Molecular Cancer Therapeutics

Selective and Concentrated Accretion of SN-38 with a CEACAM5-Targeting Antibody–Drug Conjugate (ADC), Labetuzumab Govitecan (IMMU-130)

Robert M. Sharkey, Serengulam V. Govindan, Thomas M. Cardillo, Jennifer Donnell, Jing Xia, Edmund A. Rossi, Chien-Hsing Chang, and David M. Goldenberg

Abstract

Labetuzumab govitecan (IMMU-130), an antibody–drug conjugate (ADC) with an average of 7.6 SN-38/IgG, was evaluated for its potential to enhance delivery of SN-38 to human colonic tumor xenografts. Mice bearing LS174T or GW-39 human colonic tumor xenografts were injected with irinotecan or IMMU-130 (SN-38 equivalents ∼500 or ∼16 μg, respectively). Serum and homogenates of tumors, liver, and small intestine were extracted, and SN-38, SN-38G (glucuronidated SN-38), and irinotecan concentrations determined by reversed-phase HPLC. Irinotecan cleared quickly from serum, with only 1% to 2% injected dose/mL after 5 minutes; overall, approximately 20% was converted to SN-38 and SN-38G. At 1 hour with IMMU-130, 45% to 63% injected dose/mL of the SN-38 was in the serum, with >90% bound to the ADC over 3 days, and with low levels of SN-38G. Total SN-38 levels decreased more quickly than the IgG, confirming a gradual SN-38 release from the ADC. AUC analysis found that SN-38 levels were approximately 11- and 16-fold higher in LS174T and GW-39 tumors, respectively, in IMMU-130–treated animals. This delivery advantage is amplified >30-fold when normalized to SN-38 equivalents injected for each product. Levels of SN-38 and SN-38G were appreciably lower in the liver and small intestinal contents in animals given IMMU-130. On the basis of the SN-38 equivalents administered, IMMU-130 potentially delivers >300-fold more SN-38 to CEA-producing tumors compared with irinotecan, while also reducing levels of SN-38 and SN-38G in normal tissues. These observations are consistent with preclinical and clinical data showing efficacy and improved safety.
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Introduction

The development of antibody–drug conjugates (ADC) can be traced back to early studies in the 1970s through the 1990s (1–4), when the typical drugs in clinical use were conjugated to various antibodies, and even studied in limited cases clinically (BR96; ref. 5). Two to three decades later, the science has advanced sufficiently for this class of agents to gain increased attention, measured by more than 60 ADCs in clinical development and three approved in recent years (6). In the ensuing time, each facet of the ADC, the antibody, the drug, and the linker, received attention to overcome the limitations of the early ADCs developed (6–8). Suffice it to summarize that diverse antibodies that show tumor cell internalization and relative tumor selectivity against high-density surface proteins, ultratoxic agents with subnanomolar to picomolar toxicity, and the use of serum-stable linkers became the paradigm. Despite such improvements, this first generation of ADCs still showed some off-target toxicities, free-drug toxicities even with stable linkages because of their extreme toxicity, and limited numbers of doses tolerated and a relatively low drug–antibody ratio (DAR) of less than 4 (6, 8).

Departing from this dogma, we asked whether a moderately toxic drug, with known clinical metabolism and toxicity profile, could be conjugated with a high DAR in a site-specific method, to an antibody that releases the drug both in the tumor microenvironment as well as internally at relatively high ADC doses, which can be given repeatedly because of a less toxic drug being released into the serum and in any off-target sites. The drug chosen was SN-38 (7-ethyl-10-hydroxycamptothecin), the metabolite of irinotecan (CPT-11; IRI) that has 3 logs higher potency than the prodrug, and where the pharmacological disposition and toxicity profile are well-known clinically (9), and with potency in the single-digit nanomolar level (not ultratoxic), thus not causing the off-target toxicities resulting from use of ultratoxic drugs (6). Our empirical studies also showed that a moderately stable linker would be preferred in terms of therapeutic outcome and safety (10, 11).

Using this new ADC platform, we have developed and studied two such agents clinically, one targeting Trop-2 in several epithelial cancers, sacituzumab govitecan (12–14), and the second

Immunomedics, Inc, Morris Plains, New Jersey.

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Corresponding Author: David M. Goldenberg, Immunomedics, Inc., 300 The American Road, Morris Plains, NJ 07950. Phone: 973-605-8200; Fax: 973-543-0607; E-mail: dmg.gscancer@att.net

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targeting CEACAM5 (carcinoembryonic antigen-related cell adhesion molecule-5), labetuzumab govitecan (IMMU-130), which is the conventional CEA (CD66e; ref. 15) measured in plasma as a diagnostic marker for colorectal cancer and also in a variety of epithelial cancers (16, 17). CEA is a 200-kDa glycoprotein first described in 1965 by Gold and Freedman as specific for fetal colon and colorectal adenocarcinoma (16), but later shown to be expanded in cancer expression, such as by breast, lung, pancreas, liver, stomach, medullary thyroid, bladder, prostate, and ovarian cancers (18, 19). Therefore, CEACAM5 has been a therapeutic target for radiolabeled antibodies (20–23). Labetuzumab govitecan has also been evaluated in human colonic and pancreatic cancer xenografts preclinically, showing selective therapeutic efficacy at nontoxic doses that were a fraction of the irinotecan doses given (10, 24).

