REVIEW ARTICLE

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Liposomal irinotecan (Onivyde): Exemplifying the benefits of nanotherapeutic drugs

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Abstract

Irinotecan is a topoisomerase inhibitor, widely used in treatment of malignancies including pancreatic ductal adenocarcinoma (PDAC) as part of the FOLFIRINOX regimen prescribed as a first-line treatment in several countries. However, irinotecan has not been successfully introduced as a second-line treatment for pancreatic cancer and few randomized clinical studies have evaluated its added value. Efficacy of liposomal irinotecan (nal-IRI) combined with 5-fluorouracil and leucovorin (5-FU/LV) was reported in the phase III NAPOLI-1 trial in metastatic PDAC following failure of gemcitabine-based therapy. Several features of nal-IRI pharmacokinetics (PK) could result in better outcomes versus nonliposomal irinotecan. Irinotecan is a prodrug that is converted to active SN-38 by carboxylesterase enzymes and inactivated by cytochrome P450 3A4/3A5. SN-38 is inactivated by UGT1A1 enzymes. Individual variations in their expression and activity could influence enhanced localized irinotecan activity and toxicity. Liposomal irinotecan exploits the enhanced permeability and retention effect in cancer, accumulating in tumor tissues. Liposomal irinotecan also has a longer half-life and higher area under the concentration-time curve (0–∞) than nonliposomal irinotecan, as the liposomal formulation protects cargo from premature metabolism in the plasma. This results in irinotecan activation in tumor tissue, leading to enhanced cytotoxicity. Importantly, despite the longer exposure, overall toxicity for nal-IRI is no worse than nonliposomal irinotecan. Liposomal irinotecan exemplifies how liposomal encapsulation of a chemotherapeutic agent can alter its PK properties, improving clinical outcomes for patients. Liposomal irinotecan is currently under investigation in other malignancies including biliary tract cancer (amongst other gastrointestinal cancers), brain tumors, and small-cell lung cancer.

KEYWORDS
carcinoma, pancreatic ductal, chemotherapy, drug delivery system, irinotecan, liposome

1 | INTRODUCTION

Liposomal carriers have been used to deliver anticancer drugs directly to tumors. Liposome deposition in tumors is thought to be facilitated by tumor blood vessel immaturity and leakiness, as well as by impaired lymphatic drainage at the tumor site (EPR). Reducing systemic drug exposure relative to tumor exposure in this manner might also improve safety.[1,2] Examples include liposomal doxorubicin and nal-IRI (Onivyde® Servier (outside the USA and Taiwan; Les Laborotoires Servier SAS, 50 Rue Carnot, Suresnes 92284, France); Ipsen (within the USA; 106 Allen Road, Basking Ridge, NJ 07920, USA); PharmaEngine (Taiwan; 11F, 10 Minsheng East Road, Sec. 3, Taipei 104, Taiwan)).[3,4]

Pancreatic ductal adenocarcinoma is a tumor with limited treatment options. Most patients present with metastatic disease at diagnosis and are ineligible for surgery. Advanced/metastatic pancreatic cancer is characterized by rapid clinical deterioration, thus chemotherapeutic treatment following progression on front-line therapy could be limited to palliative chemotherapy.[5] Second-line treatment options remain limited and depend on the first-line treatment used. Liposomal irinotecan is an IV liposomal formulation that encapsulates the TOP1 inhibitor irinotecan in a lipid bilayer vesicle.[6-8] Treatment with nal-IRI+5-FU/LV was associated with significantly improved outcomes for patients with mPDAC versus 5-FU/LV, including OS, median PFS, and ORR in the NAPOLI-1 study.[4] Recent data have also shown improved PFS and OS outcomes for patients with biliary tract cancer receiving nal-IRI+5-FU/LV as second-line therapy.[9]

We aim to provide an overview of the available PK data on nal-IRI, highlighting differences from nonliposomal irinotecan.

2 | IRINOTECAN AS AN ANTICANCER AGENT

In the US, irinotecan is indicated in first-line therapy for mCRC, with or without 5-FU/ LV, and in patients with recurrent or progressive disease following initial fluorouracil-based treatment.[10] Irinotecan has shown activity in other cancer types.[11-18] Clinical trial data for use of nonliposomal irinotecan as second-line therapy for PDAC after disease progression with gemcitabine-based treatment have shown varying degrees of activity.[19-22]

2.1 | METABOLISM OF IRINOTECAN

2.1.1 | Irinotecan activation and inactivation

Nonliposomal irinotecan is a water-soluble prodrug that inhibits the TOP1 cation, mainly through its active metabolite SN-38, by stabilizing the TOP1/DNA complex, leading to DNA strand breaks, cell replication inhibition, and eventual cell death.[23,24] SN-38 shows up to 1000-fold increased TOP1 inhibitory activity versus irinotecan (Figure 1).[24] Irinotecan and its metabolites are excreted through a hepatobiliary pathway into the feces and urine by ABC transporters.[25,26] The inactive SN-38 metabolite, SN-38G, can be reactivated to SN-38 by β-glucuronidases in the human colorectum. Increased levels of tumor β-glucuronidases could contribute to tumor SN-38 exposure in vivo.[27]

2.1.2 | Individual variations in enzymes involved in irinotecan metabolism

Irinotecan response varies among patients, possibly due to variation in expression of enzymes involved in its elimination. Liver CYP3A4 levels vary depending on environmental, rather than genetic, factors.[28] CYP3A4 status (defined as 6β-hydroxycortisol / cortisol ratio) was found to be a predictor of diarrhea in patients with mCRC receiving a combination of irinotecan and 5-FU.[29]

