a treatment that achieves the therapeutic effect and in a choice between two documents where one discloses actual treatment of the condition and the other discloses a proposed treatment of the condition the former should be selected as the closest prior art.

7.10. Such an approach is flawed for the reasons discussed above, i.e. the question is not which document is "closer", D13 or D15b, the question is only whether the documents are feasible to be considered as closest prior art documents. In our view, as outlined above, D15b clearly meets the established criteria to be considered as a closest prior art document. Moreover, as discussed throughout the proceedings, T0239/16 and T2506/12 are examples of decisions where Boards have selected documents which disclose proposed studies without results as closest prior art.

7.11. The PP's attempt to promote D13 as closest prior art to the exclusion of other documents, such as D15b, is also legally flawed as the Boards have rejected "*in its very principle*" the notion that a document can be excluded as a starting point only because some seemingly more promising item of prior art is available, see T0405/14, Headnote and reasons 18. In other words, whilst D13 may be a feasible starting point for the inventive step analysis this does not lessen the need to demonstrate an inventive step over other feasible starting points such as D15b.

7.12. Put another way, the PP is just attempting to use the assessment of the closest prior art as a mechanism to avoid a problematic document (D15b). If the PP really believed that D15b was not a feasible candidate as the closest prior it would just accept the opponent's choice of starting point, because it would be easier to argue that the Patent involved an inventive step from a less promising document, see, for example, T2304/16, reasons 4.2.5.

7.13. In paragraphs 5.46 of its Grounds of Appeal, the PP argues that the OD was wrong to rely on T0239/16 and T2506/12 as instances where clinical trial protocols with no results had been considered as closest prior art disclosures. In particular, the PP argues that the factual background in those cases is different from the present case because in T0239/16 and T2506/12, unlike in the present case, there were no other prior art documents which could have been taken as the closest prior art. However, the PP's analysis is wrong.

7.14. Firstly, in T0239/16 and T2506/12 the documents disclosing the clinical trial protocols, but without results, (D55 and D2, respectively) were not only feasible enough to be considered as closest prior art documents, they were feasible enough to render the claimed subject matter obvious. Thus, it is immaterial whether or not there might have been other relevant prior art documents the claimed subject matter would still have been held obvious.

7.15. Put another way, if further relevant prior art documents had existed in those cases, either the claimed subject matter would have been held obvious over such disclosures or, if the claimed subject matter had been found inventive over the further prior art documents, the problem-solution analysis would have moved on to consider D55 and D2, respectively, as closest prior art. Such an analysis would have resulted in the same outcome, i.e. revocation of the patent for lack of inventive step. In other words, the PP's argument only has any merit if the established position, discussed above, that an inventive step needs to be established in light of all feasible closest prior art documents is not followed.

7.16. Secondly, the PP is not correct in its analysis of the opposition case leading to T0239/16. According to the first instance decision, whilst the opponents argued that D55 was the most promising closest prior art document, alternative closest prior art documents put forward included D6, D9, D30/D38 and D31 (OD's Decision, pars. 1.6-1.6.5). Similarly, the PP argued that D9 or D31 should be considered as the closest prior art (OD's Decision, par. 2.7).

7.17. Further, the PP argues that D13 is structurally closer to the claimed subject matter because it discloses that MM-398 is "liposomal irinotecan"; whereas, D15b only discloses "MM-398".

7.18. Firstly, the PP is wrong because it assumes that if D13 is structurally closer than D15b that is in some way determinative; whereas, the correct question to ask is whether D13 and/or D15b are suitable closest prior art documents. Secondly, D15b references many elements of the claimed dosing regimen that are not mentioned in D13 and so in terms of the actual number of features common to the prior art and the claimed subject matter, D15b is more similar than D13. Thirdly, as elaborated further below, when read through the eyes of the skilled person D15b also discloses that "liposomal irinotecan is irinotecan sucrose octasulfate liposome injection".

7.19. The PP also argues that even if D15b is considered as a closest prior art document there are two options for what constitutes the closest embodiment, i.e. treatment Arms A and C, see Grounds of Appeal, par. 5.66-5.67. However, in our view, if D15b is considered a feasible closest prior art document then it would be nonsensical to ignore Arm C which in terms of structural features is more similar to the claimed subject matter than Arm A. Moreover, it is not the case, as contended by the PP, that the regimen in Arm C has "*seemingly come from nowhere*", see Grounds of Appeal, par. 5.61. There are a number of documents in the proceedings that highlight the effectiveness of the combination of irinotecan (liposomal and non-liposomal), leucovorin and 5-FU in treating solid tumours, including PC, see D2-D6, D12, D13, D22 and D29.

7.20. In summary, Arm C of D15b is a feasible closest prior art disclosure because it is concerned with the same purpose as the Patent and requires a minimum of structural and functional modifications to arrive at the claimed subject matter, i.e. Arm C teaches a substantially identical dosing regimen to that claimed for the treatment of the same disease as recited in the claims. Thus, even if it is believed that D13 is "closer" to the claimed subject matter than D15b (which we dispute), the established case law directs that as a feasible closest prior art disclosure the obviousness of the claimed subject matter must be considered in light of Arm C of D15b.

Distinguishing features and technical problem

7.21. We agree that D15b does not provide any therapeutic results for the described study and therefore we agree that the technical problem may initially be formulated along the lines of "*a safe and effective treatment for gemcitabine-resistant PC*." This is also the technical problem considered by the OD, see par. 5.7.2 of its Decision.

7.22. We also agree that D15b does not mention the order of administration of the drugs in the dosing regimen, i.e. the MM-398 is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU.

7.23. However, we do not agree with the other distinguishing features outlined by the PP in paragraph 5.69 of its Grounds of Appeal. In particular, we do not agree that the absence of an explicit disclosure of "liposomal irinotecan" which is "irinotecan sucrose octasulfate liposome salt injection" is a distinguishing feature because, as outlined below, at the relevant date the skilled person knew what "MM-398" was.

7.24. Moreover, whilst we agree that D15b does not mention the UGT1A1*28 allele, we do not believe that the skilled person would realistically consider the differential dosing based on whether or not a patient is homozygous for this allele as a distinguishing feature. In this regard, D10 (page 26) reports that only about 10% of the North American population is homozygous for the UGT1A1*28 allele and hence the skilled person would regard it as inconceivable that all of the patients in Arm C of the study receiving the prescribed 80 mg/m^2 of MM-398 were homozygous for the UGT1A1*28 allele, i.e. outside of the claim scope.

Could-would approach

7.25. As noted above, the technical problem may initially be formulated along the line of a safe and effective treatment for gemcitabine-resistant PC.

7.26. However, for the reasons discussed previously in relation to sufficiency of disclosure, there is no evidence in the Patent that this problem had been plausibly solved at the relevant date. As there is no plausible disclosure in the Patent, post-published evidence cannot be used as the sole basis for evidencing that the technical problem has been solved (CLBA-I.D.4.6). Thus, the technical problem must be formulated less ambitiously along the lines of providing an arbitrary treatment and the claimed subject matter therefore lacks an inventive step.

7.27. However, in the event that the Board concurs with the PP and considers that a more ambitious problem is solved, the claimed subject matter still lacks an inventive step. In particular, it is our view that the skilled person would have had a reasonable expectation that the dosing regimen described in Arm C of D15b would provide a safe and effective treatment for gemcitabine-resistant PC.

7.28. In this regard, it is important to recognise that the comments made by the PP in relation to the poor prognosis and lack of alternative treatment options for patients with gemcitabine-refractory PC apply also when considering what is a safe and effective treatment for such patients. In other words, the threshold for "success" in such a trial will be relatively low.

7.29. In summary, we largely agree with OD's analysis of the could-would approach. In light of the fact that human clinical trials (Phase III in particular) are very expensive and require authority approval it would be considered highly unlikely that the NAPOLI-1 trial would be allowed to proceed unless the trial sponsor and the concerned regulatory authorities had confidence in a successful outcome, albeit that success in this case might only be a modest therapeutic improvement. Thus, the skilled person reading D15b would also have a reasonable expectation that the trial would be successful. In particular, below we highlight the comments of the Boards in T0239/16 and T2506/12 which reflect these views:

> "Clinical trials in humans are planned scientific investigations. They require authority approval, which is only given after a risk/ benefit evaluation. For ethical (but also economic) reasons it has to be ensured that research risks are minimised and are reasonable in relation to any potential benefits. Ethical and economical considerations require that the "benefit" will arise with reasonable certainty and will not only "be hoped for"." (T0239/16, reasons 6.6)
>
> "Document D2 discloses that a clinical phase I study assessing the combination treatment of cancer with Yondelis (ET-743) and Doxil (PLD) was in progress. Thus, at the publication date of D2, the information was available that the envisaged combination treatment was considered by pharmaceutical researchers with an expectation of success sufficient to justify a clinical phase I trial. In this context it is pointed out that drug compounds to be used in a clinical trial with human subjects are not selected based on a general "try-and-see" attitude, but based on existing favourable scientific data, for both ethical and economical reasons. Thus a clinical trial is not a mere screening exercise." (T2506/12, reasons 3.10)

7.30. Furthermore, with regard to the distinguishing feature that the MM-398 is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU, we note above, in relation to sufficiency of disclosure, that the Patent provides no evidence concerning the suitability of this order of administration. Therefore, to the extent that the Patent is considered to meet the requirements of Article 83 EPC, this feature must exist in the common general knowledge.

7.31. In this regard, it appears to be routine to administer the combination in the order of irinotecan followed by leucovorin followed by 5-FU, see for example, D2, D4, D6 and D10 (par. 2.1), see also par. 5.7.7 of the OD's Decision. In any event, no specific advantage has been demonstrated for the claim feature.

7.32. Thus, overall, we believe the claimed subject matter lacks an inventive step over D15b.

Reply to Pars 5.74 to 5.87 of PP's Grounds of Appeal (Reasonable Expectation of Success)

7.33. Initially, the PP attempts to distinguish the present situation from the facts in T0239/16. However, in reaching its Decision the OD did not rely on a single case it relied on the consistent position adopted by the Boards in both T0239/16 and T2506/12. In this regard attention is drawn to the Enlarged Board's comments in, for example, R 11/08, reasons 11, and R 14/11, reasons 2.9.1, which state that differences of fact are normal in cases whereas

case law is not confined to similar or identical facts, but lies in the principles or guidance which can be extracted from earlier cases.

7.34. In paragraph 5.77 of its Grounds of Appeal, the PP highlights what it considers to be the differences in the case decided in T0239/16 that distinguish it from the present case.

7.35. The PP notes that the disease at issue in T0239/16 was osteoporosis; whereas, the subject matter of the present claims is PC. Whilst this is true, it is questionable whether it is relevant. In particular, issues of what represents providing a safe and effective treatment for a disease would take into account differences in the severity of the disease, the prognosis and the availability of alternative treatments. Moreover, the disease at issue in T2506/12, also relied on by the OD in arriving at its decision, is cancer.

7.36. The PP also highlights the background information about bisphosphonates that it argues was relevant to the decision made in T0239/16. However, in the present case there is also a number of additional prior art documents that highlight the effectiveness of the combination of irinotecan (liposomal and non-liposomal), leucovorin and 5-FU in treating solid tumours, including PC, see D2-D6, D12, D13, D22 and D29.

7.37. The PP also files new documents D23 and D24 in an attempt to undermine the OD's finding that starting from D15b the skilled person would have had a reasonable expectation of solving the above mentioned technical problem. Whilst we believe that these documents are not admissible we also note that they only deal with regulatory and technical issues and that neither deals with the economic considerations that in part underpinned the OD's Decision, see section 5.7.3, pars. 3 and 4. In particular, the OD relied on T0239/16 and T2506/12, especially the passages set out above in paragraph 7.29, which concluded that a skilled person would have a reasonable expectation that a human clinical trial would be successful because of economic considerations. In other words, the skilled person would understand that a study sponsor would not embark on an expensive clinical trial programme without a reasonable expectation that it would be successful. This evidence appears to be uncontested in the present appeal.

7.38. Nevertheless, it is an important aspect of why the skilled person in this present case would have had a reasonable expectation that the NAPOLI-1 trial (as described in D15b) would be successful. The NAPOLI-1 study was a Phase III trial involving over 400 patients at more than 70 different sites in more than 10 separate countries. In 2011 the sponsor company, Merrimack Pharmaceuticals, was a relatively new company[8] certainly not one of the big established pharmaceutical companies who could more easily afford the many millions of dollars associated with an international, multi-centre clinical trial programme.[9] In such circumstances, the skilled person would have considered it highly unlikely that the sponsor company would have undertaken the NAPOLI-1 trial without a robust technical rationale and a reasonable prospect of success.

[8] Merrimack was founded by a group of scientists from MIT and Harvard University in 2000, source Wikipedia.
[9] In 2018 Nature Reviews Drug Discovery (D35) reported on two studies that estimated the cost of Phase III clinical studies. The median cost for the studied periods (2015-2016 and 2010-2015) were $19 million and $21.4 million, respectively.

7.39. Further, in the unlikely event that the Board decides to consider D23 and D24, we address this newly submitted evidence below.

Declaration of Dr Bruce Belanger (D24)

7.40. The primary purpose of D24 appears to be its assertion that the exact claimed dosing regimen was not trialled in human patients suffering from the specific form of PC recited in the clams prior to the Phase III NAPOLI-1 trial. However, as noted above, this information which was not known to the skilled person at the relevant date and is therefore irrelevant to the assessment of inventive step.

7.41. Moreover, such an assertion is not particularly surprising since earlier clinical trials in humans (Phase I and Phase II) are designed (at least in part) to define the dose and dosing regimen that will be used in future trials. This does not mean, however, that there is no credible scientific rationale for progressing with a dosing regimen in a Phase III trial, even if not trialled previously. In this regard, whilst we have no reason to doubt Dr Belanger, we have no way of verifying what potentially relevant clinical and non-clinical work was done by the PP prior to the NAPOLI-1 trial. For example, there are well-known methods in clinical pharmacology and pharmacometrics which allow predictions to be made about the efficacy of a dose or dosage regimen using statistical simulations based upon data with somewhat different dosages or dosing regimens. We also note that Dr Belanger carefully states that the claimed regimen was not used in patients having progressed beyond gemcitabine-based therapy. However, Dr Belanger does not comment on whether the regimen was used in any other human studies e.g. in other solid tumours, or in PC as a first line therapy, or in PC failing therapies other than those that were gemcitabine-based, or whether a similar but slightly different dosage regimen was actually used in patients with gemcitabine-refractory PC. In fact it now appears that Arm C was added to the study in D15 because of promising results obtained in an ongoing study with precisely the same regimen treating colorectal cancer, see D19, page 2, col. 2, second complete paragraph.

7.42. None of this distracts from the fact that no sponsor would go to the expense of funding a large international Phase III trial, and the relevant regulatory authorities would not approve such studies unless there were good underlying scientific reasons for running the trial which equate with a reasonable expectation of the trial being successful.

7.43. In sum, even if the skilled person had been aware of the content of D24 at the relevant date (which of course s/he was not) the skilled person would still have understood that other evidence must have existed to justify the financial expense and medical risks associated with carrying out such a Phase III trial. The skilled person would equate that evidence with a reasonable expectation that the trial would be successful.

Declaration of Dr Amy McKee (D23)

7.44. As stated above, we do not believe that this Declaration should be admitted because it is late filed and concerns issues that could have been addressed (if felt relevant) during first instance proceedings. Moreover, whilst D23 does attempt to deal with some of the regulatory issues on which the OD based its decision it does so only from the perspective of the United

States when in fact the trial at issue in D15b was carried out in over 10 different countries. As such, the relevance of the Declaration is also called into question.

7.45. In paragraphs 14-20 Dr McKee discusses the role of the *clinicaltrials.gov* website. However, as a general point, the issue is not the website itself, or the role of the NLM in administering the website. The key point is that at the relevant date it was known to the skilled person that a Phase III clinical trial was underway involving over 400 patients with gemcitabine-refractory PC in over 70 sites in more than 10 different countries. The *clinicaltrials.gov* website is just one source of information concerning that trial, another would be similar websites hosted in other countries where the NAPOLI-1 trial had a centre, D15c is enclosed as an example but there are others such as in Australia (www.anzctr.org.au/). Similarly, in October 2012 review article D28 disclosed that "*Merrimack is currently recruiting participants for a randomized, open label Phase 3 study MM-398 with or without 5-fluorouracil and leucovorin, versus 5-fluorouracil and leucovorin in patients with metastatic pancreatic cancer who have failed prior gemcitabine-based therapy*."

7.46. We take issue with Dr McKee's view in paragraph 15 that "*generally speaking, an oncologist would not consider clinicaltrials.gov to be a reliable source of information as to which experimental drugs and dosage regimens are promising therapies*." In contrast, the fact that a therapy is undergoing the final stages of clinical investigation (Phase III) would be of significant interest to a clinician in a relevant field. That is why *clinicaltrials.gov* lists "health care professionals and researchers" as a key audience. It is also why the scientific literature reports that such trials are underway, for example D32 mentions the *"Ongoing studies with this agent [MM-398] include a phase III monotherapy trial in gemcitabine-refractory PC,"*, and D28 states that "*Merrimack is currently recruiting participants for a randomized, open label Phase 3 study MM-398 with or without 5-fluorouracil and leucovorin, versus 5- fluorouracil and leucovorin in patients with metastatic pancreatic cancer who have failed prior gemcitabine-based therapy*." These documents are not aimed at patients who may have an interest in joining a trial but clinicians and researchers who know that being in clinical development (especially Phase III) whilst not providing any guarantees does provide a reasonable prospect of success.

7.47. It is unclear what is meant by several of the terms used by Dr McKee in paragraph 22. e.g. the terms " a high level of efficacy and safety", and "...even if the chances of success are low, objectively speaking."

7.48. It is, however, agreed that gemcitabine-refractory PC has a poor prognosis and that such patients have few treatment options. That being the case, we agree that a clinical trial may be proposed and approved where the expected improvements are not at a high level but only modest, objectively speaking. However, in these specific circumstances, where the patient group is so poorly served, such modest improvements would be considered as a success.

7.49. Thus, it remains the case that a trial sponsor would not invest the very large sums of money necessary to conduct a Phase III trial and nor would the ethics committees and regulatory authorities, that oversee human clinical studies, allow the trial to proceed without a reasonable expectation of (that modest) success.

7.50. In paragraph 24 of her Declaration, Dr McKee states that Arm C was added to the record for clinical trial NCT01494506 on 25 January 2013. However, the existence of the trial was published elsewhere even earlier, see D15c and D28.

7.51. In paragraph 25, Dr McKee states that although ethical (IRB)[10] approval would be required to add Arm C to the clinical study it would not have been mandatory for the sponsor or investigators to liaise with the FDA, or any other regulatory body regarding this new treatment arm, and that the trial could have started without regulatory approval. This is not correct, at least outside of the US.

7.52. The NAPOLI-1 study was an international study carried out in more than 10 countries including a number of European countries, such as the UK. D15c is the entry for the NAPOLI-1 study from the EU clinical trials register for the UK, archived on 12 October 2012. As can be seen from the summary section of the document, the entry on the register was created on 30 March 2012 under EudraCT Number: 2011-004687-30 and Sponsor's Protocol Code Number: MM-398-07-03-01. The title of the trial is "*A study of MM-398 with or without 5-Fluorouracil and Leucovorin compared with 5- Fluorouracil and Leucovorin in Patients with advanced pancreatic cancer who were previously treated unsuccessfully with a gemcitabine-based therapy.*" The National Competent Authority is the UK's MHRA (Medicines and Healthcare products Regulatory Agency). From section N of the document it can be seen that the trial separately received both ethics committee (IRB) approval and competent authority approval during 2012.

7.53. The MHRA grants permission for clinical trials to be conducted in the UK in accordance with the Medicines for Human Use (Clinical Trials) Regulations (MHCTR) 2004 and 2006. Part 3, sections 12(1) and 12(3) of the MHCTR 2004 are reproduced below:

"*Requirement for authorisation and ethics committee opinion*
12. (1) No person shall-
(a) start a clinical trial or cause a clinical trial to be started; or
(b) conduct a clinical trial,
unless the conditions specified in paragraph (3) are satisfied.
...
(3) The conditions referred to in paragraphs (1) and (2) are-
(a) an ethics committee or an appeal panel appointed under Schedule 4 has given a favourable opinion in relation to the clinical trial; and
(b) the clinical trial has been authorised by the licensing authority."

7.54. Thus, before a clinical trial can start in the UK, both ethics committee (IRB) approval and licensing authority approval is required. It is not certain whether the UK part of the NAPOLI-1 trial was initially approved with three Arms or whether the trial was subsequently amended to add Arm C. In any event, the addition of Arm C would have constituted a substantial amendment to the protocol and required both ethics committee (IRB) approval and licensing authority approval, see MHCTR 2004, Part 3, section 24 (D36).

[10] The task of an Institutional Review Board (IRB) in the US is ethical oversight which is a prerequisite for every clinical study according to Good Clinical Practice. In Europe, these entities are called Ethics Committees.

7.55. Accordingly, in contrast to Dr McKee's opinion it is clear that in at least some countries in which the NAPOLI-1 trial was conducted, licensing approval from the competent health authority as well as ethics committee (IRB) approval is required before the trial can start or before any substantial amendments to the trial can be implemented.

7.56. In Paragraphs 26-29, Dr McKee opines on the factors that an IRB (ethics committee) in the United States would consider when deciding whether to approve the NAPOLI-1 trial. Dr McKee argues that the risk/benefit analysis in the patient group in question would sway the IRB to approve the trial even if the expectation of success was low, provided that the risk to the patient safety would not be excessively high.

7.57. However, Dr McKee's opinions on these issues are of limited relevance to this case.

7.58. Firstly, Dr McKee's views are only relevant to the United States – she has no apparent experience of the assessments that would be made in other countries before the trial could start, such as in the ten plus other countries in which the NAPOLI-1 trial was run. Indeed, Dr McKee does not know that in some countries in which the trial is run (e.g. the UK) both ethical committee (IRB) and licensing authority approval is required before a clinical trial can start or be substantially amended.

7.59. Secondly, Dr McKee is a clinician with experience of approving (or not approving) applications for the marketing of drugs. The test of whether a marketing application is successful is very different to the test of whether the technical problem in the problem-solution approach has been solved. In other words, when Dr McKee states that there would have been "*no reasonable expectation that Arm C would be successful*" Dr McKee is referring to the successful outcomes necessary to facilitate the granting of a marketing application for that treatment regimen. In contrast, whether the technical problem to be solved in this case, which has been formulated along the lines of providing a safe and effective treatment for gemcitabine-resistant PC, is a much lower threshold than obtaining marketing approval. Consequently the reasonable expectation of successfully solving the technical problem will be much higher than that contemplated by Dr McKee.

7.60. Thirdly, although Dr McKee was shown D15a and D15b, and informed of the subject matter of the Patent,[11] it does not appear that Dr McKee was given any other information on the clinical development of the combination of irinotecan, leucovorin and 5-FU in treating gemcitabine-refractory PC. In particular, Dr McKee's was not shown D2-D6, D7, D12, D13, D22 and D29 which describe the effectiveness of the combination of irinotecan (liposomal and non-liposomal), leucovorin and 5-FU in treating solid tumours, including PC. Had Dr McKee been given the opportunity to review these documents her opinion on the reasonable prospect of success may have been very different.

7.61. In summary, in the unlikely event that D23 is admitted it does not change the fact that it was well-known at the relevant date to the skilled person that the NAPOLI-1 trial was underway (D15 and D28), that the cost of those trials to the sponsor (Merrimack Pharmaceuticals) would be very high and that in at least some countries both ethical and licensing authority approval would be required before the trials could start. Thus, whilst recognising the unmet

[11] Paragraphs 4-6 of her Declaration.

medical need in the field of treating PC, the skilled person would not believe that the required financial support would be available nor the requisite regulatory approvals granted without a reasonable prospect of success.

7.62. Overall, therefore, we believe that starting from D15b the skilled person would have had a reasonable expectation of success for the reasons given by the OD in its Decision and set out above in paragraphs 7.25-7.32.

7.63. Even if admitted documents D23 and D24 do not change this because they do not reflect the knowledge of the skilled person at the relevant date, they do not take account of the economic costs of conducting such a Phase III trial, they do not consider that the measure of "success" in solving the technical problem at issue is different from that of successfully obtaining approval from the FDA to market a drug, and they do not consider any regulatory issues concerning the conduct of clinical trials outside of the US, which was only one of the 10 plus counties in which the NAPOLI-1 trial was conducted.

There is no teaching away

7.64. In paragraphs 5.88-5.96 of its Grounds of Appeal, the PP refers to T0239/16 and argues that the available art would have dissuaded the skilled person from believing that there was a reasonable expectation of success starting from D15b. Most of these issues were dealt with by the OD, see pars. 5.7.3-5.7.5 of its Decision.

7.65. The relevant passage from T0239/16 relied on by the PP is reproduced below:

> "The board considers that the mere fact that an active agent selected from the group of bisphosphonates is being tested in a clinical study for the treatment of osteoporosis (as disclosed in document (55)) leads to an expectation of success, due to the fact that clinical studies are based on data obtained by preclinical testing both in vitro and in animals and require authority approval which takes ethical considerations into account. This means in the present case that the skilled person would expect all study arms to treat osteoporosis effectively, unless he was dissuaded from this by the prior art." (reasons 6.4, par. 2; emphasis added)

7.66. Thus, the case law states that in light of an ongoing / approved clinical trial, such as NAPOLI-1, the skilled person would have a reasonable expectation of success unless dissuaded otherwise.

7.67. In this regard, in paragraph 5.93 of its Grounds of Appeal, the PP raises a new point: that D22 reports that the MTD for MM-398 in combination with leucovorin and 5-FU is 80 mg/m^2 (Q3W) and argues that knowing this the skilled person would seriously doubt whether the dose of MM-398 in D15b of 80 mg/m^2 (Q2W) is safe.

7.68. However, it seems highly unlikely that this single disclosure concerning such a small study (only 5 subjects received more than 80 mg/m^2 (Q3W)) in patients with a variety of solid tumours would be sufficient to dissuade the skilled person of the safety of a dosing regimen intended for a particular sub-set of patients (gemcitabine-refractory) with one particular type of cancer, i.e. PC. This is especially the case since at the relevant date the NAPOLI-1 study

had already received both ethical and licensing authority approval in multiple countries for administering MM-398 at a dose of 80 mg/m^2 (Q2W) in that particular sub-set of patients with PC. In this regard, the PP's argument appears to contradict the position of Dr McKee who states that in approving the NAPOLI-1 trial in the US the IRB's "*primary consideration would be patient safety and whether the new arm risks safety in anyway*."

7.69. Moreover, the biweekly (Q2W) MTD of MM-398 monotherapy was known to be 100 mg/m^2 (D30) and thus the OD's argument that when combining with other drugs it is common to lower the dose appears to hold true, see page 17, lines 5-6 of the OD's Decision.

7.70. In paragraph 5.96 of its Grounds of Appeal, the PP reiterates its point about combining MM-398 and 5-FU both of which are known to cause diarrhoea. This point seems to have been dealt with by the OD in its Decision, see par. 5.74. In particular, it seems highly improbable that this issue about possible cumulative side effects would be sufficient to dissuade the skilled person of the safety of a Phase III trial that had both ethical and licensing authority approval in multiple countries. Particularly, since these actives had been administered together previously on many occasions, in both liposomal and non-liposomal form, see D2-D6, D7, D12, D13, D22 and D29.

7.71. In particular, the PP refers to whether "all things being equal" the skilled person would be inclined towards monotherapy to avoid potential cumulative side effects. This is not the test applied by the Board in T0239/16 and relied on by the PP. The PP has provided no evidence that the skilled person would be dissuaded from having a reasonable expectation of success with Arm C. Indeed, the PP acknowledges that regimens comprising the co-administration of irinotecan, leucovorin and 5-FU had been used in the prior art. Moreover, things are not equal: The dose of MM-398 in Arm A is higher (albeit less frequent).

D15b inherently discloses liposomal irinotecan

7.72. At the oral proceedings before the OD, the PP raised a new argument, namely that D15b only discloses "MM-398" as part of the dosage regimen in Arm C but not for what "MM-398" stands. In particular, the PP argued, for the first time, that there are no documents on file identifying "MM-398" with "irinotecan sucrose octasulfate salt liposome injection" according to claim 1 of the MR. This argumentation is continued in paragraphs 5.48-5.50 of its Grounds of Appeal.

7.73. By way of background, it is important to recognise that identifying active ingredients by a code, such as MM-398 or CPT-11[12], or a shorthand name, such as liposomal irinotecan (CPT-11), or a generic name, such as leucovorin, and the continued use of that code and/or assigned name when publishing information about the active ingredient is very common in the pharmaceutical industry.

7.74. The code and/or assigned name are all similar, however, in that they confer no technical information about the active ingredient; this can only be obtained by using reference materials to identify the chemical structure and known therapeutic properties etc. of the active ingredient at issue. However, such a process of obtaining technical information about an

[12] CPT-11, another name for irinotecan, it is short for camptothecin-11 and signifies that irinotecan is a derivative of the naturally occurring alkaloid camptothecin, see Patent [0003].

active ingredient identified only by a code or assigned name would be very familiar to the person skilled in the art.

7.75. Thus, in the present case, the OD was right to conclude (see Decision at page 12, penultimate paragraph) that D15b inherently discloses a nanoliposomal formulation of irinotecan because this information about the identity of MM-398 could readily be obtained from the prior art, e.g. D13, heading on page 188, col. 2; page 189, col. 2; and Table 1 on page 193.

7.76. Indeed, at the relevant date it was very well-known that "MM-398" (formerly designated PEP02) is a nanoliposomal formulation of irinotecan in clinical development with Merrimack Pharmaceuticals. Not only is this information in D13, as noted by the OD, similar information can be found in other documents already on file during the first instance proceedings, such as D7, D12 and D22.

7.77. However, in case there is any doubt that at the publication date of D15b the nature of MM-398 was not well-known the following additional papers are included.

7.78. D28 is a review article describing the clinical use of nanomedicines in treating cancer. The review again describes that MM-398 and PEP02 are synonyms. That MM-398 is a "liposomal formulation of irinotecan" that is in development with Merrimack Pharmaceuticals (successor to Hermes Biosciences) and PharmaEngine, see page 3, col. 1, lines 214-229. D28 further describes that MM-398 in combination with 5-FU and leucovorin is in Phase III development in patients with metastatic PC who have failed prior gemcitabine-based therapy, see page 3, sentence bridging cols. 1 and 2, and Table 1, entry 9 which cross-references D15.

7.79. D29 is a report from a Symposium organised by the American Society of Clinical Oncology into gastrointestinal cancers, particularly treatments for refractory PC. In the section entitled "*What Did We Learn at the 2011 ASCO GI Cancer Symposium?*" (page 111, col. 1) the authors report on a presentation given by Dr Ko at the symposium detailing the clinical development of PEP02, identified as a "nanoparticle liposome formulation of irinotecan", in treating refractory PC both alone and in combination with 5-FU and leucovorin, also see Table 1, central column.

7.80. D30 describes a Phase I clinical study using a "nanoliposomal formulation of irinotecan" (identified as MM-398 and PEP02) to treat patients with metastatic colorectal cancer who had failed first line therapy.

7.81. D31 describes the results of a Phase II study involving a "nanoparticle liposome formulation of irinotecan" (identified as PEP02) as second line therapy in gastric or gastroesophageal (GEJ) adenocarcinoma.

7.82. D32 describes MM-398 as "liposomal irinotecan" where the encapsulation of irinotecan in polyethylene glycol-liposomes enhances pharmacokinetic parameters. It also reports on Phase I studies in solid tumours and ongoing Phase II and III studies, including studies in gemcitabine-refractory PC.

7.83. Moreover, it was also known at the relevant date that MM-398 is a liposomal irinotecan sucrose octasulfate salt formulation, as described in claim 1 of the Main Request.

7.84. Thus, D25 is a paper co-authored by four employees of Merrimack Pharmaceuticals including D.C. Drummond. The paper describes studies with liposomal irinotecan (MM-398) in treating glioblastoma.[13] On page 198, col. 2, the paper describes that "*Nanoliposomal irinotecan (MM-398) is a highly stabilized liposomal formulation containing nano-sized irinotecan crystals complexed with sucrose octasulfate in the liposome interior*[15]".

7.85. Thus, at the relevant date, it was already known that MM-398 is a liposomal irinotecan sucrose octasulfate salt formulation. Moreover, it appears that Merrimack Pharmaceuticals did not consider this information confidential since it generously provided the authors of D25 with supplies of MM-398 and allowed details of its composition to be published.

7.86. Moreover, reference 15, in the passage from D25 quoted above, is from 2006 (>6 years before the priority date) and is enclosed as D26. This paper is also authored by D.C. Drummond of Hermes Biosciences (predecessor of Merrimack Pharmaceuticals) and describes the preparation of nanoliposomal irinotecan formulations using sucrose octasulfate.

7.87. D33 is another paper co-authored by D.C. Drummond of Hermes Biosciences dating from 2006. Again this paper (which is also cited in D25) demonstrates that Hermes' nanoliposomal CPT-11 (irinotecan) uses a sucrose octasulfate-based method for intraliposomal loading and stabilization to provide superior drug delivery, see page 2806, col. 1, final complete sentence. Similarly, D34 is further paper co-authored by D.C. Drummond of Hermes Biosciences which was published shortly after in 2007. Again this paper is also cited in D25 and demonstrates that the Hermes' nanoliposomal CPT-11 (irinotecan) uses a sucrose octasulfate-based method for intraliposomal loading and stabilization, see page 394, col. 2.

7.88. D27 is a paper describing an international study involving clinicians from six countries using MM-398 to treat Oesophago-gastric (OG) cancer. The authors include employees of PharmaEngine, Inc. (onetime co-developers of MM-398) who was involved in funding of the study. The paper describes that MM-398 and PEP02 are synonyms and that MM-398 is a liposomal formulation of irinotecan that is in development with Merrimack Pharmaceuticals, Inc. (see title; page 1568, col. 1, par. 2; page 1571, col. 2, par. 1; and page 1573, col. 1, lines 3-4). In particular, D27 states that "*PEP02, also known as MM-398 (Merrimack Pharmaceuticals, Inc.), is a highly stable liposomal nanocarrier formulation of irinotecan hydrochloride (CPT-11) [11]*". Reference 11 in this passage is D26 mentioned above as describing the preparation of nanoliposomal irinotecan formulations using sucrose octasulfate.

7.89. Thus, there should be no doubt that at the relevant date the identity of MM-398 was well-known to the skilled person and that therefore D15b implicitly discloses liposomal irinotecan which is irinotecan sucrose octasulfate liposome injection. It should also be noted that this situation is similar to the approach adopted by the OD in finding that the Patent sufficiently

[13] D13 also describes positive results with MM-398 in glioma models; see page 189, col. 1, final complete paragraph.

discloses the treatment of gemcitabine-refractory PC by relying on the "*convergent and consistent disclosure*" of documents D3, D5 and D6 as evidence of the common general knowledge at the relevant time, see OD's Decision, at par. 3.3.6.

7.90. However, in the event that the Board considers that the common general knowledge only acknowledges MM-398 as a liposomal form of irinotecan and not necessarily as "liposomal irinotecan which is irinotecan sucrose octasulfate liposome injection", the claimed subject matter still lacks an inventive step. In particular, as noted by the OD in paragraph 5.7.8 of its Decision, there is no evidence to suggest that "liposomal irinotecan which is irinotecan sucrose octasulfate liposome injection" is anything other than an alternative form of liposomal irinotecan. As such, the choice of "liposomal irinotecan which is irinotecan sucrose octasulfate liposome injection" as disclosed in D25, D26, D33 and D34 appears arbitrary.

*Different dosing based UGT1A1*28 allele status*

7.91. As mentioned above, we do not believe that the skilled person would realistically consider the differential dosing, based on whether or not a patient is homozygous for this allele, as a distinguishing feature over D15b. However, if this is considered a distinguishing feature the following points are noted.

7.92. Example 6 of the Patent does not teach differential dosing for patients based on their UGT1A1*28 allele status and this may have implications for Article 83 EPC as outlined in the sufficiency of disclosure section above.

7.93. In the alternative, it was already common general knowledge at the relevant date to reduce the dose of irinotecan in patients who are homozygous for the UGT1A1*28 allele, see D10, section 2.3, D11, page 1290, col. 2, lines 2-7, and paragraph [0038] of the Patent. Indeed, although D15b does not mention the UGT1A1*28 allele, patients in the trial were in fact tested for the allele and 7 out of the 117 patients in Arm C were found to be homozygous, see D19, page 5, col. 2. For these patients, the initial standard dose (80 mg/m^2) was reduced to 60 mg/m^2 and then increased after the first cycle to the standard dose, see D19, page 3, col. 1, lines 12-16.

7.94. As such, to the extent it is a distinguishing feature over D15b, it would have been obvious to reduce the initial dose of MM-398 in patients who are homozygous for the UGT1A1*28 allele based on common general knowledge alone.

More ambitious technical problem not appropriate

7.95. In paragraph 5.72 of its Grounds of Appeal the PP argues that a more ambitious technical problem may be formulated starting from Arm C of D15b, namely "*the provision of a safe and effective treatment for gemcitabine-resistant pancreatic cancer which is improved relative to the monotherapy arm of D15b.*" This is clearly wrong because it is very well-established law that the technical problem is framed with respect to the closest prior art embodiment, i.e. Arm C (see OD's Decision at par. 5.7.2 and various passages throughout CLBA-I.D.4).

Conclusions

7.96. To summarise:

- Arm C of D15b is a feasible closest prior art disclosure because it concerns the same purpose as the Patent and requires a minimum of structural and functional modifications to arrive at the claimed subject matter, i.e. Arm C teaches a substantially identical dosing regimen to that claimed for the treatment of the same disease as recited in the claims.

- The only distinguishing features are the absence of concrete results in D15b and the lack of a specific disclosure of the order of drug administration, although the claimed order of administration is obvious based on common general knowledge. The identity of MM-398 was well established at the relevant date and it's inconceivable that all of the patients in Arm C were homozygous for the UGT1A1*28 allele, i.e. outside of the claim scope.

- The OD was right to conclude that starting from D15b there was a reasonable expectation of success in providing "*a safe and effective treatment for gemcitabine-resistant PC*." This conclusion is supported by the established case law (T0239/16 and T2506/12) as well as the technical background provided by documents D2-D6, D7, D12, D13, D22 and D29.

- The new evidence supplied by the PP is inadmissible as being late filed and not relevant. In particular, D24 does not reflect the knowledge of the skilled person at the relevant date and even if admitted D23 does not negate the reasonable expectation of success that the skilled person would have had in view of the extensive information available about the NAPOLI-1 trial which involved over 400 patients in at least 10 countries (D15 and D28).

7.97. As such, the claimed subject matter is obvious over the knowledge of the NAPOLI-1 trial as embodied in D15.

D12 as Closest Prior Art

7.98. We disagree with the PP in paragraph 5.3 of its Grounds of Appeal that D12 should not be considered as a closest prior art document because D13 is closer to the claimed subject matter. As explained above in relation to D15b, the correct test is whether D12 is a feasible or suitable starting point and the fact that D13 may (or may not) be "closer" is irrelevant.

7.99. We also disagree with the PP that the only embodiment to be considered in D12 (or D13) is that relating to MM-398 monotherapy and that the information concerning the combination therapy should be ignored, see pars. 5.7-5.10 of its Grounds of Appeal.

7.100. In this regard D12 discloses that the combination of MM-398, leucovorin and 5-FU is efficacious in treating gemcitabine-refractory PC. In particular, D12 states that "*In previous phase I studies, PEP02 [MM-398] either alone or in combination with 5-FU/LV demonstrated*

prolonged disease control in 5 of 7 (71%) patients (pts) with gemcitabine (GEM)-refractory advanced pancreatic cancer (PC)." (emphasis added)

7.101. Whilst the Phase I results for MM-398 monotherapy and combination therapy are pooled, it is clear from the language used in D12 (underlined) that both therapies were effective.

7.102. Thus, this embodiment of D12 is clearly directed to the same purpose as the Patent, i.e. the treatment of gemcitabine-refractory PC and has the most features in common with the claimed subject matter, i.e. it relates to the same three drugs specified in the Patent.

7.103. Moreover, whilst it is true that D12 as a whole focuses more on the Phase II study concerning MM-398 monotherapy this does not detract from the positive results reported with the combination therapy. There is no reason why the skilled person would not consider these positive results in human Phase I studies as significant.

7.104. In particular, as noted by the Board in T2506/12, reasons 3.10, human clinical studies are usually only commenced when there is already favourable existing scientific data. Therefore, in the case of D12, the skilled person would reasonably expect that there is underlying scientific data which formed the basis of the decision to commence human clinical studies with MM-398, leucovorin and 5-FU in the first place. This is in addition to the positive results in Phase I clinical studies disclosed in D12 itself. Such human clinical data would not be ignored by the skilled person. To the contrary, it is amongst the best type of evidence that is available in medical research. In other words, it is not a question of which one of the disclosures in D12 the skilled person would consider, the treatment with MM-398 monotherapy or the combination treatment with MM-398, leucovorin and 5-FU, there is no reason why the skilled person would not consider both of these relevant disclosures.

7.105. Starting from the disclosure in D12 of MM-398 combination therapy the technical problem may be formulated as the provision of an effective dosage regimen for the combination of MM-398, leucovorin and 5-FU in patients with recurring or persistent gemcitabine-refractory PC.

7.106. As discussed above under the heading of sufficiency of disclosure, there is no evidence in the Patent that this problem had been plausibly solved at the relevant date. The only potentially relevant information provided by the Patent is the dose of MM-398 (80 mg/m^2). However, there is no actual evidence in the Patent concerning: the two weekly dosing cycle for any of the drugs (including MM-398); the dose of leucovorin; the dose of 5-FU; the order of drug administration; that the PC is recurrent or persistent following primary chemotherapy; that the PC is gemcitabine refractory; or that the initial dose of MM-398 is dependent on UGT1A1*28 allele status.

7.107. As there is no plausible disclosure in the Patent that the technical problem has been solved, post-published evidence cannot be used as the sole basis for evidencing that the technical problem has been solved (CLBA-I.D.4.6). Thus, the technical problem must be formulated less ambitiously along the lines of providing an alternative or arbitrary dosing regimen and therefore the claimed subject matter clearly lacks an inventive step.

7.108. However, in the event that the Board considers that the more ambitious problem is solved, and that the Patent meets the requirements of Article 83 EPC, the claimed subject matter still lacks an inventive step.

7.109. At the relevant date, the dose of MM-398 in treating gemcitabine-refractory PC had already been investigated. D7, D13 and D30 report that the MTD for MM-398 monotherapy is 120 mg/m^2 (Q3W) or 100 mg/m^2 (Q2W). D29 reports positive results from Phase II studies in which patients with gemcitabine-refractory PC were administered MM-398 at an initial dose of 120 mg/m^2. D3 and D5 describe positive studies with a combination of non-liposomal irinotecan, leucovorin and 5-FU in treating gemcitabine-refractory PC using a bi-weekly dosing regimen also involving 400 mg/m^2 of leucovorin and 1000 or 2000 mg/m^2 of 5-FU. D22 describes a clinical study involving MM-398 in combination with 5-FU and leucovorin in treating patients with advanced solid tumours who had failed standard chemotherapy. MM-398 was administered at doses of 60, 80 100 and 120 mg/m^2, leucovorin was administered at 200 mg/m^2 and 5-FU was administered at 2000 mg/m^2. The MTD of MM-398, when given together with leucovorin and 5-FU, was determined to be 80 mg/m^2. D15b reports a Phase III study in patients with gemcitabine-refractory PC being administered MM-398 at an initial dose of 80 mg/m^2 together with leucovorin (400 mg/m^2) and 5-FU (2400 mg/m^2).

7.110. Moreover, in this regard, the Boards have previously noted that finding the optimum dosage is a matter of routine experimentation, which does not require inventive skill, see T1409/06, reasons 3.2.1, par. 2, and T0237/15, reasons 4.6.1, par. 2. The Boards have also addressed the routine nature of optimizing combination dosing regimens, see T2506/12 and the discussion of inventive step in relation to the auxiliary requests.

7.111. For issues concerning the identity of MM-398, the order of drug administration, and the differential dosing based on UGT1A1*28 allele status, see our comments above in paragraphs 7.72-7.90, 7.30-7.31, and 7.91-7.94, respectively.

7.112. As such, based on the technical information already available in the prior art, and supported by the established case law, it would have been obvious to optimise the dosing regimen of the drug combination already disclosed in D12 as effective in treating gemcitabine-refractory PC. In other words, starting from D12 the skilled person would have solved even the more ambitious technical problem mentioned above using only routine skill.

D13 as Closest Prior Art

7.113. The claimed subject matter also lacks an inventive step over D13 for reasons similar to those given above in relation to D12.

7.114. There does not appear to be any dispute that D13 is a suitable closest prior art document. However, the PP does argue that the skilled person would only consider the disclosure relating to MM-398 monotherapy and ignore the clinical data relating to the combination of MM-398, leucovorin and 5-FU in treating gemcitabine-refractory PC.

7.115. Particularly, in paragraph 5.8-5.10 of its Grounds of Appeal the PP argues that on reading the relevant passage in D13 the skilled person would ascertain that the best response from the seven patients tested in the study was with one patient who had a partial response with MM-

398 monotherapy. The PP also argues that the skilled person would note that MM-398 is to be the subject of a Phase II study but that there is no mention of the combination regimen being the subject of a Phase II study. The PP therefore concludes that only MM-398 monotherapy would be considered for further development by the skilled person.

7.116. We disagree. For reasons similar to those discussed above in connection with D12, the skilled person would not disregard the positive Phase I clinical data for the combination regimen. This type of data in human subjects is always significant. That said, the skilled person would not conclude that the better response seen in one patient in the monotherapy arm is evidence of superiority – it could also be that both progressive disease results were also in the monotherapy arm. Similarly, the skilled person would not consider the reference to Phase II studies in connection with monotherapy as evidence that the combination therapy was inadequate.

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

7.118. Particularly, the PP's comments concerning inferences the skilled person would draw from the lack of any report in D13 of a Phase II study with MM-398 combination therapy are both speculative and wrong. By the relevant date it had been demonstrated that MM-398 combination therapy showed sufficiently promising results in earlier studies to have been approved for human Phase III studies, see D15b. Similarly, D22 demonstrates that at the relevant date the combination (including 80 mg/m^2 of MM-398) had shown efficacy and acceptable safety in patients with refractory solid tumours.

7.119. A more accurate explanation of why D13 describes taking monotherapy into Phase II and not the combination therapy is simply that the combination therapy was behind monotherapy in terms of development timelines. This is apparent from D13 itself (page 198, col. 2) which describes that "*The observation [efficacy with monotherapy] was further extended in a phase I trial for nanoliposomal CPT-11 in combination with weekly 24-hour infusion of high-dose 5-FU/LV (HDFL).*", i.e. that the monotherapy trial was completed before the combination therapy trial had even started. Similarly, in December 2011 Phase III studies comparing MM-398 monotherapy against leucovorin and 5-FU were announced and subsequently during 2012 the combination treatment Arm C was added to the Phase III study (see D15a vs D15b).

7.120. Therefore, the disclosure in D13 that the combination of MM-398, leucovorin and 5-FU is efficacious in treating gemcitabine-refractory PC represents a feasible starting point for the inventive step analysis. It certainly fulfils the criteria of being "*that [disclosure] which corresponds to a similar use and requires the minimum of structural and functional modifications to arrive at the claimed invention (see T 606/89)*", GL (G-VII, 5.1).

7.121. Starting from this disclosure in D13 the problem solution analysis is essentially the same as that described above for D12.

D5 as closest prior art

7.122. As noted above, D5 describes a Phase II study in patients with PC who had failed first line gemcitabine based chemotherapy (page 1659, col. 2). The patients were treated according to a modified FOLFIRI.3 (mFOLFIRI.3) regimen which consisted of (non-liposomal) irinotecan, leucovorin 400 mg/m^2 and 5-FU and 2000 mg/m^2 every two weeks (page 1659, col. 2). The results describe the regimen as safe and effective.

7.123. Thus, the differences between the claimed subject matter and the disclosure of D5 is (i) the use of MM-398 instead of (non-liposomal) irinotecan, (ii) the dose of MM-398, and (iii) the slightly different dose of 5-FU. For issues concerning the identity of MM-398 and the differential dosing based on UGT1A1*28 allele status, see our comments above in paragraphs 7.72-7.90 and 7.91-7.94.

7.124. There is no direct comparison between the mFOLFIRI.3 regimen of D5 and the regimen claimed in the Patent and therefore the technical problem should be formulated as providing an alternative treatment regimen for gemcitabine-refractory PC. However, even if a more ambitious problem is formulated the claims still lack an inventive step.

7.125. As regards point (i), at the relevant date it was already known that MM-398 had activity against gemcitabine-refractory PC (e.g. D12 and D13) and that MM-398 was superior to the free form of irinotecan in terms of improved pharmacokinetics and tumour distribution, see D7, D12, D13, D22, D29 (page 111, col. 1), D30, D31 and D32 (page S388, col. 1).

7.126. As such, it would have been obvious to replace the (non-liposomal) irinotecan in D5 with MM-398 in the reasonable expectation of solving even a more ambitious technical problem.

7.127. As regards point (ii), at the relevant date the dose of MM-398 in treating gemcitabine-refractory PC had already been investigated. D7, D13 and D30 report that the MTD for MM-398 monotherapy is 120 mg/m^2 (Q3W) or 100 mg/m^2 (Q2W). D29 reports positive results from Phase II studies in which patients with gemcitabine-refractory PC were administered MM-398 at an initial dose of 120 mg/m^2. D22 reports that the MTD of MM-398 when given together with leucovorin and 5-FU is 80 mg/m^2. D15b reports a Phase III study in patients with gemcitabine-refractory PC being administered MM-398 at an initial dose of 80 mg/m^2 together with leucovorin (400 mg/m^2) and 5-FU (2400 mg/m^2).

7.128. As such, it would have been obvious to use MM-398 at a dose of 80 mg/m^2 in modifying the teaching of D5.

7.129. As regards point (iii), it has not been shown that this difference has any technical effect on the treatment outcome. In any event, it was already known to use the claimed dose of 5-FU (2400 mg/m^2) in combination with MM-398 and leucovorin in treating gemcitabine-refractory PC, see D15b. Hence, it would have been obvious to modify the dose of 5-FU disclosed in D5 to that known to be used with MM-398.

7.130. However, to the extent that the prior art does not explicitly teach the doses of MM-398 and 5-FU to be used in modifying the teaching of D5, the claims still lack an inventive step.

7.131. In this regard, finding the optimum dosage is a matter of routine experimentation, which does not require inventive skill, see T1409/06, reasons 3.2.1, par. 2, and T0237/15, reasons 4.6.1, par. 2. The Boards have also addressed the routine nature of optimizing combination dosing regimens, see T2506/12 and the discussion of inventive step in relation to the auxiliary requests.

7.132. Accordingly, the claims of the Main Request also lack an inventive step starting from D5.

8. AUXILIARY REQUESTS

8.1. We note from the Grounds of Appeal, paragraph 6.1, that AR1 and AR2 are primarily retained as useful fall-back positions in appeal proceedings. In any event, it seems that neither of these requests can be maintained if the MR and AR3 are not valid and hence we will focus our additional comments on AR3.

Auxiliary Request 3

8.2. AR3 includes the additional limitation that "*the patient achieves a response which is at least stable disease.*"

8.3. However, AR3 suffers the same deficiencies mentioned above in relation to Articles 83, 87 and 56 EPC. In particular, we agree with the OD's assessment in paragraph 9.4 of its Decision that the new limitation does not distinguish the claimed subject matter any further from the cited prior art, especially D15b. For instance, in Example 6 of the Patent (at paragraph [0081]) the "overall disease control rate" is reported as being 73.3% which is the sum of the PR and SD response and thus excludes PD responses. In other words the additional feature merely excludes failures from the scope of the claim.

8.4. However, in response to the Grounds of Appeal we note that the PP's definition of "success" now includes "progressive disease" and that this should be considered in determining what is meant by a reasonable expectation of success in respect of the Main Request, as discussed above.

8.5. Moreover, even if the PP is correct and that requiring at least a stable disease response is a further distinction, it does not alter the overall outcome because the skilled person would still have a reasonable expectation of success starting from the cited prior art, especially D15b.

8.6. In particular, the skilled person would reasonably expect that in any cohort of patients suffering from gemcitabine-refractory PC treated with the combination of MM-398, leucovorin and 5-FU a substantial proportion would have at least a stable disease response. In this regard, D2-D6 all report studies in which patients with gemcitabine-refractory PC are treated with a combination of non-liposomal irinotecan, leucovorin and 5-FU (FOLFIRI). D2 reports that 50% of patients had at least a stable disease response (Abstract), D3 reports that 36% of patients had at least a stable disease response (page 1641, col. 2, par. 2), D4 reports that 39.7% of patients had at least a stable disease response (page 4533, col. 2, par. 1), D5 reports that 67% of patients had at least a stable disease response (page, 1660, Table 1),

and D6 reports that 26/40 patients[14] (65%) had at least a stable disease response (page 500, col. 2, par. 1).

8.7. Given this information a skilled person starting from D15b (and knowing that MM-398 is considered superior to the free form of irinotecan in terms of improved pharmacokinetics and tumour distribution, see par. 7.123 above) would have had a reasonable expectation of success, i.e. that a substantial proportion of patients would have at least a stable disease response.

8.8. We also note that D12 reports that 71% of patients receiving MM-398 (PEP02) either alone or in combination with 5-FU/LV demonstrated prolonged disease control. Prolonged disease control would appear to relate to at least a stable disease response. It's difficult to envisage that prolonged disease control could be equated with a 25% growth in tumour dimensions as in the example given by the PP in paragraph 7.6 of its Grounds of Appeal.

8.9. Overall, therefore, AR3 also lacks an inventive step.

[14] "There was one complete response and 14 partial responses. Eleven patients (27.5%) had stable disease. Tumor progression occurred in eight patients (20%) and six patients (15%) were not assessable,.."

Annex - Comparison of Example 6 and D22

	Example 6 (PEP0203)	**D22**
Study	Phase I study involving Liposomal irinotecan + 5FU/LV	Phase I study involving Liposomal irinotecan + 5FU/LV
No. of patients	16	16
Evaluable patients	15	15
PR	2	2
SD	9	9
PD	4	Must be 4
2 PRs	Gastric and breast cancer	Gastric and breast cancer
MTD	80 mg/m2	80 mg/m2
≥Grade 3 AE	18.4%	18.4%
	Fig. 5	**D22**
Cl	0.1164 (**L**/h/m2)	116.4 (**mL**/h/m2)
Vss	2.93 (L/m2)	2.93 (L/m2)
	Fig. 6	**D22**
Cmax	7.98±4.39 (ng/mL)	7.98±4.39 (ng/mL)
AUC	354.77±145 (ng.h/mL)	354.77±145.35 (ng.h/mL)
	Claim	**D22**
Dose of 5-FU	2400 mg/m2	2000 mg/m2
Dose of LV	200 mg/m2 (l-form) 400 mg/m2 (racemate)	200 mg/m2
Cycle	Twice weekly	Q3W
	More Less often	

Summary	
EudraCT Number:	2011-004687-30
Sponsor's Protocol Code Number:	MM-398-07-03-01
National Competent Authority:	UK - MHRA
Clinical Trial Type:	EEA CTA
Trial Status:	Ongoing
Date on which this record was first entered in the EudraCT database:	2012-03-30

Index

A. Protocol Information

A.1	Member State Concerned	UK - MHRA
A.2	EudraCT number	2011-004687-30
A.3	Full title of the trial	NAPOLI 1: A Randomized, Open Label Phase 3 Study of MM-398, with or without 5-Fluorouracil and Leucovorin, versus 5-Fluorouracil and Leucovorin, in Patients with Metastatic Pancreatic Cancer Who have Failed Prior Gemcitabine-based Therapy
A.3.1	Title of the trial for lay people, in easily understood, i.e. non-technical, language	A study of MM-398 with or without 5-Fluorouracil and Leucovorin compared with 5- Fluorouracil and Leucovorin in Patients with advanced pancreatic cancer who were previously treated unsuccessfully with a gemcitabine-based therapy.
A.3.2	Name or abbreviated title of the trial where available	NAPOLI 1
A.4.1	Sponsor's protocol code number	MM-398-07-03-01
A.7	Trial is part of a Paediatric Investigation Plan	No
A.8	EMA Decision number of Paediatric Investigation Plan	

B. Sponsor Information

B.Sponsor: 1		
B.1.1	Name of Sponsor	Merrimack Pharmaceuticals Inc.
B.1.3.4	Country	United States
B.3.1 and B.3.2	Status of the sponsor	Commercial
B.4 Source(s) of Monetary or Material Support for the clinical trial:		
B.4.1	Name of organisation providing support	Merrimack Pharmaceuticals
B.4.2	Country	United States
B.5 Contact point designated by the sponsor for further information on the trial		
B.5.1	Name of organisation	MERRIMACK PHARMACEUTICALS, INC
B.5.2	Functional name of contact point	Merrimack MM398 Clinical Trial Mger

B. Sponsor Information		
B.5.3	Address:	
B.5.3.1	Street Address	One Kendall Square, Suite B7201
B.5.3.2	Town/ city	Cambridge,
B.5.3.3	Post code	MA 02139
B.5.3.4	Country	United States
B.5.4	Telephone number	001617441-1000
B.5.5	Fax number	001617812-7776
B.5.6	E-mail	clinical@merrimackpharma.com

D. IMP Identification		
D.IMP: 1		
D.1.2 and D.1.3	IMP Role	Test
D.2	Status of the IMP to be used in the clinical trial	
D.2.1	IMP to be used in the trial has a marketing authorisation	No
D.2.5	The IMP has been designated in this indication as an orphan drug in the Community	Yes
D.2.5.1	Orphan drug designation number	EU/3/11/933
D.3 Description of the IMP		
D.3.1	Product name	Nanoliposomal Irinotecan
D.3.2	Product code	MM-398
D.3.4	Pharmaceutical form	Concentrate for solution for infusion
D.3.4.1	Specific paediatric formulation	No
D.3.7	Routes of administration for this IMP	Intravenous use
D.3.8 to D.3.10 IMP Identification Details (Active Substances)		
D.3.8	INN - Proposed INN	Irinotecan Hydrochloride
D.3.9.1	CAS number	136572-09-3
D.3.9.3	Other descriptive name	Nanoliposomal Irinotecan
D.3.9.4	EV Substance Code	SUB02772MIG
D.3.10	Strength	
D.3.10.1	Concentration unit	mg/ml milligram(s)/millilitre
D.3.10.2	Concentration type	equal
D.3.10.3	Concentration number	5
D.3.11 The IMP contains an:		
D.3.11.1	Active substance of chemical origin	Yes
D.3.11.2	Active substance of biological/ biotechnological origin (other than Advanced Therapy IMP (ATIMP)	No
	The IMP is a:	
D.3.11.3	Advanced Therapy IMP (ATIMP)	No
D.3.11.3.1	Somatic cell therapy medicinal product	No
D.3.11.3.2	Gene therapy medical product	No
D.3.11.3.3	Tissue Engineered Product	No
D.3.11.3.4	Combination ATIMP (i.e. one involving a medical device)	No
D.3.11.3.5	Committee on Advanced therapies (CAT) has issued a classification for this product	No
D.3.11.4	Combination product that includes a device, but does not involve an Advanced Therapy	No
D.3.11.5	Radiopharmaceutical medicinal product	No
D.3.11.6	Immunological medicinal product (such as vaccine, allergen, immune serum)	No
D.3.11.7	Plasma derived medicinal product	No
D.3.11.8	Extractive medicinal product	No
D.3.11.9	Recombinant medicinal product	No
D.3.11.10		No

D. IMP Identification		
	Medicinal product containing genetically modified organisms	
D.3.11.11	Herbal medicinal product	No
D.3.11.12	Homeopathic medicinal product	No
D.3.11.13	Another type of medicinal product	No
D.IMP: 2		
D.1.2 and D.1.3	IMP Role	Comparator
D.2	Status of the IMP to be used in the clinical trial	
D.2.1	IMP to be used in the trial has a marketing authorisation	Yes
D.2.5	The IMP has been designated in this indication as an orphan drug in the Community	No
D.2.5.1	Orphan drug designation number	
D.3 Description of the IMP		
D.3.4	Pharmaceutical form	Concentrate for solution for injection/infusion
D.3.4.1	Specific paediatric formulation	No
D.3.7	Routes of administration for this IMP	Intravenous use
D.3.8 to D.3.10 IMP Identification Details (Active Substances)		
D.3.8	INN - Proposed INN	FLUOROURACIL
D.3.9.1	CAS number	51-21-8
D.3.9.4	EV Substance Code	SUB07721MIG
D.3.10	Strength	
D.3.10.1	Concentration unit	mg/ml milligram(s)/millilitre
D.3.10.2	Concentration type	equal
D.3.10.3	Concentration number	50
D.3.11 The IMP contains an:		
D.3.11.1	Active substance of chemical origin	Yes
D.3.11.2	Active substance of biological/ biotechnological origin (other than Advanced Therapy IMP (ATIMP)	No
	The IMP is a:	
D.3.11.3	Advanced Therapy IMP (ATIMP)	No
D.3.11.3.1	Somatic cell therapy medicinal product	No
D.3.11.3.2	Gene therapy medical product	No
D.3.11.3.3	Tissue Engineered Product	No
D.3.11.3.4	Combination ATIMP (i.e. one involving a medical device)	No
D.3.11.3.5	Committee on Advanced therapies (CAT) has issued a classification for this product	No
D.3.11.4	Combination product that includes a device, but does not involve an Advanced Therapy	No
D.3.11.5	Radiopharmaceutical medicinal product	No
D.3.11.6	Immunological medicinal product (such as vaccine, allergen, immune serum)	No
D.3.11.7	Plasma derived medicinal product	No
D.3.11.8	Extractive medicinal product	No
D.3.11.9	Recombinant medicinal product	No
D.3.11.10	Medicinal product containing genetically modified organisms	No
D.3.11.11	Herbal medicinal product	No
D.3.11.12	Homeopathic medicinal product	No
D.3.11.13	Another type of medicinal product	No
D.IMP: 3		
D.1.2 and D.1.3	IMP Role	Comparator
D.2	Status of the IMP to be used in the clinical trial	
D.2.1		Yes

D. IMP Identification		
	IMP to be used in the trial has a marketing authorisation	
D.2.5	The IMP has been designated in this indication as an orphan drug in the Community	No
D.2.5.1	Orphan drug designation number	
D.3 Description of the IMP		
D.3.4	Pharmaceutical form	Concentrate for solution for injection/infusion
D.3.4.1	Specific paediatric formulation	No
D.3.7	Routes of administration for this IMP	Intravenous use
D.3.8 to D.3.10 IMP Identification Details (Active Substances)		
D.3.8	INN - Proposed INN	Calcium folinate
D.3.9.1	CAS number	58-05-9
D.3.9.3	Other descriptive name	FOLINIC ACID
D.3.9.4	EV Substance Code	SUB13910MIG
D.3.10	Strength	
D.3.10.1	Concentration unit	mg/ml milligram(s)/millilitre
D.3.10.2	Concentration type	equal
D.3.10.3	Concentration number	10
D.3.11 The IMP contains an:		
D.3.11.1	Active substance of chemical origin	Yes
D.3.11.2	Active substance of biological/ biotechnological origin (other than Advanced Therapy IMP (ATIMP)	No
	The IMP is a:	
D.3.11.3	Advanced Therapy IMP (ATIMP)	No
D.3.11.3.1	Somatic cell therapy medicinal product	No
D.3.11.3.2	Gene therapy medical product	No
D.3.11.3.3	Tissue Engineered Product	No
D.3.11.3.4	Combination ATIMP (i.e. one involving a medical device)	No
D.3.11.3.5	Committee on Advanced therapies (CAT) has issued a classification for this product	No
D.3.11.4	Combination product that includes a device, but does not involve an Advanced Therapy	No
D.3.11.5	Radiopharmaceutical medicinal product	No
D.3.11.6	Immunological medicinal product (such as vaccine, allergen, immune serum)	No
D.3.11.7	Plasma derived medicinal product	No
D.3.11.8	Extractive medicinal product	No
D.3.11.9	Recombinant medicinal product	No
D.3.11.10	Medicinal product containing genetically modified organisms	No
D.3.11.11	Herbal medicinal product	No
D.3.11.12	Homeopathic medicinal product	No
D.3.11.13	Another type of medicinal product	No

D.8 Information on Placebo

E. General Information on the Trial		
E.1 Medical condition or disease under investigation		
E.1.1	Medical condition(s) being investigated	Metastatic Pancreatic Cancer
E.1.1.1	Medical condition in easily understood language	cancer of the pancreas that has spread to other areas
E.1.1.2	Therapeutic area	Diseases [C] - Cancer [C04]
MedDRA Classification		
E.1.2 Medical condition or disease under investigation		
E.1.2	Version	14.1

E. General Information on the Trial		
E.1.2	Level	PT
E.1.2	Classification code	10033610
E.1.2	Term	Pancreatic carcinoma metastatic
E.1.2	System Organ Class	10029104 - Neoplasms benign, malignant and unspecified (incl cysts and polyps)
E.1.2 Medical condition or disease under investigation		
E.1.2	Version	14.1
E.1.2	Level	LLT
E.1.2	Classification code	10033599
E.1.2	Term	Pancreatic adenocarcinoma metastatic
E.1.2	System Organ Class	10029104 - Neoplasms benign, malignant and unspecified (incl cysts and polyps)
E.1.2 Medical condition or disease under investigation		
E.1.2	Version	14.1
E.1.2	Level	LLT
E.1.2	Classification code	10033605
E.1.2	Term	Pancreatic cancer metastatic
E.1.2	System Organ Class	10029104 - Neoplasms benign, malignant and unspecified (incl cysts and polyps)
E.1.3	Condition being studied is a rare disease	Yes
E.2 Objective of the trial		
E.2.1	Main objective of the trial	To compare overall survival following treatment with MM-398, with orwithout 5-fluorouracil and leucovorin,versus 5-fluorouracil and leucovorin in patients with metastatic pancreatic cancer that have progressed on gemcitabine based therapy.
E.2.2	Secondary objectives of the trial	• To compare the following time-to-event efficacy endpoints between the two treatment arms: Progression-free survival (PFS) Time to treatment failure (TTF) • To compare the Objective Response Rate (ORR) between the two treatment arms • To compare the tumor marker response of CA 19-9 between the two treatment arms • To compare the Clinical Benefit Response (CBR) rate between the two treatment arms • To assess patient-reported outcomes (PROs) between the two treatment arms using the European Organization for Research and Treatment of Cancer (EORTC) quality-of-life core questionnaire (EORTC-QLQ-C30) • To compare the safety and adverse event profile between the two treatment arms •To determine the pharmacokinetic properties of MM-398, as a single agent and in combination with 5-FU and leucovorin, in this population
E.2.3	Trial contains a sub-study	Yes
E.2.3.1	Full title, date and version of each sub-study and their related objectives	Translational Research Companion Protocol for NAPOLI-1: A Randomized, Open Label Phase 3 Study of MM-398, with or without 5-Fluorouracil and Leucovorin, versus 5- Fluorouracil and Leucovorin, in Patients with Metastatic Pancreatic Cancer Who have Failed Prior Gemcitabine-based Therapy
E.3	Principal inclusion criteria	• Histologically or cytologically confirmed adenocarcinoma of exocrine pancreas • Documented metastatic disease; disease status may be measurable or non-measurable as defined by RECIST v1.1 guidelines • Documented disease progression after prior gemcitabine or gemcitabine containing therapy, in locally advanced or metastatic setting. Examples of permitted therapies include, but are not limited to: o Single agent gemcitabine o Any one gemcitabine-based regimen, with or without maintenance gemcitabine o Single agent gemcitabine to which a platinum agent, a fluoropyrimidine, or erlotinib was subsequently added o Gemcitabine administered in the adjuvant setting if disease recurrence occurred within 6 months of completing the adjuvant therapy • KPS > 70 • Adequate bone marrow reserves as evidenced by: o ANC > 1,500 cells/µl without the use of hematopoietic growth factors; and o Platelet count > 100,000 cells/µl; and

E. General Information on the Trial		
		o Hemoglobin > 9 g/dL • Adequate hepatic function as evidenced by: o Serum total bilirubin within normal range for the institution (biliary drainage is allowed for biliary obstruction) o Albumin levels ≥ 3.0 g/dL o Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 2.5 x ULN (≤ 5 x ULN is acceptable if liver metastases are present) • Adequate renal function as evidenced by a serum creatinine ≤1.5 x ULN • Normal ECG or ECG without any clinically significant findings • Recovered from the effects of any prior surgery, radiotherapy or other anti-neoplastic therapy • At least 18 years of age • Able to understand and sign an informed consent (or have a legal representative who is able to do so)
E.4	Principal exclusion criteria	• Active CNS metastases (indicated by clinical symptoms, cerebral edema, steroid requirement, or progressive disease) patients should have been off steroids for at least 28 days prior to starting study therapy • Clinically significant gastrointestinal disorder including hepatic disorders, bleeding, inflammation, occlusion, or diarrhea > grade 1 • History of any second malignancy in the last 5 years; subjects with prior history of in-situ cancer or basal or squamous cell skin cancer are eligible. Subjects with other malignancies are eligible if they have been continuously disease free for at least 5 years. • Severe arterial thromboembolic events (myocardial infarction, unstable angina pectoris, stroke) less than 6 months before inclusion • NYHA Class III or IV congestive heart failure, ventricular arrhythmias or uncontrolled blood pressure • Active infection or an unexplained fever > 38.5°C during screening visits or on the first scheduled day of dosing (at the discretion of the investigator, patients with tumor fever may be enrolled), which in the investigator's opinion might compromise the patient's participation in the trial or affect the study outcome • Known hypersensitivity to any of the components of MM-398, other liposomal products, fluropyrimidines or leucovorin • Investigational therapy administered within 4 weeks, or within a time interval less than at least 5 half lives of the investigational agent, whichever is longer, prior to the first scheduled day of dosing in this study • Any other medical or social condition deemed by the Investigator to be likely to interfere with a patient's ability to sign informed consent, cooperate and participate in the study, or interfere with the interpretation of the results • Pregnant or breast feeding; females of child-bearing potential must test negative for pregnancy at the time of enrollment based on a urine or serum pregnancy test. Both male and female patients ofreproductive potential must agree to use a reliable method of birth control, during the study and for 3 months following the last dose of study drug.
E.5 End points		
E.5.1	Primary end point(s)	Overall survival (OS)
E.5.1.1	Timepoint(s) of evaluation of this end point	The analysis for primary endpoint will take place once at least 305 death events have occurred.
E.5.2	Secondary end point(s)	Progression Free Survival Time to treatment failure Objective Response Rate Clinical Benefit Response Tumor Marker Response Patient Reported Outcome
E.5.2.1	Timepoint(s) of evaluation of this end point	On-going
E.6 and E.7 Scope of the trial		
E.6	Scope of the trial	
E.6.1	Diagnosis	No
E.6.2	Prophylaxis	No
E.6.3	Therapy	Yes
E.6.4	Safety	Yes
E.6.5	Efficacy	Yes
E.6.6	Pharmacokinetic	Yes
E.6.7	Pharmacodynamic	No
E.6.8	Bioequivalence	No
E.6.9	Dose response	No
E.6.10	Pharmacogenetic	No
E.6.11	Pharmacogenomic	Yes

E. General Information on the Trial		
E.6.12	Pharmacoeconomic	No
E.6.13	Others	No
E.7	Trial type and phase	
E.7.1	Human pharmacology (Phase I)	No
E.7.1.1	First administration to humans	No
E.7.1.2	Bioequivalence study	No
E.7.1.3	Other	No
E.7.1.3.1	Other trial type description	
E.7.2	Therapeutic exploratory (Phase II)	No
E.7.3	Therapeutic confirmatory (Phase III)	Yes
E.7.4	Therapeutic use (Phase IV)	No
E.8 Design of the trial		
E.8.1	Controlled	Yes
E.8.1.1	Randomised	Yes
E.8.1.2	Open	Yes
E.8.1.3	Single blind	No
E.8.1.4	Double blind	No
E.8.1.5	Parallel group	No
E.8.1.6	Cross over	No
E.8.1.7	Other	No
E.8.2	Comparator of controlled trial	
E.8.2.1	Other medicinal product(s)	Yes
E.8.2.2	Placebo	No
E.8.2.3	Other	No
E.8.2.4	Number of treatment arms in the trial	3
E.8.3	The trial involves single site in the Member State concerned	No
E.8.4	The trial involves multiple sites in the Member State concerned	Yes
E.8.4.1	Number of sites anticipated in Member State concerned	4
E.8.5	The trial involves multiple Member States	Yes
E.8.5.1	Number of sites anticipated in the EEA	33
E.8.6 Trial involving sites outside the EEA		
E.8.6.1	Trial being conducted both within and outside the EEA	Yes
E.8.6.2	Trial being conducted completely outside of the EEA	No
E.8.6.3	If E.8.6.1 or E.8.6.2 are Yes, specify the regions in which trial sites are planned	Argentina Australia Brazil Canada Czech Republic France Germany Hungary India Italy Korea, Republic of Russian Federation South Africa Spain Taiwan United Kingdom United States
E.8.7	Trial has a data monitoring committee	Yes
E.8.8		As per protocol

E. General Information on the Trial

	Definition of the end of the trial and justification where it is not the last visit of the last subject undergoing the trial	
E.8.9 Initial estimate of the duration of the trial		
E.8.9.1	In the Member State concerned years	2
E.8.9.1	In the Member State concerned months	0
E.8.9.1	In the Member State concerned days	0
E.8.9.2	In all countries concerned by the trial years	2
E.8.9.2	In all countries concerned by the trial months	0
E.8.9.2	In all countries concerned by the trial days	0

F. Population of Trial Subjects

F.1 Age Range		
F.1.1	Trial has subjects under 18	No
F.1.1.1	In Utero	No
F.1.1.2	Preterm newborn infants (up to gestational age < 37 weeks)	No
F.1.1.3	Newborns (0-27 days)	No
F.1.1.4	Infants and toddlers (28 days-23 months)	No
F.1.1.5	Children (2-11years)	No
F.1.1.6	Adolescents (12-17 years)	No
F.1.2	Adults (18-64 years)	Yes
F.1.2.1	Number of subjects for this age range:	150
F.1.3	Elderly (>=65 years)	Yes
F.1.3.1	Number of subjects for this age range:	255
F.2 Gender		
F.2.1	Female	Yes
F.2.2	Male	Yes
F.3 Group of trial subjects		
F.3.1	Healthy volunteers	No
F.3.2	Patients	Yes
F.3.3	Specific vulnerable populations	Yes
F.3.3.1	Women of childbearing potential not using contraception	No
F.3.3.2	Women of child-bearing potential using contraception	Yes
F.3.3.3	Pregnant women	No
F.3.3.4	Nursing women	No
F.3.3.5	Emergency situation	No
F.3.3.6	Subjects incapable of giving consent personally	No
F.3.3.7	Others	No
F.4 Planned number of subjects to be included		
F.4.1	In the member state	16
F.4.2	For a multinational trial	
F.4.2.1	In the EEA	150
F.4.2.2	In the whole clinical trial	405
F.5	Plans for treatment or care after the subject has ended the participation in the trial (if it is different from the expected normal treatment of that condition)	As per protocol

G. Investigator Networks to be involved in the Trial

N. Review by the Competent Authority or Ethics Committee in the country concerned

N. Review by the Competent Authority or Ethics Committee in the country concerned		
N.	Competent Authority Decision	Authorised
N.	Date of Competent Authority Decision	2012-03-19
N.	Ethics Committee Opinion of the trial application	Favourable
N.	Ethics Committee Opinion: Reason (s) for unfavourable opinion	
N.	Date of Ethics Committee Opinion	2012-05-09

P. End of Trial		
P.	End of Trial Status	Ongoing

Search Reset

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NEURO-ONCOLOGY

Comparing routes of delivery for nanoliposomal irinotecan shows superior anti-tumor activity of local administration in treating intracranial glioblastoma xenografts

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Department of Neurological Surgery, University of California, San Francisco, California (P.-Y.C., T.O., N.B., M.D.P., M.S.B., J.R.F., K.B., C.D.J.); Department of Neurosurgery, Chang-Gung University and Memorial Hospital, Taoyuan, Taiwan (P.-Y.C., K.-C.W.); Graduate Institute of Clinical Medical Sciences, Chang-Gung University, Taoyuan, Taiwan (P.-Y.C.); Merrimack Pharmaceuticals, Cambridge, Massachusetts (D.D., A.K., J.B.F., D.B.K.)

Background. Liposomal drug packaging is well established as an effective means for increasing drug half-life, sustaining drug activity, and increasing drug efficacy, whether administered locally or distally to the site of disease. However, information regarding the relative effectiveness of peripheral (distal) versus local administration of liposomal therapeutics is limited. This issue is of importance with respect to the treatment of central nervous system cancer, for which the blood-brain barrier presents a significant challenge in achieving sufficient drug concentration in tumors to provide treatment benefit for patients.

Methods. We compared the anti-tumor activity and efficacy of a nanoliposomal formulation of irinotecan when delivered peripherally by vascular route with intratumoral administration by convection-enhanced delivery (CED) for treating intracranial glioblastoma xenografts in athymic mice.

Results. Our results show significantly greater anti-tumor activity and survival benefit from CED of nanoliposomal irinotecan. In 2 of 3 efficacy experiments, there were animal subjects that experienced apparent cure of tumor from local administration of therapy, as indicated by a lack of detectable intracranial tumor through bioluminescence imaging and histopathologic analysis. Results from investigating the effectiveness of combination therapy with nanoliposomal irinotecan plus radiation revealed that CED administration of irinotecan plus radiation conferred greater survival benefit than did irinotecan or radiation monotherapy and also when compared with radiation plus vascularly administered irinotecan.

Conclusions. Our results indicate that liposomal formulation plus direct intratumoral administration of therapeutic are important for maximizing the anti-tumor effects of irinotecan and support clinical trial evaluation of this therapeutic plus route of administration combination.

Keywords: convection-enhanced delivery, glioblastoma, irinotecan, liposome, xenograft.

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The benefits of liposomal drug packaging have been well documented and include improved drug half-life, sustained drug activity, and increased drug efficacy. We and others have shown that liposomal formulation increases the activity of cytotoxic drugs when used in treating intracranial xenografts established

from human glioblastoma (GBM), the most common and malignant of primary brain tumors in adults, irrespective of whether the liposomal drug is administered directly into the tumor or peripherally by a vascular route.[1–8]

Prior studies, however, have not compared the relative efficacy of peripheral and local administration of liposomal therapeutics for treating brain tumors. Information from such comparisons would help increase understanding of the influence of the blood-brain barrier (BBB) in limiting drug access to tumors and the extent to which therapeutic benefit is affected by peripheral administration of liposomal drugs.[9,10] This understanding, in turn, could influence clinical trial design for testing novel therapeutics or therapeutic formulations in treating patients with brain tumors.

Although there is widespread understanding and appreciation of BBB-restricted access of therapy to intracranial tumors, this concern tends to be lessened with respect to the conduct of clinical trials by animal model studies, the results of which show activity of peripherally administered therapeutics against brain tumors. Such results, combined with the expectation of repeated administration of therapy through oral or vascular routes, to achieve comparable if not superior anti-tumor activity, relative to a single intratumoral administration of drug, have been instrumental in promoting the preferential use of peripheral administration of therapy for treating patients with brain tumor.

Convection-enhanced delivery (CED) for local administration of therapy is an alternative to vascular administration that bypasses the BBB and delivers therapeutic agents directly into the brain.[11] Previously, we have shown that the use of controlled pressure and a specially designed cannula are key to maximizing the distribution of CED-administered therapy in the brains of animal subjects,[12,13] which is a critically important issue for effective treatment of GBM, the majority of which grow in an infiltrative, disseminated manner.

Irinotecan has been extensively studied as a therapeutic agent for glioma (reviewed by Vredenburgh et al.[14]) based on promising in vitro and in vivo preclinical results, its BBB penetrance, and its distinct mechanism of action, as compared with other agents used in the treatment of these tumors. Clinical experience with irinotecan has been at least modestly promising, with this therapeutic showing activity both as a single agent and in combination with other modalities. However, the free drug is associated with complex pharmacologic interactions, less than ideal pharmacokinetic properties, and toxicity. Liposomal formulation improves on irinotecan's pharmacokinetics, reduces toxicity, and, if delivered locally, could be combined with other treatment approaches as an attractive addition to brain tumor therapy.[1,4]

In the current report, we present the results of a study in which we have conducted experiments for comparing peripheral intravascular and CED administration of nanoliposomal irinotecan for efficacy against 2 distinct orthotopic xenograft models of GBM. Our data reveal that a single administration of nanoliposomal irinotecan by CED is significantly more effective than multiple systemic intravascular administrations of the same therapeutic and is safe for animal subjects. These results provide strong support for CED administration of nanoliposomal irinotecan in the investigational treatment of GBM.

Materials and Methods

Investigational Agent

Nanoliposomal irinotecan (MM-398) is a highly stabilized liposomal formulation containing nano-sized irinotecan crystals complexed with sucrose octasulfate in the liposome interior[15] and was generously provided by Merrimack Pharmaceuticals (Cambridge, MA). The preparation of nanoliposomal irinotecan, used in the experiments that follow, had a particle size of 111 nm, as determined by dynamic light scattering, and a drug-to-phospholipid (PL) ratio of 488 g irinotecan/mol PL.

GBM Xenografts

Human GBM primary tissues, GBM43 and SF7796, are maintained as serially passaged subcutaneous xenografts in athymic mice.[16,17] Both GBM43 and SF7796 have been modified by lentiviral infection for stable expression of firefly luciferase to enable in vivo bioluminescence imaging, as previously described.[18]

To prepare tumor cells from subcutaneous xenografts for transfer to the intracranial compartment, excised subcutaneous tumors were placed in culture dishes and minced with a scalpel then mechanically dispersed by repetitive pipetting to create small cellular aggregates that were passed repeatedly through 40-micron nylon mesh filters to produce single-cell suspensions. Cell suspensions were centrifuged at a rate of 1000 rpm for 10 min at 4°C and supernatants aspirated before resuspending pellets in 1 mL of sterile DMEM media. Cells were counted and then diluted to 1×10^5 cells/μL for intracranial injection.[19]

Intracranial Tumor Establishment in Athymic Mice

Five-week-old female athymic mice (nu/nu, homozygous; Simonsen Laboratories, Gilroy, CA), housed under aseptic conditions, received intracranial tumor cell injection as previously described[17] and as approved by the University of California San Francisco Institutional Animal Care and Use Committee. In brief, mice were anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg) and then were injected with 3 μL of tumor cell suspension (300 000 cells total) into the right caudate putamen.

Bioluminescence Monitoring of Intracranial Tumor Growth

In preparation for bioluminescence imaging (BLI), mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg), then administered 150 mg/kg of luciferin (D-luciferin potassium salt; Gold Biotechnology, St. Louis,

MO) via intraperitoneal injection. Ten minutes after luciferin injection, mice were examined for tumor bioluminescence with an IVIS Lumina imaging station (Caliper Life Sciences, Alameda, CA). Regions of interest, defined using Living Image software (Caliper Life Sciences, Alameda, CA), were recorded as photons per second per steradian per square centimeter.[19] Beginning at 1 week after intracranial tumor cell injection, mice were imaged twice weekly until deaths were observed in control (untreated) mice, with data from the last imaging session used to evaluate the effect of therapy on tumor growth.

Vascular and Intratumoral Administration of Nanoliposomal Irinotecan

For vascular administration of therapy,[20] mice were warmed for 5–10 min with either a heating pad or a heat lamp to dilate tail vasculature. Injection sites were then cleaned with an alcohol swab, after which a 28 g a insulin syringe was inserted, with 50 or 100 μL liposomal drug that had been diluted in 5 mM HEPES-buffered saline (pH, 6.5) to a concentration of 0.004 mg irinotecan per microliter, and injected with steady pressure over 5–10 s. For CED administration, our approach was similar to that previously described.[21] In brief, infusion cannula were made with silica tubing (Polymicro Technologies, Phoenix, AZ) fused to a 0.1 mL syringe (Plastic One, Roanoke, VA) with a 0.5-mm stepped-tip needle that protruded from the silica guide base. Syringes were loaded with liposomal drug (0.04 mg per microliter) and attached to a microinfusion pump (Bioanalytical Systems, Lafayette, IN). The syringe with silica cannula was mounted onto a stereotactic holder then lowered through a puncture hole made in the skull[19,20] and to the same region in the caudate putamen at which tumor cells had been previously injected. Nanoliposomal irinotecan was infused at a rate of 1 μL/min until a volume of 5 or 10 μL had been delivered. Cannulae were removed 2 min after completion of infusion.

Mouse Irradiation

Mice were anesthetized via inhalation of 2.5% isoflurane with 1 L of oxygen per minute for 5 min prior to being positioned on an irradiation platform located 16.3 cm from a cesium-137 source (J. L. Shepherd & Associates, San Fernando, CA). Their eyes, respiratory tracts, and bodies were protected with lead shielding. Mice received whole brain irradiation at a dose rate of 247 cGy/min[22] until 1.5 Gy radiation had been administered. After irradiation, animals were monitored until recovery. For the experiment involving the analysis of combination therapy efficacy, mice were irradiated once daily for 5 consecutive days, Monday through Friday, with the first radiation treatment on day 7 following tumor cell implantation.

Analysis of Irinotecan Content in Intracranial Tumors

For the experiment involving analysis of tumor irinotecan content, athymic mice with intracranial GBM43 were administrated 0.4 mg of irinotecan by tail vein or directly into the tumor mass on day 13 after implantation of tumor cells and 30 min after the fifth and final of radiation fractions that had been initiated on day 9. Mice were euthanized 24 h after nanoliposomal irinotecan administration, with brains immediately resected and tumor tissue dissected prior to snap-freezing by immersion in liquid nitrogen. Analysis of irinotecan levels in tumor tissues was as described previously.[1] In brief, water was added to tissues at a 20% (w/v) ratio, and tissues were then homogenized with a mechanical homogenizer in an ice bath. Homogenates were extracted for the lactone form of irinotecan with an acidic methanol solution by vortexing and centrifugation at 13 000 rpm for 10 min, with the supernatants then transferred to autosampler vials for Dionex high-pressure liquid chromatography (HPLC) analysis.

Immunohistochemistry

Resected mouse brains were fixed in 10% buffered formalin, then paraffin-embedded and sectioned for hematoxylin and eosin (H&E) staining and immunohistochemical (IHC) analysis. To determine cleaved caspase-3 reactivity, unstained sections were processed with a Ventana BenchMark XT automated system and a protocol consisting of pretreatment with 3% ethanolic hydrogen peroxide for 32 min at room temperature, epitope retrieval in Tris buffer (pH 8) for 8 min at 90°C, and incubation with primary antibody to cleaved caspase-3 (#9661, Cell Signaling Tech., Danvers, MA) at 0.2 mg/mL for 1 h at 37°C. Total and activated caspase-3–positive cells were counted in 5 high-powered fields within the tumor for each stained tissue section, with percent positive cells averaged for all fields associated with a specific treatment and subjected to statistical analysis as described below.

Statistical Analysis

PRISM 5, version 5.03 (GraphPad Software), was used to conduct all statistical analyses. For survival analysis, significance was determined by the log-rank (Mantel- Cox) test. Animals without tumor burden that died accidentally during anesthesia were excluded from the survival analysis. For all other statistical analyses, either a 2-tailed unpaired *t* test or Tukey's multiple comparison test was applied.

Results

Comparison of Intravascular Versus CED Administration of Nanoliposomal Irinotecan for Anti-Tumor Activity

Our initial experiment for comparing intracranial GBM xenograft response to peripherally and CED administered nanoliposomal irinotecan used GBM43, which is maintained as a serially propagated subcutaneous

xenograft[16,17] and has been previously classified as a proneural GBM.[23] GBM43 cells, harvested from a disaggregated subcutaneous xenograft, produce rapidly growing intracranial tumors that have been shown to be relatively resistant to radiation therapy.[24]

Our experimental design included 3 CED and 2 intravascular administration treatment groups. CED administration was either once at 0.4 mg irinotecan, or twice at either 0.2 or 0.4 mg irinotecan each time. Intravascular administrations were 4 times at either 0.2 or 0.4 mg irinotecan administered each time. Thus, total irinotecan administered by CED was either 0.4 or 0.8 mg irinotecan, whereas total irinotecan administered by intravascular injection was either 0.8 or 1.6 mg. CED administrations were on day 5 only or on days 5 and 8, and intravascular administrations were on days 5, 8, 12, and 15 after tumor cell implantation. BLI of luciferase-modified GBM43 tumors showed an anti-tumor effect from irinotecan administration, regardless of amount or route of administration (Fig. 1A) and significantly ($P < .05$) or near significantly greater anti-tumor effect of direct over intravascular administration, irrespective of the amounts of administered irinotecan being compared (P values for all 2-way comparisons shown in Table 1).

Survival analysis from these treatments showed that all irinotecan treatment groups experienced significant survival benefit relative to control (Fig. 1B). Of importance, comparisons of all CED and intravascular administration groups showed significantly greater benefit from direct administration of therapy, even when the total amount of irinotecan administered by the vascular route was 4 times greater than that delivered directly into the tumor. More striking was the difference in number of mice that experienced apparent cure of tumor from CED of nanoliposomal irinotecan (6 of 7 in the group receiving 0.8 mg irinotecan, and a total of 4 of 18 in the 2 groups receiving 0.4 mg irinotecan), as indicated by a lack of detectable bioluminescence signal in mice at time of euthanasia. Serial sectioning of entire brains from 2 of the mice receiving CED administration of nanoliposomal irinotecan and showing lack of detectable bioluminescence at time of euthanasia revealed no detectable tumor on histopathologic analysis of H&E-stained tissues. None of the mice receiving intravascular administration of nanoliposomal irinotecan experienced cure of tumor.

Body weight monitoring of mice receiving vascular and CED of nanoliposomal irinotecan showed similar patterns of weight loss, irrespective of route of delivery (Fig. 1), with no animal losing >13% initial weight from treatment. All animals receiving liposomal therapy rapidly gained weight on completion of treatment and achieved weights comparable to or exceeding that of untreated control group mice prior to onset of symptoms indicative of tumor burden in control group mice, suggesting that administration of nanoliposomal irinotecan by either route of administration is without extended adverse effect and is well tolerated.

To investigate whether these results might prove to be generalizable to additional subtypes of GBM, we performed a second experiment with SF7796, which was

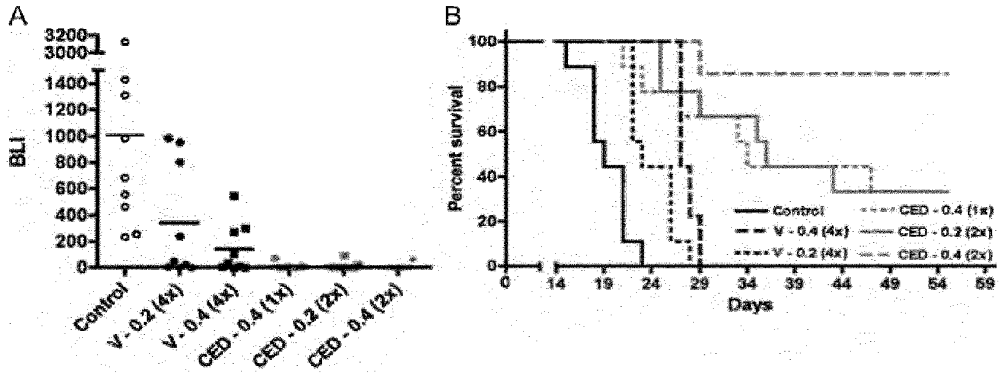

Fig. 1. Comparison of intravascular vs CED administration of nanoliposomal irinotecan for anti-tumor activity against intracranial GBM43 and corresponding survival benefit for animal subjects. (A) Treatment group day 15 normalized bioluminescence[18] (BLI) distributions (last imaging day in which all control group mice were alive). Route of administration identifiers: V, vascular administration; CED, convection enhanced delivery. Numbers following route of administration identifiers represent mg quantity of irinotecan administered with each dose; numbers in parentheses represent the number of administrations. Direct administrations were on day 5 or on days 5 and 8, and vascular administrations were on days 5, 8, 12, and 15. Student's t-test values for all 2-way comparisons are listed in Table 1s. (B) Corresponding survival plots for each treatment group. Log rank P values for all 2-way comparisons are listed in Table 2 and show that the survival benefit for mice receiving 2 CED administrations of 0.4 mg irinotecan was significantly greater ($P < .05$) than for any other treatment. Mice surviving at day 60, all of which had no detectable tumor by bioluminescence imaging, were euthanized, with analysis of serial H&E-stained sections of entire brains from 2 of these mice showing no detectable tumor. Control group mice in this experiment were untreated, which were established as valid for comparison by determining, in a separate experiment, that CED of vehicle caused no adverse or beneficial effect on animal survival relative to no treatment (Supplementary Figure 2). Number of mice included in the survival analysis for each treatment group (see Materials and Methods): Control = 9; CED 0.2 (2x) = 9; CED 0.4 (1x) = 9; CED 0.4 (2x) = 7; V 0.2 (4x) = 9; and V 0.4 (4x) = 9.

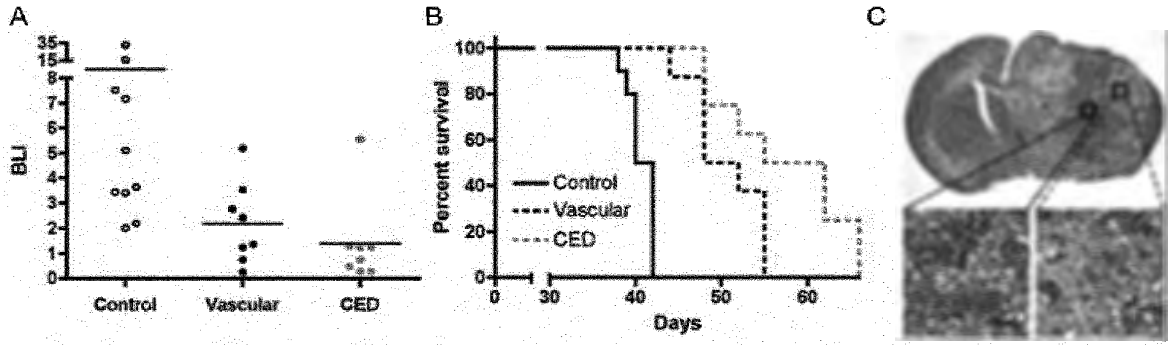

Fig. 2. Comparison of intravascular with CED administration of nanoliposomal irinotecan for anti-tumor activity and survival benefit for mice with intracranial SF7796. (**A**) Treatment group day 35 bioluminescence distributions (last imaging day for which all control group mice were alive). Mice receiving vascular administration received 0.4 mg doses of irinotecan on days 20 and 24 (0.8 mg total dose), whereas mice receiving CED administration were treated just once with 0.4 mg irinotecan on day 20 after tumor cell implantation. Student's *t*-test values for 2-way comparisons: 0.092 for control vs vascular; 0.061 for control vs CED; and 0.359 for vascular vs CED. (**B**) Corresponding survival plots for each treatment group. Log rank *P* values for 2-way comparisons: <0.001 for control vs vascular; <0.001 for control vs CED; and 0.048 for vascular vs CED. Number of mice included in the survival analysis for each treatment group: Control = 10; Vascular = 8; CED = 8. (**C**) 1.25x (upper) and 40x (lower) magnifications of an H&E-stained coronal section from the brain of a control group mouse that was euthanized on day 39 because of symptoms from increasing tumor burden. Lower left panel: high cellularity at the tumor core. Lower right panel: disseminated tumor cells at the tumor periphery.

established and has been maintained as a subcutaneous xenograft after initial implantation of a surgical specimen from a patient whose tumor had regrown after standard-of-care therapy (radiation + temozolomide[25]), which was followed by treatment of the recurrent tumor with bevacizumab[26] prior to second surgery. This GBM xenograft has been classified as mesenchymal using the classification scheme described by Verhaak et al.[23] For assessing SF7796 response to nanoliposomal therapy, we compared the anti-tumor effect and survival benefit of 0.4 mg irinotecan administered once by CED (day 20 after implantation) with 0.8 mg administered by intravascular route (0.4 mg administered twice: days 20 and 24). As for the previous experiment with GBM43, the results for SF7796 showed irinotecan anti-tumor activity by BLI (Fig. 2A) and significant survival benefit from treatment, irrespective of whether administered directly or intravascularly (Fig. 2B). SF7796 tumor cells produce a diffusely infiltrative intracranial tumor (Fig. 2C), and it is likely that this diffusely infiltrative nature, combined with the later time of treatment initiation for this experiment (day 20 vs day 5 for the initial experiment with GBM43), resulted in none of the mice with intracranial SF7796 experiencing cure of tumor from treatment. However, as before with the GBM43 model (Fig. 1), the survival benefit from one CED treatment was significantly greater than that resulting from multiple (2) intravascular administrations of liposomal irinotecan ($P = .048$).

Effect of Radiation when Combined with Intravascular or Intratumoral Nanoliposomal Irinotecan Therapy

The design for the prior 2 experiments (Figs. 1 and 2) is consistent with that which might be used in a clinical study for treating recurrent GBM and for which investigational therapies are often evaluated in isolation from co-treatment with other therapeutics and/or treatment modalities. To evaluate the activity of nanoliposomal irinotecan in a context consistent with a clinical trial for treating newly diagnosed GBM, we compared the anti-tumor activity of CED with intravascular administration of nanoliposomal irinotecan when used in combination with radiation therapy (RT). For this experiment, radiation was administered at 1.5 Gy/day × 5 (7.5 Gy total) beginning day 7 after implantation of GBM43 cells, with irinotecan administered once by CED (0.4 mg on day 7) or twice by the vascular route (0.4 mg on days 7 and 11). As with the previous GBM43 experiment (Fig. 1), CED of irinotecan outperformed intravascular administration, even though twice as much irinotecan was administered by the vascular route (Figs. 3A and B; $P = .035$ for survival benefit comparison). RT, which as a monotherapy, provided modest, albeit statistically significant, survival benefit to mice with intracranial GBM43 (Fig. 3B; $P = .011$ for RT vs control), further increased the anti-tumor effect and survival benefit from nanoliposomal irinotecan, with direct administration of irinotecan + RT providing the most extensive survival benefit of any treatment (Fig. 3B) and resulting in half (5 of 10) of the treatment group mice experiencing apparent cure of tumor. As for previous experiments, therapeutic regimens were well tolerated, with no animal subject experiencing >10% loss of pretreatment body weight at completion of therapy (data not shown).

For this latter experiment, untreated mice were included for euthanasia at day 7 to obtain samples providing indication of extent of intracranial tumor at time of treatment initiation (Fig. 4A) and at day 12 for all treatment groups (Figs. 4B–F) to allow qualitative

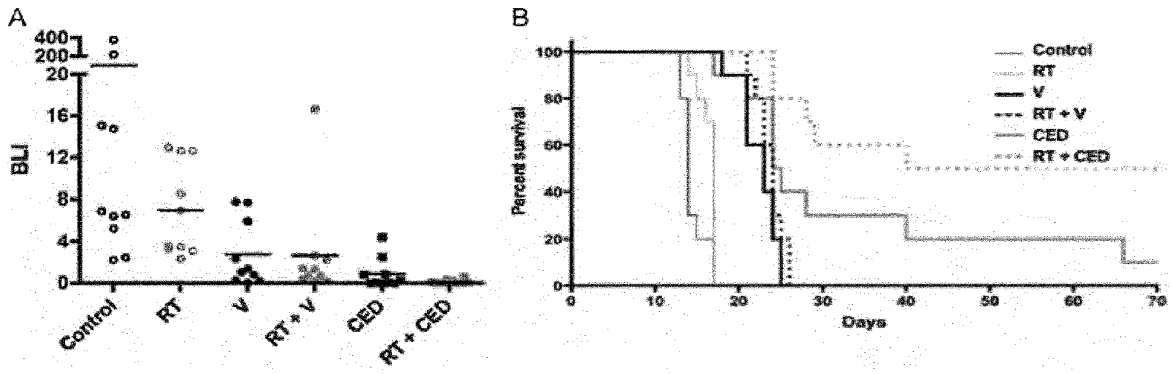

Fig. 3. Effect of radiation when combined with intravascular or CED administration of nanoliposomal irinotecan therapy. Radiation treatment for this experiment was 1.5 Gy/day x 5, beginning day 7 and ending day 11, for a total radiation dose of 7.5 Gy. Nanoliposomal irinotecan administration was 0.4 mg on day 7 for CED administration and was 0.4 mg on days 7 and 11 for intravascular administration. (A) Treatment group day 10 bioluminescence distributions (last imaging day at which all control group mice were alive). See Table 3 for all 2-way comparisons using the Student's *t*-test. (B) Corresponding survival plots for all treatment groups. Log rank *P* values for all 2-way comparisons are listed in Table 4 and show that CED administration of irinotecan as a monotherapy was significantly better than intravascular and RT monotherapies and that RT + CED of irinotecan was significantly better than all other therapies, including irinotecan monotherapy via CED. The experiment was terminated at 70 days, at which time there was no detectable bioluminescence signal in surviving mice. Ten mice included in the survival analysis for all treatment groups.