Labetuzumab govitecan has completed phase II trials in patients with advanced, heavily pretreated, colorectal cancers, where it is showing good safety and encouraging activity (25, 26). Because labetuzumab govitecan is intended for further clinical development, this study addresses its pharmacodynamics in two colonic cancer xenograft models that express copious amounts of CEACAM5, comparing the accretion of SN-38 derived from the ADC with that from its parental drug, irinotecan. Of particular interest was to determine whether an antibody to CEACAM5, which is not noted for its internalization as compared with sacituzumab govitecan, targeting Trop-2, would be comparable or poorer than the latter in delivery and accretion of SN-38 by the tumor.

Materials and Methods

Reagents

Labetuzumab govitecan (IMMU-130) was prepared from the humanized anti-CEACAM5 IgG (hMN-14; ref. 27), with a CL2A-SN-38 drug carrier conjugated at DAR of 7.6, as described previously for a similar ADC, IMMU-132 (sacituzumab govitecan; refs. 28, 29), and containing an estimated 16 μg SN-38/mg IgG) on the basis of spectrophotometric analysis. The structure of the CL2A-SN-38 linker–drug molecule, along with a schematic illustrating its attachment to the hMN-14 IgG, is shown in Supplementary Fig. S1. The lyophilized product was reconstituted in sterile saline immediately before use. Irinotecan was purchased from AREVA pharmaceuticals, Inc., and diluted in sterile saline to the desired concentration. Standards for SN-38, SN-38G, and irinotecan used for calibration of HPLC columns were purchased from Toronto Research Chemicals.

Analytic studies

SDS-PAGE profiles of the unconjugated and conjugated antibody, as well as cell binding studies for determination of dissociation constants, are described in the Supplementary Data (Materials and Methods). The rate of SN-38 release from IMMU-130 in mouse, human, and fetal calf serum (FCS) was determined as described previously (24, 28). SN-38 release also was examined in the presence of rabbit carboxylesterase (Sigma-Aldrich), comparing the ADC with irinotecan. Briefly, 120 μL of rabbit carboxylesterase (12.6 mg/mL) was added to IMMU-130 (300 μg) or irinotecan (20 μg) in 1.0 mL of 40 mmol/L PBS, pH 7.4, and incubated at 37°C. Twenty-microliter samples were removed at 1, 24, 48, 72, 96, and 168 hours, with the addition of 10 μL of 10-hydroxy camptothecin (internal standard), 20 μL of 25% human serum albumin (HSA; ZLB Bioplasma AG) to aid in precipitation, and 20 μL of the protein precipitating reagent, zinc sulfate (1 mol/L)/ethylene glycol/methanol (1:1:1). After centrifugation to remove precipitated proteins, a portion of the organic phase was analyzed for SN-38 content by reversed-phase HPLC (30). Similar procedures were used for stability determinations in other biological media. In other studies, SN-38 or the ADC was placed in PBS buffer containing HSA, and extracted with protein-precipitating-reagent for analysis of SN-38, as described previously (30). A portion of the ADC sample was hydrolyzed overnight at 50°C in an equal volume of 6.0 mol/L HCl, neutralized with NaOH, and then extracted to derive total SN-38 content.

The stability of SN-38's lactone ring was assessed by reversed-phase HPLC, using the maleimide-capped CL2A -SN-38, further amidated at the lysine ε-amine of the CL2A linker, as described in the Supplementary Fig. S2. This derivative of SN-38 was added to 40 mmol/L PBS, pH 7.4, containing DMSO and added to a temperature-controlled HPLC autosampler, where it was incubated at 37°C for the times indicated before being loaded on the HPLC column. Additional details of the analytic methodology are given in Supplementary Data (Materials and Methods).