UGT1A enzymatic activity or expression levels show interpatient variability based on genetic predisposition. Examples include individuals with Gilbert's syndrome who typically carry *UGT1A1*28* promoter variants, or *UGT1A1*6*, *UGT1A7*3*, or *UGT1A9*1b* variants.[30] These factors are typically associated with reduced SN-38 glucuronidation rates with nonliposomal irinotecan, with increased risk for gastrointestinal and bone marrow toxicities.[31-35] *UGT1A1*28* is predictive of neutropenia in patients with mCRC receiving nonliposomal irinotecan and 5-FU, and is associated with elevated plasma bilirubin.[29]

Nonliposomal irinotecan-induced delayed-type diarrhea has been correlated with the presence of at least one *UGT1A1*28* allele.[36] *UGT1A1*28* 7/7 homozygosity is a risk factor for hematological toxicity in patients receiving irinotecan, depending on administration schedule.[37]

UGT1A1*28 homozygosity also correlates with SN-38 concentrations in a nonliposomal irinotecan dose-dependent manner.[31,38] *UGT1A1*6* polymorphisms are observed in East Asians, but not in Caucasians. Glucuronidation activity of *UGT1A1*6* is decreased to a similar extent as *UGT1A1*28*, and therefore, *UGT1A1*6* is as important as *UGT1A1*28* in East Asians. There are therefore gene–dose effects of *UGT1A1*6* or **28* on glucuronidation activity, SN-38 exposure, and neutropenia, as the two polymorphisms are mutually exclusive.[35] Although the effect of *UGT1A1*28* on nonliposomal irinotecan toxicity was not suggested at lower doses in Caucasian patients,[37] Asian patients harboring *UGT1A1*6* or **28* experienced severe irinotecan toxicity at lower doses.[35,39] It is recommended to reduce irinotecan dose for *UGT1A1*6* or **28* homozygotes and those harboring both *UGT1A1*6* and **28*[30,40]; the FDA recommended dose reduction is only for patients homozygous for *UGT1A1*28*.

Asian studies have focused on *UGT1A1* polymorphism-associated toxicity. A study of 48 patients from China, including 8 with unresectable PDAC and 12 with unresectable biliary tract cancer receiving FOLFIRI or irinotecan monotherapy, used direct sequencing to identify *UGT1A1*28/*6* polymorphisms.[41] Patients homozygous or heterozygous for *UGT1A1*6* polymorphisms were more likely to develop grade III/IV neutropenia versus patients with a WT
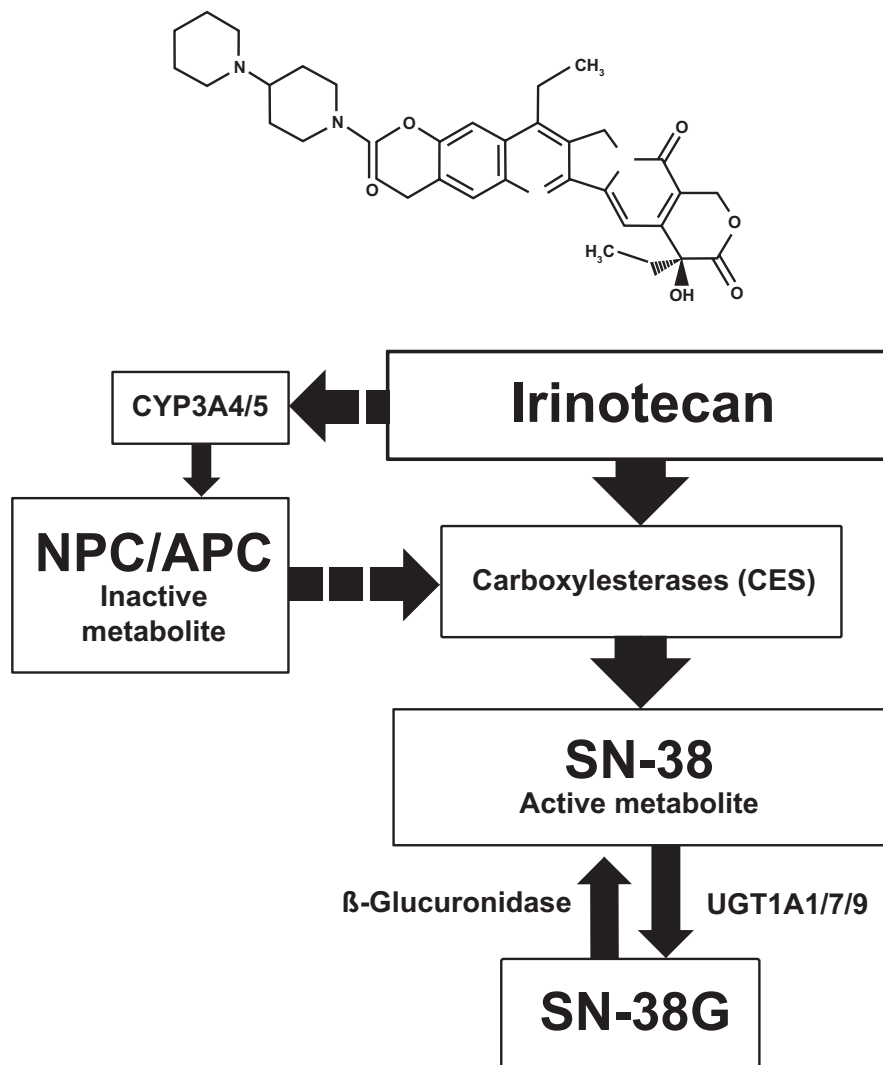
FIGURE 1 Overview of the metabolic pathway of irinotecan. In humans, the metabolic activation of irinotecan is primarily carried out through hydrolysis by carboxylesterase type 1 and 2 enzymes (CES1 and CES2, respectively), found in the liver, with activation also taking place in plasma, the small intestine, and tumor tissue.[65,66,69,70] CES2 is the major contributor of 7-ethyl-10-hydroxycamptothecin (SN-38) production from irinotecan and shows higher activity than CES1 in vitro.[71] In humans, irinotecan is metabolized and inactivated by cytochrome P450 3A4/3A5 (CYP3A4/5), which convert irinotecan to inactive 7-ethyl-10-[4-N-(5-aminopentanoicacid)-1-piperidino] carbonyloxycamptothecin (APC), 7-ethyl-10-(4-amino-1-piperidino)carbonyloxycamptothecin (NPC), and metabolites M2 and M4.[72] APC and NPC may be converted to SN-38 by CES.[73] Alternatively, SN-38 is inactivated by glucuronidation to a β-glucuronic acid conjugate (10-O-glucuronyl-SN-38 [SN-38G]) by hepatic uridine diphosphateglucuronosyl transferase 1A (UGT1A) enzymes, in particular, UGT1A1, UGT1A7, and UGT1A9.[32,74] SN-38G can be re-activated to SN-38 by β-glucuronidases present in the human colorectum, and increased levels of β-glucuronidases in tumors may contribute to tumor SN-38 exposure in vivo[27]

