Oncol Ther (2023) 11:111–128
https://doi.org/10.1007/s40487-022-00215-2

ORIGINAL RESEARCH

Liposomal Irinotecan Shows a Larger Therapeutic Index than Non-liposomal Irinotecan in Patient-Derived Xenograft Models of Pancreatic Cancer

Sandrine Barbier · Benjamin Beaufils · Ricardo de Miguel · Melissa Reyre · Yannick Le Meitour · Andreanne Lortie · Marc Hillairet de Boisferon · Sophie Chaumeron · Anne Espirito · Lina Fossati · Pauline Lagarde · Stephan Klinz · Arunthathi Thiagalingam · Stéphane Lezmi · Florence Meyer-Losic

Received: September 16, 2022 / Accepted: November 23, 2022 / Published online: January 16, 2023

ABSTRACT

Introduction: Liposomal irinotecan promotes controlled sustained release of irinotecan (CPT-11), therefore, we hypothesize that the therapeutic index (quantitative measurement of the relative efficacy/safety ratio of a drug) will be higher for liposomal than non-liposomal irinotecan.

Methods: We compared the therapeutic indexes of liposomal and non-liposomal irinotecan in mice bearing subcutaneous patient-derived xenograft (PDX) pancreatic tumors under dosing regimens approximating the clinical setting. Following preliminary drug sensitivity/antitumor activity analyses on three PDX tumor models, one model was selected for analyses of efficacy, biomarker, toxicology, pharmacokinetics in mice receiving liposomal irinotecan (2.5, 10, 50 mg/kg/week) or non-liposomal irinotecan (10, 25, 50 mg/kg/week). The maximum tolerated dose (MTD) for each treatment was 50 mg/kg/week.

Results: Using the selected IM-PAN-001 model at the MTD (both treatments, 50 mg/kg/week), antitumor activity, phospho-histone gamma-H2AX protein staining in cancer cell nuclei, histological tumor regression, and plasma levels of CPT-11 and its active metabolite SN-38 after 24 h were greater with liposomal than non-liposomal irinotecan, but tumor SN-38 levels were similar. At the lowest doses assessed, antitumor activity, histological tumor regression, and jejunum and bone marrow toxicity were similar. Based on these findings, liposomal and non-liposomal irinotecan had therapeutic indexes of 20 and 5, respectively.

Conclusion: This non-clinical study showed a fourfold broader therapeutic index with liposomal than non-liposomal irinotecan in mice bearing IM-PAN-001 PDX pancreatic tumors, even at optimal dosing for the two drugs. These findings support the clinical benefit observed with liposomal irinotecan in patients with pancreatic cancer.

Ricardo de Miguel, Arunthathi Thiagalingam and Stéphane Lezmi: At the time the study was performed.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40487-022-00215-2.

S. Barbier (✉) · B. Beaufils · R. de Miguel · M. Reyre · Y. Le Meitour · A. Lortie · L. Fossati · P. Lagarde · S. Lezmi · F. Meyer-Losic
Ipsen Innovation, 5 Avenue du Canada, Z.I. Courtaboeuf, 91940 Les Ulis, France
e-mail: sandrine.barbier@ipsen.com

M. H. de Boisferon · S. Chaumeron · A. Espirito
Oncodesign, Dijon, France

S. Klinz · A. Thiagalingam
Ipsen Bioscience, Cambridge, MA, USA

Keywords: Liposomal irinotecan; Pancreatic cancer; Patient-derived xenograft; Therapeutic index; Tumor model

Key Summary Points

Why carry out this study?

Irinotecan is an established component of combination chemotherapies used to treat cancers such as metastatic pancreatic ductal adenocarcinoma, and like many other anticancer compounds, toxicity associated with irinotecan is dose-limiting.

Encapsulation of irinotecan in a lipid bilayer vesicle protects the drug from metabolic conversion and clearance, thereby increasing the half-life of circulating liposomal irinotecan 40-fold relative to non-liposomal irinotecan.

We hypothesize that the therapeutic index will be higher for liposomal than non-liposomal irinotecan.

What was learned from the study?

Our findings demonstrate that liposomal irinotecan has a fourfold broader therapeutic index than non-liposomal irinotecan (20 vs. 5), even at optimal treatment schedules for antitumor efficacy evaluation, in this study.

These findings support the clinical benefit observed with liposomal irinotecan in patients with pancreatic cancer.

INTRODUCTION

Pancreatic adenocarcinoma remains an exception to the general trend of improvement in cancer-related mortality [1]. Irinotecan (CPT-11) is a chemotherapeutic agent used to treat pancreatic cancer. Both irinotecan and its active metabolite SN-38 (converted by non-specific carboxylesterase [CES]) [2], bind reversibly to the topoisomerase IDNA complex during S phase and prevent re-ligation of DNA single-strand breaks, leading to exposure-time-dependent double-stranded DNA damage and cell death [3, 4].

Irinotecan is an established component of various combination chemotherapies [5], including the FOLFIRINOX regimen (5-fluorouracil [5-FU], leucovorin, oxaliplatin, irinotecan) used to treat patients with metastatic pancreatic ductal adenocarcinoma (mPDAC) [6, 7]. Modified FOLFIRINOX is now recommended as the first adjuvant therapy option for select patients following resection of pancreatic cancer [7, 8], based on findings from the PRODIGE-24 trial [9]. However, this regimen is not an approved treatment for patients with mPDAC. The toxicity of irinotecan is dose-limiting [10]. Irinotecan has a complex metabolic pathway; it is rapidly cleared, leading to a short elimination half-life, and there is large inter-individual pharmacokinetic variability [10]. Preclinical animal studies suggest limited residence time of irinotecan in circulation and in tumors [11–13]. In mice implanted with human tumor xenografts, irinotecan was more effective when administered on a five-times-daily schedule compared with a twice-weekly schedule, even though the cumulative drug dose was 50% of the twice-weekly schedule [14]. Both schedules were considered equitoxic based on weight loss.

Liposomal irinotecan (ONIVYDE®; nal-IRI, MM-398, and PEP02) is irinotecan encapsulated in a lipid bilayer vesicle [15]. In combination with 5-FU/leucovorin, liposomal irinotecan is an approved treatment for mPDAC following progression with gemcitabine-based therapy [16]. The NALIRIFOX regimen (liposomal irinotecan, 5-FU, leucovorin, and oxaliplatin) is under investigation as a first-line therapy for mPDAC [17].

Liposomal encapsulation protects irinotecan from metabolic conversion and clearance, increasing the half-life of circulating liposomal irinotecan 40-fold relative to non-liposomal irinotecan [15]. The liposomal formulation facilitates passive delivery of the drug to solid tumors by the enhanced permeability and retention effect [18]. Similar systemic and intratumoral area under the curve SN-38 exposures

were achieved at a fivefold lower dose of liposomal irinotecan (10 mg/kg) than irinotecan (50 mg/kg) in mice bearing human solid-tumor xenografts [19]. In addition, the extended duration of elevated SN-38 levels within tumors conferred by the liposomal formulation (even at fivefold lower doses of liposomal irinotecan [5–10 mg/kg]) results in improved control of growth rates across a range of murine xenograft pancreatic and other tumor models compared with non-liposomal irinotecan (25–50 mg/kg) [12, 19].

Based on the pharmacological properties of liposomal irinotecan, we hypothesize that its therapeutic index will be higher than that of non-liposomal irinotecan. This study was a side-by-side comparison of the therapeutic indexes of liposomal and non-liposomal irinotecan in mice bearing subcutaneous patient-derived xenograft (PDX) pancreatic tumors.

METHODS

Ethics

Experimental procedures were approved by the Ethics Committee of Ipsen Innovation (C2EA; registration number 32) and performed in compliance with the ARRIVE guidelines, EU Directive 2010/63/EU for animal experiments, and the 2013 French Regulatory Decree. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Patient-Derived Xenograft Tumor Models

Three established PDX pancreatic tumor models were evaluated: IM-PAN-001, SA-PAN-077, and SA-PAN-083 (Innovative Models Initiative Against Cancer consortium; Oncodesign Services, Dijon, France). Xenografts were subcutaneously implanted within female CB17 severe combined immunodeficient mice (Charles River Laboratories) (more information on PDX models is provided in the Supplementary materials).