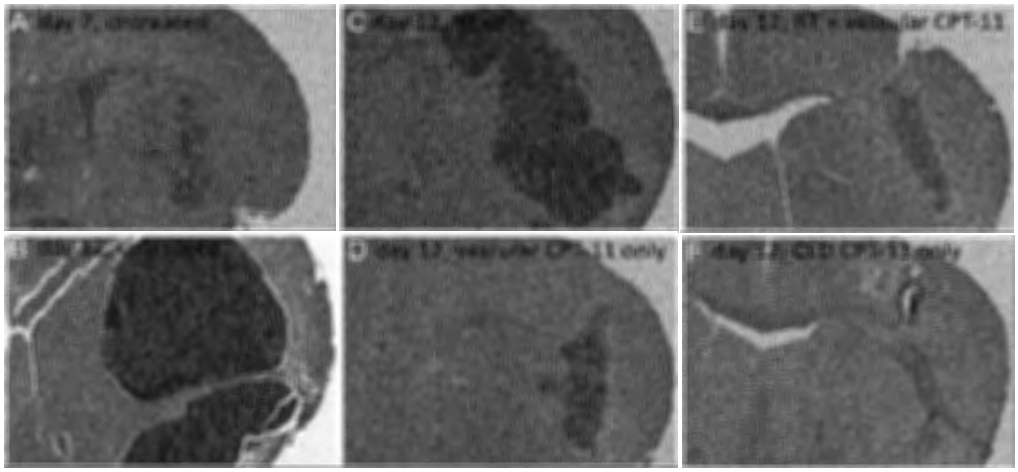

Fig. 4. H&E-stained coronal sections (2.5× magnification) from the brain of an untreated mouse that was euthanized on the first day of therapy (A) and from the brains of mice that were euthanized from each treatment group one day after the last day of therapy (day 12: panels B-F). H&E-stained sections from the brain of a mouse receiving RT + CED administration of nanoliposomal irinotecan (not shown) appeared to be similar to those of the mouse receiving local administration of liposomal therapy only (F) and showed a lack of identifiable tumor from combination therapy at day 12 after tumor cell implantation.

comparison of relative tumor size among treatment groups and quantitative IHC analysis of activated caspase 3 staining for assessing the pro-apoptotic effect of different treatments at 1 day after completion of therapy (Fig. 5). Inspection of H&E-stained tissues for sections with the largest tumor areas showed reasonable consistency between treatment effect on amount of H&E-stained tumor at time of completing therapy (Figs. 4B–F) and eventual treatment group survival (Fig. 3B). This was also the case for the activated caspase 3 IHC analysis, which showed the most extensive apoptotic response in mice receiving direct administration of nanoliposomal irinotecan + radiation therapy (Fig. 5; $P < .05$ for CED + RT vs any other treatment group; Student's *t* test results for all Fig. 5G; 2-way comparisons are shown in supplementary Table 5).

Analysis of Irinotecan Content in Intracranial Xenografts

To obtain information addressing whether radiation alters access of peripherally administered nanoliposomal irinotecan to intracranial tumor or, alternatively, alters retention of irinotecan when therapy is administered locally, additional mice received intracranial

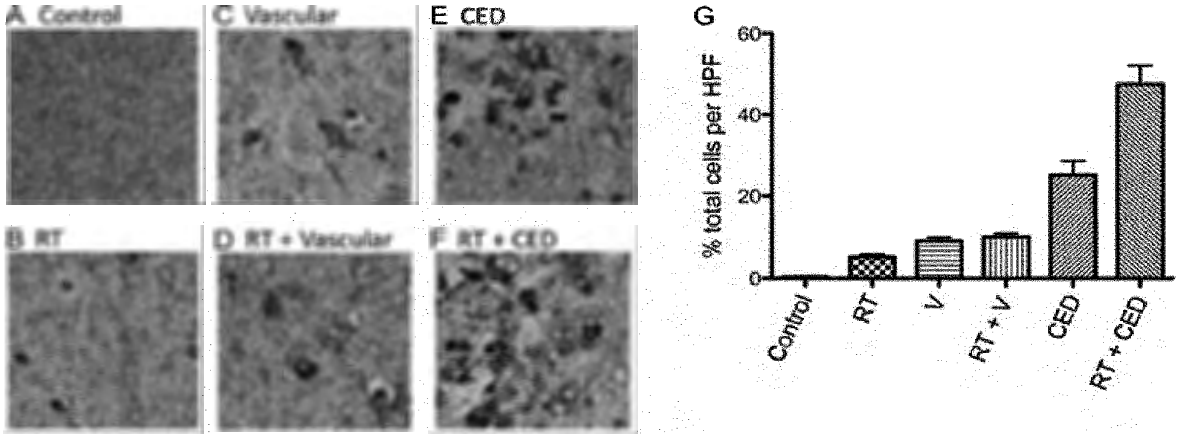

Fig. 5. (A–F) Examples of activated caspase 3 staining of tumors in the brains of mice from each of the treatment groups described in Fig. 3. Specimens were obtained from mice that were euthanized on the day after final treatments (day 12). (G) Histogram plot showing average values for percent positive cells from 5 high-powered fields examined in the brains of each of 3 mice from each treatment group: results are therefore based on a total of 15 high-powered fields per treatment group. Student's t test comparison of these values showed that radiation + CED administration of nanoliposomal irinotecan was significantly more effective in inducing apoptotic response, relative to all other treatment groups ($P < .05$).

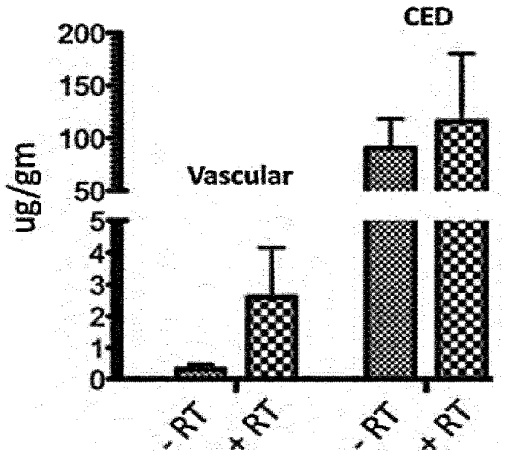

Fig. 6. Analysis of irinotecan content in intracranial GBM43 xenograft tumors 24 h after either vascular or CED administration of 0.4 mg nanoliposomal irinotecan and either in the presence or absence of radiation, with the last of 5 radiation treatments administered 30 min prior to the single irinotecan administration at day 13 subsequent to tumor cell implantation. The results show that RT does not cause a significant difference in tumor irinotecan, whether administered directly or by vascular route, but do show a significantly higher amount of irinotecan content in tumors receiving direct administration of therapy ($P < .05$). Analyzable samples were 2–4 for each treatment group.

implantation of GBM43, with tumors allowed to grow until day 9, at which time mice began receiving daily radiation for 5 consecutive days (1.5 Gy/day, 7.5 Gy total), with 0.4 mg nanoliposomal irinotecan administered either by CED or by a vascular route on day 13, the last day of radiation treatment. Twenty-four hours later, the mice were euthanized and tumor was dissected from surrounding normal brain of the euthanized mice, with dissected tumors subsequently examined for irinotecan content. The results of this analysis showed no significant difference in irinotecan content in tumors as a result of mice receiving pretreatment with radiation, although for mice receiving vascular administration of therapy, the mean value for tumor irinotecan was substantially higher in the group receiving radiation (Fig. 6). Not surprisingly, intratumoral irinotecan was substantially and significantly higher in mice receiving CED administration of liposomal therapy, irrespective of radiation pretreatment: 302-fold greater when comparing direct vs vascular administration in nonirradiated mice and 45-fold greater when comparing irinotecan content in mice from irradiated groups.

Discussion

In the current report, we have presented results addressing the relative activity and efficacy of intravascular and CED administration of nanoliposomal irinotecan in treating mice with orthotopic GBM xenografts. To the best of our knowledge, there are no previously published reports involving a route of administration comparison for liposomal therapy in treating an experimental animal model of GBM. Although our study is not exhaustive with respect to potential experimental variations, we feel that there is sufficient consistency of results for the variables tested in 2 distinct intracranial xenograft models to support the interpretation of CED administration of irinotecan nanoliposomes as being the more effective administration for maximizing anti-tumor effect of this therapy. Our cumulative experience in this area of research indicates that it is the combined effects of nanoliposomal packaging for extending the biologic half-life of active drug[1–5] and

bypassing the limiting influence of the BBB through direct intratumoral administration of therapy[1–4] that are important for maximizing the anti-tumor effect of cytotoxic chemotherapy.

Our interpretation of these results is not at odds with clinical trial designs that use intravascular administration of liposomal therapy to treat GBM, which is an approach that, according to our results, could well provide benefit to patients with brain tumors. Our results do, however, support a clinical trial design in which nanoliposomal irinotecan is administered locally.

The advantage of CED is multifactorial. The combination of cannulae that minimize reflux[21] and the liposomal formulation of irinotecan allows for more robust and uniform distribution of the therapeutic. Catheter placement into an intact tumor, confirmed by neuronavigational methods with direct imaging assessment of catheter position and subsequent convective infusion of the liposome using real-time imaging,[13] would be the optimal strategy for initial clinical studies of this agent. This clinical setting would eliminate the risk of drug reflux back into a surgical cavity seen when using CED strategies at the time of surgical resection and minimize the risk of improper placement of catheters, often seen after expected changes in the geometry of the cavity hours to days after resection.

The increased efficacy of CED administration of therapy is consistent with the substantial disparity in irinotecan content of xenograft tumors removed 24 h after treatment of animal subjects with equivalent vascular and intratumoral amounts of liposomal drug (Fig. 6). In a previous study, we showed that CED of nanoliposomal irinotecan sustains higher intracranial levels of irinotecan, relative to intracranial administration of free irinotecan, and that CED administration of nanoliposomal irinotecan outperforms direct intratumoral administration of equivalent-free irinotecan.[1] Thus, liposomal formulation is important to maximizing anti-tumor activity and, potentially, clinical benefit from CED administration of irinotecan.

For the present study, it is noteworthy that the nanoliposomal irinotecan preparation used for direct intracranial administration was the same as that used for intravascular administration, and this formulation was developed for intravascular administration of therapy. Thus, it is conceivable that alternative nanoliposomal irinotecan formulations may further improve on the intracranial distribution and efficacy of CED administration of nanoliposomal irinotecan. Despite the use of vascular-optimized nanoliposomal irinotecan, our results suggest the diffusion of locally administered liposomal therapy to an extent that is effective in eradicating, at least in some instances, tumor that occupies a substantial portion of total brain (Figs. 4A and F). Because mice experiencing apparent cure of intracranial tumor (Figs. 1 and 3) were treated with 5–10 μL of liposomal therapy, our results support the concept that this therapeutic approach involving local administration of therapy is sufficiently scalable to anticipate efficacy against brain tumor in patients with GBM.

In addition to the superior efficacy of local administration of nanoliposomal irinotecan, it is important to emphasize the apparent safety of direct intracranial administration of therapy, as indicated by body weight monitoring of treated mice (Fig. 1), and lack of an indication of neurologic deficit (e.g., ambulation, activity, and seizure) from CED of liposomal therapy. Future preclinical studies will focus on dose escalation experiments to identify maximum tolerable amounts of nanoliposomal irinotecan that can be administered directly into brain and the optimization of convection-enhanced delivery approaches for sustained CED administration of nanoliposomal irinotecan.

Finally, our results show that CED administration of nanoliposomal irinotecan can be used with radiation therapy for further improvement of anti-tumor effect and survival benefit, relative to either monotherapy, and thereby support the potential use of direct administration of liposomal therapy with subsequent standard-of-care therapy for treating GBM: RT + temozolomide.[25] With irinotecan being administered locally, one would anticipate a lack of adverse effects associated with the peripheral administration of 2 cytotoxic therapies and that local administration of nanoliposomal irinotecan could be used safely with conventional treatment for newly diagnosed GBM. Thus, our results support the clinical trial evaluation of direct intratumoral administration of nanoliposomal irinotecan, both as a single agent in the treatment of recurrent GBM and as part of a combination therapy for patients with newly diagnosed GBM.

Supplementary Material

Supplementary material is available online at Neuro-Oncology (http://neuro-oncology.oxfordjournals.org/).

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Conflict of interest statement. D. C. D., A. K., J. B. F., and D. B. K. are employees of Merrimack Pharmaceuticals, the supplier of the therapeutic investigated in the study reported herein. All other authors declare no conflict of interest.

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Development of a Highly Active Nanoliposomal Irinotecan Using a Novel Intraliposomal Stabilization Strategy

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Abstract

Liposome formulations of camptothecins have been actively pursued because of the potential for significant pharmacologic advantages from successful drug delivery of this important class of anticancer drugs. We describe nanoliposomal CPT-11, a novel nanoparticle/liposome construct containing CPT-11 (irinotecan) with unprecedented drug loading efficiency and *in vivo* drug retention. Using a modified gradient loading method featuring a sterically hindered amine with highly charged, multivalent anionic trapping agents, either polymeric (polyphosphate) or nonpolymeric (sucrose octasulfate), liposomes were capable of entrapping CPT-11 at extremely high drug-to-lipid ratios (>800 g CPT-11/mol phospholipid) and retaining encapsulated drug *in vivo* with a half-life of drug release in the circulation of 56.8 hours. CPT-11 was also protected from hydrolysis to the inactive carboxylate form and from metabolic conversion to SN-38 while circulating. The maximum tolerated dose in normal mice was determined to be 80 mg/kg for free CPT-11 and >320 mg/kg for nanoliposomal CPT-11. Nanoliposomal CPT-11 showed markedly superior efficacy when compared with free CPT-11 in human breast (BT474) and colon (HT29) cancer xenograft models. This study shows that intraliposomal stabilization of CPT-11 using a polymeric or highly charged, nonpolymeric polyanionic trapping agent results in a markedly active antitumor agent with low toxicity. (Cancer Res 2006; 66(6): 3271-7)

Introduction

Liposome-based systems have been used to enhance efficacy and/or ameliorate toxicity of certain drugs (1, 2). Thus far, the most successful approach has involved constructs engineered for long circulation times, combined with stable encapsulation of the active compound within the liposome; this allows liposomes to accumulate at sites of cancer, followed by intratumoral drug release. An example is PEGylated liposomal doxorubicin (3), which has received Food and Drug Administration approval for cancer treatment. However, the successful case of liposomal anthracyclines has not yet been matched by liposome constructs containing other anticancer drug classes, although recent progress has been made with vincristine (4–6) and certain camptothecin analogues (7–9). One of the key reasons for this has been the technical facility with which anthracyclines can be stably encapsulated in the liposome interior using remote-loading methodologies (10, 11), giving rise to stable liposome formulations that have been difficult to replicate with other classes of drugs.

CPT-11 {irinotecan; 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycampothecin} is a water-soluble camptothecin derivative currently used in cancer chemotherapy. The pharmacology of CPT-11 is complex, with extensive metabolic conversions involved in the activation, inactivation, and elimination of the drug (12, 13). CPT-11 is a prodrug that is converted by nonspecific carboxylesterases into a 100- to 1,000-fold more active metabolite, SN-38 (14). SN-38 is cleared via glucuronidation, for which major pharmacogenetic differences have been shown (15), and biliary excretion. In addition, CPT-11 and other camptothecins exist in a pH- and serum protein-dependent equilibrium between an active lactone form of the drug (predominant under acidic conditions) and an inactive carboxylate form (predominant at neutral or basic pH; ref. 16). These drug properties contribute to the marked heterogeneities in efficacy and toxicity observed clinically with CPT-11 (12, 17). Hence, drug carrier technologies represent a rational strategy to improve the pharmacokinetics and biodistribution of CPT-11 while protecting it from premature metabolism.

In this report, we describe a novel intraliposomal drug stabilization technology for encapsulation of CPT-11 into long-circulating liposome-based nanoparticles with high drug load and high *in vivo* stability, matching or surpassing previous liposomal drugs. This was achieved using polymeric or nonpolymeric highly charged anions, polyphosphate or sucrose octasulfate, as intraliposomal trapping agents in conjunction with a high-pK_a polyalkylamine gradient. The approach also allowed for preservation of the drug in its active lactone form within the liposome interior, protecting it from hydrolysis as well as premature conversion to SN-38. Here we use the term "nanoliposomal drug" to describe a nanoparticle consisting of a lipid bilayer scaffold encapsulating a nanoscale drug complex or aggregate that facilitates *in vivo* drug retention.

Materials and Methods

Liposome Preparation and Drug Loading

Solutions of triethylammonium salts of a linear poly(phosphate) (TEA-Pn, 13-18 phosphate units; Sigma Corp., St. Louis, MO) and sucrose octasulfate (TEA_8SOS) were prepared from commercially obtained sodium salts (Toronto Research Chemicals, Inc., North York, Ontario, Canada) by ion-exchange chromatography on the Dowex 50Wx8-200 resin in the H^+ form, immediately followed by titration with neat triethylamine. Residual sodium in either solution, as determined by potentiometry using a Na^+-selective electrode, was <1% of the cation content. Phosphate content was determined by inorganic phosphate assay following acid hydrolysis and was adjusted to 0.55 mol/L for TEA-Pn (osmolality, 430-480 mmol/kg). The TEA concentration was calculated from the amount of added TEA and was

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adjusted to 0.65 mol/L for TEA_8SOS solution (osmolality, 480-530 mmol/kg). The final pH for both solutions was 5.5 to 6.0.

Distearoylphosphatidylcholine (3 mol. parts), methoxypoly(ethylene)glycol (PEG2000)-derivatized distearoylphosphatidylethanolamine (0.015 mol. parts; Avanti Polar Lipids, Alabaster, AL), and cholesterol (2 mol. parts; Calbiochem, La Jolla, CA) were combined in ~50% (w/v) ethanolic solution and mixed with 10 volumes of the solution of TEA-Pn or TEA_8SOS at 60°C to 65°C. For pharmacokinetic studies, a nonexchangeable lipid label, [^{3}H] cholesteryl hexadecyl ether (Perkin-Elmer, Boston, MA), was added to the lipids in the amount of 0.5 mCi/mmol phospholipid. The lipid suspension was extruded 15 times through two stacked polycarbonate membranes (Nucleopore, Corning-Costar, Acton, MA) with 0.08-μm pore size using argon pressure at 60°C to 65°C. The extruded liposomes were 88 to 95 nm in diameter by dynamic light scattering.

Unentrapped triethylammonium polyanions were removed by chromatography on a Sepharose CL-4B size exclusion column eluted with HEPES-buffered dextrose (5 mmol/L HEPES, 5% dextrose, pH 6.5). CPT-11·HCl (kindly provided by TTY Biopharmaceuticals, Taipei, Taiwan) was added to the liposomes at a ratio of 500 g CPT-11/mol phospholipid and the pH adjusted to 6.5. The resulting solution was heated to 60°C for 30 minutes and then quenched on ice for 15 minutes. Unencapsulated CPT-11 was subsequently removed using a Sephadex G-75 column eluted with HEPES-buffered saline (5 mmol/L HEPES, 145 mmol/L NaCl, pH 6.5). The loading efficiency was determined in all preparations by quantitating both drug and phospholipid and comparing the resulting drug/phospholipid ratio to its input value. CPT-11 was determined spectrophotometrically at 372 nm in acid/methanol (20 volume % 0.5 mol/L phosphoric acid/80 volume % methanol). Phospholipid was quantitated using a standard phosphate assay (18).

Pharmacokinetic Studies

Female Sprague-Dawley rats (190-210 g) with indwelling central venous catheters were injected with a 0.2 to 0.3 mL bolus of ^{3}H-CHE-labeled CPT-11 liposomes (10 mg/kg). Blood samples (0.2-0.3 mL) were drawn at various times postinjection using a heparin-treated syringe. The withdrawn blood volume was replaced using PBS. Blood samples were diluted with 0.3 mL of ice-cold PBS containing 0.04% EDTA, weighed, and centrifuged. Plasma was assayed for CPT-11 [by fluorometry or high-performance liquid chromatography (HPLC)] and for liposome label (scintillation radioactivity counting). The percent of drug remaining in the liposomes was calculated by dividing the drug/lipid ratio in the blood samples by that of the injected liposomes (taken as 100%). Because free CPT-11 is cleared at a much faster rate than liposomes (Fig. 3*A*), a change in the CPT-11-to-liposomal lipid ratio was indicative of drug leakage from the carrier. Noncompartmental pharmacokinetics data analysis was done using PK Solutions 2.0 software (Summit Research Services, Montrose, CO).

Drug Stability and Metabolism Studies

Liposomal and free CPT-11 were administered i.v. at a dose of 25 mg/kg in female albino rats (180-220 g) as above, and blood samples were withdrawn at intervals up to 48 hours. The blood samples were mixed with ice-cold PBS containing 0.04% EDTA and quickly centrifuged. The plasma was assayed for CPT-11, SN-38, and their carboxylate forms by HPLC using a modification of the method of Warner and Burke (19). Briefly, samples were extracted with 400 μL of ice-cold methanol by vortexing and centrifugation at 14,100 × *g* for 5 minutes. The mobile phase consisted of 3% triethylammonium acetate pH 5.5 (solution A) and acetonitrile (solution B) delivered at 1.0 mL/min in a linear gradient of 20 volume % A to 50 volume % B in 14 minutes. The eluted products were detected by fluorescence with an excitation at 375 nm and emission at 500 nm. The retention times were 5.3 minutes (CPT-11 carboxylate), 6.8 minutes (SN-38 carboxylate), 9.3 minutes (CPT-11), and 11.0 minutes (SN-38).

Conversion of CPT-11 to SN-38 was assayed in macrophages isolated from the peritoneum of female NCR *nu/nu* mice and plated at a density of 150,000 cells per well in a 12-well plate. After 24 hours, nanoliposomal CPT-11 was added to macrophages at a concentration of 10 μg CPT-11/mL and incubated for 24 hours in RPMI 1640 with 10% FCS. At indicated times, the medium was removed and the cells washed twice with Hanks buffered saline. The cells were treated with 0.2 mL of 1% Triton X-100 at room temperature for 5 minutes and solubilized in 0.8 mL of 80 volume % methanol/20 volume % 0.1 mol/L H_3PO_4 with shaking for an additional 5 minutes. The cell debris was removed by centrifugation at 13,000 rpm for 10 minutes and the supernatant was assayed by HPLC as described above.

Acute Toxicity Studies

The maximum tolerated dose following single i.v. administration was evaluated in healthy female Swiss Webster mice following a protocol adapted from the protocol communicated by the National Cancer Institute (NCI) Developmental Therapeutics Program. Briefly, in the first range-seeking step, the drug was administered via the tail vein in groups of two mice, beginning with the dose of 60 mg/kg CPT-11 and continuing with the dose escalation factor of 1.8 until acute mortality or terminal morbidity (within 1 day postinjection) was observed in any animal. The second range-seeking step was similarly done using a dose escalation factor of 1.15 and starting with the highest dose at which no mortality or terminal morbidity was observed (the highest tolerated dose) in the first step. Finally, in a validation step, a group of five mice were injected at the highest tolerated dose achieved in the second step and followed for up to 11 days for signs of general health daily and body weight twice a week. If during the observation period there was no mortality, irreversible (terminal) morbidity, or weight loss in excess of 15% of the preinjection body weight, the administered dose was considered the acute single injection maximum tolerated dose.

Antitumor Efficacy Studies

BT474 tumor model. NCR *nu/nu* athymic female mice (4-6 weeks old; Taconic Farms, Germantown, NY) were s.c. implanted at the base of tail with 60-day sustained release 0.72-mg 17β-estradiol pellets (Innovative Research of America, Inc., Sarasota, FL). Two days later, 2×10^7 BT474 human breast cancer cells were implanted s.c. in the upper back area as a 0.1-mL suspension. Tumor growth was measured by caliper along the largest (length) and smallest (width) axes twice a week. Tumor volumes were calculated using the following formula (20): tumor volume = [(length) × (width)^2] / 2. At day 13 posttumor implantation (mean tumor volume, 200 mm^3), animals were randomized to three treatment groups of 13 to 15 animals per group and treated via i.v. (tail vein) injection as described in the text. The study was continued until day 60, which also represented the duration of estrogen supplementation. Animals were weighed twice weekly. If tumors reached 20% of the mouse body weight, the animals were euthanized.

HT29 tumor model. NCR *nu/nu* athymic male mice (6-week-old, weight >16 g; Charles River, Wilmington, MA) were injected s.c. in the right flank with 0.1-mL suspensions containing 5×10^6 HT-29 human colon cancer cells. Eleven days later (mean tumor volume, 150-350 mm^3), mice were randomized to six treatment groups of 11 animals per group. Starting on day 13, the animals received four tail vein injections at intervals of 4 days of various treatments as described in the text.

Results

Preparation of nanoliposomal CPT-11. A proposed novel process using a polyalkylammonium salt of a polymeric (polyphosphate) or nonpolymeric (sucrose octasulfate) highly charged multivalent anion as intraliposomal trapping agents resulted in improvement of both the encapsulation efficiency and the *in vivo* stability of the liposome-encapsulated weakly basic, amphipathic drug CPT-11. The process may involve the formation of an intraliposomal drug-polyanion complex (Fig. 1). Sucrose octasulfate is a high-charge density molecule with one strongly acidic, negatively charged sulfate group per 1.5 carbon atoms. The triethylammonium component of the salt assists drug loading as well, ensuring the charge neutrality of the liposome interior by allowing the efflux of cations accompanying the influx of the drug and possibly by formation of a self-perpetuating pH gradient to provide a driving force for progressive drug accumulation (10).

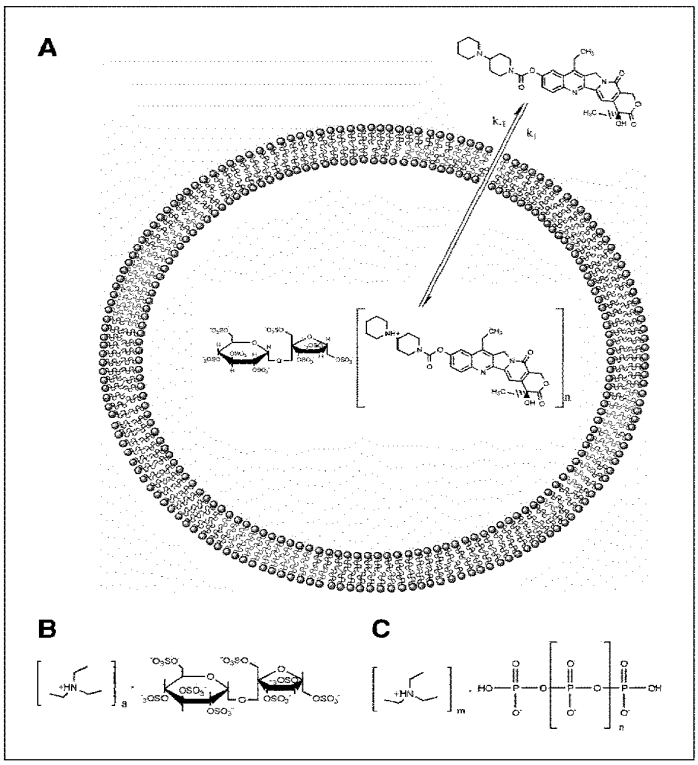

Figure 1. *A*, schematic depicting the intraliposomal stabilization strategy for CPT-11 using polyanionic trapping agents. The basic molecule CPT-11 forms a nanoscale complex with either poly(phosphate) or sucrose octasulfate in the liposome interior, thus stabilizing the liposomal formulation to increase drug retention while in circulation. Chemical structures of the triethylammonium salts of the polyanionic liposome trapping agents, poly(phosphate) (*B*) and sucrose octasulfate (*C*).

To minimize the treatment-associated lipid burden, encapsulation of CPT-11 was attempted up to drug-to-lipid ratios far exceeding the usual ratios achievable by traditional transmembrane-gradient drug loading techniques (Fig. 2). Remarkably, we found that CPT-11 encapsulation in liposomes was quantitative up to 800 g CPT-11/mol phospholipid. The final molar ratio of drug-to-phospholipid corresponds to 1.36:1 for liposomes loaded at 800 g CPT-11/mol phospholipid or 109,000 drug molecules per particle. This represents a 10- to 20-fold improvement over other liposomal formulations, including anthracyclines (3) or camptothecins lurtotecan (8) and SN-38 (21). We hypothesize that the high loading capacity of triethylammonium sucrose octasulfate liposomes is due to the formation of a stable complex between the drug and polyanion whereas the displaced triethylammonium ion dissociates and traverses the lipid bilayer as triethylamine, ensuring that the loading process continues until all added drug is encapsulated or the charge stoichiometry is achieved between the added drug and the liposomally encapsulated anion (Fig. 1).

Pharmacokinetics of nanoliposomal CPT-11. The pharmacokinetics of nanoliposomal CPT-11 formulated using either TEA-SOS or TEA-Pn were determined in normal female rats. Free CPT-11 was rapidly cleared from the circulation with $t_{1/2}$ = 0.27 hours (Fig. 3*A*). Liposome encapsulation was associated with significantly longer circulation times than free drug (Fig. 3*A* and *B*). This was especially true for liposomes loaded with TEA-SOS gradients, with blood half-lives for lipid and CPT-11 of 12.0 and 10.7 hours, respectively (Table 1).

Whereas both liposome constructs displayed long circulation for the lipid component, drug associated with TEA-SOS liposomes unexpectedly showed less rapid clearance from the blood than with TEA-Pn liposomes (Fig. 3*A* and *B*). This likely reflects that the $t_{1/2}$ of CPT-11 release from TEA-Pn liposomes was 14 hours, significantly shorter than that for TEA-SOS liposomes with a $t_{1/2}$ of CPT-11 release of 56.8 hours.

Drug stability of free and nanoliposomal CPT-11. *In vivo*, CPT-11 undergoes transformation to its more active metabolite, SN-38, and both molecules are also subject to inactivation by hydrolysis of the lactone forms to the respective carboxylate forms (Fig. 4*A* and *B*). Liposome encapsulation and delivery markedly altered these bioconversions in rats. Free CPT-11 was rapidly cleared from circulation, with only 2% of the injected dose remaining at 30 minutes and 35% of this present in the carboxylate form (Fig. 4*C*). In contrast, nanoliposomal CPT-11 showed both prolonged circulation, with 23.2% of injected dose still remaining at 24 hours, and drug protection, with no detectable conversion of CPT-11 to either SN-38 or the carboxylate form of CPT-11 (Fig. 4*D*). Thus, the high-charge density polyanionic nanoliposomal matrix provided a chaperone for the stably entrapped prodrug CPT-11, improving its pharmacokinetics and preventing its inactivation or premature conversion to the toxic metabolite SN-38.

Once deposited in tumors, liposomes are known to be taken up avidly by tumor-resident macrophages (22). To determine if macrophages could metabolically activate drug from nanoliposomal CPT-11, an *ex vivo* assay using macrophages isolated from the peritoneum of nude mice was done. Incubation of nanoliposomal CPT-11 with macrophages showed no detectable conversion to SN-38 at 24 hours but 100% conversion to SN-38 by 72 hours. This time course suggested that at least 24 hours was required for macrophage-mediated disruption of the liposome, drug release, and conversion to SN-38.

Acute toxicity of nanoliposomal CPT-11. The acute toxicity of free and nanoliposomal CPT-11 was determined in normal Swiss Webster mice using an NCI-based protocol. The maximum tolerated dose of free CPT-11 was 80 mg/kg whereas the maximum

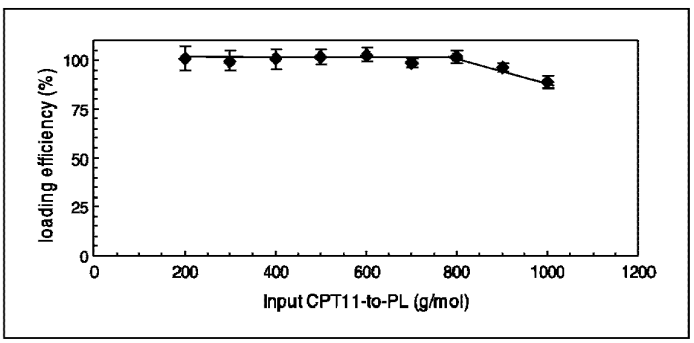

Figure 2. Liposomal loading efficiency as a function of input CPT-11-to-phospholipid (*PL*) ratio. Distearoylphosphatidylcholine/cholesterol/methoxypoly(ethylene)glycol (PEG2000)-derivatized distearoylphosphatidylethanolamine (3:2:0.015) liposomes were loaded with CPT-11 as described in Materials and Methods. The resulting CPT-11-to-phospholipid ratio following loading was determined by quantitating both CPT-11 and phospholipid in the resulting purified liposomal CPT-11 formulation, and the loading efficiency by comparing this ratio to the input ratio.

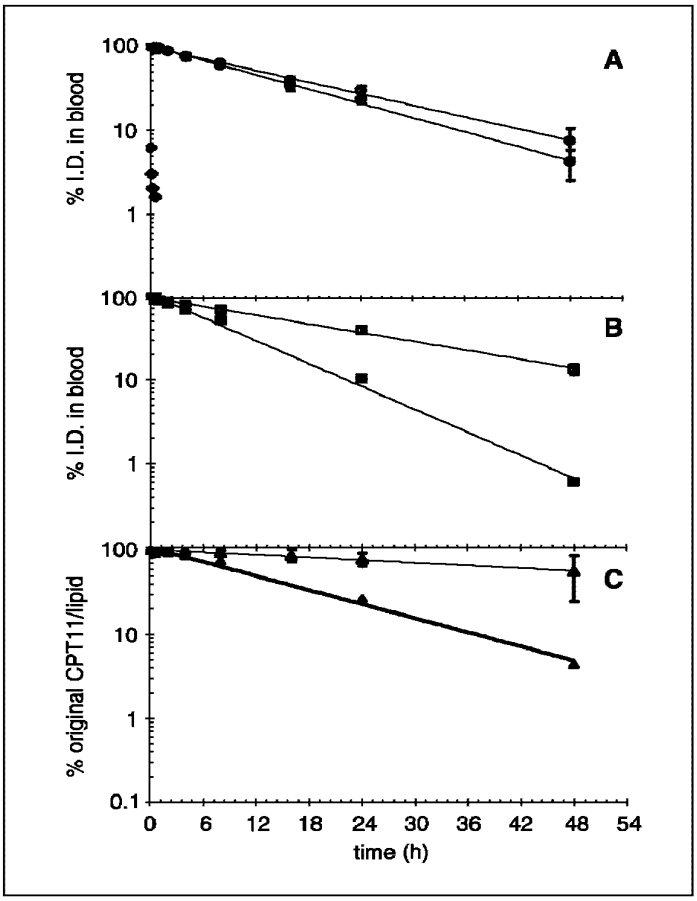

Figure 3. Pharmacokinetics of nanoliposomal CPT-11 in rats. Nanoliposomal CPT-11 prepared using either triethylammonium sucrose octasulfate (*A*) or poly(phosphate) (*B*) was administered i.v. in 9-week-old female Sprague-Dawley rats (body weight ~200 g) with indwelling central venous catheters at a dose of 10 mg CPT-11/kg (17.6 μmol phospholipid/kg). Free CPT-11 was administered i.v. as a bolus injection at 25 mg/kg (*A*, ♦). Plasma was sampled at the indicated times and analyzed for drug and liposomal lipid content. *Points*, % of injected dose (*% I.D.*) of lipid (○, □) or drug (●, ■, ♦). *C*, drug retention was calculated as percent of original drug associated with liposomal lipid at each time point for the poly(phosphate) (▲) and sucrose octasulfate (△) formulations.

tolerated dose of nanoliposomal CPT-11 formulated using a TEA-SOS gradient was not achieved even at the highest administered dose of 324 mg CPT-11/kg. A dose of >324 mg CPT-11/kg was impossible to administer because of concentration and injection volume limitations. Therefore, nanoliposomal CPT-11 delivery reduced drug toxicity in the mouse by at least 4-fold.

Efficacy of nanoliposomal CPT-11 in the BT474 breast cancer model. Treatment using nanoliposomal CPT-11, formulated using the TEA-Pn loading strategy, was evaluated in the BT474 breast tumor xenograft model (Fig. 5*A*). Free CPT-11 was clearly efficacious in this model with noticeable inhibition of tumor growth. However, treatment with nanoliposomal CPT-11 provided further advantage with dramatic regressions in tumor volumes and 100% cures of mice (defined as no residual tumor at study end).

Treatment-related toxicities were not observed. There was a slight decrease in mean body weight by 3.3% on the final treatment day in the animals receiving liposomal CPT-11; this decrease was not statistically significant compared with pretreatment weight ($P = 0.274$, Student's t test). All other weight measurements were within the expected range.

Efficacy of liposomal CPT-11 in the HT29 colon cancer model. In the HT29 colon tumor xenograft model, free CPT-11 again showed efficacy, albeit modest (Fig. 5*B*). However, both nanoliposomal CPT-11 formulations showed pronounced antitumor effects, including tumor regression during treatment followed by prolonged absence of tumor regrowth. Indeed, at 42 days postimplantation, all nanoliposomal CPT-11 treatments seemed to be equivalent and maximally efficacious.

With continued observation, tumor regrowth was observed beginning on day 47 postimplantation. At this point, all control and free CPT-11-treated mice had been sacrificed due to excessive tumor growth. Based on regrowth rates, treatment with TEA-SOS liposomes was more efficacious than TEA-Pn liposomes administered at the same CPT-11 dose. Furthermore, treatment with either liposome type at 50 mg/kg dose was more efficacious than at 25 mg/kg. In an analysis of cure rates, no mice receiving control or free CPT-11 were cured. Mice receiving TEA-Pn liposomal drug at 50 mg/kg per injection, despite initial tumor regressions, showed eventual regrowth. In the two groups receiving 25 mg/kg of either liposome formulation, one animal (9.1%) from each group was tumor-free at study end. In the group receiving 50 mg/kg of the TEA-SOS liposome formulation, 4 animals (36.4%) showed no regrowth and remained tumor-free.

Animals receiving free CPT-11, but not any of the nanoliposomal CPT-11 preparations, showed morbidity (loss of alertness, humped posture, ruffled fur, decreased mobility) for 1 hour after drug injection. Animals receiving free CPT-11 also lost 6% of weight

Table 1. Pharmacokinetic variables for free and nanoliposomal CPT-11 in rats

Formulation	$t_{1/2}$ (h)	AUC_∞ (μg h/mL)	CL (mL/h)	V_d (mL)	MRT (h)	$t_{1/2}$ CPT-11 release (h)
Free CPT-11	0.27	6.2	1,609	616.4	0.4	—
Ls-CPT-11 [TEA-Pn]	6.80	1,407.8	7.10	69.7	9.8	14.0
Ls-CPT-11 [TEA-SOS]	10.7	2,134.4	4.69	72.3	15.4	56.8

NOTE: The data used to calculate the pharmacokinetic variables for CPT-11 when formulated either in the free form or liposomal form refer to the actual drug concentrations measured in the blood that were then used to calculate the %ID values found in the corresponding curves for Fig. 3*B*.
Abbreviations: AUC_∞, area under the concentration versus time curve in plasma based on the sum of exponential terms; MRT, mean residence time calculated from exponential terms; CL, clearance calculated from exponential terms; V_d, volume of distribution.

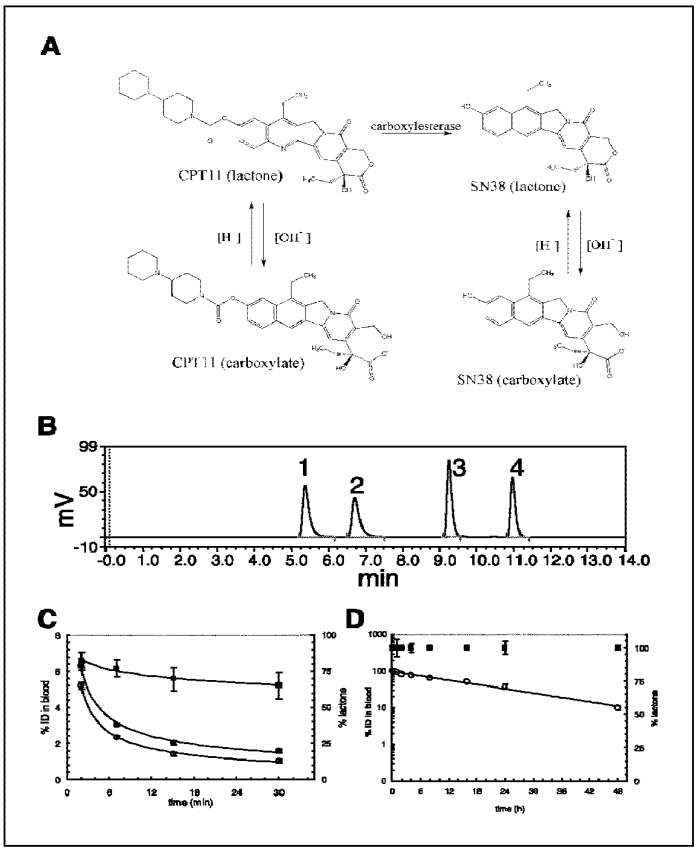

Figure 4. Drug stability of free and nanoliposomal CPT-11. *A*, CPT-11 and SN-38 exist in a pH-dependent equilibrium between closed lactone and open carboxylate configurations. CPT-11 is converted to its more active metabolite, SN-38, by carboxylesterases. *B*, HPLC chromatogram showing the separation of these species: CPT-11 carboxylate (*peak 1*), SN-38 carboxylate (*peak 2*), CPT-11 lactone (*peak 3*), and SN-38 lactone (*peak 4*). The *in vivo* drug stability of free (*C*) and nanoliposomal (*D*) CPT-11 was evaluated following single i.v. bolus administration at a dose of 25 mg CPT-11/kg in 9-week-old female Sprague-Dawley rats (body weight ~200 g). Levels of total CPT-11 (●) and CPT-11 lactone (○) in the blood were determined by HPLC analysis and expressed as percent of initial CPT-11 dose. *Right y axis*, percentage of CPT-11 in the lactone form is plotted as a function of time (■).

during treatment and did not recover, probably because of the effects of the growing tumor. Animals receiving nanoliposomal CPT-11 formulations experienced transient weight loss of 5% (at 25 mg/kg) or 9% (at 50 mg/kg) between the second and third injections as compared with pretreatment values; however, weights recovered following completion of treatment.

Discussion

Liposome delivery has been shown to improve the pharmacokinetic profile and widen the therapeutic index of certain anticancer drugs, especially the anthracycline class (1, 2). Improved efficacy is in part a result of passive targeting to tumor sites based on the enhanced permeability and retention (EPR) effect (23). To fully exploit this process, drug carriers must be engineered to retain drug while circulating, thereby preventing premature drug release before accumulating in the tumor but still allowing for release of drug once in the vicinity of the tumor. Antibody-targeted nanoparticles, such as immunoliposomes against HER2 (24) or epidermal growth factor receptor (25), represent another strategy for more efficient drug delivery to tumor cells.

Gradient-based drug loading technologies, in which electrochemical gradients drive the accumulation of drugs in the liposome interior, represent a key advance in liposome research (11, 26). This approach was further refined when transmembrane gradients of ammonium ion were proposed to form a self-sustaining pH-gradient that can load drugs inside liposomes (10). However, weakly basic anthracyclines represented the only drug class that afforded slow *in vivo* release rates when loaded using gradients involving common anionic counterions, such as sulfate or citrate. With other drug classes, gradient-based loading has been achieved with variable efficiency. To stabilize other cationic drugs against premature escape from liposomes, the use of pre-entrapped polyanionic polymers was proposed (9, 27).

In the present study, we used a drug loading transmembrane gradient system with two components, a substituted ammonium and a poly(anionic) trapping agent of either polymeric (polyphosphate) or nonpolymeric (sucrose octasulfate) nature. The use of polymeric polyanions such as heparin or dextran sulfate to improve liposomal drug retention has been reported (9, 27). Polyphosphate was effective in stabilizing intraliposomal CPT-11

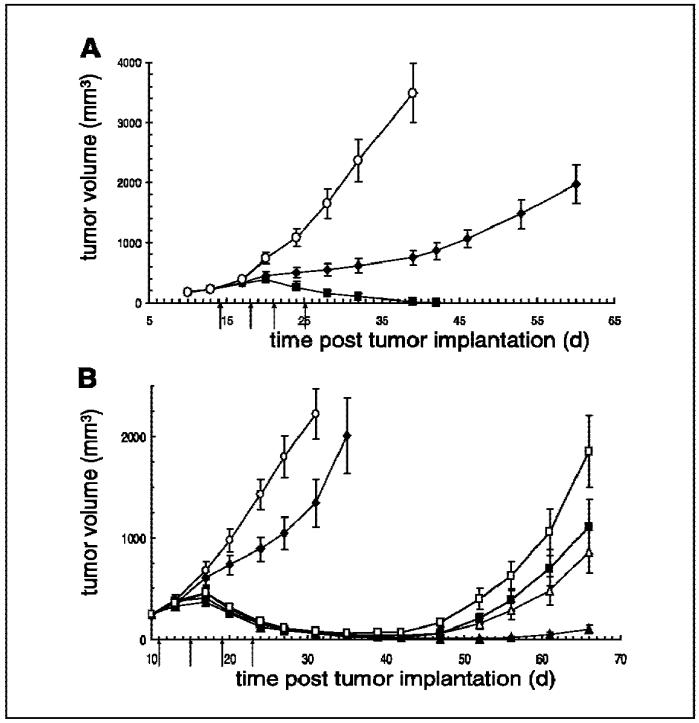

Figure 5. Antitumor efficacy of nanoliposomal CPT-11 in tumor xenograft models. *A*, BT474 breast cancer cells were implanted s.c. in nude mice along with estrogen pellets. When tumors were well established and had reached mean volumes of 200 mm^3, the following treatments were initiated: control (○), drug- and liposome-free vehicle only; free CPT-11 (◆); or nanoliposomal CPT-11 stabilized with TEA-Pn (■). Free and nanoliposomal CPT-11 were injected at 50 mg CPT-11/kg/dose i.v. twice per week for four doses (*arrows*). *B*, HT-29 colon cancer cells were implanted s.c. in nude mice. When tumors were well established and had reached mean volumes of 150 to 350 mm^3, the following treatments were administered: control (saline; ○); free CPT-11, 50 mg/kg/dose (◆); nanoliposomal CPT-11 using TEA-Pn, 25 mg/kg/dose (□); nanoliposomal CPT-11 using TEA-Pn, 50 mg/kg/dose (■); nanoliposomal CPT-11 using TEA-SOS, 25 mg/kg/dose (△); and nanoliposomal CPT-11 using TEA-SOS, 50 mg/kg/dose (▲).

against *in vivo* release, having the added advantage of being more readily biodegradable than dextran sulfate. However, polyanionic polymers such as heparin and dextran sulfate have notable anticoagulant activity and, in the case of dextran sulfate, toxic to Kupffer cells (28). The undefined chemical nature of many functionalized polymers may also contribute to variability in *in vivo* properties. Unexpectedly, we observed that a highly charged, nonpolymeric anion, such as sucrose octasulfate, provided even better drug retention than a polyanionic polymer, resulting in outstanding *in vivo* drug encapsulation stability. Sucrose octasulfate is a product of exhaustive esterification of sucrose, using chlorosulfonic acid or sulfur trioxide in pyridine or methylpyridine, and is a known pharmaceutical ingredient, the basic aluminum salt (Sucralfate) of which is widely used to treat gastric hyperacidity (29). Compared with dextran sulfate, sucrose octasulfate is chemically well defined; it does not have known anticoagulant or antimacrophage activity (29) and its salts can be produced in pure crystalline form ensuring less interlot variability.

The concept of nanoparticle delivery of camptothecins is very attractive based on potential advantages, including overcoming the solubility limitations of this class, protecting drug in the active lactone configuration, rerouting of drug from sites of toxicity such as the gastrointestinal tract, prolonging circulation time, increasing tumor accumulation via the EPR effect, and providing sustained release for a so-called metronomic effect. Using a novel intraliposomal stabilization technology, we have developed a nanoliposomal CPT-11 featuring drug loading efficiency and drug payload ($>10^5$ per particle) in far excess of that previously reported for this type of encapsulation; this agent showed marked *in vivo* retention of CPT-11 during long circulation times while simultaneously protecting the drug from lactone hydrolysis or premature activation. Compared with free CPT-11, this liposome-based nanoparticle reduced host toxicity in rats by >4-fold and greatly increased antitumor efficacy in animal models. In a separate study, we showed similar improvements in efficacy and host toxicity when nanoliposomal CPT-11 was administered locally to brain tumors using convection-enhanced delivery (30).

Previously reported liposomal camptothecin preparations have shown increased efficacy but not necessarily improved toxicity when compared with free drug (8, 9, 31). Other examples have shown prolonged circulation (32, 33), but not to the extent observed for the TEA-SOS-stabilized liposomes described here. In addition, a liposomal version of SN-38 is cleared even more rapidly with an AUC_∞ that seems to be at least 2 orders of magnitude less than that observed for nanoliposomal CPT-11 (34).

Another aspect of nanoliposomal CPT-11 is that it delivers a prodrug. Cytotoxic drugs encapsulated in liposomes are normally unable to act on their therapeutic targets or cause toxicity until they can be released from the confines of the carrier, and thus liposomal drug delivery can itself be regarded as a prodrug strategy. Hence, in this dual prodrug strategy, liposome delivery of CPT-11 chaperones the camptothecin until it reaches tumor sites where the prodrug can then be activated locally. Although local activation of CPT-11 to SN-38 has yet to be shown, carboxylesterases have a widespread distribution in different tumor types (35–37) and are active in macrophages, the principal scavenger of liposomes. Indeed, we observed that nanoliposomal CPT-11 was completely converted to SN-38 by macrophages after 72-hour incubation. We hypothesize that nanoliposomal CPT-11 may be acted on by tumor-resident macrophages, which convert drug to SN-38 with subsequent diffusion to nearby tumor cells. Alternatively, CPT-11 may be activated directly by tumor cells following release from its liposome carrier.

We conclude that nanoliposomal CPT-11 generated by novel intraliposomal drug stabilization resulted in advantageous pharmacologic properties with increased efficacy and reduced host toxicity *in vivo*. The drug-loading and stabilization technologies used for CPT-11 may also be broadly applicable to other weakly basic anticancer drugs as we have recently shown using a novel histone deacetylase inhibitor, LAQ824 (38). Nanoliposomal CPT-11 may provide a robust and useful nanoparticle-based treatment for cancer.

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Development of a Highly Active Nanoliposomal Irinotecan Using a Novel Intraliposomal Stabilization Strategy

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A randomized phase II study of PEP02 (MM-398), irinotecan or docetaxel as a second-line therapy in patients with locally advanced or metastatic gastric or gastro-oesophageal junction adenocarcinoma†

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Background: PEP02 is a novel highly stable liposomal nanocarrier formulation of irinotecan. This randomized phase II study evaluated the efficacy and safety of single agent PEP02 compared with irinotecan or docetaxel in the second-line treatment of advanced oesophago-gastric (OG) cancer.
Patients and methods: Patients with locally advanced/metastatic disease who had failed one prior chemotherapy regimen were randomly assigned to PEP02 120 mg/m^2, irinotecan 300 mg/m^2 or docetaxel (Taxotere) 75 mg/m^2 every 3 weeks. The primary end point was objective response rate (ORR). Simon's two-stage design was used and the ORR of interest was 20% ($\alpha = 0.05$, type II error $\beta = 0.10$, null hypothesis of ORR was 5%).
Results: Forty-four patients per arm received treatment, and 124 were assessable for response. The ORR statistical threshold for the first stage was reached in all arms. In the intent-to-treat (ITT) population, ORRs were 13.6% (6/44), 6.8% (3/44) and 15.9% (7/44) in the PEP02, irinotecan and docetaxel arms, respectively. The median progression-free survival (PFS) and overall survival were similar between the trial arms. Commonest grade 3–4 adverse event reported was diarrhoea in the PEP02 and irinotecan groups (27.3% versus 18.2%).
Conclusion: The ORR associated with PEP02 was comparable with docetaxel and numerically greater than that of irinotecan. PEP02 warrants further evaluation in the advanced gastric cancer setting.
Key words: docetaxel, irinotecan, liposomal irinotecan, oesophago-gastric cancer, phase II, second line

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introduction

Oesophago-gastric (OG) cancer represents a significant global health problem with an estimated one million cases diagnosed every year worldwide [1]. Several randomized trials and meta-analyses have established the role of combination chemotherapy in the first-line treatment of advanced OG cancer with prolongation of OS and improvement in the quality of life [2].

Currently there are no standard second-line treatments in this setting [3, 4], although a trend exists towards increased use of second- and third-line treatments, with a significant geographical variation seen in both the therapeutic approach and the uptake of second-line treatment. In large first-line clinical studies, the rates of uptake of subsequent chemotherapy were 14% in the UK REAL 2 study, 42% in the international ToGA trial and 75% in the Japanese SPIRITS trial [5–7]. Recent phase III trials have demonstrated a survival benefit associated with the use of irinotecan or docetaxel (Taxotere) compared with best supportive care (BSC) alone in patients who have failed one or two prior lines of treatment [8, 9]. More recently, a randomized study from Japan demonstrated comparable results with either weekly paclitaxel (Taxol) or irinotecan in second-line therapy [10].

PEP02

PEP02, also known as MM-398 (Merrimack Pharmaceuticals, Inc.), is a highly stable liposomal nanocarrier formulation of irinotecan hydrochloride (CPT-11) [11]. This liposomal formulation is associated with preferentially increased tumour exposure to irinotecan and therefore, local release and conversion to SN-38 as a result of prolonged circulation in the bloodstream, longer half-life, increased area under the curve (AUC), slower clearance and reduced volume of distribution compared with the free drug [11]. In a phase 1 study of a variety of solid tumours, the maximum tolerated dose (MTD) of PEP02 as a single agent was found to be 120 mg/m^2 once every 3 weeks [12].

This randomized three-arm phase II study was designed to assess objective response rate (ORR) with single agent PEP02, or irinotecan or docetaxel in patients with locally advanced or metastatic gastric and gastro-oesophageal (GEJ) adenocarcinomas in the second-line setting.

methods

patients

Eligible patients were aged ≥18 years of age with histologically or cytologically confirmed locally advanced or metastatic gastric or GEJ junction adenocarcinoma. Patients had to have at least one measurable lesion and have failed one prior systemic chemotherapy (including patients with disease recurrence within 6 months of (neo)adjuvant chemotherapy).

Additional eligibility criteria included Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0–2, adequate organ function, life expectancy >3 months, no concurrent uncontrolled medical condition, no other active malignancy, no known brain metastasis, no prior irinotecan/taxane treatment and no history of allergic reactions to liposomal products. The trial was conducted in accordance with the Declaration of Helsinki and had ethical approval. A written informed consent was obtained from each patient before study entry. The institutional review boards of all participating centres reviewed and approved the protocol (ClinicalTrials.gov identifier NCT00813072).

treatment

Eligible patients were randomly assigned 1:1:1 to receive PEP02: 120 mg/m^2 (90-min infusion on day 1 of each cycle), irinotecan: 300 mg/m^2 (90-min infusion on day 1 of each cycle) or docetaxel (Taxotere): 75 mg/m^2 (60-min infusion on day 1 of each cycle) intravenously as monotherapy administered every 3 weeks. In the PEP02 arm, a protocol-specified dose level increase to 150 mg/m^2 was allowed for patients who did not have a ≥grade 1 adverse event. Treatment was continued until disease progression, unacceptable toxicity or withdrawal of consent. Treatment was delayed by 1 week (maximum of 2 weeks) if the neutrophil count was $<1.5 \times 10^9$/l or the platelet count was $<100 \times 10^9$/l. The severity of adverse events was graded according to NCI-CTCAE v 3.0.

assessments

Medical history, vital signs and PS were documented within seven days before randomization, and the patients underwent ECG, urinalysis and routine blood tests (including creatinine clearance) during this timeframe. Physical examination, haematology, biochemistry and urinalysis were repeated at the beginning of each cycle.

Baseline tumour assessment [computed tomography (CT) scan of chest, abdomen, and pelvis] was carried out within 28 days before randomization and CT scans were repeated after every two treatment cycles until disease progression. Response and progression were evaluated using the RECIST version 1.0 [13] criteria and all responses were confirmed with a second CT scan carried out 1 month later. The survival status was assessed every 2 months following the completion of trial treatment. Safety assessments were carried out on the day of treatment administration and at 30 days following the last exposure to trial treatment. The severity of adverse events was graded according to NCI-CTCAE v 3.0. An independent data monitoring committee regularly reviewed study safety and efficacy data.

pharmacokinetic and pharmacogenetic analysis (non-UK sites)

Pharmacokinetic (pK) studies were carried out in the PEP02 and irinotecan arms (supplementary Appendix SA. I. 1, available at *Annals of Oncology* online). An optional pharmacogenetic (pGx) study was also conducted, with analysis being carried out on samples from consenting patients in the PEP02 or irinotecan arms (see supplementary Appendix SA. I. 2, available at *Annals of Oncology* online).

statistical considerations

The primary end point was ORR and was analysed in both the intent-to-treat (ITT) and assessable populations (AP). The ITT population was defined as all recruited subjects who received any study medication. The AP, a subset of ITT, was defined as patients who had received at least two cycles of treatment and were assessable for response.

The study was not powered to allow statistical comparison of efficacy and toxicity between the three treatment arms. For the primary end point, a Simon's two-stage design was used and the response rate of interest was set at 20% ($\alpha = 0.05$, type II error $\beta = 0.10$) with a null hypothesis rate of 5%. For each arm, two responses within the first 21 assessable patients were required to proceed to the second stage, and five responses among 41 assessable patients in both the stages were required to reject the null hypothesis. Based on these calculations, 41 assessable patients were planned to be enrolled in each arm of the study.

The secondary end points included progression-free survival (PFS; time from the date of first study treatment to the date of disease progression or death, overall survival (OS; time from the date of first study treatment to the date of death), and 1-year survival rate.

results

Between January 2008 and June 2010, 135 patients were randomly assigned from 19 sites in the UK, Spain, Taiwan, Croatia, Korea and Bosnia. Overall, 54% (73/135) of the patients were recruited from Europe and 46% (62/135) were recruited from Asia. Three patients (one per arm) were ineligible and were withdrawn before receipt of any study medication, leaving 132 patients (44 in each arm) in the ITT population (Figure 1). Eight patients did not receive at least one post-treatment tumour assessment, leaving 124 patients in the AP (PEP02 $n = 41$, irinotecan $n = 43$, docetaxel $n = 40$). The baseline characteristics were well balanced between the treatment arms, the majority of patients were male (78%), had metastatic disease (94%) and PS 0–1 (92%). (Table 1)

The mean number of treatment cycles was 4.4 in the PEP02 arm (range 1–18), 4.6 in the irinotecan arm (range 1–12) and 4.7 in the docetaxel arm (range 1–12). In the PEP02 arm, five patients without ≥grade 1 toxicity received a dose of 150 mg/m^2. The median relative dose intensity by cycle was high in all the three treatment arms (>0.90) and the proportion of patients requiring dose reduction was also similar between the treatment arms [20.5% (9 of 44) with PEP02, 25% (11 of 44) with irinotecan and 22.7% (10 of 44) with docetaxel]. The primary reason for treatment discontinuation was disease progression (68.9%) followed by adverse events (13.6%) and investigators' decision (9.8%).

efficacy

Within the first assessable 21 patients recruited to each arm, responses were noted in 4, 2 and 5 patients treated with PEP02, irinotecan and docetaxel, respectively. The ORR threshold for the first stage of Simon's two-stage was, therefore, reached in all the three arms and the trial continued to full accrual. In the ITT population, the ORR was 13.6% (6/44; 95% CI 5.2–27.4) in the PEP02 arm, 6.8% (3/44; 95% CI 1.4–18.7) in the irinotecan arm and 15.9% (7/44; 95% CI 6.6–30.1) in the docetaxel arm. (Table 2) Additionally, the response rate of PEP02 at 150 mg/m^2 ($n = 5$) was 60% (3 PR). The DCRs for the three arms were PEP02 59.1% (26/44), irinotecan 61.4% (27/44), and docetaxel 52.3% (23/44), respectively. A pre-specified subgroup analysis demonstrated a numerically better ORR in Asian versus European patients in the PEP02 and docetaxel arms [20% versus 8.3% for PEP02 and 26.3% versus 8.0% for docetaxel (supplementary Table SA.1, available at *Annals of Oncology* online)].

survival

In the ITT population, the median OS was 7.3 months (95% CI; 3.84–9.17) in the PEP02 arm, 7.8 months (95% CI; 4.90–9.20) in the irinotecan arm and 7.7 months (95% CI; 5.32, 12.32) in the docetaxel arm. Kaplan–Meier estimates of 1-year survival rates were 21.3%, 30.8% and 40.4% in those three treatment arms, respectively (Table 2, Figure 2A). Median PFS was similar in all the three arms [2.7 months (95% CI; 1.54–3.65) with PEP02, 2.6 months (95% CI; 1.48–4.34) with irinotecan and 2.7 months (95% CI; 1.41–5.45) with docetaxel] (Table 2, Figure 2B). A trend towards better overall survival was observed in Asian patients (median OS 8.9 m versus 6.0 m, HR 1.40, 95% CI 0.97–2.16, $P = 0.065$) (supplementary Figure S1, available at *Annals of Oncology* online). Median PFS and OS of patients who received PEP02 at 150 mg/m^2 were numerically higher than patients who received that at 120 mg/m^2 group (PFS: 6.0 m versus 2.5 m; OS: 7.8 m versus 6.0 m, respectively).

toxicity

Table 3 demonstrates treatment-related grade 3–4 toxic effects. Treatment was well tolerated; the overall incidence of grade

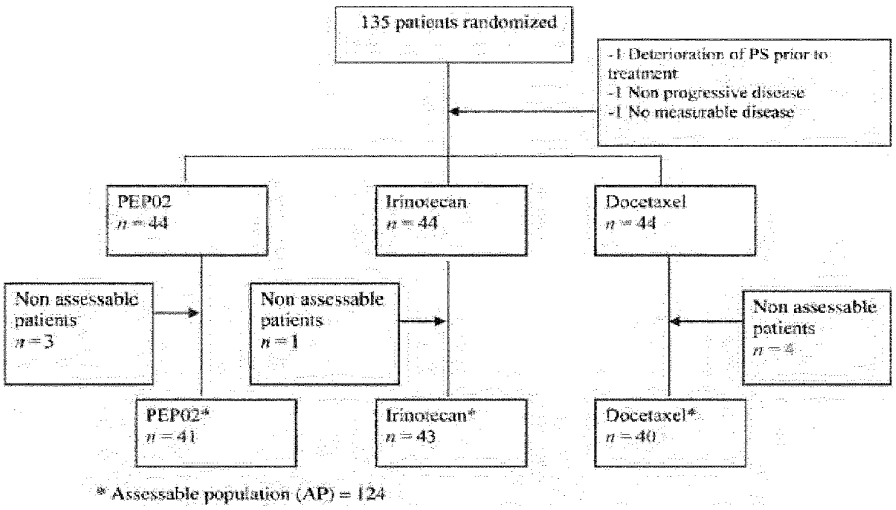

Figure 1. Consort diagram.

Table 1. Baseline characteristics

	PEP02, N = 64		Irinotecan, N = 63		Docetaxel, N = 66	
	n	%	n	%	n	%
Sex						
% of males	35	70.3%	38	77.3%	34	77.3%
Age						
Median	56		62		58	
Range	30–68		33–79		33–81	
Eastern Cooperative Oncology Group performance status (ECOG PS)						
0–1	61	93%	61	93%	60	91%
2	3	7%	3	7%	4	9%
Geographical region (n = 65 each group)						
Asia	23	47%	23	47%	20	44%
Europe	24	53%	24	53%	25	56%
Previous treatment						
Prior Radiotherapy	9	20.5%	6	13.6%	7	15.9%
Prior Surgery	30	70.5%	31	70.5%	27	61.4%
Prior Chemotherapy	44	100%	44	100%	44	100%
Primary tumour site						
Gastric	37	84%	35	80%	30	68%
GO Junction	7	16%	9	20%	14	32%
Extent of disease						
Metastatic	43	97.7%	40	91%	43	97.7%

3–4 adverse events was 38.6% in the PEP02 arm, 34.1% in the irinotecan arm and 15.9% in the docetaxel arm. No treatment-related deaths were observed. Diarrhoea was the most common toxicity noted in the PEP02 and irinotecan arms (all grade toxicity 72.7% versus 68.2%, respectively). The most frequent toxicity in the docetaxel group was alopecia (52.3% all grade toxicity). Overall, PEP02 was associated with an increased frequency of grade 3–4 diarrhoea and nausea, with similar rates of vomiting, neutropaenia and febrile neutropaenia compared with irinotecan and docetaxel. In the five patients treated at the dose of 150 mg/m^2, no clinically relevant toxicity difference was noted. Treatment-related toxic effects led to discontinuation of the study drug in six patients in each arm.

pharmacokinetic/pharmacogenetic evaluation

Sixty-four patients were included in the pK analysis. The effect of treatment on pK parameters is summarized in supplementary Table SA.1, available at *Annals of Oncology* online (supplementary Appendix, available at *Annals of Oncology* online). The pGx sub-study was undertaken in 71 patients of which 37 were treated with PEP02 and 34 with irinotecan.

pharmacokinetics of the active metabolite, SN-38

The mean T_{max} values of SN-38 were 10.2 and 2.1 h after infusion of 120 mg/m^2 PEP02 and 300 mg/m^2 irinotecan, respectively. The dose-normalized C_{max} value following PEP02 treatment was lower than that of irinotecan, and correspondingly the dose-normalized C_{max} value for the formation of SN-38 from CPT-11 following infusion of PEP02 was ~50% less than after infusion of irinotecan. However, the dose-normalized AUC_{0-t} and $AUC_{0-\infty}$ values of SN-38 in the PEP02 treatment group were 3.30 and five times higher, respectively, than those seen with irinotecan. The mean $T_{1/2}$ and $MRT_{0-\infty}$ values of PEP02 treatment were four and five times higher, respectively, than those associated with irinotecan. The pK parameters of CPT 11 and SN-38G are detailed in the supplementary Appendix, available at *Annals of Oncology* online (see supplementary Appendix SA.II, available at *Annals of Oncology* online).

pharmacogenetic analysis

The genotype frequencies of the genetic polymorphisms of the UGT1A family were analysed. Forty-three (61.4%) patients were found to be wild type for *UGT1A1*28* (TA_6TA_6), 26 (37.1%) patients had a heterozygous polymorphism (TA_7TA_6) and only one (1.4%) patient was found to have homozygous mutation (TA_7TA_7). Genotype frequencies for the other UGT1A polymorphisms are summarized in supplementary Table SA, available at *Annals of Oncology* online. 2 (supplementary Appendix, available at *Annals of Oncology* online).

UGT1A1 variants were correlated with toxicity. Thirty-six patients from the PEP02 group and 34 patients from the irinotecan group were included in this analysis. In the PEP02 arm, the frequency of grade 3–4 neutropaenia was higher for *UGT1A1*6* heterozygotes compared with the wild-type genotype [3% (1 of 30) for wild type versus 40% (2 of 5) for heterozygotes, $P = 0.0220$]. Higher rates of grade 3–4 neutropaenia was also observed in heterozygotes for the genotype *UGT1A1*27* in the irinotecan arm, when compared with wild type [13% (4 of 31) for wild type versus 66% (2 of 3) for heterozygotes, $P = 0.0197$]. No other association between gene polymorphisms and toxic effects was significant. No correlation between UGT1A gene polymorphism and PEP02/irinotecan pK was demonstrated. (supplementary Appendix SA II. 4, available at *Annals of Oncology* online)

Table 2. Summary table of main efficacy results (ITT, $n = 132$)

	ITT population	Disease control	1 Year survival rate	PFS	OS
		CR + PR n (%)	DCR n (%)	% (95% CI)	Median 95% CI	Median 95% CI
PEP02	66	4 (13.6)	26 (59.1)	71.3% (6.6, 36.0)	3.7 (3.56, 3.65)	7.3 (3.84, 9.17)
Irinotecan	66	3 (5.8)	27 (61.3)	30.8% (16.6, 45.1)	2.6 (1.85, 4.34)	7.8 (4.98, 9.20)
Docetaxel	66	7 (15.9)	23 (52.3)	61.4% (25.8, 35.1)	3.7 (3.48, 5.45)	7.7 (5.32, 12.32)

ITT, intent to treat; CR, complete response; PR, partial response; DCR, disease control rate (CR + PR + SD).

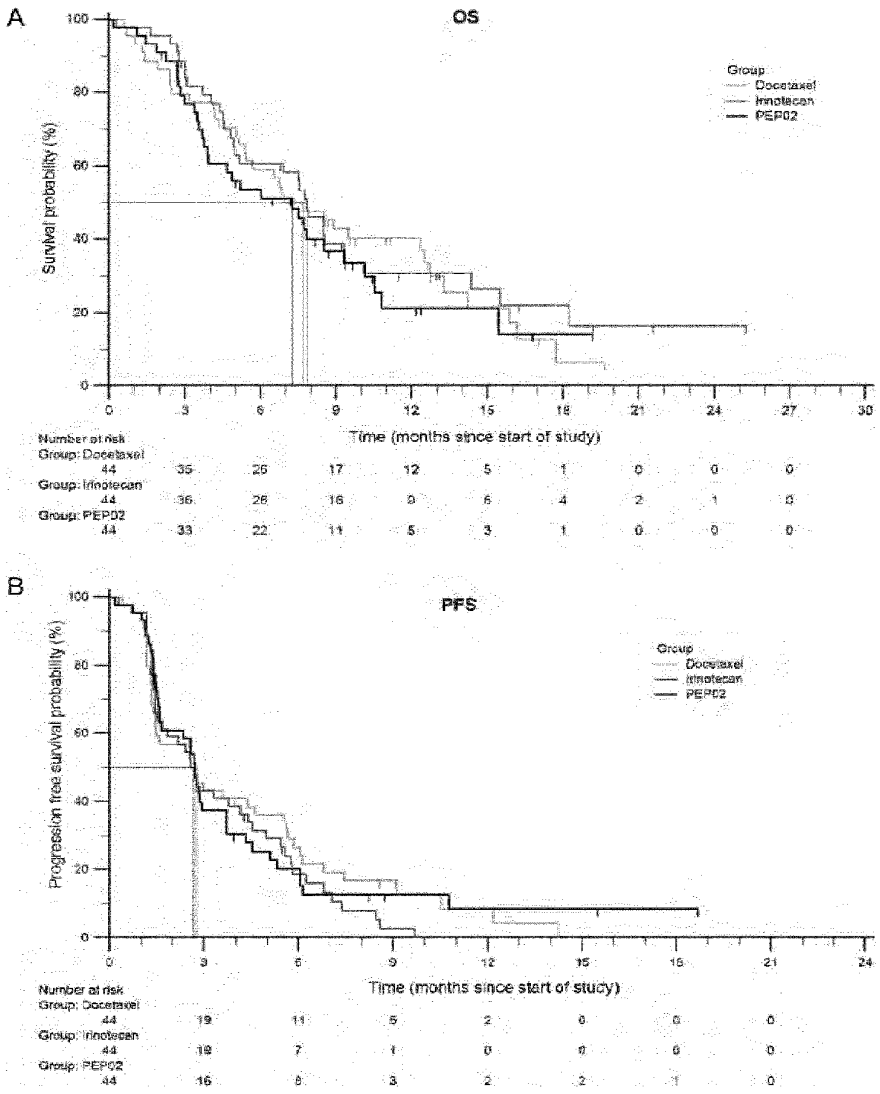

Figure 2. (A) Kaplan–Meier estimates of OS in the intent-to-treat population. (B) Kaplan–Meier estimates of PFS in the intent-to-treat population.

Table 3. Most common grade 3–5 adverse events

discussion

This randomized phase II trial represents the first study comparing a novel highly stable liposomal nanocarrier formulation of irinotecan (PEP02, MM-398) with docetaxel and irinotecan in the treatment of locally advanced or metastatic OG cancer after failure of first-line treatment.

The study's primary end point was ORR and in Simon's two-stage design only the PEP02 and docetaxel arms met the protocol-specified primary end point of five or more patients with confirmed tumour response in a total of 41 assessable patients. PFS, 1-year survival rate and OS were similar in the three arms. Other stratification factors such as geographical region, gender, ECOG and disease status (locally advanced versus metastatic) did not affect ORR or survival outcomes.

CPT-11 is mainly present in encapsulated form in the plasma after administration of PEP02 [12, 14]. In our study, pK results were consistent with previously reported profiles of PEP02 and free irinotecan [14, 15]. This study also confirms that following infusion of PEP02, there is a higher AUC, lower clearance and smaller volume of distribution for total and encapsulated irinotecan compared with the published pharmacokinetic data for free irinotecan [15, 16]. Multiple pre-clinical models have demonstrated that extended circulation of PEP02 leads to increased tumoural drug retention which permits local release and enzymatic conversion of irinotecan into SN-38. This sustained-release effect of the drug provides longer effective concentrations and AUC of the active metabolite (SN-38) in plasma and consequently a potentially beneficial longer duration of anti-tumour activity. Although the mechanism of release is not fully understood, it is assumed that once irinotecan is released from the liposomes either passively or from active breakdown potentially by Kupffer cells in the liver, it is metabolized in a similar fashion to the conventionally administered irinotecan. Therefore, genetic polymorphisms affecting toxicity and efficacy of irinotecan should be relevant to the study drug PEP02.

In this study, the percentages of observed toxic effects were consistent with the previously reported toxicity profiles of irinotecan and PEP02 while lower rates of PEP02 related diarrhoea were observed in other studies [8, 9, 12, 14, 17]. Of note, irinotecan and docetaxel doses used in our study were higher than those in the Korean and the German Arbeitsgemeinschaft Internistische Onkologie (AIO) studies. However these doses were based on the available evidence and expert clinical recommendation at the time of trial design [18, 19]. Liposomal irinotecan is not known to accumulate in many of the target organs and therefore, theoretically results in lower tissue exposure to the free drug and reduced toxicity while maintaining a greater anti-tumour potency [11]. However, overall toxicity and rates of grade 3–4 diarrhoea in our study were numerically higher than expected. We speculate that the lower clearance and higher AUC of PEP02 and SN-38 could explain this unexpected toxicity.

The frequency of homozygosity for *UGT1A1*28* allele is higher in Caucasians (5.8%–9.0%) and is associated with decreased UGT1A1 expression and activity [20–22]. The presence of homozygous mutation is known to critically impact on the glucuronidation of SN-38 resulting in severe neutropenia and diarrhoea in patients who receive irinotecan [21]. The majority of patients in this study were wild type (TA_6TA_6) for this mutation and only 1 (1.4%) Caucasian patient was found to harbor the homozygous mutation (TA_7TA_7). In Asian patients, the *UGT1A1*28* is a rare allele [23, 24] and genetic polymorphisms of *UGT1A1*6* are more frequent that may have an association with irinotecan-related grade 3–4 neutropenia and other toxic effects [25]. In this study, we found no significant associations between gene polymorphisms and pK parameters; however as previously described [26], there did seem to be an association between the heterozygote alleles of the prominent genetic polymorphisms and treatment-related grade 3–4 toxicity.

The recent phase III trial results reported by the AIO and the South Korean groups confirm the benefits associated with second-line chemotherapy in an advanced OG cancer population [8, 9]. The AIO study randomized metastatic OG cancer patients who had failed one prior line of treatment to irinotecan or BSC. The trial was terminated prematurely due to poor accrual. However, irinotecan was associated with a statistically significant OS benefit of 1.6 months (hazard ratio, HR 0.48, 95% CI 0.25–0.92, $P = 0.012$). Similarly, Kang et al. randomized 202 previously treated advanced OG cancer patients with a good PS in a 2:1 fashion to salvage chemotherapy (docetaxel or irinotecan as per investigators' choice) or BSC. In the ITT population, an OS benefit was noted in favour of chemotherapy (5.1 months versus 3.8 months, HR 0.63; $P = 0.004$) and more patients in the chemotherapy arm received further salvage chemotherapy compared with the BSC arm (40% versus 22%, respectively; $P = 0.011$). Median OS with PEP02 in this study is comparable and therefore encouraging. However, clearly with trial results demonstrating median OS of consistently <10 months, there are still significant improvements required to improve the outcomes for this patient group.

The potential advantages of nanoparticle liposomal delivery of irinotecan are several, and include bypassing solubility limitations of irinotecan, extending the circulation time, increasing tumour accumulation via the enhanced permeability and retention effect, and decreased organ toxicity. The results from recent phase I studies and this phase II study demonstrate that PEP02 is well tolerated and also has a comparable efficacy to docetaxel and irinotecan in patients with prior treatment of advanced gastric and GEJ cancer. Interestingly, patients who received PEP02 at 150 mg/m^2 had a numerically better response rate and PFS/OS compared with the patients who received 120 mg/m^2, suggesting a higher antitumour activity and this dose is worthy of further evaluation in future studies of PEP02. However, due to small numbers in this cohort and a potential selection bias for good PS patients, a significant conclusion cannot be made from these data at this time. Although toxicity especially diarrhoea associated with PEP02 appears to be high in this study, the results from ongoing studies of PEP02 as monotherapy and in combination with other cytotoxic [27] or targeted agents in other tumour types will be crucial to establish this novel agent's utility in the cancer therapeutics armamentarium.

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Scientific and Ethical committee as well as regulatory approvals were obtained at each institute and country.

PEP02 is designated as MM-398 by Merrimack Pharmaceuticals, Inc. (Cambridge, MA, USA).

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disclosure

ADG and LTC are consultants or have received honorarium from PharmaEngine. CGY holds stock of PharmaEngine, the makers of PEP02. ACR, SRP, DC, YKK, YC, CR, HYL, JT, FJR, MK and MBC have no relevant competing interest to declare.

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Clinical translation of nanomedicines

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ABSTRACT

This review describes the clinical translation of cancer nanomedicines based on three nanocarrier platforms: liposomes, polymeric micelles, and nanoparticles. A dozen nanomedicines are on the market, the majority (eight) based on the most mature liposome technology. The other marketed nanomedicines are based on polymeric micelles (one) and nanoparticles (one). Polymeric prodrugs account for the remaining two marketed products. Altogether a total of 41 nanocarrier-based formulations have translated from the bench to the bedside and are under investigation at different levels of clinical development. Many more nanocarrier-based formulations are in preclinical development. Not surprisingly, the vast majority of these nanomedicines (37) rely on passive targeting through the EPR (enhanced permeability and retention) effect, avoiding the additional regulatory, production, cost of goods, and polydispersity challenges of active, ligand-receptor based targeting. Only four actively targeting nanocarriers are in clinical development, one using PSMA and three using TfR as the target. It still needs to be demonstrated that active targeting of nanocarriers that are subjected to the EPR effect provides an advantage substantial enough to justify the additional efforts. The review clearly identifies the areas of successful translation of nanomedicines but also shows areas where the potential is still underdeveloped and opportunities for improvement are promising. Overall, the high expectation that has been placed in nanomedicines is showing progress, increasing the benefits and treatment options for cancer patients.

1. Introduction

The application of nanotechnology to medicine is the foundation for the development of nanocarrier-based nanomedicines. Nanocarriers are generally below 100 nm in size and may be constructed from a wide range of organic and inorganic materials such as emulsions, polymers, nanocapsules, nanospheres, micelles, liposomes, dendrimers, quantum dots (QDs), and fullerenes and carbon nanotubes (CNTs). These materials are being used to encapsulate or solubilize chemotherapeutic agents for improved drug delivery *in vivo* or to provide unique optical, magnetic and electrical properties for imaging and therapy. Several functional nanoparticles are being evaluated in preclinical and clinical studies and some nanoparticles have reached the patient, including clinically approved liposome drug formulations and metallic imaging agents. Nanoparticle-based research is directed at the consolidation of functions into multifunctional devices, which may ultimately facilitate the realization of personalized therapy. These multiplexed nanoparticles may be capable of (1) improved delivery of poorly water-soluble drugs; (2) identifying malignant cells via molecular detection; (3) visualizing their location in the body by providing enhanced contrast in medical imaging techniques; (4) fostering transcytosis of drugs across tight epithelial and endothelial barriers; (5) targeting and killing disease cells with minimal side effects through selective cell or tissue targeting; (6) temporal control of drug release, (7) monitoring treatment in real-time; and (8) co-delivery of multiple drugs for combination therapy [1–3].

Most drugs utilized in cancer chemotherapy are cytotoxic and cause severe side effects. In addition, these drugs are often poorly soluble in biological fluids, quickly recognized by the mononuclear phagocyte system (MPS) and cleared from the body. Some anticancer drugs, especially the newer generation of biomacromolecules such as short interfering RNA and peptides, are instable in systemic circulation due to changes in local pH or presence of enzymes. Encapsulating these drugs into nanocarriers serves two purposes: the body is protected against off-site toxicities, and the drug is protected against the body's defense system. Another benefit of nanocarriers is their improved pharmacokinetic profile compared to small molecule drugs. Small drugs distribute evenly throughout the body, resulting in very large volume of distribution (Vd). This distribution profile requires the use of high drug doses to achieve the therapeutic dose at the tumor site, leading to severe side effects including bone marrow suppression, cardiac and kidney toxicity, hair loss, and mucositis. On the contrary, nanocarriers can take advantage of the enhanced permeability and retention (EPR) effect of leaky vasculature in tumorous and inflamed tissue [4]. Poorly aligned endothelial cells in the fast growing tumor vasculature

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with fenestrations larger than 100 nm in size, and reduced lymphatic drainage in tumor tissue result in preferred accumulation of nanocarriers in these tissues over healthy tissue, reducing Vd while improving the PK profile, and consequently, the efficacy of the anticancer drug. The combination of reduced toxicity and enhanced efficacy greatly improves the therapeutic window of the drug and is a main driver for the development of nanomedicines [5,6]. Here the clinical translation of nanomedicines based on three nanocarrier platforms will be reviewed: liposomes, micelles, and nanoparticles. A fourth class of nanocarriers that is intensely studied in academia is composed of dendrimers [7,8]. The well-defined and controlled architecture of dendrimers provides hydrophobic internal voids for physical drug entrapment and multiple surface groups for chemical drug conjugation. However, despite all efforts over the last 30 years no dendrimer as a drug carrier has advanced into the clinic.

2. Nanocarrier platforms for drug delivery

2.1. Liposomes

Liposomes are spherical, closed structures composed of one or several concentric lipid bilayers surrounding an aqueous core. Depending on their membrane structure they are divided into unilamellar or multilamellar liposomes. Surface-modification with poly(ethylene glycol) (PEG) or other polymers provides them with stealth or long circulation properties. Liposomes are biocompatible and can carry hydrophilic drugs in their aqueous core and hydrophobic drugs within the lipid membrane. Liposomes are the oldest platform with regard to clinical translation. Doxil®, approved in the US in 1995 for refractory Karposi's sarcoma, is the PEGylated liposomal formulation of doxorubicin, which now has additional indications in ovarian and recurrent breast cancers [5,9,10].

2.2. Micelles

Micelles and polymeric micelles are formed by surfactants or amphiphilic block copolymers. Both molecules consist of a hydrophobic and a hydrophilic moiety, which self-assemble in water above a system- and temperature-specific critical micelle concentration (CMC) in a thermodynamically driven process. Lower CMC and therefore higher stability, and larger size (20–100 nm) favor polymeric micelles over surfactant micelles as delivery platform for hydrophobic drugs, encapsulated into the micelle core. Polymeric micelles are large enough to avoid first pass kidney elimination via glomerular filtration but are small enough to capitalize on the EPR effect. Furthermore, the small size of drug-loaded polymeric micelles provides an alternative route of cell internalization via the endosomal pathway, which is speculated to circumvent drug efflux mechanisms associated with multi-drug resistance (MDR). PEG chains are the most frequently employed structural motif for the hydrophilic component of the block copolymers due to their high aqueous solubility and non-toxicity [11–13].

2.3. Polymeric nanoparticles

The third nanocarrier platform consists of polymeric nanoparticles, which are mainly prepared through the use of emulsions or precipitation processes. Their size depends on the preparation method, ranging from 10 to up to several 100 nm. The constituent polymers of nanoparticles are not necessarily amphiphilic as those in micelles, and therefore nanoparticle formation is not caused by self-assembly but requires active processing. Drug molecules are either physically entrapped or chemically conjugated to the hydrophobic core of these nanoparticles. The solid nature of polymeric nanoparticles confers high stability and allows controlled drug release, either through diffusion or through degradation of the polymeric matrix. In addition, stimuli-responsive load-and-release modalities can be incorporated into these solid nanocarriers, activated by an external signal or changes in the local microenvironment within the body [14–16]. Polymeric prodrugs in clinical trials will also be discussed under this platform even when it remains uncertain whether these prodrugs actually assemble to form defined nanoparticles.

3. Translational nanomedicines

3.1. Liposome-based nanomedicines

The observation that phospholipids in aqueous systems can form closed bilayer structures was described in 1965 [17]. Not surprising, therefore, that liposomes are the oldest platform for nanomedicines, with Doxil® receiving approval by the US. Food and Drug Administration (FDA) in 1995. Doxil is based on three principles: (1) prolonged drug circulation time and avoidance of the MPS due to the PEGylated surface; (2) high and stable remote loading of doxorubicin driven by a transmembrane ammonium sulfate gradient; and (3) a lipid bilayer membrane in a 'liquid ordered' phase composed of phosphatidylcholine and cholesterol [18]. FDA approval of non-PEGylated DaunoXome®, AmBisome®, DepoCyt®, and Visudyne® followed in 1996, 1997, 1999, and 2000, respectively. More recent liposomal nanomedicines are Depodur®, FDA approved in 2004, and Myocet®, which is approved in Europe and has FDA approval pending as of midst 2012. A very promising extension of the proven liposome platform is ThermoDox®, currently in Phase 3 clinical trial for the treatment of hepatocellular carcinoma (HCC or primary liver cancer) using doxorubicin. The ThermoDox formulation developed by Celsion Corp. is comprised of lysolipid thermally sensitive liposomes (LTSLs) which become leaky and release the drug at 42 °C. The liposomes, delivered by IV infusion, are designed to be used in combination with hyperthermic (heat-based) treatments, such as radiofrequency thermal ablation (RFA), microwave hyperthermia, or high intensity focused ultrasound (HIFU). Heat treatment enhances the leakiness of tumor vasculature and increases the number of liposomes that enter tumor tissue. The goal of the ThermoDox approach is to capture micro-metastases which are most commonly responsible for post-treatment disease recurrence [19]. Arikace® from Insmed Inc., the liposomal formulation of amikacin, a FDA-approved aminoglycoside antibiotic, for inhalation treatment of cystic fibrosis patients with *Pseudomonas* lung infections is in Phase 3 clinical trial in Europe and Canada, and in Phase 2 for nontuberculous mycobacteria (NTM) lung infections in the US [20]. The liposomal formulation of irinotecan-HCl and floxuridine (CPX-1, Celator Pharmaceuticals) extends the platform to true combination therapy, where two anticancer drugs are combined in 1:1 ratio within a liposome for synergistic efficacy against advanced colorectal carcinoma. In addition, the liposomal combination of cytarabine and daunorubicin-HCl in 5:1 ratio has shown synergistic efficacy against acute myeloid leukemia [21,22]. The delivery of short interfering RNAs (siRNAs) using liposomes or 'lipid nanoparticles' (LNP), where a bilayer membrane encloses an aqueous core carrying the siRNA, is a very recent application of this platform. Three companies have products in Phase 1 trials, Alnylam Pharmaceuticals (ALN-VSP02), Tekmira Pharmaceuticals (TKM-PLK1), and Silence Therapeutics (Atu027) [23,24]. Delivery of highly water–soluble siRNA molecules is another extension of this platform, creating new nanomedicines with high expectations for future treatment. Eight liposome nanomedicines are on the market, three clinical candidates are in Phase 3, six are in Phase 2, and another five candidates are in Phase 1 trials (Ta-

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ble 1). However, the development of these nanomedicines is often carried by small companies, who might change ownership or close down, and therefore, the fate of some candidates in early clinical trials is uncertain not just for medical reasons. For example, OSI-211, the liposomal formulation of lurtotecan was developed by OSI Pharmaceuticals, which was bought by Astellas Pharma Inc., in 2010. Astellas sponsored two Phase 2 trials with OSI-211, both completed by October 2011; however, there is no further information about the development of this formulation. On the other hand, an example of a nanomedicine in development that successfully transferred between companies is the liposomal formulation of irinotecan, MM-398 (Merrimack Pharmaceuticals, Inc.), also known as PEP02 (PharmaEngine, Inc.). PharmaEngine licensed rights to PEP02 in Europe and Asia from Hermes Biosciences, Inc. Hermes was acquired by Merrimack in 2009. In 2011, PharmaEngine granted back to Merrimack the rights to develop, manufacture, and commercialize MM-398 in Europe and Asia with the exception of Taiwan in addition to North America. MM-398 recently achieved its primary efficacy endpoints in Phase 2 clinical trials in pancreatic and gastric cancer. In this single arm Phase 2 clinical trial of MM-398 as a monotherapy in 40 metastatic pancreatic cancer patients who had previously failed treatment with gemcitabine, patients treated with MM-398 achieved median overall survival of 22.4 weeks. Additionally, 20% of the patients in this Phase 2 trial survived for more than one year [25]. Merrimack is currently recruiting participants for a randomized, open label Phase 3 study of MM-398 with or without 5-fluorouracil and leucovorin, *versus* 5-fluorouracil and leucovorin in patients with metastatic pancreatic cancer who have failed prior gemcitabine-based therapy.

3.2. Polymeric micelle-based nanomedicines

To date there are only few nanomedicines based on polymeric micelles on the market [26]. Genexol-PM® is a cremophor-free polymeric micelle formulation using PEGylated poly(D,L-lactide) copolymer as matrix and paclitaxel as the active ingredient. Genexol-PM, developed by Samyang Pharmaceuticals, has been approved in Korea (2007) and other Asian countries for the treatment of breast cancer and non-small cell lung cancer (NSCLC). In the US, Genexol-PM has completed a Phase 2 study in patients with advanced urothelial cancer previously treated with gemcitabine and platinum, and is currently studied in a Phase 3 trial to evaluate the efficacy and safety of Genexol-PM compared to Genexol®, the conventional paclitaxel formulation with Cremorphor EL®, in patients with recurrent or metastatic breast cancer [27,28]. Besides in South Korea, the development of polymeric micelles as nanomedicine platform is a major effort in Japan, fueled by the work of the Kataoka research group at the University of Tokyo. Several polymeric micelles based on methoxy-PEG (mPEG)ylated hydrophobic poly(amino acids) carrying anticancer drugs in their hydrophobic core have been developed. NK105 is a paclitaxel formulation using modified mPEGylated poly(aspartic acid) [29]. Originally developed by NanoCarrier Co., Ltd., for stomach and breast cancers, NK105 has been licensed to Nippon Kayaku

Table 1
Liposomal nanomedicines on the market and under clinical evaluation.[a]

Product	Formulation	Application	Company	Status
AmBisome®	Liposomal amphotericin B	Fungal infections	Astellas Pharma/Gilead Sciences	Marketed
Abelcet®	Lipidic amphotericin B	Fungal infections	Sigma-Tau Pharmaceuticals	Marketed
Doxil®/ Caelyx®	PEGylated liposomal doxorubicin	Kaposi's sarcoma; metastatic breast & ovarian cancers	Janssen Pharmaceuticals	Marketed
DaunoXome®	Liposomal daunorubicin	HIV-associated Kaposi's sarcoma	Galen Ltd.	Marketed
DepoCyt®	Liposomal cytarabine	Lymphomatous meningitis	Sigma-Tau Pharmaceuticals	Marketed
DepoDur®	Liposomal morphine	Pain treatment	Pacira Pharmaceuticals	Marketed
Myocet®	Liposomal doxorubicin	Metastatic breast cancer	Cephalon Inc. (EU)/ Sopherion Therapeutics (US, CAN)	Marketed (EU)
Visudyne®	Liposomal verteporfin	Age-related macular degeneration (AMD)	Novartis AG/ QLT Inc. (US)	Marketed
ARIKACE®	Inhaled liposomal amikacin	*Pseudomonas* lung infections in cystic fibrosis	Insmed Inc.	Phase 3 (EU, CAN)
MM-398 (PEP02)	Liposomal irinotecan	Metastatic pancreatic cancer	Merrimack Pharmaceuticals, Inc.	Phase 3 NCT01494506[b]
ThermoDox®	Heat-sensitive liposomal doxorubicin	Primary liver cancer	Celsion Corp.	Phase 3 NCT00617981[b]
CPX-1	Liposomal irinotecan-floxuridine combination	Advanced colorectal carcinoma	Celator Pharmaceuticals	Phase 2 NCT00361842[b]
CPX-351	Liposomal cytarabine-daunorubicin combination	Acute myeloid leukemia	Celator Pharmaceuticals	Phase 2 NCT00788892[b]
LEP-ETU	Liposomal paclitaxel	Metastatic breast cancer	Insys Therapeutics, Inc.	Phase 2 NCT01190982[b]
LE-SN38	Liposomal SN-38	Colorectal cancer	Cancer and Leukemia Group B (NCI)	Phase 2 NCT00311610[b]
OSI-211[c]	Liposomal lurtotecan	Recurrent small cell lung cancer	Astellas Pharma Inc.	Phase 2 NCT00046787[b]
SPI-077	Stealth liposomal cisplatin	Platinum-sensitive ovarian cancer	New York University School of Medicine	Phase 2 NCT00004083[b]
ALN-VSP02	Liposomal RNAi	Solid tumors with liver involvement	Alnylam Pharmaceuticals	Phase 1 NCT01158079[b]
Atu027	Liposomal RNAi	Advanced solid tumors	Silence Therapeutics AG	Phase 1 NCT00938574[b]
IHL-305	Liposomal irinotecan	Advanced solid tumors	Yakult Honsha Co., Ltd.	Phase 1 NCT00364143[b]
NL CPT-11	Nanoliposomal CPT-11 (irinotecan)	Recurrent high-grade gliomas	University of California, San Francisco	Phase 1 NCT00734682[b]
TKM-PLK1	Liposomal RNAi	Advanced solid tumors	Tekmira Pharmaceuticals Corp.	Phase 1 NCT01262235[b]

[a] Liposomal formulations whose trials have been completed or are listed as inactive for more than 2 years with no apparent follow-up have been excluded from Table 1.
[b] ClinicalTrials.gov identifier.
[c] Last study update on OSI-211 provided in October 2011; development might have been discontinued.

Co., Ltd., and has recently opened a multi-national Phase 3 clinical study comparing NK105 *versus* paclitaxel in patients with metastatic or recurrent breast cancer. Another polymeric micelle formulation developed by Nippon Kayaku is NK012. In this formulation, SN-38 is covalently bound to a PEG-poly(glutamic acid) block copolymer via an ester bond [30]. NK012 is currently evaluated in two Phase 2 clinical trials in the US with indications in small cell lung cancer (SCLC) and triple negative breast cancer. Two polymeric micelle formulations developed by NanoCarrier are NC-6004 and NC-4016. NC-6004 is composed of cisplatin and mPEG-poly(glutamic acid), currently recruiting for a Phase 1/2 study of the combination therapy with NC-6004 and gemcitabine in patients with locally advanced or metastatic pancreatic cancer in Asian countries [31]. Similarly, NC-4016 is a polymeric micelle containing oxaliplatin (DACH-platin), which is in preclinical evaluation. NK911 is a polymeric micelle in which doxorubicin is covalently bound to a mPEG-poly(aspartic acid) block copolymer. NK911 has been evaluated in a Phase 1 trial, sponsored by Nippon Kayaku [32]. The docetaxel analogue, NC-6301, in which docetaxel is covalently bound via an ester to a mPEG-poly(aspartic acid) block copolymer, is in preclinical evaluation by NanoCarrier [33]. The evaluation of poly(amino acid) micelle nanocarriers in preclinical and clinical studies has recently been reviewed [34].

In an extension of the polymeric micelle platform SP1049C, a proprietary intravenous composition of BioMod™ polymers and doxorubicin as ingredient, was developed by Supratek Pharma Inc. The SP1049C formulation is particularly active in multidrug resistant (MDR) and metastatic cancers. BioMod polymers, including poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) triblock copolymers, have the ability to selectively disrupt mitochondrial functionality in chemoresistant cells. They penetrate cancer cells, reach mitochondrial membranes, compromise their function by depleting ATP, and release reactive oxygen species (ROS) and cytochrome C. The depletion of ATP inactivates the cell's detoxification mechanisms by inhibiting ABC transporters such as *P*-gp and MRP. The combination of BioMod polymers with an anticancer drug, therefore, results in a new drug that targets chemoresistant cancers. In the US, SP1049C is designated as an orphan drug in carcinoma of the esophagus and in gastric cancers. An international Phase 3 clinical study design has been reviewed and agreed to with the FDA under a Special Protocol Assessment (SPA) procedure [35,36]. Analogous micelle formulations containing docetaxel (SP1012C) and cabazitaxel (SP1015C) are under preclinical investigations. Nanomedicines based on polymeric micelles on the market and under clinical evaluation are listed in Table 2.

3.3. Nanoparticle-based and polymeric prodrug nanomedicines

Although nanoparticles have been discovered in the 1970s, essentially only one nanoparticle-based nanomedicine has advanced to the market [37]. Abraxane® is a paclitaxel formulation based on the nanoparticle albumin bound (nab) technology platform developed by Abraxis BioScience, a wholly-owned subsidiary of Celgene Corporation. The albumin-paclitaxel formulation consists of nanoparticles 130 nm in diameter, and is used as a colloidal suspension of lyophilized drug and human serum albumin (HSA) diluted in saline solution. Abraxane was approved by the FDA in 2005 and the European Medicines Agency (EMA) in 2008 for the treatment of breast cancer after failure of combination chemotherapy for metastatic disease or relapse within six months of adjuvant chemotherapy. Multiple clinical trials are listed on the NIH clinical trials website involving Abraxane, including Phase 3 trials in first-line NSCLC, metastatic pancreatic and melanoma cancers, and Phase 2 trials in first-line metastatic breast cancer and bladder and ovarian cancers [38,39]. The nanoparticle formulation Livatag® (doxorubicin transdrug™), developed by BioAlliance Pharma S.A., consists of doxorubicin-loaded poly(isohexyl-cyanoacrylate) nanoparticles for delivery to chemoresistant cells such as primary liver cancer cells via the hepatic intra-arterial route. Livatag was granted orphan drug status in the US and Europe. BioAlliance has begun a multicenter, randomized Phase 3 clinical trial in the US and Europe, designed to estimate the efficacy of Livatag on overall survival in nearly 400 patients suffering from hepatocellular carcinoma, resistant or intolerant to sorafenib [40]. The polymeric nanopharmaceutical formulation CRLX101 (formerly IT-101) developed by Cerulean Pharma Inc. is composed of a β-cyclodextrin-PEG alternating copolymer with camptothecin drugs conjugated to the polymeric backbone. This polymer–drug conjugate self-assembles to form nanoparticles in the 20–50 nm range [41]. CRLX101 is currently evaluated in a Phase 2 trial with patients suffering from advanced NSCLC who have failed one or two previous regimens of chemotherapy. In addition, CRLX101 is studied in a 2-stage Phase 2 trial in recurrent ovarian, tubal and peritoneal cancer, a Phase 1b study In combination with bevacizumab in the treatment of patients with advanced renal cell carcinoma, and pilot studies treating patients with advanced or metastatic stomach, gastroesophageal, or esophageal cancer that cannot be removed by surgery. CRLX101 is another example of successful transfer of a nanomedicine between companies. Originally developed by Insert Therapeutics, Inc., which then merged with Calando Pharmaceuticals, Inc., CRLX101 was licensed to Cerulean, who further develops this cyclodextrin-PEG (CDP) technology. The CDP-docetaxel nanopharmaceutical, CRLX301, is in pre-IND devel-

Table 2
Nanomedicines based on polymeric micelles on the market and under clinical evaluation.[a]

Product	Formulation	Application	Company	Status
Genexol-PM	PEG-poly(D,L-lactide) with paclitaxel	Breast cancer and NSCLC	Samyang Pharmaceuticals	Marketed (S. Korea, other Asian countries)
Genexol-PM	PEG-poly(D,L-lactide) with paclitaxel	Recurrent or meta-static breast cancer	Samyang Pharmaceuticals	Phase 3 NCT00876486[b]
NK105	Modified mPEG-poly (aspartic acid) w paclitaxel	Recurrent or meta-static breast cancer	Nippon Kayaku Co., Ltd.	Phase 3 NCT01644890[b]
NK012	PEG-poly(glutamic acid)-SN38 conjugate	SCLC and triple negative breast cancer	Nippon Kayaku Co., Ltd.	Phase 2 NCT00951613[b] Phase 2 NCT00951054[b]
SP1049C	Block copolymers with doxorubicin	Non-resectable stage IVb adenocarcinoma	Supratek Pharma Inc.	Phase 2 (UK)
NC-6004	mPEG-poly(glutamic acid) with cisplatin	Locally advanced or metastatic pancreatic cancer	NanoCarrier Co., Ltd.	Phase ½ NCT00910741[b]
NK911	mPEG-poly(aspartic acid)-doxorubicin conjugate	Solid tumors	Nippon Kayaku Co., Ltd.	Phase 1 (Japan)

[a] Polymeric micelle formulations whose trials have been completed or are listed as inactive for more than two years with no apparent follow-up have been excluded from Table 2.
[b] ClinicalTrials.gov identifier; NSCLC – Non-Small Cell Lung Cancer; SCLC – Small Cell Lung Cancer.

opment. A docetaxel-polymeric nanoparticle formulation (docetaxel-PNP) is under development by Samyang Pharmaceuticals. The nanoparticles are comprise of a mixture of monovalent metal salts of poly(lactic acid), amphiphilic diblock copolymers, and the drug. In water this mixture forms micelles, which then are stabilized by addition of divalent metal ions to form nanoparticles. This formulation is in Phase 1 clinical trial in Korea, while a Phase 1 study in the US is of unknown status as of April, 2010. Samyang has presented progress with docetaxel-PNP at the Bio Korea 2012 Conference. The status of polymeric nanoparticles is summarized in Table 3.