genotype. Patients heterozygous or homozygous for *UGT1A1*6* and *UGT1A1*28* polymorphisms were more likely to experience grade III/IV neutropenia versus patients with a double WT genotype.

The BioBank Japan project analyzed 651 patient records (102 cases and 549 controls of various malignancies), and found that *UGT1A1*6* homozygosity was predictive of adverse irinotecan reactions.[42] A meta-analysis of 1652 patient records from nine studies (eight from Asia) of patients with colorectal cancer associated *UGT1A1*6* polymorphism with late-onset diarrhea and severe neutropenia.[43] *ABCB1* (P-glycoprotein) gene polymorphisms affect renal irinotecan clearance, with the *ABCB1*8* genotype being independently associated with the irinotecan PK profile to a lesser

extent than *UGT1A1*28*.[44,45] One *CES2* promoter region SNP that appears to result in decreased enzyme activity, and therefore decreased irinotecan activation, has been identified.[46] Several SNPs identified in Japanese patients (1A>T; Met[1]Leu; 100 C>T, 424G>A, IVS8-2A>G) were found to reduce activity of CES2, either by reduced mRNA transcription or loss of enzyme activity.[47,48]

3 | LIPOSOMAL IRINOTECAN

nal-IRI consists of pegylated liposomal particles (111 nm diameter) encapsulating an irinotecan sucrosofate salt payload. The drug to

phospholipid ratio is 473 mg irinotecan-HCl/mmol phospholipid, and the phospholipid composition of the liposome is distearoylphosphatidylcholine, cholesterol, and pegylated 1,2-distearoyl-sn-glycero-3-phosphorylethanolamine in a molar ratio of 3:2:0.015.[7] Liposomal encapsulation keeps irinotecan in circulation for longer before metabolic conversion to its active metabolite SN-38, leading to an improved pharmacokinetic profile. Approximately 95% of the irinotecan payload is retained within liposomes 24 h after nal-IRI administration, allowing for high drug load and increased plasma $t_{1/2}$ versus nonliposomal irinotecan (Table 1).[7,49] Analysis of patient-derived PDAC xenografts in immunocompromised mice has shown that nal-IRI has a higher therapeutic index than nonliposomal irinotecan (20 vs. 5) and prolongs time to reach tumor volume of 600 mm^3 (90.5 vs. 60.6 days).[50]

Preclinical experiments using human histiocytic lymphoma cell lines (U937) indicated that TAMs, which express CES, can convert irinotecan to SN-38.[7] Liposomal irinotecan appears to preferentially accumulate in tumor tissue through EPR, resulting in gradual accumulation in the tumor stroma.[7] This results in continual SN-38 release through local TAM CES activity, as macrophages can take up nal-IRI liposomes, releasing their cargo and allowing access to CES enzymes. Tumor cells also express CES, and this CES might also be able to act on any irinotecan that has been locally released.[7] Variability in PDAC CES expression could thus influence the response to irinotecan-based treatments.[51] Conversely, nonliposomal irinotecan can be transported in and out of tissues with a short plasma $t_{1/2}$, reducing SN-38 duration in tumors. Additionally, neutral plasma pH results in lactone ring opening, decreasing topoisomerase inhibition.[47,49] In preclinical human colon (HT-29) and breast (BT474) cancer xenograft models, nal-IRI showed superior efficacy versus nonliposomal irinotecan.[49] In preclinical, ex vivo, time-course assays, nal-IRI conversion to SN-38 by nude mouse-derived macrophages required at least 24 h and was complete after 72 h.[49]

nal-IRI showed significant and lasting tumor growth inhibition in a preclinical HT-29 mouse xenograft model versus nonliposomal irinotecan.[7] Moreover, nal-IRI treatment resulted in longer intratumor SN-38 exposure and increased circulatory time in patient plasma compared with nonliposomal irinotecan, resulting in increased time above the tumor growth inhibition threshold. Computational PK modelling predicted that similar exposure of HT-29 mouse xenograft tumors to SN-38 from nal-IRI could be achieved at one-fifth of the dose versus nonliposomal irinotecan, with similar AUC, but longer tumor exposure above threshold and a higher efficacy (Table 2).[6,7,52] Similar effects have been seen in mouse xenograft models of breast cancer brain metastases.[53]