Treatments

Mice were randomized before administration of liposomal irinotecan (MM-398 4.3 mg/ml, Ipsen) or non-liposomal irinotecan (CAMPTO 20 mg/ml, Oncodesign) using VIVO Manager® software (Biosystemes) when their mean tumor volume was 100–200 mm^3. Treatments were injected into the caudal vein following dilution in either phosphate-buffered saline (without Mg^{2+} and Ca^{2+}) for liposomal irinotecan or 0.9% NaCl for non-liposomal irinotecan. Control mice received vehicle (0.9% NaCl) injections on the same dosing schedule as non-liposomal irinotecan. Liposomal irinotecan was administered as one injection per week (Q7D), and non-liposomal irinotecan was administered either as five injections over 5 days consecutively per week (Q1D5) (optimized exposure characteristics) or Q7D. This corresponds to one cycle of treatment for both drugs.

Using the IM-PAN-001 model, mice received three treatment cycles with liposomal irinotecan at 2.5, 10, or 50 mg/kg/week (Q7D for 3 weeks [Q7D × 3]), and non-liposomal irinotecan at 10, 25, or 50 mg/kg/week (Q1D5 over 3 weeks [Q1D5 × 3]) (eight mice per dose group) (Figs. 1, S1c). Investigator-selected doses were based on the preliminary efficacy analyses used to determine the maximum tolerated dose (MTD) (Supplementary materials).

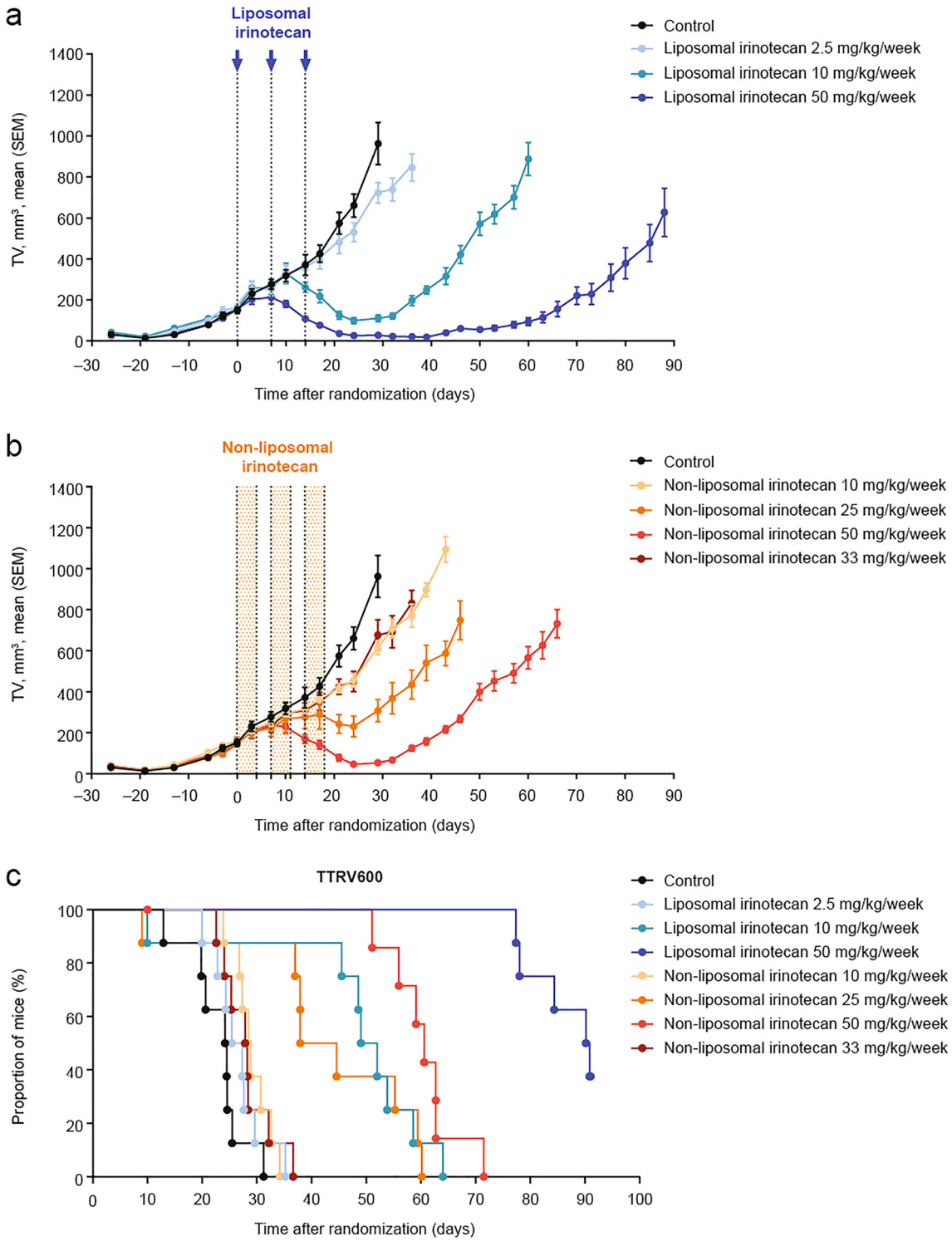
Eight additional mice received non-liposomal irinotecan at 33 mg/kg/week (Q7D × 3), which is equivalent to that used clinically in patients. The clinically equivalent dose and schedule of liposomal irinotecan is 10 mg/kg/week (Q7D × 3).

Figure S1 shows the schedule of activities. Supplementary methods and results provide further details on the rationale for and assessments conducted in all three models. The numbers of mice used in these studies were based on historical standard practice.

Monitoring and Procedures

Monitoring Regimes

Monitoring continued for ≤ 121 days after randomization (based on preliminary data from


a
Liposomal irinotecan
Control
Liposomal irinotecan 2.5 mg/kg/week
Liposomal irinotecan 10 mg/kg/week
Liposomal irinotecan 50 mg/kg/week
TV, mm³, mean (SEM)
Time after randomization (days)
b
Non-liposomal irinotecan
Control
Non-liposomal irinotecan 10 mg/kg/week
Non-liposomal irinotecan 25 mg/kg/week
Non-liposomal irinotecan 50 mg/kg/week
Non-liposomal irinotecan 33 mg/kg/week
TV, mm³, mean (SEM)
Time after randomization (days)
c
TTRV600
Control
Liposomal irinotecan 2.5 mg/kg/week
Liposomal irinotecan 10 mg/kg/week
Liposomal irinotecan 50 mg/kg/week
Non-liposomal irinotecan 10 mg/kg/week
Non-liposomal irinotecan 25 mg/kg/week
Non-liposomal irinotecan 50 mg/kg/week
Non-liposomal irinotecan 33 mg/kg/week
Proportion of mice (%)
Time after randomization (days)

◀**Fig. 1** Antitumor activity of liposomal irinotecan and non-liposomal irinotecan in the IM-PAN-001 tumor model. Tumor growth profiles after three cycles of treatment with **a** liposomal irinotecan or **b** non-liposomal irinotecan (mice per group, $n = 8$). **c** TTRV600 for all treated groups. One mouse in the liposomal irinotecan 10 mg/kg/week group reached a TV of 600 mm^3 on day 9 that was considered in the TTRV analysis; TTRV600 in this group compared with that in controls was not significant, $p = 0.2173$. *SEM* standard error of the mean, *TTRV600* time to reach a tumor volume of 600 mm^3, *TV* tumor volume

all three models; see Supplementary results, and Figures S1 and S2) or until ethical limit criteria were met, whichever came first. Animals were weighed at least twice a week, and soft stools/diarrhea patterns were assessed daily. Tumor length and width were recorded twice a week using calipers.

Humane Endpoints and Euthanasia

Table S1 lists humane endpoints selected in accordance with the Organisation for Economic Co-operation and Development. Mice were euthanized with isoflurane gas anesthesia followed by cervical dislocation/exsanguination.

Blood Collection

Intra-cardiac blood collection ($\leq$ 150 µl into K_3 ethylenediaminetetraacetic acid tubes [Sarstedt]) was performed as a terminal procedure under deep isoflurane gas anesthesia. Blood was kept on ice before centrifugation at 4 °C ($2000 \times g$) for 5 min to obtain plasma. Plasma samples were stored at − 80 °C within 10 min ready for drug-level monitoring.

Tissue and Tumor Collection

Plasma, tumor, jejunum, and bone marrow samples were collected for histopathology analysis and drug monitoring (four mice per group and timepoint) (Fig. S1c). Collection occurred during the third treatment cycle, at 24 and 168 h after administration of liposomal irinotecan, and at 1 h after the second dose and 168 h after the first dose of non-liposomal irinotecan.

After tumors were weighed, half of each tumor was snap frozen and stored at − 80 °C ready for drug-level monitoring, and the other half was fixed in isotonic formalin solution (4% formalin [VWR]) for 48 h along with jejunum and sternum with bone marrow. Fixed tissues were embedded in paraffin for histopathological and immunohistochemical analyses.