Animal studies and tissue analysis

All animal studies were approved by Rutgers School of Biomedical and Health Sciences Institutional Animal Care and Use Committee. Female NCr athymic nude (*nu/nu*) mice (4–8-weeks-old; Taconic Farms) were implanted subcutaneously with the LS174T (ATCC) or GW-39 (31) human colonic tumor cell lines. LS174T cells were authenticated by short tandem repeat (STR) assay by the ATCC and routinely tested for *Mycoplasma*. GW-39, a signet-ring colon cancer cell line (31), is maintained by serial propagation in nude mice. When tumors were well-formed (estimated to be ~0.5 g), animals were injected intravenously (0.2 mL) with a fixed amount of IMMU-130 (1.0 mg), whereas irinotecan was given at a dose of 40 mg/kg, using the average pre-study weight of the animals to determine a fixed dose for that group (animals in Study 1 bearing LS174T xenografts received 840 μg of irinotecan, while in Study 2, mice bearing GW-39 were administered 900 μg of irinotecan). By mass, 1.0 mg of irinotecan contains approximately 0.58 mg SN-38, whereas 1.0 mg of IMMU-130 contains 0.016 mg of SN-38. Necropsy intervals were based on prior experience (32), with irinotecan-treated animals examined at 5 minutes, 1, 2, and then 6 or 8 hours, whereas IMMU-130–treated animals were examined at 1, 6, 24, and 48 hours, with Study 1 also including 72 hours. Mice were anesthetized and bled by cardiac puncture, with isolation of plasma and subsequent removal of tumors, liver, and contents of a portion of the small intestine that were weighed and stored at −80°C until analyzed. On the day of analysis, serum was diluted with equal parts of water, whereas tissues were homogenized in water (10 mL water to 1 g tissue) and extracted with equal parts of the extraction media that was spiked with an internal standard, 10-hydroxy-camptothecin (10-HCPT; 9 μL 3.33 mg/mL stock solution in DMSO; Toronto Research Chemicals), as described previously (32). The solids, including precipitated serum proteins, were separated by centrifugation from the organic phase, containing irinotecan, SN-38, and SN-38G. To release the covalently bound SN-38 from the ADC, a portion of the water homogenates of samples from animals receiving IMMU-130 were subjected to an

overnight acid hydrolysis step, as described previously (32). After neutralization, the product was extracted in the same manner as above. SN-38 isolated after acid hydrolysis was designated as "Total," whereas products isolated before acid hydrolysis were considered "Free" (i.e., product that was not covalently coupled to the conjugate). Analysis was performed on a Phenomenex Chromolith high-resolution C-18 analytic column (EMD Millipore Corp.), using a linear gradient composed of 85:15 of buffer A/B to 100% buffer B (buffer A: 50 mmol/L KH_2PO_4 and 4 mmol/L sodium 1-decanesulfonate in water, pH 3.5; buffer B: 600 mL buffer A plus 400 mL acetonitrile), with a flow rate of 1 mL/min and fluorescence detection with excitation wavelength at 373 nm, and emission wavelength of 540 nm. Standard curves were generated from the ratio of the peak areas for each product compared to the internal standard, using known concentrations of irinotecan, SN-38, and SN-38G ranging from 5 to 1,000 ng/mL.

Two ELISAs used to measure protein concentrations of IMMU-130 (intact conjugate) or the hMN-14 IgG were described previously (32). Briefly, the IMMU-130 assay used an SN-38–specific monoclonal antibody to capture the intact conjugate, with its presence identified with an anti-hMN-14 idiotype antibody conjugated to horseradish peroxidase. The hMN-14 ELISA is a sandwich assay that captures and probes with an anti–hMN-14 idiotype antibody conjugated to horseradish peroxidase.

Integration of the time-activity curves (AUC) was calculated using only the trapezoidal area where detectable levels of product were measured in $\geq$3 consecutive intervals by GraphPad Prism version 6.0 for Windows (GraphPad Software). Additional statistical testing was performed using Prism software. *In vivo* clearance half-lives were determined using PK Solutions V2.0 (Summit Research Services).

Results

Physiochemical properties

The conjugation process, as illustrated in Supplementary Fig. S1, couples the CL2A-SN-38 linker–drug moiety specifically to the IgG at 8 intra-chain sulfhydryl sites that are most susceptible to mild reduction. The coupling of the linker–drug moiety to these sites results in the heavy and light chains showing up as distinct bands by SDS-PAGE under both nonreducing and reducing conditions, whereas the unconjugated IgG shows as a single band under nonreducing conditions, with the heavy and light chains dissociated under reducing conditions (Supplementary Fig. S3). Under reducing conditions, the molecular weights for IMMU-130's heavy and light chains are somewhat higher than the native IgG, reflecting the addition of the CL2A-SN-38. Antigen binding, as illustrated by the similar dissociation constants for labetuzumab and labetuzumab govitecan, is unaffected by the coupling procedure (Supplementary Fig. S4). This coupling procedure does not appear to have an appreciable impact on the clearance of the hMN-14 IgG (see below), which is consistent with studies comparing sacituzumab govitecan (IMMU-132) and hRS7 IgG in mice (29, 33). Stability studies in human serum previously found SN-38 was released with half-life of about 20 hours, while it was twice as fast in rabbit serum (24). Herein, we compared the stability in murine and human serum, finding each with a half-life of 17 to 18 hours, suggesting that the conjugate's release of SN-38 in mice should model its behavior in humans (Supplementary Fig. S5). We currently speculate that SN-38 is released from the conjugate primarily based on pH conditions rather than enzymatic degradation. Carboxylesterases are involved in the cleavage of irinotecan at the 10th position to form SN-38, with the present *in vitro* studies finding 100% of irinotecan converting to SN-38 over 24 hours (9). However, when the ADC was incubated with or without either human or rabbit carboxylesterase for up to 168 hours, levels of Free SN-38 were the same as in buffer, indicating carboxylesterase was not involved in the release of SN-38 from the ADC (not shown). We also confirmed that SN-38, which readily binds noncovalently to albumin in serum, was quantitatively isolated from buffer containing HSA using the extraction media, whereas SN-38 covalently bound to the ADC was not removed, requiring acid hydrolysis before the SN-38 content of the ADC was released (Supplementary Table S1).