3.1 | Clinical PK properties of nal-IRI

Phase I PK data from patients with advanced solid tumors receiving nal-IRI (alone or with 5-FU/LV) showed a lower C_{max}, prolonged $t_{1/2}$, and higher SN-38 AUC (all in plasma) versus patients receiving nonliposomal irinotecan. Additionally, slow release of irinotecan from liposomes over time was suggested.[52,54]

In a phase II study in patients with gastric cancer, the SN-38 $t_{1/2}$ and AUC were increased with nal-IRI versus nonliposomal irinotecan, while a lower C_{max} was maintained.[6] Further analysis indicated that nal-IRI had a tIRI (sum of irinotecan in liposomes and free irinotecan) C_{max} 13.4-times higher, a $t_{1/2}$ 2.0-times longer, and an AUC from time 0–∞ 46.2-times greater than nonliposomal irinotecan.[55]

In a clinical trial that evaluated nal-IRI-mediated tumor delivery in biopsies collected 72 h following administration (70 mg/m^2), tumor tIRI was 0.5-times higher than that observed in plasma. Tumor tSN-38 was 6-times higher than in plasma, and the tumor tSN-38 : tIRI (a measure of the extent of conversion) was 8-times higher than in plasma.[8]

3.2 | Liposomal irinotecan exposure−efficacy association

In the NAPOLI-1 nal-IRI+5-FU/LV arm, longer OS and PFS were associated with higher C_{avg} of tIRI, tSN-38, and uSN-38, as well as with longer time when SN-38 is above the threshold concentration of 0.03 ng/ml ($t_{uSN38>thr}$), with the strongest association noted for $t_{uSN38>thr}$.[55] In a population PK modelling analysis of nal-IRI using

TABLE 1 Effects of the liposomal encapsulation of irinotecan in preclinical models[7]

Advantage of nal-IRI encapsulation	Nonliposomal irinotecan	nal-IRI
Prolonged exposure in plasma	Irinotecan and SN-38 cleared from circulation within 8 h	Irinotecan and SN-38 remained in circulation within >50 h
Prolonged exposure in tumor xenograft models	>90% irinotecan cleared from tumors in 24 h; SN-38 exposure in tumors <48 h	Irinotecan persisted in tumors at >10,000 nmol/L for 168 h; prolonged SN-38 exposure above activity threshold for up to 168 h
Dose needed to achieve similar SN-38 exposure in plasma and tumors in tumor xenograft models	50 mg/kg	10 mg/kg
Enhanced tumor growth inhibition in animal models	~40%	~110%

Abbreviations: nal-IRI, liposomal irinotecan; SN-38, 7-ethyl-10-hydroxycamptothecin.

TABLE 2 Overview of pharmacokinetic parameters (total irinotecan) for nonliposomal irinotecan and liposomal irinotecan (nal-IRI) in patients with locally advanced of metastatic gastric or gastroesophageal junction adenocarcinoma

	Nonliposomal irinotecan	nal-IRI
$AUC_{0-\infty}$ (plasma), h ng/ml[6]	24,155	1,651,508
C_{max} (plasma), ng/ml[6]	4,265	60,842
$t_{1/2}$ (plasma), h[6]	7.7	21.2

Note: $AUC_{0-\infty}$ (area under the concentration–time curve between $t = 0$ and $t = infinity$) and C_{max} (maximum concentration) were normalized to dose level in the source study. $t_{1/2}$ (half-life).

plasma samples from patients with various tumors (including colorectal, gastric, and pancreatic cancer) from six studies ($n = 353$), higher C_{avg} and longer $t_{uSN38>thr}$ was associated with longer OS and PFS in patients with PDAC receiving nal-IRI+5-FU/LV. This was also associated with an increased ORR; however, C_{max} was not associated with OS in these patients.[55]

3.3 | Liposomal irinotecan exposure–safety association

In a phase II study in patients with mPDAC receiving nal-IRI, pharmacogenetic analysis of patient samples ($n = 28$) for genetic polymorphisms in *UGT1A1* and *UGT1A9* did not find any correlation with toxicities, although the patient numbers are likely too small to identify any relationship.[56] In the NAPOLI-1 trial, of seven patients positive for the *UGT1A1*28* polymorphism, five began treatment at a reduced starting dose and received the full planned dose of nal-IRI in subsequent treatment cycles.[4]

A recent population PK analysis found that *UGT1A1*28* was not a significant predictor of SN-38 levels following a nal-IRI dose, with the authors proposing that liposomal encapsulation lowered irinotecan release rate, avoiding increased plasma SN-38.[55] Additionally, a higher probability for neutropenia incidence and severity with higher uSN-38 C_{max} was observed. The association with neutropenia was stronger for uSN-38 C_{max} than for tSN-38 C_{max}.[55] It was also stronger for uSN-38 C_{max} than C_{avg}. A higher incidence and severity of diarrhea was associated with higher tIRI C_{max}. This effect was observed in Asian subpopulations but mostly in Caucasians. It is important to note that *UGT1A1*6* polymorphism was not assessed, despite 42% patients in the study being of East Asian origin.