Assessments

Body Weight

Body weight loss (BWL) relative to day 0 was presented as a heat map and categorized as: green, $\geq$ 0%; yellow, − 9.99 to − 0.01%; orange, − 14.99 to − 10.00%; red, − 30.00 to − 15.00%.

Tumor Growth

Tumor growth-related parameters included tumor volume (TV), time (days) to reach a TV of 600 or 750 mm^3 (TTRV600 or TTRV750, dependent on the analysis), and time to relapse (TTR). TV was calculated as, [(width^2 × length)/2]; with width and length reflecting the shortest and longest dimensions of the tumor, respectively. TTR was calculated when tumor regression was observed and corresponded to the last timepoint before sacrifice that the smoothened estimated tumor growth rate turned positive.

CPT-11 and SN-38 Levels

CPT-11 and SN-38 levels in plasma and tumor homogenates were determined via an exploratory liquid chromatography-triple quadrupole mass spectrometry bioanalytical method (Supplementary methods).

Histopathology and Immunohistochemistry

Tumor regression was graded according to decreased cancer cellularity and increased fibrous tissue (grade: 0, no regression; 1, minimal; 2, mild; 3, moderate; 4, marked; 5, severe) on hematoxylin–eosin-stained tissue sections. Intestinal toxicity (apoptotic cells in intestinal crypts of the jejunum) and bone marrow toxicity (decreased cellularity) were graded according to a standard toxicologic pathology scale (grade: 0, no change; 1, minimal; 2, mild; 3, moderate; 4, marked; 5, severe).

DNA damage was assessed through immunohistochemical staining for p53 binding protein 1 (53BP1 [Novus Biologicals]), phosphohistone gamma-H2AX protein (pH2AX [Abcam]) and RAD51 (Abcam) (further details in Supplementary methods). Quantification of the staining in tumors was performed using an H-score method (proportion of positive-labelled cells × staining intensity [grade: 0, no staining; 1, minimal; 2, moderate; 3, strong]). The H-score scale ranged from 0 (no staining) to 300 (strongest intensity of labeling in all cells [100%]). pH2AX immunostaining was also graded 0–5 to assess toxicity in the jejunum.

Therapeutic Index

The therapeutic index is a quantitative measurement of the relative safety of a drug. It was calculated per treatment, as the ratio of the highest exposure dose that produces the desired antitumor response for a maximum tolerated toxicity (i.e., the MTD) to the lowest dose that produces an effective antitumor response.

To evaluate therapeutic index, mice were assessed for BWL, TV, DNA damage within tumor cells, tumor regression, plasma and tumor CPT-11 and SN-38 levels (tumor CPT-11 data not shown), and toxicity within the jejunum and bone marrow.

Statistical Analyses

TV comparisons between treatments and controls were assessed using an analysis of covariance with repeated measures. The two factors assessed were (1) treatment groups, and (2) times of TV measurement. TV was normalized relative to TV at randomization. Per timepoint and pairwise comparison, a contrast was calculated followed by a Šidák correction to account for the multiplicity of tests.

TTRV comparisons between treatment groups and controls were assessed using the log-rank test with Dunnett–Hsu adjustment if the test converges, and by simulation in the opposite case. In cases where all the comparisons between the groups present in the analysis were carried out, the log-rank test with TukeyHsu adjustment if the test converged, and by simulation in the opposite case. In cases where the request only concerned certain comparisons, the log-rank test between two comparisons was adjusted by the Šidák method. Statistical analyses were performed using JMP Pro 15.0 and SAS 9.3 software.

RESULTS

PDX Model Selection

Of the three models assessed, IM-PAN-001 was selected for therapeutic index comparison, based on its sensitivity to liposomal irinotecan and the study duration; the MTD was considered to be 50 mg/kg/week for both treatments (for more details, see Supplementary results, Table S2, and Figures S2 and S3). Interestingly, the most treatment-sensitive PDX models were those presenting with the highest mean number of mitoses per three high-power fields (SA-PAN-083, 57.8 > IM-PAN-001, 38.8 > SA-PAN-007, 13.7).

Antitumor Activity After Three Treatment Cycles in IM-PAN-001

Mice were treated with three cycles of liposomal irinotecan or non-liposomal irinotecan, and TV, TTR, and TTRV600 were assessed. Significant dose-dependent reductions in tumor growth compared with controls were reported for both treatments at all doses tested ($p < 0.05$ each) (Fig. 1a, b). Compared with controls, a significant reduction in TV was observed from day 10 to day 29 when mice received 50 mg/kg/week of either liposomal irinotecan ($p \leq 0.0032$) or non-liposomal irinotecan ($p \leq 0.0279$). Regression in tumor size was observed from day 10 and day 14 with liposomal and non-liposomal irinotecan, respectively.

TTRV600 was significantly longer with liposomal irinotecan 50 mg/kg/week (90.5 days), and with non-liposomal irinotecan 25 mg/kg/week (41.3 days) and 50 mg/kg/week (60.6 days), compared with controls (24.3 days) ($p < 0.05$ each) (Fig. 1c and Table S3).

TV with liposomal irinotecan 50 mg/kg/week was significantly lower than that with 2.5 mg/kg/week from day 14 to day 39 and 10 mg/kg/week from day 10 to day 66. Similarly, non-liposomal irinotecan 50 mg/kg/week significantly reduced TV compared with 10 mg/kg/week from day 17 to day 43 and 25 mg/kg/week from day 24 to day 50. Median TTR was significantly different between liposomal irinotecan 50 and 10 mg/kg/week (39 vs. 33 days, respectively; $p = 0.0141$), but not between non-liposomal irinotecan 50 and 25 mg/kg/week (33 vs. 27 days, respectively; $p = 0.6876$) (Table S3).

At clinically equivalent doses, liposomal irinotecan (10 mg/kg/week) significantly reduced TV from day 17 to day 39 ($p \leq 0.0084$) and numerically delayed TTRV600 (50.5 vs. 28.1 days, $p = 0.6349$) compared with non-liposomal irinotecan (33 mg/kg/week) (Fig. 1c and Table S3). Changes in TV were similar with non-liposomal irinotecan 10 mg/kg/week administered Q1D5 × 3 and non-liposomal irinotecan 33 mg/kg/week administered Q7Dx3.

The MTD and lowest dose at which antitumor efficacy was observed were estimated based on previous analyses (Figures S2 and S3) and were then used to determine the therapeutic index of both treatments in the IM-PAN-001 model.

Treatment at the MTD in IM-PAN-001 (Therapeutic Index Comparison)

Antitumor Activity

At the selected MTD (50 mg/kg/week for each treatment), liposomal irinotecan demonstrated greater antitumor activity than non-liposomal irinotecan over three treatment cycles (Fig. 2a). Compared with non-liposomal irinotecan, liposomal irinotecan was associated with significantly lower TV from day 36 to day 70 ($p \leq 0.0002$), significantly longer median TTR (33 vs. 39 days, respectively; $p = 0.0044$), and numerically delayed TTRV600 (90.5 vs. 60.6 days, respectively; $p = 0.1606$) (Table S3).