We reported previously that SN-38 conjugated to the antibody was protected from glucuronidation (32), and also speculated from prior work of Zhao and colleagues (34) that the lactone ring would remain intact while SN-38 was coupled to the linker at its 20th position. This lactone ring stability was affirmed by examining a derivative of CL2A-SN-38 by reversed-phase HPLC (Supplementary Fig. S6), which showed that SN-38, incubated at 37°C in pH 7.4 PBS, readily converted to the carboxylate form, with a half-life of 30 minutes and 84% conversion at equilibrium within 2 hours (Supplementary Fig. S6). In contrast, the CL2A-SN-38 derivative showed no evidence of being converted over 17 hours; however, SN-38 released from the derivative during this incubation period was in its lactone and carboxylate forms, with most of the released SN-38 converting to the carboxylate form. As expected, when a portion of the sample taken at 7 hours was adjusted to pH 4.0, the carboxylate form was quantitatively converted back to SN-38 (lactone form; not shown).

In vivo studies: serum

Five minutes after irinotecan injection (840 or 900 μg, Studies 1 and 2, respectively; SN-38 equivalents, 486 or 521 μg), levels of irinotecan in the serum averaged 14.3 ± 2.9 μg/mL (Study 1) or 11.1 ± 2.3 μg/mL (Study 2), which represented only 1.2% to 1.7% injected dose per mL (Fig. 1A). SN-38 concentrations at 5 minutes ranged from 883 ± 555 ng/mL (Study 1) to 946 ± 242 ng/mL (Study 2), whereas levels of SN-38G ranged from 698 ± 64 ng/mL to 911 ± 84 ng/mL. Thus, within just 5 minutes, approximately 13% of irinotecan was converted to SN-38, with about half being rapidly converted to SN-38G. On the basis of the combined AUC data for SN-38 and SN-38G, mice convert approximately 20% of the administered irinotecan to SN-38 (Table 1).

Animals given 1.0 mg (16 μg SN-38 equivalents) IMMU-130 were sampled first at 1 hour after the injection, with Total SN-38 ranging from 7.2 ± 0.7 μg/mL (Study 1) to 10.2 ± 1.1 μg/mL (Study 2), which represented between 45% and 63% injected dose/mL, respectively (Study 1 clearance shown in Fig. 1B). Curve-fitting for Total SN-38 clearance suggested two-phase distribution/elimination kinetics, with the distribution phase half-life being 2.2 to 2.4 hours and an elimination phase between 14.8 and 15.3 hours. As shown in Fig. 1B, ELISA showed the IgG rate of clearance is slower (e.g., elimination half-life = 93.0 hours) than that of the Total SN-38 or the amount of IMMU-130 detected by ELISA, with the IMMU-130 ELISA showing somewhat faster clearance than Total SN-38.

In animals given IMMU-130, levels of Free SN-38 at 1 hour averaged 114 ± 29 ng/mL (Study 2) to 499 ± 100 ng/mL

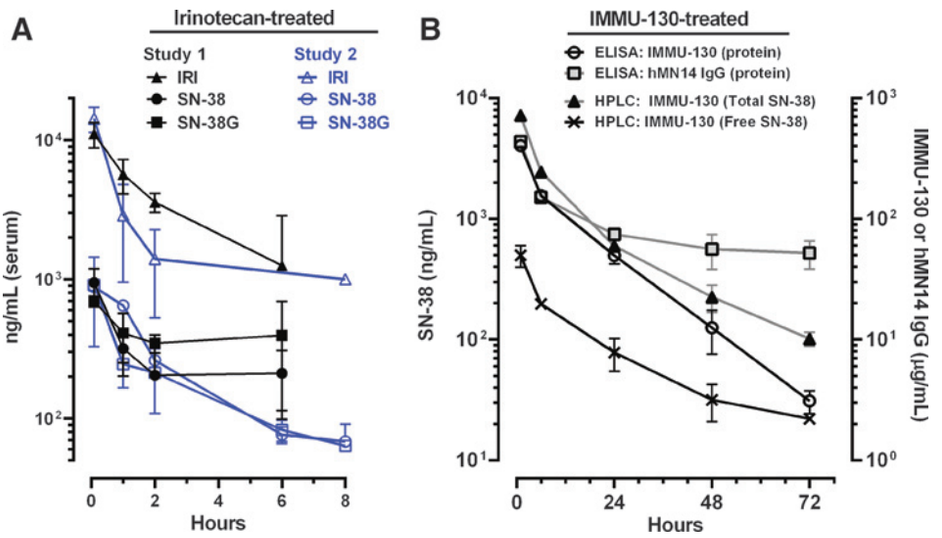

Figure 1.
Serum clearance of irinotecan (IRI) or IMMU-130 and their associated products. **A,** Irinotecan-treated animals in study 1 and 2 are shown. **B,** IMMU-130–treated animals from study 1.