In NAPOLI-1, this association was only observed in the nal-IRI monotherapy arm, presumably due to the higher nal-IRI dose used (100 mg/m^2 every 3 weeks vs. 70 mg/m^2 nal-IRI every 2 weeks in the nal-IRI+5-FU/LV combination arm), resulting in higher tIRI C_{max} for patients receiving nal-IRI monotherapy.[4,55] Differences in observed neutropenia and diarrhea rates among Caucasian and Asian patients in NAPOLI-1 can be attributed to racial differences in the tIRI and uSN-38 C_{max}, and potentially *UGT1A1*6* genotypes that were not

detected due to the study design.[55] Japanese patients experienced more FOLFIRINOX (i.e., including nonliposomal irinotecan) related toxicities than Caucasian patients despite exclusion of patients homozygous for *UGT1A1*28/*6* polymorphisms (or heterozygous for both).[57]

A phase II study of nal-IRI+5-FU/LV treatment in Japanese patients with mPDAC found no unexpected increases in rates of diarrhea or neutropenia versus the NAPOLI-1 trial.[58] Importantly, only three patients in the nal-IRI+5-FU/LV arm had relevant *UGT1A1* mutations. The nal-IRI dose was the same in both this study and NAPOLI-1 (80 mg/m^2, equivalent to 70 mg/m^2 irinotecan free base), however the nal-IRI dose was reduced for patients homozygous for *UGT1A1*28* and *UGT1A1*6* polymorphisms.

Dose modification did not significantly influence survival outcomes in a post-hoc analysis of patients from NAPOLI-1 who underwent protocol-specified dose modification; for example, OS with dose modification was 8.4 months versus 6.7 months without (hazard ratio 0.89; 95% confidence interval, 0.59–1.35),[59] suggesting nal-IRI dose modification is a feasible strategy to maintain clinical benefit. In Japan, dosing of nal-IRI is reduced according to *UGT1A1* genotype[58]; this aligns with the NAPOLI-1 study where patients homozygous for the *UGT1A1*28* allele were initially treated with a 20 mg/m^2 dose reduction of nal-IRI before building up to a full dose in the absence of any toxic effects.

3.4 | Potential for future clinical development of nal-IRI

The properties of nal-IRI have made it attractive for targeting various cancers. A retrospective study of 14 patients with metastatic biliary tract cancer showed that second-line treatment with nal-IRI+5-FU/LV resulted in half of the patients achieving disease control, suggesting efficacy in this population.[60] The phase II NIFE trial is currently underway, comparing use of nal-IRI+5-FU/LV with gemcitabine + cisplatin for treatment of locally advanced or metastatic biliary tract adenocarcinoma[61]; the NALIRICC trial is also ongoing, comparing nal-IRI+5-FU/LV with 5-FU/LV in biliary tract cancer.[60] Data from the NIFTY trial have recently been presented, with improved OS and PFS outcomes for patients with biliary tract cancer receiving nal-IRI+5-FU/LV as a second-line treatment.[9] Data from preclinical models of small-cell lung cancer has shown that nal-IRI has antitumor activity at clinically relevant dose levels, with partial or complete responses observed in tumors derived from several cell line models, and improved survival outcomes in mouse models (vs. irinotecan and topotecan).[62] Liposomal irinotecan also had activity in the second-line setting following topotecan failure.[62]

Preclinical data from animal models of breast cancer brain metastases showed that liposomes preferentially accumulate in metastatic lesions after crossing the blood–brain barrier.[53] Moreover, nal-IRI treatment results in increased accumulation of both irinotecan and SN-38 in brain metastases. A phase I study of nal-IRI in 29 patients with metastatic breast cancer showed that patients with

central nervous system disease ($n = 10$) receiving nal-IRI had an ORR of 30%, indicating that nal-IRI could be a viable treatment option in this population.[63]

A phase I dose escalation study was carried out in patients with high-grade glioma, with WT *UGT1A1* ($n = 16$) versus *UGT1A1*28* heterozygotes ($n = 18$); this found a maximum tolerated nal-IRI dose of 120 mg/m^2 for WT, and 150 mg/m^2 for heterozygous patients, with nal-IRI safety and toxicity signals similar to previous observations.[64] Further investigation will support understanding of potential nal-IRI activity in this context.

Expression profiling using a variety of human tumor tissue and liver samples showed that CES2 expression correlated with irinotecan conversion to SN-38.[65] High CES2 expression levels in tumor tissue correlated with increased OS in patients with resectable and borderline resectable PDAC receiving neoadjuvant therapy with FOLFIRINOX (combination of irinotecan, 5-FU, oxaliplatin, and LV).[51] High levels of CES activity in the small intestine suggest that irinotecan-associated delayed diarrhea is at least partly caused by local conversion of irinotecan to SN-38.[66] A better understanding of CES2 levels could support individualized dosing of nal-IRI.

Cationic liposomes can stimulate dendritic cell activation in vitro, potentially by promoting expression of costimulatory molecules.[67] This might create opportunities to combine liposomal formulations such as nal-IRI with immune checkpoint inhibitors.[68] However, immune recognition of the liposomal formulation could result in clearance of the drug and therefore reduced delivery of the irinotecan payload.

4 | CONCLUSION

The advent of liposomal agents such as nal-IRI has led to improvements in drug formulations with altered PK profiles compared with their parent compounds, which translate into different efficacy and safety profiles in clinical practice. Compared with nonliposomal irinotecan, nal-IRI generally shows increased exposure and prolonged retention (with a preference for accumulation in tumor cells), and has been shown to have increased plasma half-life among other desirable PK properties in multiple preclinical, in silico, and clinical studies. Ongoing refinement of delivery modes at the nano scale holds the promise that compounds with undesirable toxicity could become targetable at the tumor environment, reducing the incidence of negative off-target effects and improving efficacy.

CONFLICTS OF INTEREST
GM has received honoraria from Servier for advisory board meetings and conferences. FI holds a patent for *UGT1A1* genotyping and is an Abbvie employee and stockholder. HM is an editorial board member of *Cancer Science*.

AUTHOR CONTRIBUTIONS
GM, FI, and HM contributed to the literature searches, drafting the manuscript, and critically reviewing the content. GM, FI, and HM approved the final version of the manuscript for submission.