Two polymeric prodrug formulations have advanced to the market. Adagen® (pegademase bovine) is a modified enzyme used for enzyme replacement therapy for the treatment of severe combined immunodeficiency disease (SCID) associated with a deficiency of adenosine deaminase (ADA). Developed by Enzon Pharmaceuticals, Inc. (now licensed to Sigma-Tau Pharmaceuticals), Adagen received FDA approval in 1990. Adagen is a polymeric prodrug made by conjugation between methoxy-PEG (mPEG) and adenosine deaminase from bovine intestine [42]. In 2006, the FDA granted approval to Oncaspar® (pegaspargase) from Enzon (licensed to Sigma-Tau) for the first-line treatment of patients with acute lymphoblastic leukemia (ALL) as a component of a multi-agent chemotherapy regimen. Oncaspar was previously approved in 1994 for the treatment of patients with ALL who were hypersensitive to native forms of L-asparaginase. Pegaspargase is the conjugate between mPEG and native L-asparaginase. Pegylation increases the drug hydrodynamic radius, prolongs plasma retention time, decreases proteolysis, decreases renal excretion, and shields antigenic determinants from immune detection without obstructing the substrate-interaction site [43]. Currently more than 80 clinical trials are listed on the NIH clinical trials website involving Oncaspar to extend its indication and study combinations with other treatments. Following this PEGylation approach and extending it to multi-arm PEG, PEGylated SN-38 (EZN-2208), a metabolite of the camptothecin derivative irinotecan (CPT-11), was developed by Enzon Pharmaceuticals. EZN-2208 has shown increased solubility, higher exposure, and longer apparent half-life of SN-38, as well as more profound deoxyribonucleic acid (DNA) damage and inhibition of angiogenesis than irinotecan. EZN-2208 treatment in a Phase 2 metastatic breast cancer clinical trial resulted in prolonged exposure of tumors to SN-38 via preferential accumulation of EZN-2208 in the tumor and prolonged release of SN-38 in the blood [44,45]. A second Phase 2 trial with EZN-2208 alone and in combination with cetuximab for metastatic colorectal carcinoma is ongoing, as well as several Phase 1 studies. A nanomedicine based on a drug conjugate to a hydrophilic, non-PEG polymer is XMT-1001. This nanomedicine from Mersana Therapeutics is produced by conjugating camptothecin molecules to a biodegradable polyacetal, poly(1-hydroxymethylethylene hydroxymethyl formal) (Fleximer®). Drug and Fleximer backbone are connected via succinimidoglycinate or other linker molecules, selected based on the desired drug release profile [46,47]. XMT-1001 is being evaluated in an ongoing Phase 1b clinical trial in lung cancers, following successful completion of a Phase 1 trial of the safety, tolerability, and pharmacokinetics of intravenous XMT-1001 in patients with advanced solid tumors (SCLC, NSCLC). A Phase 2 clinical trial for XMT-1001 is planned for 2012. Recently Mersana extended its platform to join the small group of nanomedicines that rely on active, ligand-receptor targeting by developing a portfolio of next-generation antibody-drug conjugates (ADC). This group of nanomedicines will be discussed next. The status of polymeric prodrugs is summarized in Table 3.

4. Nanomedicines with active targeting

All nanomedicines described so far rely on the EPR effect, *i.e.*, take advantage of leaky vasculature and reduced lymphatic drainage in tumor tissue. This approach is often called 'passive targeting' in contrast to 'active targeting' which is based on the interaction between a ligand and certain receptors overexpressed on the surface of cancer cells. Small molecule drugs are not subjected to the EPR effect and distribute evenly throughout the body as measure by large volume of distribution (Vd). Adding a targeting ligand to small molecules reduces Vd and off-site toxicity, therefore improving the therapeutic window. Folic acid and other high-affinity small molecule ligands that bind to receptors overexpressed on target cells, as well as larger molecules such as antibodies, antibody fragments, and aptamers are being investigated and applied in active targeting [48]. For example, Endocyte, Inc. has completed three single arm studies of vintafolide (EC145) in patients with advanced ovarian cancer, NSCLC and solid tumors. Vintafolide delivers the very potent vinca chemotherapy directly to cancer cells by targeting the folate receptor, which is overexpressed on approximately 80–90% of ovarian and lung cancers. Vintafolide has also been evaluated in a randomized Phase 2 trial comparing vintafolide + DOXIL *versus* DOXIL alone in women with platinum-resistant ovarian cancer. While this study has completed enrollment, a Phase 3 study, also in women with platinum-resistant ovarian cancer, is now enrolling patients. The advantage of adding targeting ligands to nanomedicines, which are subjected to the EPR effect, is less obvious. Surface decoration with ligands not only complicates the regulatory pathway but also adds another layer of complexity to the production process and the cost of goods. In addition, the

Table 3
Nanomedicines based on nanoparticles and polymeric prodrugs on the market and under clinical evaluation.[a]

Product	Formulation	Application	Company	Status
Abraxane®	Albumin-bound paclitaxel	Metastatic breast cancer	Celgene Corp.	Marketed (US, EU)
Livatag®	Doxorubicin nanoparticle	Hepatocellular carcinoma	BioAlliance Pharma S.A.	Phase 3 (US,EU) NCT01655693[b]
CRLX101	β-CD-PEG-camptothecin nanopharmaceutical	Advanced NSCLC	Cerulean Pharma Inc.	Phase 2 NCT01380769[b]
Docetaxel-PNP	Docetaxel polymeric nanoparticle	Advanced solid malignancies	Samyang Pharmaceuticals	Phase 1 (S. Korea)
Adagen®	mPEG-adenosine deaminase prodrug	Enzyme replacement therapy for SCID	Sigma-Tau Pharmaceuticals	Marketed
Oncaspar®	mPEG-asparaginase prodrug	Acute lymphoblastic leukemia (ALL)	Sigma-Tau Pharmaceuticals	Marketed
EZN-2208	Multi-arm mPEG-SN38 conjugate	Metastatic breast cancer and colorectal carcinoma	Enzon Pharmaceuticals, Inc.	Phase 2 NCT01036113[b] NCT00931840[b]
XMT-1001	Polyacetal-camptothecin conjugate	SCLC and NSCLC	Mersana Therapeutics	Phase 1 NCT00455052[b]

[a] Nanoparticle and polymeric prodrug formulations whose trials have been completed or are listed as inactive for more than two years with no apparent follow-up have been excluded from Table 3.
[b] ClinicalTrials.gov identifier; NSCLC – Non-Small Cell Lung Cancer; SCID – Severe Combined Immunodeficiency Disease; SCLC – Small Cell Lung Cancer.

Table 4
Nanomedicines based on active (ligand-receptor) targeting.

Product	Formulation	Application	Company	Status
MBP-426	Transferrin-targeted NP w oxaliplatin	Metastatic gastric, gastro-esophageal junction; esophageal adenocarcinoma	Mebiopharm Co., Ltd.	Phase 2 NCT00964080[a]
BIND-014	PEG-PLA/PEG-PLA-ACUPA nanoparticle w docetaxel	Advanced or metastatic cancer	Bind Biosciences, Inc.	Phase 1 NCT01300533[a]
SGT53	Transferrin-targeted liposome w wild type p53 gene	Neoplasm	SynerGene Therapeutics, Inc.	Phase 1 NCT00470613[a]
CALAA-01	Transferrin-β-cyclodextrin polymer nanoparticle complexed w siRNA	Solid tumors	Calando Pharmaceuticals, Inc.	Phase 1 NCT00689065[a]

[a] ClinicalTrials.gov identifier.

number of ligands on the surface of single particles will be random when ligands are added by physical mixing of constituents forming the nanoparticle or by random surface modification of a preexisting nanoparticle, increasing the polydispersity of the nanomedicine. This change in surface composition can negatively affect the circulation time and recognition by the MPS, and therefore the individual clearance rate of each nanoparticle in the polydisperse mixture. Furthermore, if the targeting ligands concentrate on the nanomedicine's surface then the desired 'sticky point' of single ligand-receptor interaction can become 'superglue' due to the multivalency effect of multiple binding sites between particle and cell surfaces, in the worst case preventing intracellular uptake of the nanomedicine [49]. It is therefore not surprising that passive (EPR) targeting is the mode of choice for most current nanomedicines [50]. Following, the few examples that apply active targeting will be discussed (Table 4).

BIND-014 is a targeted nanoparticle formulation developed by Bind Biosciences, Inc. The polymeric matrix consists of PEG-poly(D,L-lactic acid) (PEG5k-PLA16k) mixed with PEG5k-PLA16k-ACUPA in a 97.5% to 2.5% ratio. The ACUPA (*S*,*S*-2-[3-[5-amino-1-carboxypentyl]-ureido]-pentanedioic acid) moiety is a PSMA (prostate-specific membrane antigen) substrate analog inhibitor. The active ingredient in this Accurin™ labeled nanoparticle is physically entrapped docetaxel. BIND-014 is completing a Phase 1 trial to assess its dose-limiting toxicities and determine the maximum tolerated dose (MTD) [51]. Contrary to Bind's PMSA targeting, the other nanomedicines are targeting the transferrin receptor (TfR) that is overexpressed on many cancer cells. MBP-426, developed by Mebiopharm Co., Ltd., is a liposome composed of N-glutaryl phosphatidylethanolamine (NGPE), conjugated with transferrin (Tf) ligands and carrying oxaliplatin entrapped in the aqueous core. MBP-426 has demonstrated anticancer preclinical activity and recently entered clinical trials [52]. The ongoing Phase 2 trial is an open-label study to evaluate the efficacy of MBP-426 at a dose of 170 mg/m^2 in combination therapy in patients with second line metastatic gastric, gastro-esophageal junction or esophageal adenocarcinoma. Similarly to MBP-426, SGT53 (SynerGene Therapeutics, Inc.) is a nanomedicine composed of a wild type p53 gene (plasmid DNA) entrapped into a liposome that is targeted to tumor cells by means of an anti-transferrin receptor single-chain antibody fragment (TfRscFv), attached to the outside of the liposome. Numerous human tumors experience loss or mutation of wild type p53 (wtp53), which plays a vital role in cell cycle control and is a critical component in cell death (apoptosis) and the regulation of angiogenesis. The loss of such critical tumor suppressor activity is believed to be responsible for p53 gene's involvement in a broad array of human tumors and resistance to chemo and radiotherapy. Preclinical studies have indicated that SGT53 could sensitize tumors to the effects of chemo and radiotherapy. The current Phase 1b clinical study is designed to evaluate the safety of SGT53 in combination with docetaxel, determine the recommended Phase 2 doses of both agents, and evaluate the combination effect of SGT53 and docetaxel on tumor size or progression. The fourth targeted nanomedicine (and third transferrin-targeted nanoparticle) is CALAA-01, developed by Calando Pharmaceuticals, Inc., CALAA-01 is composed of four components: (1) a linear polymer in which positively charged groups alternate with β-cyclodextrin; and (2) siRNA, who's negatively charged backbone complexes with the positively charged polymer segments. Several polymer-siRNA complexes self-assemble into a nanoparticle of less than 100 nm in diameter that fully protects the siRNA from degradation in serum. The resulting nanoparticles are then decorated with (3) adamantane covalently bound to PEG, and (4) adamantane covalently bound to a targeting ligand, *i.e.*, transferrin, taking advantage of the stable inclusion complex between adamantane and β-cyclodextrin. The siRNA employed in CALAA-01 inhibits tumor growth via RNA interference to reduce expression of the M2 subunit of ribonucleotide reductase (R2). CALAA-01 is investigated in a Phase 1 trial to determine its safety, toxicity, and MTD when administered intravenously to patients with relapsed or refractory cancer, characterize its PK, provide preliminary evidence of efficacy by evaluating tumor response, recommend the dose for future clinical studies, and evaluate immune response by measuring antibody and cytokine levels, and complement activation [53,54]. CALAA-01 is the only non-lipid siRNA formulation that has translated into the clinic, while siRNA nanomedicines investigated by Alnylam, Tekmira, and Silence Therapeutics are liposome-based, and therefore preferably liver bound.

5. Conclusions

The application of nanotechnology to medicine has clearly advanced beyond academic curiosity and research. A dozen nanomedicines are on the market, the majority (eight) based on the most mature liposome technology. The other marketed nanomedicines are based on polymeric micelles (one) and nanoparticles (one). Polymeric prodrugs account for the remaining two marketed products. Altogether a total of 41 nanocarrier-based formulations have translated from the bench to the bedside and are under investigation at different levels of clinical development. Many more nanocarrier-based formulations are in preclinical development, too many to be listed here. Not surprisingly, the vast majority of these nanomedicines (37) rely on passive targeting through the EPR effect, avoiding the additional regulatory, production, cost of goods, and polydispersity challenges of active, ligand-receptor based targeting. Only four actively targeting nanocarriers are in clinical development, one using PSMA and three using TfR as the

Table 5
Summary of translational activities of nanomedicines.

Status	Liposomes	Micelles	Nanoparticles	Prodrugs	Active targeting
Marketed	8	1	1	2	0
Phase 3	3	2	1	0	0
Phase 2	6	2	1	1	1
Phase 1	5	2	1	1	3

target. It still needs to be demonstrated that active targeting of nanocarriers that are subjected to the EPR effect provides an advantage substantial enough to justify the additional efforts. The summary shown in Table 5 clearly indicates the areas of successful translation of nanomedicines but also shows areas where the potential is still underdeveloped and opportunities for improvement are promising. Overall, the high expectation that has been placed in nanomedicines is showing progress, increasing the benefits and treatment options for cancer patients.

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HIGHLIGHT ARTICLE

Treatment for Refractory Pancreatic Cancer

Highlights from the "2011 ASCO Gastrointestinal Cancers Symposium". San Francisco, CA, USA. January 20-22, 2011

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Summary

While gemcitabine-based regimens are currently accepted as the standard first-line treatment of patients with locally advanced or metastatic pancreatic adenocarcinoma, there is no consensus regarding treatment in the second-line setting. This review is an update from the 2011 American Society of Clinical Oncology (ASCO) Gastrointestinal Cancers Symposium regarding recent developments in the treatment of refractory pancreatic cancer, as these were presented in Abstracts #237 and #272 of the meeting.

Introduction

Pancreatic cancer remains the fourth leading cause of cancer-related mortality with an estimated total of 43,100 new cases and 36,800 deaths in 2010 in the USA alone [1]. Overall survival remains poor despite advances in therapeutics. Gemcitabine-based regimens represent the standard systemic first-line treatment in patients with advanced pancreatic cancer, offering a better quality of life as well as a small survival benefit [2]. Only a small percentage of patients who exhibit disease progression after first-line treatment continue to receive second-line therapy, mainly because of poor performance status. Therefore, few randomized trials have been conducted and there is currently no consensus on the standard of care for refractory pancreatic cancer [3].

What Did We Know Prior to the 2011 ASCO GI Cancer Symposium?

Oettle *et al.* [4] evaluated folinic acid plus 5-FU plus oxaliplatin (FOLFOX) as second-line treatment in advanced pancreatic cancer and they were the first to establish that chemotherapy offers better overall survival to refractory patients as compared to best supportive care (21 *vs.* 10 weeks, P=0.007). According to the final results of the Charité Onkologie trial (CONKO-003), the addition of oxaliplatin to 5-FU and leucovorin improves overall survival and progression-free survival when compared to 5-FU and leucovorin [5]. Based on the above, it has been suggested that FOLFOX become a standard second-line regimen [6]. Some studies have demonstrated that the doublet of gemcitabine and oxaliplatin can be used as second-line treatment in patients refractory to standard gemcitabine regimen [7, 8]. Activity of oxaliplatin has also been shown in combination with capecitabine after gemcitabine failure [9]. These results were confirmed in a phase II study by Dr. Mane *et al.*, presented at the 2011 ASCO GI Cancer Symposium (Abstract #308) [10], but it should be noted that the latter trial enrolled patients with pancreatic or biliary adenocarcinoma and that results were reported on the total of patients.

Regarding taxanes, paclitaxel monotherapy has been suggested as an additional therapeutic option with considerable efficacy and low toxicity in second-line treatment [11]. A recent retrospective study evaluated docetaxel monotherapy as well as docetaxel-based doublets in the treatment of refractory pancreatic cancer and mild activity was shown with no grade 3 or 4 toxicity [12].

Irinotecan has been evaluated in combination with oxaliplatin in patients with advanced pretreated pancreatic cancer exhibiting modest activity and manageable toxicity [13] and offering median overall survival of 4.1 months [14].

Key words gemcitabine; irinotecan; Pancreatic Neoplasms; Treatment Failure

Abbreviations ASCO: American Society of Clinical Oncology; FOLFIRI: irinotecan with 5-FU and folinic acid; FOLFOX: folinic acid plus 5-FU plus oxaliplatin; PEP02: liposome irinotecan

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Irinotecan with 5-FU and folinic acid (FOLFIRI) showed notable activity and a good toxicity profile after gemcitabine failure [15, 6].
S-1, an oral fluoropyrimidin, has also been investigated in Japanese populations after failure of gemcitabine-based treatment. It seems that this agent is tolerable and marginally effective, offering median overall survival of 5.8 months [16, 17].
Targeted therapies are also being studied in refractory pancreatic cancer. The combination of bevacizumab and erlotinib was recently evaluated in this setting but despite good tolerability, the results were poor [18], as were the results of the use of everolimus [19] and sunitinib [20] as single agents. Bevacizumab monotherapy or its administration in combination with docetaxel did not show any antitumor activity after gemcitabine failure [21].

What Did We Learn at the 2011 ASCO GI Cancer Symposium?

With regard to treatment of refractory pancreatic cancer after failure of at least one line of therapy, two important abstracts were presented at the 2011 ASCO GI Cancer Symposium (Table 1). Both evaluated the use of irinotecan-based regimens in this treatment setting.
Dr. Ko *et al.* presented a phase II trial conducted in three centers in the USA and Taiwan (Abstract #237) [22]. They studied the use of single-agent PEP02, a novel nanoparticle liposome formulation of irinotecan, in refractory pancreatic cancer. It is characterized by improved pharmacokinetics and better tumor localization of irinotecan and of its active metabolite SN38, as compared to the free form of the drug. Favorable safety and efficacy of PEP02 were shown in previous phase I studies of patients with refractory solid tumors, such as pancreatic cancer [23, 24]. PEP02 has been studied as monotherapy [23] as well as in combination with 5-FU and leucovorin [24] and tumor response was reported. In the 2011 Ko *et al.* study [22], 37 patients with metastatic pancreatic cancer received triweekly PEP02 at a dose of 120 mg/m^2 as second-line treatment after gemcitabine failure. According to results based on the first 31 evaluable patients, a 52% disease control rate was achieved. CA 19-9 levels decreased more than 50% in one third of patients whose baseline levels were originally elevated. The study met its primary endpoint of 3-month overall survival as the latter reached 74%, with one patient surviving for more than one year. Toxicity was considered acceptable, with fatigue (31%) and neutropenia (25%) being the two most common grade equal to, or greater than, 3 adverse events.
Regimens combining irinotecan with 5-FU and folinic acid (FOLFIRI) have been administered to patients with advanced pancreatic cancer in the first- [25] and second-line setting [15] and data from phase II studies have shown modest efficacy with tolerable toxicity. Gebbia *et al.* [6] conducted a relevant retrospective study in 40 patients with refractory pancreatic cancer. A new larger retrospective study, conducted in two French institutions, was presented by Dr. Neuzillet *et al.* at the 2011 ASCO GI Cancer Symposium reporting on the use of FOLFIRI after one or more lines of treatment (Abstract #272) [26]. It included 70 patients with unresectable, locally advanced or metastatic, pancreatic cancer with an overall maspin score less than 3. These patients had previously received gemcitabine and platinum-based chemotherapies. Approximately one third of patients had been administered one prior regimen, 57% had received two lines of treatment and only 8.8% had received three or more lines. Sixty of 70 patients (85.7%) received FOLFIRI-1 (irinotecan 180 mg/m^2 day 1) and the rest were administered FOLFIRI-3 (irinotecan 100 mg/m^2 days 1 and 3). Disease control rate was 44.3%. One-year and two-year progression-free survival was 17% and 3%, respectively, whereas overall survival rates were 24% and 9%, respectively. Dosage adjustment was necessary in 21 patients (30%) and adverse events were considered tolerable with no toxic deaths reported.

Discussion

Very few options are available for patients with advanced pancreatic cancer after failure of

Table 1. Summary of the 2011 ASCO GI Cancer Symposium abstracts reporting clinical study results on treatment of refractory pancreatic cancer.

	Ko *et al.* (Abstract #237) [22]	Nauzillet *et al.* (Abstract #272) [26]
Study design	Phase II (Simon's 2-stage design) Metastatic disease	Retrospective Locally advanced or metastatic disease
Countries	USA, Taiwan	France
No. of patients	37 (31 evaluable)	70
Drugs	Liposome irinotecan (PEP02)	FOLFIRI-1 or FOLFIRI-3
Dose	120 mg/m^2 3-week cycle	Irinotecan 180 mg/m^2 day 1 Irinotecan 100 mg/m^2 days 1 and 3
Line of treatment	Second	Second or further
Previous treatment	Gemcitabine-based	Gemcitabine- and platinum-based
Disease control rate	52%	44.3%
Survival	3-month: 74% (ongoing)	Progression-free survival: 23 weeks Overall survival: 24 weeks
Grade 3/4 toxicity	Fatigue, neutropenia, nausea/vomiting, diarrhea	Hematological, digestive

Disease control rate: partial response plus stable disease

gemcitabine-based regimens. Irinotecan monotherapy has already been evaluated in patients treated with first-line gemcitabine-based chemotherapy: in 2009, Yi *et al.* [27] reported the results of a phase II trial evaluating biweekly doses of irinotecan monotherapy (150 mg/m^2) as salvage treatment in this setting. However, Ko *et al.* [22] presented the first phase II study of a novel liposomal irinotecan formulation in the second-line treatment of these patients. In both trials, disease control rates were comparable (48 *vs.* 52% in the Yi and Ko studies, respectively) as were the percentages of patients that exhibited more than 50% decrease in their CA 19-9 levels (33% in both studies). In terms of survival, three-month overall survival seems considerably higher in the liposomal irinotecan study according to the preliminary data presented at the 2011 ASCO GI Cancer Symposium (74% *vs.* approximately 40% in the Yi *et al.* trial). However, it should be noted that with regard to toxicity, the liposomal formulation of irinotecan seems to be associated with a significant greater percentage of grade 3/4 adverse events. In this study, fatigue grade equal to, or greater than, 3 is reported in 31% of patients whereas in the Yi *et al.* study this adverse event was not reported. This difference in toxicity needs to be taken into account as treatment in the second-line setting is often palliative and one of its main objectives is maintaining quality of life.

FOLFIRI regimens have been studied in the past in the treatment of gemcitabine refractory pancreatic cancer. The Yoo *et al.* [15] phase II study was the first to show favorable efficacy and toxicity profile in gemcitabine pretreated patients. Gebbia *et al.* [6] retrospectively examined 40 patients who received standard biweekly FOLFIRI after gemcitabine failure and suggested this regimen be used selectively in patients with good performance status or good response to first-line treatment. The 2011 Neuzillet *et al.* [26] study was also retrospective and showed comparable efficacy results (50% *vs.* 44.3% disease control rates in the Gebbia *et al.* and Neuzillet *et al.* studies, respectively). Estimated median overall survival was 6 months in both studies and toxicity was mainly hematological and gastrointestinal. The Neuzillet *et al.* trial is the first study to report considerable efficacy and manageable toxicity in patients receiving third- and further-line of chemotherapy. However, what needs to be noted is that patients included were of significantly better performance status (42.9% had performance status equal to 0 *vs.* 15% and 0.5% in the Yoo *et al.* and Gebbia *et al.* studies, respectively), despite the fact that more than 65% of patients had already received 2 or more lines of treatment. In the Neuzillet *et al.* trial there is also great heterogeneity in the results despite the similar median overall survival of 6 months: the range of overall survival is 0.5-36.8 months *vs.* 2-8.2 months in the Gebbia *et al.* study, respectively. Finally, the Neuzillet *et al.* study does not state whether results or toxicity differed between patients receiving FOLFIRI-1 or FOLFIRI-3 regimens.

In conclusion, little progress has been made in the field of second-line treatment of gemcitabine-refractory pancreatic cancer; therefore, there is no evidence-based treatment recommendation for these patients. There is need for larger randomized trials that will study novel agents as well as new treatment combinations in an effort to improve survival while maintaining quality of life.

Conflict of interest None

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CANCERS OF THE COLON AND RECTUM

Phase I study of biweekly liposome irinotecan (PEP02, MM-398) in metastatic colorectal cancer failed on first-line oxaliplatin-based chemotherapy.

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Abstract

613

Background: PEP02 (MM-398) is a nanoliposomal formulation of irinotecan (CPT-11) that has improved pharmacokinetics (PK) and tumor distribution of CPT-11 and its

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active metabolite, SN-38. PEP02 single agent q3w has shown preliminary efficacy and safety in Phase II pancreatic and gastric cancer studies. Since irinotecan is approved for metastatic colorectal cancer (mCRC) and biweekly regimens are widely used, the aims of this study are to determine the maximum tolerated dose (MTD), characterize the PK and pharmacogenetics (PGx), and explore the efficacy of PEP02 q2w in mCRC.

Methods: Patients (pts) with disease progression after 1st-line oxaliplatin-based chemotherapy, ECOG PS 0-1, and without prior exposure to irinotecan were eligible. PEP02 was given on day 1 and 15 of each 28 day treatment cycle. The starting dose was 80 mg/m^2 and escalated by 10 mg/m^2 to the target dose of 100 mg/m^2. PK was evaluated during the 1st cycle and the tumor response was assessed by RECIST.

Results: A total of 18 pts (M/F 9/9; median age 57.5) were enrolled, with 6 at each dose level. Dose-limiting toxicity manifested as G3 diarrhea was observed in one pt per dose level. The target dose of 100 mg/m^2 was determined to be the MTD. Nine pts had dose delayed (4, 3, 2 at 80, 90, 100 mg/m^2), mostly because of neutropenia. The PK and PGx are being analysed. As of August 2011, there are 3 pts still on study treatment and 17 pts evaluable for tumor response. Four pts (2 at 80 mg/m^2, 1 each at 90 and 100 mg/m^2) showed partial response (3 after 2 cycles and 1 after 8 cycles) and 8 pts (3 each at 80 and 90 mg/m^2, 2 at 100 mg/m^2) maintained stable disease for at least 2 cycles, which resulted in a response rate (RR) of 23.5% and a disease control rate (DCR) of 70.6%. Current median progression-free survival (PFS) is 4 months and 8 pts (47%) had PFS ≥ 6 months.

Conclusions: The MTD of biweekly PEP02 is 100 mg/m^2. As a 2nd-line monotherapy after oxaliplatin-based chemotherapy, the efficacy results indicate the potential benefit of PEP02 for mCRC (FOLFIRI-1 achieved only 4% RR, 34% DCR, and 2.5 months PFS in FOLFOX pretreated pts). A randomized Phase II study evaluating PEP02 plus 5-FU/LV (FUPEP regimen) vs. FOLFIRI

PMID: 27983424

WE RECOMMEND

A phase I/IIA pharmacokinetic (PK) and serial skin and tumor pharmacodynamic (PD) study of the EGFR irreversible tyrosine kinase inhibitor EKB-569 in combination with 5-fluorouracil (5FU), leucovorin (LV) and irinotecan (CPT-11) (FOLFIRI regimen) in patients (pts) with advanced colorectal cancer (ACC)
E. Casado et al., J Clin Oncol, 2016

A phase 1b dose-escalation trial of erlotinib, capecitabine and oxaliplatin in metastatic colorectal cancer (MCRC) patients
J. P. Delord, J Clin Oncol, 2016

Phase I Study of a Weekly Schedule of Irinotecan, High-Dose Leucovorin, and Infusional Fluorouracil as First-Line Chemotherapy in Patients With Advanced Colorectal Cancer
Udo Vanhoefer et al., J Clin Oncol, 2016

Phase II study of biweekly cetuximab (C) and irinotecan (I) as a second-line regimen for metastatic colorectal cancer (mCRC)
B. A. Carneiro, J Clin Oncol, 2016

Phase II trial of chemotherapy with high-dose FOLFIRI plus bevacizumab in the front-line treatment of patients with metastatic colorectal cancer (mCRC) and genotype UGT1A1*1/UGT1A1*1 or UGT1A1*1/UGT1A1*28 (FFCD 0504 trial). Results of a planned interim analysis
E. Mitry, J Clin Oncol, 2016

Modified FOLFOX6 With or Without Aflibercept in

is currently ongoing in France.

Guideline
Brahmer et al.

Venous Thromboembolism Prophylaxis and Treatment in Patients With Cancer: ASCO Clinical Practice Guideline Update
Key et al.

Outpatient Management of Fever and Neutropenia in Adults Treated for Malignancy: American Society of Clinical Oncology and Infectious Diseases Society of America Clinical Practice Guideline Update
Taplitz et al.

Prognostic Index for Acute- and Lymphoma-Type Adult T-Cell Leukemia/Lymphoma
Katsuya et al.

Antiemetics: American Society of Clinical Oncology Clinical Practice Guideline Update
Hesketh et al.

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CANCERS OF THE ESOPHAGUS AND STOMACH

Randomized phase II study of PEP02, irinotecan, or docetaxel as a second-line therapy in gastric or gastroesophageal junction adenocarcinoma.

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Abstract

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6

Background: PEP02 is a novel nanoparticle liposome formulation of irinotecan (CPT-11). In phase I studies, PEP02 has improved pharmacokinetics (PK) of CPT-11 and its active metabolite-SN38 with encouraging safety and tumor response in several cancer types including gastric cancer. This study evaluated the efficacy and safety of PEP02 (P), irinotecan (I) or docetaxel (D) as a single agent in gastric or gastroesophageal junction (GEJ) adenocarcinoma.

Methods: A randomized, 3 arms (1:1:1), Simon's 2-stage (2/21, 5/41 based on tumor response) study was conducted in Europe and Asia. Patients (pts) with locally advanced or metastatic disease, failed to one prior chemotherapy, ECOG PS ≤ 2, at least 1 measurable lesion, no prior CPT-11 or taxane, were treated with P - 120 mg/m^2, I - 300 mg/m^2, or D - 75 mg/m^2 every 3 weeks. PK and pharmacogenetics (PGx) samples were collected for pts in P and I arms.

Results: A total of 135 pts were randomized with 132 (44 per arm) treated between Jan 2008 and Jun 2010. Pts demographics (P/I/D): median age: 56/62/58, male (%): 79.5/77.3/77.3, Pts from Europe (%): 54.6/52.3/56.8, metastatic (%): 97.7/90.9/97.7, gastric adenocarcinoma (%): 84.1/79.6/68.2, and ECOG 0 + 1 (%): 93.2/93.2 /90.9. The confirmed responders of P/I/D were 6 (13.6%)/3 (6.8%)/7 (15.9%) and disease control were 27 (61.4%)/27 (61.4%)/24 (54.6%). These three arms have similar progression free survival and overall survival. If stratified by region, Asian pts had longer survival than European pts. Toxicities of P/I/D were: grade 3/4 neutropenia (%): 9.1/13.6/15.9. grade 3/4 diarrhea (%): 27.3/18.2/2.3, hand-foot syndromes (%): 0.0/6.8/18.2. It was notable that symptoms related to acute cholinergic syndrome were less reported in P arm than in I arm. The PK data showed the mean $T_{1/2}$, C_{max} and $AUC_{0\rightarrow\infty}$ of SN-38 in P/I arms were 88.8/22.8 hr, 8.79/44.1 ng/mL and 879/440 hr x ng/mL.

Conclusions: This randomized phase II study

suggests that PEP02 improves the PK profile and tumor response over irinotecan, and it is as efficacious as docetaxel in the 2nd-line treatment for gastric or GEJ adenocarcinoma. PEP02 is worthy of further evaluation as either 1st- or 2nd-line setting in future gastric cancer studies.

Author Disclosure

Employment or Leadership Position	Consultant or Advisory Role	Stock Ownership	Hon	ert nony	Other Remuneration
PharmaEngine	PharmaEngine	PharmaEngine	Pharm		PharmaEngine

Outpatient Management of Fever and Neutropenia in Adults Treated for Malignancy: American Society of Clinical Oncology and Infectious Diseases Society of America Clinical Practice Guideline Update
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Miscellaneous Agents—Cytotoxics and Hormonal Agents

David E. Gerber, MD

Among the 19 presentations of miscellaneous new agents at this year's meeting, several described novel cytotoxics and hormonal agents. The cytotoxic agents included microtubule inhibitors (eribulin, nanoparticle albumin-bound paclitaxel, peptide-bound paclitaxel), a topoisomerase inhibitor (nanoirinotecan), and an alkylating agent (palifosfamide). Hormonal agents included an aromatase inhibitor (anastrazole), an estrogen receptor antagonist (fulvestrant), and a selective androgen receptor modulator (GTx-024).

CYTOTOXIC AGENTS

Eribulin

Eribulin (E7389), a unique microtubule dynamics inhibitor, was presented by Dr. David Gandara from the University of California Davis. Eribulin is a synthetic analogue of the marine macrolide halichondrin B, which has been isolated from several sea sponges. It binds tubulin at a site near the vinca binding site, inhibiting microtubule growth and resulting in nonproductive tubulin aggregates. A phase III trial of eribulin versus treatment of physician's choice in patients with heavily pretreated breast cancer demonstrated a significant improvement in progression-free survival (PFS) and overall survival (OS), leading to Food and Drug Administration approval for this indication.[1] In a phase I pharmacokinetic and target-validation study, partial and minor radiographic responses were seen in patients with non–small-cell lung cancer (NSCLC).

In a phase II study in patients with advanced NSCLC previously treated with a taxane, 66 patients were treated with eribulin 1.4 mg/m^2 on days 1 and 8 every 21 days.[2] The objective response rate (RR) was 5%. Among patients with taxane-sensitive disease (n = 45; defined as progression > 90 days after taxane administration), there was a 7% partial response rate; median PFS was 2.9 months, and median OS was 12.6 months. Among patients with taxane-resistant disease (n = 21; defined as progression ≤ 90 days after taxane administration), there were no partial responses, median PFS was 1.2 months, and median OS was 8.9 months. In a separate phase II study, 103 patients with advanced NSCLC previously treated with platinum (83 taxane-naive, 20 taxane-exposed) received eribulin 1.4 mg/m^2 either on days 1 and 8 every 21 days, or on days 1, 8, and 15 every 28 days. The 28-day schedule was associated with numerous dose delays, interruptions, or omissions caused by neutropenia. Overall response rate (ORR) was 9.7%, disease-control rate was 55%, median PFS was 3.4 months, and median OS was 9.4 months.

Currently, eribulin is under investigation in numerous combination studies in advanced NSCLC, including with carboplatin (completed), erlotinib (completed), pemetrexed (ongoing), and a planned phase III trial of eribulin + gemcitabine versus cisplatin + gemcitabine.

Abraxane

Abraxane (nanoparticle albumin-bound [nab] paclitaxel) was presented by Dr. Mark Socinski from the University of Pittsburgh. Mechanistically, the drug formulation offers numerous advantages: 1) enhanced tumor penetration and retention of albumin complexes, 2) receptor-mediated transcytosis of albumin complexes across the endothelial barrier to the interstitium (via the gp60/caveolin-1 pathway), and 3) high tumor uptake via binding of albumin complexes by secreted protein acidic and rich in cysteine (SPARC). Clinically, nab-paclitaxel is more soluble than native paclitaxel and therefore, does not require cremaphor-based delivery, reducing the rate of infusion reactions and eliminating the requirement for high-dose corticosteroid premedication.

In phase II NSCLC trials, nab-paclitaxel has been administered in weekly and every 3-week combinations with carboplatin ± bevacizumab. A weekly dose of 100 mg/m^2 was better tolerated and had higher RR (47% versus 30%) compared with an every 3-week schedule.[3]

In a phase III trial, 1050 patients with previously untreated advanced NSCLC were randomized 1:1 to nab-paclitaxel 100 mg/m^2 days 1, 8, 15 plus carboplatin area under the curve (AUC) 6 day 1 (without premedication) versus paclitaxel 200 mg/m^2 day 1 plus carboplatin AUC 6 day 1 (with corticosteroid and antihistamine premedication).[4] By independent radiologic review, ORR (the primary endpoint) was 33% with nab-paclitaxel versus 25% with paclitaxel (p = 0.005). In a post hoc subset analysis, the benefit was limited to squamous tumors (n = 449): 41% versus 24%; p = 0.001. In nonsquamous tumors (N = 602), ORR was 26% versus 25%; p = 0.81. PFS was 6.3 months with nab-paclitaxel versus 5.8 months with paclitaxel (hazard ratio [HR] 0.90; 95% confidence interval [CI], 0.77–1.06; p = 0.21). OS was 12.1 months with nab-paclitaxel versus 11.2 months with paclitaxel (HR 0.92; 95% CI, 0.80–1.07; p = 0.27). In an exploratory analysis, nab-paclitaxel was associated with improved OS in patients aged 70 years or more (19.9 versus 10.4 months; p = 0.009). Nab-paclitaxel was associated with less grade 4 neutropenia, less grade 3 neuropathy, and less grade 3 myalgia. Given a favorable toxicity

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profile, ease of administration, and exploratory analyses in previous trials, nab-paclitaxel is under investigation in elderly populations, squamous tumors, and in combination with radiation therapy for stage III disease.

MM-398

Dr. Paul Bunn, from the University of Colorado, presented MM-398 (liposomal irinotecan). With MM-398, the encapsulation of irinotecan in polyethylene glycol-liposomes enhances pharmacokinetic parameters, including prolonging circulation, sustaining intratumor levels, and enhancing intratumoral activation to SN38, the active metabolite of irinotecan. In small-cell and NSCLC xenografts, MM-398–treated animals have reduced tumor burden and improved survival compared with animals treated with standard formulation irinotecan. In a phase I trial enrolling 11 patients with advanced solid tumors, the maximum tolerated dose was 120 mg/m^2 every 3 weeks. The dose-limiting toxicity was diarrhea. Ongoing studies with this agent include a phase III monotherapy trial in gemcitabine-refractory pancreatic cancer, a phase II study in combination with 5-FU for colorectal cancer, and a phase I study in glioma.

Palifosfamide

Palifosfamide, an active metabolite of ifosfamide, was presented by Dr. Larry Einhorn from Indiana University. Compared with ifosfamide, palifosfamide has more predictable kinetics, does not release acrolein (and therefore does not require coadministration of mesna), has little or no evidence of encephalopathy, and may have less hematologic toxicity. In soft tissue sarcoma, the addition of palifosfamide to doxorubicin results in improved RR and PFS. A phase I study of carboplatin, etoposide, and palifosfamide has been completed. A phase III trial of this regimen (carboplatin AUC 4 day 1, etoposide 100 mg/m^2 days 1–3, and palifosfamide 130 mg/m^2 days 1–3) for small-cell lung cancer is planned.

GRN1005

GRN1005 was presented by Dr. Ross Camidge from the University of Colorado. GRN1005 is a peptide-drug conjugate comprising three paclitaxel molecules linked to a peptide targeting the low-density lipoprotein receptor-related protein 1. This receptor is highly expressed on the surface of the blood–brain barrier and is up-regulated in malignant glioma and metastatic brain tumors from lung, breast, and melanoma primaries.[5] Binding of GRN1005 to lipoprotein receptor-related protein 1 leads to receptor-mediated transcytosis across the blood–brain barrier and results in elevated paclitaxel concentrations compared with unconjugated paclitaxel. Across two phase I monotherapy studies (enrolling 56 patients with solitary brain metastases and 61 patients with primary brain tumors), the dose-limiting toxicity was neutropenia. C_{max} and AUC were higher than seen with unconjugated paclitaxel, and GRN1005 was detected within primary brain tumor samples within hours of administration. Intracranial and systemic responses were seen in both NSCLC and small-cell lung cancer. A phase II trial of GRN1005 650 mg/m^2 every 3 weeks for patients with advanced NSCLC and progressive brain metastases after whole-brain radiation therapy or no prior whole-brain radiation therapy is underway.

HORMONAL AGENTS

Antiestrogens

Data from studies of agents targeting estrogen production or signaling were presented by Dr. Jill Siegfried from the University of Pittsburgh and Dr. Edward Garon from the University of California, Los Angeles. The role of estrogens in the development and progression of lung cancer is supported by data from the Women's Health Initiative, in which women using exogenous estrogens had increased rates of lung cancer mortality (HR 1.71; 95% CI, 1.16–2.52; $p = 0.01$).[6] Conversely, women with breast cancer treated with antiestrogens have reduced lung cancer mortality.[7] Furthermore, estrogen receptors are expressed in NSCLC from both male and female patients, and are associated with worse clinical outcomes.[8] In preclinical models, combined treatment with the aromatase inhibitor anastrazole and the estrogen receptor antagonist fulvestrant results in a 75% reduction in the number of lung tumors. A phase II study of consolidation fulvestrant + anastrozole after induction chemotherapy is currently enrolling postmenopausal women with advanced NSCLC.

The estrogen receptor interacts with epidermal growth factor receptor (EGFR) signaling in numerous ways, including direct effects on EGFR and other transmembrane growth factor receptors, effects on MAPK and PI3K/Akt signaling, and effects on DNA estrogen response elements.[9] Based on this biology and preclinical efficacy of concurrent EGFR and ER inhibition, a phase II study of erlotinib ± fulvestrant for both women and men with advanced NSCLC has been conducted and recently completed accrual.

GTx-024

GTx-024 (enobosarm) was presented by Dr. Jeffrey Crawford from Duke University. This selective androgen receptor modulator is currently under study for the treatment of cancer-related cachexia. Beneficial effects of selective androgen receptor modulators include increased muscle mass and strength, increased bone mass, and positive effects on mood, energy level, and libido. In individuals with lung cancer, GTx-024 has demonstrated improvements in lean body mass and physical function (which correlated with quality of life).[10] The most common adverse events were fatigue, anemia, nausea, and diarrhea. A randomized placebo-controlled trial of GTx-024 3 mg orally daily in patients with advanced NSCLC receiving platinum-based doublet chemotherapy is underway.

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Novel Nanoliposomal CPT-11 Infused by Convection-Enhanced Delivery in Intracranial Tumors: Pharmacology and Efficacy

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Abstract

We hypothesized that combining convection-enhanced delivery (CED) with a novel, highly stable nanoparticle/liposome containing CPT-11 (nanoliposomal CPT-11) would provide a dual drug delivery strategy for brain tumor treatment. Following CED in rat brains, tissue retention of nanoliposomal CPT-11 was greatly prolonged, with >20% injected dose remaining at 12 days for all doses. Tissue residence was dose dependent, with doses of 60 μg (3 mg/mL), 0.8 mg (40 mg/mL), and 1.6 mg (80 mg/mL) resulting in tissue half-life ($t_{1/2}$) of 6.7, 10.7, and 19.7 days, respectively. In contrast, CED of free CPT-11 resulted in rapid drug clearance (tissue $t_{1/2}$ = 0.3 day). At equivalent CED doses, nanoliposomal CPT-11 increased area under the time-concentration curve by 25-fold and tissue $t_{1/2}$ by 22-fold over free CPT-11; CED in intracranial U87 glioma xenografts showed even longer tumor retention (tissue $t_{1/2}$ = 43 days). Plasma levels were undetectable following CED of nanoliposomal CPT-11. Importantly, prolonged exposure to nanoliposomal CPT-11 resulted in no measurable central nervous system (CNS) toxicity at any dose tested (0.06-1.6 mg/rat), whereas CED of free CPT-11 induced severe CNS toxicity at 0.4 mg/rat. In the intracranial U87 glioma xenograft model, a single CED infusion of nanoliposomal CPT-11 at 1.6 mg resulted in significantly improved median survival (>100 days) compared with CED of control liposomes (19.5 days; $P = 4.9 \times 10^{-5}$) or free drug (28.5 days; $P = 0.011$). We conclude that CED of nanoliposomal CPT-11 greatly prolonged tissue residence while also substantially reducing toxicity, resulting in a highly effective treatment strategy in preclinical brain tumor models. (Cancer Res 2006; 66(5): 2801-6)

Introduction

Outcomes for brain tumor patients, particularly those with high-grade gliomas, remain suboptimal and highlight the need for novel therapeutic approaches. Because restricted access is one of the hallmarks of these tumors, strategies for improving drug delivery have attracted much interest. These strategies include regional administration approaches within the central nervous system (CNS) as well as particle-based carriers of drugs.

Convection-enhanced delivery (CED) is a local-regional drug delivery technique that uses a pressure-driven bulk-flow process to distribute agents, including macromolecules, to clinically relevant volumes of solid tissues (1, 2). CED can be used to circumvent the blood-brain barrier, which is a considerable obstacle for many systemically applied drugs (3, 4). CED represents a promising approach to treat various CNS diseases, including brain tumors, which cannot be controlled by local treatment and are poorly responsive to systemic treatment. Compared with routes of administration dependent on diffusion from the injection/implantation site, CED shows a greater volume of distribution and is designed to direct a drug to specific target volumes (5–8).

Liposomes are nano- or microscale carriers typically consisting of a phospholipid membrane shell surrounding a hollow core that can be used to encapsulate small molecules. Liposomal anthracyclines represent the first successful examples of nanoparticle-based anticancer treatment, including marketed agents pegylated liposomal doxorubicin (Doxil, Alza Pharmaceuticals, Inc., Mountain View, CA; Caelyx, Schering-Plough, Inc., Kenilworth, NJ) and liposomal daunorubicin (Daunoxome, Gilead, Inc., Foster City, CA; refs. 9–14). To encapsulate other drugs, we have recently developed a novel intraliposomal drug loading and stabilization technology: poly(anionic) polyols were used to generate new liposomal drugs with unusual drug stability and favorable preclinical pharmacokinetics (15–17). One of these new agents, nanoparticle/liposome containing CPT-11 (nanoliposomal CPT-11; refs. 18–20), encapsulates the camptothecin derivative and topoisomerase I inhibitor CPT-11/irinotecan (Camptosar, Pfizer, New York, NY). Although highly active against many cancer types, CPT-11 displays complex pharmacology characterized by diverse biochemical transformations and potential toxicities (21, 22). In particular, CPT-11 requires conversion to SN-38 for optimal activity yet must avoid inactivation via simple hydrolysis of the requisite lactone configuration to an inactive carboxylate. Furthermore, hepatic conversion to SN-38 leads to biliary excretion of this potent metabolite and resulting gastrointestinal toxicity. In principle, the pharmacologic profile of CPT-11 can be improved by tumor-directed drug delivery, including liposomal encapsulation.

We hypothesized that a combined drug delivery approach featuring nanoliposomal CPT-11 given by CED is feasible and therapeutically advantageous. In previous studies, we showed that CED can be used to achieve extensive distribution of liposomes in rodent brains, orthotopic brain tumor xenografts (23, 24), and monkey brains (25–27). We now report the pharmacology and efficacy of a novel nanoparticle/liposome-based drug given by CED in preclinical brain tumor models.

Materials and Methods

Nanoparticle/liposome constructs. Lipids included 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (PEG-DSPE; Avanti Polar Lipids, Inc., Alabaster, AL), and cholesterol (Calbiochem, San Diego, CA). Small unilamellar liposomes were composed of DSPC, cholesterol, and

Note: C.O. Noble and M.T. Krauze contributed equally to this work.
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PEG-DSPE at a molar ratio of 3:2:0.015. Liposomes were prepared by dissolving all lipids in chloroform/methanol (9:1, v/v) and removing solvent by rotary evaporation to form a dried lipid foam. After hydration, the lipid suspension was briefly vacuumized on a rotary evaporator to remove any trace of organic solvents. Unilamellar liposomes were formed by extrusion at 60°C using gas-pressure thermostatted barrel extruder (Lipex Biomembranes, Vancouver, British Columbia, Canada) through polycarbonate membranes (Whatman Nucleopore, Clifton, NJ) having a pore size of 200 nm (6 times) and 100 nm (12 times) and yielding a final diameter of 96 to 101 nm as determined by light scattering (N4Plus particle size analyzer, Beckman Coulter, Fullerton, CA). *N,N′*-bis-octadecyl-4,4,4′,4′-tetramethylindacarbocyanin iodide [$DiIC_{18}$(3); Molecular Probes, Inc., Eugene, OR] was included for fluorescent labeling.

Drug loading. To prepare triethylammonium sucrose octasulfate (TEA-SOS) as a drug-trapping agent, 0.3 mol/L sodium sucrose octasulfate (Toronto Research Chemicals, Inc., North York, Ontario, Canada) was applied to a column containing Dowex 50W-8X-200 cation exchange resin (equilibrated with 3 mol/L HCl) to convert the sodium salt of sucrose octasulfate to free acid form. Sucrose octasulfuric acid was eluted from the column with double-distilled water using an inline conductivity detector. The solution was then neutralized with neat triethylamine and diluted to a concentration corresponding to 0.65 mol/L triethylammonium with an osmolality of 480 to 530 mmol/kg (pH 5.5-6.0). Residual sodium was determined by potentiometry using a sodium-sensitive glass electrode. For TEA-SOS-containing liposomes, dried lipids were hydrated in 81 mmol/L aqueous TEA-SOS solution (0.65 mol/L triethylamine) at 60°C, and the hydrated lipid suspension was subjected to eight cycles of freezing (−80°C) and thawing (60°C). Extraliposomal TEA-SOS was removed by size exclusion chromatography on Sepharose CL-4B.

CPT-11 (a kind gift of PharmaEngine, Inc., Taipei, Taiwan) was loaded into TEA-SOS-containing liposomes by addition of a 15 mg/mL solution of CPT-11/HCl to a final drug-to-lipid ratio of 500 g CPT-11/mol phospholipid (for 0.06-0.8 mg/rat dose levels) or 800 g CPT-11/mol phospholipid (for 1.6 mg/rat dose level), with incubation of the drug-liposome mixture at 60°C (pH 6.0) for 45 minutes followed by quenching on ice for 15 minutes. Unencapsulated CPT-11 was removed by Sephadex G75 size exclusion chromatography, and the drug-loaded liposomes were stored at 4°C until use. The resulting nanoliposomal CPT-11 was concentrated on a stirred cell concentrator containing a regenerated cellulose 1×10^5 NMWL membrane (Amicon, Millipore Corp., Billerica, MA) and sterilized by passage through 0.2-μm PES syringe filter. CPT-11 concentration was determined by measuring absorbance at 375 nm of a solubilized sample. Briefly, 0.1 mL of an aqueous portion of the sample containing nanoliposomal CPT-11 or standards was added to 0.9 mL of a solution containing 72 vol % methanol, 18 vol % of 0.1 mol/L phosphoric acid, and 10 vol % chloroform. Phospholipid was measured using blue phosphomolybdate-based spectrophotometric assay (28).

Animal models. Toxicity studies used healthy male Sprague-Dawley rats weighing ~250 g (Charles-River Laboratories, Wilmington, MA). For xenograft studies, congenitally athymic, male, nude rats (*rnu/rnu*, homozygous) weighing ~150 to 200 g (National Cancer Institute Animal Production Program, Frederick, MD) were housed under aseptic conditions, which included filtered air and sterilized food, water, bedding, and cages. For the intracranial xenograft tumor model, U87 glioblastoma cells (Brain Tumor Research Center Tissue Bank, University of California at San Francisco, San Francisco, CA) were harvested by trypsinization, washed once with HBSS without Ca^{2+} and Mg^{2+}, and resuspended in HBSS for implantation. Cells (5×10^5) in 10 μL HBSS were implanted into the striatal region of athymic rat brains as follows: under deep isoflurane anesthesia, rats were placed in a small-animal stereotaxic frame (David Kopf Instrument, Tujunga, CA). A sagittal incision was made to expose the cranium and followed by a burr hole in the skull at 0.5 mm anterior and 3 mm lateral from the bregma using a small dental drill. Cell suspension (5 μL) was injected over 2 minutes at a depth of 4.5 mm from the brain surface; after a 2-minute wait, another 5 μL were injected over 2 minutes at a depth of 4 mm, and after a final 2-minute wait, the needle was removed and the wound was sutured.

Convection-enhanced delivery. CED of free CPT-11 or nanoliposomal CPT-11 was done using a volume of 20 μL as described (24, 29). Briefly, the infusion system consisted of a fused-silica needle cannula that was connected to a loading line (containing the therapeutic agent) and an olive oil infusion line. A 1-mL syringe (filled with oil) mounted onto a microinfusion pump (BeeHive, Bioanalytical Systems, West Lafayette, IN) regulated the flow of fluid through the system. Based on chosen coordinates, needle cannula was mounted onto stereotactic holders and guided to targeted region of the brain through burr holes made in the skull. The following ascending infusion rates were applied to achieve the 20-μL total infusion volume: 0.2 μL/min (15 minutes) + 0.5 μL/min (10 minutes) + 0.8 μL/min (15 minutes).

Tissue pharmacokinetics. Rats were given a single 20-μL infusion by CED of free or nanoliposomal CPT-11, and the animal was sacrificed at prescribed times. The appropriate brain hemisphere was perfused with PBS, surgically removed, and frozen. Either the tissue was ground under liquid nitrogen or water was added to the tissue at a 50% (w/w) ratio, and the tissue was homogenized using a mechanical homogenizer in an ice bath. The homogenates (0.1 mL) were extracted as the lactone form of CPT-11 and SN-38 with 0.4 mL of an acidic methanol solution (20% 0.1 mol/L phosphoric acid/80% methanol) by vortexing for 10 seconds twice and centrifugation at 13,000 rpm for 10 minutes, and the supernatants were transferred to autosampler vials for high-pressure liquid chromatography (HPLC) analysis. Blank homogenates were added to CPT-11 and SN-38 to estimate extraction efficiency. Analysis was conducted on a Dionex HPLC system using a C_{18} reverse-phase silica column preceded by a Supelco C_{18} guard column. A sample injection volume of 50 μL was used, and the column was eluted isocratically at a flow rate of 1.0 mL/min with a mobile phase consisting of 3% by volume of aqueous (pH 5.5) triethylammonium acetate and acetonitrile (73:27). CPT-11 and SN-38 were typically eluted in 5.1 and 9.8 minutes, respectively, and both were detected by fluorescence at 420 nm (365 nm excitation).

Tissue pharmacokinetics was fit to a monoexponential decay equation using the trend analysis of Microsoft Excel (Microsoft Corp., Redmond, WA). Pharmacokinetic variables, including tissue half-life ($t_{1/2}$), clearance, mean residence time in brain or brain tumor tissue, and area under the time-concentration curve (AUC_∞), were all determined by noncompartmental pharmacokinetics data analysis using PK Solutions 2.0 software (Summit Research Services, Montrose, CO).

Therapy studies. To evaluate toxicity, healthy rats received a single infusion of free or nanoliposomal CPT-11 via CED. Rats were monitored daily for survival, weekly weights, and general health (alertness, grooming, feeding, excreta, skin, fur, mucous membrane conditions, ambulation, breathing, and posture). Rats were euthanized 60 days after CED treatments, and their brains were removed, fixed in 10% buffered formalin phosphate and then in 30% sucrose, and cut into sections (25 μm) for H&E staining.

To evaluate survival, rats were randomly assigned to five groups (n = 8 rats per group) and U87 tumor cells were implanted into each rat brain. Five days after tumor implantation, a single CED infusion of 20 μL was done using different treatment conditions as described in the text. Rats were evaluated for clinical and tissue toxicity as described above.

Results

Construction of nanoliposomal CPT-11 for brain tumor treatment. CPT-11 was encapsulated in small unilamellar phospholipid vesicles at extremely high concentrations not previously attainable using intraliposomal sucrose octasulfate for high capacity binding to CPT-11, combined with effective transmembrane exchange of intraliposomal triethylammonium for the drug cation (15, 16). Drug loading efficiencies were 86% to 101% of added drug. Constructs were 96 to 101 nm in diameter and achieved drug-to-lipid ratios of 691 to 812 g CPT-11/mol phospholipid, corresponding to payloads of 0.9×10^5 to 1.0×10^5 drug molecules per nanoparticle based on 8×10^5 phospholipids/nanoparticle. The resulting nanoparticle/liposome construct

(termed nanoliposomal CPT-11 to indicate a lipid bilayer-based nanoparticle encapsulating a stably entrapped nanoscale drug complex) could be concentrated without aggregation or precipitation up to 80 mg CPT-11/mL in aqueous solution, which is ~4-fold greater than the solubility of CPT-11 as a free drug. Nanoliposomal CPT-11 showed excellent storage stability at 4°C; at 6 months, no particle size change and only 0.3% drug leakage were detected.

Tissue pharmacokinetics of nanoliposomal CPT-11 following CED in normal adult rat brains or intracranial U87 tumor xenografts. Free CPT-11 or nanoliposomal CPT-11 were given via single CED treatment into the brains of normal adult Sprague-Dawley rats, and tissue levels were determined at varying times after infusion by HPLC. Tissue retention of nanoliposomal CPT-11 was dose dependent, with doses of 60 μg (3 mg/mL), 0.8 mg (40 mg/mL), and 1.6 mg (80 mg/mL) resulting in brain tissue $t_{1/2}$s of 6.7, 10.7, and 19.7 days, respectively (Table 1). Tissue concentration versus dose was not a linear function, as even low doses (60 μg) of nanoliposomal CPT-11 resulted in substantial levels of drug: >20% injected dose (ID) remained at 12 days after infusion. In contrast, CED of free CPT-11 at the equivalent dose, which was also its highest tolerable dose tested, was cleared from the brain within 1 day (Fig. 1). Hence, at equivalent doses of nanoliposomal and free CPT-11, the AUC_∞ was increased by 25-fold for nanoliposomal CPT-11, and the tissue $t_{1/2}$ was improved from 0.3 day for free CPT-11 to 6.7 days (22-fold) for the liposomal drug. Because nanoliposomal CPT-11 could be infused at much higher doses than free CPT-11 due to its reduced toxicity (shown in the next section), nanoliposomal CPT-11 at its highest tested dose improved the AUC_∞ by 1,636-fold and tissue $t_{1/2}$ by 66-fold over that of free CPT-11 at its highest tolerable dose.

Systemic levels of CPT-11 following CED of nanoliposomal CPT-11 were measured concurrently in the plasma of healthy rats receiving 1.6 mg nanoliposomal CPT-11 (Fig. 1). Plasma CPT-11 levels were not detectable in any sample, indicating that CED abrogated significant systemic exposure.

Drug delivery to tumor tissue was evaluated in an orthotopic U87 tumor xenograft model in athymic rats, in which U87 glioma cells were implanted intracranially and allowed to grow for 10 days before treatment. In comparison with normal brain tissue, drug clearance from U87 tumors was significantly slower (Fig. 2*A*; Table 1). The tissue $t_{1/2}$ of drug (Table 1) was extended by ~4-fold (10.7 versus 43.0 days) when nanoliposomal CPT-11 was infused in the tumor xenograft model compared with normal rat brain tissue.

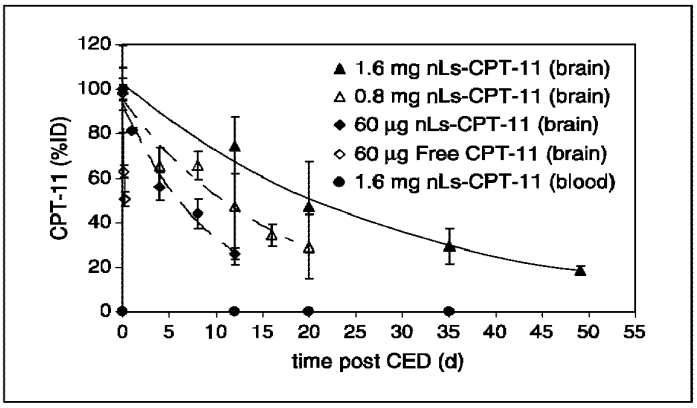

Figure 1. Tissue pharmacokinetics of free CPT-11 and nanoliposomal (nLs) CPT-11 in the normal adult rat brain and blood following single CED infusion. All values are %ID versus time after CED of 20-μL infusate. Drug concentrations were determined by HPLC assay for CPT-11/HCl. CPT-11 concentrations in blood were all below the detection limit of 1 ng/mL (~0.03%ID).

Tumor penetration of free CPT-11 given systemically (tail vein injection) in rats with intracranial U87 tumors was examined by measuring the drug in tumor tissue and plasma (Fig. 2*B*). Fifteen minutes after i.v. injection of free CPT-11 at its established maximum tolerated doses of 100 mg/kg (29.3 mg; ref. 30), drug levels in tumor were 2.7 μg/g (0.009% ID/g tissue), and at 5 hours, this had declined to 1.7 μg/g. These miniscule concentrations were much lower than the 46.2 μg/g tissue achieved 15 minutes after CED of free CPT-11 (17-fold) and, even more so, the 921 μg/g tissue after CED of 1.6 mg nanoliposomal CPT-11 (341-fold).

SN-38, the highly active metabolite of CPT-11, was also assayed in tissue and plasma samples but was undetectable in all samples. Using several different extraction methods for the detection of SN-38 by HPLC analysis (31–33), a detection limit of 1 ng/mL was established with spiked tissue recovery of >95% using an acidic methanol extraction.

Host toxicity of CED of free CPT-11 and nanoliposomal CPT-11 in rodent CNS. Free CPT-11 (60 μg or 0.4 mg) or nanoliposomal CPT-11 (0.06, 0.4, 0.8, and 1.6 mg) were given via a single 20-μL CED infusion into normal adult rat brains (Fig. 3). After sacrifice at 42 days after treatment, histologic evidence of neurotoxic injury was scored on a scale of 0 to 3+. In animals receiving CED of free CPT-11 at 60 μg, brain tissue contained evidence of minor trauma at the

Table 1. Tissue pharmacokinetics of CPT-11 formulations given by CED

	Brain			
	$t_{1/2}$ (d)	AUC_∞ (μg d/g)	Clearance (g/d)	Mean residence time (d)
60 μg free CPT-11	0.3	16.4	3.6	0.4
60 μg nanoliposomal CPT-11	6.7	417	0.14	9.6
0.8 mg nanoliposomal CPT-11	10.7	13,723	0.058	15.4
1.6 mg nanoliposomal CPT-11	19.7	26,823	0.06	28.5
	U87 Tumor			
	$t_{1/2}$ (d)	AUC_∞ (μg d/g)	Clearance (g/d)	Mean residence time (d)
0.8 mg nanoliposomal CPT-11	43.0	40,315	0.02	62.0

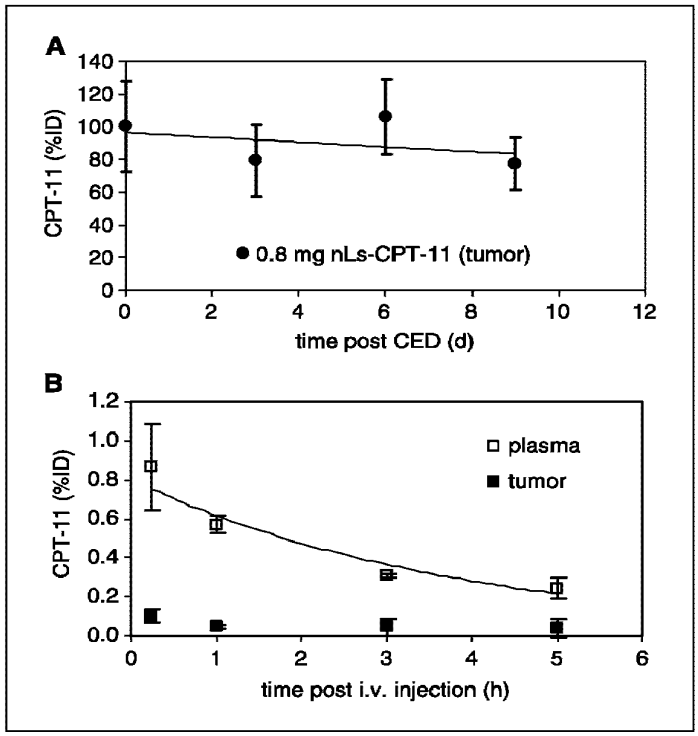

Figure 2. Tumor tissue pharmacokinetics of free CPT-11 and nanoliposomal CPT-11 in intracranial U87 tumor xenografts. Tumors were implanted and allowed to grow for 10 days before CED or i.v. treatment. *A*, nanoliposomal CPT-11, single CED treatment at 0.8 mg (20 μL). *B*, free CPT-11, following bolus i.v. injection at 100 mg/kg. Two to four rats per time point.

site of the infusion cannula (arrows) in the striatum but otherwise no apparent tissue toxicity (score = 0; Fig. 3*A*). However, all animals that received free CPT-11 at 0.4 mg/rat were observed to have extensive tissue necrosis within the CNS (score = 3+; Fig. 3*B*). In contrast, animals receiving nanoliposomal CPT-11 at all doses tested showed no evidence of CNS toxicity (score = 0 for all animals), and the only finding was minor trauma at the infusion cannula site (Fig. 3*C*).

No systemic toxicities, including weight loss or diarrhea, were observed following CED of any of the treatments (Fig. 3*D*). Furthermore, no gross neurologic or behavioral changes were noted after treatment. Indeed, no dose-limiting toxicities for nanoliposomal CPT-11 were identified up to 1.6 mg/rat, which represented the highest feasible dose. Higher doses were precluded by formulation viscosity at concentrations >80 mg/mL, although this was 4-fold greater than the solubility of free CPT-11. Taken together, these data indicated that nanoliposomal CPT-11 greatly extended the tissue tolerance and maximum tolerated doses of the drug; whereas the highest tolerable dose of free CPT-11 was 60 μg/rat, that for nanoliposomal CPT-11 was at least 1.6 mg/rat.

Efficacy of CED of free CPT-11 and nanoliposomal CPT-11. The antitumor efficacy of nanoliposomal CPT-11 (0.06, 0.8, and 1.6 mg/rat) and free CPT-11 (at the highest tolerable dose tested, 0.06 mg/rat) was evaluated following single CED infusion in the intracranial U87 tumor xenograft model. The control group received a CED infusion of "empty" liposomes of the same lipid composition as nanoliposomal CPT-11 but without any encapsulated drug and labeled with the lipophilic fluorescent marker, $DiIC_{18}$(3). As shown in Fig. 4, all animals in the control group expired due to tumor progression by day 22, and mean survival was only 20 days (median, 19.5 days). Treatment with free CPT-11 showed a slight improvement in survival, although all animals still expired by day 30 and mean survival was 28 days (median, 28.5 days). At the equivalent dose of 0.06 mg/rat, treatment with nanoliposomal CPT-11 resulted in mean survival of 36 days (median, 30 days) and one of eight rats surviving beyond 100 days; this suggested a trend toward superiority for nanoliposomal CPT-11 over free drug ($P = 0.09$, pairwise comparison). Treatment with nanoliposomal CPT-11 at 0.8 mg/rat resulted in 50% of the animals surviving beyond day 100 and mean survival of 71 days (median, 78 days). Animals treated with nanoliposomal CPT-11 at 1.6 mg/rat showed excellent survival, with five of eight rats surviving beyond day 100 and mean survival of 83 days (median, >100 days). Overall, CED infusion of nanoliposomal CPT-11 produced greatly enhanced survival compared with CED of free CPT-11 (hazard ratio = 0.39; $P = 0.01$). The improved survival associated with nanoliposomal CPT-11 treatment was dose dependent, with risk of death versus control of 90%, 24%, and 5.8% for the animals receiving the 0.06, 0.8, and 1.6 mg/rat treatments, respectively ($P < 0.001$, Cox proportional hazards model).

Histopathologic evaluation of brain tissue was done in all animals at death or after study sacrifice. Animals showing clinical signs of tumor progression were euthanized. Of the 10 animals surviving to study end at day 100, which only occurred within the groups receiving nanoliposomal CPT-11 at 0.06, 0.4, 0.8, and 1.6 mg/

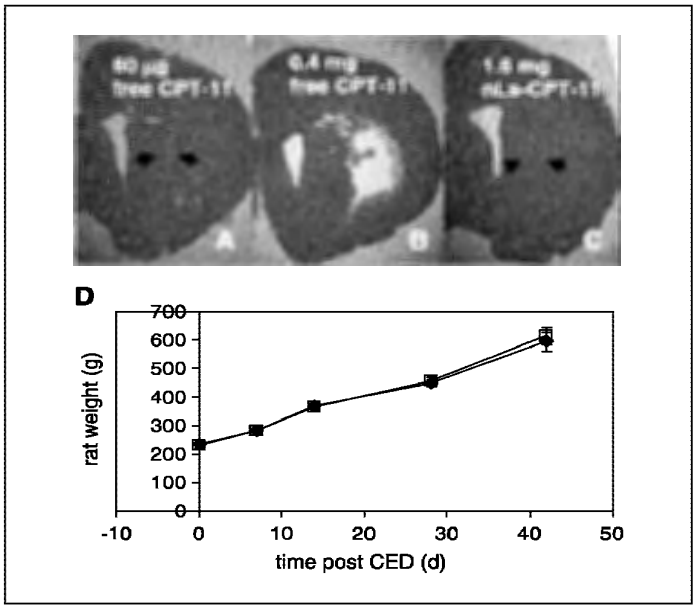

Figure 3. Tissue toxicity of free CPT-11 and nanoliposomal CPT-11 in the normal adult rat brain following CED. *A*, rats (four per group) treated with a single CED infusion of free CPT-11 at 60 μg (3 mg/mL) using an infusion volume of 20 μL. *B*, rats (four per group) treated with a single CED infusion of free CPT-11 at 0.4 mg (20 mg/mL) using an infusion volume of 20 μL. *C*, rats (four per group) treated with a single CED infusion of nanoliposomal CPT-11 at 1.6 mg (80 mg/mL) using an infusion volume of 20 μL. Six weeks after CED, animals were sacrificed and brains were processed for histopathology. Representative H&E sections from each group. Extensive tissue injury was observed in all animals treated with 0.4 mg free CPT-11. *Arrows*, rats in other treatment groups showed only focal traumatic injury at the site of the infusion cannula. *D*, serial weight measurements in control rats (□) and rats given 1.6 mg nanoliposomal CPT-11 by CED (♦). *Bars*, SD.

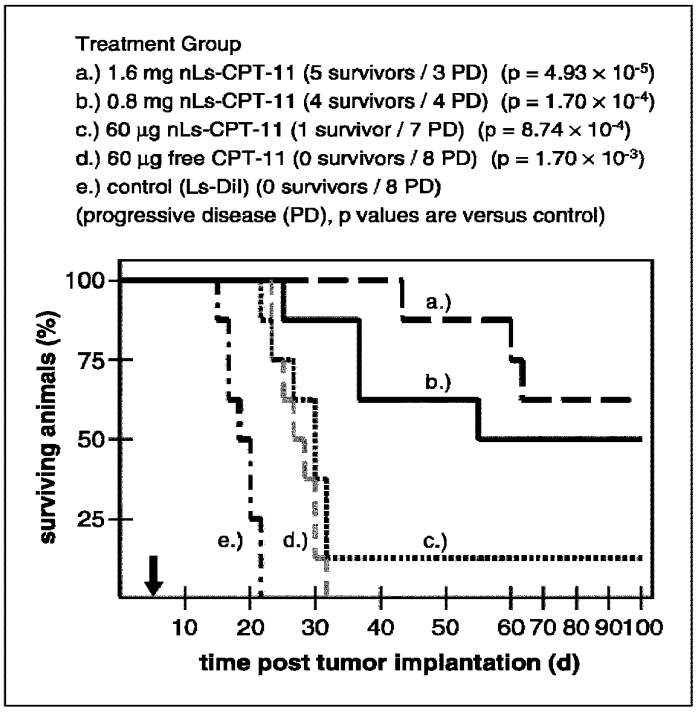

Figure 4. Treatment of rats bearing orthotopic U87 tumors with single CED infusion of free or nanoliposomal CPT-11. Five days after tumor implantation within the brain (*arrow*), rats were treated with nanoliposomal CPT-11 at 1.6 mg (80 mg/mL; *a*), nanoliposomal CPT-11 at 0.8 mg (40 mg/mL; *b*), nanoliposomal CPT-11 at 60 μg (3 mg/mL; *c*), free CPT-11 at 60 μg (3 mg/mL; *d*), and liposomal $DiIC_{18}(3)$ without encapsulated drug (empty liposomes; *e*). Eight animals per group. Median survival for each group was >100 days (*a*), 78 days (*b*), 30 days (*c*), 28.5 days (*d*), and 19.5 days (*e*).

rat, only 1 rat (0.8 mg/rat) showed histologic evidence of residual brain tumor; complete pathologic responses were noted in the 9 other survivors (Fig. 5*A* and *B*). In all cases of animal death, tumor progression was observed in the brains of the rats (Fig. 5*C* and *D*).

Discussion

We reported previously that liposomes in the range of 40 to 100 nm can be efficiently infused by CED into large volumes within the brains of rodents (23, 24) and primates (25, 26), indicating the feasibility of this approach as a drug delivery strategy. We now describe, to our knowledge, the first such example of a liposome- or nanoparticle-based drug given via CED for brain tumor treatment in preclinical models. These results indicated that combining the novel agent nanoliposomal CPT-11, which features highly efficient and stable encapsulation of CPT-11 in lipidic nanoparticles, with CED provided significant anticancer activity with a large therapeutic index against brain tumors.

These studies confirmed the difficulties associated with systemic chemotherapy for brain tumor treatment. I.v. administration of free CPT-11, a drug that seems promising against brain tumors (34), resulted in very low drug concentrations within the rat CNS due to rapid systemic clearance within hours as well as limited blood-brain barrier penetration. The problem of low therapeutic levels is compounded by the considerable systemic toxicities of this drug in its free form (35). CED is designed to provide high local-regional drug levels while reducing systemic exposure, and this was evidenced in these studies. In human brain tumors, disruption of the tumor-blood barrier could increase systemic access of this agent following CED; however, animals receiving CED of nanoliposomal CPT-11 at the highest dose tested (1.6 mg/rat, 80 mg/mL CPT-11/HCl) showed undetectable drug levels in plasma. Furthermore, this CED dose amounted to ~18-fold less total drug than an i.v. injection of free CPT-11 at 100 mg/kg (29.3 mg CPT-11/HCl). Finally, the slow and sustained release of drug from nanoliposomal CPT-11 provides an additional safety margin upon any systemic exposure.

Compared with systemic therapy, CED of free CPT-11 yielded much higher drug levels in brain tissue (17-fold) and even greater drug concentrations when CED was combined with nanoliposomal CPT-11 (341-fold). Furthermore, whereas CED of free CPT-11 was cleared from the brain quickly (<1 day), nanoliposomal CPT-11 was retained for many days in a dose-dependent manner; a single CED administration at 1.6 mg/rat was detectable over weeks (>49 days). Encapsulation of CPT-11 in these nanoliposome constructs resulted in at least 196-fold prolongation of residence time in normal tissue.

It is striking that greatly prolonged tissue retention of nanoliposomal CPT was associated with a substantial decrease in CNS toxicity compared with free drug and indeed was well tolerated up to the highest dose tested, 1.6 mg/rat. Since significant CNS toxicity was observed with free CPT-11 at 0.4 mg/rat, encapsulation in the nanoliposome construct increased tissue tolerance by

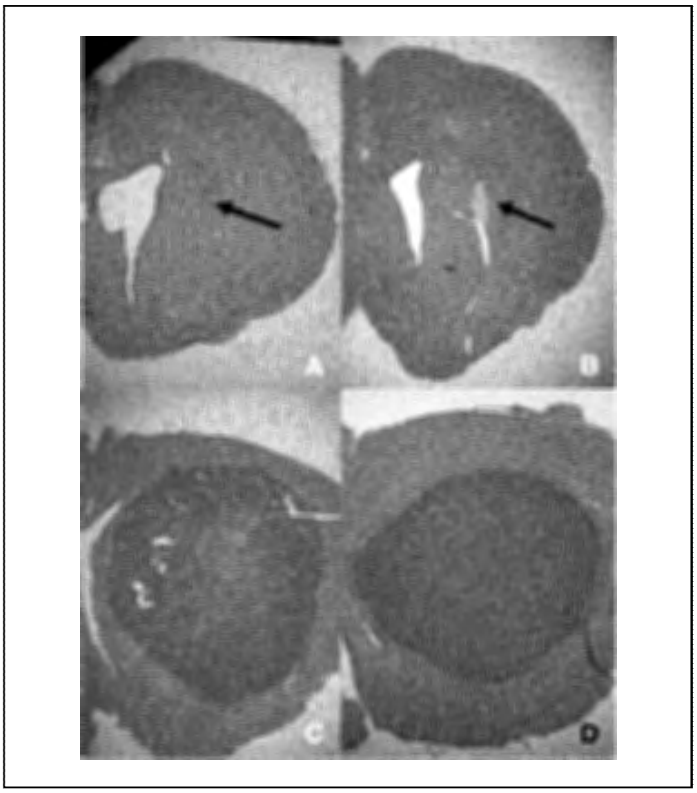

Figure 5. Representative brain sections from each experimental group in the therapy study. *A*, 1.6 mg nanoliposomal CPT-11 (obtained from a survivor and showing no residual tumor). *B*, 0.8 mg nanoliposomal CPT-11 (obtained from a survivor and show no residual tumor). *C*, 60 μg free CPT-11 (showing a typical tumor found in nonsurviving animals, in which tumor progression led to death). *D*, liposomal $DiIC_{18}(3)$ (empty liposomes; showing a typical tumor found in nonsurviving animals, in which tumor progression led to death). *Arrows*, scar from the cannula used for tumor implantation and CED.

>4-fold. This protective effect may be due to lower acute tissue exposure associated with particle encapsulation and its attendant slow rate of drug release.

Although equivalent CED doses of free CPT-11 and nanoliposomal CPT-11 indicated a slight but nonsignificant survival benefit for the latter, the treatment advantage provided by the nanoliposome construct was largely attributable to its much wider therapeutic index, enabling significantly higher doses with no added toxicity. It is possible that other mechanisms of delivery may also have contributed to the observed anticancer efficacy. Following initial distribution within brain tissue by CED, liposomes provided sustained drug levels for a prolonged period, resulting in continuous tumor exposure and a metronomic chemotherapy effect.

Nanoliposomal CPT-11 is a novel lipidic nanoparticle carrier that may be particularly well suited for brain tumor treatment. CPT-11 is very active against brain tumor cells (36, 37) and is being evaluated in clinical trials despite its pharmacologic limitations (19, 38). Using a sucrose octasulfate-based method for intraliposomal loading and stabilization, nanoliposomal CPT-11 provided clearly superior drug delivery and efficacy in conjunction with CED infusion. The highly concentrated dose of CPT-11 achieved (up to 1.6 mg/rat) was a direct result of the extremely high drug-to-lipid ratio ($\sim$800 g CPT-11/mol phospholipid, 1×10^5 CPT-11 molecules per liposome particle) attainable using this drug loading technology. This system can also, in principle, be used to deliver other drugs and/or diagnostic agents. For example, we have reported previously that similar liposomes loaded with gadolinium chelates can be readily visualized by real-time magnetic resonance imaging in the rodent (24) and monkey (25) CNS.

We conclude that nanoliposomal CPT-11 can be infused by CED into the CNS, resulting in substantial improvement in pharmacologic profile and therapeutic index. The strategy of combining a novel nanoparticle chemotherapeutic with CED may circumvent the drug delivery barriers posed by brain tumors.

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Novel Nanoliposomal CPT-11 Infused by Convection-Enhanced Delivery in Intracranial Tumors: Pharmacology and Efficacy

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Convection-enhanced delivery of nanoliposomal CPT-11 (irinotecan) and PEGylated liposomal doxorubicin (Doxil) in rodent intracranial brain tumor xenografts

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We have previously shown that convection-enhanced delivery (CED) of highly stable nanoparticle/liposome agents encapsulating chemotherapeutic drugs is effective against intracranial rodent brain tumor xenografts. In this study, we have evaluated the combination of a newly developed nanoparticle/liposome containing the topoisomerase I inhibitor CPT-11 (nanoliposomal CPT-11 [nLs-CPT-11]), and PEGylated liposomal doxorubicin (Doxil) containing the topoisomerase II inhibitor doxorubicin. Both drugs were detectable in the CNS for more than 36 days after a single CED application. Tissue half-life was 16.7 days for nLs-CPT-11 and 10.9 days for Doxil. The combination of the two agents produced synergistic cytotoxicity in vitro. In vivo in U251MG and U87MG intracranial rodent xenograft models, CED of the combination was also more efficacious than either agent used singly. Analysis of the parameters involved in this approach indicated that tissue pharmacokinetics, tumor microanatomy, and biochemical interactions of the drugs all contributed to the therapeutic efficacy observed. These findings have implications for further clinical applications of CED-based treatment of brain tumors. *Neuro-Oncology 9, 393–403, 2007 (Posted to Neuro-Oncology [serial online], Doc. D06-00150, July 24, 2007. URL http://neuro-oncology.dukejournals.org; DOI: 10.1215/15228517-2007-019)*

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Despite intensive multimodal treatment such as surgical resection, radiation therapy, and systemic chemotherapy, malignant brain tumors (e.g., glioblastoma multiforme) remain the most difficult neoplasms to treat. Poor penetration of the blood–brain barrier (BBB) by many anticancer drugs results in the need for high doses of systemic chemotherapeutics.[1,2] Systemic side effects are therefore the limiting factor in chemotherapeutic protocols for brain tumor patients. Intratumoral chemotherapy has been proposed in order to overcome these difficulties and in recognition of clinical observations that 90% of malignant gliomas recur within 2 cm of an original resection site.[3] However, many local drug delivery techniques face the persistent problem of poor distribution of infused drugs.[4] Convection-enhanced delivery (CED) was introduced by Bobo et al.[5] to overcome this difficulty. CED is a direct infusion technique that utilizes bulk flow to deliver molecules to a

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targeted site, offering an improved volume of distribution over that of simple diffusion. CED of therapeutic agents bypasses the BBB, delivers a high concentration of therapeutic agents to the infusion site, provides a wider distribution of therapeutic agents within the target site, and minimizes systemic exposure, resulting in fewer systemic toxicities.[6–9] Promising results have been reported from clinical trials with CED of several therapeutic agents in malignant brain tumors.[10–13] A major disadvantage of all these clinical trials is that delivery to the CNS cannot be monitored in real time. Therefore, side effects (e.g., chemical meningitis) can be explained only empirically.

In order to further improve the clinical prospects for CED-based therapy, we have introduced a number of innovations to CED for both current and future clinical applications. We have defined four steps essential for more efficient brain tumor therapies in the future: (1) visualized controlled local delivery of therapeutic agents to the CNS, (2) highly active therapeutic combinations that display relatively low toxicity in healthy brain, (3) optimized pharmacokinetic profile of the therapeutic agent that allows for a long half-life of the active agent in the brain in order to alleviate the need for frequent dosing and to introduce metronomic chemotherapy that targets dormant cancer cells as well as the supporting angiogenic blood vessels, and (4) establishment of therapeutic delivery parameters flexible enough to accommodate multiple tumor types.

Liposomes provide stable encapsulation for various anticancer drugs and have a number of advantages over the corresponding free drugs for the systemic treatment of cancer.[14,15] Liposomal drugs are promising candidates for local delivery within the CNS, because they are inert until the drug is made bioavailable via release from the carrier. Liposomes now also play an important role in neuro-oncology not only because of their pharmacokinetic profile but also because their distribution in the CNS can be visualized by MRI.[16,17] The introduction of a novel step-design delivery catheter in our CED studies provided for distribution of the therapeutic agent, free of detectable reflux at any infusion rate examined thus far, up to 50 μl/min.[18] We have previously shown excellent distribution of liposomes throughout the primate brain with this catheter.[17] Considerable progress has been reported in developing real-time imaging strategies with liposomal MRI contrast agents in primate CNS.[16,17,19]

Drug delivery kinetics to the brain have been optimized by encapsulation of small-molecule drugs in liposomes, thereby markedly improving the pharmacokinetic profile of these drugs in the CNS.[8] A unique intraliposomal stabilization technology has resulted in stable controlled-release formulations for a variety of difficult-to-encapsulate drugs, for example, irinotecan.[20,21] We have previously evaluated in separate studies liposomes containing either the topoisomerase I (Topo I) inhibitor doxorubicin[9] or the topoisomerase II (Topo II) inhibitors CPT-11 and topotecan, against human brain tumor xenografts.[8,22] Results from these studies have been encouraging, in part because Topo I and Topo II inhibitors exert their principal effects on the two major classes of enzymes involved in regulating DNA topology. This functional overlap suggested that a combination of both drugs could act synergistically against orthotopic brain tumor xenografts. Administration of a combination of nonliposomal Topo I and Topo II inhibitors to patients with advanced solid malignancies has been evaluated in numerous clinical studies.[23–25]

In this study, we evaluated PEGylated liposomal doxorubicin (Doxil) and nanoliposomal CPT-11 (nLs-CPT-11) in vitro and in vivo with respect to toxicity, tissue half-life, and efficacy in U87MG and U251MG xenografts. We demonstrated that only distribution within the entire tumor is able to prolong animal survival in the rodent brain tumor xenograft model.

Materials and Methods

Liposomal Therapeutics

Control "empty" liposomes, not loaded with drug, were composed of L-α-distearoylphosphatidylcholine (DSPC), choline (Chol), 1,1'-dioctadecyl-2,3,3',3'-tetramethylindocarbocyanine perchlorate [$DiIC_{18}(3)$], and polyethylene glycol–coupled 1,2-distearoyl-sn-glycero-3-phosphatidylethanolamine (PEG-DSPE) in the molar ratio of 3:2:0.03:0.015. nLs-CPT-11 was composed of DSPC, Chol, and PEG-DSPE at a molar ratio of 3:2:0.015. Liposomes were prepared by dissolution of all lipids in chloroform/methanol (9:1, vol/vol) and subsequent removal of the solvent by rotary evaporation to form a dried lipid foam. The dried lipids were hydrated in 81 mM aqueous triethylammonium sucrose octasulfate solution (0.65 M triethylamine, pH 5.2) at 60°C, and the hydrated lipid suspension was subjected to eight cycles of freezing (–80°C) and thawing (60°C). After hydration, trace organic solvent was removed from the lipid suspension on a rotary evaporator. Unilamellar liposomes were formed by extrusion at 60°C in a pressurized, thermostat-controlled barrel extruder (Lipex Biomembranes, Vancouver, Canada) through double-stacked polycarbonate membranes (Whatman Nucleopore, Clifton, NJ, USA) of pore size 200 nm (6 times) and 100 nm (12 times), yielding a final liposomal diameter of 95–110 nm as determined by dynamic light scattering (N4Plus particle size analyzer; Beckman Coulter, Fullerton, LA, USA). Extraliposomal triethylammonium sucrose octasulfate was removed by size-exclusion chromatography on a Sepharose CL-4B column eluted with HEPES-buffered dextrose (5 mM HEPES, 5% dextrose, pH 6.5).

CPT-11 was loaded into the liposomes by addition of a 15 mg/ml solution of CPT-11·HCl to a final drug-to-lipid ratio of 750 g CPT-11·HCl per mole of phospholipid, and incubation of the drug/liposome mixture at 60°C for 45 min, followed by quenching on ice for 15 min. Unencapsulated CPT-11 was removed by chromatography on a Sephadex G-75 size-exclusion column that was eluted with HEPES-buffered saline (5 mM HEPES, 145 mM NaCl, pH 6.5). Drug-loaded liposomes were stored at 4°C until use. Drug-loading efficiencies of >95% were typically observed. Nanoliposomal CPT-11 (nLs-CPT-11)

was concentrated on a stirred cell concentrator (Millipore Corp., Billerica, MA, USA) containing a regenerated cellulose 1×10^5 nominal molecular-weight-limit membrane (Millipore) and sterilized by passage through a 0.2-μm polyethersulfone syringe filter. The concentration of CPT-11 was determined by measuring the absorbance at 375 nm of a solubilized liposome sample. Briefly, 0.1 ml of an aqueous portion of the sample containing nLs-CPT-11 or standards was added to 0.9 ml of a solution containing 72 vol% methanol, 18 vol% 0.1 M phosphoric acid, and 10 vol% chloroform. Phospholipid was measured by a spectrophotometric assay.[26] All drug values for nLs-CPT-11 in this manuscript refer to equivalents of CPT-11·HCl.[8]

PEGylated liposomal doxorubicin was obtained as Doxil (Alza Pharmaceuticals, Mountain View, CA, USA). All drug values for Doxil refer to equivalents of doxorubicin·HCl.[9]

Tumor Cell Lines

Human glioblastoma multiforme cell lines U87MG and U251MG were obtained from the Brain Tumor Research Center Tissue Bank at the University of California, San Francisco (UCSF). Cells were maintained as monolayers in Eagle's minimal essential medium supplemented with 10% fetal calf serum, antibiotics, and nonessential amino acids. Cells were cultured at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

Cell Cycle Analysis

One day prior to treatment, 2×10^5 cells were seeded into each well of a six-well plate (Corning Inc., Corning, NY, USA). Cells were exposed to drug-free $DiIC_{18}(3)$-DS liposomes (control liposomes), Doxil (0.2 μg doxorubicin/ml), nLs-CPT-11 (1 μg CPT-11/ml), or both, in complete medium. After 24 h, cells were fixed in 70% ethanol, washed, digested with RNase A (Sigma, St. Louis, MO, USA), stained with propidium iodide (Sigma), and subjected to flow cytometry in a FACScan (Becton-Dickinson, San Jose, CA, USA) with 10,000 events/determination. ModFit LT software (Verity Software House, Inc., Topsham, ME, USA) analyzed cell cycle distribution.

Cell Viability Assay

Cells were seeded at 1,000 cells/well in 96-well plates (Corning Inc.), allowed to attach for 24 h, and then exposed to drug-free $DiIC_{18}(3)$-DS liposomes (control liposomes), Doxil, nLs-CPT-11, or both, in complete medium. For combination studies, the ratio of Doxil to nLs-CPT-11 was kept constant at 1:5 (wt/wt of doxorubicin to CPT-11). MTT [3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] reagent was added 48 h after initiation of treatment, and plates were read at an absorbance of 490 nm 3 h later with a SpectraMax microplate reader (Molecular Devices Corporation, Sunnyvale, CA, USA). All treatments were performed in triplicate. The background absorbance was determined by incubating media with substrate alone and subtracting the values from wells containing cells only.

Animals and Intracranial Xenograft Technique

Male Sprague-Dawley rats (250 g) were obtained from Charles River Laboratories (Wilmington, MA, USA). Congenitally athymic, male, homozygotic, nude rats (*rnu/rnu*; 150–200 g) were purchased from the National Cancer Institute Animal Production Program (Frederick, MD, USA) and were housed under aseptic conditions. All protocols were approved by the Institutional Animal Care and Use Committee at UCSF. For the intracranial xenograft tumor model, U87MG and U251MG cells were harvested by trypsinization, washed once with Hank's balanced salt solution without Ca^{2+} and Mg^{2+} (HBSS), and resuspended in HBSS for implantation. A cell suspension of 5×10^5 cells/10 μl HBSS was implanted into the striatal region of the athymic rat brains. Under isoflurane anesthesia, rats were placed in a small-animal stereotactic frame (David Kopf Instruments, Tujunga, CA, USA). A sagittal incision was made to expose the cranium, and a burr-hole was made in the skull 0.5 mm anterior and 3 mm lateral from the bregma. At a depth of 4.5 mm below the brain surface, 5 μl of cell suspension was injected. Two minutes later, the needle tip was elevated 0.5 mm, and another 5 μl was injected at a depth of 4 mm. After a further 2 min, the needle was removed and the wound sutured.

Convection-Enhanced Delivery

Throughout this study, drugs were delivered in a volume of 20 μl by CED as described previously.[6,27] Briefly, a fused-silica cannula was connected to a loading line (containing liposomes) and an oil-infusion line. A 1-ml syringe (filled with oil) was mounted onto a microinfusion pump (BeeHive; Bioanalytical Systems, West Lafayette, IN, USA) that regulated the flow of fluid through the system. Based on tumor injection site coordinates, a reflux-free and backflow-free step-design cannula[18] was mounted onto stereotactic holders and guided to the targeted region (4.5 mm depth) of the brain through burr holes made in the skull as described above. An infusion rate of 0.5 μl/min over 40 min was applied to achieve a final infusion volume of 20 μl.

Tissue Pharmacokinetics

Rats were given a single 20-μl infusion by CED of Doxil (2 mg, 0.1 mg/ml) together with nLs-CPT-11 (0.8 mg, 40 mg/ml), and the animals were sacrificed at prescribed times. The brain hemisphere was perfused with phosphate-buffered saline, surgically removed, and frozen. Water was added to the tissue at a 50% wt/wt ratio, and the tissue was homogenized mechanically in an ice bath. The homogenates (0.1 ml) were extracted with 0.9 ml of chloroform/methanol (4:1) by vortexing for 15 s, and the organic phase was collected. This process was repeated, and the combined organic extracts were evaporated to dryness with a centrifugal concentrator. The residue was

dissolved in 0.5 ml methanol, and centrifuged at 10,000 rpm for 10 min. The supernatant solution was analyzed by high-performance liquid chromatography (HPLC). Standards were prepared by extraction of spiked blank tumor tissue. Analysis was conducted on a Dionex HPLC system with a C_{18} reverse-phase silica column (Supelco C-18 column, 250 mm × 4 mm inner diameter, 5 mm particle size), preceded by a Supelco C_{18} guard column. A sample injection volume of 50 μl was used, and the column was eluted at a flow rate of 1.0 ml/min with a mobile phase consisting of 0.21 M aqueous triethylammonium acetate (pH 5.5) and acetonitrile. A linear gradient elution was used with the acetonitrile content increasing from 27% to 45% over 10 min. Each drug was detected by a fluorescence detector (excitation, 485 nm; emission, 590 nm). Typical retention times for CPT-11 and doxorubicin were 5.4 min and 7.1 min, respectively.

Pharmacokinetic parameters that included the tissue half-lives of the drug ($t_{1/2}$), clearance, the mean residence time in the brain or brain/tumor, and the area under the concentration versus time curve were all determined by noncompartmental pharmacokinetic data analysis with PK Solutions 2.0 software (Summit Research Services, Montrose, CO, USA).

Evaluation of Toxicity

Three normal Sprague-Dawley rats were evaluated for potential local toxicity after CED-mediated co-infusion of Doxil and nLs-CPT-11. Rats were monitored daily for general health (alertness, grooming, feeding, excreta, skin, fur, mucous membranes, ambulation, breathing, and posture). Animal weights were reported weekly. Doxil was used at 50% of the maximum tolerated dose (MTD), determined previously.[9] Little nLs-CPT-11 toxicity has been observed in previous studies, and the drug was used at a safe dose determined previously.[8] Sixty days after CED of a 20-μl solution containing Doxil (2 mg, 0.1 mg/ml) and nLs-CPT-11 (0.8 mg, 40 mg/ml) into the striatum, rats were euthanized and their brains fixed in 4% formaldehyde. Fixed brain tissue was subjected to paraffin sectioning (5 μm), and sections were stained with hematoxylin and eosin (H&E).

Combination Therapy in the U87MG and U251MG Intracranial Xenograft Models

Forty-two rats were implanted with U251MG tumor cells as described above. Animals treated on day 7 after tumor implantation were randomly divided into four groups: (1) a control consisting of CED of $DiIC_{18}(3)$-DS fluorescent liposomes (n = 6), (2) CED of Doxil (n = 6), (3) CED of nLs-CPT-11 (n = 6), and (4) a combination treatment of Doxil together with nLs-CPT-11 (n = 6). In a second study, animals were randomly divided into two groups on day 14 after tumor implantation: a control consisting of CED of drug-free liposomes (n = 9) and a combination treatment of Doxil and nLs-CPT-11 (n = 9).

In a third study, 24 rats implanted with U87MG tumor cells were randomly divided into four groups on day 10 after tumor implantation: (1) a control group consisting of drug-free liposomes (n = 6), (2) CED of Doxil (n = 6), (3) CED of nLs-CPT-11, and (4) a combination treatment of Doxil and nLs-CPT-11 (n = 6). CED of 20 μl of the specified drug was performed for each group in all survival studies. Rats were monitored daily for survival and general health (alertness, grooming, feeding, excreta, skin, fur, mucous membrane conditions, ambulation, breathing, and posture). Animal weights were reported weekly. The studies in U251MG were terminated 100 days after tumor implantation, and the U87MG survival study was terminated 70 days after tumor implantation. Surviving animals were euthanized, and their brains were sectioned and stained with H&E.

Distribution of Liposomes in U87MG and U251MG Brain Tumor Xenografts

Animals were implanted with U87MG (n = 3) and U251MG (n = 3) tumor cells. CED with 20 μl of $DiIC_{18}(3)$-DS fluorescent liposomes was performed on day 10 after implantation of U87MG and on day 30 after tumor implantation of U251MG. Animals were euthanized immediately after the infusion procedure. Brains were frozen and cut into 25-μm sections on a cryostat. Fluorescent images of liposome distribution were taken of each brain from rostral to caudal in 100-μm intervals. The fluorescent signal generated by $DiIC_{18}(3)$ was visualized with a fluorescence microscope equipped with a 540/25 nm band-pass filter for excitation, together with a long-pass filter at 565 nm for emission. A charge-coupled device camera with a fixed aperture was used to capture the image.

Statistical Analysis

Results for the survival studies are expressed as a Kaplan-Meier curve. Survival between the treatment groups was compared with an unpaired Student's t-test and expressed as median survival (MS).

Results

Tissue Pharmacokinetics of Doxil and nLs-CPT-11 Coadministered by CED in Rat Brain

A mixture of liposomes containing 2 mg Doxil and 0.8 mg nLs-CPT-11 was infused by a single CED treatment into the brains of adult rats, and tissue levels were determined by HPLC at various times after infusion (Fig. 1). Both drugs were detectable for more than one month after a single CED treatment. The decline in the tissue concentration of nLs-CPT-11 was exponential (R^2 = 0.9821) with a half-life of 16.7 days (Table 1). Doxil, at a 400-fold lower dose, decreased linearly (R^2 = 0.9975) with a half-life of 10.9 days. The small differences may reflect differences in drug release from the carrier, or differences in clearance of highly PEGylated (Doxil) versus mildly PEGylated liposomes (nLs-CPT-11).

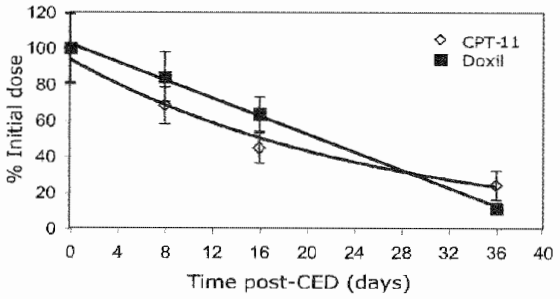

Fig. 1. Tissue pharmacokinetics of nanoliposomal CPT-11 and Doxil in the normal adult rat brain after single convection-enhanced delivery (CED) infusion. All values are percent injected dose versus time after CED of 20 μl infusate. Drug concentrations were determined by high-performance liquid chromatography assay for CPT-11·HCl and doxorubicin·HCl. Values are means ± SD of four animals per time point.

Cell Cycle Distributions In Vitro

Twenty-four hours after treatment of U87MG and U251MG cells with drug-free liposomes (control), Doxil, nLs-CPT-11, or a mixture of the two therapeutic liposomes, changes in cell cycle were recorded (Figs. 2 and 3). U87MG cells exposed to Doxil, a G2/M-active antineoplastic drug, underwent G2 arrest, with the percentage of cells in G2/M increasing from 12% (control) to 46% (Doxil; Fig. 2). U87MG cells exposed to nLs-CPT-11 entered both S-phase, to a marked extent (19% with control liposomes vs. 39% with nLs-CPT-11), and also significant G2 arrest (from 12% with control liposomes to 55% with nLs-CPT-11). After treatment with the combination of Doxil and nLs-CPT-11, both S-phase accumulation and G2 arrest were observed in U87MG cells.