Pharmacodynamic Biomarkers

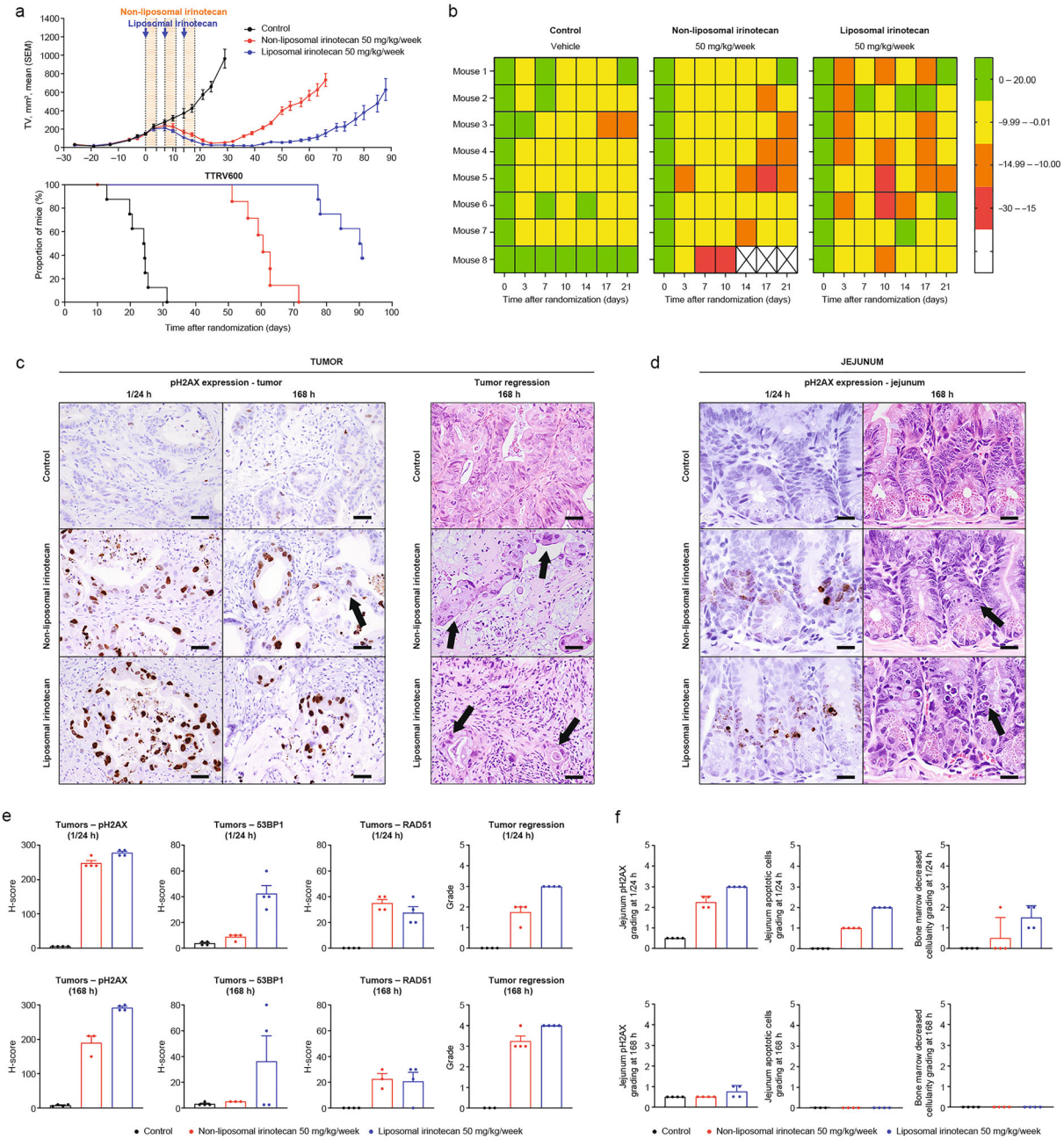
Expression of pH2AX is an early cellular response to DNA double-strand breaks and is activated when the cell undergoes mitosis. Liposomal irinotecan induced greater and more sustained DNA damage in tumors than non-liposomal irinotecan (Fig. 2c, e). During the third treatment cycle at the MTD 24 and 168 h, DNA damage was high with ≥ 95% of liposomal irinotecan-treated tumor cells showing high pH2AX expression (H-score range, 270–300). At 24 h after non-liposomal irinotecan administration, ≥ 80% of tumor cells showed high pH2AX expression (H-score range, 240–270), and this decreased by 168 h, with 60–70% of tumor cells showing moderate–high expression (H-score range, 150–210; Fig. 2c, arrow pointing at cancer cell without evidence of DNA damage). Similar trends were observed for 53BP1 expression.

Both pH2AX and 53BP1 expression were associated with histological tumor regression, which was higher with liposomal than non-liposomal irinotecan. At 168 h, fewer cancer cells were observed within tumors treated with liposomal compared with non-liposomal irinotecan (Fig. 2c, arrows). RAD51 expression was not associated with histological tumor regression, and no differences were observed between treatments.

Toxicity

Most mice receiving liposomal irinotecan (7/8) or non-liposomal irinotecan (6/8) at the MTD had moderate–severe BWL up to day 21 (Fig. 2b). BWL was transient with liposomal irinotecan and was partially reversed after the end of both treatments. One mouse receiving non-liposomal irinotecan showed severe BWL and was ethically euthanized for poor clinical condition at day 11 (no abnormalities at autopsy).

During the third treatment cycle, toxicity within the jejunum and bone marrow was observed with both treatments at 24 h (Fig. 2d, f). In the jejunum, mild levels of apoptotic cells in crypts (grade 2) and moderate associated DNA damage (pH2AX expression, grade 3) were observed with liposomal irinotecan; slightly lower levels were observed with non-liposomal



g

Treatment	Sampling timepoint (h)	Plasma CPT-11 level, nmol/l, mean (SD) ($n = 4$)	Plasma SN-38 level, nmol/l, mean (SD) ($n = 4$)	Tumor SN-38 level, nmol/kg, mean (SD) ($n = 4$)
Non-liposomal irinotecan 50 mg/kg/week	1 168	1363 (316) 8.71 (16.6)	229 (70.5) BLQ	91.8 (18.3) BLQ
Liposomal irinotecan 50 mg/kg/week	24 168	130887 (29185) BLQ	1613 (155) BLQ	73.6 (39.1) BLQ

◀**Fig. 2** Antitumor activity and toxicity after three treatment cycles at the MTD (IM-PAN-001 model). Analyses were performed during the third treatment cycle: at 24 and 168 h after the dose of liposomal irinotecan; at 1 h after the second dose and 168 h after the first dose of non-liposomal irinotecan. **a** Tumor growth profiles and survival analysis (TTRV600) in the IM-PAN-001 model treated at the MTD (mice per group, $n = 8$); charts are Fig. 1 subplots. **b** Heat map visualization of BWL in individual mice in the IM-PAN-001 model compared with their first day of treatment. Mice were weighed at least twice a week. Color grading represents the intensity of BWL (mice per group, $n = 8$); 'x' represents mouse euthanasia. **c** pH2AX immunostaining in tumor samples and H&E staining to show representative tumor regression (magnification, × 400, scale bars: 50 µm). **d** pH2AX immunostaining in intestine samples (magnification, × 1000, *scale bars*: 20 µm). During the third treatment cycle, **e** tumoral H-scores of pH2AX, 53BP1, and RAD51 immunostaining, and tumor regression grading were assessed in parallel with **f** jejunum and bone marrow toxicity (assessed by pH2AX immunostaining). **g** Plasma CPT-11, plasma SN-38 and tumor SN-38 levels (mice per group, $n = 4$). Plasma CPT-11 LLOQ = 1 nmol/l; plasma SN-38 LLOQ = 13 nmol/l; tumor SN-38 LLOQ = 3 nmol/kg. *53BP1* p53 binding protein 1, *BLQ* below the limit of quantification, *BWL* body weight loss, *CPT-11* irinotecan, *H-score* histological score, *H&E* hematoxylin–eosin, *LLOQ* lower limit of quantification, *MTD* maximum tolerated dose, *pH2AX* phospho-histone gamma H2AX, *SD* standard deviation, *SEM* standard error of the mean, *TTRV600* time (days) to reach a TV of 600 mm^3, *TV* tumor volume

irinotecan (apoptotic cells in crypts, grade 1; pH2AX expression, grade 2). In the bone marrow, decreased cellularity was mild (grade 2) with liposomal irinotecan and minimal (grade 1) with non-liposomal irinotecan. Toxicity in both tissues had reduced at 168 h.

CPT-11 and SN-38 Levels

During the third treatment cycle, mean plasma CPT-11 and SN-38 levels were 96- and 7-fold higher with liposomal (24 h post-injection) compared with non-liposomal irinotecan (1 h post second injection) at the MTD, and mean tumor SN-38 levels were similar (Fig. 2g). At the end of the third treatment cycle, plasma and tumor SN-38 levels were undetectable, and plasma CPT-11 levels were very low. Observed inter-individual variability was low–high with non-liposomal irinotecan and low–moderate with liposomal irinotecan.

Treatment at the Lowest Doses Assessed in IM-PAN-001 (Therapeutic Index Comparison)

Antitumor Activity

TV with liposomal irinotecan 2.5 mg/kg/week was similar to that with non-liposomal irinotecan 10 mg/kg/week, and TTRV600 was not significantly different (26.4 vs. 28.6 days, respectively; $p = 0.9997$) (Fig. 3a and Table S3).