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REFERENCES
1. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. *Nat Nanotechnol*. 2007;2:751-760.
2. Hashizume H, Baluk P, Morikawa S, et al. Openings between defective endothelial cells explain tumor vessel leakiness. *Am J Pathol*. 2000;156:1363-1380.
3. Barenholz Y. Doxil® — the first FDA-approved nano-drug: lessons learned. *J Control Release*. 2012;160:117-134.
4. Wang-Gillam A, Li CP, Bodoky G, et al. Nanoliposomal irinotecan with fluorouracil and folinic acid in metastatic pancreatic cancer after previous gemcitabine-based therapy (NAPOLI-1): a global, randomised, open-label, phase 3 trial. *Lancet*. 2016;387:545-557.
5. Ducreux M, Cuhna AS, Caramella C, et al. Cancer of the pancreas: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2015;26(Suppl 5):v56-68.
6. Roy AC, Park SR, Cunningham D, et al. 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. *Ann Oncol*. 2013;24:1567-1573.
7. 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;74:7003-7013.
8. Ramanathan RK, Korn RL, Raghunand N, et al. Correlation between ferumoxytol uptake in tumor lesions by MRI and response to nanoliposomal irinotecan in patients with advanced solid tumors: a pilot study. *Clin Cancer Res*. 2017;23:3638-3648.
9. Yoo C, Kim K-P, Kim I, et al. Liposomal irinotecan (nal-IRI) in combination with fluorouracil (5-FU) and leucovorin (LV) for patients with metastatic biliary tract cancer (BTC) after progression on gemcitabine plus cisplatin (GemCis): multicenter comparative randomized phase 2b study (NIFTY). *J Clin Oncol*. 2021;39:Abstract 4006.
10. Pfizer. Camptosar® (Pfizer), US Prescribing Information.
11. Verschraegen CF. Irinotecan for the treatment of cervical cancer. *Oncology (Williston Park)*. 2002;16:32-34.
12. Langer CJ. The global role of irinotecan in the treatment of lung cancer: 2003 update. *Oncology (Williston Park)*. 2003;17:30-40.
13. Perez EA, Hillman DW, Mailliard JA, et al. Randomized phase II study of two irinotecan schedules for patients with metastatic breast cancer refractory to an anthracycline, a taxane, or both. *J Clin Oncol*. 2004;22:2849-2855.
14. Bouché O, Raoul JL, Bonnetain F, et al. Randomized multicenter phase II trial of a biweekly regimen of fluorouracil and leucovorin (LV5FU2), LV5FU2 plus cisplatin, or LV5FU2 plus irinotecan in patients with previously untreated metastatic gastric cancer: a Federation Francophone de Cancerologie Digestive Group Study–FFCD 9803. *J Clin Oncol*. 2004;22:4319-4328.
15. Tsuda H, Takatsuki K, Ohno R, et al. Treatment of adult T-cell leukaemia-lymphoma with irinotecan hydrochloride (CPT-11).

CPT-11 Study Group on Hematological Malignancy. *Br J Cancer*. 1994;70:771-774.

16. Ohno R, Okada K, Masaoka T, et al. An early phase II study of CPT-11: a new derivative of camptothecin, for the treatment of leukemia and lymphoma. *J Clin Oncol*. 1990;8:1907-1912.

17. Wagner LM. Fifteen years of irinotecan therapy for pediatric sarcoma: where to next? *Clin Sarcoma Res*. 2015;5:20.

18. Palmerini E, Jones RL, Setola E, et al. Irinotecan and temozolomide in recurrent Ewing sarcoma: an analysis in 51 adult and pediatric patients. *Acta Oncol*. 2018;57:958-964.

19. Ulrich-Pur H, Raderer M, Verena Kornek G, et al. Irinotecan plus raltitrexed vs raltitrexed alone in patients with gemcitabine-pretreated advanced pancreatic adenocarcinoma. *Br J Cancer*. 2003;88:1180-1184.

20. Yoo C, Hwang JY, Kim JE, et al. A randomised phase II study of modified FOLFIRI.3 vs modified FOLFOX as second-line therapy in patients with gemcitabine-refractory advanced pancreatic cancer. *Br J Cancer*. 2009;101:1658-1663.

21. Yi SY, Park YS, Kim HS, et al. Irinotecan monotherapy as second-line treatment in advanced pancreatic cancer. *Cancer Chemother Pharmacol*. 2009;63:1141-1145.

22. Zaniboni A, Aitini E, Barni S, et al. FOLFIRI as second-line chemotherapy for advanced pancreatic cancer: a GISCAD multicenter phase II study. *Cancer Chemother Pharmacol*. 2012;69:1641-1645.

23. Kaneda N, Nagata H, Furuta T, Yokokura T. Metabolism and pharmacokinetics of the camptothecin analogue CPT-11 in the mouse. *Cancer Res*. 1990;50:1715-1720.

24. 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;51:4187-4191.

25. Slatter JG, Schaaf LJ, Sams JP, et al. Pharmacokinetics, metabolism, and excretion of irinotecan (CPT-11) following I.V. infusion of [(14) C]CPT-11 in cancer patients. *Drug Metab Dispos*. 2000;28:423-433.

26. de Jong FA, Kitzen JJ, de Bruijn P, Verweij J, Loos WJ. Hepatic transport, metabolism and biliary excretion of irinotecan in a cancer patient with an external bile drain. *Cancer Biol Ther*. 2006;5:1105-1110.

27. Tobin P, Clarke S, Seale JP, et al. The in vitro metabolism of irinotecan (CPT-11) by carboxylesterase and beta-glucuronidase in human colorectal tumours. *Br J Clin Pharmacol*. 2006;62:122-129.