When the same drug concentrations used with U87MG cells were applied to U251MG cells, complete cell cycle arrest was observed (Fig. 3). Doxil, nLs-CPT-11, and the combination of both each caused a complete elimination of cells in S-phase, with virtually all cells in G2 arrest. This difference in tumor cell sensitivity to chemotherapeutics later translated to effects on animal survival (Fig. 4).

Table 1. Tissue pharmacokinetics of nLs-CPT-11 (0.8 mg) and Doxil (2 μg)

	$t_{1/2}$ (d)	AUC (μg·d/g)	CL (g/d)	MRT (d)
nLs-CPT-11	16.7	11,600	0.069	24.1
Doxil	10.9	47.2	0.042	15.8

Pharmacokinetic data from Fig. 1 were analyzed with respect to tissue half-life ($t_{1/2}$), area under the curve (AUC), rate of clearance of either drug from brain tissue (CL), and mean residence time (MRT).

Synergistic Cytotoxic Effects of Doxil and nLs-CPT-11 Liposomes In Vitro (U87MG and U251MG)

Synergy, determined by isobologram analysis,[28] was not observed between the two agents in U87MG cells (Fig. 5). EC_{50} values (median effect doses) calculated from this experiment were 1.18 μg/ml for Doxil and 0.75 μg/ml for nLs-CPT-11. In contrast, synergy was observed between the two agents in U251MG cells (Fig. 5B). EC_{50} values from this experiment were 1.29 μg/ml for Doxil and 0.56 μg/ml for nLs-CPT-11.

Combined Effect of Doxil and nLs-CPT-11 in U251MG and U87MG Brain Tumor Xenografts

First, we studied survival in rodents with intracranial U251MG brain tumor xenografts seven days after tumor cell implantation. Rats that received the control liposomes were all euthanized 36–44 days after tumor cell implantation due to neurological symptoms indicative of tumor progression (Fig. 4). MS for this group was 38.5 days. Three of six rats that received 2 mg Doxil survived until termination of the study. However, neurological symptoms due to large tumor formations were observed in the three remaining rats, which required euthanasia 52–71 days after tumor cell implantation. Significant improvement in survival was noted in this treatment group ($p = 0.0007$), with an MS of 85.5 days. Three of six rats that received treatment at 0.8 mg nLs-CPT-11 by CED survived until termination of the study. The three remaining rats in this group were euthanized 54–62 days after tumor cell implantation due to neurological symptoms indicating tumor progression. Small tumors in the striatum were still evident in two of the surviving rats (Fig. 6D). Nevertheless, a significant survival benefit for nLs-CPT-11 over the control group was found (MS = 81 days; $p = 0.0016$). All rats in the group that received the combination treatment of Doxil and nLs-CPT-11 survived until the end of the study (MS > 100 days; $p < 0.0001$).

Next, we studied survival in rodents with U251MG brain tumor xenografts treated 14 days after tumor cell implantation. Rats in the control group that received drug-free liposomes were euthanized 38–46 days after tumor cell implantation due to neurological symptoms indicative of tumor progression (Fig. 4). The MS for the control group was 41 days. All animals receiving combination treatment of 0.8 mg nLs-CPT-11 and 2 mg Doxil survived 63–78 days, with an MS of 68 days ($p < 0.0001$).

A separate group of U87MG xenografts treated on day 10 was also evaluated. Rats in the control group that received drug-free liposomes were euthanized 18–21 days after implantation due to neurological symptoms indicative of tumor progression (MS = 19 days; Fig. 4). Five of six animals that received 2 mg Doxil were euthanized due to tumor-related symptoms 17–30 days after implantation (MS = 24 days). No significant survival benefit was observed for this group ($p = 0.215$). All animals that received 0.8 mg nLs-CPT-11 were euthanized due to neurological symptoms indicative of tumor

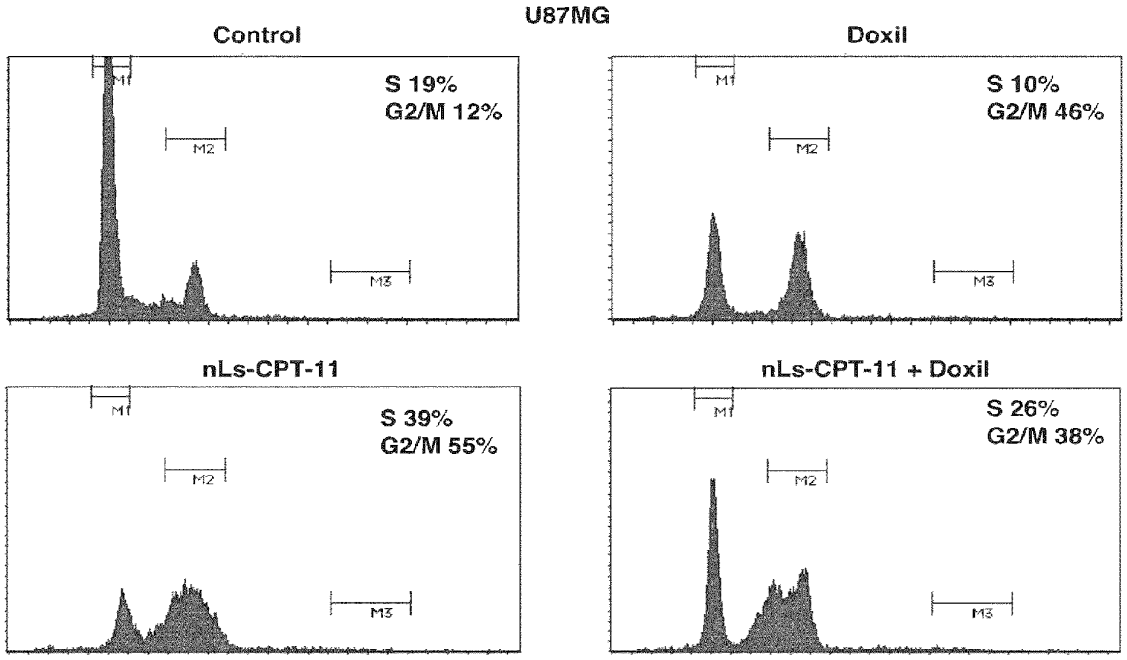

Fig. 2. Cell cycle profiles of U87MG cells examined by flow cytometry. U87MG cells were exposed to drug-free liposomes (control), Doxil (0.2 μg/ml), CPT-11 nanoliposomes (nLs-CPT-11) (5 μg/ml), or a combination of Doxil (0.2 μg/ml) and nLs-CPT-11 (5 μg/ml) for 24 h. Cells were harvested and analyzed by fluorescence-activated cell sorting as described in Materials and Methods.

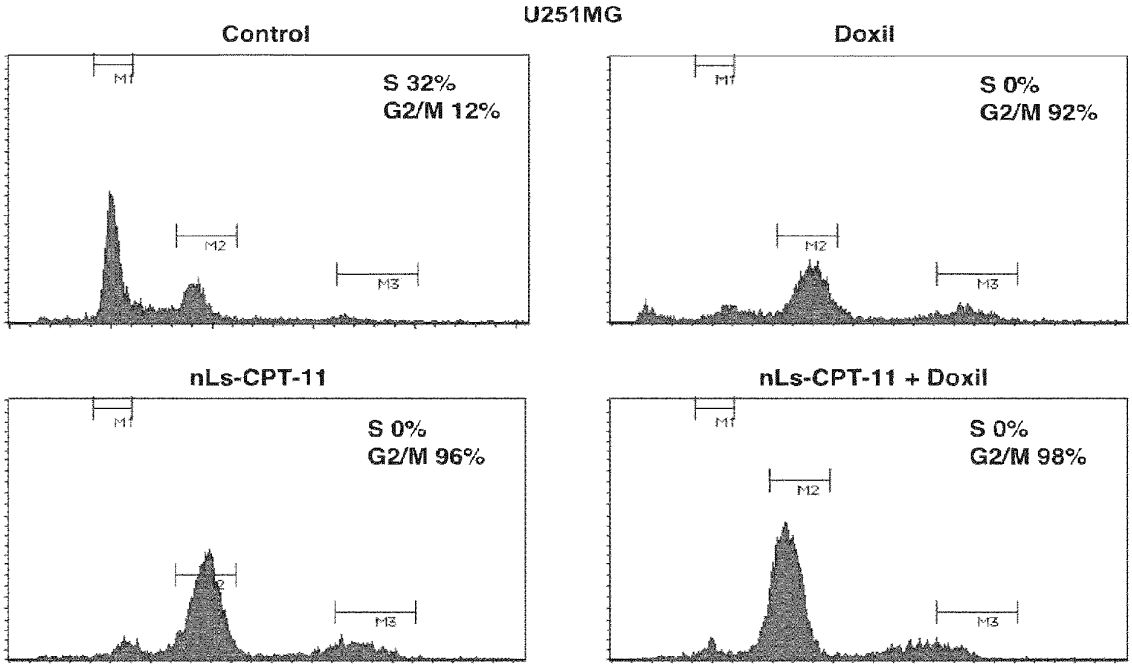

Fig. 3 Cell cycle profiles of U251MG cells examined by flow cytometry. U251MG cells were exposed to drug-free liposomes (control), Doxil (0.2 μg/ml), CPT-11 nanoliposomes (nLs-CPT-11; 5 μg/ml), or a combination of Doxil (0.2 μg/ml) and nLs-CPT-11 (5 μg/ml) for 24 h. Cells were harvested and analyzed by fluorescence-activated cell sorting as described in Materials and Methods.

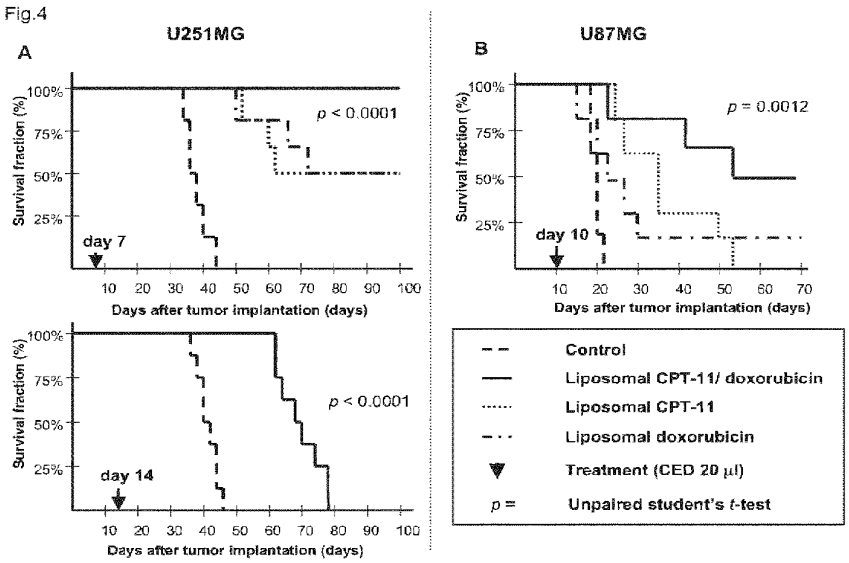

Fig. 4. Animals implanted with U251MG tumor cells. (A) Animals received liposome CED on day 7 and day 14 after tumor cell implantation. CED treatment on day 7 with nanoliposomal CPT-11 (nLs-CPT-11) and Doxil combination was able to eradicate all U251MG tumors in rodent striatum (see Fig. 5 caption). Each agent alone yielded only partial survival when delivered by CED on day 7. No animal in the day 14 combination therapy CED group survived longer than 78 days after tumor cell implantation. (B) Animals implanted with U87MG tumor cells received liposome CED on day 10 after tumor cell implantation. Three of six animals in the combination therapy group survived until termination of the study at day 70 after tumor cell implantation. Only one animal in the Doxil group survived until day 70. No animals in the nLs-CPT-11 group survived to the projected end of this survival study.

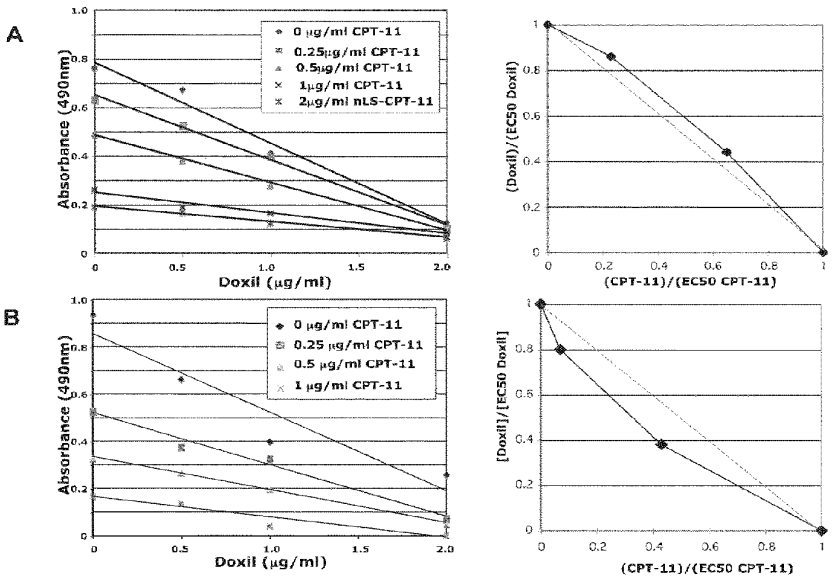

Fig. 5. Synergistic induction of cell death by Doxil and nanoliposomal CPT-11 (nLs-CPT-11) in U251MG glioma cells. (A) U87MG cells were treated for 24 h with increasing nLs-CPT-11 (0–2 µg/ml) concentrations and 24 h with increasing Doxil (0–2 µg/ml) concentrations. No synergy between the two agents was found by analysis of an isobologram (points above dotted line). (B) U251MG cells were treated identically to the U87MG cells with increasing Doxil and nLs-CPT-11 concentrations for 24 h. Synergy was determined between the two agents in the U251MG cell line as seen in the isobologram analysis (points below dotted line).

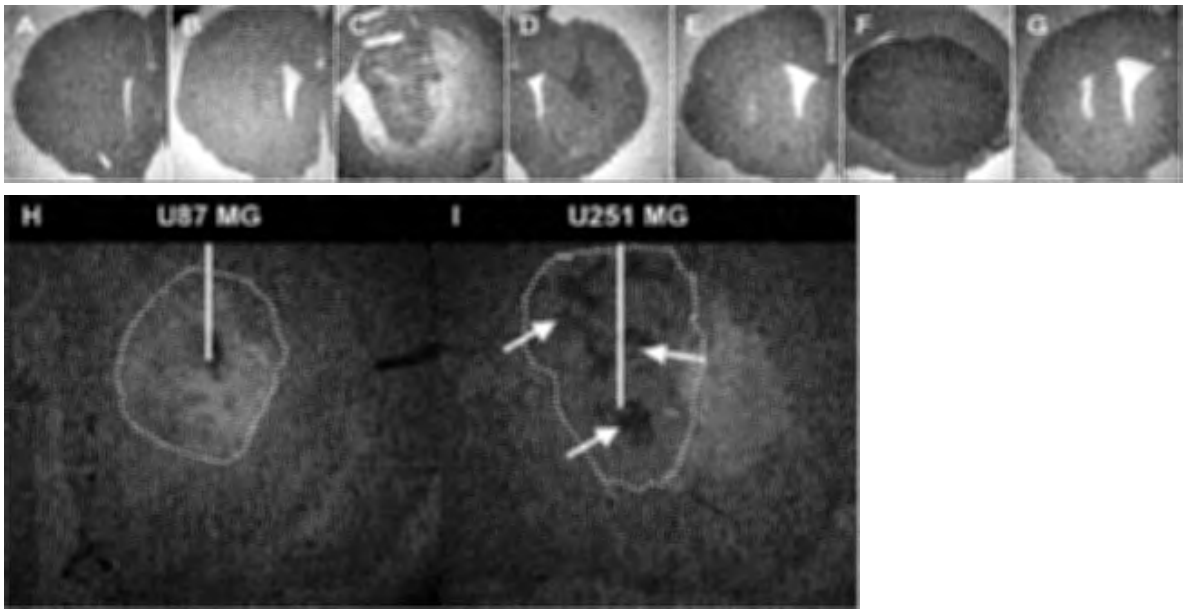

Fig. 6. Representative histology of animals used in this study. (A and B) Brain sections of animals used for toxicity study, euthanized 60 days after receiving the combination of Doxil (2.0 μg, 0.1 mg/ml) and nanoliposomal CPT-11 (nLs-CPT-11; 0.8 mg, 40 mg/ml). (C) Representative section of animals bearing U251MG xenografts. (D) Section from nLs-CPT-11 survivor of day 7 convection-enhanced delivery (CED) still bearing small U251MG tumor. (E) Survivor of day 7 CED combination therapy in U251MG at day 100. (F) Animal bearing U87MG xenografts. (G) Survivor of day 10 CED combination therapy in U87MG at day 70. (H and I) Representative sections showing $DiIC_{18}(3)$ fluorescent liposome distribution in U87MG (H) and U251MG (I) intracranial xenografts after 20 μl CED. White line represents infusion catheter placement; dotted line delineates tumor margin on histology sections; white arrows show necrotic areas in U251MG brain tumor xenografts.

progression 24–53 days after tumor cell implantation (MS = 30 days). CED treatment with nLs-CPT-11 alone resulted in a significant survival benefit ($p = 0.0048$). Three of six animals receiving combination therapy of 0.8 mg nLs-CPT-11 and 2 mg Doxil were euthanized 23–54 days after tumor cell implantation. Three animals survived until termination of the study 70 days after tumor cell implantation. A three-fold increase in mean survival was observed for the combination therapy group (MS = 62 days), compared to the control ($p = 0.0012$).

Distribution of Liposomes in U87MG and U251MG Brain Tumor Xenografts

After CED of 20 μl, liposomal $DiIC_{18}(3)$ distributed extensively throughout the U87MG brain tumor xenografts (Fig. 6H). However, the same amount of $DiIC_{18}(3)$ liposomes distributed mainly outside U251MG brain tumor xenografts and within necrotic areas (white arrows; Fig. 6I). The observed tumor-specific distribution highlights one contribution to variability in therapeutic efficacy between tumor models.

Discussion

Further improvement of current CED-based drug delivery protocols is critical for future clinical application. Clinical trials in which CED is employed for drug delivery to the brain have indicated considerable potential for this approach in neurooncology and in neurodegenerative diseases.[10,29] In this study, we have elucidated three important factors that affect overall survival: drug efficacy, drug tissue half-life, and drug distribution.

Liposomal encapsulation of chemotherapeutic drugs improves their pharmacokinetic properties.[14,20,21,30] An ideal combination of two liposomal chemotherapeutics would have similar half-lives in the CNS and rates of drug release from the carrier in order to exert maximum combined toxicity on tumor cells over extended periods of time. In our study, although the dose of Doxil used was 400-fold lower than that of nLs-CPT-11, their tissue half-lives were similar. Liposomal encapsulation not only enhances drug half-life but also improves distribution of compounds with high tissue affinity (e.g., Doxil),[9] as well as therapeutic index of the active agent (e.g., CPT-11).[8]

With respect to drug efficacy, we compared two brain tumor cell lines in our in vitro studies that are known to have different chemosensitivity in vitro.[31] The same dose of the drug combination that produced an intermediate response in U87MG caused almost complete G2 arrest in U251MG. Our drug combination showed synergy in the more chemosensitive cell line, U251MG, whereas no synergy was found in U87MG. Various mechanisms are known by which tumor cells escape G2 cell cycle arrest,[32,33] but different gene expression profiles, depending on whether cells are grown in vitro or in vivo, may be the key to the differences seen in this study.[34] Gene expression profiles in U87MG and U251MG, as determined by Camphausen et al.,[34] differ significantly in vitro but are similar when grown intracranially. We believe that contrast between the similar efficacy seen in the survival studies and the differences seen in our in vitro studies may be attributed to these effects. Moreover, the heterogeneity of malignant brain tumors warrants individual approaches to define the appropriate cancer treatment in each case.

We have shown previously that CED of liposomes easily covers an entire seven-day-old U251MG brain tumor xenograft (tumor diameter $\leq$0.5 mm).[35] The synergistic action of our liposomal therapeutic combination leads to complete eradication of U251MG xenografts, whereas Doxil or nLs-CPT-11 alone was only moderately efficacious. Interestingly, two of the three survivors in the nLs-CPT-11 group had relatively small tumor cell formations in the striatum at the termination of the study (day 100). Consistent with previous findings with nLs-CPT-11, we attribute this strong tumor growth inhibition in vivo to its central effect on the S-phase of the cell cycle.[8] In contrast, a survival study with 14-day-old U251MG brain tumor xenografts (vs. the seven-day xenografts; tumor diameter up to 1 mm) showed significant prolongation of survival. However, none of the animals survived past day 78 with the combination treatment. In order to explain this discrepancy, we conducted a series of liposome distribution studies in U251MG brain tumor xenografts. Large necrotic areas in U251MG brain tumor xenografts that increase with growth of the tumor appeared to alter liposome distribution within the tumor. As shown in Fig. 6, a large fraction of infused liposomes accumulated outside of the tumor or within necrotic areas. We conclude that the slow clearance of liposomal therapeutics inhibits tumor growth at its margins, but after eventual clearance of the therapeutic agents, tumor in areas not subject to liposome accumulation can lead to continued tumor growth. In the U87MG survival study, a significant extension of animal survival was also achieved, but only 50% of animals survived until termination of the study. As in the U251MG experiments, we also studied liposome distribution in U87MG brain tumor xenografts. Due to its rather homogeneous growth pattern, good distribution of liposomes was observed in U87MG xenografts (tumor diameter ~ 1.5 mm on day 10).[35] However, despite the better distribution of liposomes in U87MG tumors, the fast growth of U87MG, the slow release of therapeutics from liposomes, and the lower sensitivity to Doxil/nLs-CPT-11 combination in vitro, compared with U251MG, all contribute to reduced efficacy in U87MG versus U251MG tumors.

Several important conclusions can be drawn from this study. For patients with brain tumors, systemic delivery of therapeutics is usually associated with systemic side effects while achieving only marginal therapeutic concentrations in the CNS. This limits the effectiveness of systemic treatment. Thus, clinical trials to evaluate the combination of Topo I and II inhibitors via intravenous administration in patients with solid neoplasms demonstrated substantial toxicity.[23,24] The defined MTD was frequently lower than the typical dose level for the respective individual agent. CED of nonencapsulated chemotherapeutic agents has produced better outcomes but was also highly toxic upon extensive distribution of free drug within the CNS.[36] CED of a mixture of Doxil and nLs-CPT-11 has shown excellent therapeutic potential without signs of toxicity to the CNS at the doses of each liposomal drug employed. Both therapeutics were used at doses previously shown to be individually nontoxic but to be substantially therapeutic in brain tumors when delivered by CED.[8,9] The present drug combination was therefore capable of increasing therapeutic efficacy by synergistic action in U251MG without increase in CNS toxicity. Although synergy was not formally demonstrated via isobologram analysis in U87MG in vitro, the combination therapy in vivo nevertheless resulted in improved survival over each agent alone.

Another important aspect of this study is the prolonged release of the two drugs from liposomes.[8] This slow-release phenomenon might benefit patients with brain tumors, since human tumors grow far more slowly than do animal tumor xenografts. As shown by our animal experiments, eradication of tumor is a realistic possibility only when complete coverage with a liposomal drug is achieved. This is why we have developed a real-time imaging method to visualize direct liposome delivery into the CNS.[17,19] This real-time imaging technique, in combination with several infusion catheters, may permit complete liposomal coverage of tumors in human brain, because the drug may then be infused until optimal distribution is achieved while avoiding untoward leakage of the drug into healthy surrounding tissue. Currently, we are undertaking experiments in canine de novo brain tumors in order to validate the present rodent experiments in a larger mammalian brain and to strengthen the hypothesis that distribution of liposomes in tumors is greatly affected by tumor histology. Although all studies were conducted on a relatively small number of animals per group, our findings were consistent with previous rodent and primate studies. In the human setting, malignant tumors are usually more than 1 cm in diameter at time of diagnosis, and are located in various parts of the brain. Our previous studies on naive primate brains have clearly shown that our CED delivery technique, when combined with our step cannula design, allows liposome delivery into every CNS structure at any given depth.[17,19] Application of this delivery technique will probably require multiple cannulas in order to cover the entire tumor mass. We

are aware that more work needs to be done in the field of distribution characterization as a function of tumor histology, but a more complete conceptual understanding of liposome therapeutics and distribution has now been established.

Acknowledgments

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NEWS IN BRIEF

Re-assessing the rule of 5, two decades on

In 1997 Christopher Lipinski and colleagues looked at the physicochemical characteristics of approved drugs and clinical candidates at that time, and proposed that the 'rule of 5' could predict the likelihood that a given small molecule will be orally active. This guideline prioritized compounds that have molecular masses of less than 500 daltons, calculated logarithm of the octanol–water partition coefficient (clogP) of less than 5, 5 or fewer hydrogen bond donors, and 10 or fewer hydrogen bond acceptors — and red flagged compounds that have more than one parameter out of range. Novartis's Michael Shultz now argues that this drug-likeness rule of thumb does not stand the test of time.

Reporting in the *Journal of Medicinal Chemistry*, Shultz assessed the physicochemical properties of the 409 small molecules that the FDA has approved since 1997. Molecular mass in this cohort has increased considerably from the baseline, he found, and in both 2016 and 2017 the average molecular mass of FDA-approved drugs was greater than 500 daltons. An up-to-date molecular mass cut-off based on the properties of orally available small molecules approved in the past decade would now be more than 600 daltons, he found. The threshold for hydrogen bond acceptors has also increased substantially.

These data call into question the hypothesis that 'drug-like' properties exist, he concludes. "Repeating the [rule of 5] experiment today, with over twice as many oral drugs approved than were available in 1997, gives different 'rules' than those hypothesized two decades ago ... If our past future predictions have not been accurate with the [rule of 5] parameters, we must call into question our current future predictions."

With new modalities coming online — such as targeted protein degraders and constrained peptides — he urges drug developers to reconsider how they use the rule of 5. "We are in danger of repeating our past mistakes if we assume these new modalities are not 'drug-like' and cannot be oral drugs because they are not [rule of 5] compliant," writes Shultz.

Asher Mullard

How much do phase III trials cost?

Drug development has been estimated to cost anywhere from US$43 million on one controversial end of the spectrum to $2.9 billion on the other when failures, post-approval studies and opportunity cost are factored in. Taking a different approach to understanding these numbers, researchers have now focused more narrowly on the price tag for pivotal trials alone. The median expense for a single phase III trial is $19 million, they report in *JAMA Internal Medicine*, after assessing the details of 138 pivotal trials for 59 new drugs that the FDA approved from 2015 to 2016.

Trial costs — estimated on the basis of a cost calculator from the IQVIA contract research organization — vary dramatically, they found. A four-patient phase III trial to test Wellstat's uridine triacetate for the rare hereditary metabolic disorder orotic aciduria likely cost only around $2 million. But Novartis's 8,442-patient non-inferiority trial of sacubitril–valsartan versus enalapril, looking at hospitalization and cardiovascular mortality outcomes, likely cost around $347 million.

"Our study provides a different perspective to the widely held assumption that elaborate and expensive clinical trials are the main reason for the high costs of developing a new drug. These data suggest that high-cost trials occur but usually when drug effects are small or a known drug already provides clinical benefit. On the other hand, pivotal trials for novel drugs with substantial clinical benefits can be conducted at a lower cost," the authors conclude.

This analysis did not capture some of the trial costs that are borne more directly by sponsors, such as the cost to manufacture drugs or the salaries for sponsor employees who oversee trials. The study wasn't designed to assess the costs of failed trials, of earlier-stage trials or of discovery projects, and was not adjusted to include the opportunity cost of these investments.

Nevertheless, the estimate is in line with previous work from analysts at KMR Group who have assessed the clinical trial costs of more than 700 clinical trials that were run by 7 major pharmaceutical companies between 2010 and 2015. The median phase III trial in their data set cost $21.4 million, they reported last year in *Nature Reviews Drug Discovery*. The median phase II trial cost $8.6 million, and the median phase I trial cost $3.4 million, they also reported.

Asher Mullard

FDA approves first drug under new antibacterial and antifungal drug programme

The FDA's approval of Insmed's inhaled formulation of amikacin for lung disease associated with *Mycobacterium avium* marked the agency's first use of the Limited Population Pathway for Antibacterial and Antifungal Drugs (LPAD) approval pathway.

Congress established the LPAD under the 21st Century Cures Act as a means of promoting the development and approval of antibacterial and antifungal drugs for serious or life-threatening infections in limited patient populations with unmet need. Earlier in the year, the agency issued a draft guidance on the LPAD, noting that products that are reviewed under this pathway can follow streamlined clinical development strategies that may involve smaller, shorter or fewer clinical trials.

In the case of Insmed's inhaled amikacin, the regulatory green light was granted on the basis of a 336-patient, open-label phase III trial. Patients with refractory *M. avium*-associated lung disease were randomized to inhaled amikacin plus a background regimen of antibiotics, or to background antibiotics alone. At 6 months, 29% of amikacin-treated patients had no evidence of mycobacteria in their sputum, compared with 9% of patients on background therapy alone.

Injected formulations of amikacin have been in commercial use since the 1970s for the treatment of multidrug-resistant Gram-negative bacteria, non-tubercular mycobacterial infections and other indications.

"We're seeing a lot of early interest among sponsors in using this new pathway, and it's our hope that it'll spur more development and approval of antibacterial drugs for treating serious or life-threatening infections in limited populations of patients with unmet medical needs," says FDA commissioner Scott Gottlieb.

Asher Mullard

STATUTORY INSTRUMENTS

2004 No. 1031

MEDICINES

The Medicines for Human Use (Clinical Trials) Regulations 2004

Made - - - -	*31st March 2004*
Laid before Parliament	*1st April 2004*
Coming into force - -	*1st May 2004*

The Secretary of State, being a Minister designated(**a**) for the purposes of section 2(2) of the European Communities Act 1972(**b**) in relation to medicinal products, in exercise of the powers conferred by the said section 2(2), and of all other powers enabling him in that behalf, hereby makes the following Regulations:

ARRANGEMENT OF REGULATIONS

(**a**) S.I. 1972/1811.
(**b**) 1972 c.68.

PART 1

INTRODUCTORY PROVISIONS

Citation and commencement

1. These Regulations may be cited as the Medicines for Human Use (Clinical Trials) Regulations 2004 and shall come into force on 1st May 2004.

Interpretation

2.—(1) In these Regulations—

"the Act" means the Medicines Act 1968(**a**);

"adult" means a person who has attained the age of 16 years;

"adverse event" means any untoward medical occurrence in a subject to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product;

"adverse reaction" means any untoward and unintended response in a subject to an investigational medicinal product which is related to any dose administered to that subject;

"authorised health professional" means—

(a) a doctor,

(b) a dentist,

(c) a nurse, or

(d) a pharmacist;

"appropriate committee", for the purpose of any provision of these Regulations under which a function falls to be performed, means such committee established under section 4 of the Act for purposes which consist of or include any of those specified in section 4(3) of the Act as the authority performing that function considers appropriate in the circumstances;

"assemble", in relation to an investigational medicinal product, means—

(a) enclosing the product (with or without other medicinal products of the same description) in a container which is labelled before the product is sold or supplied, or used in a clinical trial, or

(b) where the product (with or without other medicinal products of the same description) is already contained in the container in which it is to be sold or supplied, or used in a clinical trial, labelling the container before the product is sold or supplied, or used in a clinical trial, in that container,

and "assembly" has a corresponding meaning;

"business", except in Schedule 2, includes a professional practice and includes any activity carried on by a body of persons, whether corporate or unincorporate;

"chief investigator" means—

(a) in relation to a clinical trial conducted at a single trial site, the investigator for that site, or

(b) in relation to a clinical trial conducted at more than one trial site, the authorised health care professional, whether or not he is an investigator at any particular site, who takes primary responsibility for the conduct of the trial;

"clinical trial" means any investigation in human subjects, other than a non-interventional trial, intended—

(a) to discover or verify the clinical, pharmacological or other pharmacodynamic effects of one or more medicinal products,

(b) to identify any adverse reactions to one or more such products, or

(c) to study absorption, distribution, metabolism and excretion of one or more such products,

with the object of ascertaining the safety or efficacy of those products;

"Commission Directive 2003/94/EC" means Commission Directive 2003/94/EC(**b**) laying down the principles and guidelines of good manufacturing practice for medicinal products for human use and for investigational medicinal products for human use;

(**a**) 1968 c.67.
(**b**) OJ No. L262, 14.10.2003, p.22.

"conditions and principles of good clinical practice" means the conditions and principles specified in Schedule 1;

"conducting a clinical trial" includes—

(a) administering, or giving directions for the administration of, an investigational medicinal product to a subject for the purposes of that trial,

(b) giving a prescription for an investigational medicinal product for the purposes of that trial,

(c) carrying out any other medical or nursing procedure in relation to that trial, and

(d) carrying out any test or analysis—

(i) to discover or verify the clinical, pharmacological or other pharmacodynamic effects of the investigational medicinal products administered in the course of the trial,

(ii) to identify any adverse reactions to those products, or

(iii) to study absorption, distribution, metabolism and excretion of those products,

but does not include any activity undertaken prior to the commencement of the trial which consists of making such preparations for the trial as are necessary or expedient;

"container", in relation to an investigational medicinal product, means the bottle, jar, box, packet or other receptacle which contains or is to contain it, not being a capsule, cachet or other article in which the product is or is to be administered, and where any such receptacle is or is to be contained in another such receptacle, includes the former but does not include the latter receptacle;

"dentist" means a person registered in the dentists register under the Dentists Act 1984(**a**) or entered in the list of visiting EEC practitioners under Schedule 4 to that Act;

"the Directive" means Directive 2001/20/EC of the European Parliament and of the Council on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use(**b**);

"Directive 2001/83/EC" means Directive 2001/83/EC of the European Parliament and of the Council on the Community code relating to medicinal products for human use(**c**), as amended(**d**);

"doctor" means a registered medical practitioner(**e**);

"EEA State" means a State which is a Contracting Party to the EEA Agreement;

"EEA Agreement" means the Agreement on the European Economic Area signed at Oporto on 2nd May 1992(**f**) as adjusted by the Protocol signed at Brussels on 17th March 1993(**g**);

"electronic signature" means data in electronic form which are attached to or logically associated with other electronic data and which serve as a method of authentication;

"European Economic Area" means the European Economic Area created by the EEA Agreement;

"the European Medicines Agency" means the European Agency for the Evaluation of Medicinal Products established by Council Regulation (EEC) No. 2309/93 laying down Community procedures for the authorization and supervision of medicinal products for human and veterinary use and establishing a European Agency for the Evaluation of Medicinal Products(**h**);

(**a**) 1984 c.24.
(**b**) OJ No. L121, 1.5.2001, p.34.
(**c**) OJ No. L311, 28.11.2001, p.67.
(**d**) *See* Article 31 of Directive 2002/98/EC (OJ No. L33, 8.2.2003, p.30) and Commission Directive 2003/63/EC (OJ No. L159, 27.6.2003, p.46).
(**e**) *See* Schedule 1 of the Interpretation Act 1978 (c.30), as amended by paragraph 18 of Schedule 5 to the Medical Act 1983 (c.54).
(**f**) OJ No. L1, 3.1.1994, p.3.
(**g**) OJ No. L1, 3.1.1994, p.572.
(**h**) OJ No. L214, 24.8.93, p.1.

"ethics committee" means—

(a) a committee established or recognised in accordance with Part 2,

(b) the Ethics Committee constituted by regulations made by the Scottish Ministers under section 51(6) of the Adults with Incapacity (Scotland) Act 2000(**a**), or

(c) the Gene Therapy Advisory Committee;

"export" means export to a third country from an EEA State, whether by land, sea or air;

"the Gene Therapy Advisory Committee" means the Gene Therapy Advisory Committee appointed by the Secretary of State to—

(a) consider and advise on the acceptability of proposals for gene therapy research on human subjects, on ethical grounds, and

(b) provide advice on developments in gene therapy research and their implications;

"Health and Social Services Board" means a Health and Social Services Board established under the Health and Personal Social Services (Northern Ireland) Order 1972(**b**);

"Health Board" means a Health Board established under the National Health Service (Scotland) Act 1978(**c**);

"health care" means services for or in connection with the prevention, diagnosis or treatment of illness;

"health care professional" means—

(a) a doctor,

(b) a dentist,

(c) a nurse,

(d) a pharmacist,

(e) a person registered in a register of ophthalmic opticians maintained under section 7 of the Opticians Act 1989(**d**),

(f) a person registered in a register established and maintained under article 5 of Health Professions Order 2001(**e**),

(g) a registered osteopath as defined by section 41 of the Osteopaths Act 1993(**f**), or

(h) a registered chiropractor as defined by section 43 of the Chiropractors Act 1994(**g**);

"health centre" means a health centre maintained under section 2 or 3 of the National Health Service Act 1977, section 36 of the National Health Service (Scotland) Act 1978 or Article 5 of the Health and Personal Social Services (Northern Ireland) Order 1972;

"health service body" means—

(a) a Strategic Health Authority, Health Board or Health and Social Services Board,

(b) a Special Health Authority, Primary Care Trust or Local Health Board established under the National Health Service Act 1977,

(c) a Special Health Board established under the National Health Service (Scotland) Act 1978,

(d) a special health and social services agency established under the Health and Personal Social Services (Special Agencies) (Northern Ireland) Order 1990(**h**),

(e) the Dental Practice Board constituted under section 37(1) of the National Health Service Act 1977,

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(**a**) 2000 asp. 4; *see* S.S.I. 2002/190.
(**b**) S.I. 1972/1265 (N.I. 14).
(**c**) 1978 c. 29.
(**d**) 1989 c. 44.
(**e**) S.I. 2002/254.
(**f**) 1993 c. 21.
(**g**) 1994 c. 17.
(**h**) S.I. 1990/247 (N.I.3)

(f) the Scottish Dental Practice Board or the Common Services Agency for the Scottish Health Service established under the National Health Service (Scotland) Act 1978,

(g) the Northern Ireland Central Services Agency for the Health and Social Services established under the Health and Personal Social Services (Northern Ireland) Order 1972,

(h) a National Health Service trust established under the National Health Service and Community Care Act 1990(**a**) or the National Health Service (Scotland) Act 1978,

(i) an NHS foundation trust within the meaning of section 1(1) of the Health and Social Care (Community Health and Standards) Act 2003(**b**), or

(j) a Health and Social Services trust established under the Health and Personal Social Services (Northern Ireland) Order 1991(**c**);

"hospital" includes a clinic, nursing home or similar institution;

"import", other than in regulation 13 and Schedule 3, means import into the United Kingdom from a third country, whether by land, sea or air;

"informed consent" shall be construed in accordance with paragraph 3 of Part 1 of Schedule 1;

"insurance or indemnity" includes provision for meeting losses or liabilities—

(a) under a scheme established under—

(i) section 21 of the National Health Service and Community Care Act 1990 (schemes for meeting losses and liabilities etc. of certain health service bodies in England and Wales)(**d**),

(ii) section 85B of the National Health Service (Scotland) Act 1978 (schemes for meeting losses and liabilities etc. of certain health service bodies in Scotland)(**e**), or

(iii) Article 24 of the Health and Personal Social Services (Northern Ireland) Order 1991 (schemes for meeting losses and liabilities etc. of certain health service bodies in Northern Ireland)(**f**), or

(b) in accordance with guidance issued by—

(i) the Secretary of State,

(ii) the Scottish Ministers,

(iii) the National Assembly for Wales, or

(iv) the Department for Health, Social Services and Public Safety,

as to the arrangements to be adopted by health service bodies for meeting the costs arising from clinical negligence (known as NHS Indemnity);

"investigational medicinal product" means a pharmaceutical form of an active substance or placebo being tested, or to be tested, or used, or to be used, as a reference in a clinical trial, and includes a medicinal product which has a marketing authorization but is, for the purposes of the trial—

(a) used or assembled (formulated or packaged) in a way different from the form of the product authorised under the authorization,

(b) used for an indication not included in the summary of product characteristics under the authorization for that product, or

(c) used to gain further information about the form of that product as authorised under the authorization;

(**a**) 1990 c.19.
(**b**) 2003 c.43.
(**c**) S.I. 1991/194 (N.I.1).
(**d**) 1990 c.19; section 21 was amended by paragraph 79 of Schedule 1 to the Health Authorities Act 1995 (c.17) and paragraph 81 of Schedule 4 to the Health Act 1999 (c.8).
(**e**) 1978 c.29; section 85 was inserted by section 41 of the National Health Service and Community Care Act 1990 (c.19) and was amended by paragraph 56 of Schedule 4 to the Health Act 1999 (c.8).
(**f**) S.I. 1991/194 (N.I. 1).

"investigational medicinal product dossier" means, in relation to an investigational medicinal product, the dossier relating to that product which accompanies a request for authorisation to conduct a trial in which that product is or is to be used, in accordance with paragraph 11 of Schedule 3;

"investigator" means, in relation to a clinical trial, the authorised health professional responsible for the conduct of that trial at a trial site, and if the trial is conducted by a team of authorised health professionals at a trial site, the investigator is the leader responsible for that team;

"investigator's brochure" means a document containing a summary of the clinical and non-clinical data relating to an investigational medicinal product which are relevant to the study of the product in human subjects;

"labelling", in relation to an investigational medicinal product, means affixing to or otherwise displaying on it a notice describing or otherwise relating to the contents, and "label" has a corresponding meaning;

"legal representative", other than in regulation 3 and Parts 2 to 4 of Schedule 3, has the meaning given by Part 1 of Schedule 1;

"licensing authority" shall be construed in accordance with section 6 of the Act;

"manufacture", in relation to an investigational medicinal product, includes any process carried out in the course of making the product, but does not include dissolving or dispersing the product in, or diluting it or mixing it with, some other substance used as a vehicle for the purposes of administering it;

"manufacturing authorisation" has the meaning given by regulation 36(1);

"marketing authorization" means—

(a) a marketing authorization granted by the licensing authority under the Medicines for Human Use (Marketing Authorisations Etc.) Regulations 1994(**a**),

(b) a marketing authorization issued by the competent authority of an EEA State, other than the United Kingdom, in accordance with Directive 2001/83/EC,

(c) a marketing authorization granted by the European Commission under Council Regulation (EEC) 2309/93(**b**), or

(d) a product licence granted by the licensing authority for the purposes of section 7 of the Medicines Act 1968(**c**);

"medicinal product" means—

(a) a medicinal product within the meaning given by Article 1 of Directive 2001/83/EC, or

(b) any product which is not a medicinal product within the meaning given by Article 1 of Directive 2001/83/EC, but which is a medicinal product within the meaning given by section 130 of the Act;

"minor" means a person under the age of 16 years;

"non-interventional trial" means a study of one or more medicinal products which have a marketing authorization, where the following conditions are met—

(a) the products are prescribed in the usual manner in accordance with the terms of that authorization,

(b) the assignment of any patient involved in the study to a particular therapeutic strategy is not decided in advance by a protocol but falls within current practice,

(c) the decision to prescribe a particular medicinal product is clearly separated from the decision to include the patient in the study,

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(**a**) S.I. 1994/3144, as amended by S.I. 1998/3105, 2000/292, 2001/795, 2002/236, 2002/542 and 2003/????.
(**b**) OJ No. L214, 24.8.1993, p.1.
(**c**) Section 7 does not apply to "relevant medicinal products" within the meaning given by S.I. 1994/3144.

(d) no diagnostic or monitoring procedures are applied to the patients included in the study, other than those which are ordinarily applied in the course of the particular therapeutic strategy in question, and

(e) epidemiological methods are to be used for the analysis of the data arising from the study;

"nurse" means a registered nurse or registered midwife;

"pharmaceutical form of an active substance" includes any substance or article to which these Regulations have effect by virtue of an order under section 104 or 105 of the Act (which relate to the application of Act to certain articles and substances which are not medicinal products);

"Pharmaceutical Society" in relation to Great Britain means the Royal Pharmaceutical Society of Great Britain, and in relation to Northern Ireland means the Pharmaceutical Society of Northern Ireland;

"pharmacist" means—

(a) in relation to Great Britain, a person registered in the register of pharmaceutical chemists established in pursuance of the Pharmacy Act 1952 and maintained in pursuance of the Pharmacy Act 1954, and

(b) in relation to Northern Ireland, a person registered in the register of pharmaceutical chemists for Northern Ireland made out and maintained under Articles 6 and 9 of the Pharmacy (Northern Ireland) Order 1976;

"Phase I trial" means a clinical trial to study the pharmacology of an investigational medicinal product when administered to humans, where the sponsor and investigator have no knowledge of any evidence that the product has effects likely to be beneficial to the subjects of the trial;

"the principles and guidelines of good manufacturing practice" means the principles and guidelines of good manufacturing practice set out in Commission Directive 2003/94/EC;

"protocol" means a document that describes the objectives, design, methodology, statistical considerations and organisation of a clinical trial;

"qualified person" means—

(a) a person who as respects qualifications and experience satisfies the requirements of Article 49 or 50 of Directive 2001/83/EC, or

(b) a person who, without satisfying the requirements referred to in paragraph (a)—

(i) has been engaged in activities equivalent to those to be performed in accordance with regulation 43(2) in respect of investigational medicinal products for a period of at least 6 months prior to 1st May 2004,

(ii) has, in accordance with paragraph 6(1) of Schedule 6, been named as a qualified person in a valid application for a manufacturing authorisation made prior to 1st May 2006, and

(iii) is—

(aa) a member of the Institute of Biology, the Pharmaceutical Society, the Royal Society of Chemistry, or such other body as may appear to the licensing authority to be an appropriate body for the purpose of this paragraph, or

(bb) the holder of a diploma, certificate or other evidence of formal qualifications awarded on completion of a university or other higher education course of study in pharmacy, chemistry, medicine, biology or a related life science, which the licensing authority have stated in a notice in writing to that person to be qualifications sufficient for the purpose of performing the functions of a qualified person;

"relevant ethics committee", in relation to a clinical trial, means—

(a) in a case where an ethics committee has given a favourable opinion in relation to that trial and paragraph 13 of Schedule 2 applies, the ethics committee which is the relevant ethics committee for that trial by virtue of sub-paragraph (5) of that paragraph;

(b) in a case where an ethics committee has given an unfavourable opinion in relation to that trial but a favourable opinion has been given by an appeal panel in accordance with paragraph 4(4) of Schedule 4, that committee, or

(c) in any other case, the ethics committee which has given a favourable opinion in relation to that trial in accordance with regulation 15;

"serious adverse event", "serious adverse reaction" or "unexpected serious adverse reaction" means any adverse event, adverse reaction or unexpected adverse reaction, respectively, that—

(a) results in death,

(b) is life-threatening,

(c) requires hospitalisation or prolongation of existing hospitalisation,

(d) results in persistent or significant disability or incapacity, or

(e) consists of a congenital anomaly or birth defect;

"sponsor" shall be construed in accordance with regulation 3;

"Strategic Health Authority" means a Strategic Health Authority established under the National Health Service Act 1977(**a**);

"subject" means, in relation to a clinical trial, an individual, whether a patient or not, who participates in a clinical trial—

(a) as a recipient of an investigational medicinal product or of some other treatment or product, or

(b) without receiving any treatment or product, as a control;

"third country" means a country or territory outside the European Economic Area;

"trial site" means a hospital, health centre, surgery or other establishment or facility at or from which a clinical trial, or any part of such a trial, is conducted;

"unexpected adverse reaction" means an adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in question set out—

(a) in the case of a product with a marketing authorization, in the summary of product characteristics for that product,

(b) in the case of any other investigational medicinal product, in the investigator's brochure relating to the trial in question.

(2) Any reference in these Regulations to the holder of a manufacturing authorisation shall be construed as a reference to the holder of such an authorisation which is for the time being in force.

(3) Any reference in these Regulations to an application, request or other document that is signed includes a reference to an application, request of other document that is signed with an electronic signature.

Sponsor of a clinical trial

3.—(1) In these Regulations, subject to the following paragraphs, "sponsor" means, in relation to a clinical trial, the person who takes responsibility for the initiation, management and financing (or arranging the financing) of that trial.

(2) If two or more persons take responsibility for the matters specified in paragraph (1) in relation to a clinical trial, those persons may—

(a) take joint responsibility for carrying out the functions of the sponsor of that trial under these Regulations; or

(b) allocate responsibility for carrying out the functions of the sponsor of that trial in accordance with paragraphs (4) to (10).

(**a**) *See* section 8 of the National Health Service Act 1977 (c.49) as substituted by section 1(2) of the National Health Service Reform and Health Care Professions Act 2002 (c. 17).

(3) If two or more persons take joint responsibility in accordance with paragraph (2)(a)—

(a) any reference to the sponsor in these Regulations shall, in relation to that trial, be construed as a reference to those persons; and

(b) paragraphs (4) to (10) shall not apply.

(4) One of the persons referred to in paragraph (2) shall be responsible for carrying out the functions of a sponsor under Part 3 (authorisation for clinical trials and ethics committee opinion) and shall make the request for authorisation to conduct the trial in accordance with regulation 17.

(5) The request for authorisation referred to in regulation 17 shall specify—

(a) who, in accordance with paragraph (4), is responsible for carrying out the functions of the sponsor under Part 3;

(b) who is to be responsible for carrying out the functions of the sponsor under Part 4 (good clinical practice and the conduct of clinical trials); and

(c) who is to be responsible for carrying out the functions of the sponsor under Part 5 (pharmacovigilance).

(6) After the clinical trial has been authorised by the licensing authority in accordance with regulation 18, 19 or 20, a different person may be specified as responsible for carrying out the functions of the sponsor under Part 3, 4 or 5 by making a substantial amendment to the terms of a clinical trial authorisation in accordance with regulations 24 to 26.

(7) Where a person is responsible for carrying out the functions of the sponsor under Part 3 by virtue of paragraph (5), or is specified in accordance with paragraph (6) as responsible for those functions, any reference to the sponsor in—

(a) that Part, except regulation 15,

(b) Parts 2 to 4 of Schedule 3,

(c) Schedule 5, in so far as it relates to decisions of the licensing authority under Part 3, and

(d) Schedule 12,

shall, in relation to the trial, be construed as a reference to that person.

(8) Where a person is specified in accordance with paragraph (5) or (6) as responsible for carrying out the functions of the sponsor under Part 4, any reference to the sponsor in—

(a) that Part, except regulation 28(1), or

(b) Schedule 5, in so far as it relates to notices under regulation 31(1),

shall, in relation to the trial, be construed as a reference to that person.

(9) Where a person is specified in accordance with paragraph (5) or (6) as responsible for carrying out the functions of the sponsor under Part 5, any reference to the sponsor in that Part shall, in relation to the trial, be construed as a reference to that person.

(10) Any reference to the sponsor in—

(a) regulations 15 and 28(1),

(b) Parts 2 and 6 to 9, and

(c) Schedules 1 and 7, and Part 1 of Schedule 3,

shall, in relation to the trial, include a reference to a person specified in accordance with paragraph (5) or (6).

(11) A person who is a sponsor of a clinical trial in accordance with this regulation must—

(a) be established in the European Community, or

(b) have a legal representative who is so established.

Responsibility for functions under the Directive

4.—(1) For the purposes of the Directive, the competent authority of the United Kingdom shall be the licensing authority.

(2) Subject to paragraph (3), the licensing authority shall perform, as respects the United Kingdom, the functions of the Member State under the Directive.

(3) Paragraph (2) shall not apply in so far as any functions fall to be performed by the exercise of any powers or duties which are conferred by any provision of these Regulations, or by any provision of the Act as applied by these Regulations, on a person or body other than the licensing authority.

PART 2

ETHICS COMMITTEES

United Kingdom Ethics Committees Authority

5.—(1) The body responsible for establishing, recognising and monitoring ethics committees in the United Kingdom in accordance with these Regulations is the United Kingdom Ethics Committees Authority, which is a body consisting of—

(a) the Secretary of State for Health;

(b) the National Assembly for Wales;

(c) the Scottish Ministers; and

(d) the Department for Health, Social Services and Public Safety for Northern Ireland.

(2) The functions of the Authority—

(a) may, by agreement between them, be performed by any one of the Secretary of State for Health, the National Assembly for Wales, the Scottish Ministers and the Department for Health, Social Services and Public Safety for Northern Ireland acting alone, or any two or more of them acting jointly; and

(b) may be performed by any one of the Secretary of State for Health, the National Assembly for Wales, the Scottish Ministers and the Department for Health, Social Services and Public Safety for Northern Ireland acting alone solely in relation to a part of the United Kingdom with respect to which the Secretary of State, the Assembly, the Ministers or the Department, as the case may be, have responsibilities.

(3) In accordance with the preceding provisions of this regulation, in these Regulations "the United Kingdom Ethics Committees Authority" ("the Authority") means any one or more of the Secretary of State for Health, the National Assembly for Wales, the Scottish Ministers and the Department for Health, Social Services and Public Safety for Northern Ireland, and, in the case of anything falling to be done by the Authority, means any one or more of them acting as mentioned in paragraph (2).

(4) The Authority may appoint such persons as they think necessary for the proper discharge by them of their functions, and those persons shall be appointed on such terms and conditions (including conditions as to remuneration, benefits, allowances and reimbursement for expenses) as the Authority think fit.

(5) Arrangements may be made between the Authority and any relevant authority for—

(a) any functions of the Authority to be exercised by, or by members of staff of, the relevant authority; or

(b) the provision of staff, premises or administrative services by the relevant authority to the Authority.

(6) Any arrangements under paragraph (5) for the exercise of any functions of the Authority shall not affect the responsibility of the Authority.

(7) In this regulation, "relevant authority" means any government department, local or public authority or holder of public office.

Establishment of ethics committees

6.—(1) The Authority may establish ethics committees to act—

(a) for the entire United Kingdom or for such areas of the United Kingdom; and

(b) in relation to such descriptions or classes of clinical trials,

as the Authority consider appropriate.

(2) The Authority may—

(a) vary the area for which any committee they have established acts or, as the case may be, the descriptions or classes of clinical trials in relation to which such a committee acts; and

(b) abolish any such committee.

Recognition of ethics committees

7.—(1) Subject to paragraph (3), the Authority may, by a notice in writing, recognise a committee as an ethics committee for the purposes of these Regulations if—

(a) an application in relation to that committee has been made in accordance with paragraph (2); and

(b) they are satisfied that the proposed arrangements for the membership and operation of that ethics committee would—

(i) enable that committee to perform the functions of an ethics committee adequately; and

(ii) comply with the provisions of Schedule 2.

(2) An application for recognition of an ethics committee shall be—

(a) made in writing to the Authority; and

(b) accompanied by such information, documents and particulars as are necessary to enable the Authority to determine the application.

(3) If any committee—

(a) was established or recognised by—

(i) the Secretary of State,

(ii) the Scottish Ministers,

(iii) the National Assembly for Wales,

(iv) the Department of Health, Social Services and Public Safety, or

(v) a Strategic Health Authority, Health Board or Health and Social Services Board,

for the purpose of advising on the ethics of research investigations on human beings, and

(b) was in existence on 30th April 2004,

the Authority may recognise that committee in accordance with paragraph (1) without an application for recognition being submitted.

(4) When recognising a committee the Authority shall specify—

(a) whether the committee may act for the entire United Kingdom or only for a particular area of the United Kingdom;

(b) the description or class of clinical trial in relation to which it may act as an ethics committee; and

(c) any other conditions or limitations that apply to that committee.

(5) The Authority may—

(a) vary the area for which a committee recognised under this regulation acts,

(b) vary the description or class of clinical trial in relation to which it may act as an ethics committee, or

(c) vary or revoke any conditions or limitations imposed under paragraph (5),

where it considers it necessary or appropriate to do so.

Revocation of recognition

8. The Authority may revoke a recognition of an ethics committee if they are satisfied that—

(a) the provisions of Schedule 2 are not complied with in relation to that committee;

(b) the committee is failing to perform its functions under these Regulations adequately or at all; or

(c) it is otherwise necessary or expedient to do so.

Constitution and operation of ethics committees

9. The provisions of Schedule 2 have effect in relation to ethics committees.

Other functions of the Authority

10.—(1) The Authority shall monitor the extent to which ethics committees adequately perform their functions under these Regulations.

(2) The Authority may provide advice and assistance to ethics committees with respect to the performance of their functions.

PART 3

AUTHORISATION FOR CLINICAL TRIALS AND ETHICS COMMITTEE OPINION

Interpretation of Part 3

11. In this Part—

"amendment to the clinical trial authorisation" means an amendment to—

(a) the terms of the request for authorisation to conduct that trial or the application for an ethics committee opinion in relation to that trial,

(b) the protocol for that trial, or

(c) the other particulars or documents accompanying that request for authorisation or application for ethics committee approval;

"substantial amendment to the clinical trial authorisation" means an amendment to the clinical trial authorisation which is likely to affect to a significant degree—

(a) the safety or physical or mental integrity of the subjects of the trial,

(b) the scientific value of the trial,

(c) the conduct or management of the trial, or

(d) the quality or safety of any investigational medicinal product used in the trial;

"valid application" means an application for an ethics committee opinion which complies with the provisions of regulation 14; and

"valid request for authorisation" means a request to the licensing authority for authorisation to conduct a clinical trial which complies with the provisions of regulation 17, and "valid amended request" shall be construed accordingly.

Requirement for authorisation and ethics committee opinion

12.—(1) No person shall—

(a) start a clinical trial or cause a clinical trial to be started; or

(b) conduct a clinical trial,

unless the conditions specified in paragraph (3) are satisfied.

(2) No person shall—

(a) recruit an individual to be a subject in a trial;

(b) issue an advertisement for the purpose of recruiting individuals to be subjects in a trial,

unless the condition specified in paragraph (3)(a) has been satisfied.

(3) The conditions referred to in paragraphs (1) and (2) are—

(a) an ethics committee or an appeal panel appointed under Schedule 4 has given a favourable opinion in relation to the clinical trial; and

(b) the clinical trial has been authorised by the licensing authority.

(4) For the purposes of these Regulations, a clinical trial has been authorised by the licensing authority if—

(a) in the case of a trial to which regulation 18 relates—

(i) the trial is to be treated as authorised by virtue of regulation 18, or

(ii) the authority has accepted the request for authorisation in accordance with the procedure specified in Schedule 5; or

(b) in the case of a clinical trial to which regulation 19 or 20 applies—

(i) the authority has given a notice of authorisation in accordance with those regulations, or

(ii) the authority has accepted the request for authorisation in accordance with the procedure specified in Schedule 5.

Supply of investigational medicinal products for the purpose of clinical trials

13.—(1) Subject to paragraphs (3) and (4), no person shall, in the course of a business carried on by him, sell or supply any investigational medicinal product to—

(a) an investigator,

(b) a health care professional who is a member of an investigator's team,

(c) a person who provides or is to provide health care under the direction or control of a person referred to in sub-paragraphs (a) and (b), or

(d) a subject,

for the purpose of administering that product in a clinical trial, unless the conditions specified in paragraph (2) are satisfied.

(2) The conditions referred to in paragraph (1) are—

(a) the licensing authority has authorised the clinical trial for the purposes of which the product is sold or supplied;

(b) in the case of an investigational medicinal product manufactured or assembled in an EEA State, other than in accordance with the terms of a marketing authorization relating to that product, or imported into an EEA State—

(i) the product has been manufactured, assembled or imported in accordance with the terms of—

(aa) a manufacturing authorisation, or

(bb) an authorisation referred to in Article 13 of the Directive granted by a competent authority of an EEA State other than the United Kingdom, and

(ii) the production batch of investigational medicinal products of which the product is a part has been checked and certified by a qualified person pursuant to Article 13(3) and (4) of the Directive.

(3) If an investigational medicinal product has been manufactured or imported prior to 1st May 2004—

(a) the condition specified in paragraph (2)(b)(i) shall apply only in relation to any assembly of that product which takes place on or after that date; and

(b) the conditions specified in paragraph (2)(b)(ii) shall not apply.

(4) The restriction in paragraph (1) shall not apply to the sale or supply of a medicinal product in accordance with the terms of a marketing authorisation relating to that product, other than a marketing authorisation issued by the competent authority of an EEA State other than the United Kingdom.

Application for ethics committee opinion

14.—(1) An application for an ethics committee opinion in relation to a clinical trial shall be made by the chief investigator for that trial.

(2) A chief investigator for a trial shall make an application for an ethics committee opinion in relation to that trial to one ethics committee only, regardless of the number of trial sites at which the trial is to be conducted.

(3) Subject to paragraphs (4) and (5), the application for an ethics committee opinion in relation to a clinical trial shall be made to an ethics committee established or recognised—

(a) for—

(i) the entire United Kingdom, or

(ii) in relation to an area of the United Kingdom in which the chief investigator is professionally based; and

(b) in relation to a description or class of clinical trial into which the proposed trial falls.

(4) If a clinical trial—

(a) is conducted at one or more trial sites in Scotland;

(b) involves adults unable by virtue of physical or mental incapacity to give informed consent; and

(c) the chief investigator is professionally based at a hospital, health centre, surgery or other establishment or facility in Scotland,

the application for an ethics committee opinion in relation to that trial shall be made to the Ethics Committee constituted by regulations made by the Scottish Ministers under section 51(6) of the Adults with Incapacity (Scotland) Act 2000(**a**).

(5) An application for an ethics committee opinion in relation to a clinical trial involving medicinal products for gene therapy, other than a trial falling within paragraph (4), shall be made to the Gene Therapy Advisory Committee.

(6) An application shall be—

(a) in writing;

(b) signed by the chief investigator making the application; and

(c) accompanied by the particulars and documents specified in Part 1 of Schedule 3.

(7) The application and any accompanying material shall be supplied in the English language.

(8) For the purposes of this regulation, a chief investigator is professionally based at the hospital, health centre, surgery or other establishment or facility at or from which he primarily conducts his professional practice.

(**a**) 2000 asp. 4; *see* S.S.I. 2002/190.

Ethics committee opinion

15.—(1) Subject to paragraphs (3) and (4), an ethics committee shall within the specified period following receipt of a valid application, give an opinion in relation to the clinical trial to which the application relates.

(2) Where following receipt of a valid application it appears to the committee that further information is required in order to give an opinion on a trial, the committee may, within the specified period and before giving its opinion, send a notice in writing to the applicant requesting that he furnishes the committee with that information.

(3) Where the committee sends a request in accordance with paragraph (2), the specified period shall be suspended pending receipt of the information requested.

(4) If the clinical trial involves a medicinal product for xenogenic cell therapy, the time limits referred to in paragraphs (1) to (3) shall not apply and the ethics committee may give an opinion in relation to that trial or send a notice under paragraph (2) at any time after receipt of the valid application.

(5) In preparing its opinion, the committee shall consider, in particular, the following matters—

(a) the relevance of the clinical trial and its design;

(b) whether the evaluation of the anticipated benefits and risks as required under paragraph 2 of Part 2 of Schedule 1 is satisfactory and whether the conclusions are justified;

(c) the protocol;

(d) the suitability of the investigator and supporting staff;

(e) the investigator's brochure;

(f) the quality of the facilities for the trial;

(g) the adequacy and completeness of the written information to be given, and the procedure to be followed, for the purpose of obtaining informed consent to the subjects' participation in the trial;

(h) if the subjects are to include persons incapable of giving informed consent, whether the research is justified having regard to the conditions and principles specified in Part 5 of Schedule 1;

(i) provision for indemnity or compensation in the event of injury or death attributable to the clinical trial;

(j) any insurance or indemnity to cover the liability of the investigator or sponsor;

(k) the amounts, and, where appropriate, the arrangements, for rewarding or compensating investigators and subjects;

(l) the terms of any agreement between the sponsor and the owner or occupier of the trial site which are relevant to the arrangements referred to in sub-paragraph (k); and

(m) the arrangements for the recruitment of subjects.

(6) If—

(a) any subject of the clinical trial is to be a minor; and

(b) the committee does not have a member with professional expertise in paediatric care,

it shall, before giving its opinion, obtain advice on the clinical, ethical and psychosocial problems in the field of paediatric care which may arise in relation to that trial.

(7) If—

(a) any subject to the clinical trial is to be an adult incapable by reason of physical and mental incapacity to give informed consent to participation in the trial; and

(b) the committee does not have a member with professional expertise in the treatment of—

(i) the disease to which the trial relates, and

(ii) the patient population suffering that disease,

it shall, before giving its opinion, obtain advice on the clinical, ethical and pyschosocial problems in the field of that disease and patient population which may arise in relation to that trial.

(8) The ethics committee shall consider, and give an opinion on, any other issue relating to the clinical trial, if—

(a) the committee has been asked by the applicant to consider the issue;

(b) it is, in the committee's opinion, relevant to the other matters considered by the committee in accordance with this regulation.

(9) Where an ethics committee gives an opinion in accordance with this regulation, it shall publish a summary of that opinion.

(10) In this regulation—

"the specified period" means—

(a) in the case of a clinical trial involving a medicinal product for gene therapy or somatic cell therapy or a medicinal product containing a genetically modified organism—

(i) where a specialist group or committee is consulted, 180 days, or

(ii) where there is no such consultation, 90 days; or

(b) in any other case, 60 days;

"specialist group or committee" means a group or committee whose functions include the provision of advice on ethical or scientific issues in relation to—

(a) in the case of medicinal products for gene therapy or somatic cell therapy, the use of such therapies in the treatment of humans; or

(b) in the case of medicinal products containing genetically modified organisms, the administration of such products to humans.

Review and appeal relating to ethics committee opinion

16.—(1) This regulation applies where a chief investigator for a trial has been notified by the ethics committee to which he made an application in accordance with regulation 13 that the committee's opinion in relation to that trial is not favourable.

(2) This regulation does not apply in relation to an opinion given by—

(a) the Ethics Committee constituted by regulations made by the Scottish Ministers under section 51(6) of the Adults with Incapacity (Scotland) Act 2000; or

(b) an ethics committee pursuant to paragraph 2 of Schedule 4.

(3) Where the opinion was given by an ethics committee other than the Gene Therapy Advisory Committee, the chief investigator may within 90 days of being notified that the committee's opinion is not favourable, give a notice to the United Kingdom Ethics Committees Authority—

(a) stating his wish to appeal against the opinion; and

(b) setting out his representations with respect to that opinion.

(4) Where the opinion was given by the Gene Therapy Advisory Committee, the chief investigator may, within 14 days of being notified of that opinion—

(a) give a notice in writing to the Committee requiring the Committee to review its opinion; or

(b) give a notice in writing to the United Kingdom Ethics Committee Authority—

(i) stating his wish to appeal against the opinion; and

(ii) setting out his representations with respect to that opinion.

(5) Where the Gene Therapy Advisory Committee is required by a notice under paragraph (4) to review its opinion, it must do so within 60 days of receipt of the notice.

(6) On a review pursuant to paragraph (5), the Gene Therapy Advisory Committee may vary or confirm their opinion and shall give notice in writing to the chief investigator of the variation or confirmation.

(7) If the Gene Therapy Advisory Committee confirm their opinion pursuant to paragraph (6), a chief investigator may within the 14 days of being notified of the confirmation give notice in writing to the United Kingdom Ethics Committees Authority—

(a) stating his wish to appeal against the Committee's opinion; and

(b) setting out his representations with respect to that opinion

(8) Schedule 4 shall have effect to regulate the procedure where the Authority receives a notice in accordance with paragraph (3), (4) or (7).

Request for authorisation to conduct a clinical trial

17.—(1) A request for authorisation to conduct a clinical trial shall be made to the licensing authority by the sponsor of the trial.

(2) A request shall—

(a) be in writing and signed by or on behalf of the sponsor; and

(b) be accompanied by—

(i) the particulars and documents specified in Part 2 of Schedule 3, and

(ii) any fee which may be payable in connection with that application under the Medicines (Products for Human Use—Fees) Regulations 1995(**a**).

(3) The request and any accompanying material shall be supplied in the English language.

Authorisation procedure for clinical trials involving general medicinal products

18.—(1) This regulation applies to clinical trials involving medicinal products other than those to which regulations 19 and 20 apply.

(2) The licensing authority may, within the period of 30 days from the date of receipt of a valid request for authorisation of a clinical trial to which this regulation applies, give written notice to the sponsor—

(a) setting out the licensing authority's grounds for not accepting the request;

(b) stating that the licensing authority accepts the request for authorisation; or

(c) stating that the licensing authority accepts the request for authorisation, subject to the conditions specified in the notice.

(3) Subject to paragraph (4), if—

(a) a notice is given in accordance with paragraph (2)(b); or

(b) no notice is given in accordance with paragraph (2),

the clinical trial is to be treated as authorised.

(4) If a notice is given in accordance with paragraph (2)(c), the clinical trial is to be treated as authorised only if the conditions specified in the notice are satisfied.

(5) If the sponsor is given a notice in accordance with paragraph (2)(a) or (c), he may, within the period of 14 days, or such extended period as the licensing authority may in any particular case allow, from the date on which the notice was received, send an amended request to the licensing authority for further consideration.

(6) The licensing authority shall consider a valid amended request and may, within the period of 60 days from the date on which the original request was received give a written notice to the sponsor—

(a) setting out the licensing authority's grounds for not accepting the amended request;

(b) stating that the licensing authority accepts the amended request; or

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(**a**) S.I. 1995/1116.

(c) stating that the licensing authority accepts the amended request, subject to the conditions specified in the notice.

(7) Subject to paragraph (8), if a valid amended request has been received and—

(a) a notice is given in accordance with paragraph (6)(b); or

(b) no notice is given in accordance with paragraph (6),

the clinical trial is to be treated as authorised.

(8) If a valid amended request has been received and a notice is given in accordance with paragraph (6)(c), the clinical trial is to be treated as authorised only if the conditions specified in the notice are satisfied.

(9) If—

(a) the licensing authority gives written notice to the sponsor of grounds for non-acceptance in accordance with paragraph (2)(a) and the sponsor does not submit an amended request in accordance with paragraph (5), or

(b) the sponsor has submitted an amended request in accordance with paragraph (5), but the licensing authority gives written notice to the sponsor of grounds for non-acceptance in accordance with paragraph (6)(a),

the request is to be treated as rejected and the authority shall not consider any further amendments to the request.

Authorisation procedure for clinical trials involving medicinal products for gene therapy etc.

19.—(1) This regulation applies to clinical trials involving—

(a) medicinal products for gene therapy and somatic cell therapy, including xenogenic cell therapy; or

(b) medicinal products containing genetically modified organisms.

(2) Subject to the following provisions of this regulation, the licensing authority may, within the period of 30 days from the date of receipt of a valid request for authorisation of a clinical trial to which this regulation applies—

(a) issue a written authorisation to the sponsor; or

(b) give a notice in writing to the sponsor setting out the grounds for not accepting the request.

(3) The licensing authority shall not authorise a clinical trial involving products for gene therapy if the use of those products in that trial would result in modifications to any subject's germ line genetic identity.

(4) If the licensing authority considers that it is appropriate to do so, they may consult the relevant committee before deciding whether to authorise a clinical trial.

(5) Where the authority consults the relevant committee in accordance with paragraph (4), the period specified in paragraph (2) shall be extended by a further 90 days.

(6) Where a sponsor is given a notice in accordance with paragraph (2)(b), he may, within the period of 30 days, or such extended period as the licensing authority may in any particular case allow, from the date on which the notice was received, send an amended request to the licensing authority for further consideration.

(7) The licensing authority shall consider a valid amended request and, not later than 90 days, or, in a case falling within paragraph (5), 180 days, from the date on which the original request was received—

(a) issue a written authorisation to the sponsor; or

(b) give a notice in writing to the sponsor setting out the grounds for not accepting the request.

(8) A written authorisation issued under this regulation may contain such conditions as the licensing authority consider appropriate.

(9) If the clinical trial involves a medicinal product for xenogenic cell therapy, the time limits set out in paragraphs (2), (5) and (7) shall not apply and the authority may issue an authorisation or notice under those paragraphs at any time after receipt of the request.

(10) In this regulation, "the relevant committee" means—

(a) the Committee on Safety of Medicines(**a**); or

(b) such other body or committee as the licensing authority may consider appropriate in relation to the application under consideration.

Authorisation procedure for clinical trials involving medicinal products with special characteristics

20.—(1) This regulation applies to clinical trials—

(a) involving medicinal products—

(i) which do not have a marketing authorization and are referred to in Part A of the Annex to Regulation (EEC) No. 2309/93(**b**), or

(ii) which have an active ingredient—

(aa) that is a biological product of human or animal origin,

(bb) containing biological components of human or animal origin, or

(cc) the manufacturing of which requires such components,

other than products falling within regulation 19; or

(b) where the licensing authority, within 7 days from the date of receipt of a valid request for authorisation of the trial, issues a notice to the sponsor specifying that by virtue of the special characteristics of the medicinal product to which the trial relates, written authorisation for that trial is required.

(2) The licensing authority may, within the period of 30 days from the date of receipt of a valid request for authorisation of a clinical trial to which this regulation applies—

(a) issue a written authorisation to the sponsor; or

(b) give a notice in writing to the sponsor setting out the grounds for not authorising the trial.

(3) Where a sponsor is given a notice in accordance with paragraph (2)(b), he may, within the period of 14 days, or such extended period as the licensing authority may in any particular case allow, from the date on which the notice was received, send an amended request to the licensing authority for further consideration.

(4) The licensing authority shall consider a valid amended request and, not later than 60 days from the date on which the original request was received—

(a) issue a written authorisation to the sponsor; or

(b) give a notice in writing to the sponsor setting out the grounds for not accepting the request.

(5) A written authorisation issued under this regulation may contain such conditions as the licensing authority consider appropriate.

Clinical trials conducted in third countries

21.—(1) If the licensing authority receives a valid request for authorisation relating to a clinical trial which is or is to be conducted in a third country as well as the United Kingdom, the licensing authority may, if they think fit, require the production by the sponsor of any one or more of the following—

(**a**) The Committee on Safety of Medicines was established under section 4 of the Act, by S.I. 1970/1257, for the purposes set out in that instrument.

(**b**) OJ No. L214, 24.8.93, p.1.

(a) an undertaking, given by the sponsor, to permit their premises in that country to be inspected by or on behalf of the licensing authority for the purpose of establishing whether the conditions and principles of good clinical practice are satisfied or adhered to in relation to that trial; or

(b) an undertaking, given by the owner or occupier of any premises in that country at which the clinical trial is or is to be conducted, to permit those premises to be inspected by or on behalf of the licensing authority for the purpose of establishing whether the conditions and principles of good clinical practice are satisfied or adhered to in relation to that trial.

(2) If a sponsor fails to produce an undertaking required by the licensing authority in accordance with paragraph (1), that failure constitutes a ground for not accepting the request for authorisation, for the purposes of regulations 18 to 20.

Amendments to clinical trial authorisation

22. Subject to regulation 30, an amendment to a clinical trial authorisation may be made—

(a) by the licensing authority, in accordance with regulation 23; or

(b) by the sponsor, in accordance with regulation 24 or 25.

Amendments by the licensing authority

23.—(1) Subject to paragraphs (1) and (2), the licensing authority may make amendments to a clinical trial authorisation if it appears to the authority to be necessary to ensure—

(a) the safety or scientific validity of the clinical trial; or

(b) that the conditions and principles of good clinical practice are satisfied or adhered to in relation to the clinical trial.

(2) Where the licensing authority propose to make an amendment in accordance with paragraph (1), the authority shall, at least 14 days before the date on which it is proposed the amendment should take effect, serve a notice on the sponsor stating their proposal and the reasons for it.

(3) If, within 14 days of the date a notice is served in accordance with paragraph (2), the sponsor makes representations in writing to the licensing authority, the authority—

(a) shall take those representations into account before deciding whether to make the amendment; and

(b) may delay the date the proposed amendment is to take effect, in order to allow time for them to consider those representations.

Amendments by the sponsor

24.—(1) A sponsor may make an amendment to a clinical trial authorisation, other than a substantial amendment, at any time.

(2) A sponsor shall—

(a) keep records of the amendments made in accordance with paragraph (1); and

(b) send those records, or copies of such records, to the licensing authority, where the authority send him a notice in writing requiring him to provide those records, or copies of such records.

(3) If the sponsor proposes to make a substantial amendment to a clinical trial authorisation which consists of, or includes, an amendment to—

(a) the terms of the request for authorisation of the clinical trial; or

(b) the particulars or documents that accompanied that request,

he shall send a valid notice of amendment to the licensing authority, whether or not he is also required to send a notice in accordance with paragraph (4).

(4) If the sponsor proposes to make a substantial amendment to a clinical trial authorisation which consists of, or includes, an amendment to—

(a) the terms of the application for an ethics committee opinion in relation to the clinical trial; or

(b) the particulars or documents that accompanied that application,

he shall send a valid notice of amendment to the relevant ethics committee, whether or not he is also required to send a notice in accordance with paragraph (3).

(5) The licensing authority may, within the period of 35 days from the date of receipt of a valid notice of amendment, give written notice to the sponsor—

(a) setting out the licensing authority's grounds for not accepting the proposed amendment; or

(b) stating that the licensing authority accepts the application for amendment, subject to any conditions which may be specified in the notice.

(6) A relevant ethics committee shall, within the period of 35 days from the date of receipt of a valid notice of amendment, give an opinion to the sponsor.

(7) Subject to paragraph (8), if the sponsor has sent a notice in accordance with paragraph (3), he may make the amendment only if—

(a) the licensing authority have given him a notice in accordance with paragraph (5)(b); or

(b) no notice has been given by the licensing authority in accordance with paragraph (5).

(8) If the sponsor has been given a notice in accordance with paragraph (5)(b), he may make the amendment subject to the conditions, if any, specified in the notice.

(9) If the sponsor has sent a notice in accordance with paragraph (4), he may make the amendment only if the relevant ethics committee has given a favourable opinion.

(10) In this regulation—

"valid notice of amendment" means a notice that is—

(a) in writing; and

(b) accompanied by—

(i) the particulars specified in Part 3 of Schedule 3, and

(ii) any fee which may be payable in connection with that notice under the Medicines (Products for Human Use—Fees) Regulations 1995(**a**).

Modifying or adapting rejected proposals for amendment

25.—(1) Subject to the following provisions of this regulation, if—

(a) the ethics committee opinion on a proposed amendment to the protocol is not favourable; or

(b) the sponsor has been notified by the licensing authority of any grounds for non-acceptance of a proposed amendment to the protocol,

and it is possible to modify or adapt the proposed amendment in order to meet the concerns of ethics committee or the licensing authority as set out in the opinion or, as the case may be, the grounds for non-acceptance, the sponsor may amend the protocol accordingly.

(2) If a sponsor proposes to amend the protocol in accordance with paragraph (1), the sponsor shall, at least 14 days before the amendment is to be made, give a notice in writing to the licensing authority and the relevant ethics committee.

(3) The licensing authority may, within the period of 14 days from the date of receipt of a notice under paragraph (1), give written notice to the sponsor setting out the licensing authority's further grounds for not accepting the modified or adapted amendment.

(**a**) S.I. 1995/1116.

(4) The relevant ethics committee may, within the period of 14 days from the date of receipt of a notice under paragraph (1), give a written notice to the sponsor stating that its opinion of the modified or adapted amendment is unfavourable.

(5) If—

(a) the sponsor receives a written notice under paragraphs (3) or (4), he may not make the amendment; and

(b) if he receives no such notice, he may make the modified or adapted amendment.

Reference to the appropriate committee or the Medicines Commission

26.—(1) If—

(a) a sponsor has been notified by the licensing authority that—

(i) there are grounds for not accepting a request for authorisation, or

(ii) in accordance with regulation 18(2) or (6), 19(8) or 20(5), the trial is authorised subject to specified conditions;

(b) the licensing authority has amended a clinical trial authorisation under regulation 23; or

(c) the sponsor who has been notified by the licensing authority in accordance with regulation 24(4) or 25(3) that—

(i) the authority does not accept a proposed, modified or adapted amendment to the clinical trial authorisation, or

(ii) the authority accepts such an amendment subject to conditions,

the sponsor may, within 28 days, or such extended period as the licensing authority may in any particular case allow, of the notice being given, give notice in writing to the licensing authority of his wish to make written or oral representations to the appropriate committee or, if for the time being there is no such committee, the Medicines Commission(**a**).

(2) Schedule 5 shall have effect to regulate the procedure for reference to the appropriate committee, or as the case may be, the Medicines Commission following receipt of a notice in accordance with paragraph (1).

Conclusion of clinical trial

27.—(1) Subject to paragraph (2), within 90 days of the conclusion of a clinical trial the sponsor shall notify the licensing authority and the relevant ethics committee in writing that the trial has ended.

(2) If a trial is terminated—

(a) before the date for the conclusion of the trial specified in the protocol for that trial, or

(b) before the event specified in the protocol as the event which indicates the end of the trial has occurred,

the sponsor shall notify the licensing authority and the relevant ethics committee in writing of the termination of the trial within 15 days of the date of termination.

(3) A notification made in accordance with paragraphs (1) or (2) shall contain the particulars specified in Part 4 of Schedule 3.

(**a**) *See* section 2 of the Act.

PART 4

GOOD CLINICAL PRACTICE AND THE CONDUCT OF CLINICAL TRIALS

Good clinical practice and protection of clinical trial subjects

28.—(1) No person shall—

(a) conduct a clinical trial; or

(b) perform the functions of the sponsor of a clinical trial (whether that person is the sponsor or is acting under arrangements made with that sponsor),

otherwise than in accordance with the conditions and principles of good clinical practice.

(2) Subject to paragraph (5), the sponsor of a clinical trial shall put and keep in place arrangements for the purpose of ensuring that with regard to that trial the conditions and principles of good clinical practice are satisfied or adhered to.

(3) Subject to paragraphs (4) and (5), the sponsor of a clinical trial shall ensure that—

(a) the investigational medicinal products used in the trial, and

(b) any devices used for the administration of such products,

are made available to the subjects of the trial free of charge.

(4) The restriction in paragraph (3) shall not apply in relation to any charge payable by a subject under regulations made under—

(a) the National Health Service Act 1977(**a**);

(b) the National Health Service (Scotland) Act 1978(**b**); or

(c) the Health and Personal Social Services (Northern Ireland) Order 1972(**c**),

in respect of any medicinal products or devices provided in pursuance of those Acts or that Order.

(5) If—

(a) a clinical trial is conducted at more than one trial site; and

(b) the request for authorisation to conduct that trial specifies that in relation to one or more trial sites the duties of the sponsor under paragraphs (2) and (3) are to be performed by a person other than the sponsor,

those duties shall, in relation to that site or those sites, be performed by the person so specified.

Conduct of trial in accordance with clinical trial authorisation etc.

29. Subject to regulation 30, no person shall conduct a clinical trial otherwise than in accordance with—

(a) the protocol relating to that trial, as may be amended from time to time in accordance with regulations 22 to 25;

(b) the terms of—

(i) the request for authorisation to conduct that trial,

(ii) the application for an ethics committee opinion in relation to that trial, and

(iii) any particulars or documents, other than the protocol, accompanying that request or that application,

as may be amended from time to time in accordance with regulations 22 to 25; and

(c) any conditions imposed by the licensing authority under regulation 18(2) or (6), 19(8), 20(5), 24(4) or Schedule 5.

(**a**) 1977 c.49.
(**b**) 1978 c.29.
(**c**) S.I. 1972/1265 (N.I. 14).

Urgent safety measures

30.—(1) The sponsor and investigator may take appropriate urgent safety measures in order to protect the subjects of a clinical trial against any immediate hazard to their health or safety.

(2) If measures are taken pursuant to paragraph (1), the sponsor shall immediately, and in any event no later than 3 days from the date the measures are taken, give written notice to the licensing authority and the relevant ethics committee of the measures taken and the circumstances giving rise to those measures.

Suspension or termination of clinical trial

31.—(1) If, in relation to a clinical trial—

(a) the licensing authority have objective grounds for considering that—

(i) any condition, restriction or limitation which applies to the conduct of the trial and is set out in the request for authorisation or the particulars or documents accompanying that request, or

(ii) any condition imposed by the licensing authority under regulation 18(2) or (6), 19(8), 20(5), 24(4) or Schedule 5,

is no longer satisfied (either generally or at a particular trial site); or

(b) the licensing authority have information raising doubts about the safety or scientific validity of the trial, or the conduct of the trial at a particular trial site,

the licensing authority may, by a notice served in accordance with paragraph (2), require that the trial, or the conduct of the trial at a particular trial site, be suspended or terminated.

(2) A notice in accordance with paragraph (1) shall be served—

(a) in a case where the suspension or termination applies to the trial generally, on—

(i) the sponsor, or

(ii) the investigator at each trial site;

(b) in a case where the suspension or termination applies to the conduct of a trial at a particular trial site, on—

(i) the sponsor, or

(ii) the investigator at that trial site.

(3) The notice shall specify—

(a) whether the notice applies to the trial generally or to one or more of the trial sites;

(b) whether the notice requires suspension or termination of the trial;

(c) if the notice requires suspension of the trial—

(i) whether the suspension applies until further notice from the licensing authority or for such period as may be specified in the notice, and

(ii) any conditions which are to be satisfied before the trial or, as the case may be, the conduct of the trial at a particular site, may be recommenced; and

(d) whether suspension or termination is to take effect immediately on receipt of the notice or on such date as may be specified in the notice.

(4) If the licensing authority issues a notice under paragraph (1), they shall forthwith inform—

(a) where the notice has not been served on the sponsor, the sponsor;

(b) competent authorities of each EEA State, other than the United Kingdom;

(c) the relevant ethics committee;

(d) the European Medicines Agency; and

(e) the European Commission.

(5) Subject to paragraph (6), at least one week before issuing a notice under paragraph (1) the licensing authority shall, by a notice in writing to the sponsor or the investigator—

(a) inform him that the authority is minded to issue a notice suspending or terminating the trial, or the conduct of a trial at a particular site, and of the reasons why they are so minded; and

(b) advise him that they may, within one week of the date of the notice, furnish the authority with written representations as to whether the trial, or the conduct of the trial at a particular site, should be so suspended or terminated.

(6) Paragraph (5) shall not apply where it appears to the licensing authority that there is an imminent risk to the health or safety of any of the subjects of the clinical trial.

(7) A person on whom a notice has been served in accordance with paragraphs (1) and (2) may, within 28 days, or such extended period as the licensing authority may in any particular case allow, of the notice being given, give notice of his wish to make written or oral representations to the appropriate committee or, if for the time being there is no such committee, the Medicines Commission.

(8) Schedule 5 shall have effect to regulate the procedure for reference to the appropriate committee or, as the case may be, the Medicines Commission(**a**) following receipt of a notice in accordance with paragraph (7).

(9) Where the notice of suspension or termination is referred to an appropriate committee or the Medicines Commission it shall remain in force unless revoked in accordance with Schedule 5.

PART 5

PHARMACOVIGILANCE

Notification of adverse events

32.—(1) An investigator shall report any serious adverse event which occurs in a subject at a trial site at which he is responsible for the conduct of a clinical trial immediately to the sponsor.

(2) An immediate report under paragraph (1) may be made orally or in writing.

(3) Following the immediate report of a serious adverse event, the investigator shall make a detailed written report on the event.

(4) Paragraphs (1) to (3) do not apply to serious adverse events specified in the protocol or the investigator's brochure as not requiring immediate reporting.

(5) Adverse events, other than those to which paragraphs (1) to (3) apply, that are identified in the protocol as critical to evaluations of the safety of the trial shall be reported to the sponsor in accordance with the reporting requirements, including the time periods for such reporting, specified in that protocol.

(6) The reports made under paragraphs (1), (3) and (5) shall identify each subject referred to in the report by a number assigned to that subject in accordance with the protocol for the trial.

(7) The number assigned to a subject in accordance with the protocol must be different from the number of any other subject in that trial, including any subject at a trial site outside the United Kingdom.

(8) Where the event reported under paragraph (1) or (5) consists of, or results in, the death of a subject, the investigator shall supply—

(a) the sponsor; and

(b) in any case where the death has been reported to the relevant ethics committee, that committee,

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(**a**) *See* section 2 of the Act.

with any additional information requested by the sponsor or, as the case may be, the committee.

(9) The sponsor shall keep detailed records of all adverse events relating to a clinical trial which are reported to him by the investigators for that trial.

(10) The licensing authority may, by sending a notice in writing to the sponsor, require him to send the records referred to in paragraph (9), or copies of such records, to the authority.

Notification of suspected unexpected serious adverse reactions

33.—(1) A sponsor shall ensure that all relevant information about a suspected unexpected serious adverse reaction which occurs during the course of a clinical trial in the United Kingdom and is fatal or life-threatening is—

(a) recorded; and

(b) reported as soon as possible to—

(i) the licensing authority,

(ii) the competent authorities of any EEA State, other than the United Kingdom, in which the trial is being conducted, and

(iii) the relevant ethics committee,

and in any event not later that 7 days after the sponsor was first aware of the reaction.

(2) A sponsor shall ensure that within 8 days of a report in accordance with paragraph (1)(b), any additional relevant information is sent to the persons or bodies listed in that paragraph.

(3) A sponsor shall ensure that a suspected unexpected serious adverse reaction which occurs during the course of a clinical trial in the United Kingdom, other than those referred to in paragraph (1), is reported as soon as possible to—

(a) the licensing authority;

(b) the competent authorities of any EEA State, other than the United Kingdom, in which the trial is being conducted; and

(c) the relevant ethics committee,

and in any event not later that 15 days after the sponsor is first aware of the reaction.

(4) For the purposes of paragraphs (1) to (3), the sponsor may fulfil his obligations to report or provide information to the licensing authority and the competent authorities of any EEA State, other than the United Kingdom, by entering the report or information in the European database established in accordance with Article 11 of the Directive.

(5) A sponsor shall ensure that, in relation to each clinical trial in the United Kingdom for which he is the sponsor, the investigators responsible for the conduct of a trial are informed of any suspected unexpected serious adverse reaction which occurs in relation to an investigational medicinal product used in that trial, whether that reaction occurs during the course of that trial or another trial for which the sponsor is responsible.

(6) The licensing authority shall—

(a) keep a record of all suspected unexpected serious adverse reactions relating to an investigational medicinal product which are brought to its attention, whether pursuant to paragraphs (1) or (3) or otherwise; and

(b) ensure that the details of those reactions are entered in the European database established in accordance with Article 11 of the Directive, whether by the sponsor or the authority.

Clinical trials conducted in third countries

34. If a clinical trial is being conducted at a trial site in a third country in addition to sites in the United Kingdom, the sponsor of that trial shall ensure that all suspected unexpected serious adverse reactions occurring at that site are entered into the European database established in accordance with Article 11 of the Directive.

Annual list of suspected serious adverse reactions and safety report

35.—(1) As soon as practicable after the end of the reporting year, a sponsor shall, in relation to each investigational medicinal product tested in clinical trials in the United Kingdom for which he is the sponsor furnish the licensing authority and the relevant ethics committees with—

(a) a list of all the suspected serious adverse reactions which have occurred during that year in relation to—

(i) those trials, whether at trial sites in the United Kingdom or elsewhere, or

(ii) any other trials relating to that product which are conducted outside the United Kingdom and for which he is the sponsor,

including those reactions relating to any investigational medicinal product used as a placebo or as a reference in those trials; and

(b) a report on the safety of the subjects of those trials.

(2) In paragraph (1), "reporting year", in relation to an investigational medicinal product, means the year ending on the anniversary of—

(a) in the case of a product which has a marketing authorization, the earliest date on which any such authorization relating to that product was granted or issued; or

(b) in any other case, the earliest date on which any clinical trial—

(i) relating to that product, and

(ii) for which the person responsible for making the report was the sponsor,

was authorised in an EEA State.

(3) For the purposes of paragraph (2)(b), the date on which a clinical trial was authorised in an EEA State is—

(a) in the case of the United Kingdom, the date on which the trial was authorised by the licensing authority in accordance with these Regulations, or

(b) in the case of any other EEA State, the date on which the trial was authorised by the competent authority of that EEA State in accordance with the Directive.

PART 6

MANUFACTURE AND IMPORTATION OF INVESTIGATIONAL MEDICINAL PRODUCTS

Requirement for authorisation to manufacture or import investigational medicinal products

36.—(1) Subject to paragraph (2) and regulation 37, no person shall manufacture, assemble or import any investigational medicinal product except in accordance with an authorisation granted by the licensing authority for the purposes of this regulation ("a manufacturing authorisation").

(2) The restriction in paragraph (1) shall not apply to the manufacture or assembly of a medicinal product to the extent that such manufacture or assembly is in accordance with the terms and conditions of a marketing authorization relating to that product.

Exemption for hospitals and health centres

37.—(1) The restriction imposed by regulation 36(1) shall not apply to the assembly of an investigational medicinal product where the conditions specified in paragraph (2) are satisfied.

(2) The conditions referred to in paragraph (1) are that—

(a) the assembly is carried out in—

(i) in a hospital or health centre, and

(ii) by a doctor, a pharmacist or a person acting under the supervision of a pharmacist; and

(b) the investigational medicinal products are assembled exclusively for use in—

(i) that hospital or health centre, or

(ii) any other hospital or health centre which is a trial site for the clinical trial in which the product is to be used.

Application for manufacturing authorisation

38.—(1) An application for the grant of a manufacturing authorisation shall be—

(a) made to the licensing authority;

(b) in writing; and

(c) signed by or on behalf of the applicant.

(2) Every application for the grant of a manufacturing authorisation shall specify which, if any, of the standard provisions referred to in regulation 40(4) it is desired shall be excluded or modified in relation to the grant of the authorisation.

(3) Every application for the grant of a manufacturing authorisation shall be accompanied by—

(a) the particulars specified in Schedule 6 to these regulations; and

(b) any fee which may be payable in connection with that application under the Medicines (Products for Human Use—Fees) Regulations 1995(**a**).

(4) The application and any accompanying material shall be supplied to the licensing authority in the English language.

Consideration of application for manufacturing authorisation

39.—(1) Subject to paragraph (3) and regulation 40, the licensing authority shall consider a valid application for a manufacturing authorisation and grant, or refuse to grant, an authorisation within a period not exceeding 90 days from the date the application is received.

(2) Following receipt of an application, the licensing authority may give a notice in writing to the applicant requesting him to provide further information relating to—

(a) the particulars referred to in regulation 38(3); or

(b) the qualified person referred to in regulation 43.

(3) Where the licensing authority give a notice pursuant to paragraph (2), the period specified in paragraph (1) shall be suspended from the date the notice is given and shall recommence only on receipt of the information requested.

(4) If the application for a manufacturing authorisation relates (wholly or partially) to the importation of investigational medicinal products, the licensing authority may, if they think fit, require the production by the applicant of an undertaking, given by the manufacturer of any such products, to permit—

(a) the premises where they are or are to be manufactured; and

(b) the operations carried on or to be carried on in the course of manufacturing them,

to be inspected by or on behalf of the licensing authority.

(5) In this regulation, "valid application" means an application which complies with the provisions of regulation 38.

Grant or refusal of manufacturing authorisation

40.—(1) The licensing authority shall grant a manufacturing authorisation only if—

(**a**) S.I. 1995/1116.

(a) the applicant—

(i) has complied with the requirements of regulation 38,

(ii) has at his disposal suitable and sufficient premises, technical equipment and control facilities complying with the requirements of Commission Directive 2003/94/EC, as regards the manufacture or import, and control, of the products to which the authorisation relates and the storage of such products,

(iii) has at his disposal the services of at least one qualified person, and

(iv) if a notice has been given under regulation 39(2), has provided the information requested by the licensing authority; and

(b) they have established that the particulars supplied pursuant to regulation 38(3) are accurate.

(2) Subject to paragraph (1), the licensing authority may grant a manufacturing authorisation in respect of any or all of—

(a) the descriptions of investigational medicinal products;

(b) the manufacturing, assembling or importation operations; or

(c) the premises,

specified in the application made pursuant to regulation 38.

(3) The licensing authority may grant a manufacturing authorisation containing—

(a) any provisions to be incorporated in the authorisation in accordance with paragraph (4); or

(b) such other provisions as the licensing authority consider appropriate.

(4) The provisions specified—

(a) in the case of a manufacturing authorisation relating to the manufacture or assembly of investigational medicinal products, in Part 2 of Schedule 7; and

(b) in the case of a manufacturing authorisation relating to the importation of investigational medicinal products, in Part 3 of Schedule 7,

may be incorporated by the licensing authority in any manufacturing authorisation, with or without modifications and either generally or in relation to investigational medicinal products of any particular class.

(5) The provisions of Schedule 8 shall have effect where the licensing authority propose—

(a) to refuse to grant a manufacturing authorisation; or

(b) to grant a manufacturing authorisation otherwise than in accordance with the application.

(6) Where the licensing authority—

(a) refuse to grant a manufacturing authorisation; or

(b) grant a manufacturing authorisation otherwise than in accordance with the application,

and the applicant requests the authority to state their reasons, the licensing authority shall give the applicant a notice in writing stating the reasons for their decision.

Application and effect of manufacturing authorisation

41. A manufacturing authorisation shall apply only in relation to—

(a) the descriptions of investigational medicinal products;

(b) the manufacturing, assembling or importation operations; and

(c) the premises,

specified in the application made pursuant to regulation 38 and in respect of which the authorisation is granted.

Obligations of manufacturing authorisation holder

42. The holder of a manufacturing authorisation shall comply with—

(a) the principles and guidelines of good manufacturing practice; and

(b) the provisions referred to in regulation 40(3).

Qualified persons

43.—(1) Subject to paragraphs (4) and (5), the holder of a manufacturing authorisation must have at his disposal the services of at least one qualified person who is responsible for carrying out the duties referred to in paragraph 2.

(2) A qualified person shall be responsible for carrying out the duties specified in Article 13(3) and (4) of the Directive, in accordance with that Article, in respect of the investigational medicinal products manufactured, assembled or imported in accordance with the authorisation in question.

(3) A qualified person shall perform his functions under these Regulations in accordance with the Code of Practice for Qualified Persons in the Pharmaceutical Industry, published jointly by the Institute of Biology, the Royal Pharmaceutical Society of Great Britain and the Royal Society of Chemistry in March 2004(**a**).

(4) If the holder of the authorisation satisfies the requirements as to qualifications and experience specified in paragraph (a) or (b) of the definition of "qualified person" in regulation 2(1), he may act as the qualified person in accordance with paragraph (2) for the purposes of that authorisation.

(5) For the purposes of this paragraph, but without prejudice to paragraph (6) below, the holder of the authorisation may regard a person as satisfying the provisions of the said Article 49 or 50, as respects formal qualifications if he produces evidence that—

(a) he is a member of—

(i) the Institute of Biology,

(ii) the Pharmaceutical Society,

(iii) the Royal Society of Chemistry, or

(iv) such other body as may appear to the licensing authority to be an appropriate body for the purpose of this paragraph; and

(b) he is regarded by the body of which he is a member as so satisfying those provisions.

(6) Where, after giving the holder of the authorisation and the person acting as a qualified person the opportunity of making representations to them (orally or in writing), the licensing authority are of the opinion that—

(a) the person so acting does not satisfy—

(i) the provisions of the said Articles 49 and 50 of Directive 2001/83/EC as respects qualifications and experience, or

(ii) the requirements as to qualifications and experience specified in paragraph (b) of the definition of "qualified person" in regulation 2(1); or

(b) he is failing to carry out the duties referred to in paragraph (2) adequately or at all,

and have notified the holder of the authorisation accordingly in writing, the holder of the authorisation shall not permit that person to act as a qualified person.

(**a**) A copy of the Code of Practice may be obtained by writing to the Institute of Biology, 20 Queensbury Place, London SW7 2DZ, the Royal Pharmaceutical Society of Great Britain, 1 Lambeth High Street, London SE1 7JN or the Royal Society of Chemistry, Burlington House, Piccadilly, London W1V 0BN.

Variation of manufacturing authorisation

44.—(1) The licensing authority may vary a manufacturing authorisation, whether on the application of the holder of the authorisation or otherwise.

(2) Subject to the following provisions of this regulation, if the holder of a manufacturing authorisation makes a valid application to vary the manufacturing authorisation the licensing authority shall consider the application and—

(a) in a case where the effect of the variation would be to change the—

(i) the types of investigational medicinal products,

(ii) the manufacturing, assembling or importation operations,

(iii) the premises,

(iv) the technical equipment and control facilities,

in respect of which the authorisation has been granted, may vary or refuse to vary the authorisation within a period not exceeding 30 days from the date the application is received;

(b) in any other case, may vary or refuse to vary the authorisation within such period as the licensing authority consider appropriate.

(3) If the application falls within paragraph (2)(a), but it appears to the licensing authority to be necessary to conduct an inspection of any premises to which the variation relates, the authority may vary or refuse to vary the authorisation within a period not exceeding 90 days from the date the application is received.

(4) Following receipt of a valid application to vary a manufacturing authorisation, the licensing authority may give a notice in writing to the applicant requesting him to provide further information relating to the contents of the application or any particulars relevant to the application.

(5) Where the licensing authority give a notice pursuant to paragraph (4), and a period specified in paragraph (2)(a) or paragraph (3) applies, that period shall be suspended from the date the notice is given and shall recommence only on receipt of the information requested.