Pharmacodynamic Biomarkers

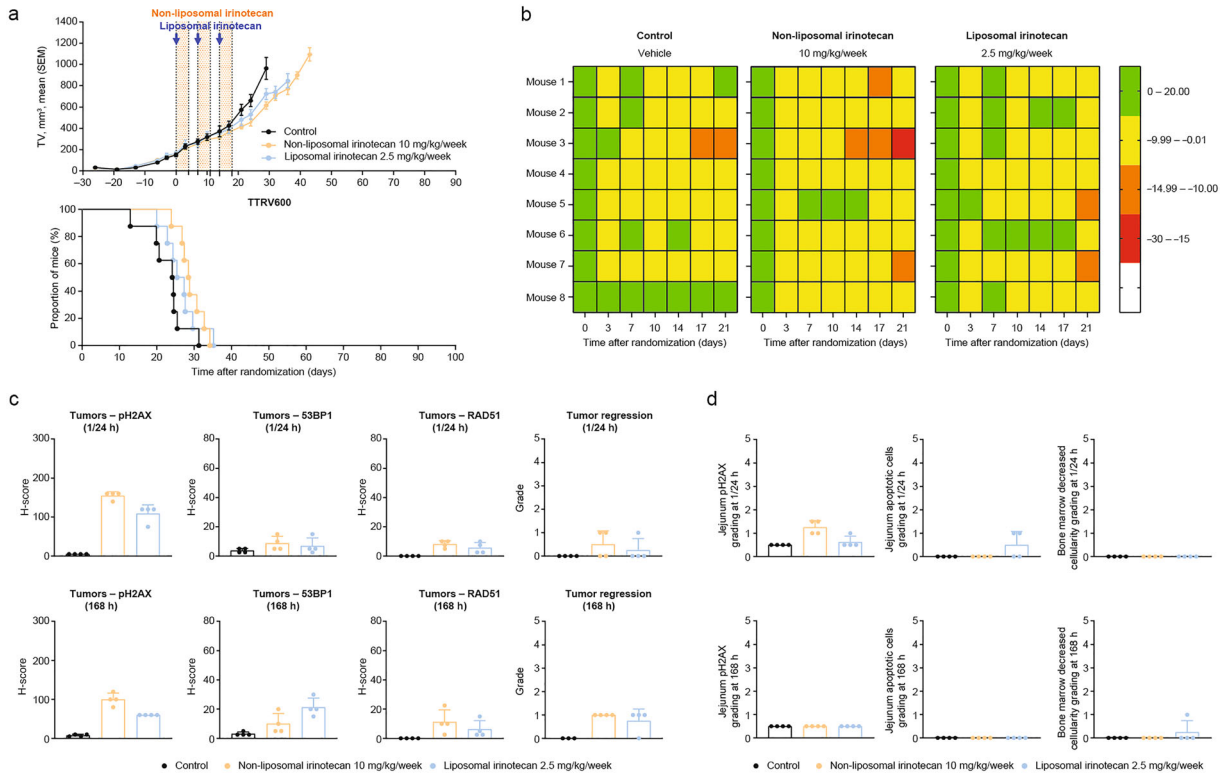
During the third treatment cycle, liposomal irinotecan induced low–moderate pH2AX expression (H-score range, 75–120) in 50–60% of tumor cells at 24 h, and low pH2AX expression (H-score range, 60–60) in 30–30% of tumor cells at 168 h (Fig. 3c). Levels of pH2AX expression were higher with non-liposomal irinotecan than liposomal irinotecan. Similar trends were observed with 53BP1 and RAD51 expression.

Tumor regression was minimal at 24 and 168 h with liposomal (1/4 and 3/4 mice, respectively) and non-liposomal irinotecan (2/4 and 4/4 mice, respectively) (Fig. 3c).

Toxicity

All mice receiving liposomal irinotecan 2.5 mg/kg/week or non-liposomal irinotecan 10 mg/kg/week had minor BWL up to day 21 (Fig. 3b). Four mice receiving liposomal irinotecan had transient moderate–severe BWL after ending treatment, but partially recovered until ethical euthanization due to TV. Less than half of the mice (3/8) receiving non-liposomal irinotecan had moderate–severe BWL up to day 21 (Fig. 3b). After ending non-liposomal irinotecan treatment, two mice showed transient moderate BWL with partial recovery in one mouse, and four animals with severe BWL did not recover before ethical euthanization due to TV.

Toxicity was minimal or lower within the jejunum at 24 h (grade range, 0–1.5) and



e

Treatment	Sampling timepoint (h)	Plasma CPT-11 level, nmol/l, mean (SD) ($n = 4$)	Plasma SN-38 level, nmol/l, mean (SD) ($n = 4$)	Tumor SN-38 level, nmol/kg, mean (SD) ($n = 4$)
Non-liposomal irinotecan 10 mg/kg/week	1 168	160 (60.0) 3.21 (1.57)	112 (47.2) BLQ	38.3 (13.6) BLQ
Liposomal irinotecan 2.5 mg/kg/week	24 168	16.1 (12.0) BLQ	16.5 (19.2) BLQ	12.7 (17.9) BLQ

Fig. 3 Antitumor activity and toxicity after three treatment cycles at the lowest doses assessed (IM-PAN-001 model). Analyses were performed during the third treatment cycle: at 24 and 168 h after the dose of liposomal irinotecan; at 1 h after the second dose and 168 h after the first dose of non-liposomal irinotecan. **a** Tumor growth profiles and survival analysis (TTRV600) in the IM-PAN-001 model treated at the lowest doses (mice per group, $n = 8$); charts are Fig. 1 sub-plots. **b** Heat map visualization of BWL in individual mice in the IM-PAN-001 model compared with their first day of treatment. Color grading represents the intensity of BWL (mice per group, $n = 8$). During the third treatment cycle, **c** tumoral H-scores of pH2AX, 53PB1, and RAD51 immunostaining, and tumor regression grading were assessed in parallel with **d** jejunum and bone marrow toxicity. **e** Plasma CPT-11, plasma SN-38, and tumor SN-38 levels (mice per group, $n = 4$). Plasma CPT-11 LLOQ = 1 nmol/l; plasma SN-38 LLOQ = 13 nmol/l; tumor SN-38 LLOQ = 3 nmol/kg. Heat map data presented here for the control group is repeated from Fig. 2b for comparison with both treatments at their respective lowest doses. *CPT-11* irinotecan, *BWL* body weight loss, *H-score* histological score, *LLOQ* lower limit of quantification, *pH2AX* phospho-histone gamma H2AX, *SD* standard deviation, *SEM* standard error of the mean, *TTRV600* time (days) to reach a TV of 600 mm^3, *TV* tumor volume

minimal within the bone marrow at 168 h (1/4 mice) (Fig. 3d).

CPT-11 and SN-38 Levels

During the third treatment cycle, mean plasma CPT-11 and plasma and tumor SN-38 levels were 10-, 7- and 3-fold higher with non-liposomal (1 h post second injection) than with liposomal irinotecan (24 h post-injection) (Fig. 3e). At the end of the third treatment cycle, plasma and tumor SN-38 levels were undetectable, and plasma CPT-11 levels were undetectable with non-liposomal irinotecan and low

with liposomal irinotecan. Observed inter-individual variability was moderate with non-liposomal irinotecan and high with liposomal irinotecan.

Findings at the equivalent cumulative dose (ECD) of 10 mg/kg/week for either formulation are reported in Supplementary results and Figure S4; findings at the ECD of 50 mg/kg/week are reported in the preceding MTD section.

Based on these findings at the MTD and lowest doses assessed, the therapeutic indexes of liposomal and non-liposomal irinotecan were 20 and 5, respectively.

DISCUSSION

Patients with pancreatic cancer have a poor prognosis, with a reported 5-year survival rate of only 10.8% [20]. Therapy options are limited and there is a need for new effective treatments. Currently, the NALIRIFOX regimen is under investigation as a first-line therapy for patients with mPDAC [17].

To date, there are no clinical studies comparing non-liposomal and liposomal irinotecan in pancreatic cancer treatment. This non-clinical study provides important data comparing these two formulations that can be used to supplement existing clinical data. In this study in mice with subcutaneously implanted IM-PAN-001 PDX pancreatic tumors, the therapeutic index was fourfold greater with liposomal irinotecan Q7D than non-liposomal irinotecan Q1D5 (20 vs. 5, respectively), supporting the effectiveness of liposomal irinotecan in the clinical setting in mPDAC. Transient dose-dependent BWL was observed with both formulations, with greater tolerance for liposomal irinotecan at the MTD and the clinically equivalent dose. At the MTD (50 mg/kg/week), liposomal irinotecan was associated with greater efficacy than non-liposomal irinotecan. Similar trends were observed at the lowest doses assessed (liposomal irinotecan 2.5 mg/kg/week, non-liposomal irinotecan 10 mg/kg/week), as well as at the ECD of 10 mg/kg/week (Supplementary results and Figure S4).