(Study 1), whereas levels of SN-38G were 31 ± 10 ng/mL (Study 2) to 43 ± 0.4 (Study 1). Collectively, the levels of SN-38 and SN-38G represent only a small fraction (1.1% to 6.9%) of the Total SN-38 in the serum. In both studies with IMMU-130, SN-38G was detectable only over the first 6 hours, and even at 6 hours, remained 2 to 3 times lower than SN-38 levels. Because SN-38G levels only could be detected on 2 occasions, an AUC estimate for SN-38G is not provided. At 72 hours, approximately 20% of the Total SN-38 was in the Free SN-38 form. In Study 1, AUC estimates revealed concentrations of Total SN-38 were about 10-fold higher than Free SN-38, whereas in Study 2, where lower levels of SN-38 were detected over a shorter period, this ratio increases to approximately 80-fold difference.

In vivo studies: tumors

Irinotecan concentrations in both tumor xenografts were always substantially higher than SN-38 levels (Fig. 2A). SN-38 levels were higher initially in LS174T versus GW-39 (997 ± 179 vs. 503 ± 169 ng/g, respectively), with a distribution half-life of 0.25 and 0.5 hours and an elimination half-life of 5.0 and 5.5 hours (LS174T and GW-39, respectively). AUC data showed LS174T with 1.6-fold higher levels of SN-38 than GW-39, but SN-38G levels in LS174T were substantially lower than in GW-39 (Table 1).

In IMMU-130–treated animals, the only compound detected over the entire monitoring period was Total SN-38; no Free SN-38 or Free SN-38G were detected (Table 2). Both tumors had peak levels at 6 hours (894 ± 68 vs. 736 ± 154 ng/g in LS174T and GW-39, respectively), with AUC suggesting similar concentrations over their respective monitoring period (e.g., if LS174T data were truncated to 48 hours to mimic GW-39 monitoring period, AUC would be 24.919 μg/g/h).

In vivo studies: liver and small intestine contents

Irinotecan is processed to SN-38 and SN-38G primarily in the liver, with elimination through the intestines (9). These studies concurred, with irinotecan concentrations in the liver at 5 min representing about 9% to 10% injected dose (ID)/g (84.4 ± 4.9 μg/g and 79.8 ± 13.8 μg/g in study 1 and 2, respectively; Fig. 3A). AUC data show that the level of SN-38 in the liver exceeded SN-38G by 7- to 10-fold. In contrast, the AUC data for the contents of the upper portion of small intestine, where the bile duct drains, showed irinotecan and SN-38G levels peaking at 1 to 2 hours (Fig. 3C and D), but with SN-38G levels exceeding that of SN-38 by 7- to 10-fold, suggesting a rapid transit of SN-38G from the liver to the intestine. These results were similar to those reported previously with the anti–Trop-2-SN-38 conjugate (30).

For animals given IMMU-130, only Total and Free SN-38 were detected in the liver; no SN-38G was detected (Table 2). Total SN-38 levels in the liver at 1 hour represented 4.2 ± 1.1% ID/g (677 ± 171 ng/g) and 10.9 ± 1.3% ID/g (1749 ± 213 ng/g) for Study 1 and 2, respectively (Fig. 3B). Free SN-38 levels at 1 hour averaged between 316 and 342 ng/g, but decreased rapidly, being undetected after 6 hours. Samples of the small intestinal contents were not processed for Total SN-38, because previous studies found Free and Total concentrations were the same in animals given IgG-conjugated SN-38, indicating

Table 1. Concentrations of products found in tissues of nude mice bearing LS 174T (Study 1) or GW-39 (Study 2) human colonic tumor xenografts given irinotecan

	Serum		Liver		SI contents		Tumors	
	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2	LS174T	GW-39
SN-38	1.974	1.670	16.804	15.083	9.203	4.962	2.776	1.706
SN-38G	1.497	2.370	1.512	1.934	67.247	54.782	0.629	1.572
Irinotecan	17.186	21.963	74.969	134.876	216.482	185.654	48.083	24.261

NOTE: In Study 1, animals were given 840 μg of irinotecan (486 μg SN-38 equivalents), and were necropsied at 5 minutes, 1, 2, 6, and 8 hours. Study 2, animals were given 900 μg of irinotecan (521 μg SN-38 equivalents), with tissues taken at the same intervals, excluding 8 hours. AUC expressed as μg/g/ṡh (tumors, liver) or μg/mL/ṡh (serum), with small intestinal (SI) contents expressed μg/h. Integration for AUC determination was performed only when products were detected in at least 3 intervals and are not extrapolated beyond the testing interval.