28. Lamba JK, Lin YS, Schuetz EG, Thummel KE. Genetic contribution to variable human CYP3A-mediated metabolism. *Adv Drug Deliv Rev*. 2002;54:1271-1294.

29. Rouits E, Charasson V, Pétain A, et al. Pharmacokinetic and pharmacogenetic determinants of the activity and toxicity of irinotecan in metastatic colorectal cancer patients. *Br J Cancer*. 2008;99:1239-1245.

30. Fujiwara Y, Minami H. An overview of the recent progress in irinotecan pharmacogenetics. *Pharmacogenomics*. 2010;11:391-406.

31. Iyer L, Das S, Janisch L, et al. UGT1A1*28 polymorphism as a determinant of irinotecan disposition and toxicity. *Pharmacogenomics J*. 2002;2:43-47.

32. Iyer L, King CD, Whitington PF, et al. Genetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. *J Clin Invest*. 1998;101:847-854.

33. Rivory LP, Haaz MC, Canal P, Lokiec F, Armand JP, Robert J. Pharmacokinetic interrelationships of irinotecan (CPT-11) and its three major plasma metabolites in patients enrolled in phase I/II trials. *Clin Cancer Res*. 1997;3:1261-1266.

34. Zhang X, Yin JF, Zhang J, Kong SJ, Zhang HY, Chen XM. UGT1A1*6 polymorphisms are correlated with irinotecan-induced neutropenia: a systematic review and meta-analysis. *Cancer Chemother Pharmacol*. 2017;80:135-149.

35. Minami H, Sai K, Saeki M, et al. Irinotecan pharmacokinetics/pharmacodynamics and UGT1A genetic polymorphisms in Japanese: roles of UGT1A1*6 and *28. *Pharmacogenet Genomics*. 2007;17:497-504.

36. de Jong FA, Kehrer DF, Mathijssen RH, et al. Prophylaxis of irinotecan-induced diarrhea with neomycin and potential role for UGT1A1*28 genotype screening: a double-blind, randomized, placebo-controlled study. *Oncologist*. 2006;11:944-954.

37. Hoskins JM, Goldberg RM, Qu P, Ibrahim JG, McLeod HL. UGT1A1*28 genotype and irinotecan-induced neutropenia: dose matters. *J Natl Cancer Inst*. 2007;99:1290-1295.

38. Stewart CF, Panetta JC, O'Shaughnessy MA, et al. UGT1A1 promoter genotype correlates with SN-38 pharmacokinetics, but not severe toxicity in patients receiving low-dose irinotecan. *J Clin Oncol*. 2007;25:2594-2600.

39. Han JY, Lim HS, Shin ES, et al. Comprehensive analysis of UGT1A polymorphisms predictive for pharmacokinetics and treatment outcome in patients with non-small-cell lung cancer treated with irinotecan and cisplatin. *J Clin Oncol*. 2006;24:2237-2244.

40. Pharmaceutical and Medical Devices Agency. PMDA Review Report of Pembrolizumab; 2013.

41. Yang C, Liu Y, Xi WQ, et al. Relationship between UGT1A1*6/*28 polymorphisms and severe toxicities in Chinese patients with pancreatic or biliary tract cancer treated with irinotecan-containing regimens. *Drug Des Devel Ther*. 2015;9:3677-3683.

42. Hikino K, Ozeki T, Koido M, et al. Comparison of effects of UGT1A1*6 and UGT1A1*28 on irinotecan-induced adverse reactions in the Japanese population: analysis of the Biobank Japan Project. *J Hum Genet*. 2019;64:1195-1202.

43. Zhu X, Ma R, Ma X, Yang G. Association of UGT1A1*6 polymorphism with irinotecan-based chemotherapy reaction in colorectal cancer patients: a systematic review and a meta-analysis. *Biosci Rep*. 2020;40:BSR20200576.

44. Sai K, Kaniwa N, Itoda M, et al. Haplotype analysis of ABCB1/MDR1 blocks in a Japanese population reveals genotype-dependent renal clearance of irinotecan. *Pharmacogenetics*. 2003;13:741-757.

45. Mathijssen RH, de Jong FA, van Schaik RH, et al. Prediction of irinotecan pharmacokinetics by use of cytochrome P450 3A4 phenotyping probes. *J Natl Cancer Inst*. 2004;96:1585-1592.

46. Charasson V, Bellott R, Meynard D, Longy M, Gorry P, Robert J. Pharmacogenetics of human carboxylesterase 2, an enzyme involved in the activation of irinotecan into SN-38. *Clin Pharmacol Ther*. 2004;76:528-535.

47. Kubo T, Kim SR, Sai K, et al. Functional characterization of three naturally occurring single nucleotide polymorphisms in the CES2 gene encoding carboxylesterase 2 (HCE-2). *Drug Metab Dispos*. 2005;33:1482-1487.

48. Kim SR, Sai K, Tanaka-Kagawa T, et al. Haplotypes and a novel defective allele of CES2 found in a Japanese population. *Drug Metab Dispos*. 2007;35:1865-1872.

49. Drummond DC, Noble CO, Guo Z, Hong K, Park JW, Kirpotin DB. Development of a highly active nanoliposomal irinotecan using a novel intraliposomal stabilization strategy. *Cancer Res*. 2006;66:3271-3277.

50. Barbier S, Lezmi S, Beaufils B, et al. Differentiation of liposomal irinotecan from dose-dense non-liposomal irinotecan in patient-derived pancreatic cancer xenograft tumor models. *J Clin Oncol*. 2020;38:e16724.