Worldwide, 85% of preclinical agents entering oncology clinical trials fail to demonstrate sufficient safety or efficacy to gain regulatory approval [21]. Because cancers are heterogeneous diseases with biological, genetic, and histopathological differences between patients, tumors, and even within individual tumors, it is not difficult to understand why animal models based on established cell lines can have limited preclinical and clinical translational value, due to their clonal nature and adaptations to defined culture media. Compared with models based on established cell lines, xenografts from patient tumors implanted into immunodeficient or humanized mice better preserve the characteristics of the original tumor and provide tools for translational and clinical research. Primary tumor xenograft explants may recapitulate the heterogeneity and genetic diversity observed in patients [22, 23], and PDXs reportedly retain sufficient histology, transcriptome, and genome fidelity with their parental tumor [24]. This has been demonstrated in pancreatic cancer models [25]. Use of PDX models in new cancer treatment screening has been shown in a prospective study in PDAC, which demonstrated the efficacy of gemcitabine + nab-paclitaxel in a PDX model [26], correlating with the known clinical efficacy of gemcitabine + nab-paclitaxel. Similarly, a lack of efficacy in PDX models has been shown to correlate with negative clinical results [27].

The IM-PAN-001 PDX model used in this study came from a tumor biopsy in a treatment-naive patient who later received FOLFIRINOX. Although FOLFIRINOX is associated with increased survival, this patient did not show an objective response and died 3.44 months after diagnosis. Similarly, in this study, non-liposomal irinotecan (a component of FOLFIRINOX) at the clinically equivalent dose was associated with only a marginal response.

Despite the strengths of PDX models, implantation within rodents is a major limitation because rodents can tolerate significantly greater systemic exposure to camptothecin analogues (i.e., irinotecan) than patients [28]. Additionally, irinotecan conversion by plasma CES is observed in mice but not humans. Consequently, studies with syngeneic or human xenograft models in mice may overpredict the potential clinical activity of this class of

anticancer drug. Based on rodent data, one study predicted that the estimated MTDs for eight topoisomerase I inhibitors would result in clinical trials having to initiate with doses close to or exceeding the MTD in humans [29]. In the present study, this difference in camptothecin tolerance had no impact on our findings because the respective MTDs in humans for the two formulations in the clinical setting are already known, while any potential MTD benefit for the liposomal formulation in the mouse models was mitigated by assuring optimal efficacy of the non-liposomal irinotecan.

As both formulations should yield data that accurately reflect response characteristics in patients despite greater tolerances found in mouse models, we administered non-liposomal and liposomal irinotecan using schedules and doses that were similar to those used in clinical settings. The dosing schedule for each treatment was selected to optimize within the mouse models the balance between tumor exposure to SN-38, antitumor activity, and toxicity, compared to traditional administration schedules. Liposomal irinotecan Q7D accounted for the prolonged residence time of the drug [30]. Owing to irinotecan pharmacokinetics and clearance in plasma and tumor, Q1D5 has emerged as a popular schedule for new irinotecan-containing regimens in non-clinical studies [14, 31, 32], and has been used in pediatric studies for irinotecan trials [33]. Data suggest that smaller doses administered frequently (protracted irinotecan) may result in greater antitumor activity and reduced toxicity than larger doses administered intermittently [34, 35]. Consequently, we selected the Q1D5 schedule for non-liposomal irinotecan to allow sufficient time for tumor exposure to SN-38. Use of this schedule (5 mg/kg/day for 5 days) improved efficacy compared with irinotecan administered as a single clinically relevant dose (33 mg/kg/week), demonstrating the optimized exposure characteristics for the Q1D5 schedule.

Using these doses and optimal treatment regimens, liposomal irinotecan was associated with greater efficacy than non-liposomal irinotecan at all dose levels, despite optimal exposure for non-liposomal irinotecan. Local conversion of the payload and prolonged residence time of SN-38 within tumors after liposomal irinotecan administration may explain the greater efficacy observed. The impact of residence time has been evaluated in clinical trials and in the literature using different schedules of treatment. According to a post-hoc analysis of clinical observations from six trials, a high and sustained circulating SN-38 level after liposomal irinotecan was associated with longer overall survival and progression-free survival, and a higher objective response rate [36]. This is consistent with preclinical findings after liposomal irinotecan administration that showed associations between the longer residence time of SN-38 within tumors and antitumor efficacy, compared with non-liposomal irinotecan administered once a week [19]. In addition, prolonged SN-38 exposure with liposomal irinotecan compared with non-liposomal irinotecan in PDX pancreatic cancer models has been reported previously [13]. Liposomal irinotecan has been compared with non-liposomal irinotecan in other cancer models such as a mouse model of Ewing's sarcoma, where liposomal irinotecan administered twice in a week showed greater antitumor activity compared with non-liposomal irinotecan at the same dosing regimen [37]. This supports an ongoing phase 2 clinical trial of liposomal irinotecan in children and young adults with recurrent solid malignancies and Ewing's sarcoma.

To exert maximum cytotoxic effects across different cell-cycle phases, tumor cells have to be exposed to SN-38 across multiple cell cycles, with TV doubling time and percentage of cells in the S phase of the cell cycle being described as important markers of efficacy [19, 38]. The amount of DNA damage within tumors, detected through pH2AX, 53BP1, and RAD51 expression, in the present study was associated with histological tumor regression. At the end of the third treatment cycle, when the majority of tumor cells had strongly positive pH2AX expression, persistent tumor regression and DNA damage were observed; this was associated with a greater in vivo response to treatment. The most treatment-sensitive PDX models were those presenting with high mean numbers of mitoses per three high-power fields (SA-PAN-

083, 57.8 > IM-PAN-001, 38.8 > SA-PAN-007, 13.7), and basal expression of pH2AX, which suggests that DNA repair mechanisms were already altered or that cells were prone to present with spontaneous DNA damage. Expression of Schlafen 11 (SLFN11), which has been linked with response to DNA-damaging agents [39], may correlate with the different responses to SN-38 in our PDX models, but this remains to be evaluated.

Although treatment was associated with tumor shrinkage, after three cycles with non-liposomal irinotecan 25 and 50 mg/kg/week and liposomal irinotecan 10 and 50 mg/kg/week, residual viable tumor cells eventually began to proliferate, but this tumor relapse was delayed for liposomal irinotecan. In the literature, local recurrence of a tumor after treatment could be related to the presence of cancer stem cells (CSCs), a group of poorly differentiated cells. These CSCs are highly treatment-resistant and are characterized by both increased renewal capacity and low proliferation capacity (the stem cell pool is not mitotically active), which is associated with a more aggressive phenotype [40]. In the IM-PAN-001 tumor model, we showed that three treatment cycles may have inadvertently constituted a selection pressure for a sub-population of cells within tumors that could resume tumor growth once treatment ended. Alternatively, or in addition, tumor relapse may have stemmed from residual tumor cells that did not exhibit DNA damage, whether by treatment resilience or limited/non-uniform treatment exposure throughout the tumor mass. Similar to CSCs, chemo-resistant cells may have higher energy availability to regrow the tumor mass. Studying the signaling pathway characteristics of the tumor cells responsible for tumor relapse could help to understand mechanisms implicated in tumor recurrence after topoisomerase I treatment or associated with the topoisomerase I resistance phenotype [41]. This would help gather more evidence for (1) the differentiation of liposomal from non-liposomal irinotecan, or (2) combination regimens that take advantage of the prolonged exposure and improved antitumor effect of liposomal irinotecan.

As well as demonstrating efficacy associated with non-liposomal and liposomal irinotecan, our study also presents a rigorous analysis of the associated safety of these compounds, with BWL assessments, histological analyses of target organs, and therapeutic index calculations based on the correlation to antitumor effect at several doses for both drugs. At the time of this study, this level of analysis in the context of a therapeutic index comparison is not currently well documented in the scientific literature. As part of the safety analysis, the defined MTD criteria (based on BWL and clinical condition) enabled selection of appropriate dose-levels in the optimal treatment regimen (repeated cycles) for the PDX models. These criteria are aligned with published guidelines on humane endpoints [42], including the Organisation for Economic Co-operation and Development guidelines. Our evaluation of MTD is in agreement with published data [43] as well as non-clinical safety data of liposomal irinotecan presented in the Food and Drug Administration's New Drug Application.