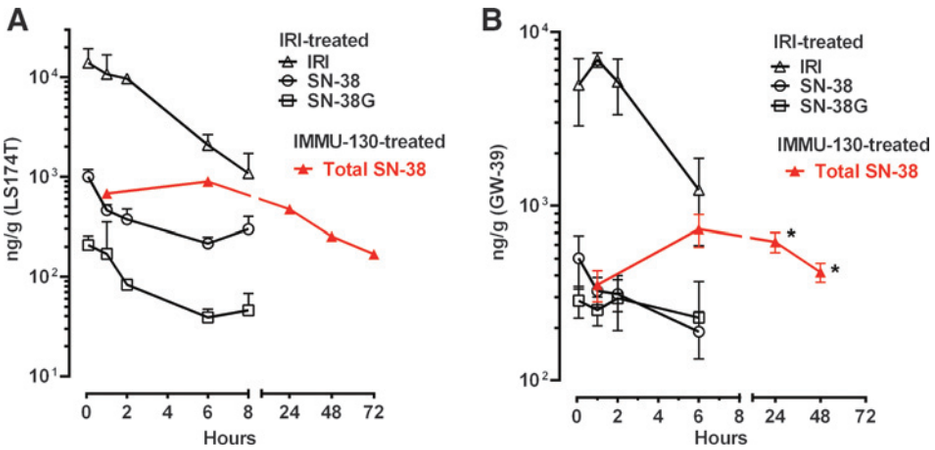

Figure 2.
Concentrations of constitutive products in LS174T (**A**) or GW-39 (**B**) human colonic tumor xenografts from animals given IRI or IMMU-130. Animals (LS174T, $N = 3$; GW-39, $N = 4$) were injected with IRI or IMMU-130 and necropsied at the times indicated. Asterisks (*) show where levels of total SN-38 were significantly higher in GW-39 than LS174T (e.g., 623 ± 82 and 418 ± 53 ng/g for GW-39 at 24 and 48 hours versus 472 ± 26 and 253 ± 31 ng/g for LS174T at 24 and 48 hours, respectively).

that the intact conjugate is not eliminated in this manner (30). However, Free SN-38 and SN-38G were detected in the intestinal contents, but at levels much lower than in animals given irinotecan (Fig. 3C and D; Table 2), which largely reflected the smaller number of SN-38 equivalents given with IMMU-130. For example, peak levels of SN-38 in the small intestine contents for irinotecan-treated animals in the 2 studies were between 1,500 and 2,000 ng, which represented 0.3% to 0.4% of SN-38 equivalents administered (i.e., 486 to 521 μg SN-38 equivalents). For IMMU-130-treated animals, peak levels of SN-38 from 90 to 215 ng were found in the 2 studies at 1 hour, which represented 0.5% to 1.3% of the administered SN-38 equivalents (16 μg).

Discussion

CEA is one of the first tumor markers discovered, and over the years, it has served a variety of purposes in colorectal and other cancers (16, 17). The family of CEACAM molecules is very diverse, with some co-expressed on neutrophils, while others, such as CEACAM5, are more highly selective for cancer (15). This selectivity is most eloquently illustrated from studies involving radiolabeled antibodies, where despite expression in the normal colon, targeting is absent with antibodies to CEACAM5, with localization restricted to cancer, whereas antibodies to other CEA epitopes localize differently (20, 21, 35, 36). Although not considered a target that encourages internalization, several antibody–drug and toxin conjugates were developed, with evidence that the conjugates internalize (4, 37–40). Internalization is considered one of the hallmark properties for a successful ADC, particularly if a highly stable conjugate is used. Stable linkages were essential for the recently successful ADCs, primarily because these conjugates rely on highly potent drugs that would be too toxic if released in the circulation (6, 41). This is an important distinction for the class of SN-38 conjugates represented by labetuzumab govitecan or sacituzumab govitecan, where early studies using labetuzumab found linkers that released SN-38 in serum too soon (e.g., <12 hours), or that were highly stable (>7 days), were less effective than linkages that had a more intermediate stability (e.g., 1–2-day half-life; refs. 10, 11, 24). This linkage strategy also relies on the fact that SN-38 held by the conjugate is delivered to the tumors in a fully active form, yet when released outside the tumor, it is tolerated at the recommended clinical dose, with well-known and manageable side effects (12–14). The SN-38-released half-life also appears to be well matched with peak levels of antibody localization, which radiolabeled labetuzumab studies showed occurred between 6 and 24 hours (27).

Similar to the results reported previously with sacituzumab govitecan (30), no Free SN-38 or SN-38G were found in the tumor of animals given IMMU-130, only covalently- bound SN-38 (i.e., Total SN-38). We hypothesize Free SN-38 is liberated gradually, and therefore levels were too low for detection. However, if we assume all the Total SN-38 will eventually be converted to Free SN-38, then the AUC analysis suggests that labetuzumab govitecan could potentially deliver 11- to 16-fold more SN-38 to the tumor xenografts than irinotecan. Indeed, this enhancement would increase by at least 30-fold when the difference in the amount of SN-38 administered was considered (e.g., ~500 μg SN-38 for irinotecan dose vs. ~16 μg IMMU-130 dose). In addition, consideration must be given the fact that mice convert irinotecan to SN-38 more efficiently than humans (42), and therefore the levels of SN-38 in these xenografts is likely an over-estimate of the SN-38 delivery