(6) The provisions of Schedule 8 shall have effect where the licensing authority propose to vary a manufacturing authorisation otherwise than on the application of the holder of the authorisation.

(7) Where the licensing authority—

(a) vary a manufacturing authorisation, otherwise than in accordance with a valid application by the holder of the authorisation; or

(b) after consideration of such an application, refuse to vary a manufacturing authorisation,

the licensing authority shall notify the holder of that authorisation in writing, stating the reasons for their decision.

(8) In this regulation, "valid application" means an application—

(a) made to the licensing authority;

(b) in writing and signed by or on behalf of the applicant;

(c) specifying the variation requested by the applicant;

(d) accompanied by—

(i) such particulars as are necessary to enable the licensing authority to consider the application, and

(ii) any fee which may be payable in connection with that application under the Medicines (Products for Human Use—Fees) Regulations 1995(**a**); and

(e) where the application, and any accompanying material, is in the English language.

(**a**) S.I. 1995/1116.

Suspension and revocation of manufacturing authorisation

45.—(1) The licensing authority may by a notice in writing to the holder of a manufacturing authorisation, forthwith or from a date specified in the notice, suspend the authorisation for such period as the authority may determine, or revoke the authorisation, on one or more of the following grounds—

(a) the holder is not carrying out, or has indicated by a notice in writing that he is no longer to carry out, the manufacturing, assembly or importation operations to which the authorisation relates;

(b) the particulars accompanying the application in accordance with regulation 38(3), were false or incomplete in a material particular;

(c) a material change of circumstances has occurred in relation to any of those matters or particulars;

(d) the holder of the authorisation has failed to any material extent to comply with his obligations under regulation 42 or 43(1);

(e) the holder has manufactured, assembled or, as the case may be, imported investigational medicinal products otherwise than in accordance with the terms of the authorisation;

(f) the holder has manufactured or assembled investigational medicinal products otherwise than in accordance with—

(i) in the case of products manufactured before a request for authorisation to conduct the clinical trial involving those products has been made in accordance with regulation 17 or any equivalent provisions in any EEA State other than the United Kingdom, the specification for the product provided by the person who is to act as the sponsor of the proposed clinical trial,

(ii) in the case of products manufactured for the purpose of export, the specification for the product provided by the person to whose order the products are manufactured, or

(iii) in any other case, the specification for the product contained in the investigational medicinal product dossier for that product;

(g) the qualified person has failed to carry out the duties referred to in regulation 43(2), adequately or at all; and

(h) the holder of the authorisation does not have the staff, premises, equipment or facilities necessary for carrying out properly—

(i) the manufacture or assembly operations to which the authorisation relates, or

(ii) the importation operations to which the authorisation relates,

including any handling, storage or distribution activities relating to those operations.

(2) The suspension or revocation of an authorisation under this regulation may be—

(a) total; or

(b) limited to investigational medicinal products—

(i) of one or more descriptions, or

(ii) manufactured, assembled or stored on any particular premises or in a particular part of any premises.

(3) The provisions of Schedule 8 shall have effect where the licensing authority propose to suspend or revoke a manufacturing authorisation in accordance with this regulation.

(4) Where the licensing authority suspend or revoke a manufacturing authorisation in accordance with this regulation, they shall notify the holder of that authorisation in writing, stating the reasons for their decision to suspend or revoke the authorisation.

PART 7

LABELLING OF INVESTIGATIONAL MEDICINAL PRODUCTS

Labelling

46.—(1) An investigational medicinal product shall be labelled in accordance with Article 15 of Commission Directive 2003/94/EC(**a**).

(2) Paragraph (1) shall not apply where the investigational medicinal product is—

(a) for use in a clinical trial with the characteristics specified in the second paragraph of Article 14 of the Directive;

(b) dispensed to a subject in accordance with a prescription given by an authorised health care professional; and

(c) labelled in accordance with the requirements of Schedule 5 to the Medicines for Human Use (Marketing Authorisations Etc.) Regulations 1994(**b**) that apply in relation to dispensed relevant medicinal products.

PART 8

ENFORCEMENT AND RELATED PROVISIONS

Application of enforcement provisions of the Act

47.—(1) Sections 107 to 116, 118, 119, 121 to 125, 127, 129, 131 and 132(1) of, and Schedule 3 to, the Act shall apply for the purposes of these Regulations, but with the modifications specified in Schedule 9.

(2) In those provisions as applying by virtue of paragraph (1), a reference to any part of those provisions or a part of any of them is a reference to the provision or part as so applying.

Infringement notices

48.—(1) If an enforcement authority have objective grounds for considering that any person has contravened any provision to which this regulation applies, they may serve upon that person a notice in writing (in these Regulations referred to as an "infringement notice")—

(a) informing him of the authority's grounds for considering that the person has contravened one or more of those provisions;

(b) specifying the relevant provision of these Regulations;

(c) specifying the measures which the person must take in order to ensure that the contravention does not continue or, as the case may be, does not recur;

(d) requiring the person to take those measures, within such period as may be specified in the notice;

(e) warning the person that unless the requirements of sub-paragraph (d) are met, further action may be taken in respect of the contravention.

(2) An infringement notice may include directions as to the measures to be taken by the person on whom the notice is served to ensure that the contravention does not continue or, as the case may be, does not recur, including the different ways of securing compliance.

(3) If an enforcement authority serves an infringement notice in accordance with paragraph (1), they shall forthwith inform—

(**a**) OJ No. L262, 14.10.2003, p.22.

(**b**) S.I. 1994/3144; Schedule 5 was amended by S.I. 1998/3105, 2000/292 and 2002/542; "dispensed relevant medicinal product" is defined in paragraph 1 of Schedule 5.

(a) the competent authorities of each EEA State, other than the United Kingdom;

(b) the relevant ethics committee; and

(c) the European Commission.

(4) This regulation applies to regulations 22(b), 27, 28(1) to (3), 29, 30(2) and 32 to 35.

(5) In this regulation, "enforcement authority" means any Minister or body on whom a duty or power to enforce any provisions of these Regulations is imposed or conferred by or under sections 108 to 110 of the Act as applied by regulation 47.

Offences

49.—(1) Any person who contravenes any of the following provisions—

(a) regulation 12(1) and (2);

(b) regulation 13(1);

(c) regulation 27;

(d) regulation 28(1) to (3);

(e) regulation 29;

(f) regulation 30(2);

(g) regulation 32(1), (3), and (5) to (9)

(h) regulation 33(1) to (5)

(i) regulation 34

(j) regulation 35(1);

(k) regulation 36(1);

(l) regulation 42; and

(m) regulation 43(1) and (6),

shall be guilty of an offence.

(2) Any person who has in his possession a medicinal product for the purpose of selling or supplying it in contravention of regulation 13(1) shall be guilty of an offence.

(3) Any person who fails to comply with a notice of suspension or termination served on him under regulation 31, unless that notice has been withdrawn or revoked by the licensing authority, shall be guilty of an offence.

(4) Where an investigational medicinal product is manufactured, assembled or imported in contravention of regulation 36(1), any person who sells or supplies the product for the purposes of a clinical trial knowing or having reasonable cause to suspect that it was so manufactured, assembled or imported shall be guilty of offence.

(5) Where an investigational medicinal product is imported in contravention of regulation 36(1), any person who, otherwise than for the purpose of performing or exercising a duty or power imposed or conferred by or under these Regulations, the Act or any other enactment, is in possession of the product knowing or having reasonable cause to suspect that it was so imported shall be guilty of offence.

(6) Any sponsor who sells or supplies, or procures the sale or supply, of an investigational medicinal product—

(a) to a subject for the purposes of a clinical trial; or

(b) to a person for the purpose of administering the product to such a subject,

the labelling of which does not comply with regulation 46, shall be guilty of an offence.

(7) Any person who sells or supplies an investigational medicinal product—

(a) to a subject for the purposes of a clinical trial; or

(b) to a person for the purpose of administering the product to such a subject,

the labelling of which does not comply with regulation 46, knowing, or having reasonable cause to believe, that the labelling does not so comply, shall be guilty of an offence.

False or misleading information

50.—(1) Any person who in the course of—

(a) making an application for an ethics committee opinion;

(b) making a request for authorisation to conduct a clinical trial; or

(c) making an application for the grant or variation of a manufacturing authorisation,

provides to the licensing authority or an ethics committee any relevant information which is false or misleading in a material particular shall be guilty of an offence.

(2) Any person who—

(a) is conducting a clinical trial authorised in accordance with these Regulations;

(b) is a sponsor of such a clinical trial;

(c) while acting under arrangements made with a sponsor of such a clinical trial, performs the functions of that sponsor; or

(d) holds a manufacturing authorisation,

and who, for the purposes of these Regulations, provides to the licensing authority or an ethics committee any relevant information which is false or misleading in a material particular shall be guilty of an offence.

(3) Any person who, for the purpose of being engaged as a qualified person in accordance with regulation 43, provides to the licensing authority or to the holder of a manufacturing authorisation any information which is false or misleading in a material particular shall be guilty of an offence.

(4) In this regulation, "relevant information" means any information which is relevant to an evaluation of—

(a) the safety, quality or efficacy of an investigational medicinal product;

(b) the safety or scientific validity of a clinical trial; or

(c) whether, with regard to a clinical trial, the conditions and principles of good clinical practice are being satisfied or adhered to.

Defence of due diligence

51.—(1) A person does not commit an offence under these Regulations if he took all reasonable precautions and exercised all due diligence to avoid the commission of that offence.

(2) Where evidence is adduced which is sufficient to raise an issue with respect to that defence, the court or jury shall assume that the defence is satisfied unless the prosecution proves beyond reasonable doubt that it is not.

Penalties

52. A person guilty of an offence under these Regulations shall be liable—

(a) on summary conviction to a fine not exceeding the statutory maximum or to imprisonment for a term not exceeding three months or to both;

(b) on conviction on indictment to a fine or to imprisonment for a term not exceeding two years or to both.

PART 9

MISCELLANEOUS PROVISIONS

Construction of references to specified publications

53.—(1) Where any authorisation granted under these Regulations refers to a specified publication, but not to any particular edition of that publication, then, for the purpose of determining whether anything done, at a time when the authorisation is in force, is done in accordance with the authorisation, the reference shall, unless the authorisation otherwise expressly provides, be construed as a reference to the current edition of that publication as in force at that time.

(2) In this regulation any reference to the current edition of a specified publication as in force at a particular time is a reference to the edition of that publication in force, under whatever title, at that time together with any amendments, additions and deletions made to it up to that time.

(3) In this regulation, "specified publication" has the meaning given by section 103(1) of the Act(**a**).

Consequential and other amendments to enactments

54. The provisions of the enactments specified in Schedule 10 are amended as there specified.

Revocations

55. The enactments specified in column (1) of Schedule 11 are revoked to the extent specified in column (3) of that Schedule.

Transitional provisions

56. The transitional provisions set out in Schedule 12 shall have effect.

Signed by authority of the Secretary of State for Health

Warner
Parliamentary Under Secretary of State,
Department of Health

31st March 2004

(**a**) Section 103 was amended by section 22(1) of the Health and Medicines Act 1988 (c.49).

SCHEDULE 1

Regulation 2(1)

CONDITIONS AND PRINCIPLES OF GOOD CLINICAL PRACTICE AND FOR THE PROTECTION OF CLINICAL TRIAL SUBJECTS

PART 1

APPLICATION AND INTERPRETATION

1.—(1) The conditions and principles specified in Part 2 apply to all clinical trials.

(2) If any subject of a clinical trial is—

(a) an adult able to give informed consent, or

(b) an adult who has given informed consent to taking part in the clinical trial prior to the onset of incapacity,

the conditions and principles specified in Part 3 apply in relation to that subject.

(3) If any subject of a clinical trial is a minor, the conditions and principles specified in Part 4 apply in relation to that subject.

(4) If any subject—

(a) is an adult unable by virtue of physical or mental incapacity to give informed consent, and

(b) did not, prior to the onset of incapacity, give or refuse to give informed consent to taking part in the clinical trial,

the conditions and principles specified in Part 5 apply in relation to that subject.

(5) If any person—

(a) is an adult unable by virtue of physical or mental incapacity to give informed consent, and

(b) has, prior to the onset of incapacity, refused to give informed consent to taking part in the clinical trial,

that person cannot be included as a subject in the clinical trial.

2. In this Schedule—

"Declaration of Helsinki" means the Declaration of Helsinki adopted by the World Medical Assembly in June 1964, as amended by the General Assembly of the Association in October 1975, October 1983, September 1989 and October 1996;

"guardian" shall be construed in accordance with section 51(8) of the Adults with Incapacity (Scotland) Act 2000(**a**);

"legal representative" means, in relation to a minor or to an adult unable by virtue of physical or mental incapacity to give informed consent, and who is, or is being considered as, a subject for a clinical trial—

(a) in relation to adults and minors in England, Wales and Northern Ireland, and minors in Scotland—

(i) a person, other than a person involved in the conduct of the trial, who—

(aa) by virtue of their relationship with that adult or that minor, is suitable to act as their legal representative for the purposes of that trial, and

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(**a**) 2000 asp 4.

(bb) is available and willing to so act for those purposes, or

(ii) if there is no such person, a person, other than a person connected with the conduct of the clinical trial, who is—

(aa) the doctor primarily responsible for the medical treatment provided to that adult, or

(bb) a person nominated by the relevant health care provider; and

(b) in relation to adults in Scotland—

(i) any guardian or welfare attorney who has power to consent to the adult's participation in research, or

(ii) if there is no such guardian or welfare attorney, the adult's nearest relative, or

(iii) if it is not reasonably practicable to contact a guardian or welfare attorney or the adult's nearest relative before the decision to enter the adult as a subject of the clinical trial is made, a person, other than a person connected with the conduct of the clinical trial, who is—

(aa) the doctor primarily responsible for the medical treatment provided to that adult, or

(bb) a person nominated by the relevant health care provider;

"nearest relative" has the meaning given by section 87(1) of the Adults with Incapacity (Scotland) Act 2000;

"parental responsibility"—

(a) in relation to England and Wales, has the same meaning as in the Children Act 1989(**a**),

(b) in relation to Scotland, has the same meaning as in the Children (Scotland) Act 1985(**b**), and

(c) in relation to Northern Ireland, has the same meaning as in the Children (Northern Ireland) Order 1995(**c**);

"person connected with the conduct of the trial" means—

(a) the sponsor of the trial,

(b) a person employed or engaged by, or acting under arrangements made with, the sponsor and who undertakes activities in connection with the management of the trial,

(c) an investigator for the trial,

(d) a health care professional who is a member of an investigator's team for the purposes of the trial, or

(e) a person who provides health care under the direction or control of a person referred to in paragraphs (c) and (d) above, whether in the course of the trial or otherwise;

"relevant health care provider" means—

(a) in relation to a person receiving services in pursuance of the National Health Service Act 1977(**d**), the National Health Service (Scotland) Act 1978(**e**), or the Health and Personal Social Services (Northern Ireland) Order 1972(**f**)—

(i) in a case where a health service body is providing those services, that body, or

(ii) in any other case, the health service body which entered the arrangements under which those services are provided, or

(b) in relation to any other person receiving health care, the person primarily responsible for providing that health care; and

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(**a**) 1989 c.41; *see*, in particular, sections 3(1) and 5(6).
(**b**) 1995 c.36; *see*, in particular, sections 1(3) and 7(5).
(**c**) S.I. 1995/755 (N.I.2); *see*, in particular, article 6.
(**d**) 1977 c.49.
(**e**) 1978 c. 29.
(**f**) S.I. 1972/1265 (N.I. 14).

"welfare attorney" shall be construed in accordance with section 51(8) of the Adults with Incapacity (Scotland) Act 2000.

3.—(1) For the purposes of this Schedule, a person gives informed consent to take part, or that a subject is to take part, in a clinical trial only if his decision—

(a) is given freely after that person is informed of the nature, significance, implications and risks of the trial; and

(b) either—

(i) is evidenced in writing, dated and signed, or otherwise marked, by that person so as to indicate his consent, or

(ii) if the person is unable to sign or to mark a document so as to indicate his consent, is given orally in the presence of at least one witness and recorded in writing.

(2) For the purposes of this Schedule, references to informed consent—

(a) shall be construed in accordance with paragraph (1); and

(b) include references to informed consent given or refused by an adult unable by virtue of physical or mental incapacity to give informed consent, prior to the onset of that incapacity.

PART 2

CONDITIONS AND PRINCIPLES WHICH APPLY TO ALL CLINICAL TRIALS

Principles based on International Conference on Harmonisation GCP Guideline(a)

1. Clinical trials shall be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with good clinical practice and the requirements of these Regulations.

2. Before the trial is initiated, foreseeable risks and inconveniences have been weighed against the anticipated benefit for the individual trial subject and other present and future patients. A trial should be initiated and continued only if the anticipated benefits justify the risks.

3. The rights, safety, and well-being of the trial subjects are the most important considerations and shall prevail over interests of science and society .

4. The available non-clinical and clinical information on an investigational medicinal product shall be adequate to support the clinical trial.

5. Clinical trials shall be scientifically sound, and described in a clear, detailed protocol.

6. A trial shall be conducted in compliance with the protocol that has a favourable opinion from an ethics committee.

7. The medical care given to, and medical decisions made on behalf of, subjects shall always be the responsibility of an appropriately qualified doctor or, when appropriate, of a qualified dentist.

8. Each individual involved in conducting a trial shall be qualified by education, training, and experience to perform his or her respective task(s).

9. Subject to the other provisions of this Schedule relating to consent, freely given informed consent shall be obtained from every subject prior to clinical trial participation.

10. All clinical trial information shall be recorded, handled, and stored in a way that allows its accurate reporting, interpretation and verification.

(a) *See* Section 2 of the Note for Guideline on Good Clinical Practice (CPMP/ICH/135/95) published by the European Agency for the Evaluation of Medicinal Products in July 2002.

11. The confidentiality of records that could identify subjects shall be protected, respecting the privacy and confidentiality rules in accordance with the requirements of the Data Protection Act 1998 and the law relating to confidentiality.

12. Investigational medicinal products used in the trial shall be—

(a) manufactured or imported, and handled and stored, in accordance with the principles and guidelines of good manufacturing practice, and

(b) used in accordance with the approved protocol.

13. Systems with procedures that assure the quality of every aspect of the trial shall be implemented.

Conditions based on Article 3 of the Directive

14. A trial shall be initiated only if an ethics committee and the licensing authority comes to the conclusion that the anticipated therapeutic and public health benefits justify the risks and may be continued only if compliance with this requirement is permanently monitored.

15. The rights of each subject to physical and mental integrity, to privacy and to the protection of the data concerning him in accordance with the Data Protection Act 1998 are safeguarded.

16. Provision has been made for insurance or indemnity to cover the liability of the investigator and sponsor which may arise in relation to the clinical trial.

PART 3

CONDITIONS WHICH APPLY IN RELATION TO AN ADULT ABLE TO CONSENT OR WHO HAS GIVEN CONSENT PRIOR TO THE ONSET OF INCAPACITY

1. The subject has had an interview with the investigator, or another member of the investigating team, in which he has been given the opportunity to understand the objectives, risks and inconveniences of the trial and the conditions under which it is to be conducted.

2. The subject has been informed of his right to withdraw from the trial at any time.

3. The subject has given his informed consent to taking part in the trial.

4. The subject may, without being subject to any resulting detriment, withdraw from the clinical trial at any time by revoking his informed consent.

5. The subject has been provided with a contact point where he may obtain further information about the trial.

PART 4

CONDITIONS AND PRINCIPLES WHICH APPLY IN RELATION TO A MINOR

Conditions

1. Subject to paragraph 6, a person with parental responsibility for the minor or, if by reason of the emergency nature of the treatment provided as part of the trial no such person can be contacted prior to the proposed inclusion of the subject in the trial, a legal representative for the minor has had an interview with the investigator, or another member of the investigating team, in which he has been given the opportunity to understand the objectives, risks and inconveniences of the trial and the conditions under which it is to be conducted.

2. That person or legal representative has been provided with a contact point where he may obtain further information about the trial.

3. That person or legal representative has been informed of the right to withdraw the minor from the trial at any time.

4. That person or legal representative has given his informed consent to the minor taking part in the trial.

5. That person with parental responsibility or the legal representative may, without the minor being subject to any resulting detriment, withdraw the minor from the trial at any time by revoking his informed consent.

6. The minor has received information according to his capacity of understanding, from staff with experience with minors, regarding the trial, its risks and its benefits.

7. The explicit wish of a minor who is capable of forming an opinion and assessing the information referred to in the previous paragraph to refuse participation in, or to be withdrawn from, the clinical trial at any time is considered by the investigator.

8. No incentives or financial inducements are given—

(a) to the minor; or

(b) to a person with parental responsibility for that minor or, as the case may be, the minor's legal representative,

except provision for compensation in the event of injury or loss.

9. The clinical trial relates directly to a clinical condition from which the minor suffers or is of such a nature that it can only be carried out on minors.

10. Some direct benefit for the group of patients involved in the clinical trial is to be obtained from that trial.

11. The clinical trial is necessary to validate data obtained—

(a) in other clinical trials involving persons able to give informed consent, or

(b) by other research methods.

12. The corresponding scientific guidelines of the European Medicines Agency are followed.

Principles

13. Informed consent given by a person with parental responsibility or a legal representative to a minor taking part in a clinical trial shall represent the minor's presumed will.

14. The clinical trial has been designed to minimise pain, discomfort, fear and any other foreseeable risk in relation to the disease and the minor's stage of development.

15. The risk threshold and the degree of distress have to be specially defined and constantly monitored.

16. The interests of the patient always prevail over those of science and society.

PART 5

CONDITIONS AND PRINCIPLES WHICH APPLY IN RELATION TO AN INCAPACITATED ADULT

Conditions

1. The subject's legal representative has had an interview with the investigator, or another member of the investigating team, in which he has been given the opportunity to understand the objectives, risks and inconveniences of the trial and the conditions under which it is to be conducted.

2. The legal representative has been provided with a contact point where he may obtain further information about the trial.

3. The legal representative has been informed of the right to withdraw the subject from the trial at any time.

4. The legal representative has given his informed consent to the subject taking part in the trial.

5. The legal representative may, without the subject being subject to any resulting detriment, withdraw the subject from the trial at any time by revoking his informed consent.

6. The subject has received information according to his capacity of understanding regarding the trial, its risks and its benefits.

7. The explicit wish of a subject who is capable of forming an opinion and assessing the information referred to in the previous paragraph to refuse participation in, or to be withdrawn from, the clinical trial at any time is considered by the investigator.

8. No incentives or financial inducements are given to the subject or their legal representative, except provision for compensation in the event of injury or loss.

9. There are grounds for expecting that administering the medicinal product to be tested in the trial will produce a benefit to the subject outweighing the risks or produce no risk at all.

10. The clinical trial is essential to validate data obtained—

(a) in other clinical trials involving persons able to give informed consent, or

(b) by other research methods.

11. The clinical trial relates directly to a life-threatening or debilitating clinical condition from which the subject suffers.

Principles

12. Informed consent given by a legal representative to an incapacitated adult in a clinical trial shall represent that adult's presumed will.

13. The clinical trial has been designed to minimise pain, discomfort, fear and any other foreseeable risk in relation to the disease and the cognitive abilities of the patient.

14. The risk threshold and the degree of distress have to be specially defined and constantly monitored.

15. The interests of the patient always prevail over those of science and society.

SCHEDULE 2 Regulations 7(1)(b), 8(a) and 9

ADDITIONAL PROVISIONS RELATING TO ETHICS COMMITTEES

Interpretation

1. In this Schedule—

"appointing authority" means—

(a) in relation to an ethics committee established under regulation 6, the Authority,

(b) in relation to an ethics committee recognised by the Authority after an application in accordance with regulation 7(1), the person who applied for recognition, or

(c) in relation to an ethics committee recognised without an application for recognition being submitted in accordance with regulation 7(3), the Authority;

"expert member" means a member of an ethics committee who—

(a) is a health care professional,

(b) has professional qualifications or experience relating to the conduct of, or use of statistics in clinical trials, unless those professional qualifications or experience relate only to the ethics of clinical research or medical treatment, or

(c) is not a health care professional, but has been a registered medical practitioner or a person registered in the dentists register under the Dentists Act 1984;

"financial year" means the twelve months ending with 31st March; and

"lay member" means a member of an ethics committee, other than an expert member.

Application of provisions of the Schedule

2.—(1) The provisions of this Schedule shall not apply in relation to the Ethics Committee constituted by regulations made by the Scottish Ministers under section 51(6) of the Adults with Incapacity (Scotland) Act 2000.

(2) The provisions of this Schedule, other than paragraph 13, shall not apply before 1st May 2005 in relation to an ethics committee established or recognised solely for the purpose of considering Phase I trials.

Membership

3.—(1) An ethics committee shall consist of—

(a) expert members; and

(b) lay members.

(2) An ethics committee shall have no more than 18 members.

(3) Subject to paragraph 7, the members of an ethics committee shall be appointed by the appointing authority.

(4) A person shall not be eligible for appointment as a lay member of an ethics committee if, in the course of his employment or business, he—

(a) provides medical, dental or nursing care, or

(b) conducts clinical research.

(5) An appointing authority shall, in relation to an ethics committee, exercise their power under sub-paragraph (3) so as to ensure that—

(a) at least one third of the total membership shall be lay members; and

(b) at least half of the lay members must be persons who are not, or who never have been—

(i) health care professionals,

(ii) persons involved in the conduct of clinical research, other than as a subject of such research, or

(iii) a chairman, member or director of—

(aa) a health service body, or

(bb) a body, other than a health service body, which provides health care.

4. A member of an ethics committee shall hold and vacate office as a member in accordance with the terms of the instrument appointing him as a member.

Chairman, vice-chairman and alternate vice-chairman

5.—(1) The appointing authority shall appoint—

(a) one of the members of each ethics committee to be chairman of the committee;

(b) another member to be vice-chairman; and

(c) another member to be alternate vice-chairman.

(2) The members appointed as chairman, vice-chairman and alternate vice-chairman shall each be appointed for such period, not exceeding the remainder of his term as a member, as the appointing authority may specify on appointing him.

(3) Any member so appointed may at any time resign from the office of chairman, vice-chairman or alternate vice-chairman.

(4) Where the chairman has died or has ceased to hold office, or where he is unable to perform his duties as chairman owing to illness, absence or any other cause, references to the chairman in this Schedule shall, so long as there is no chairman available to perform his duties, be taken to include references to—

(a) the vice-chairman;

(b) if the vice-chairman is also is unable to perform his duties, the alternate vice-chairman; or

(c) if all three individuals are unavailable, a member appointed by the appointing authority for the purposes of acting as chairman until one of those individuals is available to perform his duties.

Committees, meetings and proceedings

6.—(1) An ethics committee may—

(a) appoint sub-committees consisting of members of the committee; and

(b) make arrangements for the exercise, on behalf of the committee, of any of its functions by such a sub-committee,

in accordance with the standing orders and operating procedures adopted under sub-paragraph (3).

(2) Subject to sub-paragraph (4), the meetings and proceedings of an ethics committee and its sub-committees shall be conducted in accordance with the standing orders made, and standing operating procedures adopted, under sub-paragraph (3).

(3) An ethics committee—

(a) shall, subject to approval by the Authority, make standing orders, and adopt standing operating procedures, for the regulation of its proceedings and business; and

(b) may, subject to approval by the Authority, vary or revoke such orders or procedures,

including provision for the suspension of the standing orders or operating procedures or any of them.

(4) No business shall be transacted at a meeting of an ethics committee, or a sub-committee of an ethics committee, to determine, in accordance with regulation 15, the opinion of an ethics committee in relation to a clinical trial, unless at least seven members of the committee (including any members co-opted under paragraph 8) are present, including at least—

(a) one lay member who is not and never has been—

(i) a health care professional, or

(ii) a chairman, member, director, officer or employee of a health service body; and

(b) one expert member.

Deputies and co-opted members

7.—(1) An ethics committee may appoint a person to act as the deputy of an expert member or a lay member provided that the person would be eligible for appointment as an expert member or, as the case may be, a lay member.

(2) A deputy shall hold and vacate office as a deputy member in accordance with the terms of the instrument appointing him as a deputy.

(3) A deputy may vote as a member of the committee only if the member for which he acts as deputy is absent.

(4) A deputy member and the member for which he is deputy shall count as one member for the purposes of paragraphs 3(2) and (4) and 6(4).

8.—(1) At any meeting of an ethics committee, the committee may co-opt up to 2 additional members for the purposes of that meeting.

(2) At any meeting of a sub-committee of an ethics committee, the sub-committee may co-opt an additional member for the purposes of that meeting.

(3) Subject to sub-paragraph (4), a person shall be eligible to be co-opted as a member only if he is or has been a member of an ethics committee.

(4) Paragraph (3) shall not apply in relation to the Gene Therapy Advisory Committee.

(5) A co-opted member shall hold office only in relation to the meeting for which he is co-opted.

(6) A member co-opted under this paragraph shall not count as a member for the purposes of paragraphs 3(2) and (4).

Staff, premises and facilities

9.—(1) The appointing authority shall make arrangements for the appointment of such administrative and other staff for an ethics committee as they consider necessary to enable the committee to perform its functions.

(2) The appointing authority shall—

(a) secure the provision to an ethics committee of such accommodation and facilities as they consider necessary to enable the committee to perform its functions; and

(b) secure that arrangements are made for such administration, maintenance, cleaning and other services as may, in their opinion, be necessary for such accommodation and facilities.

(3) To enable an ethics committee to perform its functions, a health service body may make staff, premises and facilities available to an ethics committee under arrangements made with the appointing authority.

Expenses

10.—(1) The appointing authority shall, in respect of each financial year, pay to an ethics committee sums equal to the amount approved as the amounts of expenditure which they consider

may be reasonably incurred by the committee in that year for the purpose of performing its functions.

(2) An ethics committee shall not incur expenses in excess of the amounts approved for that committee by the appointing authority under this paragraph.

11. The appointing authority may pay to members of ethics committees such travelling and other allowances as the authority may determine.

Annual report

12.—(1) Within the period six months from the end of each financial year, every ethics committee shall prepare a report on the committee's activities during that year, which shall include a list of—

(a) the applications made to the committee in accordance with regulation 14; and

(b) the decisions made by the committee in relation to those applications.

(2) The ethics committee shall send a copy of the report to the Authority and, if the Authority is not the appointing authority for that committee, to its appointing authority.

Transfer of functions

13.—(1) This paragraph applies where—

(a) recognition of an ethics committee is revoked in accordance with regulation 8; or

(b) an ethics committee is abolished or ceases operation.

(2) If the person who was the appointing authority before revocation, abolition or the ceasing of operation of the committee ("the old committee") is the Authority, that person may nominate another ethics committee as responsible for the work of the committee.

(3) If the person referred to in sub-paragraph (2) was not the Authority, that person may only nominate an ethics committee with the approval of the Authority.

(4) If the person referred to in sub-paragraph (2) no longer exists or if that person fails to nominate another ethics committee, the Authority shall nominate such a committee.

(5) Where an ethics committee is nominated in accordance with the preceding sub-paragraphs—

(a) that committee shall consider any applications made to the old committee in accordance with regulation 14, if the old committee had not given an opinion before the date of revocation, abolition or ceasing of operation;

(b) that committee shall be the relevant ethics committee for any clinical trial in relation to which the old committee had given a favourable opinion in accordance with regulation 15.

SCHEDULE 3

Regulation 14(6), 17(2), 24(9) and 27(3)

PARTICULARS AND DOCUMENTS THAT MUST ACCOMPANY AN APPLICATION FOR AN ETHICS COMMITTEE OPINION, A REQUEST FOR AUTHORISATION, A NOTICE OF AMENDMENT AND A NOTIFICATION OF THE CONCLUSION OF A TRIAL

PART 1

APPLICATION FOR ETHICS COMMITTEE OPINION

1. An application document including the following information or, in each case, an explanation of why that information is not being provided—

(a) the reference number of the ethics committee to which the application is made;

(b) particulars identifying the trial including—

(i) the number allocated to the trial on the European database referred to in Article 11 of the Directive, and

(ii) full and short titles of the trial;

(c) the following particulars relating to the trial design—

(i) a summary of the trial, including justification and relevance, and the methodology to be used,

(ii) the primary, and any secondary, research hypothesis,

(iii) statistical analysis and justification for the numbers of subjects to be recruited for the trial, and

(iv) details of the process for peer review of the scientific value of the trial;

(d) brief details of any plans to conduct the trial outside the UK and any authorisation given in relation to the trial by a competent authority of an EEA State in accordance with Article 9 of the Directive;

(e) the name and address of the sponsor;

(f) details of any arrangements under which the sponsor has delegated any of his responsibilities in relation to the proposed trial;

(g) the financial arrangements for the trial, in particular—

(i) sources of funding for the trial and information on financial or other interests of the applicant relevant to the trial,

(ii) the arrangements for remuneration of, or re-imbursement of expenses incurred by, subjects,

(iii) any provision for compensation in the event of injury or death attributable to the trial,

(iv) details of any insurance or indemnity to cover the liability of the sponsor and investigator, and

(v) summary details of any financial arrangements between—

(aa) the sponsor or person funding the trial and the investigator, and

(bb) the sponsor or person funding the trial and the owner or occupier of the trial site;

(h) arrangements for the recruitment of subjects, including the materials to be used;

(i) the criteria for inclusion and exclusion of patients, including justification for recruiting from vulnerable groups;

(j) in the case of Phase I trials, methods for recording and verifying health status for healthy volunteers;

(k) procedures for checking simultaneous or recent involvement of potential subjects in other trials;

(l) details of any relationship between subject and investigator which may be relevant for the purposes of an ethical opinion;

(m) details of—

(i) any proposed additional investigational procedures or other interventions over and above those required for normal clinical care, and

(ii) any aspect of normal clinical care to be withheld or other deviation from normal treatment, and

(iii) the plan for treatment or care of subjects once their participation in the trial has ended;

(n) the procedures for—

(i) providing information to potential subjects, including a contact point where additional information can be obtained about the trial and the rights of trial subjects,

(ii) providing subjects with updated information during and (where relevant) after the trial, and

(iii) obtaining informed consent;

(o) details of the arrangements for access to confidential data about the subjects and the arrangements to protect subjects' privacy;

(p) the rules for terminating or concluding the trial before—

(i) the date for the conclusion of the trial specified in the protocol, or

(ii) the event specified in the protocol as the event which indicates that the end of the trial has occurred;

(q) any agreement on—

(i) the access by the investigator or his team to the data produced by the trial, and

(ii) the policy for publication of that data;

(r) an assessment of the ethical issues relating to the trial, including—

(i) the importance of the trial and of the new knowledge to be gained,

(ii) an assessment of the potential benefits, and

(iii) an assessment of the possible risks for the subjects;

(s) details relating to the chief investigator and each investigator, including—

(i) experience in conducting research, and

(ii) any potential conflicts of interest; and

(t) details of any proposed trial site and its suitability for conducting the trial.

2. A document containing the particulars specified in paragraphs 1 to 4 and 6 to 9 of Part 2 of this Schedule.

3. The following documents or, in each case, an explanation of why that document is not being provided—

(a) the protocol;

(b) the investigator's brochure for the proposed trial or, where the investigational medicinal product has a marketing authorisation and the product is to be used in accordance with the

terms of that authorisation, the summary of product characteristics relating to that product;

(c) any document providing evidence of any insurance to cover the liability of the sponsor and investigator;

(d) copies of the advertisement material for recruitment of research participants;

(e) in the case of advertising contained on video or audio cassettes, a copy of the script for that advertising;

(f) a copy of any letter inviting a subject to participate in the trial;

(g) a copy of any questionnaire, diary or sample card to be completed by the subject in writing;

(h) a copy of all written information to be given to a potential subject or their legal representative prior to seeking informed consent;

(i) a copy of the form to be used to record the consent of a subject or their legal representative;

(j) a copy of any letters or other written information to be sent to any person who normally provides a subject's clinical care;

(k) a summary curriculum vitae for the chief investigator and each investigator.

PART 2

REQUEST FOR AUTHORISATION

1. The name and address of—

(a) the sponsor,

(b) if the sponsor is not established in the European Community, his legal representative,

(c) if any person has been authorised by the sponsor to make the request on his behalf, that person,

(d) if the persons taking responsibility for the initiation, management and financing (or arranging the financing) of the clinical trial have allocated responsibility in accordance with regulation 3(4), any person responsible for carrying out the functions of the sponsor under Part 4 or 5 of these Regulations, and

(e) any other person to whom the sponsor has delegated any of his responsibilities in relation to the proposed trial.

2. If any person is specified as a person responsible for the duties of the sponsor under regulation 28(2) and (3) in relation to the trial—

(a) the name and address of that person; and

(b) the trial sites in relation to which they are so responsible.

3. The address of each trial site and the names and address of the investigator responsible for the conduct of the trial at each site.

4. Where the trial is to be conducted at trial sites in another EEA State, a list of the competent authorities to which a request for authorisation has been made.

5. A copy of the ethics committee opinion in relation to that trial, if available.

6. A description of any investigational medicinal product to be used in the trial.

7. The name and address of the person responsible for the manufacture or importation of any finished investigational medicinal product to be used in the trial and the details of any authorisation referred to in Article 13 of the Directive held by that person.

8.—(1) The address of any premises at which any batch of finished investigational medicinal products to be used in the clinical trial has been, or is to be, checked in accordance with Article 13(3) of the Directive.

(2) If an investigational medicinal product to be used in the clinical trial has been, or is to be, imported from a third country, a statement from the qualified person at the disposal of the person holding the authorisation referred to in Article 13 of the Directive in relation to that importation specifying—

(a) the address of any premises outside the European Economic Area at which the product was manufactured or assembled; and

(b) the manufacturing or assembling operations performed at those premises.

9. A description of the proposed clinical trial.

10. The protocol for the proposed trial.

11.—(1) Subject to sub-paragraph (7), a dossier on each investigational medicinal product to be used in the trial ("investigational medicinal product dossier"), compiled in accordance with the following sub-paragraphs.

(2) In all cases the dossier must contain a summary assessment of the potential risks and benefits of the use of the product in the proposed trial.

(3) In the case of an investigational medicinal product, other than a product referred to in sub-paragraphs (4) to (7), the dossier must contain—

(a) summaries of the chemical, pharmaceutical and biological data on the active substance and the finished product;

(b) summaries of the non-clinical pharmacology and toxicology data on that product, if available; and

(c) summaries of the available data from previous clinical trials of, and human experience with, that product.

(4) In the case of an investigational medicinal product which has a marketing authorization, the dossier must contain—

(a) a copy of the summary of product characteristics;

(b) if there has been a change—

(i) to the process of manufacture of the product or its active substance, or

(ii) of manufacturer of that product or substance,

the summaries referred to in sub-paragraph (3)(a);

(c) if the product is to be used in the trial after it has been blinded, the summaries referred to in sub-paragraph (3)(a), in so far as they relate to the blinded product; and

(d) if the product is to be used other than in accordance with the terms of the summary of product characteristics under that authorization, the summaries referred to in sub-paragraphs (3)(b) and (c), in so far as that data relates to such use.

(5) In the case of an investigational medicinal product which does not have a marketing authorization, but where—

(a) another pharmaceutical form or strength of that product has a marketing authorization; and

(b) the investigational medicinal product is supplied by the holder of that authorization,

the dossier must contain the summaries referred to in sub-paragraph (3)(a), in so far as they relate to the finished product to be used in the trial, and the summaries referred to in sub-paragraph (3)(b) and (c), in so far as they relate to the product to be used in the trial.

(6) In the case of an investigational medicinal product which does not have a marketing authorization, but where—

(a) another medicinal product containing the same active substance has a marketing authorization; and

(b) the investigational medicinal product is supplied by the manufacturer of that other product,

the dossier must contain the summaries referred to in sub-paragraph (3)(a), in so far as they relate to that other product, and the summaries referred to in sub-paragraph (3)(b) and (c), in so far as they relate to the product to be used in the trial.

(7) Where the investigational medicinal product is a placebo, the dossier must contain the summaries referred to in sub-paragraph (3)(a), in so far as they relate to that product.

(8) A dossier relating to an investigational medicinal product is not required if—

(a) the product has been used in a clinical trial that has been authorised, or is to be treated as having been authorised, by the licensing authority for the purposes of these Regulations; and

(b) the sponsor of that trial authorises the licensing authority to refer to the dossier submitted in relation to that trial.

12. A description or sample of the labelling which is to appear on each investigational medicinal product when supplied to a subject in the trial.

PART 3

NOTICE OF AMENDMENT

1. The name and address of—

(a) the sponsor,

(b) if the sponsor is not established in the European Community, his legal representative, and

(c) if any person has been authorised by the sponsor to send the notice on his behalf, that person.

2. Particulars identifying the trial, including—

(a) the title of the trial; and

(b) the number allocated to the trial on the European database referred to in Article 11 of the Directive.

3. A description of the proposed amendment.

4. A statement of the reasons for proposing that amendment.

5. A copy of the proposed changes to—

(a) the clinical trial protocol; or

(b) any other particulars or documents accompanying the request for authorisation or the application for an ethics committee opinion.

6. Summaries of—

(a) any data submitted in support of the proposed amendment; and

(b) any change to the assessment referred to in paragraph 11(2) of Part 2.

PART 4

NOTIFICATION OF CONCLUSION OF A CLINICAL TRIAL

1. The name and address of—

(a) the sponsor, and

(b) if the sponsor is not established in the European Community, his legal representative.

2. Particulars identifying the trial, including—

(a) the title of the trial; and

(b) the number allocated to the trial on the European database referred to in Article 11 of the Directive.

3. The investigational medicinal product tested in the trial.

4.—(1) The date on which the trial ended in the United Kingdom.

(2) If the trial was conducted at more than one trial site in the United Kingdom, the dates on which the trial was ended at those sites, if different from the date referred to in sub-paragraph (1).

(3) If the trial was conducted at any trial sites outside the United Kingdom, a statement as to whether the trial has ended at any of those sites and, if so, the date on which the trial was so ended.

5. If the trial is terminated as specified in regulation 27(2), the reasons for terminating the trial early.

SCHEDULE 4 Regulation 16(6)

APPEAL AGAINST UNFAVOURABLE ETHICS COMMITTEE OPINION

1.—(1) Subject to the following sub-paragraphs, where the United Kingdom Ethics Committee Authority ("the Authority") receive a notice pursuant to regulation 16(3) or (7) that a chief investigator wishes to appeal against an ethics committee opinion which is not favourable, the Authority shall, subject to sub-paragraph (2)—

(a) direct that the application for that opinion may be considered by another ethics committee specified in the direction; or

(b) appoint a panel in accordance with paragraph 3 ("an appeal panel") and refer the opinion to that panel.

(2) Subject to sub-paragraph (4), the Authority may refuse to give a direction or appoint a panel pursuant to sub-paragraph (1) where it considers that the grounds for appealing against the opinion are unfounded.

(3) Where the Authority refuse to give a direction or appoint a panel pursuant to sub-paragraph (1), the Authority shall send a notice to the chief investigator setting out their reasons for refusal.

(4) Where the opinion was given by the Gene Therapy Advisory Committee, the Authority must appoint a panel in accordance with paragraph 3 and refer the opinion to that panel.

2. Where a direction is given in accordance with paragraph 1(1)(a)—

(a) the ethics committee which gave the unfavourable opinion shall—

(i) send the application for that opinion, and

(ii) any additional information provided by the chief investigator,

to the ethics committee specified in the direction; and

(b) that committee shall consider the application in accordance with regulation 15.

3.—(1) An appeal panel appointed pursuant to paragraph 1(1)(b) shall consist of a chairman and at least 6 other members.

(2) One of the members shall be a person who is not—

(a) a health care professional,

(b) a person having professional qualifications or experience relating to the conduct of, or use of statistics in, clinical trials, unless those professional qualifications or experience relate only to the ethics of clinical research or medical treatment, or

(c) a person who, although not a health care professional, has been a registered medical practitioner or a person registered in the dentists register under the Dentists Act 1984.

4.—(1) An appeal panel shall consider an ethics committee opinion referred to it in accordance with the following sub-paragraphs.

(2) The appeal panel shall consider—

(a) the opinion;

(b) the application for that opinion;

(c) the particulars and documents accompanying that application;

(d) the matters specified in regulation 15(6);

(e) any representations set out in the notice to the Authority; and

(f) in a case where the opinion has been confirmed by the Gene Therapy Advisory Committee on a review pursuant to regulation 16(5), the reasons given by the Committee for that confirmation.

(3) The panel may, if the chief investigator so requests, hold a hearing to consider the opinion, at which the chief investigator may make oral representations.

(4) The panel shall within 30 days of the opinion being referred to the panel, or such extended period as the Authority may in any particular case allow, either confirm the opinion or give a favourable opinion.

5. If an appeal panel gives a favourable opinion, the condition specified in regulation 11(3)(a) shall be deemed to have been satisfied.

6. The Authority may pay to members of an appeal panel such travelling and other allowances as the Authority may determine.

SCHEDULE 5 Regulations 26(2) and 31(8)

PROCEDURAL PROVISIONS RELATING TO THE REFUSAL OR AMENDMENT OF, OR IMPOSITION OF CONDITIONS RELATING TO, CLINICAL TRIAL AUTHORISATION AND THE SUSPENSION OR TERMINATION OF CLINICAL TRIALS

1.—(1) Where the licensing authority are notified of the sponsor's wish to make representations in accordance with regulation 26(1) or 31(7) the authority shall afford an opportunity for the sponsor to make written or oral representations to the appropriate committee or, if for the time being there is no such committee, the Medicines Commission.

(2) After considering the representations, the appropriate committee or the Medicines Commission shall report their findings and advice, and the reasons for their advice, to the licensing authority.

(3) In the case of a decision not to accept a request for authorisation or an amendment to the clinical trial authorisation, the licensing authority shall, after considering the report of the appropriate committee or the Commission—

(a) confirm that they have grounds for not accepting the request or amendment; or

(b) accept the request for authorisation or amendment to the clinical trial authorisation, subject to such conditions as the licensing authority may consider appropriate.

(4) In the case of a decision to impose a condition following a request for authorisation or notice of amendment, the licensing authority shall, after considering the report of the appropriate committee or the Commission—

(a) confirm their decision; or

(b) remove or alter the condition in question.

(5) In the case of a notice to suspend or terminate a trial, the licensing authority shall, after considering the report of the appropriate committee or the Commission, confirm or revoke the notice.

(6) The licensing authority shall give notice to the sponsor of—

(a) the findings and advice of the appropriate committee or the Medicines Commission and the reasons for it; and

(b) their decision in accordance with sub-paragraphs (3), (4) or (5).

2.—(1) If a person to whom a notice is given under paragraph 1(6) is dissatisfied and he has not made representations to the Medicines Commission under paragraph 1(1), he may give notice in writing to the licensing authority within 28 days, or such extended period as the licensing authority may in any particular case allow, of the notice being given of his wish to make written or oral representations to the Medicines Commission.

(2) On receipt of a notice under sub-paragraph (1) the licensing authority shall afford an opportunity for the sponsor to be heard by the Medicines Commission or, as the case may be, for his written representations to be considered by them.

(3) After considering the representations the Medicines Commission shall report their findings and advice, and the reasons for their advice, to the licensing authority.

(4) After considering the report of the Medicines Commission, the licensing authority shall—

(a) confirm or alter their decision under paragraph 1(3), (4) or (5); and

(b) give notice to the person of—

(i) the findings and advice of the Medicines Commission and the reasons for it, and

(ii) the licensing authority's confirmation or alteration of their decision under paragraph 1(3) to (5).

3.—(1) If a decision notified in accordance with paragraphs 1(6) or 2(4) is a decision to which this paragraph applies, the sponsor may within the time allowed after the notification was given, give notice of his wish to appear before and be heard by a person appointed for the purpose by the licensing authority, or of making representations in writing to the licensing authority with respect to the decision referred to in the notification.

(2) Where the sponsor gives notice under sub-paragraph (1) of his wish to appear before and be heard by a person appointed for the purpose by the licensing authority, the licensing authority shall make that appointment and—

(a) the person so appointed shall not, except with the consent of the applicant or holder, be an officer or servant of any of the Ministers specified in paragraphs (a) and (b) of section 1(1) of the Act;

(b) if the applicant or holder so requests, the hearing shall be in public; and

(c) if the applicant or holder so requests, the licensing authority shall furnish to him a copy of the report of the person so appointed.

(3) The licensing authority shall take into account the report of the person appointed and decide whether to confirm or alter their decision.

(4) The decisions to which this paragraph applies are decisions of the licensing authority—

(a) to confirm—

(i) that they have grounds for not accepting a request for authorisation or an amendment to the clinical trial authorisation,

(ii) their decision to impose a condition, or

(iii) the notice to suspend or terminate the trial,

against the advice of the Medicines Commission under paragraph 1(2);

(b) to impose conditions in accordance with paragraph 1(3)(b) or alter a condition in accordance with paragraph 1(4)(b), in a way which differs from the advice given by the Medicines Commission under paragraph 1(2); or

(c) to confirm a decision under paragraph 1(3), (4) or (5) against the advice of the Medicines Commission under paragraph 2(3);

(d) to alter a decision under paragraph 1(3), (4) or (5) in a way which differs from the advice of the Medicines Commission under paragraph 2(3).

SCHEDULE 6

Regulation 38(3)

PARTICULARS THAT MUST ACCOMPANY AN APPLICATION FOR A MANUFACTURING AUTHORISATION

1. The name and address of the applicant, and, where the applicant is not the proposed holder of the authorisation, the name and address of the proposed holder.

2. A statement of the types of investigational medicinal products in respect of which the authorisation is required.

3. A statement of the manufacturing, assembling or importation operations to which the authorisation is to relate, including a statement whether they include one or more of the following—

(a) the manufacture of investigational medicinal products;

(b) the assembly of investigational medicinal products; or

(c) the importation of investigational medicinal products.

4.—(1) The address of each of the premises where the manufacturing, assembling or importation operations to which the application relates, including any testing associated with manufacture, assembly or import, are or are to be carried out.

(2) The address of each of the premises where the proposed holder of the authorisation proposes to store investigational medicinal products or from which he proposes to distribute them.

(3) A statement indicating the facilities and equipment available at each of the premises referred to in sub-paragraphs (1) and (2), for storing the investigational medicinal products on, and distributing them from or between, such premises.

(4) A separate statement in respect of each of the premises referred to in sub-paragraphs (1) and (2), of the manufacturing, assembling or importation operations capable of being carried out at those premises with their existing facilities. Each statement shall specify the classes of investigational medicinal products to which the operations are relevant.

(5) A separate statement in respect of each of the premises referred to in sub-paragraphs (1) and (2), of the facilities and equipment available at those premises for carrying out each stage of the manufacturing, assembling or importation operations described in sub-paragraph (4) of this paragraph.

5. A statement of any manufacturing operations, other than those to which the manufacturing authorisation is to relate, that are carried on by the proposed authorisation holder on or near each of the premises referred to in paragraph 4, and of the substances or articles which are the subject of any such operation.

6.—(1) The name and address and qualifications and experience of the qualified person who is to carry out the duties referred to in regulation 43(2).

(2) In the case of an authorisation relating to manufacture or assembly, the name and qualifications and experience of the production manager or other person whose duty it will be to supervise the production operations at each of the premises referred to in paragraph 4 of this Schedule, and the name and function of the person to whom he is responsible.

(3) In the case of an authorisation relating to manufacture or assembly—

(a) the name and degrees, diplomas or other qualifications and experience of the person to be in charge of quality control over all the premises referred to in paragraph 4 of this Schedule;

(b) the extent of the authority to be delegated to him to reject unsatisfactory batches of investigational medicinal products, and

(c) the name and function of the person to whom he is responsible.

7. A description of the arrangements for the identification and storage of materials and ingredients before and during manufacture and for the storage of investigational medicinal products after manufacture, assembly or importation.

8. A description of the arrangements at each of the premises where the holder of the authorisation stores or proposes to store investigational medicinal products for ensuring, so far as practicable, whether by maintaining records or other means, a satisfactory turn-over of stocks of investigational medicinal products.

9. A description of the arrangements—

(a) for maintaining production or importation records;

(b) for maintaining records of analytical and other testing procedures applied in the course of manufacture, assembly or importation for ensuring compliance of materials used in the manufacture of any investigational medicinal products with the specification of such materials or medicinal products; and

(c) for keeping reference samples of materials used in the manufacture of any investigational medicinal products and of the investigational medicinal products.

SCHEDULE 7

Regulation 40(4)

STANDARD PROVISIONS FOR MANUFACTURING AUTHORISATIONS

PART 1

INTERPRETATION

In this Schedule, "product specification" means—

(a) in the case of an investigational medicinal product manufactured before a request for authorisation to conduct the clinical trial involving those products has been made in accordance with regulation 17 or any equivalent provisions in any EEA State other than the United Kingdom, the specification for that product provided by the person who is to act as the sponsor of the proposed clinical trial,

(b) in the case of an investigational medicinal product manufactured for the purpose of export, the specification for that product provided by the person to whose order the products are manufactured, or

(c) in any other case, the specification for an investigational medicinal product contained in the investigational medicinal product dossier for that product.

PART 2

PROVISIONS WHICH MAY BE INCORPORATED IN AN AUTHORISATION RELATING TO THE MANUFACTURE OR ASSEMBLY OF INVESTIGATIONAL MEDICINAL PRODUCTS

1. The holder of the authorisation shall—

(a) provide and maintain such staff, premises and plant (including technical equipment) as are necessary for the carrying out, in accordance with his authorisation and the product specification, of such stages of the manufacture and assembly of the investigational medicinal products as are undertaken by him; and

(b) not carry out any such manufacture or assembly except at the premises specified in his manufacturing authorisation.

2. The holder of the authorisation shall—

(a) provide and maintain such staff, premises, equipment and facilities for the handling, storage and distribution of the investigational medicinal products which he handles, stores or distributes under his authorisation as are necessary to maintain the quality of the investigational medicinal products;

(b) not use for such purposes premises other than those specified in the authorisation or which may be approved from time to time by the licensing authority; and

(c) ensure that any arrangements he makes with a person for the storage and distribution of the investigational medicinal products are adequate to maintain the quality of those products.

3. The holder of the authorisation shall place the quality control system referred to in Article 11(1) of Commission Directive 2003/94/EC under the authority of the person notified to the licensing authority in accordance with paragraph 6(3) of Schedule 6 as being responsible for quality control.

4. The holder of the authorisation may use a contract laboratory pursuant to Article 11(2) of Commission Directive 2003/94/EC if operated by a person approved by the licensing authority.

5. The holder of the authorisation shall provide such information as may be requested by the licensing authority for the purposes of these Regulations or the Act—

(a) about the products currently being manufactured or assembled under his authorisation; and

(b) of the operations being carried out in relation to such manufacture or assembly.

6. The holder of the authorisation shall—

(a) inform the licensing authority before making any material alteration in the premises or plant used under his authorisation, or in the operations for which they are used; and

(b) inform the licensing authority of any change that he proposes to make in any personnel named in his authorisation as respectively—

(i) responsible for supervising the production operations, or

(ii) responsible for quality control of the investigational medicinal products being manufactured or assembled including the person named as the qualified person for the purposes of regulation 43 and paragraph 14.

7. The holder of the authorisation shall—

(a) keep readily available for inspection by a person authorised by the licensing authority the batch documentation referred to in Article 9(1) of Commission Directive 2003/94/EC; and

(b) permit the person authorised to take copies or make extracts from such documentation.

8. The holder of the authorisation shall keep readily available for examination by a person authorised by the licensing authority the samples of each batch of bulk formulated products referred to in Article 11(4) of Commission Directive 2003/94/EC.

9. Where the holder of the authorisation has been informed by the licensing authority that any batch of any investigational medicinal product to which his authorisation relates has been found not to conform as regards strength, quality or purity with—

(a) the specification of the relevant product; or

(b) the provisions of these Regulations, the Act or any regulations under the Act that are applicable to the investigational medicinal product,

he shall, if so directed, withhold such batch from distribution for use in clinical trials, so far as may be reasonably practicable, for such a period not exceeding six weeks as may be specified by the licensing authority.

10. The holder of the authorisation shall ensure that any tests for determining conformity with the standards and specifications applying to any particular product used in the manufacture shall, except so far as the conditions of the product specification for that product otherwise provide, be applied to samples taken from the investigational medicinal product after all manufacturing processes have been completed, or at such earlier stage in the manufacture as may be approved by the licensing authority.

11. Where the authorisation relates to the assembly of an investigational medicinal product, and the holder of the authorisation supplies that investigational medicinal product at such a stage of assembly that does not fully comply with the provisions of the product specification that relate to labelling, that holder of the authorisation shall communicate the particulars of those provisions to the person to whom that investigational medicinal product has been so supplied.

12. Where—

(a) the manufacturing authorisation relates to the assembly of an investigational medicinal product;

(b) that investigational medicinal product is not manufactured by the holder of the authorisation; and

(c) particulars as to the name and address of the manufacturer of, or of the person who imports, that investigational medicinal product had been given by the holder of the authorisation to the licensing authority,

the holder of the authorisation shall forthwith notify the licensing authority in writing of any changes in such particulars.

13. The holder of the authorisation, for the purpose of enabling the licensing authority to ascertain whether there are any grounds—

(a) for suspending, revoking or varying any authorisation or licence granted under these Regulations or Part II of the Act;

(b) amending the clinical trial authorisation in accordance with regulation 23 or 24; or

(c) suspending or terminating any clinical trial in accordance with regulation 31,

shall permit, and provide all necessary facilities to enable, any person duly authorised in writing by the licensing authority, on production if required of his credentials, to carry out such inspection or to take such samples or copies, in relation to things belonging to, or any business carried on by, the holder of the authorisation, as such person would have the right to carry out or take under the Act for the purpose of verifying any statement contained in an application for an authorisation or licence.

14. The holder of the authorisation shall at all times provide and maintain such staff, premises, equipment and facilities as will enable the qualified person who is at his disposal pursuant to regulation 43(1) to carry out the duties referred to in regulation 43(2).

PART 3

PROVISIONS WHICH MAY BE INCORPORATED IN AN AUTHORISATION RELATING TO THE IMPORTATION OF INVESTIGATIONAL MEDICINAL PRODUCTS

1. The holder of the authorisation shall—

(a) provide and maintain such staff, premises, equipment and facilities for the handling, storage and distribution of the investigational medicinal products which he handles, stores or distributes under his authorisation as are necessary to avoid deterioration of the investigational medicinal products;

(b) not use for such purposes premises other than those specified in the authorisation or which may be approved from time to time by the licensing authority; and

(c) ensure that any arrangements he makes with a person for the storage and distribution of the investigational medicinal products are adequate to maintain the quality of those products.

2. The holder of the authorisation may use a contract laboratory pursuant to Article 11(2) of Commission Directive 2003/94/EC if operated by a person approved by the licensing authority.

3. The holder of the authorisation shall provide such information as may be requested by the licensing authority concerning the type and quantity of any investigational medicinal products which he imports.

4. The holder of the authorisation shall—

(a) inform the licensing authority before making any structural alterations to, or discontinuance of the use of, premises to which his authorisation relates; and

(b) inform the licensing authority if he changes the person named as the qualified person for the purposes of regulation 43 and paragraph 9.

5. The holder of the authorisation shall—

(a) keep readily available for inspection by a person authorised by the licensing authority the batch documentation referred to in Article 9(1) of Commission Directive 2003/94/EC; and

(b) permit the person authorised to take copies or make extracts from such documentation.

6. Where the holder of the authorisation has been informed by the licensing authority that any batch of any investigational medicinal product to which his authorisation relates has been found not to conform as regards strength, quality or purity with—

(a) the specification of the relevant product; or

(b) the provisions of these Regulations, the Act or any regulations under the Act that are applicable to the investigational medicinal product,

he shall, if so directed, withhold such batch from distribution for use in clinical trials, so far as may be reasonably practicable, for such a period not exceeding six weeks as may be specified by the licensing authority.

7. If the holder of the authorisation is not the sponsor of the clinical trial for which the investigational medicinal product is manufactured or assembled, he shall comply with the provisions of the product specification that relates to the supply of that investigational medicinal product for use in the trial.

8. The holder of the authorisation, for the purpose of enabling the licensing authority to ascertain whether there are any grounds—

(a) for suspending, revoking or varying any authorisation or licence granted under these Regulations or Part II of the Act;

(b) amending the conduct of a clinical trial in accordance with regulation 23 or 24; or

(c) suspending or terminating any clinical trial in accordance with regulation 31,

shall permit, and provide all necessary facilities to enable, any person duly authorised in writing by the licensing authority, on production if required of his credentials, to carry out such inspection or to take such samples or copies, in relation to things belonging to, or any business carried on by, the holder of the authorisation, as such person would have the right to carry out or take under the Act for the purpose of verifying any statement contained in an application for an authorisation or licence.

9. The holder of the authorisation shall at all times provide and maintain such staff, premises, equipment and facilities as will enable the qualified person who is at his disposal pursuant to regulation 43(1) to carry out the duties referred to in regulation 43(2).

SCHEDULE 8 Regulations 40(5) and 44(6)

PROCEDURAL PROVISIONS RELATING TO PROPOSALS TO GRANT, REFUSE TO GRANT, VARY, SUSPEND OR REVOKE MANUFACTURING AUTHORISATIONS

1. In this Schedule—

"authorisation" means a manufacturing authorisation; and

"time allowed" means the period of 28 days or such extended period as the licensing authority may in any particular case allow.

2. Subject to paragraph 6, if the licensing authority propose—

(a) not to grant an authorisation;

(b) to grant an authorisation other than in accordance with the application; or

(c) to revoke, vary or suspend an authorisation,

the licensing authority shall notify the applicant or holder accordingly.

3. Any notification given under paragraph 2 shall include a statement of the proposals of the licensing authority and of the reasons for them.

4. A person to whom notification has been given under paragraph 2 may, within the time allowed after the notification was given, give notice of his wish to appear before and be heard by a person appointed for the purpose by the licensing authority, or of making representations in writing to the licensing authority with respect to the decision or proposal referred to in the notification.

5.—(1) Where an applicant or the holder gives notice under paragraph 4 of his wish to appear before and be heard by a person appointed for the purpose by the licensing authority, the licensing authority shall make that appointment and—

(a) the person so appointed shall not, except with the consent of the applicant or holder, be an officer or servant of any of the Ministers specified in paragraphs (a) and (b) of section 1(1) of the Act;

(b) if the applicant or holder so requests, the hearing shall be in public; and

(c) if the applicant or holder so requests, the licensing authority shall furnish to him a copy of the report of the person so appointed.

(2) The licensing authority shall take into account the report of the person appointed and decide whether to grant the authorisation, revoke, vary or suspend the authorisation or confirm or alter their decision, as the case may be.

6.—(1) Paragraph 2 shall not apply to the suspension of an authorisation where it appears to the licensing authority that, in the interests of safety, it is necessary to suspend the authorisation with immediate effect for a period not exceeding 3 months.

(2) If, after the suspension has taken effect, it appears to the licensing authority that the authorisation should be further suspended or revoked, the licensing authority shall proceed in accordance with the provisions of paragraphs 2 to 5.

SCHEDULE 9 Regulation 47(1)

MODIFICATIONS OF THE ENFORCEMENT PROVISIONS OF THE ACT SUBJECT TO WHICH THOSE PROVISIONS ARE APPLIED FOR THE PURPOSES OF THESE REGULATIONS

1. The modifications of the Act mentioned in regulation 47 are as follows.

2.—(1) Amendments in section 107 (validity of decisions and related proceedings) as follows.

(2) In subsection (1), for "Part II of this or of a Minister under section 75 of this Act, and the validity of any licence or certificate" substitute "the Clinical Trials Regulations, and the validity of any authorisation".

(3) In subsections (2)(a) and (3)(b), for "this Act" substitute "the Clinical Trials Regulations".

(4) In subsection (2)(b), for "this Act or of any regulations made under this Act" substitute "the Clinical Trials Regulations".

(5) In subsection (4) (effect of quashing a decisions) substitute—

"(4) Subsections (4A) and (4B) of this section apply where a decision—

(a) to grant or issue an authorisation, or

(b) to give notice accepting a request for an authorisation, is quashed under this section.

(4A) Any authorisation granted or issued, or notice given, in pursuance of the decision shall be void.

(4B) Any proceedings on the application, or request, for the authorisation may be continued as if not such decision had been made.".

3. For section 108 (enforcement in England and Wales) substitute—

"(1) It shall be the duty of the Secretary of State to enforce in England, or to secure the enforcement in England of, the provisions of the Clinical Trials Regulations.

(2) It shall be the duty of the National Assembly for Wales to enforce in Wales, or to secure the enforcement in Wales of, the provisions of the Clinical Trials Regulations ".

4. In section 109 (enforcement in Scotland), for subsections (1) to (3), substitute—

"(1) It shall be the duty of the Scottish Ministers to enforce in Scotland, or to secure the enforcement in Scotland of, the provisions of the Clinical Trials Regulations.".

5. For section 110 (enforcement in Northern Ireland) substitute—

"It shall be the duty of the Department for Health, Social Services and Public Safety to enforce in Northern Ireland, or to secure the enforcement in England and Wales of, the provisions of the Clinical Trials Regulations.".

6.—(1) Amendments in section 111 (rights of entry) as follows.

(2) In subsection (1)(a) (entry to ascertain whether Act etc. contravened), for the words after "contravention" substitute "of any provisions of the Clinical Trials Regulations, or".

(3) In subsection (1)(b) (entry for purposes of functions under the Act etc.), for "this Act or any such regulations or order" substitute "those Regulations or any of the provisions of this Act applied by regulation 47 or those Regulations".

(4) In subsection (2)(a) (right to enter craft), for the words from "this Act or of any regulations" onwards substitute "the Clinical Trials Regulations;".

(5) In subsection (3) (rights to enter premises conferred on persons authorised by licensing authority)—

(a) for "an applicant for a licence under Part II of this Act" substitute "a person applying for or requesting an authorisation under the Clinical Trials Regulations", and

(b) for "application for the licence or certificate" substitute "application or request for the authorisation".

7.—(1) Amendments in section 112 (power to inspect, take samples and seize goods and documents) as follows.

(2) In subsection (1) (inspection for purpose of ascertaining whether Act etc. contravened), for the words before paragraph (a) substitute—

"For the purposes of ascertaining whether there is or has been a contravention of the Clinical Trials Regulations, any person duly authorised in writing by an enforcement authority shall have a right to inspect—".

(3) In subsection (2) (items of which samples may be taken), before the word "or" at the end of paragraph (a) insert—

"(aa) a medicinal product used or intended to be used in a clinical trial,".

(4) In subsection (3) (right to require production etc. of books and documents), after paragraph (a) insert—

"(a) to require—

(i) the sponsor of a clinical trial,

(ii) any person who, under arrangements made with the sponsor of a clinical trial, carries out functions of the sponsor of the trial,

(iii) an investigator for a trial,

(iv) any person, other than an investigator, who conducts a trial,

(v) any person occupying premises at which a clinical trial is being conducted, or

(vi) any person who, in the courses in the course of their employment with a person of a description specified in any of sub-paragraphs (i) to (v) of this paragraph, undertakes activities in connection with a clinical trial,

to produce any books or documents relating to the clinical trial which are in his possession or under his control;".

(5) In subsection (3)(b) (powers to take copies of documents produced), for "the preceding paragraph" substitute "paragraph (a) or (aa) of this subsection;".

(6) In subsection (3), after paragraph (b) insert—

"(c) to take possession of any book or document produced under paragraph (a) or (aa) of this subsection.".

(7) In subsection (4) (right to seize items and documents), for "offence under this Act is" substitute—

"offence—

(a) under the Clinical Trials Regulations, or

(b) under section 114, 118 or 123 of this Act,

is".

(8) In that subsection, for "under this Act" (in the second place) substitute "under those Regulations or under any of the provisions of this Act applied by regulation 47 of those Regulations".

(9) In subsection (5) (opening of containers), for "this Act and any regulations or order made thereunder" substitute "the Clinical Trials Regulations".

(10) In subsection (7) (rights of persons authorised by licensing authority)—

(a) for "a licence or certificate under Part II of this Act" substitute "an authorisation under the Clinical Trials Regulations", and

(b) for "the application for the licence or certificate" substitute "the application or request for the authorisation".

(11) In subsection (9) (Schedule 3 to have effect in relation to samples obtained for purposes of the Act), for "this Act" (in the second place) substitute "the Clinical Trials Regulations".

(12) After subsection (9) insert—

"(10) In this section "clinical trial", and "investigator" and "sponsor" in relation to a clinical trial, have the meaning given by the Clinical Trials Regulations".

8.—(1) Amendments in section 115 (analysis of samples) as follows.

(2) In subsection (7) (certificate to be in prescribed form and signed), for "form prescribed by the Ministers" substitute "prescribed form".

(3) Omit subsection (9) (regulations under subsection (5) to be made by the Ministers).

9.—(1) Amendments in section 116 (liability to forfeiture under the Customs and Excise Management Act 1979) as follows.

(2) For "this Act" (in both places) substitute "the Clinical Trials Regulations".

(3) After subsection (3) insert—

"(4) In this section "the Ministers" means the Secretary of State and the Department for Health, Social Services and Public Safety, acting jointly.".

10. In section 118(1)(b) (restrictions on disclosing of information obtained in pursuance of the Act), for "this Act" substitute "the Clinical Trials Regulations or any provision of this Act applied by regulation 47 of those Regulations".

11.—(1) Amendments in section 119 (protection for officers of enforcement authorities) as follows.

(2) In each of subsections (1) and (2) (relief from personal liability and power of authority to indemnify officer)—

(a) for "this Act" (in the first place) substitute "relevant legislation", and

(b) for "this Act" (in the second place) substitute "that legislation".

(3) In subsection (3) (meaning of "officer"), for "this Act" substitute "relevant legislation".

(4) After subsection (3) insert—

"(4) In this section "relevant legislation" means—

(a) the Clinical Trials Regulations, or

(b) any provision of this Act applied by regulation 47 of those Regulations".

12.—(1) Amendments in section 121 (contravention due to fault of other person) as follows.

(2) In subsection (1) (where a person is guilty of an offence due to act or default of another, the other is also guilty of the offence), for "to which this section applies constitutes an offence under this Act" substitute "of the Clinical Trials Regulations constitutes an offence under those Regulations".

(3) In subsection (2) (defence of due diligence where contravention due to act or default of another), for "this Act in respect of a contravention of a provision to which this section applies" substitute "the Clinical Trials Regulations in respect of a contravention of a provision of those Regulations".

(4) Omit subsection (4) (provisions to which section applies).

13.—(1) Amendments in section 122 (warranty as defence) as follows.

(2) In subsection (1), for "this Act in respect of a contravention of a provision to which this section applies" substitute "the Clinical Trials Regulations in respect of a contravention of regulation 46 of those Regulations".

(3) Omit subsection (2) (provisions to which section applies).

14.—(1) Amendments in section 124 (offences by bodies corporate) as follows.

(2) In subsection (1), for "this Act" substitute "the Clinical Trials Regulations, or under section 114, 118 or 123 of this Act,".

(3) After subsection (2) insert—

"(2A) In subsections (1) and (2) of this section "body corporate" includes a Scottish partnership and "director", in relation to such a partnership, includes any of its partners".

15.—(1) Amendments in section 125 (prosecutions) as follows.

(2) In each of subsections (1) and (2) (time limits in England and Wales, and in Scotland), for "under this Act" substitute "under the Clinical Trials Regulations, or for an offence under section 114, 118 or 123 of this Act,".

(3) Omit subsections (3) to (7).

16. In section 127 (service of documents)—

(a) for "any provision of this Act" substitute "relevant legislation", and

(b) at the end add—

"In this section "relevant legislation" means any provision of the Clinical Trials Regulations or any provision of this Act applied by regulation 47 of those Regulations".

17.—(1) Amendments in section 129 (orders and regulations) as follows.

(2) Omit subsection (1) (powers to make regulations exercisable by the Ministers where not expressed to be otherwise exercisable).

(3) In subsection (2) (powers to make orders and regulations under the Act exercisable by statutory instrument), for the words from "this Act (other" to "section 120 of this Act)" substitute "any provision of this Act applied by regulation 47 of the Clinical Trials Regulations".

(4) In subsection (3) (instruments which are subject to negative procedure), for paragraphs (a) to (c) substitute "an order or regulations made under any provision of this Act applied by regulation 46 of the Clinical Trials Regulations".

(5) In subsection (4) (powers to make orders that include power to revoke or vary), for the words from ", other than" to "69(3), of this Act" substitute "of this Act applied by regulation 47 of the Clinical Trials Regulations".

(6) In subsection (5) (powers to make regulations include power to make differential provision etc.), for "this Act" substitute "any provision of this Act applied by regulation 47 of the Clinical Trials Regulations".

(7) In subsection (6) (duty to consult), for the words from the beginning to "effect) the Ministers" substitute—

"Before making any regulations or order under any provision of this Act applied by regulation 47 of the Clinical Trials Regulations, the persons".

(8) Omit subsections (6A) and (7) (which apply only in relation to veterinary products or instruments made otherwise than under Part 8 of the Act).

18.—(1) Amendments in section 131 (meaning of "wholesale dealing" and "retail sale" etc).

(2) Omit subsection (1) (meaning of "wholesale dealing").

(3) In subsection (2) (purposes referred to in subsections (1) and (3)), for "the preceding subsection" substitute "subsection (3) of this section".

(4) In subsection (3) (meaning of "retail sale"), for "In this Act" substitute "In the provisions of this Act applied by regulation 47 of the Clinical Trials Regulations,".

(5) Omit subsection (4) (meaning of "supply in circumstances corresponding to retail sale").

19.—(1) Amendments in section 132(1) (interpretation) as follows.

(2) At the beginning, for "In this Act," substitute "In the provisions of this Act applied by regulation 47 of the Clinical Trials Regulations,".

(3) In the definition of "enforcement authority", for the words from "power" to "or under" substitute "to enforce the provisions of the Clinical Trials Regulations is imposed by".

(4) After the definition of "manufacture" insert—

""medicinal product" means—

(a) anything that is a medicinal product within the meaning given by Article 1 of Directive 2001/83/EC, and

(b) anything that is an investigational medical product for the purposes of the Clinical Trials Regulations;".

(5) Omit the definition of "offence under this Act".

(6) For the definition of "prescribed" substitute—

""prescribed" means prescribed by regulations made by the Secretary of State and the Department for Health, Social Services and Public Safety, acting jointly;".

20.—(1) Amendments in Schedule 3 (sampling) as follows.

(2) In paragraph 1(1)(a), for the words from "this Act or of any regulations" onwards substitute "the Clinical Trials Regulations, or".

(3) In paragraph 1(1)(b), for the words from "of their functions" onwards substitute "(in this Schedule referred to as "the relevant enforcement authority") of their functions under those Regulations or under any provision of this Act applied by regulation 47 of those Regulations,".

(4) In paragraph 16, omit "the relevant enforcement officer is a Minister or the Pharmaceutical Society, and".

(5) Omit paragraph 17.

(6) In paragraph 19(3), for "form prescribed by the Ministers" substitute "prescribed form".

(7) Omit paragraph 20(2).

(8) In each of paragraphs 21 and 22, for "under this Act" substitute "under the Clinical Trials Regulations, or under section 114, 118 or 123 of this Act,".

(9) In paragraph 24(1), for "under this Act," substitute "under the Clinical Trials Regulations or under section 114, 118 or 123 of this Act,".

(10) In paragraph 27 (power to apply Schedule with modifications), for "Ministers" substitute "Secretary of State and the Department for Health, Social Services and Public Safety, acting jointly,".

SCHEDULE 10

Regulation 54

CONSEQUENTIAL AND OTHER AMENDMENTS OF ENACTMENTS

PART 1

ACTS OF PARLIAMENT

The Act

1.—(1) Section 3 of the Act (general functions of the Medicines Commission)(**a**) is amended as follows—

(2) In subsection (1), for the words from "advice" to "products, where" substitute—

"advice on matters—

(a) relating to the execution of this Act,

(b) relating to the exercise of any power conferred by this Act,

(c) relating to the execution of the Clinical Trials Regulations,

(d) relating to the exercise of any power conferred by those regulations, or

(e) otherwise relating to medicinal products,

where".

(3) In subsection (2), after "by or under this Act" insert "or the Clinical Trials Regulations".

(4) For subsection (2)(d) substitute—

"(d) to advise the licensing authority in cases where the authority—

(i) are required by the provisions of Part II of this Act, or by the provisions of the Clinical Trial Regulations, to consult the Commission with respect to any matter arising under those provisions; or

(ii) without being required to do so, elect to consult the Commission with respect to any matter arising under any of those provisions."

2. In section 4 of the Act (establishment of committees)(**b**), in subsection (2), for the words from "connected with" onwards substitute—

"connected with—

(a) the execution of this Act or the Clinical Trials Regulations, or

(b) the exercise of any power conferred by this Act or those regulations,

either generally or in relation to any particular class of substances or articles to which any provision of this Act or those regulations applies.".

3. In section 7 of the Act (restrictions as to dealings with medicinal products)(**c**), after subsection (3), insert the following subsection—

(**a**) Section 3 has effect as if any reference to the Act included a reference to the Medicines for Human Use (Marketing Authorisations Etc) Regulations 1994 (S.I. 1994/3144) ("the 1994 Regulations"); *see* regulation 9(1) of the 1994 Regulations.

(**b**) Section 4 has effect as if any reference to the Act included a reference to the Medicines for Human Use (Marketing Authorisations Etc) Regulations 1994 (S.I. 1994/3144) ("the 1994 Regulations"); *see* regulation 9(1) of the 1994 Regulations

(**c**) Section 7 does not apply to "relevant medicinal products" within the meaning of regulation 1(2) of the 1994 Regulations; *see* regulation 9(2) of the 1994 Regulations.

"(3A) The restrictions imposed by subsections (2) and (3) of this section shall not apply where the medicinal product concerned is an investigational medicinal product within the meaning of the Clinical Trials Regulations.".

4.—(1) Section 8 of the Act (provisions as to manufacture and wholesale dealing) shall be amended as follows.

(2) At the beginning of subsection (2), insert "Subject to subsection (2A) of this section".

(3) After subsection (2) insert the following subsections—

"(2A) In the case of a medicinal product that is an investigational medicinal product, the restrictions imposed by subsection (2) of this section only apply—

(a) if the product has a product licence or marketing authorization, and

(b) to the extent that the manufacture or assembly of the product is in accordance with the terms and conditions of that licence or authorization.

(2B) In subsection (2A) of this section—

"investigational medicinal product" has the meaning given by the Clinical Trials Regulations; and

"marketing authorization" means—

(a) a marketing authorization issued by a competent authority in accordance with Directive 2001/83/EC, or

(c) a marketing authorization granted by the European Commission under Council Regulation (EEC) 2309/93(**a**).".

(4) In subsections (3) and (3A)(**b**), for "subsection (3C)", in both places those words appear, substitute "subsections (3C) and (3D)".

(5) After subsection (3C), insert the following subsection—

"(3D) The restrictions imposed by subsections (3) and (3A) of this section do not apply where the product concerned is an investigational medicinal product within the meaning given by the Clinical Trials Regulations.".

5.—(1) Section 23 of the Act (special provisions as to the effect of manufacturer's licence)(**c**) shall be amended as follows.

(2) In subsection (1)—

(a) omit "clinical trials and";

(b) for paragraph (b), substitute the following paragraph—

"(b) the products are manufactured or assembled to the order of—

(i) a person who is the holder of such a product licence, or

(ii) if the products are to be used for the purposes of a clinical trial, the sponsor of that trial,".

(3) After subsection (5), insert the following subsection—

"(6) In this section, "clinical trial" and "sponsor", in relation to a clinical trial, have the meaning given by Clinical Trials Regulations.".

6. Section 31 of the Act shall be omitted

7.—(1) Section 35 of the Act (supplementary provisions as to clinical trials and medicinal test on animals) shall be amended as follows.

(2) In subsection (1), omit "a clinical trial certificate or".

(**a**) OJ No. L214, 24.8.1993, p.1.
(**b**) Subsections (3A) to (3C) of section 8 were inserted by regulation 2(4) of S.I. 1993/834
(**c**) Section 23 of the Act has effect as if any reference in subsection (1) to a product licence included a reference to a marketing authorization; *see* regulation 9(1) of the 1994 Regulations.

(3) In subsection (2), omit paragraph (a).

(4) In subsection (4), omit the words from the beginning to "; and".

(5) In subsection (5)—

(a) omit "a clinical trial or";

(b) for paragraph (a), substitute the following paragraph—

"(a) an animal test certificate has been issued and is for the time being in force in respect of that test, and the test is to be carried out in accordance with that certificate, and";

(c) in paragraph (b), omit "trial or".

(6) In subsection (7)—

(a) for "sections 31 and 32" substitute "section 32";

(b) omit "of a clinical trial or"; and

(c) in paragraph (a), omit "trial or".

(7) In subsection (8), omit paragraph (a).

(8) In subsection (10), omit "any of the provisions of subsections (5) to (8) of section 31 of this Act, or".

8. In section 36 of the Act (application for, and issue of, certificate)—

(a) in subsection (1), omit "a clinical trial certificate or";

(b) in subsection (2), omit "clinical trial or";

(c) in subsection (3), omit "clinical trial certificates or".

9.—(1) Section 37 of the Act (transitional provisions as to clinical trials and medicinal tests on animals) shall be amended as follows.

(2) In subsection (1), omit "31, ".

(3) In subsection (2), for "sections 31 and 32" substitute "section 32".

(4) In subsection (3)—

(a) omit paragraph (a);

(b) for "section 31 or section 32 of this Act do not apply to anything done in relation to medicinal products of that description or (as the case may be)" substitute "section 32 of the Act do not apply to anything done".

(5) In subsection (4)—

(a) omit "a clinical trial certificate or";

(b) in paragraph (a), for the words from the beginning to "so specified" substitute "substances or articles specified in the application".

10. In section 38 of the Act (duration and renewal of certificate)—

(a) in subsections (1) and (4), omit "clinical trial certificate or";

(b) in subsections (5) and (6), for "a clinical trial certificate or animal test certificate" substitute "an animal test certificate".

11. In section 39 of the Act (suspension, revocation or variation of certificate)—

(a) in subsections (1), (3) and (4), for "a clinical trial certificate or animal test certificate" substitute "an animal test certificate";

(b) in subsection (2)(c) and (e), omit "clinical trial or".

12. In section 44 of the Act (provision of information to licensing authority), in subsections (1) and (2), for "a clinical trial certificate or animal test certificate" substitute "an animal test certificate".

13. In section 45 of the Act (offences under Part II)—

(a) in subsections (1) and (2), omit "section 31,";

(b) in subsection (3), for "a clinical trial certificate or animal test certificate" substitute "an animal test certificate".

14. In section 46 of the Act (special defences under section 45), for "a clinical trial certificate or animal test certificate" (in each place) substitute "an animal test certificate".

15. In section 47 of the Act (standard provisions for licences or certificates), in subsection (2) and (4), omit "clinical trial certificate or".

16. In section 50 of the Act (certificates for exporters of medicinal products), after paragraph (b) insert ", and

(c) to the provisions of the Clinical Trials Regulations and to any authorisation granted or other thing done by virtue of those regulations.".

17. In section 104 of the Act (application of Act to certain articles and substances), in subsection (1), after "such provisions of this Act" insert ", or the Clinical Trials Regulations,".

18. In section 105 of the Act (application of Act to certain other substances which are not medicinal products), in subsection (1), after "such provisions of this Act" insert ", or the Clinical Trials Regulations,".

19. In section 132 of the Act (general interpretation provisions)—

(a) in subsection (1)—

(i) omit the entry defining "clinical trial" and "clinical trial certificate", and

(ii) before the definition of "the Commission" insert the following definition—

""the Clinical Trials Regulations" means the Medicines for Human Use (Clinical Trials) Regulations 2004;"; and

in subsection (3), omit "a clinical trial certificate or".

The Medicines Act 1971

20. In section 1 of the Medicines Act 1971 (fees payable for the purposes of Part II of the Act)(**a**) after subsection (2) insert the following subsection—

"(2A) In subsections (1) and (2)(b) above, any reference to a licence under Part II of the principal Act shall be taken to include a reference to a manufacturing authorisation under the Medicines for Human Use (Clinical Trials) Regulations 2004.".

The Adults with Incapacity (Scotland) Act 2000

21. Section 51 of the Adults with Incapacity (Scotland) Act 2000(**b**) (authority for research) shall be amended as follows—

(a) in subsection (2), at the beginning of paragraph (b) insert "Subject to subsection (3A),";

(b) after subsection (3), insert the following subsection—

"(3A) Where the research consists of a clinical trial of a medicinal product, the research may be carried out—

(**a**) Section 1 of the Medicines Act 1971 has effect as if any reference in subsection (1) to any application in pursuance of the Act for a licence under Part II of the Act (or for the variation or renewal of such a licence) included a reference to any application under the Medicines for Human Use (Marketing Authorisations Etc) Regulations 1994 (S.I. 1994/3144) for a marketing authorization (or for the variation or renewal of such an authorization) and any reference in subsection (2)(b) to a licence under Part II of the Act included a reference to a marketing authorization; *see* regulation 9(12) of the those Regulations.

(**b**) 2000 asp 4.

(a) without being approved by the Ethics Committee, if a favourable opinion on the trial has been given by an ethics committee, other than the Ethics Committee, in accordance with regulation 15 of the Medicines for Human Use (Clinical Trials) Regulations 2004; and

(b) without the consent of any guardian or welfare attorney, or the adult's nearest relative, if—

(i) it has not been practicable to contact any such person before the decision to enter the adult as a subject of the clinical trial is made, and

(ii) consent has been obtained from a person, other than a person connected with the conduct of the clinical trial, who is—

(A) the doctor primarily responsible for the medical treatment provided to that adult, or

(B) a person nominated by the relevant health care provider."; and

(c) at the end insert the following subsection—

"(9) In this section—

"clinical trial on a medicinal product" means a clinical trial as defined by regulation 2(1) of the Medicines for Human Use (Clinical Trials) Regulations 2004;

"an ethics committee" has the meaning given by that regulation;

"person connected with the conduct of the trial" and "relevant health care provider" have the meanings given by Schedule 1 to those regulations.".

PART 2

ORDERS AND REGULATIONS

1. In the Medicines (Standard Provisions for Licences and Certificates) Regulations 1971(**a**)—

(a) in regulation 2 (interpretation), in the definition of "clinical trial certificate of right" and "animal test certificate of right", omit ""clinical trial certificate of right" and";

(b) in regulation 3 (standard provisions for licences and certificates), omit paragraph (2); and

(c) in Schedule 1, omit Part II (standard provisions for clinical trial certificates and clinical trial certificates of right).

2. In the Medicines (Surgical Materials) Order 1971(**b**), in article 3, for the words from ", the provisions contained in Parts I and II of the Act" to the end substitute—

"(a) the provisions contained in Parts I and II of the Act, sections 62, 64, 65 and 67 of Part III of the Act, and the provisions contained in Parts V, VI and VIII of the Act shall have effect in relation to the said articles or substances described in the Schedule to this Order, as those provisions have effect in relation to medicinal products; and

(b) the provisions of the Clinical Trials Regulations shall have effect in relation to the said articles or substances.".

3.—(1) In the Medicines (Exemption from Licences) (Special Cases and Miscellaneous Provisions) Order 1972(**c**), article 4 shall be amended as follows.

(2) In paragraph (1)—

(a) for "sections 7, 31(2) and 32" substitute "sections 7 and 32";

(**a**) S.I. 1971/972; regulation 3(3) and Part III of Schedule 1 are revoked insofar as they apply to animal test certificates by S.I. 2003/3309.
(**b**) S.I. 1971/1267, as amended by S.I. 1994/3119.
(**c**) S.I. 1972/1200.

(b) in subparagraph (a), omit "a clinical trial, or, as the case may be,".

(3) In paragraph (2)—

(a) in subparagraph (i)—

(i) in paragraph (a), omit "a clinical trial, or, as the case may be,",

(ii) in paragraph (b), omit "clinical trial or";

(b) in subparagraph (iii)—

(i) omit "the clinical trial or, as the case may be,",

(ii) omit the words from "the doctor or dentist" to "as the case may be,";

(c) in subparagraph (iv)—

(i) omit the words from "that the doctor or dentist" to "as the case may be,",

(ii) omit the words "the trial, or, as the case may be".

(4) Omit paragraph (3).

4. In the Medicines (Dental Filling Substances) Order 1975(**a**), in article 2, in paragraph (1), for the words from "the following provisions of the Act" to the end substitute—

"(a) the provisions contained in Parts I, II, III, V, VI and VIII of the Act shall have effect in relation to dental filling substances as those provisions have effect in relation to medicinal products; and

(b) the provisions of the Clinical Trials Regulations shall have effect in relation to those substances.".

5. In the Medicines (Specified Articles and Substances) Order 1976(**b**), in article 2, in paragraph (1), for the words from "the provisions of the Act" to the end substitute—

"(a) the provisions of the Act set out in Part I of the said Schedule 2 shall have effect in relation to such articles or substances as those provisions have effect in relation to medicinal products; and

(b) the provisions of the Clinical Trials Regulations shall have effect in relation to those articles or substances.".

6.—(1) The Medicines (Labelling) Regulations 1976(**c**) shall be amended as follows.

(2) In regulation 1 (citation and scope)(**d**), after "apply", insert "or a medicinal product which is an investigational medicinal product within the meaning of the Medicines for Human Use (Clinical Trials) Regulations 2003".

(3) In regulation 2 (commencement), in paragraph (b), in sub-paragraph (i), omit ", clinical trial certificate".

(4) Omit regulation 6 (clinical trials).

(5) In regulation 10 (surgical materials), omit the words from ", except that" to the end.

(6) In regulation 16 (provisions in licences, clinical trial certificates and animal test certificates)—

(a) in paragraph (1), for "a clinical trial certificate or animal test certificate " substitute "an animal test certificate";

(b) in paragraph (2), omit ", clinical trial certificate".

(7) Omit Schedule 2 (particulars required in the labelling of containers and packages of medicinal products for clinical trials).

(**a**) S.I. 1975/533, as amended by S.I. 1994/3119.
(**b**) S.I. 1976/968, as amended by S.I. 1994/3119.
(**c**) S.I. 1976/1726; the Regulations were revoked in so far as they relate to the labelling of containers and packages of medicinal products for administration in certain medicinal tests on animals by S.I. 1996/2194.
(**d**) As amended by S.I. 1994/3144.

7. In the Medicines (Fluted Bottles) Regulations 1978(**a**), in regulation 3 (exceptions)—

(a) after paragraph (e), insert the following paragraph—

"(ee) where medicinal products are investigational medicinal products within the meaning given by the Medicines for Human Use (Clinical Trials) Regulations 2004;"; and

(b) in paragraph (g), omit "clinical trial certificate or".

8. In Schedule 1 to the Medicines (Fixing of Fees Relating to Medicinal Products for Human Use) Order 1989(**b**), after paragraph 9A(**c**) insert the following paragraph—

"**9B.** Functions of the licensing authority which are functions of theirs by virtue of the Medicines for Human Use (Clinical Trials) Regulations 2004 and the functions of any person appointed under Schedule 5 or 8 to those Regulations.".

9. In the Medicines Act 1968 (Application to Radiopharmaceutical-Associated Products) Regulations 1992(**d**), in the Schedule—

(a) in the entry relating to section 44 of the Act, for "", or of a clinical trial certificate or animal test certificate,"" substitute "", or of an animal test certificate,"";

(b) in the entry relating to section 45 of the Act, omit "section 31,", in both places those words appear;

(c) in the entry relating to section 46 of the Act, for ""or of a clinical trial certificate or animal test certificate"" substitute ""or of an animal test certificate""; and

(d) in the entry relating to section 47 of the Act—

(i) for ""any clinical trial certificate or animal test certificate"" substitute ""any animal test certificate"", and

(ii) for "", or any clinical trial certificate or animal test certificate,"" substitute "", or any animal test certificate,"".

10. In the Medicines (Homoeopathic Medicinal Products for Human Use) Regulations 1994(**e**), in Schedule 4 (application of the provisions of the Act)—

(a) in the entry relating to section 23 of the Act, omit "clinical trials and";

(b) in the entry relating to section 44 of the Act, for "a clinical trial certificate or animal test certificate", in both places those words appear, substitute "an animal test certificate";

(c) in the entry relating to section 45 of the Act—

(i) omit "section 31,", and

(ii) for ""or of a clinical trial certificate or animal test certificate"" substitute ""or of an animal test certificate""; and

(d) in the entry relating to section 46 of the Act, for ""or of a clinical trial certificate or animal test certificate"" substitute ""or of an animal test certificate"".

11. In the Dangerous Substances and Preparations (Safety) (Consolidation) Regulations 1994(**f**), in regulation 1 (citation, commencement and interpretation), in paragraph (2), in the definition of "medicinal product"—

(a) for sub-paragraph (ii), substitute the following sub-paragraph—

"(ii) which is an "investigational medicinal product" within the meaning of regulation 2(1) of the Medicines for Human Use (Clinical Trials) Regulations 2003, or"; and

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(**a**) S.I. 1978/40; regulation 3 was amended by S.I. 1994/3142 and 3144.
(**b**) S.I. 1989/684.
(**c**) Paragraph 9A was inserted by S.I. 1995/871.
(**d**) S.I. 1992/605.
(**e**) S.I. 1994/105.
(**f**) S.I. 1994/2844; regulation 1(2) was substituted by S.I. 1996/2635.

(b) omit sub-paragraph (iii).

12. In the Medicines for Human Use (Marketing Authorisations Etc.) Regulations 1994(**a**)—

(a) in regulation 1—

(i) in paragraph (2), after the definition of "the Act" insert the following definition—

""the Clinical Trials Directive" means Directive 2001/20/EC of the European Parliament and of the Council on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use;",

(ii) in paragraph (5), omit "and except in the case of "clinical trial,""; and

(b) in Schedule 1, in paragraph 2, for sub-paragraph (e)(**b**) substitute the following sub-paragraph—

"(e) the relevant medicinal product—

(i) is manufactured, assembled or imported by the holder of an authorization referred to in Article 40 of the 2001 Directive which relates specifically to the manufacture, assembly or import of relevant medicinal products to which paragraph 1 applies; or

(ii) has been manufactured, assembled or imported as an investigational medicinal product by the holder of an authorization referred to in Article 13 of the Clinical Trials Directive; and".

13. In the Prescription Only Medicines (Human Use) Order 1997(**c**)—

(a) in article 1 (citation, commencement and interpretation), in paragraph (2), for the definition of "clinical trial exemption" substitute the following definition—

""clinical trial" has the meaning given by regulation 2(1) of the Medicines for Human Use (Clinical Trials) Regulations 2003;"; and

(b) in article 3B (prescribing and administration by supplementary prescribers), in paragraph (3), in sub-paragraph (b), in head (ii), for the words from "and—" to the end substitute "which has been authorised, or is to be treated as having been authorised, by the licensing authority in accordance with the Medicines for Human Use (Clinical Trials) Regulations 2003".

14. In the Ionising Radiation (Medical Exposure) Regulations 2000(**d**), in regulation 2 (interpretation), in paragraph (1), after the definition of "ionising radiation", insert the following definition—

""Local Research Ethics Committee" means—

(a) an ethics committee established or recognised in accordance with Part 2 of the Medicines for Human Use (Clinical Trials) Regulations 2004,

(b) the Ethics Committee constituted by regulations made by the Scottish Ministers under section 51(6) of the Adults with Incapacity (Scotland) Act 2000, or

(c) any other committee established to advise on the ethics of research investigations in human beings, and recognised for that purpose by or on behalf of the Secretary of State, the National Assembly for Wales or Scottish Ministers;".

15. In the Private and Voluntary Health Care (England) Regulations 2001(**e**), in regulation 24 (research), for paragraph (2) substitute the following paragraph—

"(2) For the purposes of paragraph (1)(a), "appropriate Research Ethics Committee" means—

(**a**) S.I. 1994/3144.
(**b**) Paragraph 2(e) of Schedule 1 was amended by SI 2002/236.
(**c**) S.I. 1997/1830; the relevant amending instrument is S.I. 2003/696.
(**d**) S.I. 2000/1059.
(**e**) S.I. 2001/3968.

(a) an ethics committee established or recognised in accordance with Part 2 of the Medicines for Human Use (Clinical Trials) Regulations 2004; or

(b) any other committee established to advise on the ethics of research investigations in human beings, and recognised for that purpose by or on behalf of the Secretary of State;".

16. In the Misuse of Drugs Regulations 2001(**a**), in regulation 18 (marking of bottles and other containers), for paragraph (3) substitute the following paragraph—

"(3) In this regulation—

"clinical trial" has the same meaning as in the Medicines for Human Use (Clinical Trials) Regulations 2003;

"medicinal test on animals" has the same meaning as in the Medicines Act 1968.".

17. In the Health Service (Control of Patient Information) Regulations 2002(**b**), in regulation 1 (citation, commencement, interpretation and extent), in paragraph (2), for the definition of "research ethics committee" substitute the following definition—

""research ethics committee" means—

(a) an ethics committee established or recognised in accordance with Part 2 of the Medicines for Human Use (Clinical Trials) Regulations 2004, or

(b) any other committee established to advise on the ethics of research investigations in human beings, and recognised for that purpose by or on behalf of the Secretary of State or the National Assembly for Wales;".

18. In the National Health Service (Functions of Strategic Health Authorities and Primary Care Trusts and Administration Arrangements) (England) Regulations 2002(**c**), in regulation 2 (interpretation), in paragraph (1), for the definition of "research ethics committee" substitute the following definition—

""research ethics committee" means—

(a) an ethics committee established or recognised in accordance with Part 2 of the Medicines for Human Use (Clinical Trials) Regulations 2004, or

(b) any other committee established to advise on the ethics of research investigations on human beings and recognised for that purpose by or on behalf of the Secretary of State;".

(**a**) S.I. 2001/3998.
(**b**) S.I. 2002/1438.
(**c**) S.I. 2002/2375.

SCHEDULE 11

Regulation 55

REVOCATIONS

Regulations and orders	*S.I. number*	*Extent of revocation*
The Medicines (Standard Provisions for Licence and Certificates) Regulations 1971	S.I. 1971/972	The whole Regulations in so far as they relate to clinical trial certificates
The Medicines (Applications for Product Licences and Clinical Trial and Animal Test Certificates) Regulations 1971	S.I. 1971/973	The whole Regulations in so far as they relate to applications for clinical trial certificates
The Medicines (Exemption from Licences) (Clinical Trials) Order 1974	S.I. 1974/498	The whole Order
The Medicines (Renewal Applications for Licences and Certificates) Regulations 1974	S.I. 1974/832	The whole Regulations in so far as they relate to renewal applications for clinical trial certificates
The Medicines (Exemption from Licences) (Clinical Trials) Order 1995	S.I. 1995/2808	The whole Order
The Medicines (Exemption from Licences and Certificates) (Clinical Trials) Order 1995	S.I. 1995/2809	The whole Order
The National Health Service Reform and Health Care Professions Act 2002 (Supplementary, Consequential Etc Provisions) Regulations 2002	S.I. 2002/2469	Schedule 1, Part 2, paragraph 67.

SCHEDULE 12

Regulation 56

TRANSITIONAL PROVISIONS

Ethical approval given before 1st May 2004

1.—(1) This sub-paragraph applies where—

(a) a clinical trial is conducted after 30th April 2004;

(b) no ethics committee has given a favourable opinion in relation to that trial in accordance with regulation 15; and

(c) a committee established or recognised for the purpose of advising on the ethics of research investigations on human beings has before 1st May 2004 given a favourable ethical opinion in relation to that trial.

(2) Subject to the following sub-paragraphs, where sub-paragraph (1) applies—

(a) the trial shall be treated for the purposes of these Regulations as if an ethics committee has given a favourable opinion in relation to that trial in accordance with regulation 15;

(b) regulations 12, 24(3) and 29 shall apply in relation to the trial with the modification that references to the application for an ethics committee opinion shall be read as references to the application for approval made to the committee referred to in sub-paragraph (1)(c); and

(c) regulations 24, 25, 27, 30 to 35 and 48 shall apply in relation to the trial with the modification that references to the relevant ethics committee shall be read as references to the committee referred to in sub-paragraph (1)(c).

(3) This sub-paragraph applies where the committee referred to in sub-paragraph (1)(c) has not been recognised by the Authority in accordance with regulation 7—

(a) for the area in which the trial sites are situated, or

(b) for the description or class of clinical trial into which the trial falls,

before 1st September 2004.

(4) Where sub-paragraph (3) applies—

(a) the sponsor of the clinical trial may make an application to an ethics committee established or recognised by the Authority in accordance with Part 2—

(i) for the area in which the trial sites are situated, or

(ii) for the description or class of clinical trial into which the trial falls,

for an amendment to the protocol for the trial within the meaning of Part 3 of these Regulations; or

(b) the chief investigator may make an application to an ethics committee in accordance with regulation 14.

(5) Where an ethics committee receives an application for an amendment in accordance with sub-paragraph (4)(a), it shall consider the amendment as if it was a valid notice of amendment under regulation 24.