Studies of liposomal irinotecan in healthy rats and dogs have identified the gastrointestinal tract and hematologic system as target organs of dose-dependent toxicity, with possibly resultant diarrhea, consistent with the known effects of irinotecan; lower body weight gain and reduction in food intake were also recorded, though these effects were reversible [44]. Drug-related diarrhea in rodents receiving high-dose liposomal irinotecan may be attributed to structural and functional injuries to the intestinal tract resulting from the cytotoxic activity of SN-38 [16, 45]. Indeed, CPT-11 or conjugated SN-38 may be further converted or processed to SN-38 by CES or the β-glucuronidase activity of microflora resident within the large intestine [46]. In one study using a rat model, histological damage was most severe in the cecum, with the duration of exposure to both CPT-11 and SN-38 in the intestinal epithelium, and the CPT-11 plasma C_{max} closely related to the incidence and severity of CPT-11-induced delayed-onset diarrhea [47]. Intraperitoneal administration of irinotecan in mice has induced diffuse mucosal damage, with correlations reported between

diarrhea grade and (1) the amount of irinotecan administered, and (2) both irinotecan and SN-38 concentrations in the intestinal wall [48].

In the present study, no diarrhea was observed and only minimal–mild toxicity in the jejunum and bone marrow was observed compared to in the tumor, despite BWL at the MTD. This result cannot be explained by low exposure to the drug because SN-38 is reportedly widely distributed throughout the body as well as in tumors (based on published and unpublished internal data [19]). Because irinotecan and SN-38 are S-phase-dependent DNA damage inducers [49], and because H2AX phosphorylation is part of cell-cycle checkpoints allowing time for repair mechanisms to correct genetic lesions or to induce apoptosis, pH2AX foci were only detected within dividing cells. As a consequence, owing to the lower doubling time of cancer cells, a greater cytotoxic effect of treatment, depicted by pH2AX expression, could have been anticipated in tumors compared with the jejunum and bone marrow.

It is important to consider potential study limitations when interpreting our findings. No formal statistical calculations were performed to determine sample sizes across studies. Sample sizes were determined heuristically based on historical standard practice with preclinical investigations at the participating research centers. Only female mice were used in this study, which were selected for logistical reasons owing to their less aggressive nature; no sex-related differences were anticipated. Although our investigations initially assessed three PDX models for dose-range finding purposes, only one model was carried through for the full analysis. Further investigation in a larger population of PDX models is warranted.

CONCLUSIONS

In this study, we took a global approach to compare the therapeutic indexes of liposomal and non-liposomal irinotecan in a preclinical model of pancreatic PDX, which included toxicology, pharmacokinetic, efficacy and pharmacodynamic biomarker analyses. The Q1D5 schedule used for non-liposomal irinotecan was selected to mimic the extended exposure of liposomal irinotecan and approximate the clinical setting used for example in pediatric patients. Even at its optimal treatment schedules for antitumor efficacy evaluation in this study, liposomal irinotecan (Q7D) had a greater therapeutic index than non-liposomal irinotecan (Q1D5) with 20 vs. 5, respectively. Our findings showed a fourfold broader therapeutic index of a Q7D schedule of liposomal irinotecan over a Q1D5 schedule of non-liposomal irinotecan. These findings suggest that liposomal irinotecan could potentially be more beneficial to patients with pancreatic cancer than non-liposomal irinotecan. The efficacy of irinotecan has been evaluated previously in a single ‘mouse clinical trial’ using PDX models for biomarker discovery and mechanism of action analyses [50]. This type of non-clinical approach, which mimics clinical trials, could be used to document the therapeutic index of liposomal irinotecan and its differentiation from non-liposomal irinotecan in a large panel of indications (not limited to pancreatic cancer), in order to explore the heterogeneity of clinical response among patients.

ACKNOWLEDGEMENTS

The authors would like to thank Jean-Luc Blachon for conducting the statistical analyses.

Funding. This study was funded by Ipsen Innovation (Les Ulis, France), and the study publication, including the Rapid Service Fee, was funded by Ipsen.

Medical Writing and Editorial Assistance. The authors thank David Gothard, PhD, and Tamzin Gristwood, PhD, of Oxford PharmaGenesis, Oxford, UK, for providing medical writing support, which was sponsored by Ipsen, in accordance with Good Publication Practice (GPP3) guidelines.

Author Contributions. Sandrine Barbier, Benjamin Beaufils, Ricardo de Miguel, Melissa Reyre, Yannick Le Meitour, Andreanne Lortie,

Marc Hillairet de Boisferon, Sophie Chaumeron, Anne Espirito, Lina Fossati, Pauline Lagarde, Stephan Klinz, Arunthathi Thiagalingam, Stéphane Lezmi, and Florence Meyer-Losic contributed to study conception/design, acquisition/analysis/interpretation of the data, drafting the publication or revising it critically for scientific accuracy and important intellectual content, and final approval of the publication.

Prior Presentation. Presented at American Society of Clinical Oncology (ASCO) 2020, Virtual Scientific Program: Barbier S, Lezmi S, Beaufils B, Lortie A, Hillairet de Boisferon M, Chaumeron S, Espirito A, Klinz SG, Thiagalingam A, Meyer-Losic F. Differentiation of liposomal irinotecan from dose-dense non-liposomal irinotecan in patient-derived pancreatic cancer xenograft tumor models. J Clin Oncol. 2020;38(suppl 15):e16724 (https://doi.org/10.1200/jco.2020.38.15_suppl.e16724).

Disclosures. Sandrine Barbier, Benjamin Beaufils, Ricardo de Miguel, Melissa Reyre, Yannick Le Meitour, Andreanne Lortie, Lina Fossati, Pauline Lagarde, Stephan Klinz, Arunthathi Thiagalingam and Florence Meyer-Losic are employees or former employees of Ipsen. Stéphane Lezmi was an employee of Ipsen at the time of the study, and is now an employee of Excilone Services (Jouy-en Josas, France) at the time of this publication. Marc Hillairet de Boisferon, Sophie Chaumeron and Anne Espirito are employees of Oncodesign. Ricardo de Miguel (University of Zaragoza, Spain), Melissa Reyre (Oniris-Nantes, France), Yannick Le Meitour (University of Strasbourg, France) were masters students in the investigative pathology group, financially supported by Ipsen at the time the study was performed.

Compliance with Ethics Guidelines. All experimental procedures were approved by the Ethics Committee of Ipsen Innovation (C2EA; registration number 32) and were performed in full compliance with the ARRIVE guidelines, EU Directive 2010/63/EU for animal experiments, and the 2013 French Regulatory Decree. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Data Availability. The datasets generated during and/or analyzed during the current study are available from the study sponsor (Ipsen) on reasonable request. Study data that underlie the results reported in this publication may be made available to researchers who provide a research proposal. Additional relevant information such as the study protocol and study report may also be made available. Data are available beginning 6 months after publication. Further details on Ipsen's sharing criteria, eligible studies and process for sharing are available here (https://vivli.org/members/ourmembers/). Any requests should be submitted to www.vivli.org for assessment by an independent scientific review board.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Cancer J Clin. 2018;68:394–424.

2. Kawato Y, Aonuma M, Hirota Y, Kuga H, Sato K. Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. Cancer Res. 1991;51:4187–91.

3. Ko AH. Nanomedicine developments in the treatment of metastatic pancreatic cancer: focus on nanoliposomal irinotecan. Int J Nanomedicine. 2016;11:1225–35.

4. Ewesuedo RB, Ratain MJ. Topoisomerase I inhibitors. Oncologist. 1997;2:359–64.

5. Bailly C. Irinotecan: 25 years of cancer treatment. Pharmacol Res. 2019;148: 104398.

6. Sohal DPS, Kennedy EB, Cinar P, et al. Metastatic pancreatic cancer: ASCO guideline update. J Clin Oncol. 2020. https://doi.org/10.1200/JCO.20.01364:JCO2001364.

7. ESMO Guidelines Committee, eUpdate—Cancer of the pancreas treatment recommendations https://www.esmo.org/guidelines/guidelines-by-topic/gastrointestinal-cancers/pancreatic-cancer. Accessed 7 July.

8. Khorana AA, McKernin SE, Berlin J, et al. Potentially curable pancreatic adenocarcinoma: ASCO clinical practice guideline update. J Clin Oncol. 2019;37:2082–8.

9. Conroy T, Hammel P, Hebbar M, et al. FOLFIRINOX or gemcitabine as adjuvant therapy for pancreatic cancer. N Engl J Med. 2018;379:2395–406.

10. De Man FM, Goey AKL, Van Schaik RHN, Mathijssen RHJ, Bins S. Individualization of irinotecan treatment: a review of pharmacokinetics, pharmacodynamics, and pharmacogenetics. Clin Pharmacokinet. 2018;57:1229–54.