51. Capello M, Lee M, Wang H, et al. Carboxylesterase 2 as a determinant of response to irinotecan and neoadjuvant FOLFIRINOX therapy in pancreatic ductal adenocarcinoma. *J Natl Cancer Inst*. 2015;107:djv132.

52. Chiang NJ, Chao TY, Hsieh RK, et al. A phase I dose-escalation study of PEP02 (irinotecan liposome injection) in combination with 5-fluorouracil and leucovorin in advanced solid tumors. *BMC Cancer*. 2016;16:907.

53. 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.

54. Chang TC, Shiah HS, Yang CH, et al. Phase I study of nanoliposomal irinotecan (PEP02) in advanced solid tumor patients. *Cancer Chemother Pharmacol.* 2015;75:579-586.

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

56. Ko AH, Tempero MA, Shan YS, et al. A multinational phase 2 study of nanoliposomal irinotecan sucrosofate (PEP02, MM-398) for patients with gemcitabine-refractory metastatic pancreatic cancer. *Br J Cancer.* 2013;109:920-925.

57. Okusaka T, Ikeda M, Fukutomi A, et al. Phase II study of FOLFIRINOX for chemotherapy-naïve Japanese patients with metastatic pancreatic cancer. *Cancer Sci.* 2014;105:1321-1326.

58. Ueno M, Nakamori S, Sugimori K, et al. nal-IRI+5-FU/LV versus 5-FU/LV in post-gemcitabine metastatic pancreatic cancer: randomized phase 2 trial in Japanese patients. *Cancer Med.* 2020;9:9396-9408.

59. Chen LT, Macarulla T, Blanc JF, et al. Early dose reduction/delay and the efficacy of liposomal irinotecan with fluorouracil and leucovorin in metastatic pancreatic ductal adenocarcinoma (mPDAC): a post hoc analysis of NAPOLI-1. *Pancreatology.* 2021;21:192-199.

60. Taghizadeh H, Unseld M, Schmiderer A, et al. First evidence for the antitumor activity of nanoliposomal irinotecan with 5-fluorouracil and folinic acid in metastatic biliary tract cancer. *Cancer Chemother Pharmacol.* 2020;86:109-115.

61. Perkhofer L, Berger AW, Beutel AK, et al. Nal-IRI with 5-fluorouracil (5-FU) and leucovorin or gemcitabine plus cisplatin in advanced biliary tract cancer – the NIFE trial (AIO-YMO HEP-0315) an open label, non-comparative, randomized, multicenter phase II study. *BMC Cancer.* 2019;19:990.

62. Leonard SC, Lee H, Gaddy DF, et al. Extended topoisomerase 1 inhibition through liposomal irinotecan results in improved efficacy over topotecan and irinotecan in models of small-cell lung cancer. *Anticancer Drugs.* 2017;28:1086-1096.

63. Sachdev JC, Munster P, Northfelt DW, et al. Phase I study of liposomal irinotecan in patients with metastatic breast cancer: findings from the expansion phase. *Breast Cancer Res Treat.* 2021;185:759-771.

64. Clarke JL, Molinaro AM, Cabrera JR, et al. A phase 1 trial of intravenous liposomal irinotecan in patients with recurrent high-grade glioma. *Cancer Chemother Pharmacol.* 2017;79:603-610.

65. Xu G, Zhang W, Ma MK, McLeod HL. Human carboxylesterase 2 is commonly expressed in tumor tissue and is correlated with activation of irinotecan. *Clin Cancer Res.* 2002;8:2605-2611.

66. Khanna R, Morton CL, Danks MK, Potter PM. Proficient metabolism of irinotecan by a human intestinal carboxylesterase. *Cancer Res.* 2000;60:4725-4728.

67. Vangasseri DP, Cui Z, Chen W, Hokey DA, Falo LD Jr, Huang L. Immunostimulation of dendritic cells by cationic liposomes. *Mol Membr Biol.* 2006;23:385-395.

68. Inglut CT, Sorrin AJ, Kuruppu T, et al. Immunological and toxicological considerations for the design of liposomes. *Nanomaterials (Basel).* 2020;10:190.

69. Guichard S, Terret C, Hennebelle I, et al. CPT-11 converting carboxylesterase and topoisomerase activities in tumour and normal colon and liver tissues. *Br J Cancer.* 1999;80:364-370.

70. Shingyoji M, Takiguchi Y, Watanabe-Uruma R, et al. In vitro conversion of irinotecan to SN-38 in human plasma. *Cancer Sci.* 2004;95:537-540.

71. Senter PD, Beam KS, Mixan B, Wahl AF. Identification and activities of human carboxylesterases for the activation of CPT-11, a clinically approved anticancer drug. *Bioconjug Chem.* 2001;12:1074-1080.

72. Santos A, Zanetta S, Cresteil T, et al. Metabolism of irinotecan (CPT-11) by CYP3A4 and CYP3A5 in humans. *Clin Cancer Res.* 2000;6:2012-2020.

73. Sanghani SP, Quinney SK, Fredenburg TB, Davis WI, Murry DJ, Bosron WF. Hydrolysis of irinotecan and its oxidative metabolites, 7-ethyl-10-[4-n-(5-aminopentanoic acid)-1-piperidino] carbonyloxycamptothecin and 7-ethyl-10-[4-(1-piperidino)-1-amino]-carbonyloxycamptothecin, by human carboxylesterases ces1a1, CES2, and a newly expressed carboxylesterase isoenzyme, CES3. *Drug Metab Dispos.* 2004;32:505-511.

74. de Jong FA, de Jonge MJ, Verweij J, Mathijssen RH. Role of pharmacogenetics in irinotecan therapy. *Cancer Lett.* 2006;234:90-106.