(6) Where an ethics committee gives a favourable opinion in relation an application for amendment made pursuant to sub-paragraph (4)(a)—

(a) sub-paragraph (2)(c) shall cease to apply; and

(b) regulations 24, 25, 27, 30 to 35 and 48 shall apply in relation to the trial with the modification that references to the relevant ethics committee shall be read as references to the committee which gave that favourable opinion.

(7) Where an ethics committee gives a favourable opinion in relation to an application pursuant to sub-paragraph (4)(a), sub-paragraph (2) shall cease to apply in relation to that trial

(8) Where sub-paragraph (3) applies and before 1st May 2006 no favourable opinion has been given as specified in sub-paragraphs (6) and (7), sub-paragraph (2) and (4) shall cease to apply from that date.

(9) If the committee referred to in sub-paragraph (1)(b) is abolished or ceases operation before 1st May 2006—

(a) the Authority shall nominate an ethics committee as responsible for the work of the committee which is abolished or which ceases operation; and

(b) regulations 24, 25, 27, 30 to 35 and 48 shall apply in relation to the trial with the modification that references to the relevant ethics committee shall be read as references to the committee so nominated.

Applications for ethical approval prior to 1st May 2004

2.—(1) This sub-paragraph applies where a person has made an application for an ethical opinion in relation to a clinical trial before 1st May 2004 to a committee established or recognised for the purpose of advising on the ethics of research investigations on human beings.

(2) Where—

(a) sub-paragraph (1) applies;

(b) the committee has not given its opinion before 1st May 2004; and

(c) the committee has been recognised by the Authority in accordance with regulation 7 for the area in which the trial sites are situated, or for the description or class of clinical trial into which the trial falls,

the committee shall consider the application as if it had been made in accordance with regulation 14.

Clinical trial exemptions or notifications prior to 1st May 2004

3.—(1) This sub-paragraph applies where—

(a) a clinical trial is conducted after 30th April 2004; and

(b) immediately before 1st May 2004, a clinical trial certificate was in force and the trial was being conducted in accordance with that certificate.

(2) Where sub-paragraph (1) applies—

(a) the trial shall be treated for the purposes of these Regulations as having been authorised by the licensing authority;

(b) regulations 17 to 21 shall not apply in relation to the trial; and

(c) regulations 11, 24(2), 29, 31 and 45(1), and Schedule 7, shall apply in relation to the trial with the modification that references to the request for authorisation shall be read as references to the application for the clinical trial certificate.

4.—(1) This sub-paragraph applies where—

(a) a clinical trial is conducted after 30th April 2004; and

(b) immediately before 1st May 2004, the exemption conferred by article 3 of the Medicines (Exemption from Licences) (Clinical Trials) Order 1995(**a**) applied in respect of the sale or supply of medicinal products for the purposes of that trial.

(2) Where sub-paragraph (1) applies—

(a) the trial shall be treated for the purposes of these Regulations as having been authorised by the licensing authority;

(**a**) S.I. 1995/2808.

(b) regulations 17 to 21 shall not apply in relation to the trial; and

(c) regulations 11, 24(2), 29, 31 and 45(1), and Schedule 7, shall apply in relation to the trial with the modification that references to the request for authorisation shall be read as references to the notice to the licensing authority specified in article 4(1)(a) of the Medicines (Exemption from Licences) (Clinical Trials) Order 1995 .

5.—(1) This sub-paragraph applies where—

(a) a clinical trial is conducted after 30th April 2004;

(b) the investigational medicinal product used in the trial is a product with a marketing authorization;

(c) the trial has before 1st May 2004 been notified to the licensing authority by the person supplying the product for the purposes of that trial; and

(d) the licensing authority has before 1st May 2004 notified that person that—

(i) the trial appeared to fall within the terms of the Medicines (Exemption from Licences) (Clinical Trials) Order 1974(**a**), and

(ii) the authority agreed to the trial proceeding.

(2) Where sub-paragraph (1) applies—

(a) the trial shall be treated for the purposes of these Regulations as having been authorised by the licensing authority;

(b) regulations 17 to 21 shall not apply in relation to the trial; and

(c) regulations 11, 24(2), 29, 31 and 45(1), and Schedule 7, shall apply in relation to the trial with the modification that references to the request for authorisation shall be read as references to the notification referred to in sub-paragraph (1)(c).

6.—(1) This sub-paragraph applies where—

(a) a clinical trial is conducted 30th April 2004; and

(b) immediately before 1st May 2004, the exemption conferred by article 2(2) of the Medicines (Exemption from Licences) (Special Cases and Miscellaneous Provisions) Order 1972(**b**) applied in respect of the sale or supply of medicinal products for the purposes of that trial.

(2) Where sub-paragraph (1) applies—

(a) the trial shall be treated for the purposes of these Regulations as having been authorised by the licensing authority;

(b) regulations 17 to 21 shall not apply in relation to the trial; and

(c) regulations 11, 24(2), 29, 31 and 45(1), and Schedule 7, shall apply in relation to the trial with the modification that references to the request for authorisation shall be read as references to the notification to the licensing authority specified in article 2(3)(c) or (4)(a) of the Medicines (Exemption from Licences) (Special Cases and Miscellaneous Provisions) Order 1972.

Applications for clinical trial exemptions or notifications prior to 1st May 2004

7.—(1) This sub-paragraph applies where—

(a) an application for a clinical trial certificate has been made in accordance with section 36 of the Act and the licensing authority has not before 1st May 2004 determined whether to issue a certificate;

(b) the licensing authority has received a notice pursuant to article 4(1)(a) of the Medicines (Exemption from Licences) (Clinical Trials) Order 1995 and on 1st May 2004—

(**a**) S.I. 1974/498.
(**b**) S.I. 1972/1200.

(i) the specified period within the meaning of article 4(2) of that Order has not expired, and

(ii) the authority has not given or sent a notice pursuant to article 4(1)(b); or

(c) the licensing authority has received a notice pursuant to article 4(2)(iv) of the Medicines (Exemption from Licences) (Special Cases and Miscellaneous Provisions) Order 1972 and on 1st May 2004—

(i) the period specified in article (2)(v) of that Order has not expired, and

(ii) the authority has not given a direction pursuant to that article.

(2) Where sub-paragraph (1) applies the licensing authority shall treat the application or notice as a valid request for authorisation to conduct the clinical trial to which the application or notice relates under regulation 17.

EXPLANATORY NOTE

(This note is not part of the Regulations)

These Regulations implement Directive 2001/20/EC on the approximation of laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use ("the Directive").

The Regulations provide that the licensing authority established in accordance with the Medicines Act 1968 for the purpose of Part II of that Act (licences and certificates relating to medicinal products) shall exercise the functions of the competent authority under the Directive and certain functions falling to be performed by Member States under that Directive (regulation 4), unless those functions are conferred on any other person or body (for example, enforcement functions are conferred on the Secretary of State for Health, the National Assembly for Wales, the Scottish Ministers and the Department for Health, Social Services and Public Safety in Northern Ireland).

Regulations 5 to 10, and Schedule 2, make provision for ethics committees in the United Kingdom, which are to be responsible, amongst other things, for giving opinions on the ethics of clinical trials involving medicinal products. Regulation 5 provides for the United Kingdom Ethics Committees Authority, which is to be responsible for establishing, recognising, and monitoring ethics committees.

Regulations 11 to 27, and Schedules 3 to 5, make provision for clinical trial authorisations by the licensing authority and for ethics committee opinions. In particular: regulation 12 provides that a clinical trial may be conducted only if it has been authorised by the licensing authority and an ethics committee has given a favourable opinion; regulation restricts the supply of medicinal products for the purposes of clinical trials; regulations 14 to 16 and Schedules 3 and 4 make provision for applications for ethics committee opinions; and regulations 17 to 21 and Schedule 3 deal with requests to the licensing authority for authorisation. Regulations 22 to 25 make provision for amendments to clinical trial authorisations; and regulation 26 and Schedule 5 make provision for the reference to the appropriate committee or the Medicines Commission of decisions to refuse authorisations, amendments etc. Regulation 27 makes provision for the conclusion of a trial.

Regulations 28 to 31, and Schedules 1 and 5, make provision for: the conduct of a clinical trial, including the requirement to adhere to the principles of Good Clinical Practice; urgent safety measures to protect trial subjects from immediate hazards; and the suspension and termination of a trial. Schedule 5 includes provisions for referral to the appropriate committee or the Medicines Commission where a trial is suspended or terminated by the licensing authority.

Regulations 32 to 35 make provision for pharmacovigilance; i.e. the recording and reporting of adverse events and reactions to medicinal products being used in a clinical trial.

Regulations 36 to 45, and Schedules 6 to 8, make provision for the manufacture and importation of medicinal products to be used in clinical trials. In particular they make provision for: authorisations for manufacture, assembly and importation (regulations 36 and 37); the applications for, consideration of and grant or refusal of such authorisations (regulations 38 to 40 and Schedules 6 to 8); the application and effect of authorisations (regulations 41 and 42); the qualified persons responsible for checking the quality of products being manufactured, assembled or imported (regulation 43); and the variation, suspension and revocation of authorisations (regulations 44 and 45 and Schedule 8).

Regulation 46 concerns the labelling of such medicinal products.

Regulations 47 to 52, and Schedule 9, make provision for enforcement and related matters, including powers of inspection, infringement notices, offences and penalties for breaches of the Regulations.

Regulations 53 to 56, and Schedules 10 to 12, contain miscellaneous provisions for the construction of references in authorisations to pharmacopoeias and other publications, for the consequential amendment and revocation of legislation, and for transitional arrangements.

A full regulatory impact assessment of the effect that this instrument will have on the costs of business is available from the Medicines and Healthcare products Regulatory Agency, Room 10-202, Market Towers, 1 Nine Elms Lane, London SW8 5NQ. A copy of that assessment, and a Transposition Note in relation to the implementation of Directive 2001/20/EC, have been placed in the libraries of both Houses of Parliament.

Abstract ID: TPS4145(222793), Poster Board # 327a

Liposomal irinotecan (nal-IRI) plus 5-fluorouracil (5-FU) and leucovorin (LV) or gemcitabine plus cisplatin in advanced cholangiocarcinoma: The AIO-NIFE-trial, an open label, randomized, multicenter phase II trial

Thomas J. Ettrich[1], Andres W. Berget[1], Thomas Seufferlein[1], Lukas Perkhofer[1]
[1]Ulm University, Department of Internal Medicine I, Ulm, Germany

Background

Biliary tract cancer (CCC) is associated with a poor prognosis due to mostly advanced stages at diagnosis. Overall survival (OS) does not exceed 6 months and the 5-year OS rate is less than 5% for patients with advanced or metastatic disease. Advanced CCC shows response to chemotherapy resulting in an improved disease control, improved OS and quality of life (QoL). In the ABC-02 phase III trial, gemcitabine/cisplatin compared with gemcitabine alone prolonged PFS (8.0 vs. 5.0 mo.) and OS (11.7 vs. 8.1 mo.) and is considered as standard of care (Valle et al., NEJM 2010). So far this regimen has not been compared with other active combination regimen. Irinotecan in combination with 5-FU showed promising results in 1st- and 2nd-line therapy in many GI cancers. In pancreatic adenocarcinomas, the combination of liposomal irinotecan (nal-IRI) plus 5-FU/LV improves survival in a post gemcitabine-based treatment setting. Our research hypothesis is that this regimen compares well with respect to clinical endpoints with the standard of care gemcitabine/cisplatin in patients with advanced CCC.

Timelines:

- Enrollement started in January 2018 in 25 german centers
- The Scheduled recruitment period is 18 months

Statistics:

Simon's optimal two-stage design: H_0: less than 40% of patients are progression-free by 4 months of NAL-IRI plus 5-FU/leucovorin. Alternative hypothesis: ≥60% of patients are progression-free by 4 months of nal-IRI plus 5-FU/leucovorin.

Scheduled interim analyses:

A planned interim analysis will be conducted after 18 patients have been evaluated in the experimental arm. If 7 or less of the first 18 patients assigned to Nal-IRI plus 5-FU/leucovorin have a disease control (CR, PR or SD) at 4 months, H_0 will be accepted and the study will be terminated. If 8 or more patients with disease control are observed, another 28 patients in each treatment group are to be included.

Trial design

- NIFE is a randomized study for patients (to be enrolled: n = 92) with locally advanced or metastatic, non-resectable, adenocarcinoma of the intra- or extrahepatic biliary tract (no carcinoma of gallbladder or papilla of Vater)
- NIFE is an open label, non-comparative, multicenter, two-sided phase II study with an unconnected analysis of the results in both arms against a fixed PFS rate (< 40% at 4 months). The randomization (1:1) is eminent to achieve two comparable patient groups
- Arm A (experimental): Nal-IRI 80 mg/m2, leucovorin 400 mg/m2, 5-FU 2400 mg/m2, on day 1, cycle q2w)
- Arm B (standard): Cisplatin 25 mg/m2 and Gemcitabine 1000 mg/m2 on day 1 and 8, cycle q3w

- There will be a retrospective central surgical and radiological review
- Tissue and blood sample collection will be mandatory for biomarker analyses

Primary endpoint:

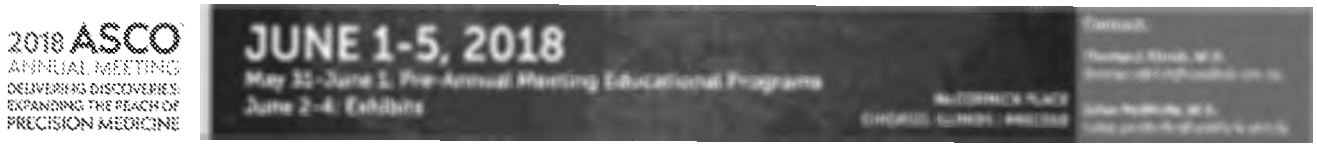
- PFS-rate at 4 months according to RECIST 1.1

Secondary endpoints:

- Progression free survival (PFS)
- 3-years-Overall-survival
- Disease control rate (DCR) after 8 weeks
- Safety
- Quality of life (QoL)
- Time until definitive deterioration (TUDD) in QoL score

Exploratory endpoints:

- Exploratory biomarkers analysis (ctDNA exome sequencing, transcriptome, miRNA-arrays)
- Potential correlation of PFS with ctDNA load or mutational pattern
- Predictive/Prognostic biomarker profiles (ctDNA exome sequencing, transcriptome, miRNA-arrays)



Key inclusion Criteria:
Eligible for 1st-line treatment
Locally advanced or metastatic adenocarcinoma of the intrahepatic or extrahepatic bile ducts
ECOG 0 or 1
Measurable disease (RECIST 1.1)
Mandatory participation in translational research
SCREENING
n=120
Stratification:
intrahepatic vs. extrahepatic
advanced vs. metastatic
age ≤ 70 vs. > 70 years
ECOG 0 vs. 1
male vs. female
R 1:1
Arm A n=46
Arm B n=46
2 mo
4 mo
STAGING
CYCLE
...until progression or intolerable toxicity
ARM A: Nal-IRI/5-FU/leucovorin, q 2 weeks
Nal-IRI 80 mg/m², 1.5 h iv infusion (d1)
Leucovorin 400 mg/m², 0.5 h iv infusion (d1)
5-FU 2400 mg/m², 46 h iv infusion (start d1)
ARM B: Cisplatin/Gemcitabin, q 3 weeks
Cisplatin 25 mg/m², 1 h iv infusion (d1, 8)
Gemcitabine 1000 mg/m², 0.5 h iv infusion (d1, 8)
Samples for translational research:
PanGenerateDNA
EDTA blood
Serum
Tumor tissue
HR-Quality of life-Assessment:
EORTC QLQ-C30
EORTC BIL21
HADS-D

Liposomal irinotecan (nal-IRI) plus 5-fluorouracil (5-FU) and leucovorin (LV) or gemcitabine plus cisplatin in advanced cholangiocarcinoma: The AIO-NIFE-trial, an open label, randomized, multicenter phase II trial.

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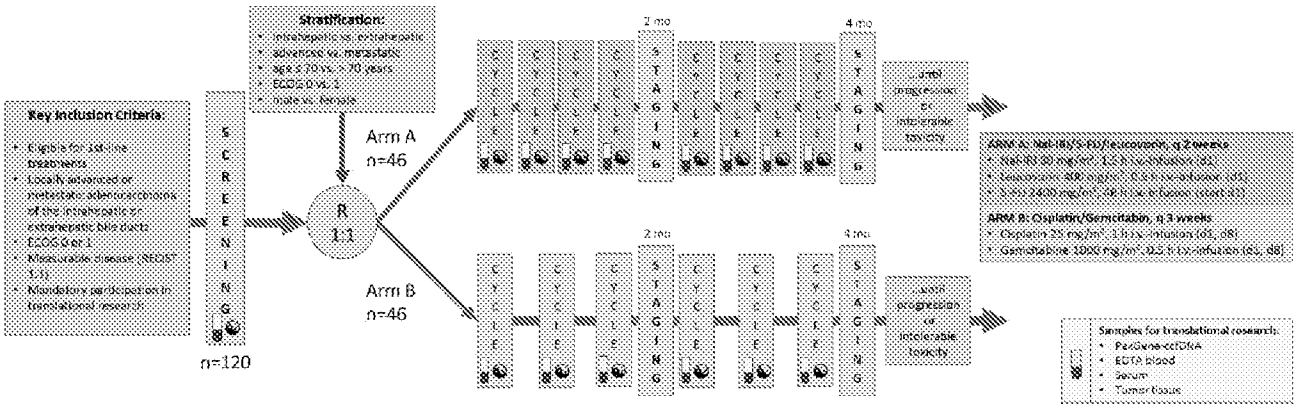

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ASCO
JUNE 1-5, 2018

GASTROINTESTINAL (NONCOLORECTAL) CANCER

Liposomal irinotecan (nal-IRI) plus 5-fluorouracil (5-FU) and leucovorin (LV) or gemcitabine plus cisplatin in advanced cholangiocarcinoma: The AIO-NIFE-trial, an open label, randomized, multicenter phase II trial.

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Show Less

Ulm University, Ulm, Germany; Ulm University, Department of Internal Medicine I, Ulm, Germany

Abstract Disclosures

Abstract

TPS4145

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The randomization (1:1) is eminent to achieve two comparable patient groups. Primary objective is PFS at 6 months. Key secondary objectives are 3-year OS, PFS, ORR, DCR and QoL/TUDD. There will be a retrospective surgical and radiological review. Tissue and blood sample collection will be mandatory for biomarker analyses (microdissection and exome sequencing of tumor tissue, ctDNA exome sequencing, transcriptome, miRNA-arrays). Start was in Q I/2018 in 25 centers in Germany. Clinical trial information: NCT03044587.

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

21 July 2016
EMA/CHMP/589179/2016
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Onivyde

International non-proprietary name: irinotecan

Procedure No. EMEA/H/C/004125/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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Table of contents

List of abbreviations

AE	Adverse Event
AESI	Adverse Event of Special Importance
ALT	Alanine aminotransferase
APC	7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin
API	Active Pharmaceutical Ingredient
AST	Aspartate Aminotransferase
AUC	Area under the plasma concentration time curve
BMI	Body Mass Index
BSA	Body Surface Area
CA 19-9	Carbohydrate antigen 19-9
CBR	Clinical Benefit Response
CEP	Certificate of Suitability of the EP
CHMP	Committee for Medicinal Products for Human use
CI	Confidence Interval
CL	Clearance
Cmax	Maximum plasma concentration
Cmin	Minimum plasma concentration
CR	Complete Response
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
CYP3A4	Cytochrome P450 3A4
dL	Deciliter
DMSO	Dimethyl sulfoxide
DSC	Differential Scanning Calorimetry
DSPC	1,2-Distearoyl-sn-glycero-3-phosphocholine
EC	Ethics Committee
eGFR	Estimated Glomerular Filtration Rate
ELSD	Evaporative Light Scattering Detector
EORTC	European Organization for Research and Treatment of Cancer

EP	Evaluable Population
5-FU	5-Fluorouracil
FDA	U.S. Food and Drug Administration
FID	Flame Ionisation Detection
FMEA	Failure mode effects analysis
FOLFIRI	5-FU+leucovorin+irinotecan
FOLFIRINOX	5-FU+leucovorin +irinotecan+oxaliplatin
FOLFOX	Oxaliplatin+5-FU+ leucovorin
GC	Gas Chromatography
GCP	Good Clinical Practice
GERCOR	Groupe Coopérateur Multidisciplinaire en Oncologie
GMP	Good Manufacturing Practice
HEPES	2-[4-(2 Hydroxyethyl)piperazin-1-yl] ethanesulfonic acid
HPLC	High performance liquid chromatography
HR	hazard ratio
ICH	International Conference on Harmonisation
INR	International Normalized Ratio
IPC	In-process Control
IQR	Inter-Quartile Range
IR	Infrared
ISS	Integrated Safety Summary
ITT	Intent-To-Treat
IV	Intravenous
IWRS	Interactive Web Response System
KF	Karl Fischer titration
KPS	Karnofsky Performance Score
L	Liter
LLOQ	Lower Limit of Quantification
LV	Leucovorin
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram

ml	millilitre
mM	millimolar
Mono	Monotherapy
MPEG-2000-DSPE	N-(carbonyl-methoxypolyethlyeneglycol-2000)-1,2-distearoly-sn-glycero-3-phosphoethanolamine
MTD	Maximum Tolerated Dose
NAPOLI	NAnoliPOsomal Irinotecan
NMR	Nuclear Magnetic Resonance
NPC	7-ethyl-10-(4-amino-1-piperidino) carbonyloxycamptothecin
OFF	Oxaliplatin+5-FU+Leucovorin
ORR	Objective Response Rate
OS	Overall Survival
PC	Process Control
PD	Progressive Disease
PEG	Polyethylene glycol
PFS	Progression Free Survival
Ph. Eur.	European Pharmacopoeia
PK	Pharmacokinetic
pKa	acid dissociation constant
PP	Per Protocol
PPQ	Process Performance Qualification
PR	Partial Response
PT	Preferred Term
q2w	Every 2 weeks
q3w	Every 3 weeks
QbD	Quality by design
QOL	Quality Of Life
RECIST	Response Evaluation Criteria in Solid Tumours
RMSE	Root mean square error
SAE	Serious Adverse Event
SD	Stable Disease

SmPC	Summary of Product Characteristics
SMQ	Standard MedDRA Queries
SOC	System Organ Class
SOS	Sucrose Octasulphate
t1/2	Half life
TEAE	Treatment emergent adverse event
TEA-Pn	Triethylammonium polyphosphate
TEA-SOS	Triethylammonium Sucrose Octasulphate
TEM	Transmission Electron Microscopy
TGA	Thermo-Gravimetric Analysis
Tm	gel-liquid crystal transition temperature
TSE	Transmissible Spongiform Encephalopathy
TTF	Time to Treatment Failure
TTP	Time to Progression
UGT	Uridine 5'-diphospho-glucuronosyltransferase
ULN	Upper Limit of Normal
UV	Ultraviolet
VAS	Visual Analog Scale
Vd	Volume of distribution
Vss	Volume of distribution at steady state

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Baxalta Innovations GmbH submitted on 30 April 2015 an application for marketing authorisation to the European Medicines Agency (EMA) for Onivyde, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 20 November 2014.

Onivyde was designated as an orphan medicinal product EU/3/11/933 on 09 December 2011. Onivyde was designated as an orphan medicinal product in the following indication: Treatment of pancreatic cancer.

The applicant applied for the following indication:

Treatment of metastatic adenocarcinoma of the pancreas, in combination with 5-fluorouracil and leucovorin in patients who have been previously treated with gemcitabine. Onivyde is indicated in adults.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Onivyde as an orphan medicinal product in the approved indication. The outcome of the COMP review can be found on the Agency's website: ema.europa.eu/Find medicine/Rare disease designations.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that irinotecan was considered to be a known active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision CW/1/2011 on the granting of a class waiver.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

Protocol Assistance

The applicant received Protocol Assistance from the CHMP on 6 December 2012. The Protocol Assistance pertained to clinical aspects of the dossier.

Licensing status

The product was not licensed in any country at the time of submission of the application.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Filip Josephson Co-Rapporteur: Daniela Melchiorri

- The application was received by the EMA on 30 April 2015.
- The procedure started on 28 May 2015.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 17 August 2015. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 14 August 2015. The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on 27 August 2015.
- During the meeting on 24 September 2015, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 25 September 2015.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 21 December 2015.
- With the responses to the CHMP consolidated List of Questions the applicant informed the Agency of the change of the applicant's company name from Baxter Innovations GmbH to Baxalta Innovations GmbH (effective as of 1st May 2015).
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 1 February 2016.
- During the PRAC meeting on 11 February 2016, the PRAC agreed on the PRAC Assessment Overview and Advice to CHMP. The PRAC Assessment Overview and Advice was sent to the applicant on 16 February 2016.
- During the CHMP meeting on 25 February 2016, the CHMP agreed on a list of outstanding issues to be addressed in writing and by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 21 June 2016.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of outstanding issues to all CHMP members on 6 July 2016.
- The following GMP and GCP inspection(s) were requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product:

- GCP inspections at three clinical investigator sites in South Korea and Australia were conducted between September and October 2015. The outcome of the inspection carried out was issued on 18 December 2015.
- A GMP inspection at one finished product manufacturing site in USA conducted between 12-14 January 2016. The outcome of the inspection carried out was issued on 24th February 2016.

- During the meeting on 21 July 2016, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive scientific opinion to Onivyde.

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Pancreatic cancer is a malignant neoplasm of the pancreas (ICD-9, 2014). More than 80% of exocrine pancreatic cancers are infiltrating ductal adenocarcinomas, a majority of which exhibit KRAS mutations, predominantly G12V or G12D mutations (Seufferlein, et al, 2012), and the remaining types include adenosquamous carcinomas, squamous cell carcinomas, signet ring cell carcinomas, acinar cell carcinomas, undifferentiated carcinomas, undifferentiated carcinomas with giant cells, and solid pseudopapillary neoplasms of the pancreas. Exocrine pancreatic tumors are far more common than pancreatic neuroendocrine tumors, which make up about 3-5% of all pancreatic malignancies (Krampitz, 2013). Hereditary conditions account for ~5-10% of pancreatic cancer (Seufferlein et al., 2012).

2.1.2. Epidemiology

In Europe, cancer of the pancreas is the seventh most frequent cancer, accounting for some 2.9% of cancer in men and 3.2% in women) and the fifth leading cause of cancer-related death in Europe (GLOBOCAN 2012). The age-standardized mortality rates (ASR) per 100,000 are 8.0 for men and 5.5 for women (Malvezzi et al, 2013).

2.1.3. Clinical presentation, diagnosis and stage/prognosis

Mainly a disease of the elderly, pancreatic adenocarcinoma accounts for 85-95% of all pancreatic cancers. More than 50% of pancreatic cancers are identified in metastatic stage, with overall survival ranging from 7-11 months. In 30%-40% of patients the disease is localized, but not surgically resectable, with OS of 11-18 months. The overall 1-year survival rate ranges from 11% to 28.3% (Seufferlein T, Annals of Oncology 2012). Chemo- and radio-resistant, unresectable pancreatic adenocarcinoma has a dire prognosis, with a 5--year OS of 6 %, i.e. with an increase in OS of only 1% in the past three decades.

2.1.4. Management

Despite 5-fluorouracil/leucovorin with irinotecan and oxaliplatin (FOLFIRINOX) and gemcitabine/nab-paclitaxel significantly improving outcomes for metastatic cancer (response rates between 23% and 31%, PFS of 5.5--6.6 months, OS between 8.5 and 11 months), refractory disease still poses significant challenges.

Novel chemotherapeutics, stroma and immune-targeted agents are currently being developed (Drug Design, Development and Therapy 2015:9 3529--3545). However, there is no consensus on second-line treatment after failure on first-line therapy. The OFF regimen (oxaliplatin/5FU/LV) showed an OS advantage of 2.6 months in CONKO-003, a randomized study in patients with disease progression after first-line gemcitabine therapy.

About the product

Onivyde (also referred to as MM-398) is developed for the treatment of metastatic adenocarcinoma of the pancreas, in combination with 5-fluorouracil (5-FU) and leucovorin (LV), in patients who have been previously treated with gemcitabine.

The active substance in Onivyde is irinotecan (topoisomerase I inhibitor) encapsulated in a lipid bilayer vesicle or liposome. Irinotecan is a derivative of camptothecin. Camptothecins act as specific inhibitors of the enzyme DNA topoisomerase I. Irinotecan and its active metabolite SN-38 bind reversibly to the topoisomerase I-DNA complex and induce single-strand DNA lesions which block the DNA replication fork and are responsible for the cytotoxicity. Irinotecan is metabolized by carboxylesterase to SN-38. SN-38 is approximately 1000 times as potent as irinotecan as an inhibitor of topoisomerase I purified from human and rodent tumour cell lines.

The applicant applied for the following indication:

Treatment of metastatic adenocarcinoma of the pancreas, in combination with 5-fluorouracil and leucovorin in patients who have been previously treated with gemcitabine.

The recommended indication is:

Treatment of metastatic adenocarcinoma of the pancreas, in combination with 5 fluorouracil (5 FU) and leucovorin (LV), in adult patients who have progressed following gemcitabine based therapy.

Onivyde must only be prescribed and administered to patients by healthcare professionals experienced in the use of anti cancer therapies.

Onivyde is not equivalent to non-liposomal irinotecan formulations and should not be interchanged.

Onivyde, leucovorin and 5 fluorouracil should be administered sequentially. The recommended dose and regimen Onivyde is 80 mg/m^2 intravenously over 90 minutes, followed by LV 400 mg/m^2 intravenously over 30 minutes, followed by 5 FU 2,400 mg/m^2 intravenously over 46 hours, administered every 2 weeks. Onivyde should not be administered as a single agent.

All dose modifications should be based on the worst preceding toxicity. LV dose does not require adjustment. For Grade 1 and 2 toxicities there are no dose modifications recommended. Dose adjustments, are recommended to manage Grade 3 or 4 toxicities related to Onivyde.

For patients who start treatment with 60 mg/m^2 Onivyde and do not dose escalate to 80 mg/m^2, the recommended first dose reduction is to 50 mg/m^2 and the second dose reduction is to 40 mg/m^2. Patients who require further dose reduction should discontinue treatment.

Table 1: Recommended dose modifications for ONIVYDE+5-FU/LV for Grade 3-4 toxicities for patients not homozygous for UGT1A1*28

Toxicity grade (value) by NCI CTCAE v 4.0[1]	**ONIVYDE/5-FU adjustment (for patients not homozygous for UGT1A1*28)**	
Haematological toxicities		
Neutropenia	A new cycle of therapy should not begin until the absolute neutrophil count is ≥ 1500/mm^3	
Grade 3 or Grade 4 (< 1000/mm^3)	***First occurrence***	Reduce ONIVYDE dose to 60 mg/m^2 Reduce 5-FU dose by 25% (1800 mg/m^2).

Toxicity grade (value) by NCI CTCAE v 4.0[1]	**ONIVYDE/5-FU adjustment (for patients not homozygous for UGT1A1*28)**	
or Neutropenic fever	***Second occurrence***	Reduce ONIVYDE dose to 50 mg/m^2 Reduce 5-FU dose by an additional 25% (1350 mg/m^2).
	Third occurrence	Discontinue treatment
Thrombocytopenia Leukopenia	A new cycle of therapy should not begin until the platelet count is ≥ 100,000/mm^3 Dose modifications for leukopenia and thrombocytopenia are based on NCI CTCAE toxicity grading and are the same as recommended for neutropenia above.	
Nonhaematological toxicities[2]		
Diarrhoea	A new cycle of therapy should not begin until diarrhoea resolves to ≤ Grade 1 (2-3 stools/day more than pre-treatment frequency).	
Grade 2	A new cycle of therapy should not begin until diarrhoea resolves to ≤ Grade 1 (2-3 stools/day more than pre-treatment frequency).	
Grade 3 or 4	***First occurrence***	Reduce ONIVYDE dose to 60 mg/m^2 Reduce 5-FU dose by 25% (1800 mg/m^2)
	Second occurrence	Reduce ONIVYDE dose to 50 mg/m^2 Reduce 5-FU dose by an additional 25% (1350 mg/m^2)
	Third occurrence	Discontinue treatment
Nausea/vomiting	A new cycle of therapy should not begin until nausea/vomiting resolves to ≤ Grade 1 or baseline	
Grade 3 or 4 *(despite antiemetic therapy)*	***First occurrence***	Optimise antiemetic therapy Reduce ONIVYDE dose to 60 mg/m^2
	Second occurrence	Optimise antiemetic therapy Reduce ONIVYDE dose to 50 mg/m^2
	Third occurrence	Discontinue treatment
Hepatic, renal, respiratory or other[3] toxicities ***Grade 3 or 4***	A new cycle of therapy should not begin until the adverse reaction resolves to ≤ Grade 1	
	First occurrence	Reduce ONIVYDE dose to 60 mg/m^2 Reduce 5-FU dose by 25% (1800 mg/m^2)
	Second occurrence	Reduce ONIVYDE dose to 50 mg/m^2 Reduce 5-FU dose by an additional 25% (1350 mg/m^2)
	Third occurrence	Discontinue treatment
Anaphylactic reaction	***First occurrence***	Discontinue treatment

1 NCI CTCAE v 4.0 = National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0

2 Excludes asthenia and anorexia; Asthenia and Grade 3 anorexia do not require dose adjustment.

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as concentrate for solution for infusion containing 5 mg/ml of irinotecan hydrochloride trihydrate (as irinotecan sucrosofate salt) as active substance. The active substance is formulated in pegylated liposomes.

Other ingredients are:

Liposome forming lipids:

1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and N-(carbonyl-methoxypolyethylene glycol-2000)-1, 2-distearoly-sn-glycero-3-phosphoethanolamine (MPEG 2000 DSPE).

Other excipients:

Sucrose octasulphate, 2-[4-(2 Hydroxyethyl)piperazin-1-yl] ethanesulfonic acid (HEPES buffer), sodium chloride, and water for injections.

The product is available in type I glass vial with a grey chlorobutyl stopper and an aluminium seal with a flip-off cap, containing 10 ml of concentrate, as described in section 6.5 of the SmPC. Each pack contains one vial.

2.2.2. Active Substance

General information

The chemical name of irinotecan hydrochloride trihydrate is (S)-[1,4'-bipiperidine]-1'-carboxylic acid 4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl ester hydrochloride trihydrate corresponding to the molecular formula $C_{33}H_{38}N_4O_6 \cdot HCl \cdot 3H_2O$. It has a relative molecular mass of 677.18 and has the following structure:

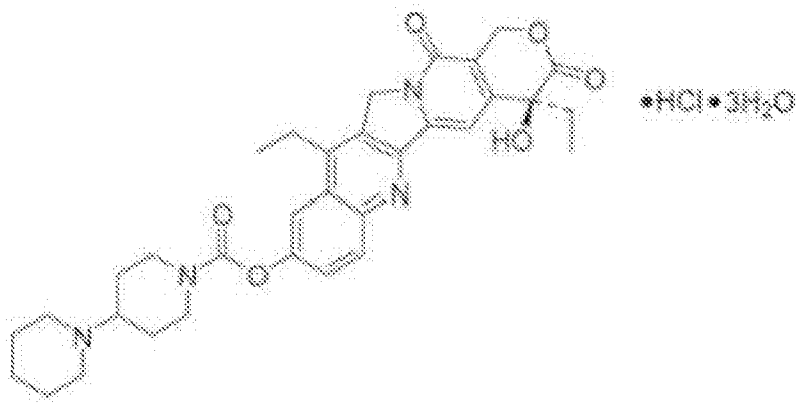


Figure 1. Structural formula of irinotecan hydrochloride trihydrate.

The molecular structure of irinotecan hydrochloride trihydrate has been confirmed by elemental analysis, IR spectroscopy, UV spectroscopy, mass spectrometry, ^{13}C and ^{1}H NMR spectroscopy, thermal analysis (TGA and DSC) and X-ray powder diffraction analysis.

The active substance is a pale yellow to yellow crystalline powder; it is hygroscopic and light sensitive; freely soluble in DMSO and anhydrous acetic acid, slightly soluble in ethanol, sparingly soluble in aqueous buffer at pH 4 and slightly soluble in aqueous buffer at pH 2.

Irinotecan exhibits stereoisomerism due to the presence of a chiral centre. The source of stereoisomerism is camptothecin starting material. Enantiomeric purity of the active substance is controlled routinely by chiral HPLC.

The active substance manufacturer has demonstrated that a single crystalline form of irinotecan hydrochloride trihydrate is consistently produced. Polymorphism has no relevance on the performance of the finished product, as the active substance is dissolved during the manufacturing process of the finished product.

Manufacture, characterisation and process controls

The active substance is currently sourced from a single manufacturer. Detailed information on the manufacturing of the active substance has been provided in the restricted part of the ASMF and it was considered satisfactory.

Irinotecan hydrochloride trihydrate is a semi-synthetic active substance. It is synthesized from well-defined starting materials. The originally proposed starting material was redefined during the assessment procedure, since the CHMP considered it necessary in support of a more exhaustive control strategy. The concerns raised were related to missing information on the source of the material, the supplier of the material, extraction process, control of the material, and the potential carry-over of impurities to the active substance. As a result of this redefinition, the route of synthesis of the active substance was expanded to include these additional steps under GMP and the requested information was provided. A revised process will be implemented once the necessary analyses and comparability studies have been finalised and a new active substance supplier is approved. This commitment is considered a legally binding post-authorisation measure, with the submission expected by 31 December 2016. As an additional measure until the new process is fully implemented, the CHMP required more information on the control strategy for aflatoxin, mycotoxin, pesticide, residual solvents, and elemental impurities, for the batches of intermediate initially defined as the starting material which was considered satisfactory.

The specifications and control methods for current starting materials and reagents have been presented and only those batches conforming to the specifications can be used in the manufacture of the active substance.

The CHMP considered that the information presented, the steps taken, and the commitments provided by the Applicant are sufficient to ensure that the quality of the product is warranted and raises no concerns that could impact the safety of the medicinal product.

The active substance is synthesized in four main steps, using well defined starting materials with acceptable specifications.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products have been presented.

Reprocessing procedure of the active substance is described in the dossier and the reprocessing activities were considered acceptable.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances.

Potential and actual impurities were well discussed with regards to their origin and characterised.

The active substance is packaged in two-layer polyethylene bag as the inner package, and the pharmaceutical aluminium tin as the outer package. Plastic materials in contact with the active substance comply with the EC directive 2002/72/EC and EC 10/2011 as amended.

Specification

The active substance specification includes tests for: appearance, assay (HPLC), identity (IR, HPLC), chiral purity (UV, HPLC), chloride identification (Ph. Eur.), impurities (HPLC), residual solvents (Ph. Eur.), water content (KF), heavy metals (Ph. Eur.), pH (Ph. Eur.), solubility (Ph. Eur.), residue on ignition (Ph. Eur.), microbial limits (Ph. Eur.), and bacterial endotoxins (Ph. Eur.).

Due to its mechanism of action, irinotecan is potentially genotoxic and carcinogenic. Mutagenic and carcinogenic potential of the impurities of starting materials, intermediates generated during the synthesis or degradation products cannot be excluded. In line with ICH M7, exposure to a mutagenic impurity in these cases would not significantly add to the cancer risk of the active substance. Therefore, these impurities are controlled in the active substance at acceptable levels for non-mutagenic impurities, complying with the identification (0.10%) and qualification thresholds (0.15%) described in the ICH Q3A guideline.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay, identification, and impurities testing has been presented.

Batch analysis results for three consecutive commercial scale batches used for process validation and fifteen batches used in process performance qualification, stability, nonclinical, and clinical studies of the active substance have been provided. The results are within the specifications and consistent from batch to batch.

Stability

Stability data on nine commercial scale batches and three additional batches of pilot or lab scale of active substance from the proposed manufacturer stored in the intended commercial package for 48 months under long term conditions at 25 ºC / 60% RH and for up to 6 months under accelerated conditions at 40 ºC / 75% RH according to the ICH guidelines were provided. Photostability testing following the ICH guideline Q1B was performed on one batch. Results on stress conditions including high temperature, high humidity, acidity, alkalinity, oxidation, reduction, and hydrolysis were also provided on one batch.

The following parameters were tested: appearance, assay, chiral purity, impurities, and pH. The analytical methods used were the same as for release and were stability indicating.

All tested parameters of the active substance stored under long term and accelerated conditions were within the specifications. No trends in the formation of impurities or water absorption were observed. The active substance was stable with respect to assay and impurity levels when tested under the stress conditions of high temperature, and high humidity. Water levels increased under both stress conditions demonstrating that the active substance is hygroscopic. Testing under acidic, alkaline, oxidative, reductive, and hydrolytic stress conditions showed the instability of the active substance, resulting in the increase in the level of impurities and

the decrease in assay. During the photostability testing the levels of impurities increased demonstrating that the active substance is sensitive to light.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable when protected from light and moisture. The stability results justify the proposed retest period of 3 years when stored in the proposed container with no special storage conditions.

2.2.3. Finished Medicinal Product

Description of the product and pharmaceutical development

The finished product is a white to slightly yellow opaque sterile concentrate for solution for infusion. It consists of an isotonic dispersion of liposomes containing irinotecan hydrochloride trihydrate.

The liposomes are small unilamellar lipid bilayer vesicles, approximately 110 nm in diameter, enclosing an aqueous compartment that contains irinotecan in a gelated or precipitated state, as sucrosofate salt. The lipid membrane is composed of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and a N-(carbonylmethoxypolyethlyene glycol-2000)-1,2-distearoly-sn-glycero-3-phosphoethanolamine (MPEG-2000-DSPE). The liposomes are dispersed in an aqueous buffered solution.

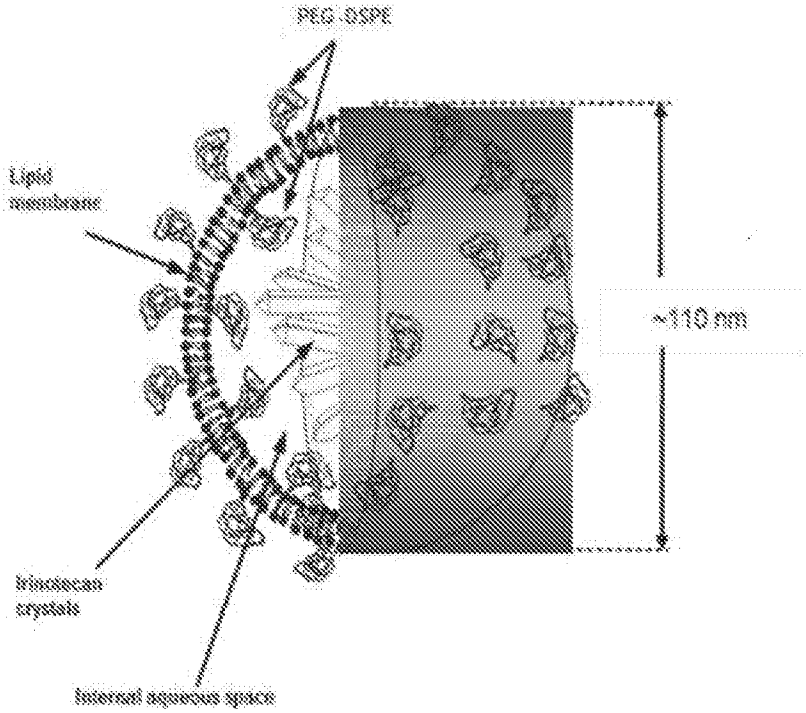


Figure 2. Schematic representation of the liposome.

As mentioned above, irinotecan hydrochloride trihydrate is present in the liposomes in the form of irinotecan sucrosofate salt. During pharmaceutical development, a number of formulations were screened where the anion of the drug entrapping solution was varied. Development identified sucrose octasulphate (SOS) as a superior anion of the drug entrapping solution. Sucrose octasulphate is added in the finished product manufacturing process as a triethylammonium salt, i.e. as triethylammonium sucrose octasulphate (TEA-SOS). It maintained

the encapsulation efficiency and had superior *in vitro* irinotecan retention in liposomes compared with the other formulation. Therefore this formulation was chosen for clinical and commercial use.

Sucrosofate potassium salt (K-SOS) is a novel excipient and it is listed in the composition of the finished product as a free base. It is used as the raw material for the preparation of sucrosofate triethylammonium salt (TEA-SOS) used in liposome formation. The excipient K-SOS is transformed into TEA-SOS by ion-exchange. TEA-SOS and the lipids form the liposomes by passive encapsulation following polycarbonate membrane extrusion. An electrochemical gradient is subsequentiy formed by removal of TEA-SOS from the exterior of the liposomes by diafiltration. Triethylamine is removed during the manufacturing process. Detailed information on the manufacture, characterization and controls, justification of the use and a summary of supporting toxicology data for sucrosofate potassium salt was requested by the CHMP during the assessment procedure and the provided data was considered satisfactory.

The lipid membrane is composed of 1, 2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoly-sn-glycero-3-phosphoethanolamine (MPEG-2000-DSPE). The function of the liposomal excipients is to produce the liposomal bilayer membrane which forms small unilamellar vesicles encapsulating and retaining the active substance until it is passively delivered to the tumour site. The liposomal excipients were selected for their properties when combined to produce liposomes capable of actively loading and retaining the active substance, while maintaining low protein binding *in vivo* and consequently prolonging their circulation lifetime.

DSPC is the major lipid component in the liposome bilayer. It was selected based on its high purity. It is a synthetic lipid with a well-defined fatty acid composition. It has a relatively high phase transition (Tm of 55°C) and is present in the highly ordered structure termed the gel phase, which resists permeability to small molecule drugs at physiological temperature (37°C).

Cholesterol is another main component of the liposome bilayer. It is incorporated to stabilize liposomal phospholipid membranes from disruption by plasma proteins, to decrease binding of plasma opsonins responsible for rapid clearance of liposomes from the circulation, and to decrease permeability of solutes/drugs in combination with bilayer forming phospholipids.

MPEG-2000-DSPE is a minor component of the liposome bilayer. Its presence on the surface provides a minimal steric barrier preventing liposome aggregation. MPEG-2000-DSPE coated liposomes are shown to be stable with respect to size and drug-encapsulation. Mean molecular weight and polydispersity index is included in the specification. Stability of MPEG-2000-DSPE has been discussed and considered acceptable.

The liposomes are dispersed in an aqueous buffered solution consisting of HEPES buffer, sodium chloride, and water for injections.

HEPES is a novel excipient. Detailed information on the manufacture, characterization and controls, justification of its use and a summary of supporting toxicology data for HEPES was requested by the CHMP during the assessment procedure and the provided data was considered satisfactory.

The list of excipients is included in section 6.1 of the SmPC and in paragraph 2.1.1 of this report.

In the initial stages of the manufacturing process of the finished product, irinotecan hydrochloride trihydrate active substance is dissolved in a dextrose solution. It is subsequently mixed with formed liposomes and precipitated and encapsulated as irinotecan sucrose octasulphate (sucrosofate) salt within a liposome, using an active drug loading process. The hydrochloride counter ion is replaced with sucrosofate resulting in the formation of irinotecan sucrosofate salt within the liposome. Sucrosofate is encapsulated in the liposome with the active substance. A scientific discussion on physicochemical characterisation of liposomes was presented in

the dossier. It included entrapment volume, morphology using cryoTEM analysis, particle size, particle size distribution, percent encapsulated drug, drug to phospholipid ratio, lipid impurity, DSPC to cholesterol ratio, *in vitro* release, zeta potential, and pH.

The formulation used during phase 3 clinical studies is the same as that intended for marketing.

The results of the comparability study between the two formulations (used in phase 1 and phase 2 studies, and phase 3 studies and commercial manufacture, respectively) demonstrate that products manufactured by both processes meet release criteria with no significant differences in quality attributes. Additional physico-chemical characterization and purity analysis (including lipid analysis, *in vitro* drug release assay, liposome morphology and size analysis) found no significant differences between both processes except for improved lower levels of lysophosphatidylcholine impurity. Stability studies of the drug products were performed to help detect differences that are not readily detectable by the characterization studies. There were no significant differences in quality attributes between the initial process and the phase 3 process after four weeks of storage under stress stability conditions (30 ± 2°C). The provided comparability study between the initial process and the process used to manufacture phase 3 and commercial batches was found acceptable.

Pharmaceutical development of the finished product contains QbD elements.

The Quality Target Product Profile was to develop a medicinal product conforming to: an intravenous infusion of sterile liposomes, that is white to slightly yellow, opaque, packaged in a 10 mL single use glass vial. Irinotecan is encapsulated as irinotecan sucrose octasulphate salt, in a long circulating liposome composed of cholesterol, DSPC, and mPEG-2000-DSPE. Each 10 mL vial contains the equivalent of 50 mg irinotecan (reported on the hydrochloride trihydrate basis) at a concentration of 5 mg/mL irinotecan (4.3 mg/mL as reported on the anhydrous free base form). It is stable for 24-36 Months at 2-8°C.

The critical quality attributes (CQAs) identified were: visual appearance, irinotecan identity, lipid identity, cholesterol identity, irinotecan concentration, percent encapsulated drug, irinotecan impurities, lipid impurity, residual solvents, bacterial endotoxins, bioburden and sterility, drug to phospholipid ratio, DSPC to cholesterol ratio, extractable volume in container, *in vitro* release, osmolality, particle size, particle size distribution, particulate matter in injections, pH, and zeta potential.

The manufacturing process has been evaluated through the use of risk assessments to identify the critical product quality attributes and critical process parameters. A risk analysis was performed using the failure mode effect analysis (FMEA) method in order to define critical process steps and process parameters that may have an influence on the finished product quality attributes. The critical process parameters have been adequately identified.

The *in vitro* release method was designed to detect defective, incorrectly formulated or degraded liposomes; which could potentially affect the release of the active substance from the encapsulating vesicle. Discriminating ability of the method has been demonstrated with a series of studies which included a generation of defective liposomes and use of the model independent approach using similarity and difference factors applied to compare the *in vitro* release results.

The product is manufactured using sterile filtration and aseptic filling. Due to the nature of the finished product which is a liposome formulation with a phase transition temperature of the lipid membrane of 55 °C, terminal sterilisation by moist heat in line with decision trees for sterilisation choices for aqueous products is not possible. The sterilisation method was therefore appropriately justified.

The primary packaging is type I glass vial with a grey chlorobutyl stopper. The container closure of the finished product is sealed by an aluminium seal with a flip-off cap. The material complies with Ph. Eur. and EC

requirements. Compatibility studies were performed and demonstrated the compatibility of the finished product diluted in 5% dextrose and 0.9% saline at concentrations ranging from 0.2 mg/mL to 2 mg/mL with standard intravenous bags and infusion sets. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

Manufacture of the product and process controls

The manufacturing process consists of five main steps: liposome formation (lipid dissolution, multi lamellar vesicle (MLV) formation and small unilamellar vesicle (SUV) formation and sizing), active drug loading (diafiltration, drug loading), bulk drug product formulation, filling finish including bioburden reduction and sterile filtration, and labelling and packaging. The process is considered to be a non-standard manufacturing process.

Process validation for the manufacture of the finished product has been performed on three commercial scale batches in two discrete steps (bulk product manufacturing process and filling finish manufacturing step) in line with the traditional approach to process validation. In addition three scale down validation runs with bacterial challenge were performed in support of the validation of filters used to sterilise the product prior to the filling process. Additional validation studies included aseptic process validation, steam sterilisation validation (for the container closure of the finished product, and the components and equipment parts required for filling of the vials), dry oven validation (used to depyrogenate non-heat liable materials used in the manufacturing process), and container closure validation.

It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process and pharmaceutical form.

Product specification

The finished product release specifications include appropriate tests for this kind of dosage form: appearance (Ph. Eur.), irinotecan identity (HPLC, UV), DSPC identity (HPLC-ELSD), cholesterol identity (HPLC-ELSD), MPEG2000-DSPE identity (HPLC-ELSD), irinotecan concentration (assay) (HPLC-UV), percent encapsulated drug (HPLC-UV), irinotecan impurities (HPLC-UV), lipid impurity (HPLC-ELSD), residual solvents (GC), residual trimethylamine (GC-FID), bacterial endotoxins (Ph. Eur.), sterility (Ph. Eur.), drug to phospholipid ratio (calculation), DSPC to cholesterol ratio (calculation), extractable volume (Ph. Eur.), in vitro release (HPLC-UV), osmolality (Ph. Eur.), particle size (Ph. Eur.), particle size distribution (Ph. Eur.), particulate matter in injections (Ph. Eur.), pH (Ph. Eur), and zeta potential (in house).

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing of the active substance and of the excipients has been presented.

Batch analysis results are provided for 11 commercial scale batches and additional 23 pilot or lab scale batches, confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

Stability of the product

Stability data of three commercial scale batches and three additional pilot or lab scale batches of finished product stored under long term conditions for up to 30 months at 2 °C – 8 °C and for up to 6 months under accelerated conditions at 25 ± 2 °C / 60±5% RH according to the ICH guidelines were provided. The batches of medicinal product are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Samples were tested for appearance (Ph. Eur.), irinotecan concentration (assay) (HPLC-UV), percent encapsulated drug (HPLC-UV), irinotecan impurities (HPLC-UV), lipid impurity (HPLC-ELSD), bacterial endotoxins (Ph. Eur.), sterility (Ph. Eur.), container closure integrity (in house), in vitro drug release (HPLC-UV), osmolality (Ph. Eur.), particle size (Ph. Eur.), particle size distribution (Ph. Eur.), particulate matter in injections (Ph. Eur.), and pH (Ph. Eur).

The analytical procedures used are stability indicating. No significant changes have been observed during long term and accelerated stability testing. Additional data from bulk finished product batches stored under accelerated conditions showed an increase in osmolality and irinotecan concentration, which was not observed in the vialled finished product. These results are consistent with a small loss of water from the bulk polycarbonate stability containers. For both bulk and vialled finished product, irinotecan impurities and lipid impurities increased slightly, but remained within the specification limit. These results confirm the expected irinotecan degradation and lipid hydrolysis seen at higher temperatures under temperature stress conditions.

In addition, three batches of both bulk and vialled finished product were exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. The results from this study showed a decrease in pH (related to LysoPC impurity and associated stearic acid increase) and irinotecan concentration, and an increase in irinotecan and lipid impurities. No significant change was observed in percent encapsulated drug, in vitro release, osmolality or particle size distribution. These data demonstrate that the finished product is sensitive to light and should be protected from light exposure.

Additional results on three batches of the finished product used in stress studies were provided. These included freeze-thaw cycling (3 cycles of overnight exposure at -20°C followed by a minimum of 3 hours at ambient room temperature (~20°C)), demonstrating a decrease in percent encapsulated drug outside the specification limit. An increase in irinotecan impurities, lipid impurities and particle size was observed, but they remained within the specification limits. Particle size distribution showed variable results likely caused by freeze/thaw stress. No significant change was observed in irinotecan concentration, in vitro release, pH or osmolality. The data are consistent with expected loss of liposome integrity, potential liposome aggregation and leakage of the irinotecan from the liposome during freeze-thawing. The product is sensitive to freezing and thawing and therefore should be protected from freezing.

Finally, results from three batches of the finished product on thermal stress conditions (40 ± 2°C or 50 ± 2°C for 4 weeks) were provided. A decrease in percent encapsulated drug outside the specification limit, and an increase in irinotecan and lipid impurities within specification limits were observed, demonstrating that the finished product is heat sensitive.

Based on available stability data, the proposed shelf-life of 30 months for an unopened vial when stored in a refrigerator (2 °C – 8 °C) and in the outer carton in order to protect from light, as stated in the SmPC (section 6.3) are acceptable. The medicinal product must not be frozen.

Chemical and physical stability for the diluted solution for infusion has been demonstrated at 15-25°C for up to 6 hours or in the refrigerator (2ºC - 8ºC) for no more than 24 hours. The finished product was diluted in 5%

dextrose or 0.9% saline to 0.2, 0.48 (in dextrose only) and 2.0 mg/mL and held for 24 hours at 2-8 °C or 6 hours at room temperature prior to being infused for 90 minutes using an infusion pump. The samples were analyzed for visual appearance, pH, concentration, purity and identity of irinotecan, drug encapsulation ratio, particle size and for di(2-ethylhexyl)phthalate (DEHP) from the PVC infusion bags.

DEHP levels were below the detection limit in samples with lower irinotecan hydrochloride concentrations (0.2mg/mL and 0.48mg/mL), which corresponds to the commercial dose. The levels of DEHP detected in the high concentration samples correspond to a worst case exposure of 500 µg of DEHP per day, assuming an infusion volume of 500 mL. These levels were below (1% or less of) the parenteral tolerable intake level of 0.6 mg/kg/day (U.S. Food and Drug Administration, Center for Devices and Radiological Health. Safety Assessment of Di(2-ethylhexyl)phthalate (DEHP) Released from PVC Medical Devices, July 2002). Exposure of patients to DEHP at these doses is not expected to result in adverse effects.

The data demonstrate the compatibility of the drug product diluted in 5% dextrose or 0.9% saline at concentrations ranging from 0.2 mg/mL to 2 mg/mL with standard intravenous bags and infusion sets.

Adventitious agents

Cholesterol obtained from sheep wool is used in the product. Valid TSE CEP from the suppliers of the cholesterol used in the manufacture is provided. No other excipients derived from animal or human origin have been used.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

Onivyde is a concentrate for solution for infusion with irinotecan hydrochloride trihydrate, a semi synthetic active substance in a liposomal formulation.

These liposomes are unilamellar lipid bilayer vesicles, approximately 110 nm in diameter, which encapsulate irinotecan in a gelated or precipitated state, as sucrosofate salt. Sucrosofate salt is considered a novel excipient and it has been chosen as the optimal counter ion for precipitating, loading and retaining of the active substance within the liposome. The liposomes are dispersed in an aqueous buffered solution, containing HEPES which is a zwitterionic buffer and a novel excipient.

The liposomal formulation of irinotecan is designed to prolong circulation of the active substance in plasma and increase the delivery of the active substance in tumours to take advantage of the compromised vasculatures of tumours.

The pharmaceutical development was focused on 1) the selection of an optimal counter ion for active drug loading, 2) the selection of an optimal ratio of DSPC to cholesterol components of the lipid membrane which influence its physical properties and the capability of retaining the active substance in the liposome, 3) the selection of MPEG-2000-DSPE component of the liposome bilayer whose presence on the surface provides a steric barrier to avoid liposome aggregation, and 4) the selection of an appropriate buffer capable of retaining the optimal pH range for the concentrate for solution for infusion. At the time of the CHMP opinion, there was a minor unresolved quality issue having no impact on the Benefit/Risk ratio of the product. The Applicant is

requested to resolve the requirement of GMP compliance for the entire route of synthesis of the active substance, as a legally binding measure:

To introduce the revised and agreed synthetic process for the manufacture of the active substance from the agreed starting materials by 31/12/2016.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

2.2.6. Recommendation(s) for future quality development

Not applicable.

2.3. Non-clinical aspects

2.3.1. Introduction

Onivyde (also referred to as MM-398) is a liposome of ~100 nm average diameter that encapsulates the active pharmaceutical ingredient irinotecan hydrochloride (CPT-11). Irinotecan is a known active substance and its mechanism of action was previously established in primary pharmacology studies. No such studies have been performed with the new formulation Onivyde.

2.3.2. Pharmacology

Primary pharmacodynamic studies

In vitro studies
Three in vitro studies on murine and human cell lines, and tumour tissue lysates from patients or mouse models were provided, aiming at investigating MM-398 liposomal uptake/release at the cellular level and irinotecan (CPT-11) conversion into its active metabolite (SN-38). Furthermore, a recent publication profiling MM-398 PK/PD model in a colon cancer model was provided (Kaira et al., 2014).

Murine and human macrophage-like cell lines were shown to accumulate 10-40 times higher levels of liposomes as compared to tumour cell lines. Uptake of liposomes led to the release of irinotecan from macrophage cell lines. In experiments on tumour tissue lysates, including pancreatic, and human macrophage-like cells, enzymatic conversion of irinotecan to the active metabolite SN-38 (100-1000x more active than irinotecan) was demonstrated.

In vivo studies

In a series of *in vivo* pharmacology studies in xenograft tumour models of human breast, gastric, pancreatic, cervical, brain and colon cancer, the Applicant compared the efficacy of MM-398 relative to equivalent or higher

dosing levels of free irinotecan. Generally, increased efficacy (measured as significant inhibition of tumour growth) was achieved with MM-398. In view of the present indication, the pancreatic tumour studies are of special interest. The efficacy of MM-398 was evaluated in several orthotopic and ectopic xenograft models of pancreatic cancer. MM-398 showed more potent anti-tumour activity, including durable tumour regression, compared to the equivalent dose of free irinotecan, in an orthotopic L3.6pl-T tumour model. Best effect in this model was obtained when using EGFR-targeted MM-398. Treatment with MM-398 also caused reduced tumour growth and a lower metastatic burden in an orthotopic BxPC3 tumour model, and decreased tumour hypoxia preceding tumour regression in the high-hypoxia OCIP51 orthotopic model.

During the procedure, the Applicant submitted a new pharmacology study report (MM-398-NC-N-Ph-027), evaluating antitumor activity of MM-398 against ectopic patient-derived pancreatic cancer xenografts in SCID mice. The results have been summarized by the Applicant in the table below.

Table 2: Tumour growth inhibition after treatment with Onivyde or gemcitabine in PDX tumour-explant models of pancreatic cancer

PDX model	Passage	Treatment	Dose (mg/kg)[a]	TGI (%)	# doses; TGI day	2-way ANOVA[b]	Gemcitabine sensitive
12424	8	MM-398	5	28.6	4; d35	N	N
		MM-398	10	67.2		Y	
		Gemcitabine	100	-13.9		N	
12424	9	MM-398	10	76.4	4; d36	Y	N
14244	9	MM-398	5	68.7	4; d28	Y	Y
		MM-398	10	93.8		Y	
		Gemcitabine	100	78.4		Y	
14312	4	MM-398	20	96.2	4; d35	Y	N
		Gemcitabine	100	28.9		Y	
15010	4	MM-398	5	94.9	4; d35	Y	Y
		MM-398	10	97.8		Y	
		Gemcitabine	100	81.6		Y	
19015	7	MM-398	5	-13.4	4; d42	N	N
		MM-398	10	61.6		Y	
		Gemcitabine	100	-8.5		N	
		Gemcitabine	200	34.4		Y	
18254[c]	7	MM-398	10	70.8	4; d28	Y	Y
		MM-398	20	78.9		Y	
		MM-398	40	75.0		Y	
18254	8	MM-398	20	67.4	4; d28	Y	Y
18268	7	MM-398	20	39.4	4; d30	N	[d]
OCIP51	9	MM-398	20	71.6	3; d21	Y	NA

[a] Doses are given as bolus intravenously q7d

[b] Post-hoc test, simple effects within rows with Sidak's multiple comparisons test. Y indicates statistical significance ($p < 0.05$), N: no statistical significance; NA not available

[c] Evaluated in Rnf[-] SCID mice without plasma CES activity

[d] Patient showed partial response to gemcitabine

Dosing schedules
In studies using a q4d schedule, body weight losses in the range of 10-20% were observed. This effect was diminished when a q7d schedule was applied, which more closely resembles the clinical treatment regimen. No study employing the clinical q14d schedule was conducted.

Combination studies
Combination studies with Onivyde and 5-fluorouracil (5-FU) or cisplatin, in xenograft models of colon and cervical cancer, respectively, demonstrated more potent effects than when the substances were administered as single agents. No combination studies were performed in pancreatic cancer models.

Mechanistic studies
Onivyde distribution and uptake were investigated in an orthotropic intracranial xenograft model of glioblastoma in rats. Intracranial delivery of MM-398 caused increased retention time (22 times higher t ½) in the brain as compared with free irinotecan. This correlated with increased survival. In contrast, iv administration of MM-398 in the same tumour model did not result in increased brain tissue retention, although the tissue exposure in terms of C_{max} and AUC was higher than that achieved with free irinotecan. Survival was increased in rats treated with MM-398.

Further investigation of the relationship between tissue pharmacokinetics and tumour treatment efficacy was conducted in a xenograft colon cancer model in mice, comparing Onivyde and free irinotecan (iv administration). Despite equal SN-38 exposure (plasma, tumour) the liposomal formulation resulted in 2-2.7 times better tumour growth control.

Tumour hypoxia is linked to aggressive disease progression and resistance to therapy. To investigate effects on hypoxia the Applicant conducted a non-invasive FAZA PET study in the HT-29 colon cancer model, monitoring changes in the tumour microenvironment after treatment with MM-398 or free irinotecan at similar SN-38 exposure in the tumour. Tumours in mice treated with MM-398 at 10 mg/kg bw showed significantly lower levels of hypoxia compared to those treated with CPT-11 at 50 mg/kg bw. Tumour growth was also significantly reduced in mice treated with MM-398 at 10 mg/kg bw, as compared with treatment with CPT-11 at 50 mg/kg bw.

In a final mechanistic study the Applicant performed flow cytometry analysis on cell suspensions from tumour tissue to investigate the distribution of fluorescently-tagged liposomes in various animal tumour models. The results showed that F4/80-positive mature macrophages accumulated a larger proportion of the overall cellular liposomal load relative to their population size compared to other myeloid or non-myeloid cell populations. Macrophage and myeloid populations accounted for 78-94% of the cellular liposomal uptake.

Secondary pharmacodynamic studies

No secondary pharmacodynamics studies were performed.

Safety pharmacology programme

A study in conscious telemetered Beagle dogs was conducted to assess the effect of MM-398 on the cardiovascular and respiratory systems. Single iv infusion with MM-398 at doses up to 21 mg/kg (420 mg/m^2) had no effect on cardiovascular, hemodynamic, electrocardiographic, respiratory parameters, or body temperature. The maximum plasma concentrations of irinotecan and SN-38 at the end of infusion were 7.5x and 3.6x above therapeutic C_{max} respectively.

No CNS safety pharmacology study was conducted.

Pharmacodynamic drug interactions

No studies were performed with Onivyde to evaluate pharmacodynamic drug interactions.

2.3.3. Pharmacokinetics

The pharmacokinetics and toxicokinetics of irinotecan and the active metabolite SN-38 have been studied in relevant animal species, i.e. rats, dogs and tumour bearing mice, following IV administration of single and repeat doses of liposome encapsulated irinotecan (MM-398) and unencapsulated irinotecan (CPT-11). The TK analysis of the two pivotal toxicity studies in rats and dogs was not GLP compliant and is thus not considered valid to support the interpretation of these studies (see further under Toxicology).

In all studies, total irinotecan levels (i.e. liposome encapsulated irinotecan and free irinotecan) were measured and used for the evaluation of the pharmacokinetics of irinotecan. Determination of encapsulated irinotecan in dogs following an IV single bolus dose of 10 mg/kg Onivyde showed a stable degree of 75 to 86% encapsulation up to the last determination 36 hours post-dose which indicates that the concentrations of total irinotecan represent encapsulated irinotecan to a corresponding degree.

Absorption

Single doses of 90 min IV infusions of MM-398 to SD rats and Beagle dogs showed some deviations from dose proportional plasma exposure (in terms of AUC) for irinotecan at the dose levels studied (higher than dose proportional from the low [6 mg/kg] to the mid [20 mg/kg] dose in rats and from the mid [30 mg/kg] to the high [50 mg/kg] in dogs. For SN-38 the increase in AUC was overall approximately dose proportional in dogs but generally less than dose proportional in rats.

When compared at the same dose level (20 mg/kg in rats and 30 mg/kg in dogs) the AUC of irinotecan and SN-38 was higher for MM-398 than for unencapsulated irinotecan in rats (approximately 1700-fold and 90-fold, respectively) and dogs (approximately 40-fold and 3-fold, respectively). Terminal elimination half-life of irinotecan and SN-38 was longer for Onivyde (10 and 15h, respectively, in rats; 13h for both analytes in dogs) than following unencapsulated irinotecan (0.7 and 11h, respectively, in rats; 4.3 and 6.2h, respectively in dogs).

Repeated doses of 90 min IV infusions of MM-398 once every third week for 18 weeks to rats and dogs showed approximately dose proportional plasma exposure (in terms of AUC) for irinotecan at the dose levels studied in rats (30, 75 and 190 mg/kg) and dogs (9, 15 and 21 mg/kg). For SN-38 the increase in total AUC was overall approximately dose proportional in dogs but generally less than dose proportional in rats. The accumulation of irinotecan was about 2-fold in rats but there was no evidence for accumulation of SN-38 in rats or of irinotecan and SN-38 in dogs.

When compared at the same dose level (75 mg/kg in rats and 21 mg/kg in dogs) the AUC of irinotecan and SN-38 was higher for MM-398 than for unencapsulated irinotecan in rats (approximately 1000-fold and 50-fold, respectively) and dogs (approximately 150-fold and 3-fold, respectively). Terminal elimination half-life of irinotecan and SN-38 was longer for Onivyde (33 and 323h, respectively, in rats; 12 and 35h, respectively, in dogs) than following unencapsulated irinotecan (4.9 and 6.1h, respectively, in rats; 2.8 and 5.1h, respectively in dogs). MRT_{0-168} values for irinotecan and SN-38 were also longer for MM-398 (22.0 to 22.9h and 40.1 to 53.8h, respectively, in dogs) than for unencapsulated CPT-11 (3.4 to 4.1h and 4.8 to 6.0h, respectively, in

dogs). The very long half-life of SN-38 in rats is likely due to the presence in rodent species of a plasma carboxylesterase activity that contributes to the systemic levels of SN-38 after administration of MM-398.

Distribution

Quantitative whole-body autoradiography following a single 90 min IV infusion of 10 mg/kg ^{14}C-MM-398 and unencapsulated ^{14}C-CPT-11 in Long Evans rats showed a wide distribution to tissues for both formulations with the highest concentrations generally observed at the end of the infusion. For several of the tissues (e.g. including CNS, pigmented skin, liver [and bile], kidney, epididymis, testis, lung, trachea, heart, bone marrow, blood and spleen) the concentrations of radioactivity was higher for ^{14}C-MM-398 than for unencapsulated ^{14}C-CPT-11 with the highest levels for ^{14}C-MM-398 in plasma, bile and spleen. Tissue levels generally remained higher for a longer period following ^{14}C-MM-398. These results were supported by another study which showed increased levels and prolonged duration in plasma, spleen, and liver of total irinotecan and/or SN-38 for MM-398 as compared to unencapsulated CPT-11 following a single bolus IV infusion of 10 mg/kg.

The radioactive levels in the gastro-intestinal contents of rats were much higher for unencapsulated ^{14}C-CPT-11 than for^{14}C-MM-398 and the levels of irinotecan and SN-38 in intestines (with contents) in tumour bearing mice after a single IV bolus administration of 40 mg/kg were in general higher for unencapsulated CPT-11 than for MM-398. Together the results indicate a slower elimination of MM-398 than of unencapsulated CPT-11. This is a possible explanation for the increased and prolonged plasma and tissue levels observed for MM-398.

While the relative proportions of radioactivity in brain versus plasma were lower for ^{14}C-MM-398 than for unencapsulated drug, the absolute radioactive concentrations were 18x higher in the CNS of rats given ^{14}C-MM-398. This indicates that MM-398 may be able to pass the blood brain barrier Alternatively, the higher CNS concentrations could be due to a longer plasma half-life of MM-398. Retention of ^{14}C-MM-398-derived radioactivity in the uvea of the eye may indicate binding to ocular melanin. A lower distribution of radioactivity into the cellular fraction of blood following ^{14}C-MM-398 than following unencapsulated ^{14}C-CPT-11 was also indicated.

In tumour bearing mice, highest tissue exposure to irinotecan and SN-38 for MM-398 in terms of C_{max} and $AUC_{0-\infty}$ was obtained in the liver whereas tumours had the lowest C_{max} of irinotecan, and lowest C_{max} and $AUC_{0-\infty}$ of SN-38. The AUCs of irinotecan and SN-38 in plasma, organs and tumours were increased for MM-398 as compared to unencapsulated CPT-11. For irinotecan the largest increase was observed in tumours (28-fold) whereas for SN-38, the increase in tumours (6-fold) was lower than in the liver (16-fold) and the kidney (10-fold). Following MM-398, C_{max} of irinotecan was increased in tumours but to a slight extent also in the liver, 1.6-fold and 1.1-fold, respectively. For SN-38, C_{max} was increased in liver and kidney but decreased in tumours and in small and large intestine. The MRT and $t_{½}$ of irinotecan and SN-38 were increased for MM-398 as compared to unencapsulated CPT-11 and were longer in tumours than in the other tissues (at least approximately 3-fold for irinotecan and 2-fold for SN-38). According to the Applicant the increase in C_{max} of irinotecan in tumours together with a longer duration in tumours as compared to other tissues indicate favourable pharmacokinetic characteristics for MM-398 both with respect to efficacy and safety.

Metabolism

After release from the Onivyde liposomes, irinotecan is expected to follow the same metabolic fate as unencapsulated irinotecan. The active metabolite SN-38 is reported to be formed from irinotecan via carboxylesterase-mediated cleavage in the liver and intestines. Plasma carboxylesterase activity and possibly more efficient carboxylesterases in rodents suggest that exposure comparisons from dog studies may be more relevant for the estimation of margins to human exposure. The active SN-38 is further metabolised to the inactive SN-38 glucuronide (SN-38G) via uridine-5′-diphosphate-glucuronosyltransferase UGT1A isoforms in

the liver and subsequent deglucuronidation by intestinal β-glucuronidase. Irinotecan is also metabolised by CYP3A4 to various oxidative metabolites, including APC and NPC. While NPC can be converted to SN-38 by carboxylesterases, APC is not and does most likely not contribute directly to the activity and toxicity profile of irinotecan *in vivo*.

Excretion

A mass balance study of a single 90 min IV infusion of 5 mg/kg encapsulated ^{14}C-CPT-11 in intact and bile duct cannulated SD rats showed that faecal excretion was the major route for excretion in intact rats with 78.3 to 83.4% and 16.5 to 22.9% of the radioactive dose detected in faeces and urine, respectively, and that biliary excretion represented 77% of the faecal excretion.

2.3.4. Toxicology

The toxicity profile of Onivyde (MM-398) was characterized in mice, rats and dogs. The in life-phase of the pivotal toxicity studies in rats and dogs was GLP compliant; however, the TK/bioanalysis part was not (see below). The study package comprised single-dose and repeat-dose toxicity studies up to 18-weeks duration (dosing once every third weeks, in total 6 cycles), and an *in vitro* blood compatibility study. The genotoxic, carcinogenic, reproductive and developmental toxicity potential of the API irinotecan has been determined previously. Ninety (90)-minute intravenous (iv) dosing has been used in all *in vivo* studies, as this is the administration route that will be used clinically.

The general scientific integrity and validity of the pivotal toxicity studies was called into question, considering a number of GLP issues that needed to be addressed. The Applicant clarified that at the time of the initiation of the two pivotal studies, the test facility where the *in life phase* of both pivotal toxicity studies [6-cycle rat (PEP02-NC-G-Tx-010, 1005-3071) and dog (PEP02-NC-G-Tx-011, 1006-2162)] has been performed, was under OECD compliant GLP monitoring. Moreover, the full GLP compliance *status* of the *in life phase* of the two studies has been confirmed by a dedicated GLP Study Audit (Study audit report 15842) performed at the CiToxLAB facility, in November 2015 as part of a biannual routine inspection program. However, the Monitoring Authority could not comment on the GLP status of the TK/bioanalytical phase of the studies performed at non-OECD subcontractor sites, therefore the audit does not cover the GLP status of the TK/bioanalytical analyses and this data cannot be used to support the interpretation of the study results.

Single dose toxicity

Single-dose studies were performed in ICR mice, Sprague Dawley rats and Beagle dogs. The approximate lethal dose in mice was 200 mg/kg for both MM-398 and CPT-11. Gastrointestinal (GI) toxicity was evident. Doses of MM-398 at ≥400mg/kg caused thymus atrophy, spleen atrophy and pale liver.

In rats, 960 mg/kg and 200 mg/kg were lethal doses in MM-398 and CPT-11 dosed animals, respectively. Main clinical signs were dose-dependent GI toxicity, hypoactivity and irregular respiration. Body weight reductions were evident in all dose groups. Rats dosed with MM-398 at ≥480 mg/kg showed reductions in leukocytes that partly recovered before termination of the study.

Dogs exposed to MM-398 and CPT-11 showed pronounced GI toxicity including emesis and diarrhoea. All treatment groups showed dose-dependent reductions in leukocytes. Preterminally euthanized animals had small spleens and thymuses. All exposed groups showed reductions in body weight and food consumption. The maximum tolerated dose (MTD) was 15 mg/kg bw (300 mg/m^2). The exposure margin from MTD to human therapeutic dose (80 mg/m^2) was 3.8x.

Table 3: Single dose toxicity studies with Onivyde

Study ID/ GLP	Species/ Sex/Number/ Group	Dose/Route (i.v.) MM-398 mg/kg / empty liposome phospholipids concentration mM / Conventional CPT-11	Approx. lethal dose / observed max non-lethal dose (mg/kg)	Major findings
PEP02-NC-G-Tx-003 GLP	ICR Mouse/5/sex	LIIA06 200, 400, 800 LIIA06P 30.91mM CPT-11 200	Estimated LD50 237 (M), 336 (F)	Mortality M+F placebo 0/10, CPT-11: 2/10, MM-398 1/10, 10/10, and 10/10. Delayed onset of severe adverse clinical signs and death observed at 6 to 8 days post-dosing For CPT-11 rapid onset and death on the day of dosing
PEP02-NC-N-Tx-004 (PS00006) non GLP	SD male rats 3 /group	LIIA02 and LIIA03 200, 400 LIIA03P 32mM CPT-11 200	MM-398: 200 CPT 11: 200	CPT-11: 2 deaths on day 1; hypoactivity, exopthalmus, soft faeces, watery faeces, yellowish urine stain around the genital area, tremors and chromodacryrrhea MM-398 200 and 400 mg/kg: brown soft faeces on day 3 (2/3 and 3/3 rats. No clinical signs in controls groups. LIIA02 400 low bw gain
PEP02-NC-G-Tx-002 (No. 7082) GLP	SD male rats 5/sex/group	LIIA05, LIIA06 480, 720, 960 LIIA06P 30.91 mM CPT-11 320	About 960	Toxicity due to effects on GI tract responsible for the deaths of animals at 960 mg/kg. CPT-11 induce GI toxicity at 320 mg/kg
Study PEP 02-NC-N-TX001 (No. 6171) GLP	Beagle dogs	CPT-11 dose escalation 4 day wash out 30, 40 and 60.	MTD 30 (F), 40 (M)	Vomiting an severe GI alterations
PEP02-NC-G-Tx-005 (No. 6160)	Beagle dogs 3 /sex	LIIA05 dose escalation 4 day wash out 40, 50, 65 or 100 (infusion 90 min) Control 5 % dextrose in water	MTD 50	Severe, irreversible GI toxicity $\geq$ 65. Transient GI toxicity (loss of appetite, decrease in body weight, evidence of emesis and loose stool) 40 and 50
Protocol PEP02-NC-G-Tx-007 (6151) GLP	Beagle dogs 3 /sex	LIIA09; 15, 30, and 50 LIIA10P (41.56 mM) CPT-11 30	MTD 15 (300 mg/m^2) On this basis the dose for 4 weeks study was determined to be <30 mg/kg/day.	Emesis and GI toxicity. Neurologic events, such as tremors, along with the GI effects, were noted with CPT-11

Repeat dose toxicity

The potential toxicity and the TK profile of MM-398 was investigated in 4 studies:

-in Sprague-Dawley rats [PEP02-NC-G-Tx-006 (7083)] and Beagle dogs [PEP02-NC-G-Tx-009 (6152)] by i.v. infusion once each week during a 28-day period, followed by a 14-day recovery/observation period (4 weeks study),

- in Sprague-Dawley rats [PEP02-NC-G-Tx-010 (1005-3071)] and Beagle dogs [PEP02-NC-G-Tx-011 (1006-2162)] administered as 6 cycles (each cycle comprised of a single dose followed by a 3-week observation period (6 cycles study) via i.v. infusion, each over a period of 90 minutes.

Table 4: Repeat dose toxicity studies with Onivyde

Report	MM-398 batch (Drug/pho ratio mg/mmol)	Liposom concentr (mg/mL)	phospholipid content (mM)	Size (nm) (drug encapsulation %)	Placebo Batches Phosp. Concen (mM)	SOS amount dose/total (mg/kg)	TEA amount dose/total (mg/kg)
RATS							
PEP02-NC-G-Tx-006 (7083) 4 wks	LIIA07 (505)	17.36	29.8	87.9 (99.7%)	LIIA05-liposome (47.2)	57.9/231	51.7/207
	LIIA08 (501)	16.93	29.3	90.8 (99.7%)	LIIA08P placebo (23.8)	36.3/145.2	67.8/271.2
[a] Animals received ~ 357 - 447 mg/kg phospholipids. [b] animals received or ~ 282 - 353 mg/kg phospholipids. * used only when LIIA05-liposome had been exhausted							
No. PEP02-NCG-Tx-010 (1005-3071) 6 cyc	LIIAS3001 (510)	5	8.39	128 (98.75%)	LIIA12P (43.64)		
	LIIA12 (481)	18.7	33.4	104.7 (99%)	LIIA14P (45.26)		
	LIIA14 (514.6)	24.61	41.34	104.7 (99%)	LIIA14P-2 (40.22)		
DOGS							
PEP02-NC-G-Tx-009 (6152) 4 wks	LIIAS3001 (510)	5	8.39	128 (98.75%)	LIIA011 (19.33)	1.6/6.4	4.4/17.8
PEP02-NC-G-Tx-011, ITR 1006-2126 6 cyc	LIIPS5001 (517.5)	5	12.2	104.9 (99.68%)	LIIPS 5001 (12.2)		
	LIIAS4002 (498.3)	5	Not reported	116 (99.53%)			

Mortalities

Preterminal mortalities and unscheduled sacrifice of animals occurred in all repeat-dose toxicity studies with CPT-11 and MM-398. A clear species difference in sensitivity was observed. Despite high exposure to irinotecan and SN-38, only occasional rats treated with MM-398 or CPT-11 were found dead or preterminally sacrificed. In most cases, the cause of death was unrelated to the compound. In the 4-week study, one female rat died after treatment with MM-398 at a dose of 260mg/kg (1560 mg/m^2) on SD 13. No specific cause of death was determined. In the 18-week study, one male dosed with MM-398 at 190 mg/kg (1140 mg/m^2) was euthanized during the recovery period due to deteriorating condition. The margins between these mortalities and the human therapeutic dose are in the range of 14-19x.

In contrast, dogs showed severe GI toxicity at relatively low doses, causing preterminal sacrifice of two males treated at 16 mg/kg (320 mg/m^2) in the 4-week study. Both dogs had evidence of GI bleeding, and

histopathology revealed mucosal hemorrhage and atrophy, and crypt epithelial necrosis. The margin between the dose of irinotecan causing mortality in the 4-week dog study and the human therapeutic dose is 4x.

In the dog 6-cycle study, a total of 13 dogs were found dead and an additional 6 dogs were removed from the study early. Changes in the bone marrow, GI tract and lymphoid tissues accounted for the mortality in the majority of dogs (see below). At LOAEL for mortality (21 mg/kg bw = 420 mg/m^2) the margin to human therapeutic dose of irinotecan was 5x.

Gastrointestinal tract

The GI tract was a target of toxicity for MM-398 and CPT-11 in the repeat-dose toxicity testing. The effects were not as dramatic as in the single-dose studies, since the doses used were lower. In rats, the GI-effects were mainly limited to reductions in food consumption and body weight decrease. Body weights were dose-dependently decreased immediately following the 90-minute infusions but returned to control levels within 9 days in the 6 cycle study. Food consumption decreased in all dose-groups but returned to control levels in males during recovery. CPT-11 caused less pronounced effects on body weight and food consumption as compared to MM-398 at the same dose.

In dogs, the high dose group (16mg/kg) in the 4--week study showed reductions in food intake with accompanying reductions in body weight. Two preterminally sacrificed dogs showed GI haemorrhage, intestinal mucosal atrophy and crypt epithelial necrosis (see above). In the 6-cycle study, all dose groups showed transient reduction in food intake, but no significant reduction in body weight. Pre-terminally dead/euthanized dogs at ≥ 21 mg/kg showed intestinal crypt epithelium necrosis and dilatation, mucosal villous atrophy, and congestion/haemorrhage. MM-398 and CPT-11 caused similar GI toxicity in dogs when given at the same dose.

Bone marrow

Bone marrow hypocellularity, reflected by peripheral haematology effects, was consistently found in the repeat-dose toxicity studies. In the 4-week rat study, red blood cells (RBC), haemoglobin (HGB) and white blood cells (WBC) were dose-dependently decreased and platelets (in males) were dose-dependently increased at MM-398 doses from 65 to 260 mg/kg. Similar effects were seen with CPT-11 at the same dose (130 mg/kg). Bone marrow hypocellularity was observed in MM-398 high dose (260 mg/kg) rats in the 4-week study, and at 190 mg/kg in the 18-week study. Female dogs exposed to 8 mg/kg MM-398 in the 4-week study showed reversible neutropenia. In the 18-week study, bone marrow hypocellularity was observed in pre-terminally dead/euthanized dogs treated with MM-398 or CPT-11 at ≥ 21 mg/kg bw. Dogs treated with MM-398 at ≥15mg/kg and with CPT-11 at 21 mg/kg had decreased levels of RBC, HGB, haematocrit and WBC.

Lymphoid organs

Lower thymus weights (32-80%) were observed in rats exposed to MM-398 ≥65mg/kg once weekly for 4 weeks. These effects correlated with thymic atrophy that was dose-dependent in severity and frequency. Dogs exposed to 16mg/kg MM-398 or CPT-11 for 4 weeks showed slightly reduced thymus weights, but without microscopic correlation. In the 18-week study, pre-terminally dead/euthanized dogs treated with MM-398 or CPT-11 at ≥ 21 mg/kg showed lower thymus weights correlated with lymphoid atrophy/necrosis.

Lymphoid atrophy/necrosis was present in the spleen, lymph nodes and Peyer´s patches of dogs treated with MM-398 at ≥ 15 mg/kg, and with CPT-11 at 21 mg/kg, for 18 weeks. Atrophy of Peyer´s patches was also present in dogs treated at ≥ 8 mg/kg in the 4-week study.

Spleen

Extramedullary haematopoiesis (EMH) was present in rats treated with CPT-11 (130mg/kg) in the 4-week study. In the 18-week rat study, all MM-398 exposed groups showed EMH in the spleen. In the 4-week dog study, splenic EMH was noted in the high dose MM-398 and CPT-11 groups. In the 18-week dog study, EMH was observed both in the spleen and liver in dogs treated with MM-398 at ≥ 15 mg/kg and with CPT-11 at ≥ 21 mg/kg.

Liver

Liver weights were 6-24% lower in rats exposed to MM-398 ≥65mg/kg in the 4-weeks study. No microscopic correlation was found. Minimal to mild multifocal liver degeneration/necrosis (without effect on liver weight or serum chemistry parameters) was reported for all MM-398 exposure groups in the 6-cycle study in rats. No similar findings were present in dogs. The margin between LOAEL (30 mg/kg = 180 mg/m^2, incidence 1/40 rats) and human therapeutic dose of irinotecan is 2x.

Vehicle-associated effects

In the repeat-dose toxicity studies in rats, vacuolation and histiocytosis in multiple organs were observed in all MM-398 dose groups, as well as in the vehicle control groups. These findings are considered to be related to phagocytosis of the liposome. On the whole, similar findings were seen in MM-398 treated animals and controls. Histiocytosis in the heart was only observed in rats treated with MM-398 at ≥ 190 mg/kg (1140 mg/m^2). In dogs, minimal histiocytosis in the spleen was present in vehicle controls and all MM-398 treated groups in the 18-week study.

Other findings

Other findings related to administration of MM-398 included renal medullary tubular hypertrophy in rats at 260 mg/kg in the 4-week study, decreased activated partial thromboplastin (APTT) in male rats at ≥ 65 mg /kg in the 4-week study, lower platelets, PCT and reticulocytes in dogs treated at ≥ 15 mg/kg MM-398 or 21 mg/kg CPT-11 in the dog 18-week study, and dental effects treated with MM-398 or CPT-11 in the rat 18 week-study.

Reversibility

All major findings related to treatment with MM-398 or CPT-11, and the vehicle-associated histiocytosis, were fully or partly reversible upon cessation of treatment.

Genotoxicity

No genotoxicity studies have been conducted with Onivyde.

Carcinogenicity

No carcinogenicity studies have been conducted with Onivyde.

Reproduction Toxicity

No reproductive and developmental toxicity studies have been conducted with Onivyde.

In the 4 week rat study, moderately lower prostate weights were reported in males at ≥65mg/kg MM-398. Histopathology showed mild atrophy of both prostate and seminal vesicles. In the 18 week study, 1 male rat in the high dose group had necrosis and atrophy of the testis unilaterally and severe aspermia of epididymis.

Dogs exposed to 16mg/kg MM-398 or CPT-11 in the 4-week study showed slightly lower prostate and testis weights, but no histological correlation could be made. In dogs that were euthanized/removed early from the study in the 6-cycle study (≥ 21 mg/kg MM-398 or CPT-11), prostate and testes weight reductions of 45-73%

were observed. Microscopically, the lower prostate and testes weights correlated with acinar atrophy and degeneration/necrosis of seminiferous epithelium, respectively.

Ovary weight reduction of 16-24% without any obvious histopathological correlation was reported in the rat 4-week study in females exposed to ≥65 mg/kg MM-398. In the 4-week dog study, lower uterus weight without any histopathological correlation was present at 16 mg/kg MM-398 or CPT-11. Decreased ovary and uterus weight, correlated with reduced number of follicles and atrophy, respectively, was observed in the 18-week dog study at ≥ 21 mg/kg MM-398 or CPT-11.

Toxicokinetic data

Toxicokinetic evaluations have been performed for both CPT-11 and MM-398 in single- and repeat-dose studies. These analyses were not GLP compliant and thus cannot be used to calculate margins to human therapeutic exposure. Some of the TK data suggests that the rat conversion of irinotecan to the active metabolite SN-38 is more efficient in the rat than in the dog. A possible explanation is that rodent species have a plasma carboxylesterase activity that contributes to the systemic levels of SN-38 after administration of irinotecan, while dogs and humans do not express plasma carboxylesterase enzymes.

Local Tolerance

Transitory skin reactions, including reddening and/or swelling and slow skin edema were seen in rats and/or dogs treated with MM-398 at ≥21 mg/kg. Similar findings were recorded also in animal receiving CPT-11 and empty liposomes, suggesting these effects were procedure-related.

Other toxicity studies

Haemolytic and flocculation potential

The haemolytic and flocculation potential of MM-398 was tested *in vitro* using human whole blood and plasma. No haemolytic effect of MM-398 was observed, and although some flocculation was seen in the samples spiked with liposome or MM-398, this is not considered an issue for further consideration.

Phototoxicity

No phototoxicity studies were performed with MM-398.

2.3.5. Ecotoxicity/environmental risk assessment

The Applicant has performed a Phase I assessment, as specified in the ERA guideline. Since irinotecan is an ionisable compound, Log Dow values were applied instead of Log Kow. These values were determined experimentally according to the OECD 107 guideline. All three values were below the PBT action limit of 4.5, as specified in the ERA guideline.
The original ERA document was updated in terms of recalculated PECsw based on refined Fpen value for the sought indication. The refined Fpen was calculated to 0.00001. Applying this value to the formula, the resultant PECsw is 0.00064 µg/l, which is below the threshold of 0.01 µg/l. Thus there is no need for a Phase II environmental fate and effect analysis, provided that the Log Dow values can be supported by experimental data.

Table 5. Summary of main study results

Substance (INN/Invented Name):			
CAS-number (if available):			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD107	Log D_{ow} (pH 4.0) = -2.03 ± 0.01 Log D_{ow} (pH 7.0) = 0.27 ± 0.02 Log D_{ow} (pH 9.0) = -0.81 ± 0.02	Not PBT
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
PEC $_{surfacewater}$, default or refined (e.g. prevalence, literature)	$6.4 \cdot 10^{-04}$ µg/L	µg/L	<0.01 threshold
Other concerns (e.g. chemical class)		N	N

2.3.6. Discussion on non-clinical aspects

Anti-tumour efficacy and proof of principle have been demonstrated in various rodent tumour models, including pancreatic cancer. The results of these studies have provided the basis for dose setting and schedule in the clinic. Comparisons between liposome encapsulated irinotecan (MM-398) and non-liposomal irinotecan (CPT-11) suggest that the liposomal formulation exerts a more potent anti-tumour effect.

Questions were raised about the role of tumour-associated macrophages (TAMs) as drug deposits for MM-398 drug uptake/release, the distribution of Onivyde in tumours and the liposomal uptake capabilities by pancreatic cancer cells. The Applicant clarified that TAMs were not proposed to be the sole provider of tumour-based drug release. Fluorescent immunohistochemistry (IHC) analysis on several pancreatic cancer models showed liposome "hotspots" in perivascular areas of the tumour stroma, which is known to be enriched with TAMs in pancreatic cancer. Despite this, DNA damage was localized to tumour cells, proving drug release from the tumour stroma.

Lack of proof-of-concept of sufficient tumour SN-38 level achievement after TAM drug uptake/release was raised as a concern. The Applicant provided data showing that tumour SN-38 levels at 72 h were consistent with concentrations needed to elicit cell death as determined from in vitro experiments. The relationship between SN-38 concentration and cell death was measured in a panel of pancreatic cell lines and found IC50 levels of less than 10 nM for a large number of cell line models.

To address some uncertainties regarding the effect of Onivyde in animal models of pancreatic cancer, the Applicant submitted a new pharmacology study showing efficacy of Onivyde in three gemcitabine-resistant PDX models (#12424, #14312, #19015) as well as in two gemcitabine-sensitive PDX models (#14244, #15010). These results strengthen the non-clinical proof of concept for Onivyde. A comparison between MM-398 and unencapsulated irinotecan in a gemcitabine-sensitive PDX model showed significantly improved tumour growth inhibition (TGI) with Onivyde relative to unencapsulated irinotecan. Unfortunately, no comparison was made in a gemcitabine-resistant PDX model, which is the most relevant model for the sought indication. However, the Applicant submitted supportive data, showing consistently greater TGI upon treatment with Onivyde at lower

doses versus conventional irinotecan in cell-line derived pancreatic cancer models, including the gemcitabine-resistant AsPC-1 model. In summary, Onivyde at doses of 10-20 mg/kg bw (iv administration, q7d, 3-5 doses in total) showed efficacy in several different mouse models of pancreatic cancer. This corresponds to an efficacious dose of 30-60 mg/m2 in mice, which is somewhat lower than the recommended starting dose of 80 mg/m2 in patients.

In a xenograft colon cancer model in mice, comparing Onivyde and free irinotecan (iv administration) a much better tumour growth control despite equal SN-38 exposure (plasma, tumour) was observed with the liposomal formulation. The key factor behind this effect was proposed to be the longer duration of SN-38 exposure in the tumour obtained with Onivyde as compared with free irinotecan administration. This notion is somewhat contradictory to the results obtained in the rat glioblastoma model, suggesting differences between tumours and/or species.

Since irinotecan is a known active substance, the lack of secondary pharmacology studies is accepted. However, the Applicant's assertion that topoisomerase I is the exclusive target for irinotecan is not agreed with. There are several reports in the literature, describing the inhibition of acetylcholinesterase by irinotecan, at clinically relevant concentrations. This mechanism has been proposed as an explanation for the cholinergic syndrome observed in some patients treated with irinotecan, although other mechanisms have also been implicated. Cholinergic syndrome is listed as an adverse reaction in section 4.8 of the proposed SmPC.

In safety pharmacology studies in dogs, ONIVYDE had no effect on cardiovascular, hemodynamic, electrocardiographic, or respiratory parameters at doses up to 21 mg/kg (420 mg/m2). The lack of CNS safety pharmacology study can be accepted based on the already known safety pharmacology profile of irinotecan, and the fact that clinical CNS effects (cholinergic syndrome) have already been identified. Furthermore, in a limited evaluation of CNS effects in the 18-week repeat dose toxicity study in rats doses of MM-398 up to 190 mg/kg bw did not produce any CNS-related signs, in contrast to free irinotecan at 75 mg/kg bw, which caused uncoordinated gait and tremor (see section 5.3 of the SmPC). According to the Applicant, this difference may be explained by the prevention of entry into the brain due to the size of the liposome being too large to pass through the blood brain barrier. On the other hand, iv administration of Onivyde gave higher concentrations of irinotecan and SN-38 in the brain as compared with CPT-11 in the rat glioblastoma model.

The Applicant was asked to clarify the pharmacokinetics of Onivyde with regard to passage over the blood-barrier. It was suggested that the longer plasma half-life of Onivyde, causing extended systemically released irinotecan, was the most likely explanation for the higher concentrations of Onivyde in the brain versus non-liposomal irinotecan.

Since irinotecan has been co-administered with 5-FU/LV in clinical practice, non-clinical studies on pharmacodynamic drug interactions are not considered needed.

The Applicant was asked to clarify and discuss possible reasons for the large differences in half-life of SN-38 between MM-398 and CPT-11. The Applicant clarified that in both non-clinical and clinical studies the half-life of SN-38, as measured from the time of injection of both liposomal and unencapsulated irinotecan formulations, was increased by liposomal encapsulation. This was considered to be the key mechanism behind drug retention in tumours.

Single and repeat-dose non-GLP pharmacokinetic and toxicokinetic studies in rat and dogs showed that MM-398 results in higher plasma exposure (in terms of AUC) and a prolonged period in plasma of irinotecan and its active metabolite SN-38 as compared to non-liposomal irinotecan (CPT-11) when given at the same dose levels. This was related to slower CL, lower Vd, longer $t_{½}$ and longer MRT for the MM-398 formulation. A wide distribution to tissues was observed and for several tissues the levels were increased and remained higher for a longer period

following MM-398 as compared to CPT-11. In tumour bearing mice, the largest increase for MM-398 in tissue exposure in terms of AUC was observed in tumours for irinotecan, whereas for SN-38, the increase in tumours was lower than in the liver and the kidney. The MRT and $t_{½}$ of irinotecan and SN-38 were also increased and were at least 2 to 3-fold longer in tumours than in the other tissues. While C_{max} of irinotecan was increased in tumours, and to a slight extent also in the liver, C_{max} of SN-38 was decreased in tumours but increased in liver and kidney following MM-398.

Irinotecan is metabolised to SN-38 via carboxylesterase-mediated cleavage in the liver and intestines and CYP3A4 to APC and NPC, which can be converted to SN-38 by carboxylesterases. The active metabolite SN-38 is further metabolised to the inactive SN-38 glucuronide via UGT1A isoforms in the liver and subsequent deglucuronidation by intestinal β-glucuronidase. Faecal excretion, of which a major part is represented by biliary excretion, was shown to be the major route for excretion of MM-398-derived radioactivity in rats.

The doses chosen for the studies are appropriate to characterize the toxicity of Onivyde and to make proper hazard evaluations and risk assessments.

Single dose and repeat-dose toxicity studies with MM-398 in mice, rats and dogs have identified the GI system and bone marrow as main target organs for toxicity. The severity of effects was dose related and reversible. The no observed adverse effect level (NOAEL) in rats and dogs following 90 min intravenous infusion of ONIVYDE once every 3 weeks for 18 weeks was at least 180 mg/m2. GI toxicity is a well-known adverse effect of irinotecan. Diarrhoea has been included as an important identified risk in the RMP for Onyvide, and reflected in sections 4.4 and 4.8 of the proposed SmPC. Bone marrow adverse effects have also previously been reported for irinotecan. Leukopenia/neutropenia and anaemia are included as important identified risks in the RMP for Onyvide, and have been included under sections 4.4 and 4.8 of the proposed SmPC.

In addition, adverse effects have been observed in lymphoid organs, liver, and male and female reproductive organs. Some of the effects on lymphoid organs may be related to stress, while others appear to be secondary to bone marrow toxicity. The inclusion of Leukopenia as an important identified risk in the RMP for Onyvide is considered to cover the effects on lymphoid organs observed in the non-clinical repeat dose toxicity studies. The observed EMH in repeat dose toxicology studies is most likely a compensatory effect secondary to bone marrow toxicity and is not considered to be an adverse finding.

Histiocytosis in multiple organs of animals administered MM-398 as well as in animals given vehicle control (empty liposome) reflects phagocytosis of the liposome and, in the absence of other associated toxicity, is not considered an adverse finding. Nevertheless, the Applicant was asked to discuss findings of non-reversible accumulation of foamy histiocytes in the lungs of control dogs and presence of foamy histiocytes in the spleen of recovery animals. The Applicant clarified that foamy histiocytes in the lungs were observed also in dogs treated with unencapsulated irinotecan, and that interstitial pulmonary disease (IPD) has been reported in patients treated with non-liposomal irinotecan. For that reason, ILD is included as an important potential risk in the RMP for Onivyde. With regard to foamy histiocytes in the spleen, this was concluded to be of negligible clinical relevance.

All major toxicity findings, and the vehicle-associated histiocytosis, were fully or partially reversible upon cessation of treatment. Histiocytosis in the absence of signs of other toxicity (e.g. necrosis, inflammation) is not considered to be an adverse finding.