11. Baker JH, Lam J, Kyle AH, et al. Irinophore C, a novel nanoformulation of irinotecan, alters tumor vascular function and enhances the distribution of 5-fluorouracil and doxorubicin. Clin Cancer Res. 2008;14:7260–71.

12. Klinz SG, Leonard SC, Paz N, et al. DNA damage with liposomal irinotecan (nal-IRI) in pancreatic cancer xenografts: multimodal analysis of deposition characteristics. J Clin Oncol. 2018;36(Suppl):e16205.

13. Leonard SC, Paz N, Klinz SG, et al. Deposition characteristics and resulting DNA damage patterns of liposomal irinotecan (nal-IRI) in pancreatic cancer xenografts. J Clin Oncol. 2018;36(Suppl):335.

14. Jansen WJ, Kolfschoten GM, Erkelens CA, et al. Anti-tumor activity of CPT-11 in experimental human ovarian cancer and human soft-tissue sarcoma. Int J Cancer. 1997;73:891–6.

15. Drummond DC, Noble CO, Guo Z, et al. Development of a highly active nanoliposomal irinotecan using a novel intraliposomal stabilization strategy. Cancer Res. 2006;66:3271–7.

16. Ipsen Biopharmaceuticals, Inc. Prescribing Information, ONIVYDE® (irinotecan liposome injection). US Food and Drug Administration https://www.ipsen.com/websites/Ipsen_Online/wp-content/uploads/sites/9/2019/01/21083350/ONIVYDE_USPI.pdf. Accessed 29 June 2021.

17. Wainberg ZA, Bekaii-Saab T, Boland PM, et al. First-line liposomal irinotecan with oxaliplatin, 5-fluorouracil and leucovorin (NALIRIFOX) in pancreatic ductal adenocarcinoma: a phase I/II study. Eur J Cancer. 2021;151:14–24.

18. Maeda H. Toward a full understanding of the EPR effect in primary and metastatic tumors as well as issues related to its heterogeneity. Adv Drug Deliv Rev. 2015;91:3–6.

19. Kalra AV, Kim J, Klinz SG, et al. Preclinical activity of nanoliposomal irinotecan is governed by tumor deposition and intratumor prodrug conversion. Cancer Res. 2014;74:7003–13.

20. Surveillance, Epidemiology, and End Results Program—Cancer Stat Facts: Pancreatic Cancer https://seer.cancer.gov/statfacts/html/pancreas.html. Accessed 28 June 2021.

21. Arrowsmith J, Miller P. Trial watch: phase II and phase III attrition rates 2011–2012. Nat Rev Drug Discov. 2013;12:569.

22. Biankin AV, Hudson TJ. Somatic variation and cancer: therapies lost in the mix. Hum Genet. 2011;130:79–91.

23. Witkiewicz AK, McMillan EA, Balaji U, et al. Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. Nat Commun. 2015;6:6744.

24. Xu C, Li X, Liu P, Li M, Luo F. Patient-derived xenograft mouse models: a high fidelity tool for individualized medicine. Oncol Lett. 2019;17:3–10.

25. Izumchenko E, Paz K, Ciznadija D, et al. Patient-derived xenografts effectively capture responses to oncology therapy in a heterogeneous cohort of patients with solid tumors. Ann Oncol. 2017;28:2595–605.

26. Von Hoff DD, Ramanathan RK, Borad MJ, et al. Gemcitabine plus nab-paclitaxel is an active

regimen in patients with advanced pancreatic cancer: a phase I/II trial. J Clin Oncol. 2011;29:4548–54.

27. Hidalgo M, Amant F, Biankin AV, et al. Patient-derived xenograft models: an emerging platform for translational cancer research. Cancer Discov. 2014;4:998–1013.

28. Thompson J, Stewart CF, Houghton PJ. Animal models for studying the action of topoisomerase I targeted drugs. Biochim Biophys Acta. 1998;1400:301–19.

29. Juvekar AS, Thompson J, Stewart CF, Hougton PJ. Preclinical models for evaluating topoisomerase I-targeted drugs. In: Adams VR, Burke TG, editors. Camptothecins in cancer therapy. Cancer Drug Discovery and Development. Totowa: Humana Press; 2005. p. 127–51.

30. Mohammad AS, Griffith JI, Adkins CE, et al. Liposomal irinotecan accumulates in metastatic lesions, crosses the blood-tumor barrier (BTB), and prolongs survival in an experimental model of brain metastases of triple negative breast cancer. Pharm Res. 2018;35:31.

31. Rothenberg ML, Kuhn JG, Schaaf LJ, et al. Alternative dosing schedules for irinotecan. Oncology (Williston Park). 1998;12:68–71.

32. Thompson J, Zamboni WC, Cheshire PJ, et al. Efficacy of oral irinotecan against neuroblastoma xenografts. Anticancer Drugs. 1997;8:313–22.

33. Blaney S, Berg SL, Pratt C, et al. A phase I study of irinotecan in pediatric patients: a pediatric oncology group study. Clin Cancer Res. 2001;7:32–7.

34. Gerrits CJ, Schellens JH, Burris H, et al. A comparison of clinical pharmacodynamics of different administration schedules of oral topotecan (Hycamtin). Clin Cancer Res. 1999;5:69–75.

35. Houghton PJ, Cheshire PJ, Hallman JD 2nd, et al. Efficacy of topoisomerase I inhibitors, topotecan and irinotecan, administered at low dose levels in protracted schedules to mice bearing xenografts of human tumors. Cancer Chemother Pharmacol. 1995;36:393–403.

36. Adiwijaya BS, Kim J, Lang I, et al. Population pharmacokinetics of liposomal irinotecan in patients with cancer. Clin Pharmacol Ther. 2017;102:997–1005.

37. Kang MH, Wang J, Makena MR, et al. Activity of MM-398, nanoliposomal irinotecan (nal-IRI), in Ewing's family tumor xenografts is associated with high exposure of tumor to drug and high SLFN11 expression. Clin Cancer Res. 2015;21:1139–50.

38. Goldwasser F, Bae I, Valenti M, Torres K, Pommier Y. Topoisomerase I-related parameters and camptothecin activity in the colon carcinoma cell lines from the National Cancer Institute anticancer screen. Cancer Res. 1995;55:2116–21.

39. Winkler C, Armenia J, Jones GN, et al. SLFN11 informs on standard of care and novel treatments in a wide range of cancer models. Br J Cancer. 2021;124:951–62.

40. Nassar D, Blanpain C. Cancer stem cells: basic concepts and therapeutic implications. Annu Rev Pathol. 2016;11:47–76.

41. Yang Y, Wang G, Zhu D, et al. Epithelial-mesenchymal transition and cancer stem cell-like phenotype induced by Twist1 contribute to acquired resistance to irinotecan in colon cancer. Int J Oncol. 2017;51:515–24.

42. Aston WJ, Hope DE, Nowak AK, et al. A systematic investigation of the maximum tolerated dose of cytotoxic chemotherapy with and without supportive care in mice. BMC Cancer. 2017;17:684.

43. Minderman H, Cao S, Rustman YM. Rational design of irinotecan administration based on preclinical models. Oncology (Williston Park). 1998;12:22–30.

44. ONIVYDE (Irinotecan liposome injection) pharmacology/toxicology NDA review and evaluation https://www.accessdata.fda.gov/drugsatfda_docs/nda/2015/207793Orig1s000PharmR.pdf. Accessed 29 June 2021.

45. Pfizer. Prescribing Information, CAMPTOSAR (irinotecan) injection, for intravenous use. US Food and Drug Administration https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/020571s051lbl.pdf. Accessed 29 June 2021.

46. Yamamoto M, Kurita A, Asahara T, et al. Metabolism of irinotecan and its active metabolite SN-38 by intestinal microflora in rats. Oncol Rep. 2008;20:727–30.

47. Kurita A, Kado S, Kaneda N, et al. Modified irinotecan hydrochloride (CPT-11) administration schedule improves induction of delayed-onset diarrhea in rats. Cancer Chemother Pharmacol. 2000;46:211–20.

48. Ikuno N, Soda H, Watanabe M, Oka M. Irinotecan (CPT-11) and characteristic mucosal changes in the mouse ileum and cecum. J Natl Cancer Inst. 1995;87:1876–83.

49. Voigt W, Matsui S, Yin MB, et al. Topoisomerase-I inhibitor SN-38 can induce DNA damage and chromosomal aberrations independent from DNA synthesis. Anticancer Res. 1998;18:3499–505.

50. Guo S, Jiang X, Mao B, Li QX. The design, analysis and application of mouse clinical trials in oncology drug development. BMC Cancer. 2019;19:718.