Table 2. Concentrations of products found in tissues of nude mice bearing LS174T (Study 1) or GW-39 (Study 2) human colonic tumor xenografts given IMMU-130

	Serum		Liver		SI contents		Tumors	
	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2	LS174T	GW-39
Total SN-38	65.396	80.595	24.411	19.604	NT	NT	29.972	27.452
Free SN-38	6.183	1.091	ND	ND	5.441	6.647	ND	ND
Free SN-38G	ND	ND	ND	ND	4.703	0.808	ND	ND

NOTE: In Study 1 and 2, animals were given 1.0 mg IMMU-130 (SN-38 equivalents = 16 μg), and were necropsied at 1, 6, 24, and 48 hours (Study 2), and through 72 hours for study 1. AUC expressed as μg/g/h, except for small intestinal (SI) contents, which are expressed μg/h. Integration for AUC determination was performed only when products were detected in at least 3 intervals and are not extrapolated beyond the testing interval.
Abbreviations: ND, not determined; either not detected or there were <3 data points for integration; NT, not tested.

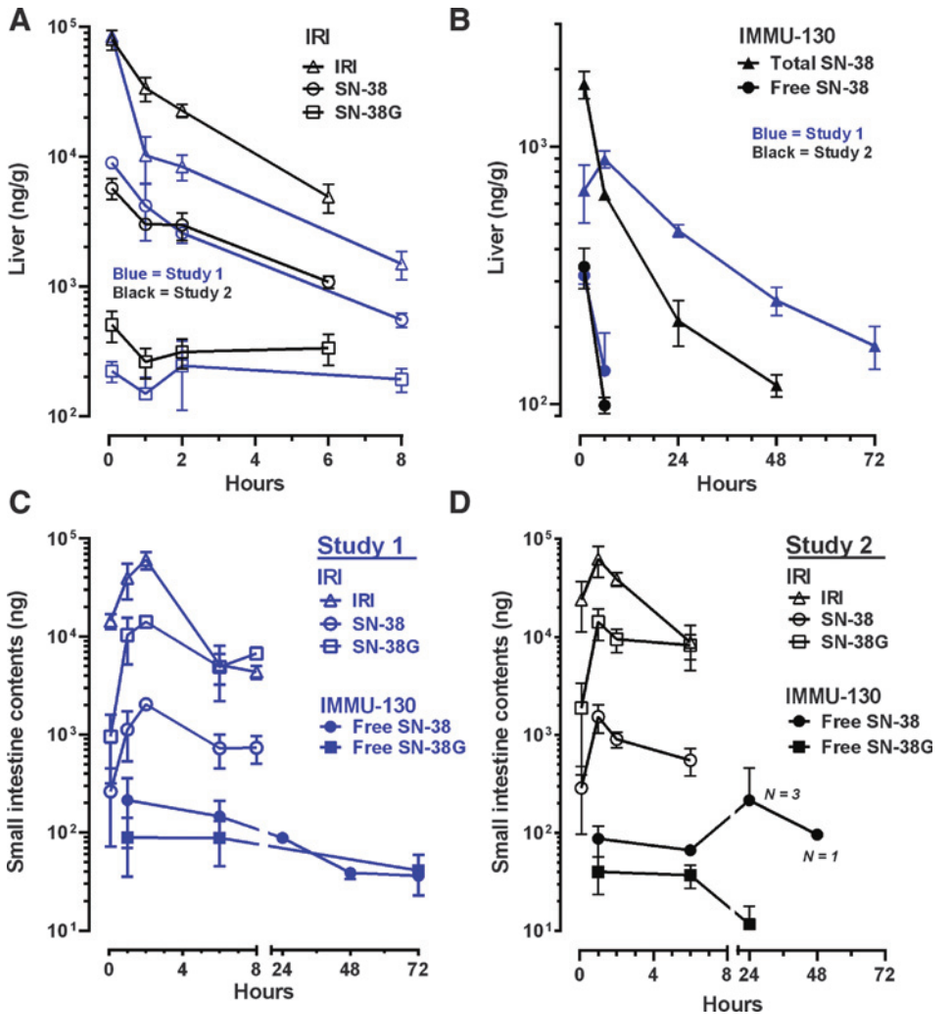

Figure 3.
Concentrations of constitutive products in the liver and small intestine of animals given IRI or IMMU-130. The top shows liver concentration data from the 2 studies for IRI (**A**) and IMMU-130 (**B**) separately. The bottom shows the contents of the small intestine data for IRI- or IMMU-130–treated animals in study 1 (**C**) and study 2 (**D**). In study 2 (**D**), where 4 animals were included at each interval, the number of animals with detectable levels represented at later times for the IMMU-130–treated animals is shown.

potential of irinotecan in humans. Furthermore, the irinotecan dose given to the mice in this study closely approximated the human-equivalent dose (e.g., ~3.5 mg/kg), whereas, on the basis of the 10 mg/kg dose currently recommended for patients, we could have increased the IMMU-130 dose nearly 3-fold. When each of these factors is considered, the tumor AUC differences noted here can be magnified by as much as approximately 450-fold ($30 \times 5 \times 3$). It also is important to emphasize that these estimates were derived from subcutaneously implanted tumors, whereas an orthotopically grown tumor might have provided different results. Irrespective of the potential delivery advantage claims made by these measurements and estimates, the important observation is that IMMU-130 improves therapeutic responses when compared with irinotecan in a variety of xenograft models (10, 24).