Unencapsulated irinotecan (CPT-11), included for comparison in these studies, showed a similar though less pronounced toxicity profile. The reason for the more prominent toxicity seen with MM-398 is most likely the considerably higher exposures achieved with this formulation.

Non liposomal irinotecan and SN 38 were genotoxic in vitro in the chromosomal aberration test on CHO cells as well as in the in vivo micronucleus test in mice. However, in other studies with irinotecan they have been shown to be devoid of any mutagenic potential in the Ames test. For non liposomal irinotecan, in rats treated once a week during 13 weeks at the maximum dose of 150 mg/m², no treatment related tumours were reported 91 weeks after the end of treatment. Under these conditions, there was a significant linear trend with dose for the incidence of combined uterine horn endometrial stromal polyps and endometrial stromal sarcomas. Due to its mechanism of action, irinotecan is considered a potential carcinogen (see section 5.3 of the SmPC).

Non liposomal irinotecan was teratogenic in rats and rabbits at doses below the human therapeutic dose. In rats, pups born from treated animals and having external abnormalities showed a decrease in fertility. This was not seen in morphologically normal pups. In pregnant rats there was a decrease in placental weight and in the offspring a decrease in foetal viability and increase in behavioural abnormalities.

Non liposomal irinotecan caused atrophy of male reproductive organs both in rats and dogs after multiple daily doses of 20 mg/kg and 0.4 mg/kg, respectively. These effects were reversible upon cessation of treatment.

Adequate information has been reflected in sections 4.6 and 5.3 of the SmPC for Onivyde.

According to the distribution studies, MM-398 is distributed to the uvea. There is also UV absorbance at 360nm. The Applicant provided clinical data, emphasizing the lack of phototoxic events with unencapsulated irinotecan as well as the absence of such findings in the NAPOLI study. Based on this data it is concluded that there is no specific concern regarding a risk for photosensitivity reactions associated with treatment using MM-398.

2.3.7. Conclusion on the non-clinical aspects

No new toxicity issues have been identified with the liposomal formulation of irinotecan. Non-clinical GI and bone marrow toxicity of irinotecan are known to translate to the clinic. Both are included as important identified risks in the RMP for Onivyde.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

Type of Study	Study Identifier	Location of Study Report	Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Bioanalytical Methods	Bioanalytical methods for human studies across multiple studies	5.3.1.4	To validate measurements of concentration of analytes including total irinotecan, encapsulated irinotecan, SN-38, SN-38G, 5-FU, and the interference among the measurements	NA	NA	NA	NA	NA	Complete; Bioanalytical Methods Report
PK	Human Plasma Protein Binding to Nanoliposomal Irinotecan (PNU-IRI, MM-398, PEP02)	5.3.1.1	To determine the levels of protein binding and identify the major binding proteins associated with the MM-398 liposomes	NA	NA	NA	NA	NA	Complete; Protein Binding Report
Safety and PK	PEP0201	5.3.3.3	Safety, MTD, PK and preliminary efficacy	Phase 1, open label, multi center, dose escalation study, non-comparative	MM-398 (PEP02), 60, 120, 180 mg/m^2 q3 w IV	31	Solid tumors	Maximum of 6 cycles	Complete; Legacy Study Report
Safety and PK	PEP0202	5.3.3.2	Phase 1: To define the recommended phase II dose of MM-398 (PEP02) in combination with cisplatin, safety, PK and pharmacogenetics Phase 2: To determine response rates of MM-398 single-agent and in combination with cisplatin, duration of tumor response	Phase 1/2 study; Phase 1: dose escalation study; Phase 2: MM-398 vs. MM-398 plus cisplatin	Phase 1: MM-398 in combination with cisplatin: MM-398: 60 and 80 mg/m^2 q3w IV Cisplatin: 60 mg/m^2 q3 w IV Phase 2: not performed	6	Metastatic cervical cancer	Maximum of 6 cycles	Complete; Legacy Study Report
			Observe control rate, progression-free survival, the overall survival, tumor marker response, safety, PK, pharmacogenetics and QoL						
Safety and PK	PEP0203	5.3.3.2	To determine the recommended phase 2 dose of MM-398 (PEP02) in combination with 5-FU/LV, safety, PK, preliminary efficacy and pharmacogenetics	Phase 1, open label, multi center, dose escalation study of MM-398 in combination with 5-FU/LV; non-comparative	MM-398: 80, 56, 100, 120 mg/m^2 q3w IV 5-FU: 2600 mg/m^2 c.i. Day 1 and 8 q3w IV Levucovorin: 200 mg/m^2 c.i. Day 1 and 8 q3w IV	15	Solid tumors	Maximum of 6 cycles	Complete; Legacy Study Report
Safety and PK	PEST-CRC	5.3.3.2	To determine the MTD of MM-398, safety, PK, tumor response and pharmacogenetics	Phase 1, open label, dose escalation study; non-comparative	MM-398: 80, 60 and 100 mg/m^2 q3w IV	18	Colorectal cancer	Until disease progression	Complete; Legacy Study Report

Type of Study	Study Identifier	Location of Study Report	Objective(s) of the Study	Study Design and Type of Control	Test Productivity; Dosage Regimen; Route of Administration	Number of Subjects	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
PK	Population Pharmacokinetics and Exposure-Response Analysis of MM-398	5.3.3.3	• To develop population pharmacokinetic models to describe the PK profiles for MM-398 (total irinotecan and $SN-38_L$) in patients with advanced solid cancer; • To evaluate the impact of intrinsic (body size, demographics, and measurement of hepatic and renal functions, and UGT1A1*28 homozygosity) and extrinsic factors (co-administration with 5-FU, and concomitant use) on the PK of MM-398; • To evaluate the relationship between exposure and safety endpoints of interest of diarrhea, neutropenia, and anemia; and • To evaluate the relationship between exposure and efficacy endpoints.	Cross-study analysis	MM-398: multiple doses IV	350	Cancer patients	NA	Population Pharmacokinetics and Exposure-Response Analysis Report
Feasibility and safety	MM-398-01-01-02 (CITTE)	5.3.A.2	Pilot phase: Evaluate the feasibility of Fe-MP2 to bioavailably	Open-label Phase 1 study;	Pharmacokinetic: single dose of 5 mg/kg (not to exceed 320	33	Solid tumors	Until disease progression	Ongoing, Interim Synopsis Report
			tumor-associated macrophages; measure tumor levels of irinotecan and SN-38; evaluate the correlation between PD markers and administration of MM-398. Expansion phase: Further investigate the feasibility of ferumoxytol (FMDI) pretreatment in tumor lesions; characterize the relationship between FMDI tumor uptake & tumor response to MM-398. All phases: Safety; tumor response and PK	non-comparative	mg total) IV MM-398: 80 mg/m^2 q2w IV				
Efficacy and Safety	MM-398-07-03-01 (Napoli 3)	5.3.5.1	OS, PFS, TTF, ORR, tumor marker response, QoL, safety and PK	Open-label, randomized, Phase 3 study of MM-398, with or without 5-FU/LV, vs. 5-FU/LV alone; active control (5-FU/LV)	Arm A: MM-398 120 mg/m^2 q3w Arm C: MM-398 80 mg/m^2 + 5-FU 2,000 mg/m^2 + LV 400 mg/m^2 q2w 5U	417	Metastatic pancreatic cancer	Until progression	Complete; eCTD Study Report
Efficacy and Safety	MEP0204	5.3.5.1	Tumor response, PFS, duration of response, time to progression, TTF, disease control rate, 1 year OS, OS, safety, dose intensity, PK, pharmacogenetics	Open-label, randomized, Phase 2 study; non-comparative	Arm 1: MM-398 120 mg/m^2 q3 w IV Arm 2: Irinotecan 300 mg/m^2 q3w IV Arm 3: Docetaxel 75 mg/m^2 q3w IV	131	Gastric or GEJ cancer	Until progression	Complete; Legacy Study Report
Efficacy and Safety	DEP0702	5.3.5.3	3 month survival rate, objective tumor response, PFS, duration of response, OS, tumor marker response, clinical benefit response, safety and pharmacogenetics	Open-label, single arm, Phase 2 multicenter study; non-comparative	MM-398 120 mg/m^2 q3w IV	40	Metastatic pancreatic cancer	Until progression	Complete; Legacy Study Report

Type of Study	Study Identifier	Location of Study Report	Objective(s) of the Study	Study Design and Type of Control	Test Product(s), Dosage Regimen, Route of Administration	Number of Subjects +	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Efficacy and Safety	PEPCOL	5.4	Objective response rate, safety, PFS, OS, QoL and pharmacogenetic	Open label, randomized, multicenter Phase 2 study comparing a continuation of MM-398, 5-FU/LV & bevacizumab vs. 5-FU/LV, irinotecan and bevacizumab	Arm 1: Bevacizumab 5 mg/kg, irinotecan 180 mg/m^2, LV 400 mg/m^2, 5-FU bolus 400 mg/m^2, 5-FU continuous infusion 2400 mg/m^2, q2w IV OR Day 1: Bevacizumab 5 mg/kg, irinotecan 90 mg/m^2, LV 400 mg/m^2, 5-FU continuous infusion 2400 mg/m^2 Day 3: irinotecan 90 mg/m^2 Q2w IV	55	Colorectal cancer	Until disease progression	Ongoing; Published Literature

+ Number of patients enrolled in MM-398 studies as of October 24, 2014

2.4.2. Pharmacokinetics

The plasma pharmacokinetics of total irinotecan and total SN 38 were evaluated in patients with cancer who received Onivyde, as a single agent or as part of combination chemotherapy, at doses between 60 and 180 mg/m^2.

Bioanalysis
LC/MS/MS methods for determination of total irinotecan (encapsulated+un-encapsulated), the active metabolite SN-38 and the SN-38-glucuronide have been developed and pre-validated. A validated method for determination of liposome-encapsulated irinotecan and separation of un-encapsulated irinotecan is also reported.

Absorption

N/A

Distribution

Plasma exposure of total (encapsulated+un-encapsulated) irinotecan consists almost only of encapsulated irinotecan (the encapsulated form was 95.4% of the total and the un-encapsulated irinotecan, while not measured directly, was estimated to be <5%).

The volume of distribution (Vd) estimates in patients administered with MM-398 were approximately 2 L/m^2 (see table below).

The pharmacokinetic parameters of total irinotecan and SN 38 analytes, following the administration of Onivyde 80 mg/m^2 are presented in the table below.

Table 6: Summary of mean (±standard deviation) total irinotecan and total SN 38

Analyte	PK parameters	Unit	ONIVYDE geomean (95% CI)[a] 80 mg/m²(n=353)[b]	Non-liposomal irinotecan mean (SD) 125 mg/m² (n=99)[c]
Total irinotecan	AUC	h ng/ml	919228 (845653-999204)	10529 (3786)
	C_{max}	ng/ml	28353 (27761-28958)	1492 (452)
	Clearance (CL)	l/h/m2	0.087 (0.080-0.094)	13.0 (5.6)
	Volume (V)	l/m2	2.6 (2.6-2.7)	138 (60.9)
	$t_{1/2\ effective}$	h	20.8 (19.4-22.3)	6.07 (1.19)
Total SN-38	AUC	h ng/ml	341 (326-358)	267 (115)
	C_{max}	ng/ml	3.0 (2.9-3.1)	27.8 (11.6)
	$t_{1/2\ effective}$	h	40.9 (39.8-42.0)	11.7 (4.29)

SD= standard deviation
AUC= area under the plasma concentration curve (extrapolated to infinity for ONIVYDE and AUC_{last} for non-liposomal irinotecan)
C_{max}= maximum plasma concentration
$t_{1/2\ effective}$= effective half-lives
[a]Values are estimated from population PK analysis
[b]N=353 refers to all the subjects included in the population PK analysis
[c]Values are obtained from published data [Schaaf LJ et al. *Clin Cancer Res.* 2006 Jun 15;12:3782-91]

The plasma protein binding of Onivyde is negligible (< 0.44% of total irinotecan in Onivyde). The plasma protein binding of non-liposomal irinotecan is moderate (30% to 68%), and SN 38 is highly bound to human plasma proteins (approximately 95%).

Table 7: In vitro plasma protein binding of MM-398

Method	Sample number	N	Mean (95%CI) Protein Bound (pg/nmol Phospholipid)	Mean (95%CI) Protein Bound (µg/mg CPT-11)
size-exclusion chromatography	1780	15	0.76 (-0.69, 2.21)	1.23 (-1.20, 3.66)
affinity capture on the magnetic carrier coated with anti-poly (ethylene glycol) antibody	1780	9	-1.79 (-4.66, 1.09)	-4.48 (-11.68, 2.73)
	1781	9	-1.32 (-4.44, 1.81)	-3.17 (-10.70, 4.36)
	1782	9	-3.33 (-6.18, -0.48)	-8.90 (-16.51, -1.29)
	Positive control	9	166.16 (132.8, 199.5)	NA

Source: MM-398 Protein Binding Report, Table 6, Table 11

Elimination

The metabolic conversion of irinotecan to the active metabolite SN-38 is mediated by carboxylesterase enzymes. *In vitro* studies indicate that irinotecan, SN-38 and another metabolite aminopentane carboxylic acid (APC) do not inhibit cytochrome P-450 isozymes. SN-38 is subsequently conjugated predominantly by the enzyme UDP-glucuronosyl transferase 1A1 (UGT1A1) to form a glucuronide metabolite.

The systemic exposure of the active metabolite SN-38, following single iv infusions of 60-120 mg/m2 was <0.1% of the exposure of total irinotecan.

Preliminary data on systemic and local concentration in the tumour 72h post a single iv infusion of 80 mg/m2 Onivyde, show that the local concentration in the tumour of SN-38 is about 4-fold higher compared to the plasma level of SN-38 (Study MM-398-01-01-02). The plasma level of total irinotecan is about double as high as the level in the tumour.

SN-38 reached its C_{max} after about 21h following treatment with Onivyde, its $t_{1/2}$ varied between *ca* 60-75h.

UGT1A1 activity is reduced in individuals with genetic polymorphisms that lead to reduced enzyme activity such as the UGT1A1*28 polymorphism. Genotype frequency of genetic polymorphism of the UGT1A1 family has been performed for UGT1A1*28, *93 (UGT1A1 G3156A), *6, *27, *7 (UGT1A1T-3279G), *29, UGT1A9*22 (*1b) and DPYD*2A. No correlation between the different genes studied and PK of irinotecan/SN-38 were reported.

In the population pharmacokinetic analysis in patients with ONIVYDE using the results of a subset with UGT1A1*28 genotypic testing, in which the analysis adjusted for the lower dose administered to patients homozygous for the UGT1A1*28 allele, patients homozygous (N=14) and non-homozygous (N=244) for this allele had total SN-38 average steady-state concentrations of 1.06 and 0.95 ng/ml, respectively.

Both irinotecan and SN-38 are known to exist in an active lactone form and an inactive carboxylate form.

The half-life ($t_{1/2}$) of total irinotecan following infusion of Onivyde varies between 12–55 h. In study PEP0206, the $t_{1/2}$ was calculated to 21 and 8 h following infusion with Onivyde and the commercial available irinotecan, respectively

The urinary excretion of non-liposomal irinotecan is 11% to 20%; SN-38, <1%; and SN-38 glucuronide, 3%. The cumulative biliary and urinary excretion of irinotecan and its metabolites (SN-38 and SN-38 glucuronide) over a period of 48 hours following administration of irinotecan in two patients ranged from approximately 25% (100 mg/m^2) to 50% (300 mg/m^2) (see section 5.2 of the SmPC).

Unchanged drug was the major excretion product in both urine and faeces (Table xxx).

Table 8: Overview of excretion pattern (percentage of radioactive dose) in urine and faeces following an iv dose of 125 mg/m^2 of 14C-irinotecan to patients diagnosed with solid tumours

Excretion Matrix	Drug or Metabolite Name				
	SN-38G (M3)	NPC (M5)	APC (M11)	CPT-11	SN-38 (M17)
All patients (n = 7)					
Urine	3.02 ± 0.77	0.14 ± 0.08	2.23 ± 1.53	22.40 ± 5.50	0.43 ± 0.12
Feces	0.27 ± 0.17	1.36 ± 0.94	8.29 ± 2.95	32.31 ± 4.47	8.24 ± 2.51
Total	3.29	1.50	10.5	54.7	8.67

Dose proportionality and time dependencies

Dose-proportionality
In the literature, the exposure of irinotecan is reported to increase in a dose-proportional manner following iv infusion of a sterile solution (Mathijssen *et al*, 2001).

Following single doses of Onivyde 60-180 mg/m^2, a dose proportional increase in exposure of total irinotecan was observed. C_{max} of SN-38 increased dose-proportionally, but AUC increased less than dose-proportional.

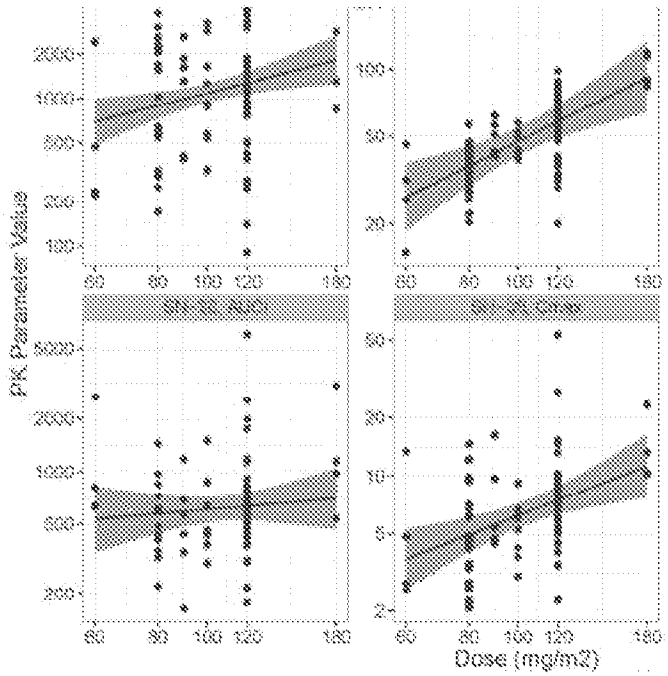


Figure 3: Exposure of total irinotecan and SN-38 across studies treated with Onivyde 60-180 mg/m²

Time-dependency

No PK following repeated dosing with Onivyde has been studied.

Special populations

Impaired renal function

No dedicated pharmacokinetic study has been conducted in patients with renal impairment. In a population pharmacokinetic analysis, mild-to-moderate renal impairment had no effect on the exposure of total SN-38 after adjusting for BSA. The analysis included 68 patients with moderate (CLcr 30 - 59 mL/min), 147 patients with mild (CLcr 60 - 89 ml/min) renal impairment, and 135 patients with normal renal function (CLcr > 90 ml/min). There was insufficient data in patients with severe renal impairment (CLcr < 30 ml/min) to assess its effect on pharmacokinetics (see sections 4.2, 4.4 and 5.2 of the SmPC).

Impaired hepatic function

No dedicated pharmacokinetic study has been conducted in patients with hepatic impairment. In a population pharmacokinetic analysis, patients with baseline total bilirubin concentrations of 1-2 mg/dl (n=19) had average steady state concentrations for total SN-38 that were increased by 37% (0.98 [95%CI: 0.94 - 1.02] and 1.29 [95%CI: 1.11 - 1.5] ng/ml, respectively) compared to patients with baseline bilirubin concentrations of < 1 mg/dl (n=329); however, there was no effect of elevated ALT/AST concentrations on total SN-38 concentrations. No data are available in patients with total bilirubin more than 2 times the ULN (see sections 4.2, 4.4 and 5.2 of the SmPC).

Race

The population pharmacokinetic analysis suggest that Asians have 56 % lower total irinotecan average steady state concentration (3.93 [95 %CI: 3.68- 4.2] and 1.74 [95 %CI: 1.58-1.93] mg/l, respectively) and 8 % higher

total SN-38 average steady state concentration (0.97 [95 %CI: 0.92-1.03] and 1.05 [95 %CI: 0.98-1.11] ng/ml, respectively) than Caucasians.

Gender

Gender was found as a covariate impacting the SN-38 exposure. Higher SN-38 concentrations were observed in females than in males: SN-38 Converted Cavg were 0.8 ng/mL in females and 0.7 ng/mL. The Applicant claimed this observation is likely confounded by the interaction between sex and BSA, as females had lower BSA compared to males (mean BSA of 1.8 m2 in males and 1.6 m2 in females).

The effect of gender was not reported for un-encapsulated irinotecan.

Weight

In the PPK analysis, there was no association between BSA and race-adjusted concentrations for irinotecan. For SN 38, increased BSA was associated with lower SN-38 exposure (despite higher doses administered in patients with higher BSA).

In the clinical studies, Onivyde was administered using BSA-based dosing. The proposed BSA-based dosing was further supported through simulations. Comparison of the simulated pharmacokinetic parameters for irinotecan and SN-38 was evaluated by simulating C_{avg} and C_{max} if patients had been dosed with either a BSA-based dose of 80mg/m^2 or an equivalent fixed dose of 135.73 mg (i.e., the nominal dose for a subject with median BSA). The analysis showed reduced variability in SN-38 C_{max} with BSA-based dosing (in line with the results from the covariate analysis). The interquartile range for SN-38 C_{max} was 74% with flat dose and 57% with BSA-based dose.

Elderly

The PopPK suggests that the effect of the covariate "age" had no clinically meaningful effect on the exposure of irinotecan and SN-38. No effect of age has been reported for un-encapsulated irinotecan.

Intra- and inter-individual variability
Variability of pharmacokinetic parameters was assessed in the popPK analysis. Total irinotecan was modelled as a two-compartmental model with central clearance inter-individual variability (IIV) of 88% and central volume IIV of 49%. SN-38 was modelled as a one-compartmental model with central clearance IIV of 55%.

The standard deviation of residual error was 0.30 and 0.16, for irinotecan and SN-38, respectively.

High inter-individual variability, up to 75%, was seen in total exposure following 90-min iv infusions of Onivyde.

PK in target population
The PK following treatment with Onivyde has been studied in patients with solid tumours including patients with the proposed indication metastatic pancreatic cancer. The exposure of total irinotecan, both Cmax and AUC, were much higher following an iv infusion of 120 mg/m^2 Onivyde compared to an infusion of 300 mg/m^2 with the un-encapsulated irinotecan. The C_{max} of SN-38 was about 5-fold higher after infusion with the commercial formulation compared to with Onivyde although the dose was just 2.5-fold higher. The total exposure of SN-38 after treatment with the un-encapsulated irinotecan was about half compared to with Onivyde.

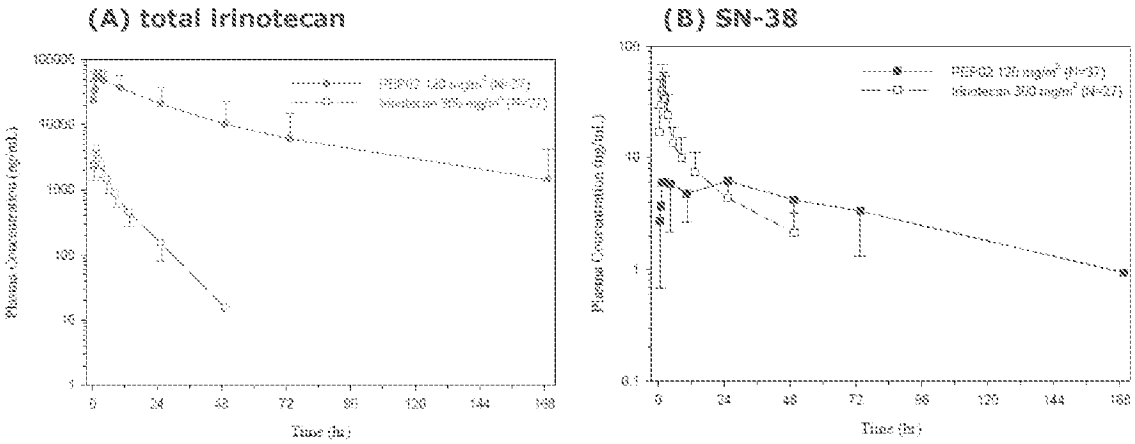


Figure 4: Mean plasma concentrations of total irionotectan (A) and SN-38 (B) versus time following iv infusions of 120 and 300 mg/m2 of Onivyde and commercial irinotecan, respectively, to patients

Table 9: PK of total irinotecan and SN-38 following 90- and 60-min iv infusion of Onivyde (120 mg/m^2) and commercial irinotecan (300 mg/m^2), respectively, in patients

	Onivyde 120 mg/m^2	Irinotecan 300 mg/m^2
$C_{max,\ irinotecan}$ (µg/ml)	61	4.3
$C_{max,\ SN-38}$ (µg/ml)	0.009	0.044
$AUC_{0-\infty,\ irinotecan}$ (µg/ml.h)	1812	26
$AUC_{0-\infty,\ SN-38}$ (ng/ml.h)	0.9	0.4
$t_{1/2,\ irinotecan}$ (h)	21	8
$t_{1/2,\ SN-38}$ (h)	89	23

Population pharmacokinetics analysis

PPK analysis was conducted with the objectives of describing the PK profiles of total irinotecan (CPT11) and the active metabolite SN-38, and to evaluate the impact of intrinsic and extrinsic factors.

Data from six studies were used to build the model, and included 1800 factors samples (355 subjects) and 1773 SN-38 samples (353 subjects).

Methods

Parameter estimations were performed using NONMEM version 7.3, with default setting FOCEI with Laplacian method. Measured concentrations below the limit of quantification were modelled as mixed continuous and categorical method (M3 method; Bergstrand & Karisson, 2009). The M3 method was implemented using log-transformed values of concentration and the Laplacian estimation method.

The covariates investigated for influence on the pharmacokinetic parameters of irinotecan included BSA and CL on central volume and on clearance, respectively. Other covariates explored were:

- BSA: body surface area;
- Measurements of hepatic function: AST, ALT, albumin, liver metastasis status, bilirubin, UGT1A1*28);

- Renal function: creatinine clearance;
- Demographics: sex, age, race;
- External functions: co-administration with 5-FU and manufacturing site.

The covariate model was similar for SN-38, with the exception that the estimated clearance and volume of distribution of irinotecan, and manufacturing site were added as to the SN-38 input flux.

Covariate relationships were investigated using a full-covariate method (Gastonguay 2011). Highly correlated covariates were evaluated, but only the most significant was retained and considered for further evaluation.

Results

The final model for irinotecan was a two-compartment model. The final model for SN-38 pharmacokinetic was a one-compartmental model with two input fluxes: 1) initial amount administered with Onivyde as an impurity within liposomes, and 2) in vivo conversion of irinotecan released from Onivyde. Final estimated parameters of irinotecan and SN-38 are shown in Table and Table .

Table 10: Final estimated parameters of irinotecan pharmacokinetics (no units were provided in the PPK analysis report)

Parameter	Estimated Values (final model)	Estimated Values from Bootstrapping (N=497)		
		Median	2.5%	97.5%
Objective Function	-1196.3895	-1238.53	-2100.37	-506.661
Fixed effects				
Volume (V1)	4.498	4.498	4.1604	4.6668
Clearance (CL)	15.44	15.438	11.705	21.403
Q	0.05413	0.054	0.0254	0.6766
V2	0.06817	0.068	0.0524	43.9466
V1-BSA	0.3749	0.375	0.2004	0.534
CL-(race==Asian)	0.7172	0.715	0.5704	0.8842
CL-(treatment contains 5FU)	0.0331	0.029	-0.0982	0.1986
CL-(manufacturing site)	0.04327	0.037	-0.218	0.2572
CL-(liver metastasis)	-0.03806	-0.031	-0.228	0.1456
CL-(ALT)	-0.03428	-0.343	-0.6404	-0.0492
CL-(Albumin)	-1.731	-1.731	-3.5044	-0.5054
CL-(Bilirubin)	0.1716	0.172	-0.1128	0.5266
CL-(Creatinine Clearance)	0.002168	0.002	-0.001	0.004
Random effects				
Omega(V1)	0.2388	0.057	0.015	0.0966
Omega(CL)	0.7712	0.127	0.0474	0.2378
Omega(V1-CL)(off-diagonal)	0.6869	0.596	0.377	1.043
Residuals				
Standard deviation of residual error	0.3012	0.301	0.2008	0.3856

Table 11: Final estimated parameters of SN-38 pharmacokinetics (no units were provided in the PPK analysis report)

Parameter Name	Estimated Values (Final Model)	Estimated Values from Bootstrap (n=499)		
		Median	2.5%	97.5%
Objective Function	-2863.2540	-2897.94	-3191.04	-2560.65
Fixed effects				
Clearance (CL)	13.3	13.27	12.0126	14.0213
K13 conversion flux from CPT11	0.0683	0.07	0.064	0.077
Impurity (IMP)	0.0541	0.054	0.051	0.061
CL-(race==Asian)	-0.0733	-0.071	-0.1312	-0.00835
CL-(UGT1A1*28==homozygote)	-0.00259	-0.002	-0.003	-0.002
CL-(treatment contains 5FU)	0.00272	0.003	0.001	0.005
K13-(manufacturing site)	0.000557	0.001	0.001	0.001
CL-(liver metastasis)	-0.00551	-0.004	-0.008	-0.00045
CL-(ALT)	-0.000124	0	0	0
CL-(Albumin)	-0.0403	-0.023	-0.046	0.001
CL-(Bilirubin)	-0.571	-0.545	-0.95955	-0.22785
CL-(Creatinine Clearance)	-0.145	-0.11	-0.21	-0.00625
K13-CPT11clearance	1.97	1.966	1.7518	2.20155
K13-CPT11volume	-0.0552	-0.042	-0.083	0.011
K13-BSA	-1.24	-1.195	-1.733	-0.70485
Random effects				
Omega(CL)	0.2993	0.092	0.076	0.107
Omega(CL-K13) (off diagonal)	-0.1974	-0.013	-0.021	0.01555
Omega(K13)	0.4637	0.224	0.18145	0.275
Omega(CL-impurity) (off diagonal)	-0.0811	-0.007	-0.015	0
K13-impurity (off diagonal)	0.2512	0.091	0.0239	0.14955
Omega(Impurity)	0.795	0.61	0.462	0.67065
Residuals				
Standard deviation of residual error	0.156	0.157	0.139	0.17565

Pharmacokinetic interaction studies

No formal drug/drug interaction studies were performed. Information about drug interactions with Onivyde is referenced from the published scientific literature for nonliposomal irinotecan.

Strong CYP3A4 inducers

Patients receiving concomitant non-liposomal irinotecan and CYP3A4 enzyme-inducing anticonvulsants phenytoin, phenobarbital or carbamazepine have substantially reduced exposure to irinotecan (AUC reduction by 12% with St John's wort, 57%-79% with phenytoin, phenobarbital, or carbamazepine) and SN-38 (AUC reduction by 42% with St John's wort, 36%-92% with phenytoin phenobarbital, or carbamazepine).

Strong CYP3A4 inhibitors and UGT1A1 inhibitors

Patients receiving concomitant non-liposomal irinotecan and ketoconazole, a CYP3A4 and UGT1A1 inhibitor, have increased SN-38 exposure by 109 %. Therefore, co-administration of Onivyde with other inhibitors of CYP3A4 (e.g. grapefruit juice, clarithromycin, indinavir, itraconazole, lopinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telaprevir, voriconazole) may increase systemic exposure of Onivyde.

In study MM-398-07-03-01(NAPOLI 1) Onivyde was administered in combination with 5-Fluorouracil and Leucovorin. Co-administration of Onivyde with 5-fluorouracil/leucovorin does not alter the pharmacokinetics of Onivyde based on the population pharmacokinetic analysis.

2.4.3. Pharmacodynamics

Mechanism of action

The active substance of Onivyde is irinotecan which is encapsulated in long-circulating liposomes. The drug product liposome is a small unilamellar lipid bilayer vesicle, approximately 110 nm in diameter, which encapsulates an aqueous space which contains irinotecan in a gelated or precipitated state, as sucrosofate salt.

Onivyde has been shown to extend circulation of irinotecan and prolong the duration of active therapy at the site of tumour cells to inhibit tumour growth.

Irinotecan (irinotecan hydrochloride trihydrate) is an antineoplastic agent of the topoisomerase I inhibitor class. Irinotecan is a semi-synthetic derivative of camptothecin, an alkaloid extract from plants such as *Camptotheca acuminata*. Camptothecins interact specifically with the enzyme topoisomerase I, which relieves torsional strain in DNA by inducing reversible single-strand breaks. Irinotecan and its active metabolite SN-38 bind to the topoisomerase I - DNA complex and prevent religation of these single-strand breaks. Irinotecan serves as a water-soluble precursor of the lipophilic metabolite SN-38, which is formed from irinotecan primarily by liver carboxylesterase enzymes. The SN-38 metabolite is approximately 1000 times more potent than irinotecan as an inhibitor of topoisomerase I purified from human and rodent tumour cell lines. The precise contribution of SN-38 to the activity of IRINOTECAN in humans has not been completely defined. Both irinotecan and SN-38 exist in an active lactone form and an inactive hydroxy acid anion form. An acidic pH promotes the formation of the lactone whereas a basic pH favours the hydroxy acid anion form.

Primary and Secondary pharmacology

No primary or secondary pharmacodynamics studies were performed. Topoisomerase I is the exclusive target for the API of MM-398, irinotecan.

PKPD relationship
The PK is considered descriptive in the current submission. However, the exposure-efficacy analysis indicates that increased exposure to irinotecan and SN-38 are associated with longer overall survival and progression-free survival.

Higher irinotecan exposures were associated to a higher probability of incidence and severity of diarrhoea and higher SN-38 exposures with higher probability and severity of neutropenia, and with higher probability of anaemia.

2.4.4. Discussion on clinical pharmacology

Onivyde is a liposomal formulation of irinotecan with different pharmacokinetic properties compared to non-liposomal irinotecan. The dose concentration and strength are different in comparison to non-liposomal irinotecans. Onivyde is not equivalent to other non-liposomal irinotecan formulations and should not be interchanged.

Once released from the liposome-based particles, irinotecan is hypothesized to follow the same excretion route as the un-encapsulated irinotecan. The Applicant has therefore not further characterised the elimination of irinotecan, drug-drug interactions and PK of irinotecan in special populations.

The PK following treatment with Onivyde has been studied in patients with solid tumours including patients with the proposed indication of metastatic pancreatic cancer. Analytes measured in clinical trials include total irinotecan (which includes encapsulated and un-encapsulated irinotecan), its active metabolite SN-38 and its inactive glucuronidated form SN-38G. In one study (PEP0201), encapsulated irinotecan and total irinotecan were directly measured.

Irinotecan released from liposome encapsulation follows a similar metabolic pathway reported with non-liposomal irinotecan. Liposome encapsulation of irinotecan extends circulation and limits distribution relative to those of the non-liposomal irinotecan. The small volume of distribution suggests that Onivyde is largely confined to vascular fluid (see section 5.2 of the SmPC).

The disposition of Onivyde and non-liposomal irinotecan has not been fully elucidated in humans.

Linear PK of irinotecan is reported following iv infusion with un-encapsulated irinotecan solutions as well as following single doses with Onivyde (60-180 mg/m^2).

The systemic exposure of total irinotecan (C_{max} and AUC) was much higher following an iv infusion of 120 mg/m^2 Onivyde compared to an infusion of 300 mg/m^2 with the un-encapsulated irinotecan. A smaller Vss can be expected for a liposomal formulation. The C_{max} of SN-38 was about 5-fold higher with the commercial formulation compared to with Onivyde although the dose was just 2.5-fold higher. The total exposure of SN-38 after the commercial formulation was about half of exposure compared to after Onivyde.

Preliminary data on local concentration of irinotecan and SN-38 in tumours, following a single dose of 80 mg/m^2 Onivyde have been presented. Data on systemic and local concentration in the tumour 72h post a single iv infusion of 80 mg/m^2 Onivyde, show that the local concentration in the tumour of SN-38 is about 4-fold higher compared to the plasma level of SN-38 (the plasma level of total irinotecan is about double as high as the level in the tumour). However, the same comparison of tumour concentration and plasma levels following treatment with the un-encapsulated irinotecan is unknown.

No correlation between the different genes of the UGT1A1 family studied and PK of irinotecan/SN-38 were reported however, the data set was relatively limited and it is uncertain whether the study was too small to detect a difference in SN-38 exposure.

The PPK analysis appears to have been reasonably well performed.

No *in vivo* data following repeated dosing have been provided. But considering the long $t_{1/2}$ for SN-38 and the high inter-individual variability, patients might still be exposed at the time for next dose. Simulation of expected steady state exposure using single dose data will not predict any potential time-dependency in the PK. Patients have been repeatedly dosed and efficacy/safety data are available.

The systemic exposure of parent compound and/or metabolites are unknown in patients with different degrees of decreased renal function.

No data on influence of renal impairment on the PK of irinotecan have been presented however data from popPK analysis in patients with mild to moderate renal impairment indicated that no dose adjustment is recommended in those patients. Onivyde is not recommended for use in patients with severe renal impairment (CLcr < 30 ml/min) (see sections 4.2, 4.4 and 5.2 of the SmPC).

Neither has any dedicated study on PK of Onivyde in patients with different degrees of hepatic impairment been performed. Based on nonclinical data, an increased tissue distribution of 14C-irinotecan was seen in the rat, with higher levels in the liver, following administration with the liposomal compared to un-encapsulated solution for injection. The use of Onivyde should be avoided in patients with bilirubin > 2.0 mg/dl, or aspartate aminotransferase (AST) and alanine aminotransferase (ALT) > 2.5 times upper limit of normal (ULN) or > 5 times ULN if liver metastasis is present (see sections 4.2, 4.4 and 5.2 of the SmPC).

The effect of hepatic impairment was explored. Higher baseline bilirubin was associated with higher SN-38 concentration following the administration of Onivyde. Albumin has a weak association to the exposure of total irinotecan. The Applicant claims no significant associations were observed between total irinotecan or SN-38 and ALT, AST, and liver metastasis status. However, a significant association between total irinotecan (Composite Cavg P=0.0001) and CPT 11 (Cavg P=0.0002) were found.

The association between UGT1A1*28 homozygosis to SN-38 exposure is evaluated stratified by race. While the effect of UGT1A1*28 homozygosis to SN-38 exposure was well-documented in Caucasians, the effect was less well-studied in Asians (Beutler1998), who have a relatively low incidence of UGT1A1*28 homozygosity. Data from Study MM-398-07-03-01 was consistent with the reduced incidence of UGT1A1*28 homozygosity in Asians compared to Caucasians: homozygosity was observed in 23/243 (9.5%) Caucasians, 2/129 (1.5%) Asians, and 2/26 (7.6%) other races. Because race was a strong covariate for CPT11, the evaluation of the association between the maximum concentration of SN-38 converted and UGT1A1*28 homozygous was performed separately for each race group. Caucasians UGT1A1*28 homozygous have higher level of SN-38 converted compared to non-homozygous Caucasians. However, this difference is not statistically significant.

Total and converted SN-38 Cavg is higher in Caucasian UGT1A1*28 homozygous due to reduced UGT1A1 enzymatic activity.

Co-administration of Onivyde with inducers of CYP3A4 may reduce systemic exposure of Onivyde. Onivyde should therefore not be administered with strong CYP3A4 enzyme inducers such as anticonvulsants (phenytoin, phenobarbital or carbamazepine), rifampin, rifabutin and St. John's wort uniess there are no therapeutic alternatives. The appropriate starting dose for patients taking these anticonvulsants or other strong inducers has not been defined. Consideration should be given to substituting with non-enzyme inducing therapies at least 2 weeks prior to initiation of Onivyde therapy (see sections 4.4 and 4.5 of the SmPC).

Onivyde should not be administered with strong CYP3A4 enzyme inhibitors (e.g. grapefruit juice, clarithromycin, indinavir, itraconazole, lopinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telaprevir, voriconazole). Strong CYP3A4 inhibitors should be discontinued at least one week prior to starting Onivyde therapy Onivyde should not be administered with strong UGT1A inhibitors (e.g. atazanavir, gemfibrozil, indinavir) uniess there are no therapeutic alternatives (see sections 4.4 and 4.5 of the SmPC).

No interaction of Onivyde with other medicinal products is known.

The population pharmacokinetic analysis in patients aged 28 to 87 years, of whom 11% were ≥75 years suggests that age had no clinically meaningful effect on the exposure to irinotecan and SN 38 (see section 5.2 of the SmPC).

The population pharmacokinetic analysis in 196 male and 157 female patients suggests that gender had no clinically meaningful effect on the exposure to irinotecan and SN 38 after adjusting for body surface area (BSA).

2.4.5. Conclusions on clinical pharmacology

Irinotecan, the active substance of Onivyde, is a known active substance and overall the clinical pharmacology study package is considered sufficient. The PK of irinotecan/SN-38 following administration of Onivyde is considered descriptive.

2.5. Clinical efficacy

In support of the MAA, the applicant submitted one pivotal study (NAPOLI-1) and several supportive studies:

- **PEP0208**: phase II study of MM-398 in patients with metastatic pancreatic cancer previously treated with gemcitabine containing regimens (main supporting study)

- **PEP0201:** phase I, DLT and MTD in solid tumours

- **PEP0202**: phase I Dose Escalation Study Followed by Multi-National, Open-Label Randomized Phase II Study Evaluating the Efficacy and Tolerability of PEP02 with or without Cisplatin in Patients with Recurrent or Metastatic Squamous Carcinoma of the Uterine Cervix). Phase II not performed, study terminated.

- **PEP0203:** A multi-center, open-label phase I dose-escalation study of PEP02 in combination with 5-fluorouracil (5-FU) and leucovorin (LV) in advanced solid tumours

- **PIST-CRC:** Phase I and Pharmacokinetic Study of Biweekly PEP02 (Liposome Irinotecan) in Patients with Metastatic Colorectal Cancer Refractory to First-line Oxaliplatin-based Chemotherapy

- **PEP0206:** A Randomized Phase II Study of PEP02, Irinotecan or Docetaxel as a Second Line Therapy in Patients with Locally Advanced or Metastatic Gastric or Gastroesophageal Junction Adenocarcinoma

· Interim results for ongoing studies **CITS** (MM-398-01-01-02; A Pilot Study in Patients Treated with MM-398 to Determine Tumour Drug Levels and to Evaluate the Feasibility of Ferumoxytol Magnetic Resonance Imaging to Measure Tumour Associated Macrophages and to Predict Patient Response to Treatment) and **PEPCOL**.

2.5.1. Dose response study(ies)

The single-agent MM-398 dose and schedule is based on results from a phase I dose escalating trial in advanced solid tumour patients (PEP0201). An accelerated titration design based on single-patient cohorts was used, starting from an initial MM-398 dose of 60 mg/m^2 (i.e 1/10 of the LD_{10} in mice) and escalated to 120 mg/m^2, 180 mg/m^2, 240 mg/m^2, 300 mg/m^2, and then subsequently escalated by 50 mg/m^2 with every 3 weeks infusions, until MTD was reached. Because the dose-toxicity correlation of irinotecan has been well established and documented, one to two patients cohorts were employed at the lower dose levels (up to 240 mg/m^2), followed by the three-patient cohort at the higher dose level (≥300 mg/m^2). A total of 11 patients were enrolled, including 1 patient at dose level 0 (60 mg/m2), 6 patients at dose level 1 (120 mg/m2) and 4 patients at dose level 2 (180 mg/m2). At this latter dose level, two out four patients had DLTs (i.e, grade 4 leucopenia and neutropenia lasting for longer than 3 days; grade 3 febrile neutropenia and grade 3 diarrhoea). The dose was then reduced (120 mg/m^2), and 1 patient among a total of 6 treated at this dose level experienced a DLT (Grade 3 infection). Therefore, based on these results 120 mg/m^2 was determined as MTD for MM-398 single agent (see further details in Section 2.1.3). Overall, 2 patients with advanced pancreatic cancer patients, both treated at 180 mg/m^2 dose level, were enrolled in study PEP0201, including 1 patient who achieved PR and 1 patient who reported PD.

This single-agent MM-398 regimen (120 mg/m^2 every 3 weeks) was then used in a phase II study, conducted in pancreatic cancer patients previously treated with gemcitabine (PEP0208), and based on its efficacy and safety results, the NAPOLI-1 study was originally designed and started enrolment.

The recommended dose of MM-398 in combination with 5-FU/LV when administered every 3 weeks was investigated in a dose-escalation study conducted in advanced solid tumours (PEP0203), with a MM-398 starting dose of 60 mg/m^2, subsequently escalated by increments of 20 mg/m^2 between dose levels. The 5-FU/LV was given on days 1 and 8 of each cycle at a fixed dose of 2,000 mg/m^2 and 200 mg/m^2, respectively. Overall, 16 patients were enrolled, including 3 patient at dose level 60 mg/m^2, 6 patients at dose level 80 mg/m^2, 5 patients at dose level 100 mg/m^2 and 2 patients at dose level 120 mg/m^2. Both patients treated with MM-398 120 mg/m^2 experienced DLTs (i.e, Grade III diarrhoea, Grade IV neutrophil count decreased, and Grade III infection in one patient and Grade III diarrhoea, Grade III leucopenia, and Grade III neutropenia in the other). Therefore, the dose level was decreased to 100 mg/m^2 and additional patients were included. Overall, two patients treated with MM-398 100 mg/m^2 experienced DLTs (i.e, fatigue, anaemia, white blood cell count decreased and neutrophil count decreased, all Grade III, in one patient; grade III anorexia, diarrhoea, abdominal pain, gastric ileus, febrile neutropenia and infection, and grade IV leucopenia, and neutropenia in the other patient). No DLTs were reported in the 6 patients treated at 80 mg/m^2 dose level that was considered the MTD of MM-398 in combination with 5-FU/LV given every 3 weeks. In total, 5 patients with advanced previously treated pancreatic cancer were enrolled in study PEP0203 across dose levels (1 at 60 mg/m^2, 3 at 80 mg/m^2, and 1 at 120 mg/m^2). Stable disease was reported in all patients, with the exception of 1 patient in which a PD was recorded after 2 cycles of MM-398 80 mg/m^2 treatment (see further details in Section 2.1.3). This schedule was not incorporated in the NAPOLI-1 study.

A combination arm of MM-398 + 5-FU/LV was only added when the NAPOLI-1 study had already started, based on the availability of acceptable safety data from an investigator initiated randomized phase II study, conducted by the GERCOR French cooperative group to compare MM-398 versus irinotecan in combination with 5-FU/LV as second line therapy in unresectable metastatic colorectal cancer patients (PEPCOL). In this study, the dose of MM-398 was 80 mg/m^2 administered every 2 weeks in combination with with 5-FU/LV at 2400/400 mg/ m^2.

2.5.2. Main study(ies)

NAPOLI -1: A randomized, open Label phase 3 study of MM-398, with or without 5-Fluorouracil and Leucovorin, versus 5-Fluorouracil and Leucovorin in patients with metastatic pancreatic cancer who have failed prior gemcitabine-based therapy.

Methods

NAPOLI-1 is a 3-arm (initially 2-arm), open-label, randomized (1:1:1 ratio), active-controlled study.

Study Participants

Inclusion criteria

In order to be included in the study, patients were required to have:

1. Histologically or cytologically confirmed adenocarcinoma of exocrine pancreas

2. Documented metastatic disease; disease status was permitted to be measurable or non-measurable as defined by RECIST v. 1.1 guidelines

3. Documented disease progression after prior gemcitabine or gemcitabine-containing therapy, in locally advanced or metastatic setting. Examples of permitted therapies included, but were not limited to:

- Single agent gemcitabine
- Any one gemcitabine-based regimen, with or without maintenance gemcitabine
- Single agent gemcitabine to which a platinum agent, a fluoropyrimidine, or erlotinib was subsequently added
- Gemcitabine administered in the adjuvant setting, if disease recurrence occurred within 6 months of completing the adjuvant therapy

4. Karnofsky Performance Status (KPS) ≥ 70

5. Adequate bone marrow reserves as evidenced by:

- ANC > 1,500 cells/µL without the use of hematopoietic growth factors; and
- Platelet count > 100,000 cells/µL; and
- Haemoglobin > 9 g/dL (blood transfusions were permitted for patients with haemoglobin levels below 9 g/dL)

6. Adequate hepatic function as evidenced by:

- Serum total bilirubin within normal range for the institution (biliary drainage was allowed for biliary obstruction)
- Albumin levels ≥ 3.0 g/dL
- Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 2.5 x ULN (≤ 5 x ULN was acceptable if liver metastases were present)

7. Adequate renal function as evidenced by a serum creatinine ≤ 1.5 x ULN

8. Normal ECG or ECG without any clinically significant findings

9. Recovered from the effects of any prior surgery, radiotherapy or other anti-neoplastic therapy

10. At least 18 years of age

11. Able to understand and sign an informed consent (or have a legal representative who is able to do so)

Exclusion criteria

Patients were required to meet all the inclusion criteria listed above and none of the following exclusion criteria:

1. Active CNS metastases (indicated by clinical symptoms, cerebral oedema, steroid requirement, or progressive disease); patient should have been off steroids for at least 28 days prior to starting study therapy

2. Clinically significant gastrointestinal disorder including hepatic disorders, bleeding, inflammation, occlusion, or diarrhoea > Grade 1

3. History of any second malignancy in the last 5 years; subjects with prior history of in-situ cancer or basal or squamous cell skin cancer were eligible. Subjects with other malignancies were eligible if they had been continuously disease free for at least 5 years.

4. Severe arterial thromboembolic events (myocardial infarction, unstable angina pectoris, stroke) less than 6 months before inclusion.

5. NYHA Class III or IV congestive heart failure, ventricular arrhythmias or uncontrolled blood pressure.

6. Active infection or an unexplained fever > 38.5°C during Screening visits or on the first scheduled day of dosing (at the discretion of the investigator, patients with tumour fever were permitted to be enrolled), which in the investigator's opinion might have compromised the patient's participation in the trial or affected the study outcome.

7. Known hypersensitivity to any of the components of Onivyde, other liposomal products, fluoropyrimidines, or leucovorin.

8. Investigational therapy administered within 4 weeks, or within a time interval less than at least 5 half-lives of the investigational agent, whichever was longer, prior to the first scheduled day of dosing in this study.

9. Any other medical or social condition deemed by the Investigator to be likely to interfere with a patient's ability to sign informed consent, cooperate and participate in the study, or interfere with the interpretation of the results

10. Pregnant or breast feeding; females of child-bearing potential were required to test negative for pregnancy at the time of enrolment based on a urine or serum pregnancy test. Both male and female patients of reproductive potential were required to agree to use a reliable method of birth control, during the study and for 3 months following the last dose of study drug.

Treatments

MM-398, dose and mode of administration

· **Arm A** (MM-398):

Patients not homozygous for the UGT1A1*28 allele: 120 mg/m^2.

Patients homozygous for the UGT1A1*28 allele: initial dose of 80 mg/m^2 that could be increased in increments of 20 mg/m^2 (absent toxicity at the first administration) from cycle 2 onwards to a maximum dose of 120 mg/m^2.

MM-398 was administered every 3 weeks as IV infusion over 90 minutes.

- **Arm C** (MM-398 + 5-FU/LV):

Patients not homozygous for the UGT1A1*28 allele: 80 mg/m^2.

Patients homozygous for the UGT1A1*28 allele: initial dose of 60 mg/m^2 that could be increased (absent toxicity at the first administration) to a maximum dose of 80 mg/m^2.

MM-398 was administered every 2 weeks as an IV infusion over 90 minutes.

Reference therapy, dose and mode of administration

· **Arm B** (5-FU/LV): 5-FU 2,000 mg/m^2 by IV infusion over 24 hours (± 30 minutes) every week for 4 weeks (Study Days 1, 8, 15, and 22), followed by 2 weeks of rest in a 6-week cycle; LV 200 mg/m^2 of the *l* + *d* racemic form by IV infusion over 30 minutes every week for 4 weeks (Study Days 1, 8, 15, and 22) followed by 2 weeks of rest in a 6-week cycle.

Background 5FU/LV, dose and mode of administration

- **Arm C** (MM-398+5-FU/LV): 5-FU 2,400 mg/m^2 by IV infusion over 46 hours (± 60 minutes) every 2 weeks; LV 400 mg/m^2 of the *l* + *d* racemic form by IV infusion over 30 minutes every 2 weeks.

Anti-emetic premedication as per standard institutional practices was administered in all arms. Prophylactic atropine could be prescribed to patients treated with Onivyde who experienced acute cholinergic symptoms in the previous cycles.

In all arms, patients were to be treated until disease progression.

Objectives

Primary objective: To compare overall survival (OS) following treatment with MM-398, with or without 5-FU and leucovorin (5-FU/LV), versus 5-FU/LV, in patients with metastatic pancreatic cancer that had progressed on gemcitabine based therapy.

Secondary objective: To compare the following between the experimental and control arms: progression free survival (PFS), time to treatment failure (TTF), objective response rate (ORR), tumour marker response of CA19-9, clinical benefit response (CBR) rate, quality of life (QOL), safety and adverse event (AE) profiles, pharmacokinetic (PK) properties.

Outcomes/endpoints

Primary endpoint: OS defined as the time from the date of patient randomization to date of death or the date last known alive. For each patient who was not known to have died as of the cut-off date for a particular analysis, OS was censored for that analysis at the date of last contact prior to the data cut-off date.

Secondary endpoints:

- PFS defined as the time in months from the date of patient randomization to the date of death or disease progression, whichever occurred earlier.

- TTF defined by the occurrence of discontinuation of treatment for any reason, including disease progression, treatment toxicity, and death.

- ORR defined by the percentage of patients in the study population with a best overall response of Complete Response (CR) or Partial Response (PR) as assessed by the investigator. The best overall response was defined as the best response per RECIST (version 1.1) recorded from randomization until progression or end of study.

- Tumour marker response of CA19-9 defined as a decrease of ≥50% of CA19-9 in relation to the baseline level at least once during the treatment period. Only patients with elevated baseline CA19-9 value (> 30 U/mL), i.e. the TMRE population, were included in the calculation of tumour marker response rate.

- CBR is based on pain assessment and analgesic consumption. All patients were asked to complete a daily pain assessment and analgesic consumption diary throughout their participation in the study.

Sample size

For the sample size calculations, it was assumed that the median Overall Survival times were 4.5 months for the MM 398 monotherapy (Arm A), 3 months for the control arm (Arm B), and 6 months for the combination arm

(Arm C), corresponding to hazard ratios (HR) of 0.67 and 0.5 in favor of Arm A and Arm C relative to Arm B, respectively. Both Arm A and Arm C were compared to Arm B.

A total of 305 events provided at least 85% power to detect the hypothesized OS advantage for Arm A relative to Arm B. The planned study size also provided at least 99% power to detect the OS advantage for Arm C relative to Arm B. With a 14 month patient accrual and up to 3 months follow up time, it was expected that a total of approximately 405 patients would be randomized.

The sample size and power calculations also assumed that approximately 65 patients were randomized in protocol version 1 and that the remaining patients were randomized under the 3-arm versions of the protocol.

These power statements were based on the corresponding two pairwise unstratified logrank tests using a Bonferroni-Holm testing procedure which strongly controls the family wise error rate for the planned comparisons at the two-sided 0.05 level, i.e. one sided 0.025 level.

The primary analysis was to take place when at least 305 death events have occurred. The final number of patients enrolled and included in the analyses included all patients randomized under protocol version 1.1 (and later).

Randomisation

Patients have been randomized to treatment arms using an Interactive Web Response System (IWRS) at a central location, based on stratified factors such as baseline albumin levels (≥ 4.0 g/dL *vs* < 4.0 g/dL), KPS (70 and 80 *vs* ≥ 90), and ethnicity (Caucasian *vs* East Asian *vs* All Others).

In the original protocol, randomization in a 1:1 ratio to MM-398 monotherapy vs 5-FU/LV control arm was performed. Starting from protocol version 2, a third arm was added to investigate MM-398 in combination with 5-FU/LV, and patients were assigned to treatment arms via randomized blocks within a stratum (1:1:1 ratio).

Blinding (masking)

N/A

Statistical methods

Planned statistical analyses were conducted on the following 8 patient populations.

Table 12: Summary of the planned statistical analysis - study NAPOLI-1

Population	Definition	Analyses
Intent-to-Treat (ITT)	all patients randomized after confirmation of successful allocation of a randomization number through the IWRS	primary population for all efficacy parameters
Per-protocol population (PP)	patients who received treatment for at least 6 weeks and did not violate any inclusion/exclusion criteria nor significantly deviate from the protocol	Sensitivity analyses of OS, PFS, ORR, TTF
Evaluable Patient (EP) population for tumor response	all randomized and treated patients, who met all inclusion/exclusion criteria, had measurable disease at baseline and were evaluable for response°	PFS, ORR, TTF
Tumor marker	patients with elevated CA19.9 level	Tumor Marker (CA19.9)

response-evaluable (TMRE) population	(> 30 U/mL) at baseline	Response
CBR-evaluable (CBRE) population	patients with at least one of the following at baseline: • pain intensity ≥ 20 (out of 100) • morphine consumption ≥ 10 mg/day PO morphine equivalents • KPS of 70 to 90 points	Clinical Benefit Response
PRO population	all ITT patients with baseline and at least one subsequent EORTC-QLQ-C30 assessment	Quality of Life analyses
PK population	all treated patients with at least one PK assessment on treatment	pharmacokinetic analyses
Safety population	patients that received at least one dose (including a partial dose) of study medication	all safety analyses

° i.e. patients with at least one tumor evaluation while on treatment and those with early (≤ 12 weeks) disease progression, including symptomatic deterioration and death.

Primary Endpoint Analysis:

The primary analysis involved 2 pairwise comparisons of survival in the ITT population using un-stratified logrank test. The testing was carried out using a Bonferroni-Holm testing procedure to strongly control the family wise Type I error rate at the 2-sided 0.05 level. Kaplan-Meier analyses were performed on each treatment group to obtain nonparametric estimates of the survival function and the median survival time. Corresponding 95% confidence intervals were computed using the log-log method. Unstratified Cox proportional hazards regression were used to estimate hazard ratios and their corresponding 95% confidence intervals. The proportional hazards assumptions were examined by Cox regression models with treatment as a time-dependent covariate.

Secondary Endpoint Analysis:

PFS was compared pairwise (Arm A v. Arm B, Arm C v. Arm B) using un-stratified logrank tests. Kaplan-Meier analyses were performed on each treatment group to obtain nonparametric estimates of the PFS function and the median PFS time. Corresponding 95% confidence intervals were computed using the log-log method. Unstratified Cox proportional hazards regressions were used to estimate hazard ratios and their corresponding 95% confidence interval.

The number and percentage of patients in the ITT, PP, and EP populations experiencing objective response (confirmed CR + PR) at the time of analysis was presented and the 95% confidence interval for the proportion was calculated based on the normal approximation. Differences in objective response rates between treatment arms were compared pairwise using Fisher's exact tests. Analyses of ORR were based on unconfirmed tumour response assessments per investigator under RECIST version 1.1 criteria, as well as confirmed tumour responses.

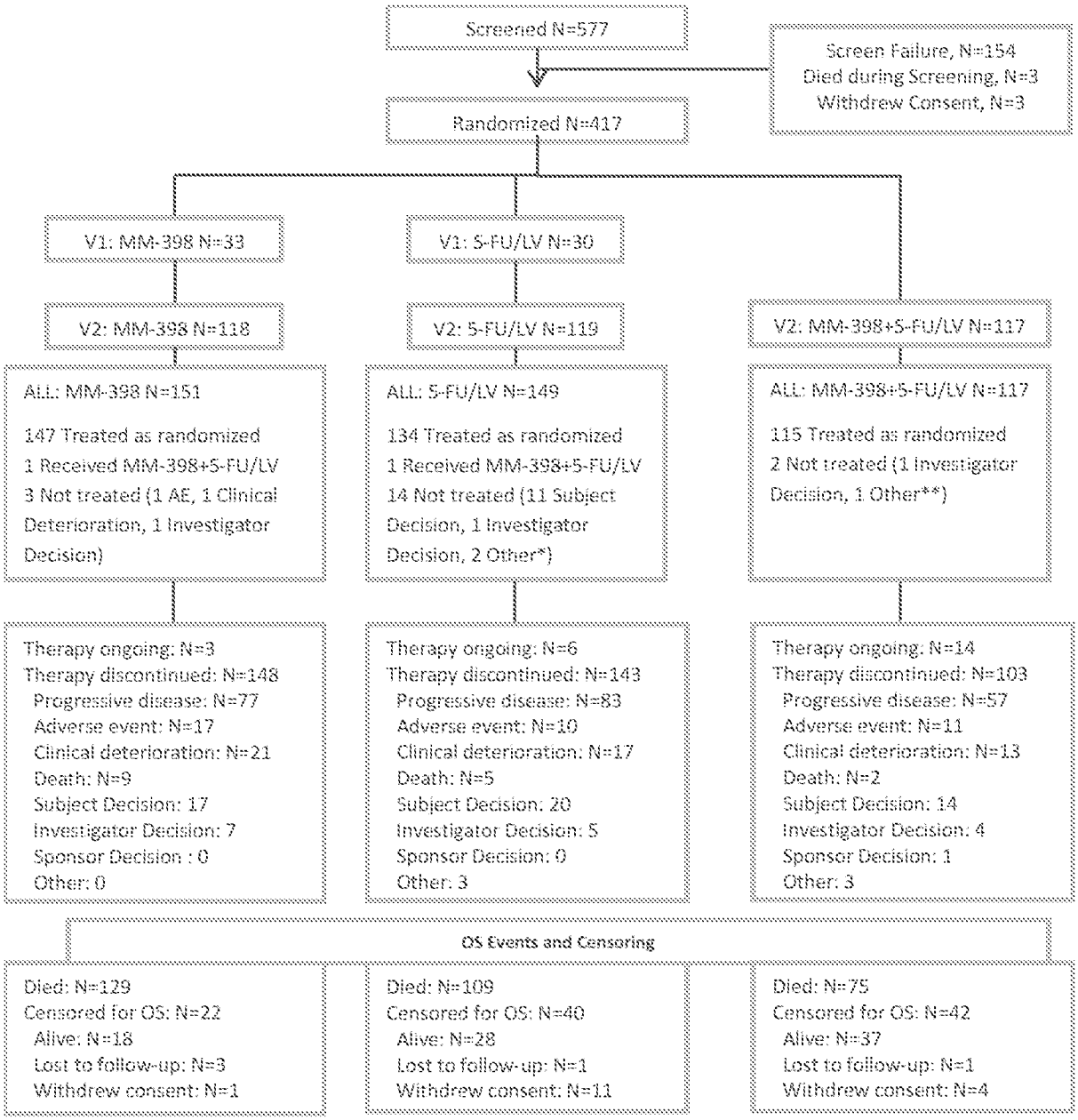
Clinical benefit response rates were compared pairwise on the CBRE population using Fisher's Exact Tests. Contingency tables for pain classification, KPS, and overall clinical benefit response, were also presented for each treatment group. Median time to clinical benefit response and median duration of clinical benefit response were computed using data from patients with clinical benefit response.

Regarding the Tumour Marker Response Analysis, CA 19-9 serum levels were measured within 7 days before the start of treatment (baseline), and subsequently every 6 weeks. Tumour marker response was evaluated by the change in CA19-9 serum levels and defined as a decrease of 50% of CA19-9 in relation to the baseline level at least once during the treatment period. Only patients with elevated baseline CA19-9 value (> 30 U/mL), i.e. the

TMRE population, were included in the calculation of tumour marker response rate. Tumour marker response rates from the treatment arms were compared pairwise using Fisher's exact tests.

Results

Participant flow



Recruitment

Of the 577 patients screened for inclusion, 417 patients were randomized and are included in the Intent-to-Treat (ITT) population. These patients were enrolled in 76 study sites in North America (20 sites), Europe (30 sites),

Asia (12 sites), South America (8 sites), and Oceania (6 sites). Out of the 417 patients included, 151 (36%) were enrolled in Europe, 125 patients (30%) in Asia, and 68 (16%) in North America, while the remaining part (18%) was from Australia and South America.

Conduct of the study

Overall, 2 protocol amendments were implemented to the original version of NAPOLI-1 study (dated 6 Oct 2011).

The main changes introduced by protocol amendments are reported in the following Table:

Amendment #	Protocol version (date)	Changes
01	2.1 (14 June 2012)	The study design was changed by the addition of the third treatment arm (MM-398 and 5-FU/LV combination). Based on statistical assumption for the 3-arm study, the total number of patients required to be enrolled was increased from 270 to 405; moreover, the primary analysis for overall survival was to take place after 305 death events instead of 220 initially planned. Requirement for a formal interim analysis, for safety and futility, was removed. Instead, a requirement for an intensive safety review of the first 15 subjects enrolled in each arm by the independent DSMB was added to ensure the safety of the new combination arm. The restriction excluding patients previously treated with irinotecan from participating in the study was removed to be consistent with the absence of restriction in case of previous treatment with 5-FU/LV. Inclusion of such patients was left to the investigator's discretion. Dose modification guideline for patients who were homozygous for UGT1A1*28 and had already had their dose increased was clarified. To keep the response assessment consistent with the RECIST 1.1. guidelines, confirmation of a PR or CR was no longer required. In case of study treatment discontinuation for reasons other than disease progression, tumour assessment every 6 weeks was continued during the follow-up period until documentation of objective disease progression. Since post-study therapy can affect the tumour response status, censoring for tumour response analysis was applied at the time of new anti-neoplastic therapy starts, and, not further tumour assessments was required from then onwards. In addition to Arm A, PK assessments were now required for patients on Arm B and C as well. The requirement of an optional PK sample was added to be collected in Cycle 1, any time between 8 and 72 hours following administration of MM-398, from patients in Arm A and C patients only.
02	2.2 (19 October 2012)	The dose for the *l* isomeric form of leucovorin was added, due to the ongoing global shortages for oncology drugs. Clarification that all efficacy comparisons between Arm A and Arm B would include all patients randomized to either arm, under all versions of the protocol. The efficacy comparisons between Arm B and Arm C would include only patients randomized under protocol version 2 or later. An additional sensitivity analysis was added to censor the overall survival at a date where any post-treatment anti-cancer therapy was first administered.

Baseline data

Demographic and baseline characteristics of the 417 patients enrolled in NAPOLI-1 study are summarized in the following tables:

Table 13: Demographics-ITT Population

Characteristic	MM-398 Mono N=153	5-FU/LV Mono Control N=149	MM-398+5-FU/LV Combo N=117	5-FU/LV Combo Control N=116*	*All ITT** N=417*
Gender, n (%)					
Female	64 (42.4)	68 (45.6)	48 (41.0)	52 (43.7)	*180 (43.2)*
Male	87 (57.6)	81 (54.4)	69 (59.0)	67 (56.3)	*237 (56.8)*
Race, n (%)					
American Indian Or Alaska Native	1 (0.7)	0	0	0	*1 (0.2)*
Asian	52 (34.4)	50 (33.6)	34 (29.1)	36 (30.3)	*136 (32.6)*
Black Or African American	3 (2.0)	3 (2.0)	4 (3.4)	3 (2.5)	*10 (2.4)*
White	89 (58.9)	92 (61.7)	72 (61.5)	78 (65.5)	*253 (60.7)*
Other	6 (4.0)	4 (2.7)	7 (6.0)	4 (3.4)	*17 (4.1)*
Age (yrs)					
Mean (SD)	63.6 (10.13)	63.8 (9.65)	63.2 (9.86)	61.0 (9.46)	*62.8 (9.88)*
Median	65.0	65.0	63.0	62.0	*65.0*
Min, Max	31, 87	34, 83	41, 81	34, 80	*31, 87*
Height (cm)					
Mean (SD)	166.6 (10.73)	166.2 (10.15)	167.5 (9.64)	166.7 (10.10)	*166.7 (10.21)*
Min, Max	144, 193	145, 193	142, 189	147, 193	*142, 193*
Weight (kg)					
Mean (SD)	84.7 (14.15)	85.6 (17.87)	85.9 (14.87)	86.3 (18.33)	*85.3 (15.66)*
Min, Max	38, 118	37, 151	40, 123	37, 151	*37, 151*
BMI (kg/m^2)					
Mean (SD)	23.09 (3.468)	23.57 (4.915)	23.33 (4.134)	23.57 (5.094)	*23.39 (4.195)*
Min, Max	15.1, 34.9	16.7, 42.9	16.0, 43.5	16.7, 42.9	*15.1, 43.5*

Baseline defined as last observation prior to study treatment

* This group is a subset of 5-FU/LV mono control group that was enrolled in the study after protocol Version 2 was activated.

** Rows do not add across because of the presentation of the control groups (mono controls are all patients treated with 5-FU/LV only; combo controls include a subset of these patients who were enrolled after protocol Version 2, which added the third treatment arm). Patients are counted only once in the All column.

5-FU= 5-fluorouracil; LV=leucovorin

Source: Table 14.1.3.1

Table 14: Baseline Characteristics-ITT Population

Characteristic	MM-398 Mono N=151	5-FU/LV Mono Control N=149	MM-398+5-FU/LV Combo N=117	5-FU/LV Combo Control N=119*	*All ITT** N=417*
Baseline KPS Level, n (%)					
50	0	0	1 (0.9)	0	*1 (0.2)*
60	0	0	2 (1.7)	0	*2 (0.5)*
70	15 (9.9)	11 (7.4)	7 (6.0)	10 (8.4)	*33 (7.9)*
80	58 (38.4)	61 (40.9)	38 (32.5)	51 (42.9)	*148 (35.5)*
90	64 (42.4)	54 (36.2)	51 (43.6)	40 (33.6)	*166 (40.5)*
100	22 (14.6)	23 (15.4)	18 (15.4)	17 (14.3)	*63 (15.8)*
Baseline Albumin (g/dL)					
Mean (SD)	3.97 (0.442)	3.96 (0.502)	3.97 (0.459)	3.98 (0.506)	*3.96 (0.468)*
Min, Max	2.6, 4.8	2.4, 5.1	2.6, 5.1	2.4, 5.0	*2.4, 5.1*
Measurable lesions at baseline	144 (95.4)	144 (96.6)	113 (96.6)	114 (95.8)	*401 (96.2)*
No measurable lesions at baseline	7 (4.6)	5 (3.4)	4 (3.4)	5 (4.2)	*16 (3.8)*
Measurable metastatic lesions at baseline	128 (84.8)	129 (86.6)	97 (82.9)	103 (86.6)	*354 (84.9)*
No measurable metastatic lesions at baseline	23 (15.2)	20 (13.4)	20 (17.1)	16 (13.4)	*63 (15.1)*
Number of subjects at baseline with , n (%)					
1 measurable metastatic lesion	36 (23.8)	26 (17.4)	18 (15.4)	22 (18.5)	*82 (19.4)*
2 measurable metastatic lesion	63 (41.7)	72 (48.3)	48 (41.0)	58 (48.7)	*184 (44.1)*
3 measurable metastatic lesion	33 (14.6)	21 (14.1)	22 (18.8)	15 (12.6)	*67 (15.8)*
>3 measurable metastatic lesion	7 (4.6)	10 (6.7)	7 (6.0)	8 (6.7)	*34 (8.0)*
Anatomical location of lesion at baseline***, n (%)					
Distant Lymph node	44 (29.1)	40 (26.8)	33 (27.4)	31 (26.1)	*116 (27.9)*
Liver	103 (68.2)	108 (72.5)	75 (64.1)	83 (69.7)	*284 (68.1)*
Lung	49 (32.5)	44 (29.5)	36 (30.8)	36 (30.3)	*129 (30.9)*
Pancreas	99 (65.6)	97 (65.1)	75 (64.1)	72 (60.5)	*271 (65.0)*
Peritoneal	48 (31.8)	39 (26.2)	30 (25.6)	33 (27.7)	*115 (27.6)*
Regional Lymph node	19 (12.6)	20 (13.4)	13 (11.1)	14 (11.8)	*53 (12.7)*
Other	38 (25.2)	48 (32.2)	27 (23.1)	39 (32.8)	*113 (27.1)*
Prior lines of treatment					
1st line advanced/metastatic	17 (11.3)	18 (12.8)	15 (12.8)	15 (12.6)	*51 (12.2)*
2nd line advanced/metastatic	86 (57.0)	86 (57.7)	62 (53.0)	67 (56.3)	*234 (56.1)*
3rd+ line advanced/metastatic	48 (31.8)	44 (29.5)	40 (34.2)	37 (31.1)	*132 (31.7)*

Baseline defined as last observation prior to study treatment

* This group is a subset of 5-FU/LV mono control group that was enrolled in the study after protocol Version 3 was activated.

** Rows do not add across because of the presentation of the control groups. Patients are counted only once in the All column.

*** Investigator reported.

5-FU= 5-fluorouracil; LV=leucovorin

Source: Table 14.1.3.3, Table 14.1.4.2.1

Further details and data on time since first cytological or histopathological diagnosis and first metastatic diagnosis are reported in the table below:

Table 15: Cancer Diagnosis-ITT Population

Characteristic Category/Statistic	MM-398 Mono (N=[illegible])	5-FU/LV Mono Control (N=[illegible])	MM-398+5-FU/LV Combo (N=[illegible])	5-FU/LV Combo Control (N=[illegible])	All ITT (N=[illegible])
Location of Pancreatic Tumor at Diagnosis, n(%)					
Head only	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Body only	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Tail only	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Multi-locations include head	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Multi-locations not include head	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Unknown	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Disease Stage, n(%)					
IA	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
IB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
IIA	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
IIB	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
III	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
IV	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Missing	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Time since first cytological or histo-pathological diagnosis (months)					
N	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Mean (SD)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Median	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Q1, Q3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Min, Max	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Time since first metastatic diagnosis (months)					
N	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Mean (SD)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Median	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Q1, Q3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Min, Max	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Note: Denominator for percentage corresponds to the N in each column.

Details on prior anticancer therapies and on response to previous treatments are shown below:

Table 16: Prior Anticancer Therapy-ITT Population

Characteristic Category/Statistic	MM-398 Mono (N=[illegible])	5-FU/LV Mono Control (N=[illegible])	MM-398+5-FU/LV Combo (N=[illegible])	5-FU/LV Combo Control (N=[illegible])	All ITT (N=[illegible])
Prior Lines of Treatment					
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Anticancer therapy with special interest [1]					
Gemcitabine alone	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Gemcitabine combination	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
5-FU based	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Irinotecan based	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Platinum based	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Best Response to prior therapy					
Complete Response (CR)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Partial Response (PR)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Stable Disease (SD)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Progressive Disease (PD)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Not Evaluable (NE)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Unknown	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Best Response - prior gemcitabine therapy					
Complete Response (CR)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Partial Response (PR)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Stable Disease (SD)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Progressive Disease (PD)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Not Evaluable (NE)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Unknown	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Note: Denominator for percentage corresponds to the N in each column.
[1]: Subjects may receive more than one therapy and could appear in more than one category.

At study entry, the median time since the last any prior anticancer treatment was 1.3 months, with a median time of 1.4 months from the last prior gemcitabine therapy.

Numbers analysed

Analyses were conducted in 8 different patient populations (see also statistical analysis plan).

The number of patients included in each analysis population is reported in the table below:

Table 17: Analysis population

Population	MM-398 Mono N=151 (%)	5-FU/LV Mono Control N=149 (%)	MM-398+5-FU/LV Combo N=117 (%)	5-FU/LV Combo Control N=119* (%)	*All ITT N=417 (%)*
Intent-to-Treat (ITT)	151 (100)	149 (100)	117 (100)	119 (100)	*417 (100)*
Safety	147 (97.4)	134 (89.9)	117 (100)	105 (88.2)	*398 (95.4)*
Per-Protocol (PP)	116 (76.8)	95 (63.8)	66 (56.4)	71 (59.7)	*277 (66.4)*
Evaluable Patient (EP) for Tumor Response	133 (88.1)	120 (80.5)	104 (88.9)	92 (77.3)	*357 (85.6)*
Tumor Marker Response-evaluable (TMRE)	123 (81.5)	105 (70.5)	97 (82.9)	81 (68.1)	*325 (77.9)*
Clinical Benefit Response (CBR) Population	92 (60.9)	80 (53.7)	78 (66.7)	80 (50.4)	*250 (60.0)*
Patient Reported Outcome (PRO) Population	105 (69.5)	82 (55.0)	72 (61.5)	56 (47.1)	*259 (62.1)*
PK Population	144 (95.4)	85 (57.0)	116 (99.1)	85 (71.4)	*345 (82.7)*

* This group is a subset of 5-FU/LV mono control group that was enrolled in the study after protocol Version 2 was activated.
There were 2 subjects who received study drug not as randomized. The MM-398 and 5-FU/LV randomized groups each had 1 subject who received MM-398+5-FU/LV.
5-FU= 5-fluorouracil; LV=leucovorin; PP=Per Protocol
Source: Table 14.1.1.9

Outcomes and estimation

Primary efficacy endpoint: OS

Table 18: NAPOLI-1 CSR: Study Primary Efficacy Analysis (Overall Survival)

Primary Efficacy Analysis: Overall Survival	Monotherapy Comparison				Combination Therapy Comparison			
	MM-398	5-FU/LV	p-value [a]	Hazard Ratio[b]	MM-398 + 5-FU/LV	5-FU/LV	p-value [a]	Hazard Ratio[b]
ITT Population								
N	151	149			117	119		
Median OS, months (95% CI)[c]	4.9 (4.23, 5.62)	4.2 (3.58, 4.86)	0.9416	0.99	6.1 (4.76, 8.87)	4.2 (3.29, 5.32)	0.0122	0.67
Died, n (%)	129 (85.4)	109 (73.2)			75 (64.1)	80 (67.2)		
Reason for Censoring								
Alive, n (%)	18 (11.9)	28 (18.8)			37 (31.6)	27 (22.7)		
Lost to Follow-Up, n (%)	3 (2.0)	1 (0.7)			1 (0.9)	1 (0.8)		
Subject Withdrew Consent from Follow-Up, n (%)	1 (0.7)	11 (7.4)			4 (3.4)	11 (9.2)		

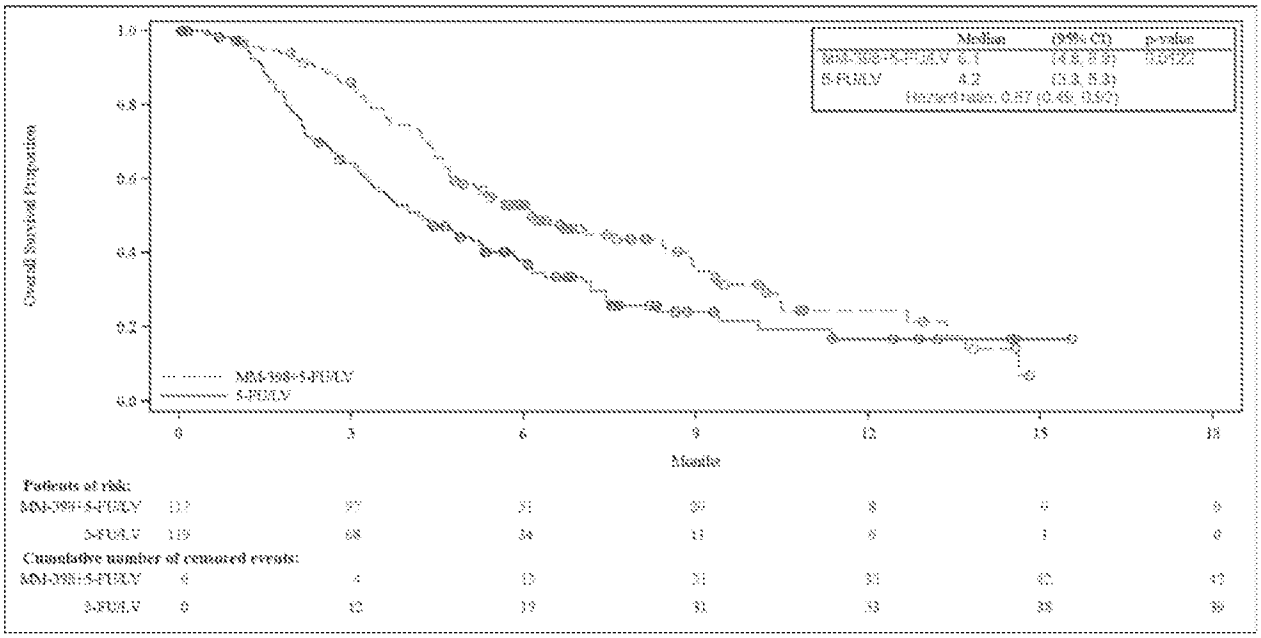


Figure 5: NAPOLI-1 CSR: Kaplan-Meier Plot for Overall Survival (Months) - MM-398+5-FU/LV versus 5-FU/LV Control (ITT Population)

Table 19: Sensitivity analyses of the primary efficacy endpoint (overall survival) – NAPOLI-1

Sensitivity Analyses of Overall Survival	Monotherapy Comparison				Combination Therapy Comparison			
	MM-398	5-FU/LV	p-value[1]	Hazard Ratio[2]	MM-398 + 5-FU/LV	5-FU/LV	p-value[1]	Hazard Ratio[2]
Stratified Analysis on ITT Population								
N	151	149			117	119		
Median OS, months (95% CI)[3,4]	4.9 (4.24, 5.62)	4.2 (3.58, 4.86)	0.5545	0.93	6.1 (4.76, 8.87)	4.2 (3.29, 5.32)	0.0039	0.57
Safety Population								
N	147	134			117	105		
Median OS, months (95% CI)[4]	4.9 (4.27, 5.62)	4.2 (3.58, 4.86)	0.2372	0.87	6.2 (4.86, 8.87)	4.2 (3.29, 5.29)	0.0108	0.66
Per Protocol Population								
N	116	85			66	71		
Median OS, months (95% CI)[4]	5.4 (4.80, 6.28)	4.8 (3.98, 5.88)	0.3174	1.11	8.9 (6.44, 10.5)	5.1 3.98, 7.16)	0.0106	0.57
ITT Population (Censoring at Change in Therapy)								
N	151	149			117	119		
Median OS, months (95% CI)[4]	4.8 (4.11, 5.39)	3.9 (3.12, 5.22)	0.7460	0.9506	6.1 (4.70, 12.68)	4.0 (3.06, 5.88)	0.0033	0.5665
ITT Population (Subjects Enrolled under Protocol Version 3)								
N	118	119						
Median OS, months (95% CI)[4]	4.8 (4.11, 5.63)	4.2 (3.29, 5.32)	0.6512	1.07				

1 Two-sided p-value from log-rank test.
2 Hazard ratios and the associated p-values are derived using Cox's proportional hazards model with treatment as the independent variable.
3 For the Stratified Analysis on the ITT population, p-value is derived from the two-sided stratified log rank test, incorporating randomization strata. Hazar ratios are derived using the stratified Cox's proportional hazards model with treatment as the independent variable.

Secondary efficacy endpoints

- Progression Free Survival (PFS)

Table 20: Study Secondary Efficacy Analysis (PFS) - NAPOLI-1

Secondary Efficacy Analysis: Progression Free Survival	Monotherapy Comparison				Combination Therapy Comparison			
	MM-398	5-FU/LV	p-value[a]	Hazard Ratio[b]	MM-398 + 5-FU/LV	5-FU/LV	p-value[a]	Hazard Ratio[b]
ITT Population								
N	151	149			117	119		
Median PFS Time, months (95% CI)[c]	2.7 (2.13, 2.89)	1.6 (1.41, 1.84)	0.1001	0.81	3.1 (2.69, 4.17)	1.5 (1.41, 1.84)	0.0001	0.56
Progressed, n (%)	89 (58.9)	89 (59.7)			65 (55.6)	69 (58.0)		
Died before progression, n (%)	38 (25.2)	31 (20.8)			18 (15.4)	23 (19.3)		
Reason for Censoring								
Clinical Deterioration, n (%)	4 (2.7)	3 (2.0)			3 (2.6)	2 (1.7)		
Last non-PD Assessment within 12 Weeks of Cutoff Date, n (%)	4 (2.7)	7 (4.7)			15 (12.8)	7 (5.9)		
Not Treated and No Post-Baseline Tumour Assessment, n (%)	1 (0.7)	10 (6.7)			1 (0.9)	10 (8.4)		
Other, n (%)	15 (9.9)	9 (6.0)			15 (12.8)	8 (6.7)		

[a]p-value is derived from the two-sided unstratified log rank test
[b]Hazard ratios are derived using unstratified Cox's proportional hazards model with treatment as the independent variable
[c]Median PFS time is the Kaplan-Meier estimate of the median survival time
Source: 5.3.5.1 NAPOLI-1 CSR, Section 7.4.1.2, Table 7-9

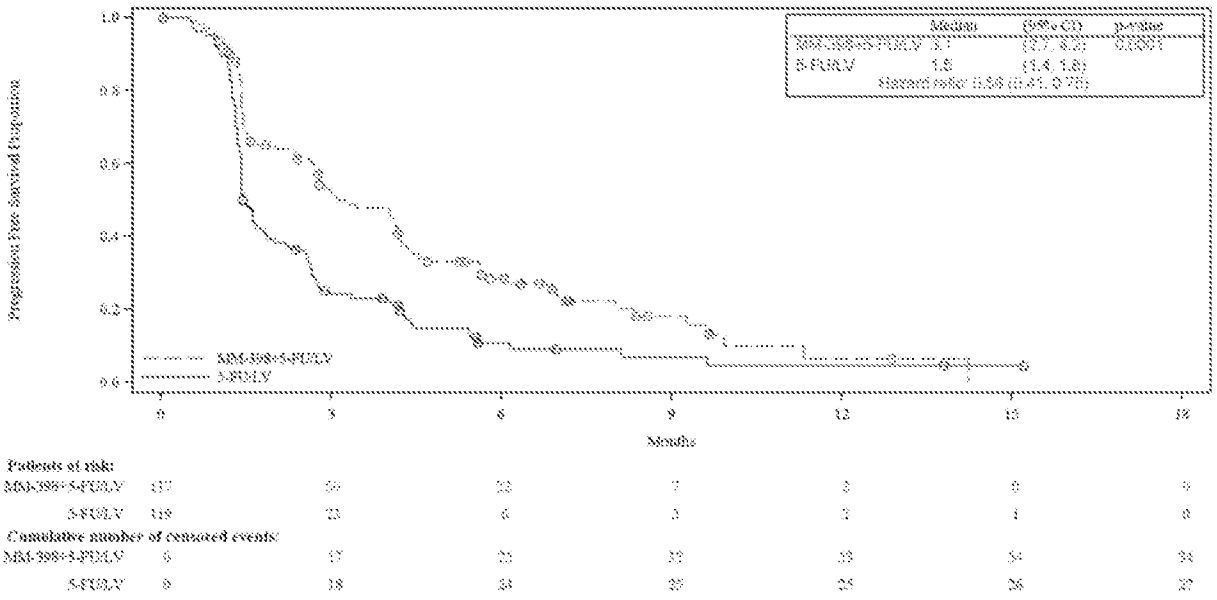


Figure 6: Kaplan-Meier Plot for PFS (Months) - MM-398+5-FU/LV versus 5-FU/LV Control (ITT Population) - NAPOLI-1

The combination of MM-398+5-FU/LV achieved a median PFS of twice that of the control arm of 5-FU/LV, 3.1 vs 1.5 months, HR 0.56, p=0.0001. However, the PFS was assessed by the investigator, not by IRC.

The HR for monotherapy comparison (MM-398 vs 5FU/LV) is 0.81, p-value=0.1, while the HR for the combination therapy comparison is 0.56, p-value=0.0001.

Table 21: Sensitivity analyses of PFS – NAPOLI-1

Sensitivity Analyses of Progression Free Survival	Monotherapy Comparison				Combination Therapy Comparison			
	MM-398	5-FU/LV	p-value[1]	Hazard Ratio[2]	MM-398 + 5-FU/LV	5-FU/LV	p-value[1]	Hazard Ratio[2]
Stratified Analysis on the ITT Population								
N	151	149			117	119		
Median PFS Time, months (95% CI)[3,4]	2.7 (2.14, 2.89)	1.6 (1.41, 1.84)	0.0346	0.78	3.1 (2.69, 4.17)	1.5 (1.41, 1.84)	<0.0001	0.51
Per Protocol Population								
N	116	95			66	71		
Median PFS Time, months (95% CI)[3]	2.8 (2.63, 3.71)	1.6 (1.41, 2.56)	0.1116	0.79	4.3 (3.06, 5.72)	1.6 (1.41, 2.60)	<0.0001	0.46
Evaluable Population								
N	133	120			104	92		
Median PFS Time, months (95% CI)[4]	2.8 (1.77, 2.92)	1.5 (1.41, 1.81)	0.0907	0.79	3.1 (2.66, 4.21)	1.4 (1.41, 1.81)	<0.0001	0.53
ITT Population (Early Discontinuation)								
N	151	149			117	119		
Median PFS Time, months (95% CI)[4]	2.6 (1.77, 2.79)	1.5 (1.41, 1.71)	0.1363	0.83	3.1 (2.66, 4.14)	1.4 (1.41, 1.68)	<0.0001	0.55
ITT Population (Missing Data)								
N	151	149			117	119		
Median PFS Time, months (95% CI)[3]	2.7 (2.14, 2.83)	1.6 (1.41, 1.84)	0.1021	0.81	3.1 (2.69, 4.17)	1.5 (1.41, 1.84)	0.0001	0.56
ITT Population (Progression Directly Derived from Lesion Data)								
N	151	149			117	119		
Median PFS Time, months (95% CI)[4]	2.8 (2.14, 2.92)	1.5 (1.41, 1.81)	0.0969	0.80	3.3 (2.66, 4.21)	1.4 (1.41, 1.84)	0.0001	0.56
ITT Population (Subjects Enrolled under Protocol Version 2)								
N	118	119						
Median PFS Time, months (95% CI)[4]	2.6 (1.68, 2.89)	1.5 (1.41, 1.84)	0.1724	0.82				

[1] Two-sided p-value from log-rank test.
[2] Hazard ratios and the associated p-values are derived using Cox's proportional hazards model with treatment as the independent variable.
[3] For the Stratified Analysis on the ITT population, p-value is derived from the two-sided stratified log rank test, incorporating randomization strata. Hazard ratios are derived using the stratified Cox's proportional hazards model with treatment as the independent variable.
[4] Median PFS time is the Kaplan-Meier estimate of the median survival time.
Abbreviations: 5-FU/LV=5-fluorouracil/leucovorin; CI=confidence interval; ITT=Intent-to-Treat; PFS=progression free survival

- Time to treatment failure (TTF)

Table 22: Time to Treatment Failure - NAPOLI-1

Secondary Efficacy Analysis: Time to Treatment Failure	Monotherapy Comparison				Combination Therapy Comparison			
	MM-398	5-FU/LV	p-value[a]	Hazard Ratio[b]	MM-398 + 5-FU/LV	5-FU/LV	p-value[a]	Hazard Ratio[b]
ITT Population								
N	151	149			117	119		
Median TTF, months (95% CI)[c]	1.7 (1.48, 2.66)	1.4 (1.31, 1.41)	0.1008	0.82	2.3 (1.58, 2.79)	1.4 (1.31, 1.41)	0.0002	0.60
Death, n (%)	9 (6.0)	5 (3.4)			1 (0.9)	5 (4.2)		
Progressive disease, n (%)	77 (51.0)	84 (56.4)			61 (52.1)	65 (54.6)		
Other Reason for Treatment Termination, n (%)	62 (41.1)	54 (36.2)			41 (35.0)	43 (36.1)		

Table 23: Time to treatment discontinuation: adverse event discontinuations

Treatment Group	n (% of ITT)	Time to discontinuation (weeks)		
		Mean	Median	Min - Max
MM-398+5-FU/LV	11 (9.4)	5.2	5.1	1.7 – 9.1
5-FU/LV (protocol V2)	7 (5.9)	7.1	6.3	3.3 – 15.9

Table 24: Time to treatment discontinuation: clinical deterioration discontinuations

Treatment Group	n (% of ITT)	Time to discontinuation (weeks)		
		Mean	Median	Min - Max
MM-398+5-FU/LV	13 (11.1)	11.3	10.1	1.3 – 32.4
5-FU/LV (protocol V2)	12 (10.1)	9.5	8.5	1.3 – 25.7

Table 25: Time to treatment discontinuation: investigator decision discontinuations

Treatment Group	n (% of ITT)	Time to discontinuation (weeks)		
		Mean	Median	Min - Max
MM-398+5-FU/LV	4 (3.4)	14.8	12.9	9.9 – 23.3
5-FU/LV (protocol V2)	4 (3.4)	3.4	3.2	1.1 – 6.1

Table 26: Time to treatment discontinuation: subject decision discontinuations

Treatment Group	n (% of ITT)	Time to discontinuation (weeks)		
		Mean	Median	Min - Max
MM-398+5-FU/LV	14 (12.0)	6.7	5.4	0.6 – 14.1
5-FU/LV (protocol V2)	19 (16.0)	6.4	0.3	0.1 – 66.1

Table 27: Time to treatment discontinuation: all non-PD discontinuations (i.e. discontinuations for RECIST 1.1 PD or death are excluded)

Treatment Group	n (% of ITT)	Time to discontinuation (weeks)		
		Mean	Median	Min - Max
MM-398+5-FU/LV	44 (37.6)	8.6	7.8	0.6 – 32.4
5-FU/LV (protocol V2)	44 (37.6)	7.0	3.5	0.1 – 66.1

Table 28: Time from treatment discontinuation to PD: all non-PD discontinuations (i.e. discontinuations for RECIST 1.1 PD or death are excluded)

Treatment Group	# progressed/# discontinued	Time from discontinuation to progression (weeks) Kaplan-Meier estimates		
		25th percentile	Median	75th percentile
MM-398+5-FU/LV	25/44	8.1	5.1	2.6
5-FU/LV (protocol V2)	23/44	5.1	3.0	1.3

- Confirmed objective response rate, ORR

Table 29: Objective Response (ITT Population) - NAPOLI-1

Factor	Monotherapy Comparison		Combination Therapy Comparison	
	MM-398 (N=151)	5-FU/LV (N=149)	MM-398 + 5-FU/LV (N=117)	5-FU/LV (N=119)
Confirmed (≥ 4 weeks After Investigator Assessment of PR or CR)				
Best Overall Response, n (%)				
• Partial Response	5 (3.3)	1 (0.7)	9 (7.7)	1 (0.8)
• Stable Disease	57 (37.7)	35 (23.5)	47 (40.2)	26 (21.8)

Factor	Monotherapy Comparison		Combination Therapy Comparison	
	MM-398 (N=151)	5-FU/LV (N=149)	MM-398 + 5-FU/LV (N=117)	5-FU/LV (N=119)
• Non-Complete Response/ Non-Progressive Disease[a]	3 (2.0)	2 (1.3)	3 (2.6)	2 (1.7)
• Progressive Disease	51 (33.8)	71 (47.7)	35 (29.9)	56 (47.1)
• Not Evaluable	35 (23.2)	40 (26.8)	23 (19.7)	34 (28.6)
Objective Response Rate				
N	5	1	9	1
Rate (%)	3.31	0.67	7.69	0.84
95% CI of Rate[b]	0.46, 6.17	(0.0, 1.98)	2.86, 12.52	0.0, 2.48
Rate Difference (95% CI)	2.64 (-0.50, 5.78)		6.85 (1.75, 11.95)	
p-value[c]	0.2141		0.0097	

[a]Applies only to those patients without measurable disease at Baseline who could only be classified as CR, non-CR/non-PD, or PD
[b]95% CI is of Overall Response Rate for individual treatment arms and for the rate difference (treatment vs control) were calculated based on the normal approximation
[c]Two-sided p-values from pairwise (MM-398 monotherapy vs. Control; MM-398 combination therapy vs. Control) Fisher's exact test
Source: 5.3.5.1 NAPOLI-1 CSR, Section 7.4.1.2, Table 7-13

The ORR sensitivity analyses (PP and evaluable population) were consistent with the results in the ITT population.

- Tumour marker response (CA19-9)

A summary of tumour marker (CA19-9) response is presented below:

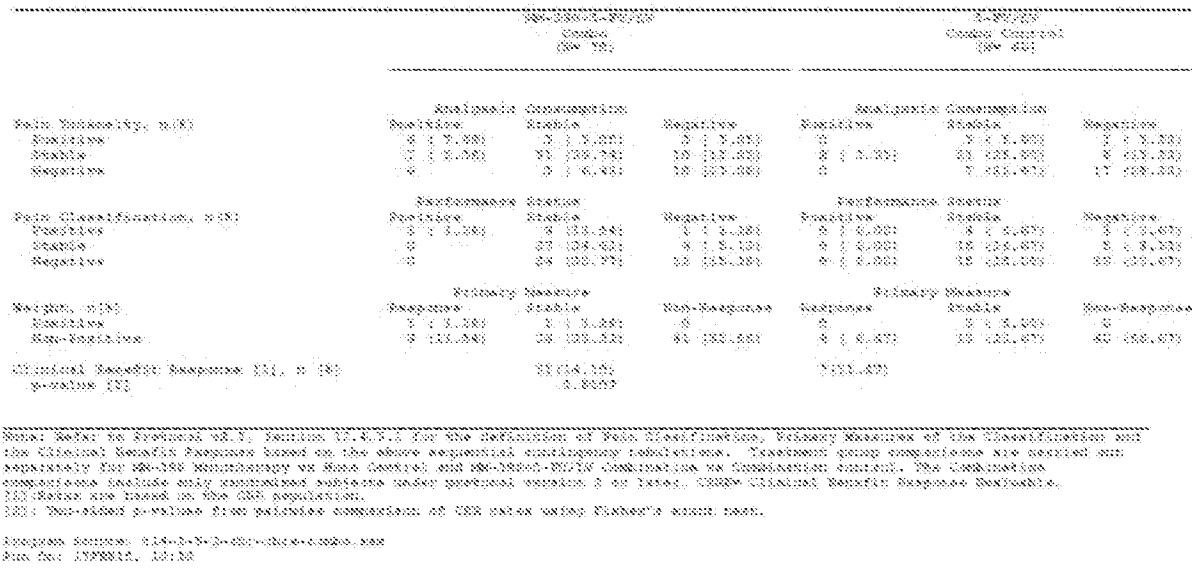
Table 30: Summary of Tumor Marker (CA19-9) Response (TMRE Population)

	Monotherapy Comparison		Combination Therapy Comparison	
	MM-398 (N=113)	5-FU/LV (N=105)	MM-398 + 5-FU/LV (N=87)	5-FU/LV (N=81)
Tumor Marker Response Eval. n (%)	29 (25.6)	12 (11.4)	28 (28.9)	7 (8.6)
p-value [1]	0.0238		0.0006	
Median Time to First Tumor Marker Response [2] (months), (95% CI)	4.4 (3.19, -)	3.81, -	4.3 (2.92, -)	3.91, -
Log-rank p-value [3]	0.1859		0.0392	
Wilcoxon p-value [3]	0.1590		0.0180	

[1] Two-sided p-values from pairwise comparisons of Tumor Marker Response rates using Fisher's exact test.
[2] Median time to First Tumor Response is Kaplan-Meier estimate of the median time to First Tumor Marker Response, in months.
[3] Two-sided p-values from pairwise comparisons of Time to First Tumor Marker Response.
Abbreviations: 5-FU/LV=5-fluorouracil/leucovorin; CI=confidence interval; TMRE=tumor marker response-evaluable
Source: Table 14.2.6

- Clinical Benefit Response (CBR):

Table 31: Clinical Benefit Response (pairwise comparison: combination therapy vs control) CBRE Population



- Quality of life (QoL):

The status of QoL (improved, stable, or worsened) is registered through the EORTC QLQ-C30 questionnaire.

Baseline median Global Health Status, Functional Scale and Symptoms Scale scores were similar among treatment arms. For Physical Functioning, Emotional Functioning, and Cognitive Functioning median scores at baseline were ≥75, indicating a high/healthy level of functioning. At Week 6 and Week 12 no appreciable changes from baseline were registered for both domains.

Regarding symptom scales, median baseline scores were in the range 0-33, indicating low levels of symptomatology. No appreciable change from baseline in median scores were reported at Week 6 and Week 12 for pain, dyspnea, insomnia, appetite loss, and constipation. A post-baseline increase in median symptom scores from 0 (i.e., no symptomatology) to 16.7-33.3 was registered for nausea/vomiting and diarrhoea.

Ancillary analyses

Subgroup OS analyses:

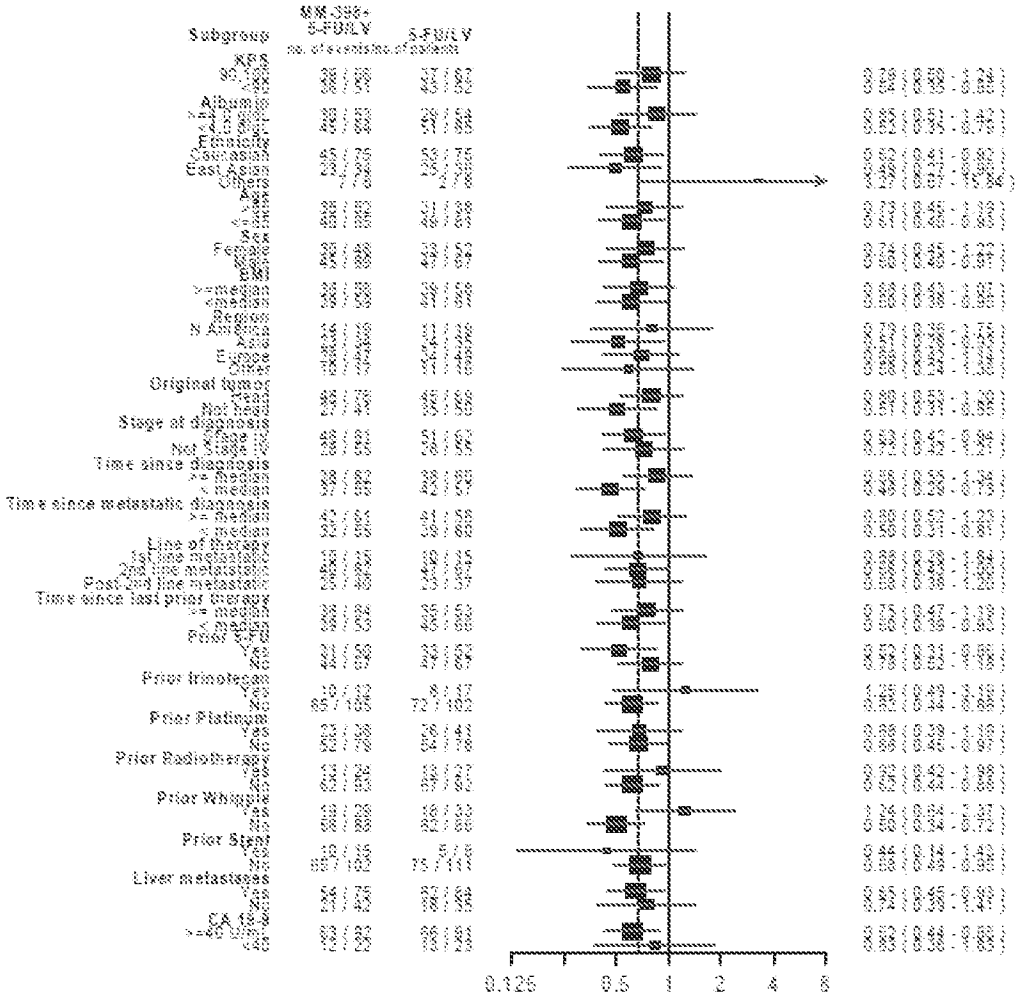


Figure 7: Pre-planned subgroup analysis of Overall Survival – NAPOLI-1

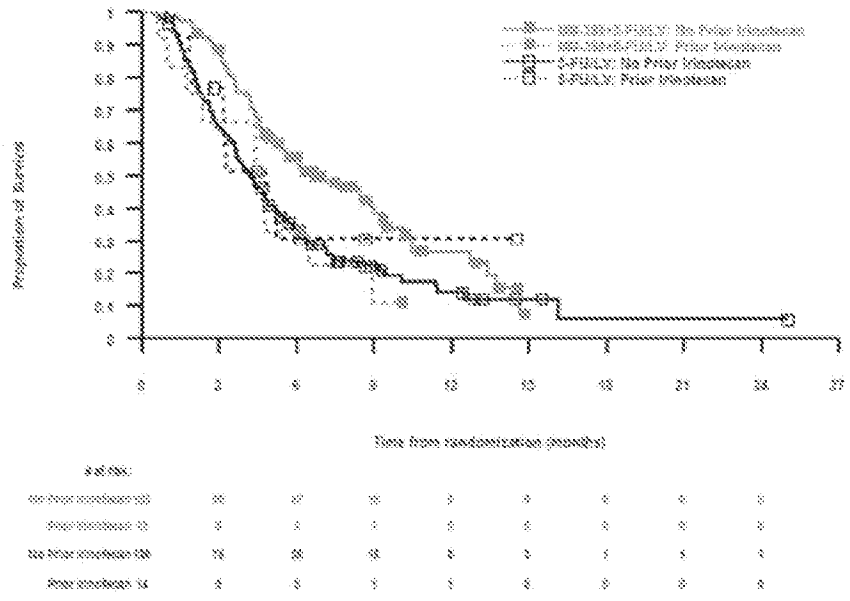


Figure 8: Analysis of OS in treated patients by prior irinotecan exposure (MM-398+5-FU/LV treatments)

Summary of main study

The following table summarises the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 32: Summary of Efficacy for trial MM-398-07-03-01 (NAPOLI-1)

Title: A Randomized, Open Label Phase 3 Study of MM-398, With or Without 5-Fluorouracil and Leucovorin, Versus 5-Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer Who Have Failed Prior Gemcitabine-Based Therapy			
Study identifier	MM-398-07-03-01 NAPOLI-1		
Design	Multicenter, multinational open label, randomized, three-arm study		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		Until disease progression not applicable not applicable
Hypothesis	Superiority		
Treatments groups	MM-398		120 mg/m^2 IV over 90 minutes, every 3 weeks. In case of homozygosity UGT1A1*28, dose was reduced at 80 mg/m^2 for the first cycle of therapy. If not drug related toxicity, from Cycle 2 onwards, the dose could be increased by 20 mg/m^2, up to a maximum of 120 mg/m^2. 151 randomized patients
	5-FU/LV		5-FU 2000 mg/m^2 IV over 24-hours, every week for 4, followed by 2 weeks of rest, in a 6 week cycle LV 200 mg/m^2 IV over 30 minutes, every week for 4 weeks, followed by 2 weeks of rest, in a 6 week cycle. 149 randomized patients
	MM-398+5-FU/LV		MM-398 80 mg/m^2 IV over 90 minutes, every 3 weeks. 5-FU 2400 mg/m^2 IV over 46-hours, every 2 weeks LV 400 mg/m^2 IV over 30 minutes, every 2 weeks In case of homozygosity UGT1A1*28, MM-398 dose was reduced at 60 mg/m^2 for the first cycle of therapy. If not drug related toxicity, from Cycle 2 onwards, the dose could be increased to 80 mg/m^2. 117 randomized patients
Endpoints and definitions	Primary endpoint	OS	time from the date of patient randomization to date of death or the date last known alive.
	Secondary endpoint	PFS	time in months from the date of patient randomization to the date of death or disease progression, whichever occurred earlier.
	Secondary endpoint	TTF	occurrence of discontinuation of treatment for any reason, including disease progression, treatment toxicity, and death.
	Secondary endpoint	ORR	percentage of patients in the study population with a best overall response of CR or PR as assessed by the investigator.

	Secondary endpoint	Tumor marker response (CA19-9)	decrease of 50% of CA19-9 in relation to the baseline level at least once during the treatment period.
Data cutoff date	14 February 2014		
Results and Analysis			
Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent to treat 14 February 2014		

Descriptive statistics and estimate variability	Treatment group	MM-398	5-FU/LV	MM-398+ 5-FU/LV*	5-FU/LV*
	Number of subject	151	149	117	119
	Primary endpoint				
	OS N. events (%)	129 (85.4)	109 (73.2)	75 (64.1)	80 (67.2)
	Median OS months (95% CI)	4.9 (4.23, 5.62)	4.2 (3.58, 4.86)	**6.1** (4.76, 8.87)	**4.2** (3.29, 5.32)
	Unstratified HR p-value (two-sided, un stratified Log-Rank Test)	0.99 (0.77,1.28) 0.94		**0.67** (0.49, 0.92) **0.01**	
	Secondary endpoints				
	PFS N. events (%)	127 (84.1)	120 (80.5)	83 (70.9)	92 (77.3)
	Median PFS months (95% CI)	2.7 (2.13, 2.89)	1.6 (1.41, 1.84)	3.1 (2.69, 4.17)	1.5 (1.41, 1.84)
	Unstratified HR p-value (two-sided, un stratified Log-Rank Test)	0.81 0.10		0.56 0.0001	
	TTF N. events n (%)	86 (56.9)	89 (59.7)	62 (52.9)	70 (58.8)
	Median TTF months (95% CI)	1.7 (1.48, 2.66)	1.4 (1.31, 1.41)	2.3 (1.58, 2.79)	1.4 (1.31, 1.41)
	Unstratified HR p-value (two-sided unstratified Log-Rank Test)	0.82 0.10		0.60 0.0002	
	ORR (CR+PR) n(%) (95% CI)	5 (3.3) (0.46, 6.17)	1 (0.6) (0.0, 1.98)	9 (7.6) (2.86, 12.5)	1 (0.84) (0.0, 2.48)
	p-value (2-sided Fisher's exact test)	0.21		0.009	
	Tumor Marker Response (CA19.9) TMRE Population N	123	105	97	81
	Tumor Marker response n(%)	29 (23.6)	12 (11.4)	28 (28.9)	7 (8.6)
	p-value (2-sided Fisher's exact test)	0.023		0.0006	
Notes	*patients enrolled under Version 2 of the protocol TMRE= Tumor Marker Response Evaluable				

Analysis performed across trials (pooled analyses and meta-analysis)

Clinical studies in special populations

- Age

Table 33: Patients enrolled by study and age groups

	Age Group [1]		
Study	65 - 74 n (%)	75 - 84 n (%)	85 - n (%)
Controlled Trials			
Across All Controlled Trials	181 (33)	50 (9)	2 (<1)
MM-398-07-03-01	149 (36)	41 (10)	2 (<1)
MM-398+5-FU/LV	40 (34)	14 (12)	0
MM-398 monotherapy	58 (38)	18 (12)	2 (1)
5-FU/LV control	51 (34)	9 (6)	0
PEP0206	32 (24)	9 (7)	0
MM-398	11 (24)	3 (7)	0
IRINOTECAN	13 (29)	4 (9)	0
DOCETAXEL	8 (18)	2 (4)	0
Non-controlled Trials			
Across All Non-Controlled Trials	19 (18)	7 (7)	0
PEP0201	1 (9)	0	0
PEP0202	1 (17)	0	0
PEP0203	2 (13)	0	0
PEP0208	9 (23)	3 (8)	0
PIST-CRC-01	2 (11)	3 (17)	0
MM-398-01-01-02	4 (31)	1 (8)	0

[1]: Denominator for % is study total. Total is per randomization for controlled studies.

Intra-arm median OS comparison of patients ≤65 vs >65 years of age

-In MM-398+5FU/LV arm, the mOS was 8.87 and 5.42 months, respectively, with a HR 1.55.

-In the control arm, the HR was 0.97.

-For monotherapy with MM-398, the mOS were similar for patients under and over 65y (HR 0.90).

Despite poorer outcome in patients >65 y vs. ≤65 in the combination arm, the HR versus 5FU/LV was 0.73.

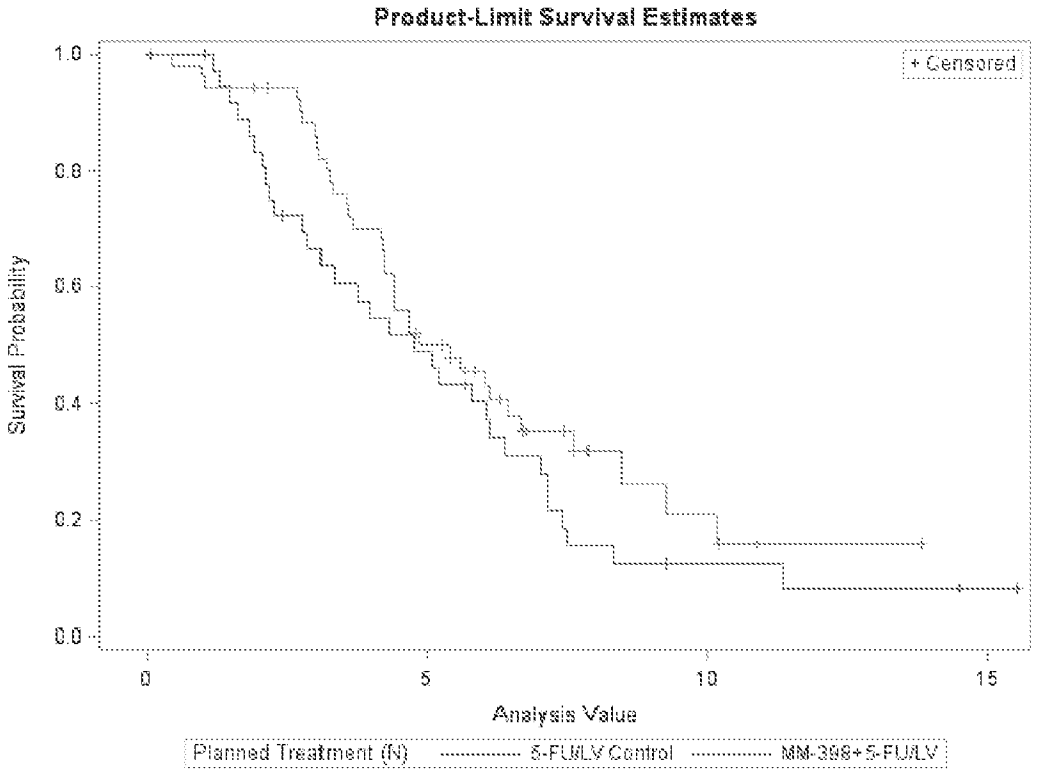


Figure 9: Kaplan Meier curves for patients age > 65 in NAPOLI-1 (MM-398 + 5-FU/LV vs its corresponding control)

Table 34: Survival estimates for patients age >65 in NAPOLI-1 (MM-398 + 5-FU/LV vs its corresponding control)

	MM-398+5-FU/LV (N=52)					5-FU/LV (N=38)			
	Survival					Survival			
Month	Estimate	Std Err	Number Failed	Number at risk		Estimate	Std Err	Number Failed	Number at risk
1	0.96	0.027	2	50		1	0	0	37
2	0.94	0.032	3	48		0.83	0.062	6	30
3	0.88	0.045	6	44		0.66	0.079	12	23
4	0.70	0.065	15	35		0.55	0.084	16	19
5	0.50	0.071	25	24		0.49	0.084	18	17
6	0.46	0.071	27	18		0.40	0.083	21	13
7	0.35	0.072	31	11		0.31	0.080	24	10
8	0.32	0.073	32	6		0.15	0.063	29	5
9	0.26	0.077	33	5		0.12	0.058	30	4
10	0.21	0.078	34	4		0.12	0.058	30	3
11	0.16	0.074	35	1		0.12	0.058	30	3
12	0.16	0.074	35	1		0.08	0.051	31	2

- Sex

The gender-related differences in OS are minimal.

- Ethnicity

No statistically significant difference in OS is observed between Caucasians and East-Asians, HR 0.62 and 0.49, respectively.

Supportive studies

- **PEP0208:** a phase II study of liposomal irinotecan (formerly PEP02) in patients with metastatic pancreatic cancer previously treated with gemcitabine containing regimens.

The results of PEP0208 led to the development of the phase 3 NAPOLI-1 study, which is the basis for this marketing authorization application. Forty (40) patients were enrolled to receive single-agent MM-398 120 mg/m^2 q3w until disease progression. Median OS was 5.2 months. Tumour response rate in the PP population was 7.5% and involved 3 patients with PR.

- **PEP02** (human plasma protein binding to liposomal irinotecan)
- **PEP0201** (phase I, DLT and MTD in solid tumours).

The MTD was determined as a dose of 120mg/m^2 while in PEP02 MM-398 was given as a 90-minute infusion on a q3w schedule, and chosen as recommended dose of MM398 single agent in future studies.

- **PEP0202:** a Phase I Dose Escalation Study Followed by Multi-National, Open-Label Randomized Phase II Study Evaluating the Efficacy and Tolerability of PEP02 with or without Cisplatin in Patients with Recurrent or Metastatic Squamous Carcinoma of the Uterine Cervix).

When the dose was escalated to the second level (MM-398 80 mg/m2 + cisplatin 60 mg/m2), two of the patients in this cohort developed SAEs which led to death. The study was terminated before the MTD of MM-398 in combination with cisplatin 60 mg/m2 given every 3 weeks could be determined.

- **PEP0203:** a multi-center, open-label phase I dose-escalation study of PEP02 in combination with 5-fluorouracil (5-FU) and leucovorin (LV) in advanced solid tumours.

MTD of MM-398 was determined to be 80 mg/m2. All treatment-related AEs and grade III or above at the MTD dose level were 51.1% and 10.6%, respectively.
Among the 15 efficacy evaluable patients with advanced solid tumour refractory to standard systemic treatment, the best tumour response were confirmed as PR in 2, SD in 9 and PD in 5. The overall tumour response rate and disease control rate were 13.3% and 73.3%, respectively. The dose of 80 mg/m^2 of PEP02 in combination with D1 and D8 infusion of 5-FU/LV in q3w schedule was recommended for the future phase II studies.

- **PIST-CRC:** a phase I and Pharmacokinetic Study of Biweekly MM-398 in Patients with Metastatic Colorectal Cancer Refractory to First-line Oxaliplatin-based Chemotherapy)

Of the 18 treated patients, the overall response rate achieved 22.2%. There were 4 patients with partial response (PR) (2 in 80mg/ m^2, 1 in 90 mg/ m^2, 1 in 100 mg/ m^2), 9 patients with stable disease (SD), and 5 with progressive disease (PD). The disease control rate was 72.2%.

- **PEP0206** : a randomized phase II Study of MM-398, Irinotecan or Docetaxel as a Second Line Therapy in Patients with Locally Advanced or Metastatic Gastric or Gastroesophageal Junction Adenocarcinoma

The number of patients with confirmed partial or complete response (PR or CR) was 6 in the MM-398 arm (objective tumour response rate of 13.6%), 3 in the irinotecan arm (6.8%), and 7 in the docetaxel arm (15.9%). No differences were seen in the mPFS: MM-398, 81 days, irinotecan 79.5 days, docetaxel 82 days. The 3 treatments showed similar efficacy in terms of the survival parameters.

- **PEPCOL:** a randomized phase II study of MM-398 or irinotecan in combination with leucovorin and 5-fluorouracil as second-line therapy in patients with unresectable metastatic colorectal cancer

Fifty-five patients with unresectable mCRC who had failed one prior oxaliplatin-based first-line therapy were randomly assigned to FUPEP (MM-398 80 mg/m² d1, folinic acid (FA) 400 mg/m² d1, 5-FU 2,400 mg/m² d1-2) or FOLFIRI (FOLFIRI1: irinotecan 180 mg/m² d1, FA 400 mg/m² d1, 5-FU bolus 400 mg/m² d1, 5-FU infusion 2,400 mg/m² d1-2; or modified FOLFIRI3: irinotecan 90 mg/m² d1 and 3, FA 400 mg/m² d1, 5-FU infusion 2,400 mg/m² d1-2). Bevacizumab q2w (5 mg/kg) was allowed in both arms as of June 2012 (TML study report). The primary endpoint was the objective tumour response (OR). OR rate were 16.7% (n=4/24) and 11.5% (n=3/26) in the FUPEP and the FOLFIRI arms, respectively. Most common grade 3-4 adverse events reported in the respective FUPEP and the FOLFIRI arms were diarrhoea (21% vs 33%), neutropenia (11% vs 30%), mucositis (11% vs 11%), and alopecia (G2: 25% vs 26%).

Translational research

Translational samples have been collected from a subset of consenting patients: archival tumour material has been collected from 26% of the patients, while plasma and whole blood PAXgene material has been collected from 47% of the patients. Discussions with vendors to evaluate best approaches, prioritization of sample use and methodologies as well as method development are ongoing.

2.5.3. Discussion on clinical efficacy

The MAA is based on one pivotal trial, NAPOLI-1, and several supportive studies.

The dose-escalation trial PEP0203 established the irinotecan dose of 80 mg/m^2, in combination with day 1 and day 8 5FU (2000 mg/m^2) / LV (200 mg/m^2) infusion q3w as the recommended schedule for further studies.

The PEPCOL trial (a randomized phase II study of MM-398 or irinotecan in combination with leucovorin and 5-fluorouracil as second-line therapy in patients with unresectable metastatic colorectal cancer) was the basis for the introduction of the combination arm (MM-398+5FU/LV) in the pivotal NAPOLI-1 trial; in PEPCOL, a combination of MM-398 (given q2w at a dose of 80 mg/m^2 to patients with metastatic colorectal cancer previously treated with oxaliplatin) with 5-FU (2400 mg/m^2 day1-2) / LV (400 mg/m^2 day 1) was administered with encouraging tumour response and less AEs (namely diarrhoea and neutropenia) than in the FOLFIRI arm.

Design and conduct of clinical studies

The study was subject to CHMP advice when a third arm (MM-398 + 5FU/LV) was proposed to be added to the ongoing MM-398 vs. 5FU/LV study. In the advice it was recommended that the design should be simplified, comparing MM-398 vs. standard irinotecan both on top of 5FU/LV, meaning that a completely new study was recommended. For obvious reasons this design would have provided a more straight forward possibility to assess the benefit of MM-398 compared with standard irinotecan.

Irinotecan in regimens such as FOLFIRI, however, has not been demonstrated to be beneficial in pancreatic cancer after failure on gemcitabine. Whilst not considered to be the most informative, the design, including 5FU/LV alone as reference therapy, is accepted from a regulatory perspective.

The 5FU/LV regimens in the control arm B versus the experimental arm C are dissimilar enough to warrant further discussion:

In the experimental arm (MM-398+5-FU/LV, arm C), the nominal dose of 5-FU was 2,400 mg/m^2 IV over 46 hours every 2 weeks, per 6 weeks 7,200 mg/m^2. Over a mean of 15 weeks of exposure, this led to a 6-week average dose intensity of 5065 mg/m^2 and relative dose intensity for 5FU of 83.9%.

In the control arm (5-FU/LV, arm B), the nominal dose of 5-FU was 2,000 mg/m^2 IV over 24 hours, administered weekly for the first 4 weeks, followed by 2 weeks' rest, per 6 weeks 8,000 mg/m^2. Over a mean of 10 weeks of exposure; the 6-week average dose intensity was 6,710 mg/m^2. The relative dose intensity for 5FU is 95.7%.

The nominal 6-week dose of 5FU was thus slightly higher in the control arm (8,000 vs. 7,200), but the infusion time was shorter (24 vs. 46 h). To this may be added that LV dose also differed: 200 mg/m^2 (arm B) vs 400 mg/m^2 (arm C). Qualitatively, longer infusion time and higher LV dose would favour the experimental arm, whilst the higher dose would favour the control group. Quantitatively, however, the differences are considered minor and highly unlikely per se to result in a difference in PFS/OS between arms B and C.

The 6-week average dose intensity over the mean period of exposure is a function of variability in exposure time/completion of cycles and dose reductions; the main factor in both study arms being variable exposure time, most notable for the control arm, where the relative dose intensity was very high (96%). The high relative dose intensity in the control arm, however, shows that there was room for a more intensive 5FU/LV regimen.

The number of different 5FU/LV regimens still in clinical use is large, meaning that there is no "gold standard"; here illustrated by two second-line studies conducted in patients with pancreatic cancer who have received gemcitabine first-line. In the phase III PANCREOX trial mFOLFOX6 was compared with 5FU/LV. The selected control regimen was LV 400 mg/m^2 and 5FU 2400mg/m^2 over 46 hours q2w, i.e. the same as the background 5FU/LV regimen in arm C. This was a negative trial (OS HR 1.8, p=0.02).

In the phase III CONKO-003 trial, a combination of oxaliplatin with 5-FU/LV (OFF regimen) was compared with 5FU/LV. The control regimen was LV 200 mg/m^2 followed by a continuous IV infusion of fluorouracil 2,000 mg/m^2 over 24 hours on days 1, 8, 15, and 22. After a 3-week rest period from day 23 to 42, the second course was initiated on day 43 (i.e. day 1 of the second cycle). The 5FU/LV regimen was the same for the experimental and the control arm; the difference with the control arm in NAPOLI-1 lies in the more spaced administration of 5FU/LV in CONKO, i.e. a less intensive regimen. A survival benefit related to oxaliplatin add-on was shown, HR 0.68 p=0.01. Efficacy wise the two regimes, OFF and MM398+5FU/LV, are indeed similar. However, oxaliplatin causes long lasting neurotoxicity.

Table 35: Summary of various R-FU/LV regimens used

		NAPOLI-1		CONKO-003	PANCREOX
		Control	Combination regimen	Control	Control
LV	Dose (mg/m^2)	200	400	200	400
	Infusion time	30min	30min	30min	30min
5-FU	Dose (mg/m^2)	2000	2400	2000	2400
	Infusion time	24h	46h	24h	46h
Regimen		d1, 8, 15, 22	q2w	d1, 8, 15, 22	q2w
Rest period		2w		3w	

Although it would have facilitated the assessment of the add-on benefit of MM-398 if the same 5FU/LV regimen had been used as background and control, the control regimen in NAPOLI-1 cannot be viewed as too non-intensive.

In the monotherapy arm, MM-398 was administered at 120 mg/m^2 every 3 weeks and in the combination arm 80 mg/m^2 every 2 weeks, i.e. the same dose intensity over a 6-week period. It cannot be excluded that this difference influences the anti-tumour activity. Therefore the add-on benefit of 5FU/LV to MM-398 cannot be disentangled, but it is considered acceptable from a regulatory perspective.

The study population was heterogeneous, mainly in terms of prior lines of therapy and therefore also in terms of exposure to different cytotoxic agents. Apart from prior exposure to irinotecan (46 patients, 10%), this apparently did not influence to a major degree the relative activity of the combination regimen.

Efficacy data and additional analyses

The primary efficacy analysis of OS was performed when 305 deaths had occurred (data cut-off 14 February 2014).

The primary endpoint assessment showed a median OS gain of 1.9 months in favour of the experimental arm (6.1 vs 4.2 months, HR 0.67, p=0.0122). A median OS benefit of two months (i.e. an about 50% increase from 4 to 6 months) in this clinical setting is relevant from a regulatory perspective. However, the p-value is borderline significant, bearing in mind that the alpha was split at a significance level of 0.025.

The primary analysis was non-stratified although it is normally expected that the primary analysis is stratified according to the factors used for stratification (EMA/CHMP/295050/2013). KPS was the only notable imbalance in stratification factors and seemingly favoured the experimental arm. It should be noticed, however, that the HR in patients with KPS <90 is 0.54 vs. 0.79 in those with KPS ≥90.

As a sensitivity analysis, a stratified analysis is reported and shows a lower hazard ratio and a lower p-value: HR 0.57 vs 0.67, p-value 0.0009 vs 0.012. These results, in the CHMP preferred analysis, make the "borderline concern" less of an issue.

Fourteen (14) patients in the control group did not receive any study drug but were analysed as if they had received 5 FU/LV causing a possible OS bias. A more conservative analysis with the imputation of OS times for patients who did not have observed OS time due to early discontinuation from OS follow-up was provided. The outcome of the imputed dataset simulations both stratified and non-stratified, yielded similar values, therefore supporting the robustness of the primary OS analysis.

Among the pre-planned OS subgroup analyses, a treatment effect favouring the 5-FU/LV over the combination arm has been observed for ethnicity other than caucasian and east asian, prior Whipple and prior irinotecan. This observation is also confirmed by univariate and multivariate analyses conducted to identify possible prognostic factors for both OS and PFS, which consistently showed prior irinotecan together with age>65 to negatively impact on the prognosis of patients treated with the combination arm.

Despites the limitations of subgroup analyses, the observation in patients pre-treated with irinotecan raises concerns due to the increasing use of irinotecan-containing regimen as first line therapy. Based on the limited data provided in patients with prior exposure to irinotecan, moreover showing no advantage (if not a detrimental effect) over 5FU/LV, the benefit of Onivyde in patients previously treated with an irinotecan-based regimen has not been demonstrated (see sections 4.4 and 5.1 of the SmPC).

The PFS was assessed by the investigator, not by IRC. The combination of MM-398+5-FU/LV achieved a median PFS of twice that of the control arm of 5-FU/LV (3.1 vs 1.5 months, HR 0.56, p=0.0001).

The combination of MM-398+5-FU/LV achieved a median TTF of 2.3 months compared to 1.4 months for the control arm of 5-FU/LV, HR 0.60, p=0.0002. TTF results are thus consistent with PFS outcomes.

The confirmed ORR for MM-398+5-FU/LV was 7.7% (95% CI: 2.86, 12.52) compared to 0.8% for 5-FU/LV. The confirmed ORR for MM-398 monotherapy was 3.3%, vs 0.7% for 5-FU/LV. As expected, the unconfirmed ORRs were higher than the confirmed ORRs: 16.2% for MM-398+5-FU/LV vs 0.8% of 5-FU/LV control arm; 6.0% for MM-398 vs 0.7% for 5-FU/LV.

When comparing Onivyde monotherapy to 5FU/LV, the OS results are very similar with a HR 0.99. A trend towards better PFS results was observed, however the HR was 0.8, p=0.1. The unconfirmed ORR (RECIST 1.1) was 6% vs. 0.7% in the 5FU/LV arm. This trend towards higher activity of MM-398 is of some importance as it underlines that the survival benefit seen in the combination arm cannot be explained by the differences in the 5FU/LV regimens and is caused by the add-on effect of MM-398.

The decreases in the CA19-9 levels are consistent with the findings of other efficacy endpoints.

The main objective of CBR assessment was to show an improvement in pain intensity in the combination arm which was not observed.

Due to a very high attrition rate (only 60% had at least one post-baseline assessment), a conclusion on the effect of the addition of MM-398 to 5-FU/LV on the quality of life cannot be drawn.

In the combination arm, the OS outcome is clearly worse in patients above 65 years of age, but put in relation to the control arm, there is still a likely benefit in this group of patients (OS HR 0.7).

Discussions with participating investigators, key opinion leaders and internal experts are ongoing to determine priorities for the optimal studies on the translational samples collected in the context of the available results of the main study, NAPOLI-1, and emerging research in pancreatic cancer. Clinically relevant results will be communicated appropriately when they become available. Complete results will be provided as soon as possible, but are likely to be available in 2017, at the earliest. The Applicant is recommended to submit the results of the translational research as soon as available.

2.5.4. Conclusions on the clinical efficacy

The efficacy results observed with Onivyde in terms of OS benefit and supportive evidence are considered clinically meaningful and statistically sufficiently robust to support approval in metastatic pancreatic cancer.

The place of Onivyde in the algorithm of treatment in metastatic pancreatic cancer should be carefully considered given the increased use of FOLFIRINOX as first line therapy and the limited number of patients in the pivotal NAPOLI-1 study previously treated with both gemcitabine and irinotecan.

The CHMP considers the following measures necessary to address issues related to efficacy:

The Applicant is recommended to conduct and submit the results of a translational research program in pancreatic cancer based on samples collected from completed and ongoing studies.

2.6. Clinical safety

The table below summarises the safety database used for the assessment of Onivyde.

Table 36: Clinical Studies with MM-398 for Assessment of Safety

Study Identifier	Study Design	Test Product(s); Dosage Regimen	N	Diagnosis of Patients	MM-398 Treated Patients
MM-398-07-03-01 (NAPOLI-1)[a]	Open label, randomized, phase 3	Arm A: MM-398 120 mg/m^2 q3w Arm B: 5-FU 2000 mg/m^2+LV 200 mg/m^2 qw Arm C: MM-398 80 mg/m^2 + 5-FU 2400 mg/m^2 + LV 400 mg/m^2 q2w	417	Metastatic pancreatic cancer	264
PEP0208[a]	Open label, single arm, phase 2	MM-398 120 mg/m^2 q3w	40	Metastatic pancreatic cancer	40
PEP0206[a]	Open label, randomized, phase 2 study	Arm 1: MM-398 120 mg/m^2 q3w Arm 2: irinotecan 300 mg/m^2 q3w Arm 3: docetaxel 75 mg/m^2 q3w	132	Gastric & GEJ cancer	44
PEP0201[a]	Phase 1	MM-398 60, 120, 180 mg/m^2 q3w	11	Solid tumours	11
PEP0202[a]	Phase 1/2 study	MM-398: 60 and 80 mg/m^2 q3w + cisplatin: 60 mg/m^{2}q3w	6	Metastatic cervical cancer	6
PEP0203[a]	Phase 1 of MM-398 in combination with 5-FU/LV	MM-398: 60, 80, 100, 120 mg/m^2 q3w 5-FU: 2000 mg/m^2 on Day 1 and 8 q3w Leucovorin: 200 mg/m^2 on Day 1 and 8 q3w	16	Solid tumours	16
PIST-CRC-01[a]	Phase 1	MM-398: 80, 90 and 100 mg/m^2 q2w	18	Colorectal cancer	18
MM-398-01-01-02 (CITS)[a] (ongoing)	Open-label	80 mg/m^2 every 2 weeks	13	Solid tumour	13
PEPCOL[b] (ongoing)	Open label, randomized, phase 2	Arm 1 FOLFIRI1 or modified FOLFIRI-3 +/- bevacizumab 5 mg/kg Arm 2 MM-398 80 mg/m^2 + 5-FU 2400 mg/m^2 + LV 400 mg/m^2 q2w IV +/- bevacizumab 5mg/kg	55	Colorectal cancer	28[c]

N=patients enrolled; FOLFIRI: folinic acid; 5-FU and irinotecan; 5-FU: 5-fluorouracil; LV: leucovorin; IV: intravenous; qw: every week; q2 week: every other week; q3w: every third week;
[a]Merrimack has access to clinical database and study reports
[b]Safety is summarized based on published report
[c]Among the 28 patients receiving MM-398 in combination with 5-FU/LV, 12 patients also received bevacizumab. In the irinotecan in combination with 5-FU/LV group, 13 out of 27 patients received bevacizumab.
Source: 2.7.4 Summary of Clinical Safety Table 3

Patient exposure

To assess the safety of Onivyde 80 mg/m^2 plus 5-FU 2400 mg/m^2 and leucovorin (LV) 400 mg/m^2 administered every 2 weeks (q2w) in the proposed indication for pancreatic cancer, the most relevant data source is the safety analysis of the Phase 3, randomized controlled NAPOLI-1 study, focusing on the MM-398+5- FU/LV combination arm. The monotherapy Onivyde (120mg/m^2) was also analysed in detail in order to identify undesirable effects associated with Onivyde that may not been uncovered from the Onivyde +5-FU/LV combination arm.

Other than NAPOLI-1, only two studies contain Onivyde safety data in combination with 5-FU and LV, albeit in different patient populations:

1. PEPCOL, an investigator sponsored study in colorectal cancer

2. PEP0203 Phase 1 study in solid tumours

PEPCOL employed the same dose and schedule as studied in NAPOLI-1, whereas the PEP0203 study employed an every-3-week (Q3W) MM-398 dosing schedule. MM-398 doses were escalated: 60, 80, 100 and 120 mg/m^2. The dose and schedule for PEP0203 5-FU/LV administration was 5-FU 2000 mg/m2 mixed with leucovorin 200 mg/m2 as a 24-hour continuous infusion on Days 1 and 8, every 21 days (3 weeks).

All other studies with Onivyde (including an experimental arm of Onivyde 120 mg/m2 IV Q3W in the NAPOLI-1 study) were conducted as a monotherapy or in combination with agents other than 5-FU and leucovorin (PEP0201, PIST-CRC, PEP0206, PEP0208, CITS) or in combination with cisplatin (PEP0202). For these reasons, no formal pooling of the safety data from the NAPOLI-1 study with other studies was conducted.

Overall, 440 patients have received Onivyde in the completed and ongoing studies as of 14 February 2014. The 440 patients treated with Onivyde in these clinical trials had a variety of advanced solid tumours with high unmet medical need. The largest patient exposure to Onivyde has been in patients with advanced metastatic pancreatic cancer (a total of 304 patients), primarily in the Phase 3 study, NAPOLI-1 (264 patients).

Table 37: Clinical Studies with MM-398 for Assessment of Safety

Study Identifier	Study Design	Test Product(s); Dosage Regimen	N	Diagnosis of Patients	MM-398 Treated Patients
MM-398-07-03-01 (NAPOLI-1)[a]	Open label, randomized, phase 3	Arm A: MM-398 120 mg/m^2 q3w Arm B: 5-FU 2000 mg/m^2+LV 200 mg/m^2 qw Arm C: MM-398 80 mg/m^2 + 5-FU 2400 mg/m^2+ LV 400 mg/m^2 q2w	417	Metastatic pancreatic cancer	264
PEP0208[a]	Open label, single arm, phase 2	MM-398 120 mg/m^2 q3w	40	Metastatic pancreatic cancer	40
PEP0206[a]	Open label, randomized, phase 2 study	Arm 1: MM-398 120 mg/m^2 q3w Arm 2: irinotecan 300 mg/m^2 q3w Arm 3: docetaxel 75 mg/m^2 q3w	132	Gastric & GEJ cancer	44
PEP0201[a]	Phase 1	MM-398 60, 120, 180 mg/m^2 q3w	11	Solid tumors	11
PEP0202[a]	Phase 1/2 study	MM-398: 60 and 80 mg/m^2 q3w + cisplatin: 60 mg/m^{2}q3w	6	Metastatic cervical cancer	6
PEP0203[a]	Phase 1 of MM-398 in combination with 5-FU/LV	MM-398: 60, 80, 100, 120 mg/m^2 q3w 5-FU: 2000 mg/m^2 on Day 1 and 8 q3w Leucovorin: 200 mg/m^2 on Day 1 and 8 q3w	16	Solid tumors	16
PIST-CRC-01[a]	Phase 1	MM-398: 80, 90 and 100 mg/m^2 q2w	18	Colorectal cancer	18
MM-398-01-01-02 (CITS)[a] (ongoing)	Open-label	80 mg/m^2 every 2 weeks	13	Solid tumor	13
PEPCOL[a] (ongoing)	Open label, randomized, phase 2	Arm 1: FOLFIRI or modified FOLFIRI-3 +/- bevacizumab 5 mg/kg Arm 2: MM-398 80 mg/m^2 + 5-FU 2400 mg/m^2 + LV 400 mg/m^2 q2w IV +/- bevacizumab 5mg/kg	55	Colorectal cancer	28[c]

N=patients enrolled
FOLFIRI: folinic acid
5-FU and irinotecan; 5-FU: 5-fluorouracil
LV: leucovorin
IV: intravenous
qw: every week
q2 week: every other week
q3w: every third week
[a]Merrimack has access to clinical database and study reports
[b]Safety is summarized based on published report
[c]Among the 28 patients receiving MM-398 in combination with 5-FU/LV, 12 patients also received bevacizumab. In the irinotecan in combination with 5-FU/LV group, 13 out of 27 patients received bevacizumab.

Due to the differences in dose/dose regimen among study arms, the patient exposure was presented and discussed in the clinical efficacy section.

Adverse events

The Safety Analysis Population from the pivotal study NAPOLI-1 includes **398** of the 417 patients randomized in this study, who received at least one dose of study drug.

Adverse events (AEs) are coded using MedDRA version 14.1 Treatment emergent adverse events (TEAEs) are defined as events that occurred or worsened on or after the day of first dose of the study drug and within 30 days after last administration of study drug.

Table 38: Summary of All Adverse Events (Safety Population) – NAPOLI-1

	MM-398 120 mg/m^2 (N=147) n (%)	MM-398 80mg/m2+5-FU/LV (N=117) n (%)	5-FU/LV (N=134) n (%)
Subjects with at least one AE	146 (99.3)	116 (99.1)	132 (98.5)
Subjects with at least one TEAE	145 (98.6)	116 (99.1)	132 (98.5)
Subjects with CTCAE grade 3 or higher TEAE	112 (76.2)	90 (76.9)	75 (56.0)
Subjects with TEAE related to study drug	128 (87.1)	107 (91.5)	93 (69.4)
Subjects with drug related AE of CTCAE grade 3 or higher	76 (51.7)	63 (53.8)	24 (17.9)
Subjects with Grade 3 as most severe toxicity [a]	54 (36.7)	53 (45.3)	21 (15.7)
Subjects with Grade 4 as most severe toxicity [b]	18 (12.2)	9 (7.7)	3 (2.2)
Subjects with Grade 5 as most severe toxicity [c]	4 (2.7)	1 (0.9)	0
Subjects with serious TEAE	90 (61.2)	56 (47.9)	60 (44.8)
Subjects with TEAE leading to any dose modification	81 (55.1)	83 (70.9)	48 (35.8)
Subjects with TEAEs resulting in dose delay	49 (33.3)	72 (61.5)	43 (32.1)
Subjects with TEAE leading to dose reduction	46 (31.3)	39 (33.3)	5 (3.7)
Subjects with TEAE leading to dose discontinuation	17 (11.6)	13 (11.1)	10 (7.5)

Source: 5.3.5.1 NAPOLI-1 CSR Table 8-5

TEAEs by SOC

Table 39: Summary of Most Common Treatment Emergent Adverse Events by System Organ Class – NAPOLI-1

System Organ Class- - MedDRA version 14.1	MM-398 (N=147) n (%)	MM-398+5-FU/LV (N=117) n (%)	5-FU/LV (N=134) n (%)
Number of Subjects With Any TEAE(s)	145 (98.6)	116 (99.1)	132 (98.5)
Gastrointestinal disorders	140 (95.2)	108 (92.3)	109 (81.3)
General disorders and administration site conditions	107 (72.8)	84 (71.8)	80 (59.7)
Metabolism and nutrition disorders	106 (72.1)	73 (62.4)	67 (50.0)
Blood and lymphatic system disorders	68 (46.3)	**67 (57.3)**	36 (26.9)
Investigations	69 (46.9)	56 (47.9)	35 (26.1)
Infections and infestations	54 (36.7)	45 (38.5)	35 (26.1)
Nervous system disorders	41 (27.9)	36 (30.8)	27 (20.1)
Skin and subcutaneous tissue disorders	49 (33.3)	33 (28.2)	39 (29.1)
Respiratory, thoracic and mediastinal disorders	42 (28.6)	27 (23.1)	30 (22.4)
Musculoskeletal and connective tissue disorders	24 (16.3)	26 (22.2)	36 (26.9)
Psychiatric disorders	21 (14.3)	17 (14.5)	20 (14.9)
Injury, poisoning and procedural complications	6 (4.1)	15 (12.8)	13 (9.7)
Vascular disorders	15 (10.2)	14 (12.0)	18 (13.4)
Hepatobiliary disorders	18 (12.2)	9 (7.7)	11 (8.2)
Renal and urinary disorders	19 (12.9)	7 (6.0)	10 (7.5)
Cardiac disorders	8 (5.4)	4 (3.4)	5 (3.7)
Eye disorders	3 (2.0)	4 (3.4)	5 (3.7)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	6 (4.1)	4 (3.4)	6 (4.5)
Immune system disorders	1 (0.7)	3 (2.6)	1 (0.7)
Ear and labyrinth disorders	3 (2.0)	2 (1.7)	2 (1.5)
Reproductive system and breast disorders	3 (2.0)	2 (1.7)	0

For most SOCs, the AEs that occurred/worsened from the first day of drug administration till up to 30 days to the last dose were more frequent in the MM-398 monotherapy arm, followed closely by the combination arm.

The most striking difference between the experimental arms in the opposite sense is 'blood': 46.9% AEs in the monotherapy arm, compared to 57.3% in the combination arm.

TEAE causality

Table 40: Most Common Treatment Emergent Adverse Events Related to Study Drug (≥10% of Patients in any Group)

MedDRA Preferred Term	MM-398 (N=147) n (%)	MM-398+5-FU/LV (N=117) n (%)	5-FU/LV (N=134) n (%)
Number of Subjects With Any Related TEAE(s)	**128 (87.1)**	**107 (91.5)**	**93 (69.4)**
Diarrhoea	91 (61.9)	55 (47.0)	20 (14.9)
Nausea	69 (46.9)	53 (45.3)	35 (26.1)
Vomiting	63 (42.9)	50 (42.7)	22 (16.4)
Fatigue	40 (27.2)	36 (30.8)	22 (16.4)
Decreased appetite	44 (29.9)	32 (27.4)	16 (11.9)
Neutropenia	22 (15.0)	**25 (21.4)**	3 (2.2)
Anaemia	27 (18.4)	20 (17.1)	12 (9.0)
Asthenia	20 (13.6)	18 (15.4)	5 (3.7)
White blood cell count decreased	10 (6.8)	**17 (14.5)**	2 (1.5)
Neutrophil count decreased	15 (10.2)	**16 (13.7)**	1 (0.7)
Alopecia	30 (20.4)	14 (12.0)	6 (4.5)
Weight decreased	12 (8.2)	14 (12.0)	3 (2.2)
Stomatitis	4 (2.7)	14 (12.0)	6 (4.5)
Abdominal pain	17 (11.6)	7 (6.0)	5 (3.7)

The TEAE causality is in line with the known safety profile of standard irinotecan, i.e. gastrointestinal AEs and hematotoxicity.

AEs' incidence and severity was proportional with dose intensity in the MM-398 containing arms, with the exception of the PTs 'neutropenia', 'WBC decreased', 'neutrophil count decreased'. 'Fatigue' and 'asthenia' were also more frequently observed in the combination arm. With regards to common TEAEs ≥ gr 3 (see also AESI), diarrhoea and vomiting were the leading AEs in MM-398 containing arms, and the incidence was proportional with the exposure to drug.

Adverse drug reactions

In order to determine the adverse drug reactions of MM-398 and to derive the frequencies of these adverse drug reactions for labelling, the following analyses of NAPOLI-1 study were performed.

Any grade treatment emergent adverse events that occurred 3% higher frequency and/or Grade 3 or higher TEAES that occurred 2% higher frequency in either the MM-398 monotherapy or MM-398+5-FU/LV combination arms compared to the 5-FU-LV control arms were tabulated using MedDRA Preferred Terms.

A similar analysis was performed using clustering of certain single MedDRA PT into medically relevant groups in order to avoid underreporting of adverse reactions frequencies due to the similarities between some single MedDRA Preferred Terms. In addition to the cumulative frequencies of treatment emergent adverse events, an exposure adjusted adverse event rate was also calculated per person-years as n/T, where n=number of subjects with specified event and T=total person-years. Person-years were calculated as the time from the first dose date to:

- the onset date of first event for subjects with event (patients with the event)

- the minimum of (date of last dose + 30 days, date of death, February 14, 2014) (patients without the event)

The following table of ADRs has been established on the basis of the criteria described above.

Table 41: Adverse Reactions Reported with Onivyde therapy in NAPOLI-1

MedDRA* System Organ Class	Adverse reaction	Frequency (%)
Infections and infestations	Septic shock	2.3
	Sepsis	3.0
	Pneumonia	3.4

MedDRA* System Organ Class	Adverse reaction	Frequency (%)
	Febrile neutropenia	3.8
	Gastroenteritis	3.0
	Oral candidiasis	3.8
	Biliary sepsis	0.8
Blood and lymphatic system disorders	Neutropenia	18.6
	Leukopenia	10.3
	Anaemia	34.8
	Thrombocytopenia	4.3
	Lymphopenia	1.9
Immune system disorders	Hypersensitivity	0.8
Metabolism and nutrition disorders	Hypokalaemia	17.4
	Hypomagnesaemia	10.2
	Dehydration	10.2
	Decreased appetite	47.0
	Hypoglycaemia	3.4
	Hyponatraemia	5.7
	Hypophosphataemia	3.4
Psychiatric disorders	Insomnia	8.0
Nervous system disorders	Dizziness	12.1
	Cholinergic syndrome	0.7
	Dysgeusia	3.8
Cardiac disorders	Hypotension	4.9
Vascular disorders	Pulmonary embolism	3.4
	Embolism	1.4
	Deep vein thrombosis	1.9
	Thrombosis	0.8
Respiratory, thoracic and mediastinal disorders	Dyspnoea	7.6
	Dysphonia	2.7
	Hypoxia	0.8
Gastrointestinal disorders	Diarrhoea	65.2
	Vomiting	53.4
	Nausea	56.4
	Abdominal pain	29.2
	Stomatitis	13.7
	Colitis	1.1
	Haemorrhoids	3.4
	Oesophagitis	0.8
	Proctitis	0.8
Hepatobiliary disorders	Hypoalbuminaemia	12.9
Skin and subcutaneous tissue disorders	Alopecia	18.2
	Rash maculo-papular	0.8
	Nail discolouration	0.8
Renal and urinary disorders	Acute renal failure	2.3
General disorders and administration site conditions	Pyrexia	21.2
	Peripheral oedema	15.5
	Mucosal inflammation	10.3
	Fatigue	38.3

MedDRA* System Organ Class	Adverse reaction	Frequency (%)
	Asthenia	22.3
	Infusion related reaction	2.3
	Oedema	1.9
Investigations	Weight decrease	18.6
	Increased bilirubin	3.8
	Increased alanine aminotransferase	4.9
	Increased aspartate aminotransferase	4.2
	Increased international normalized ratio	1.5

Serious adverse event/deaths/other significant events

SAEs

The incidence of SAEs in MM-398 containing arms was generally proportional with the exposure to liposomal irinotecan, with exception of 'infections and infestations'.

AESI: the AESIs were defined based on the known safety profile of Onivyde and on Camptosar label.

Longitudinal data for AESI were requested and provided by the Applicant, as well as the prevalence/incidence of AESI overall and by grade ≥3, however not per treatment cycle. Information on any repeated gr3 or 4 AESI were provided.

Most AESIs are early or very early events, according to the data; they wane / disappear after an average of six administrations. In the context of NAPOLI-1, six doses make up for 3 months (MM-398 administered q2w) of combination therapy and 4.5 moths for MM-398 monotherapy.

A brief look at the line listings reveals that the patients still in treatment after 6 doses are about a third from the total. So it is these patients who begin to benefit from a milder AESI profile of the treatment.

Table 42: Frequency of Treatment Emergent Adverse Events of Special Importance, NAPOLI-1 Study

AESI	MM-398 (N=147) n (%)		MM-398+5-FU/LV (N=117) n (%)		5-FU/LV (N=134) n (%)	
	Any grade	Grade 3 or higher	Any grade	Grade 3 or higher	Any grade	Grade 3 or higher
Any AESI	140 (95.2)	91 (61.9)	113 (96.6)	71 (60.7)	115 (85.8)	46 (34.3)
Neutropenia	37 (25.2)	22 (15.0)	46 (39.3)	32 (27.4)	7 (5.2)	2 (1.5)
Leukopenia	41 (27.9)	24 (16.3)	53 (45.3)	34 (29.1)	10 (7.5)	2 (1.5)
Anaemia	49 (33.3)	16 (10.9)	45 (38.5)	12 (10.3)	31 (23.1)	9 (6.7)
Thrombocytopenia	8 (5.4)	1 (0.7)	15 (12.8)	3 (2.6)	9 (6.7)	-
Neutropenic fever/sepsis	7 (4.8)	6 (4.1)	4 (3.4)	3 (2.6)	1 (0.7)	-
diarrhoea	105 (71.4)	34 (23.1)	69 (59.0)	17 (14.5)	35 (26.1)	6 (4.5)
Nausea	89 (60.5)	8 (5.4)	60 (51.3)	9 (7.7)	46 (34.3)	4 (3.0)
Vomiting	80 (54.4)	20 (13.6)	61 (52.1)	13 (11.1)	35 (26.1)	4 (3.0)
Stomatitis	17 (11.6)	-	37 (31.6)	5 (4.3)	16 (11.9)	1 (0.7)
Gastrointestinal nonspecific inflammation	73 (49.7)	18 (12.2)	50 (42.7)	12 (10.3)	65 (48.5)	12 (9.0)
Colitis	5 (3.4)	1 (0.7)	1 (0.9)	-	-	-
Ileus	6 (4.1)	3 (2.0)	2 (1.7)	0	5 (3.7)	4 (3.0)
Cholinergic events	8 (5.4)	-	4 (3.4)	0	11 (8.2)	1 (0.7)
Acute pancreatitis	1 (0.7)	-	2 (1.7)	2 (1.7)	2 (1.5)	2 (1.5)
Hand-foot syndrome	3 (2.0)	-	3 (2.6)	0	5 (3.7)	0
Acute renal failure	10 (6.8)	4 (2.7)	6 (5.1)	-	6 (4.5)	1 (0.7)
Pulmonary toxicity (interstitial lung disease)	2 (1.4)	1 (0.7)	-	-	-	-
Thrombotic events[3]	21 (14.3)	10 (6.8)	7 (6.0)	4 (3.4)	12 (9.0)	9 (6.7)
Thrombotic events[4]	19 (12.9)	10 (6.8)	6 (5.1)	3 (2.6)	11 (8.2)	8 (6.0)
Infusion associated reactions	15 (10.2)	-	14 (12.0)	-	18 (13.4)	-
Infusion associated reactions, acute	3 (2.0)	-	8 (6.8)	-	8 (6.0)	-
Sepsis/bacteraemia	11 (7.5)	9 (6.1)	9 (7.7)	7 (6.0)	8 (6.0)	6 (4.5)

Diarrhoea

Severe diarrhoea occurred in 13% of patients in the combination arm. Diarrhoea starts early, after the first dose, but new onset diarrhoea is observed up to 4 doses. Early diarrhoea (onset day1, consistent with cholinergic hyperstimulation) occurred twice as frequently in the combination arm (29.9%) than in the other two arms. Late events (after day1) were more frequent in the monotherapy arm (66.7%) and in the combination arm (42.7%) than in the control arm. One third of the patients in the combination arm experience diarrhoea (mostly gr1-2) throughout the treatment, the prevalence of diarrhoea remains constant over time in MM-398 containing arms, however treatment discontinuation due to diarrhoea was very low. Diarrhoea is more frequent and more severe in Caucasians. However, diarrhoea was manageable with atropine/loperamide and supportive therapy.

Leukopenia/neutropenia

Neutropenia had the highest frequency reported after the initial two doses in the MM-398+5-FU/LV arm (any grade 13.7% and 16.3% respectively; ≥grade 3 neutropenia was 9.4% after the first and second dose). Neutropenia was generally present throughout MM-398 treatment with the highest any grade and ≥grade 3 prevalence after the second dose in the MM-398+5-FU/LV combination arm. In the MM-398+5-FU/LV arm the prevalence of any grade neutropenia ranged between 0-26.2 % while the prevalence of ≥grade 3 neutropenia was between 0-11.4%.

Neutropenic fever/sepsis: The incidence and prevalence frequencies by dose are similar for these acute events

which tended to occur early in the treatment. In the MM-398+5-FU/LV arm, 2 patients (1.9%) had an event after the second dose, 1 patient (1.3%) after the 3rd dose and 1 patient after the 9th dose. In the MM-398 monotherapy arm, 5 patients (3.4%) experienced and event after the first dose, 1 (0.8%) after the second dose and 1 patients (1.5%) after the 3rd dose. Almost all events of neutropenic fever/sepsis were grade 3 or higher severity.

Severe infections:

The incidence and prevalence of these acute events are similar regardless of the dose. All events were noted within the initial 5 doses of Onivyde administered in either Onivyde arms with one exception (1 patient was reported to have experienced septic shock after the 12th dose of Onivyde+5-FU/LV administration). The majority of the sepsis/bacteraemia events had a severity of grade 3 or higher.

Overall, severe neutropenia occurred in 20% of patients in the combination arm; severe neutropenic fever/sepsis in 3%; and fatal neutropenic sepsis in 0.8%. The cases of simultaneous occurrence of diarrhoea and neutropenia are especially complicated.

Patients with baseline serum bilirubin levels ≥1.0 mg/dL, or with deficient glucuronidation of bilirubin e.g. Gilbert's syndrome, may be at greater risk for leukopenia/neutropenia.

Patients with obstructing pancreatic head lesions and indwelling biliary stents experienced infectious complications such as ascending cholangitis and biliary sepsis that could be potentially life threatening in the setting of profound myelosuppression.

There is an increased risk of infections and haematological toxicity in patients with severe diarrhoea; the clinician should be aware of the risk and complete blood cell counts should be performed in these patients.

Acute infusion reactions:

Acute infusion reactions (consisting of rash, urticaria, periorbital oedema or pruritus) were reported in 8 of 117 patients (6.8%) in the Onivyde+5-FU/LV arm, 3 of 147 patients (2.0%) in the Onivyde monotherapy arm, and 8 of 134 patients (6.0%) in the 5-FU/LV arm. New events (all grade 1 or grade 2) occurred generally early during Onivyde treatment, with only 2 out of 10 patients noted with events after the fifth dose.

Thromboembolic events

In NAPOLI-1, thromboembolic events were reported in up to 6% of the patients in the combination arm and up to 14.3% of patients treated Onivyde monotherapy. In clinical studies with Onivyde, deep vein thrombosis, pulmonary embolism, and embolism were considered common AEs.

Deaths attributed to treatment including Onivyde

The PFS event distribution is shown below:

	Monotherapy comparison		Combination therapy comparison	
	MM-398	5FU/LV	MM-398+5FU/LV	5FU/LV
Progressed	89 (58.9)	89 (59.7)	65 (55.6)	69 (58.0)
Died before progression	38 (25.2)	31 (20.8)	18 (15.4)	23 (19.3)

A trend towards fewer deaths before disease progression can be observed for the Onivyde+5FU/LV combination therapy.

In NAPOLI-1, most "treatment-related deaths" were observed in the Onivyde monotherapy arm, 4/147 (GI toxicity, DIC, septic shock and infectious colitis); in the combination arm, there was one drug-related death/117, namely septic shock.

Laboratory findings

Haematology

In NAPOLI-1, any grade decreased haemoglobin and decreased platelets were observed across arms, predominantly in the monotherapy and the combination arm; one case of gr4 thrombocytopenia was observed in the MM-398 arm. Any grade neutropenia was more frequent in the combination arm (51.8%), followed by monotherapy (35.6%), with control at 6%. Gr3 neutropenia had the same disposition: 15.8 vs 8.9 vs 2.3%. Gr4 neutropenia was at 7.5% in the MM-398 group, 4.4% in the combination arm, with no cases registered in the control arm.

Chemistry

In NAPOLI-1, changes in liver function tests and electrolytes were observed across arms: increased alkaline phosphatase, ALT, AST and decreased albumin; decreased K, Mg and Na. Gr3 (11 patients) and 4 (1 patient) hypopotassaemia were observed in MM-398 monotherapy arm; in the combination arm there was one gr 4 hypoK. As for hypoNa, in the monotherapy arm 14 patients experienced gr3 events and one a gr4 event.

The supportive studies seem to exhibit a similar pattern of laboratory findings.

Safety in special populations

Age

TEAEs by age group in all patients receiving Onivyde are summarised in the table below.

Table 43: Summary of Treatment-Emergent Adverse Events by Age Group - All patients receiving any MM-398: NAPOLI-1,PEP0201,PEP0202,PEP0203,PEP0206,PEP0208,PIST-CRC,398-01-01-02

Event type category	<65 N=242 n (%)	65-74 N=127 n (%)	75-84 N=41 n (%)	85+ N=2 n (%)
Any AE	240 (99.2)	125 (98.4)	41 (100.0)	2 (100.0)
Any Serious AE	121 (50.0)	60 (47.2)	26 (63.4)	1 (50.0)
Fatal	14 (5.8)	10 (7.9)	6 (14.6)	0
Hospitalization	112 (46.3)	57 (44.9)	26 (63.4)	1 (50.0)
Life-threatening	8 (3.3)	4 (3.1)	3 (7.3)	0
Disability	1 (0.4)	2 (1.6)	1 (2.4)	0
Other Med Signif.	9 (3.7)	6 (4.7)	2 (4.9)	0
AE leading to dropout	23 (9.5)	25 (19.7)	5 (12.2)	0
Psychiatric disorders	46 (19.0)	19 (15.0)	5 (12.2)	0
Nervous system disorders	75 (31.0)	42 (33.1)	12 (29.3)	1 (50.0)
Accidents and injuries	7 (2.9)	3 (2.4)	3 (7.3)	0
Cardiac disorders	15 (6.2)	6 (4.7)	3 (7.3)	0
Vascular disorders	27 (11.2)	14 (11.0)	4 (9.8)	0
Cerebrovascular disorders	1 (0.4)	5 (3.9)	0	0
Infections and infestations	93 (38.4)	47 (37.0)	17 (41.5)	0
Anticholinergic syndrome	93 (38.4)	46 (36.2)	13 (31.7)	1 (50.0)

	<65 N=242	65-74 N=127	75-84 N=41	85+ N=2
Event type category	n (%)	n (%)	n (%)	n (%)
Sum of items[1]	37 (15.3)	21 (16.5)	7 (17.1)	1 (50.0)

Adverse events coded using MedDRA version 14.1.
[1]: Preferred terms: ORTHOSTATIC HYPOTENSION, FALL, LOSS OF CONSCIOUSNESS, DIZZINESS, SYNCOPE, AT AXIA, and any FRACTURE.
[2]: Preferred terms where (incidence in patients 65 and older)-(incidence in patients younger than 65) > 5.

Decrease in Quality of life by age group related to Onivyde treatment is reflected in the table below.

Table 44: Summary of Quality of Life Worsened by Age Group MM-398+5-FU/LV treated patients in NAPOLI-1, PRO Population

	<65 N=41	65-74 N=24	75-84 N=7	85+ N=0
Event type category	n (%)	n (%)	n (%)	n (%)
Global Health Status Worsened	16 (39.0)	14 (58.3)	2 (28.6)	0

Based on changes in EORCTC-QLQ-C30 Global Health Status during the treatment period.
Global Health Status Worsened: Patient did not meet improvement criteria, defined as achievment of a 10 percentag e point increase
from baseline with improvement from baseline lasting at least 6 weeks,and either died OR had scores that decreased by percentage
points at a post baseline time point.

Overall, no major clinical differences in safety or efficacy were reported between patients ≥65 years and patients <65 years, although a higher frequency of discontinuation (14.8% vs. 7.9%) was noted in the former group treated with Onivyde+5-FU/LV in the NAPOLI-1 study and in some cases the adverse reactions did not resolve. Grade 3 or higher and serious treatment emergent adverse reactions were more frequent in patients < 65 years (84.1% and 50.8%) compared to patients ≥ 65 years (68.5 % and 44.4%). Conversely, patients > 75 years (n=12) experienced more frequent serious adverse reactions, dose delay, dose reduction and discontinuation compared to patients ≤ 75 years (n=105) when treated with Onivyde+5-FU/LV in the pancreatic adenocarcinoma study (see section 4.8 of the SmPC).

Table 45: Treatment emergent adverse events leading to treatment discontinuation

Group	Pt ID	Adverse Event Leading to Discontinuation	Age	Drug-related	Onset Date(Day)/ Resolution Date	Grade	Recovered
MM-398+5-FU/LV	134-0549	Gangrene and Pelvic Fracture	75	No	2013-06-07(18)	2	No
	130-0195	RENAL AND URINARY DISORDERS/ Acute Prerenal Failure	72	No	2013-03-27(57)/ 2013-04-03(64)	2	Yes
	451-0303	Vomiting, Diarrhea, Leukopenia etc., considered as related to study	72	Yes	2013-04-25(16)	2	No
	617-0106	GASTROINTESTINAL DISORDERS/ Vomiting	67	Yes	2012-09-25(2)/ 2012-10-05(12)	3	Yes
	617-0185	Cerebrovascular Accident	70	Yes	2013-02-03(14)	3	No
	823-0197	Sepsis	66	Yes	2013-04-09(61)	3	Yes
	886-0285	Septic Shock/Neutropenia	80	Yes	2013-04-08(11)/ 2013-04-08(13)	5	No
5-FU/ LV	103-0008	ASTHENIA/weakness	75	No	2012-12-06(116	3	Yes
	131-0374	INVESTIGATIONS/NEUTROPHIL COUNT DECREASED	50	No	2013-05-03(15)/2013-05-12(35)	3	Yes
	151-0078	GASTROINTESTINAL DISORDERS	83	No	2012-11-16(72)/2012-11-30(87)	3	Yes

Hepatic Impairment

No dedicated hepatic impairment study has been conducted with Onivyde. In clinical studies of non-liposomal irinotecan administered on a weekly dosage schedule, patients with modestly elevated baseline serum total bilirubin levels (1.0 to 2.0 mg/dl) had a significantly greater likelihood of experiencing first cycle Grade 3 or Grade 4 neutropenia than those with bilirubin levels that were less than 1.0 mg/dl. No data are available for patients with total bilirubin > 2.0 mg/dl.

Renal Impairment

There are no safety data in patients with moderate to severe renal impairment.

Body Weight/BMI

Onivyde is dosed based on body surface area that is calculated using body weight. No further dose adjustment is recommended based on body weight or BMI. In NAPOLI-1 study, 5 of 8 underweight patients experienced a grade 3 or 4 adverse reaction, mostly myelosuppression, while 7 of the 8 patients required dose modification such as dose delay, dose reduction or dose discontinuation (see sections 4.4 and 4.8 of the SmPC).

Race

Compared to Caucasians, Asian patients were observed with a lower incidence of diarrhoea [14 (19.2%) out of 73 Caucasians had a ≥ Grade 3 diarrhoea, and 1 out of 33 (3.3%) Asians had a ≥ Grade 3 diarrhoea], but a higher incidence and higher severity of neutropenia. In patients receiving Onivyde+5-FU/LV, the incidence of ≥ Grade 3 neutropenia was higher among Asian patients [18 of 33 (55%)] compared to White patients [13 of 73 (18%)]. Neutropenic fever/neutropenic sepsis was reported in 6% of Asian patients compared to 1% of White patients (see section 4.8 of the SmPC).

Table 46: Observed incidence of grade ≥3 diarrhoea and neutropenia by race and by treatment in NAPOLI-1

Adverse Events	Treatment	Asians	Caucasians	Source
Diarrhea grade≥3, single MedDRA PT term	MM-398+5-FU/LV	3.0% (1/33)	19.2% (14/73)	NAPOLI-1 Clinical Study Report Table 14.3.2.8.2.3.1 and Table 14.3.2.8.2.3.2
	MM-398 alone	15.4% (8/52)	23.5% (20/85)	
Neutropenia grade≥3 (defined by product specific grouping for labeling)	MM-398+5-FU/LV	54.5% (18/33)	17.8% (13/73)	2.7.4. Summary Clinical Safety Section 2.7.4.2.1.8
	MM-398 alone	32.7% (17/52)	5.9% (5/85)	

Table 47: Predicted incidence of grade ≥3 diarrhoea and neutropenia by race based on population PK and exposure response analysis

Race	N	PK Param	Predicted Concentration with 80 mg/m^2 q2w			Predicted Incidence Rate of TEAEs Grade ≥ 3[a]		
			GLS mean	95%CI		Neu[b]	Neu[c]	Diarrhea
Caucasian	182	Total Irinotecan Cmax	29.76	29.29	30.24	NA	NA	14.7%
Asian	150		27.03	26.3	27.78	NA	NA	12.7%
Caucasian	182	SN-38 Converted Cmax	1.78	1.70	1.87	14.6%	25.8%	NA
Asian	150		2.76	2.62	2.90	21.4%	32.1%	NA

[a] Prediction of incidence rates of TEAEs were computed based on the estimates of univariate logistic regression obtained from exposure-safety relationship combined with the estimates of the predicted concentration of each subgroup from covariate analysis. The exposure used for neutropenia and anemia TEAEs was SN-38 Converted C_{max}, and the exposure for diarrhea TEAEs was CPT-11 C_{max}

[b] Predicted incidence of grade ≥ 3 neutropenia based on logistic regression model estimated from all dataset (combined MM-398 monotherapy and MM-398+5FU/LV arm)

[c] Predicted incidence of grade ≥ 3 neutropenia based on logistic regression model estimated from MM-398+5FU/LV arm of Study MM-398-07-03-01

Additional notable clinically relevant differences in the frequency of other AEs include a higher rate of nausea, vomiting, decreased appetite and alopecia were reported for Asians compared to Caucasians in Onivyde containing arms. Fatigue was reported with higher frequency in the Caucasians compared to Asians.

Patients with prior Whipple procedure

In the clinical study evaluating Onivyde+5 FU/LV, patients with a prior Whipple procedure had a higher risk of serious infections following treatment with Onivyde+5 FU/LV [9 of 29 (30%) compared] to 11 of 88 (12.5%) patients with no prior Whipple procedure (see section 4.8 of the SmPC).

Patients with UGT1A1 allele

Individuals who are 7/7 homozygous for the UGT1A1*28 allele are at increased risk for neutropenia from non-liposomal irinotecan. In the clinical study evaluating Onivyde+5-FU/LV, the frequency of ≥ Grade 3 neutropenia in these patients [2 of 7 (28.6%)] was similar to the frequency in patients not homozygous for the UGT1A1*28 allele who received a starting dose of Onivyde of 80 mg/m^2 [30 of 110 (27.3%)] (see sections 4.8 and 5.1 of the SmPC).

The Applicant provided data from NAPOLI-1 regarding the incidence /severity grade of leukopenia/neutropenia in UGT1A1*28 homozygous vs heterozygous patients during the first therapy cycle.

Table 48: Incidence and grade of neutropenia and diarrhoea AESI, NAPOLI-1 study, safety population, cycle 1, MM-398 + 5-FU/LV arm

Event	UGT1A1*28 6/6 (non-UGT1A1*28) N=63 n (%)		UGT1A1*28 6/7 or 6/8 (heterozygous UGT1A1*28) (N=47) n (%)		UGT1A1*28 7/7 (homozygous for UGTA1*28) (N=7) n (%)	
	Any grade	Grade 3 or higher	Any grade	Grade 3 or higher	Any grade	Grade 3 or higher
Neutropenia	11 (17.5)	9 (14.3)	4 (8.5)	1 (2.1)	1 (14.3)	1 (14.3)
Diarrhea	23 (36.5)	4 (6.3)	14 (29.8)	3 (6.4)	1 (14.3)	0

Refer to Table 3.15.14.7, Table 3.15.14.5, Table 3.15.14.8, Table 3.15.14.1, Table 3.15.14.3 (in attachment)

Table 49: Incidence and grade of neutropenia and diarrhoea AESI, NAPOLI-1 study, safety population, cycle 1, MM-398 monotherapy arm

Event	UGT1A1*28 6/6 (non-UGT1A1*28) N=84 n (%)		UGT1A1*28 6/7 or 6/8 (heterozygous UGT1A1*28) (N=47) n (%)		UGT1A1*28 7/7 (homozygous for UGTA1*28) (N=7) n (%)	
	Any grade	Grade 3 or higher	Any grade	Grade 3 or higher	Any grade	Grade 3 or higher
Neutropenia	15 (17.9)	10 (11.9)	10 (18.5)	4 (7.4)	1 (14.3)	0
Diarrhea	47 (56.0)	8 (9.5)	30 (55.6)	9 (16.7)	4 (57.1)	2 (28.6)

Refer to Table 3.15.14.7, Table 3.15.14.5, Table 3.15.14.8, Table 3.15.14.1, Table 3.15.14.3

The patients with UGT1A1*28 6/6 have been treated with higher doses than those with UGT1A1*28 7/7 (homozygous), leading to higher rates of neutropenia and diarrhoea in the former group.

Long-term safety data

Based on the limited number of patients who received MM-398 for more than 1 year (8 patients), and considering the across different characteristics (race, age, sex, dose exposure, TEAEs, grades, seriousness, dose modification), no specific patterns in frequency or types of AEs emerged in this long term safety population compared to the overall safety analysis population.

Safety related to drug-drug interactions and other interactions

No formal drug/drug interaction studies were performed. Interaction with other medicinal products has previously been described in the EU SmPC of irinotecan. In study MM-398-07-03-01(NAPOLI 1) Onivyde was administered in combination with 5-Fluorouracil and Leucovorin.

Compared to monotherapy administration, co-administration with 5-FU/LV in the Study MM-398-07-03-01 resulted in a reduced total irinotecan and SN-38 exposure.

Dose delays, dose reductions and discontinuations due to AEs

Table 50: Summary of dose reductions and treatment duration – NAPOLI-1

	MM-398 Mono (N=147)	5-FU/LV Mono Control (N=134)	MM-398+ 5-FU/LV Combo (N=117)	5-FU/LV Combo Control (N=105)
Subjects who had ≥1 dose reduction, n (%)	51 (34.7)	5 (3.7)	50 (42.7)	3 (2.9)
Subjects who had ≥2 dose reduction, n (%)	15 (10.2)	1 (0.7)	11 (9.4)	1 (1.0)
Subjects who had ≥3 dose reduction, n (%)	0	0	3 (2.6)	0
Treatment Duration ≥6 weeks, n (%)	118 (80.3)	100 (74.6)	84 (71.8)	76 (72.4)
Treatment Duration ≥12 weeks, n (%)	58 (39.5)	39 (29.1)	48 (41.0)	30 (28.6)
Treatment Duration ≥18 weeks, n (%)	33 (22.4)	21 (15.7)	41 (35.0)	16 (15.2)

The patients in MM-398+5FU/LV combination arm had more 1-dose and 3-dose reductions than the monotherapy arm, but also a treatment duration over 18 weeks in 35% of the patients (compared with 22.4 % in the monotherapy arm).

Dose delays

MM-398 monotherapy: 49 patients (33.3%) experienced TEAEs that required dose delay, mostly due to GI disorders (12.2%), followed by blood disorders (6.8%). The dose delay ranged from 14-33% during the first 10 doses administered, with delay of 7 days.

MM-398+5-FU/LV: dose delays were required in almost two thirds of the patients (61.5%). Neutropenia (14.5 %), WBC (12.0%), neutrophil count decreased (9.4%), diarrhoea (7.7%), fatigue (6.8%), vomiting (6.0%), and platelet decrease (5.1%) were the most common events requiring a dose delay; all other events were reported in less than 5% of patients. In the MM-398+5-FU/LV arm, 27% of patients required a dose delay in the beginning of the treatment with a subsequent gradual decrease in the frequency of patients who needed dose delay (range 8-17% during the first 10 doses administered). The median dose delay for most doses administered was 14 days, i.e. the investigators most often delayed treatment until the next scheduled treatment date.

5-FU/LV: 43 patients (32.1%) required dose delay due to TEAEs; mostly due to GI disorders (10.4%), and 'General Disorders and Administration Site Conditions (8.2%).

Dose reductions

MM-398 monotherapy: 46 patients (31.3%) experienced TEAEs that required dose reductions (34.7% subjects with more than one dose reduction). Diarrhoea was reported in 17 patients (11.6%) and vomiting in 9 patients (6.1%); all other events in this treatment group that required dose reductions were reported in less than 5% of patients.

MM-398+5-FU/LV: 39 patients (33.3%) experienced TEAEs that required dose reductions (42.7% subjects with more than one dose reduction in table 8.3). Neutropenia (10 patients, 8.5%), neutrophil count decreased (8 patients, 6.8%), diarrhoea (7 patients, 6.0%), and white blood cell count decreased (6 patients, 5.1%) were the most common events requiring a dose reduction; all other events were reported in less than 5% of patients. Dose reductions occurred in the first 6 cycles in the combination arm.

5-FU/LV: 5 patients (3.7%) required dose reductions due to TEAEs.

Discontinuations

Overall, 17 patients (11.6%) treated with MM-398 monotherapy experienced TEAEs leading to dose discontinuation, while with MM-398+5-FU/LV combination therapy, 13 patients (11.1%) required dose discontinuation; 7.5% of patients treated with 5-FU/LV required dose discontinuation.

Table 51: Adverse event discontinuations

Treatment Group	n (% of ITT)	Time to discontinuation (weeks)		
		Mean	Median	Min - Max
MM-398+5-FU/LV	11 (9.4)	5.2	5.1	1.7 – 9.1
5-FU/LV (combination control)	7 (5.9)	7.1	6.3	3.3 – 15.9
MM-398 monotherapy	17 (11.3)	9.5	5.0	1.7 – 29.7
5-FU/LV (monotherapy control)	10 (6.7)	8.3	6.5	3.0 – 17.0

Table 52: Clinical deterioration discontinuations

Treatment Group	n (% of ITT)	Time to discontinuation (weeks)		
		Mean	Median	Min - Max
MM-398+5-FU/LV	13 (11.1)	11.3	10.1	1.3 – 32.4
5-FU/LV (combination control)	12 (10.1)	9.5	8.5	1.3 – 25.7
MM-398 monotherapy	21 (13.9)	11.9	12.1	1.0 – 39.6
5-FU/LV (monotherapy control)	17 (11.4)	10.9	8.9	1.3 – 39.0

Table 53: Investigator decision

Treatment Group	n (% of ITT)	Time to discontinuation (weeks)		
		Mean	Median	Min - Max
MM-398+5-FU/LV	4 (3.4)	14.8	12.9	9.9 – 23.3
5-FU/LV (combination control)	4 (3.4)	3.4	3.2	1.1 – 6.1
MM-398 monotherapy	7 (4.6)	8.7	6.3	0.3 – 21.3
5-FU/LV (monotherapy control)	5 (3.4)	3.6	3.4	1.1 – 6.1

Table S4: Subject decision

Treatment Group	n (% of ITT)	Time to discontinuation (weeks)		
		Mean	Median	Min - Max
MM-398+5-FU/LV	14 (12.0)	6.7	5.4	0.6 – 14.1
5-FU/LV (combination control)	19 (16.0)	6.4	0.3	0.1 – 66.1
MM-398 monotherapy	17 (11.3)	9.1	7.0	2.1 – 20.3
5-FU/LV (monotherapy control)	20 (13.4)	6.2	0.5	0.1 – 66.1

Table S5: All reasons not related to PD (i.e. Discontinuations for progressive disease or death excluded)

Treatment Group	n (% of ITT)	Time to discontinuation (weeks)		
		Mean	Median	Min - Max
MM-398+5-FU/LV	44 (37.6)	8.6	7.8	0.6 – 32.4
5-FU/LV (combination control)	44 (37.0)	7.0	3.5	0.1 – 66.1
MM-398 monotherapy	62 (41.1)	10.1	6.8	0.3 – 39.6
5-FU/LV (monotherapy control)	55 (36.9)	7.7	4.0	0.1 – 66.1

2.6.1. Discussion on clinical safety

The safety database consists of 440 patients from 9 clinical studies. As the safety profile of the active substance is known, the size of the safety database is considered sufficient to assess the risks associated with MM-398/5FU/LV combination in the proposed indication. The pivotal study NAPOLI-1 was used to derive the Safety Analysis Population including 264 patients.

The most common AEs in the Onivyde-containing arms are similar to the known safety profile of standard irinotecan in cancer patients.

Diarrhoea is a very common adverse reaction leading to colitis, ileus, gastroenteritis, fatigue, dehydration, weight loss, renal toxicities, hyponatraemia, and hypokalaemia. Renal impairment and acute renal failure have been identified, usually in patients who became volume depleted from severe vomiting and/or diarrhoea. In the pivotal clinical study, Grade 3 or 4 diarrhoea occurred in 15 out of 117 patients (12.8%) receiving Onivyde +5-FU/LV. For patients experiencing late diarrhoea (> 24 hours), the median time to late diarrhoea onset was 8 days from the previous dose of Onivyde. Early onset diarrhoea, typically appearing ≤24 hours after dose administration, can occur and is usually transient. Early onset diarrhoea may also be accompanied by cholinergic symptoms that can include rhinitis, increased salivation, flushing, diaphoresis, bradycardia, miosis and hyperperistalsis that can induce abdominal cramping. In the pivotal clinical study, early diarrhoea onset occurred in 35 patients (29.9%) and cholinergic events occurred in 4 patients (3.4%) receiving Onivyde +5-FU/LV. In patients experiencing early diarrhoea, therapeutic and prophylactic atropine should be considered unless contraindicated. Patients should be made aware of the risk of delayed diarrhoea which can be debilitating and, on rare occasions, life threatening since persistent loose or watery stools can result in dehydration, electrolyte imbalance, colitis, gastrointestinal (GI) ulceration, infection or sepsis. As soon as the first liquid stool

occurs, the patient should start drinking large volumes of beverages containing electrolytes. Patients should have loperamide (or equivalent) readily available to begin treatment for late diarrhoea. Loperamide should be initiated at first occurrence of poorly formed or loose stools or at the earliest onset of bowel movements more frequent than normal. Loperamide should be given until patient is without diarrhoea for at least 12 hours. If diarrhoea persists while patient is on loperamide for more than 24 hours, adding oral antibiotic support (e.g. fluoroquinolone for 7 days) should be considered. Loperamide should not be used for more than 48 consecutive hours due to risk of paralytic ileus. If diarrhoea persists for more than 48 hours, stop loperamide, monitor and replace fluid electrolytes and continue antibiotic support until resolution for accompanying symptoms. Onivyde treatment should be delayed until diarrhoea resolves to ≤ Grade 1 (2 3 stools/day more than pre-treatment frequency). Onivyde must not be administered to patients with bowel obstruction and chronic inflammatory bowel disease, until it is resolved. Following Grade 3 or 4 diarrhoea, the subsequent dose of Onivyde should be reduced (see sections 4.2, 4.4 and 4.8 of the SmPC).

Myelosuppression, especially neutropenia, was more frequent and severe in the Onivyde-containing arms compared to the control arm, and were most frequent in the combination arm. Dose delay, dose reduction, and colony stimulating factors were used to manage myelosuppression. Complete blood cell count monitoring is recommended during Onivyde treatment. Patients should be aware of the risk of neutropenia and the significance of fever. The median time to nadir for ≥ Grade 3 neutropenia is 23 (range 8 - 104) days post first dose of treatment with Onivyde. Febrile neutropenia (body temperature > 38°C and neutrophil count ≤1,000 cells/mm^3) should be urgently treated in the hospital with broad spectrum intravenous antibiotics. Onivyde should be withheld if neutropenic fever occurs or the absolute neutrophil count drops below 1500/mm3. Sepsis with neutropenic fever and consequent septic shock with fatal outcome has been observed in patients with metastatic pancreatic adenocarcinoma treated with Onivyde. In patients who experienced severe haematological events, a dose reduction or treatment discontinuation is recommended (see sections 4.2 and 4.4). Patients with severe bone marrow failure should not be treated with Onivyde.

History of prior abdominal radiation increases the risk of severe neutropenia and febrile neutropenia following Onivyde treatment. Close monitoring of blood counts is recommended, and the use of myeloid growth factors should be considered for patients with a history of abdominal radiation. Caution should be exercised in patients receiving concurrent administration of Onivyde with irradiation.

Patients with deficient glucuronidation of bilirubin, such as those with Gilbert's syndrome, may be at greater risk of myelosuppression when receiving therapy with Onivyde.

Thrombocytopenia was infrequent, as has been documented with non-liposomal irinotecan.

Administration of live or live-attenuated vaccines in patients immunocompromised by chemotherapeutic medicinal products including Onivyde may result in serious or fatal infections; therefore vaccination with a live vaccine should be avoided. Killed or inactivated vaccines may be administered; however, the response to such vaccines may be diminished (see section 4.4 of the SmPC).

In patients treated with Onyvide, higher total irinotecan C_{max} is associated with higher probability of diarrhoea (gastrointestinal toxicity), and higher SN-38 C_{max} is associated with higher probability of developing neutropenia (bone marrow suppression).

As reported in the PK results, race was a strong covariate in the Onivyde treatment. In NAPOLI-1, Asians showed higher frequency of Grade ≥3 drug related TEAEs in the Onivyde combination arm compared to Caucasians **(72.7% vs 45.2%).** This is mainly due to an increased frequency of Grade 3 or higher neutropenia and febrile neutropenia in Asians compared to Caucasians. Diarrhoea was less frequent and less severe in Asians compared

to Caucasians in the MM-398 combination arm. This observation could be related to the high prevalence of UGT1A1*6 variant in Asian patients, and unfortunately this was not tested in the study.

The Applicant provided data from NAPOLI-1 regarding the incidence /severity grade of leukopenia/neutropenia in UGT1A1*28 homozygous vs heterozygous patients during the first therapy cycle.

A reduced starting dose of Onivyde (liposomal irinotecan) of 60 mg/ m2 should be considered for patients known to be homozygous for the UGT1A1*28 allele. Patients without drug related toxicities during the first cycle of therapy may have the dose of Onivyde increased to a total dose of 80 mg/m2 in subsequent cycles based on individual patient tolerance (see sections 4.2, 4.8 and 5.1 of the SmPC). In these patients, a new cycle of therapy should not begin until adverse event resolves to ≤ Grade 1. At a first occurrence of a Grade 3 or 4 adverse reaction (ADR), the Onivyde dose should be reduced to 50 mg/m^2. At a second occurrence of a Grade 3 or 4 ADR, the Onivyde dose should be reduced to 40 mg/m^2. At a third occure, treatment should be discontinued.

Overall, the safety profiles of Onivyde monotherapy and Onivyde+5-FU/LV combination therapy were consistent with the safety profile of standard irinotecan and 5-FU.

In comparison with the reference product, certain known AEs of irinotecan have so far not been observed with Onivyde: anaphylaxis and anaphylactoid reactions; interstitial lung disease; acute pancreatitis, either because liposomal irinotecan does not exhibit these AEs or due to the limited safety database available.

The Onyvide+5-FU/LV combination was generally better tolerated than the Onyvide monotherapy (mostly due to less frequent and less severe GI adverse reactions), with the exception of higher incidence of neutropenia.

In the Onyvide+5-FU/LV arm, 27% of patients required a dose delay in the beginning of the treatment with a subsequent gradual decrease in the frequency of patients who needed dose delay (range 8-17% during the first 10 doses administered). The median dose delay for most doses administered was 14 days, i.e. the investigators most often delayed treatment until the next scheduled treatment date. For the Onyvide monotherapy arm the dose delay ranged from 14-33% during the first 10 doses administered, with delay of 7 days. Dose reductions occurred in the first 6 cycles in the combination arm. Regarding discontinuations, with the exception of a shorter mean time to discontinuation due to AEs in the combination arm, swift investigator decision in the control arm and subject's decision not to participate in the control arm (all expected), the results were overall balanced.

Hypersensitivity reactions, including acute infusion reaction may occur and Onivyde should be discontinued in case of severe hypersensitivity reactions.

In clinical studies of non-liposomal irinotecan administered on a weekly dosage schedule, patients with modestly elevated baseline serum total bilirubin levels (1.0 to 2.0 mg/dl) had a significantly greater likelihood of experiencing first cycle Grade 3 or Grade 4 neutropenia than those with bilirubin levels that were less than 1.0 mg/dl. Regular monitoring of complete blood counts should be conducted in patients with total bilirubin of 1.0 2.0 mg/dl due to possible increase of the concentration of SN 38 and thus increased risk of neutropenia in this population. The use of Onivyde should be avoided in patients with bilirubin > 2.0 mg/dl, or aspartate aminotransferase (AST) and alanine aminotransferase (ALT) > 2.5 times upper limit of normal (ULN) or > 5 times ULN if liver metastasis is present. In addition, caution is required when Onivyde is given in combination with other hepatotoxic medicinal products, especially in patients with pre-existing hepatic impairment (see sections 4.2, 4.4 and 5.2 of the SmPC).

Patients with a history of a Whipple procedure have a higher risk of serious infections following Onivyde treatment. Patients should be monitored for signs of infections (see sections 4.4 and 4.8 of the SmPC).

Interstitial Lung Disease (ILD)-like events leading to fatalities have occurred in patients receiving non-liposomal irinotecan. No cases of ILD-like events have been reported with Onivyde therapy in clinical studies. Risk factors include pre-existing lung disease, use of pneumotoxic medicinal products, colony stimulating factors or having previously received radiation therapy. Patients with risk factors should be closely monitored for respiratory symptoms before and during Onivyde therapy. A reticulo-nodular pattern on chest X-ray was observed in a small percentage of patients enrolled in a clinical study with irinotecan. New or progressive dyspnoea, cough, and fever should prompt interruption of Onivyde treatment, pending diagnostic evaluation. Onivyde should be discontinued in patients with a confirmed diagnosis of ILD.

Because of the increased risk of ADRs (including Grade 3/4), caution should be exercised when using Onivyde in patients with body mass index <18.5 kg/m^2.

Forty one percent (41%) of patients treated with Onivyde across the clinical program were ≥ 65 years. No dose adjustment is recommended.

It is recommended that patients receive premedication with standard doses of dexamethasone (or an equivalent corticosteroid) together with a 5-HT3 antagonist (or other antiemetic) at least 30 minutes prior to Onivyde infusion (see section 4.2 of the SmPC).

Women of childbearing potential should use effective contraception during Onivyde treatment and 1 month thereafter. Males should use condoms during Onivyde treatment and 4 months thereafter.

There are no adequate data on the use of Onivyde in pregnant women. Onivyde can cause harm to the foetus when administered to the pregnant woman, as the main ingredient irinotecan has been shown to be embryotoxic and teratogenic in animals. Therefore, based on results from animal studies and the mechanism of action of irinotecan, Onivyde should not be used during pregnancy unless clearly necessary. If Onivyde is used during pregnancy or if the patient becomes pregnant while receiving therapy, the patient should be informed about the potential hazard to the foetus.

It is unknown whether Onivyde or its metabolites are excreted into human milk. Because of the potential for serious adverse reactions of Onivyde in breast-feeding infants, Onivyde is contraindicated during breast-feeding (see sections 4.3 and 4.6 of the SmPC). Patients should not breast-feed until one month after the last dose.

There are no data on the impact of Onivyde on human fertility. Non-liposomal irinotecan was shown to cause atrophy of male and female reproductive organs after multiple daily irinotecan doses in animals (see sections 4.6 and 5.3 of the SmPC).

In clinical trials, Onivyde was administered at doses up to 240 mg/m^2 to patients with various cancers. The adverse reactions in these patients were similar to those reported with the recommended dosage and regimen. There have been reports of overdose with non-liposomal irinotecan at doses up to approximately twice the recommended therapeutic dose of irinotecan, which may be fatal. The most significant adverse reactions reported were severe neutropenia and severe diarrhoea. There is no known antidote for overdose of Onivyde. Maximum supportive care should be instituted to prevent dehydration due to diarrhoea and to treat any infectious complications (see section 4.9 of the SmPC).

Onivyde has moderate influence on the ability to drive and use machines. During treatment patients should observe caution when driving or using machines (see section 4.7 of the SmPC).

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics

2.6.2. Conclusions on the clinical safety

Irinotecan hydrochloride trihydrate, contained in Onivyde, is a known active substance, whose safety profile is well established. In NAPOLI-1, most TEAEs of Onivyde in combination with 5FU/LV were manageable with supportive therapy, dose delays or both. No unexpected safety findings have so far emerged from the liposomal irinotecan development program to challenge what is previously known from standard irinotecan.

2.7. Risk Management Plan

The CHMP received the following PRAC Advice on the submitted Risk Management Plan (RMP):

The PRAC considered that the RMP version 1.0 (dated 21 April 2015) could be acceptable if the Applicant implements the changes to the RMP as described in the PRAC endorsed PRAC Rapporteur assessment report dated 10 September 2015.

The CHMP endorsed this advice.

The Applicant implemented all changes to the RMP as requested by the PRAC and the CHMP.

The CHMP endorsed the RMP version 1.0 (dated 27 April 2016) with the following content:

Table 56 - Summary of Safety Concerns

Important identified risks	Diarrhoea Leukopenia/Neutropenia Anaemia Acute infusion reactions Thromboembolic events
Important potential risks	Embryotoxicity/teratogenicity Hypersensitivity reactions Medication error related to drug/dose confusion with non-liposomal irinotecan Interstitial lung disease
Missing information	Use in patients with hepatic impairment Use in patients with renal impairment

Pharmacovigilance plan

Not applicable

Table 57 – Summary Table of Risk Minimisation Measures

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Important identified risks		
Diarrhoea	Wording in SmPC section 4.2, 4.4 and 4.8.	None
Leukopenia/Neutropenia	Wording in SmPC section 4.2, 4.3, 4.4, 4.8.	None
Anaemia	Wording in SmPC section 4.8.	None
Acute infusion reactions	Wording in SmPC section 4.4, 4.8.	None
Thromboembolic events	Wording in SmPC section 4.8.	None
Important potential risks		
Embryotoxicity/teratogenicity	Wording in SmPC section 4.6, 5.3.	None
Hypersensitivity reactions	Wording in SmPC section 4.3, 4.4, 4.8.	None
Medication error related to drug/dose confusion with non-liposomal irinotecan	Wording in SmPC section 4.2.	None
Interstitial lung disease	Wording in SmPC section 4.4.	None
Missing information		
Use in patients with hepatic impairment	Wording in SmPC section 4.2, 4.4, 5.2.	None
Use in patients with renal impairment	Wording in SmPC section 4.2, 4.4, 5.2.	None

Conclusion

The CHMP and PRAC considered that the risk management plan version 1.0 (dated 27 April 2016) is acceptable.

The MAH is reminded that, within 30 calendar days of the receipt of the Opinion, an updated version of Annex I

of the RMP template, reflecting the final RMP agreed at the time of the Opinion should be submitted to h-eurmp-eviinterface@emea.europa.eu.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.9. New Active Substance

The CHMP, based on the available data, considers that irinotecan hydrochloride trihydrate is not a new active substance, as it is a constituent of a medicinal product previously authorised within the European Union.

2.10. Product information

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use*.

2.10.2. Additional expert consultation

During the evaluation procedure for Onivyde, a Healthcare Professional consultation was launched to check whether there could be a potential risk of medication errors between the liposomal and non-liposomal formulation of irinotecan on the basis of the proposed product information and what measures could be implemented in the packaging or SmPC to minimise such risk.

The comments received in this consultation prompted the PRAC and CHMP to request further changes to the labelling (mainly to clearly differentiate both formulations and indicating that they are not equivalent). The MAH adequately addressed these concerns by amending the labelling and SmPC.

3. Benefit-Risk Balance

3.1. Favourable effects

In the pivotal trial, conducted in a heterogeneous population with respect to prior therapy, the primary endpoint of OS, resulted in a median 1.9 month survival benefit in favour of the experimental arm (6.1 vs 4.2 months, HR 0.67, p=0.0122, cut-off 0.025). As a sensitivity analysis, a stratified analysis by baseline stratification factors is reported and shows more convincing results with an HR of 0.57 (p-value = 0.0009). As this stratified analysis would be the CHMP preferred analysis, the concerns related to the statistically borderline character of the primary analysis are alleviated.

An improved survival of median 2 months, or a 50% prolongation of median survival, is considered clinically and regulatory meaningful in patients with relapsed/refractory pancreatic cancer.

The combination of Onivyde+5-FU/LV achieved a median investigator-assessed PFS of 3.1 vs 1.5 months for the control arm, HR 0.56, p=0.0001. Delayed tumour progression in pancreatic cancer is expected to delay symptom progression in this condition characterised by severe symptoms: mainly pain, weight loss and fatigue.

The confirmed ORR for Onivyde+5-FU/LV was 7.7% (95% CI: 2.86, 12.52) compared to 0.8% for 5-FU/LV.

3.2. Uncertainties and limitations about favourable effects

The 5FU/LV regimens in the control arm and the experimental arm were dissimilar enough to warrant further discussion. It would have facilitated the assessment of the add-on benefit of Onivyde if the same 5FU/LV regimen had been used as background and control, but the differences are considered too small to be of relevance in terms of survival.

With reference to the CONKO-003 trial, the control 5FU/LV regimen in NAPOLI-1 cannot be viewed as too non-intensive.

A trend towards improved PFS (HR 0.8, p=0.1) was shown in the comparison of Onivyde vs. 5FU/LV, at very similar OS (HR 0.99). In this context it is acknowledged that it is unknown whether 5FU/LV provides a survival benefit in this population.

In the monotherapy arm, Onivyde was administered at 120 mg/m^2 every 3 weeks and in the combination arm 80 mg/m^2 every 2 weeks, i.e. the same dose intensity over a 6-week period. The importance of this difference from a benefit/risk perspective is unknown. Therefore the add-on benefit of 5FU/LV to Onivyde cannot be disentangled, however this is not a regulatory concern.

Due to a too high early attrition rate, informative HRQoL data are not available.

Among the pre-planned OS subgroup analyses, a treatment effect favouring the 5-FU/LV over the combination arm has been observed for prior irinotecan use. This observation is also confirmed by univariate and multivariate analyses conducted to identify possible prognostic factors for both OS and PFS, which consistently showed that prior irinotecan, together with age>65, negatively impacted on the prognosis of patients treated with the combination arm.

The lack of benefit (if not a detrimental effect) in patients pre-treated with irinotecan raises concerns due to the increasing use of irinotecan-containing regimen as first line therapy. Due to the limited number of patients with prior exposure to non-liposomal irinotecan, the benefit of Onivyde has not been established in this population. This information has been reflected in section 4.4 of the SmPC.

3.3. Unfavourable effects

The TEAE causality seems in line with the known safety profile of irinotecan, i.e. gastrointestinal AEs and hematotoxicity. Whilst the dose intensity per 6 weeks was the same (120 mg/m^2 x 2 vs 80 mg/m^2 x 3), the 80 mg/m^2 regimen in combination with 5FU/LV resulted in more myelosuppression, but gastrointestinal adverse reactions were more commonly observed in the 120 mg/m^2 arm. 'Fatigue' and 'asthenia' were also more frequently observed in the combination arm.

In comparison with the 5-FU/LV control arm almost all adverse reactions were more commonly observed in the combination arm. These differences resulted in more dose reductions (33% vs. 4%) and discontinuations (11% vs. 8%).

Early diarrhoea (onset day1, consistent with cholinergic hyper-stimulation) occurred twice as frequently in the combination arm (29.9%) than in the other two arms. Late events (after day1) were more frequent in the combination arm (42.7%) than in the control arm. However, diarrhoea was manageable with supportive therapy.

Patients with baseline serum bilirubin levels ≥1.0 mg/dL, or with deficient glucuronidation of bilirubin e.g. Gilbert's syndrome, may be at greater risk for neutropenia. Patients who are homozygous for UGT1A1*28 have a greater risk of haematological toxicity with irinotecan.

In patients treated with Onyvide, diarrhoea (gastrointestinal toxicity) is associated with total irinotecan C_{max}, and neutropenia (bone marrow suppression) is associated with unencapsulated SN-38 C_{max}. The observed incidence of grade≥3 diarrhoea and neutropenia by race are also consistent with the difference in the pharmacokinetics.

There is an increased risk of infections and haematological toxicity in patients with severe diarrhoea. Close clinical monitoring is advised.

3.4. Uncertainties and limitations about unfavourable effects

In comparison with standard irinotecan, certain known AEs of irinotecan have so far not been observed with Onivyde, such as interstitial lung disease and acute pancreatitis; either because liposomal irinotecan does not exhibit these AEs or due to the limited safety database available. These events will be monitored through routine pharmacovigilance.

3.5. Effects Table

Table 58: Effects Table for Onivyde in adenocarcinoma of the pancreas (data cut-off: 14 February 2014)

Effect	Short description	Unit	Treatment	Control	Uncertainties / Strength of evidence
Favourable Effects					
OS	Overall survival (median)	months	6.1	4.2	Modest increase in OS

Effect	Short description	Unit	Treatment	Control	Uncertainties / Strength of evidence
PFS	Progression free survival (median)	months	3.1	1.5	Supportive of OS (Investigator-assessed)
TTF	Time to treatment failure (median)	months	2.3	1.4	Supportive of OS
ORR	Overall response rate (confirmed RECIST 1.1)	%	7.69	0.84	Supportive of OS, PFS
DOR	Duration of response	weeks	10	6	In order to contextualise: OS benefit, 1.9 months Rates of discontinuation: 11% more PD in the control arm Open-label
Unfavourable Effects					
Total AE, excl. PD	Discontinuation Dose reduction	%	9.4 33.3	6.7 3.7	
Related AE	According to investigator	%	91.5	69.4	
SAE	GI disorders Infections Blood	%	22.2 17.1 6.0	15.7 11.2 2.2	
Diarrhoea	Grade all Grade 3/4	%	59 14.5	26.1 4.5	In order to contextualise: More, yet manageable, AEs in the Onivyde combination arm, and consistent with the safety profile of standard irinotecan/FOLFIRI.
Neutropenia	Grade all Grade 3/4	%	39.3 27.4	5.2 1.5	

Abbreviations: AE: Adverse event; GI: Gastro-intestinal; PD: progressive disease

3.6. Benefit-risk assessment and discussion

3.6.1. Importance of favourable and unfavourable effects

The newer approaches to the first-line treatment of metastatic pancreatic adenocarcinoma – e.g. 5-FU/LV with irinotecan and oxaliplatin (FOLFIRINOX) or gemcitabine/nab-paclitaxel have improved the outcomes in this patient group, with response rates between 23% and 31%, PFS of 5.5–6.6 months, and OS between 8.5 and 11 months. Due to good tolerability, however, gemcitabine monotherapy remains a viable treatment option.

In the next-line setting the prognosis is very poor and tolerability becomes an even more important issue. No patient reported outcome data are available to inform the assessment with regard to the perceived side effects of therapy. Therefore conventional adverse event reporting has to be used in the assessment of tolerability.

With respect to efficacy, survival is the best overall measure of treatment benefit, but delayed progression is likely to be related to delayed symptomatic progression.

3.6.2. Balance of benefits and risks

In view of the survival benefit of Onivyde in combination with 5-FU/LV and the identified risks of irinotecan, the benefit/risk balance of Onivyde in patients with adenocarcinoma of the pancreas previously treated with a gemcitabine based therapy is considered favourable.

3.7. Conclusions

The overall B/R of Onivyde is positive.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of Onivyde is favourable in the following indication:

Treatment of metastatic adenocarcinoma of the pancreas, in combination with 5 fluorouracil (5 FU) and leucovorin (LV), in adult patients who have progressed following gemcitabine based therapy.

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Other conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription

Conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable

New Active Substance Status

The CHMP, based on the available data, considers that irinotecan hydrochloride trihydrate is not a new active substance, as it is a constituent of a medicinal product previously authorised within the European Union.