With sacituzumab govitecan, where Trop-2 binding leads to rapid internalization (43), the tumor AUC comparison found a 20- to 135-fold advantage over irinotecan-treated animals, whereas with labetuzumab govitecan, the tumor AUC were 11- to 16-fold higher than irinotecan-treated animals. We believe it is unlikely that differences in the internalization properties of the two ADCs account for the higher ratio found with sacituzumab govitecan. Instead, we note from the previous study (30) that the AUC for SN-38 uptake in animals given irinotecan and bearing the Capan-1 human pancreatic tumors were lower than irinotecan-delivered SN-38 found in the 2 colonic cancer xenografts, while in the NCI-N87 human gastric cancer xenografts, irinotecan-derived SN-38 was the same as these colonic cancer xenografts. In the latter model, the tumor AUC advantage over irinotecan-treated animals was just 20-fold compared with sacituzumab govitecan, which is more consistent with what we found with labetuzumab govitecan in the GW-39 tumor xenograft model. Thus, not only will there be differences in SN-38 delivery to tumors based on irinotecan treatment, but treatment with ADCs could result in SN-38 delivery differences based on the internalization properties of an antibody, as well as a variety of other factors, such as antigen expression level, antigen heterogeneity within the tumor, and/or its accessibility.

The bioavailability issues for irinotecan are well documented (9), starting with its rapid clearance, but more important is its low conversion rate and its detoxification, primarily through glucuronidation and the lactone ring conversion to the carboxylate form. Strategies for extending the half-life of irinotecan or SN-38 by PEG modification appeared promising in preclinical models, but did not have clinical success

(42, 44–46); however, a liposomal formulation of irinotecan is approved for use in pancreatic cancer (47). The SN-38 ADCs differ from these agents principally by their ability to bind selectively to tumor sites expressing the target antigen, but as mentioned above, a key strength is the protection from SN-38 detoxification afforded by these ADC formulations. We previously showed protection from glucuronidation, and herein we confirm that lactone ring integrity is maintained while bound to the CL2A linker. The gradual release from the conjugate (~90% cleared over 3 days) also contributes to maintaining low levels of Free SN-38 in the serum, which in turn minimizes SN-38G generation, a likely reason why clinically the incidence of severe diarrhea is lower than that experienced with irinotecan (12–14). Evaluation of serum samples taken from patients given labetuzumab or sacituzumab govitecan have confirmed SN-38G levels are about 2-fold lower than SN-38 (25, 48), which contrasts with data in patients given irinotecan, where SN-38G levels can be 4- to 30-fold higher than SN-38 (49, 50).

Liver homogenates from animals given the ADC also contained Free SN-38 only over the first 6 hours, with no SN-38G, whereas Total SN-38 levels were detected over 2 to 3 days. Irinotecan-treated animals had much higher SN-38 levels in the liver than IMMU-130–treated animals, with much lower levels of SN-38G. Interestingly, levels of SN-38G were much higher in the small intestine than SN-38 in the irinotecan-treated animals, a finding consistent in both studies, as well as the earlier studies (30), which likely suggests an enhanced transit of SN-38G into the small intestine compared with SN-38. Importantly, with IMMU-130, levels of Free SN-38 and SN-38G were somewhat similar and much lower than seen with irinotecan. These results are consistent with the finding that serum levels of SN-38G are much lower with the ADC than with irinotecan, supporting the clinical observation of a lower incidence of severe diarrhea with these conjugates (12–14, 25).

In conclusion, despite being a less actively internalizing antibody, the SN-38 conjugation platform applied to a CEACAM5-targeting antibody allows for enhanced delivery of SN-38 when compared with irinotecan treatment. Given the specificity of the CEACAM5 antibody combined with CEACAM5's presence in several epithelial cancers, labetuzumab govitecan (IMMU-130) is a promising ADC, as confirmed recently clinically (26).

Disclosure of Potential Conflicts of Interest

D.M. Goldenberg has ownership interest (including patents) in Immunomedics, Inc. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: R.M. Sharkey, S.V. Govindan, D.M. Goldenberg
Development of methodology: R.M. Sharkey, S.V. Govindan, E.A. Rossi
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S.V. Govindan, T.M. Cardillo, J. Donnell, J. Xia
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R.M. Sharkey, E.A. Rossi, D.M. Goldenberg
Writing, review, and/or revision of the manuscript: R.M. Sharkey, S.V. Govindan, T.M. Cardillo, J. Donnell, J. Xia, E.A. Rossi, C.-H. Chang, D.M. Goldenberg
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R.M. Sharkey, J. Donnell, J. Xia, C.-H. Chang
Study supervision: R.M. Sharkey, D.M. Goldenberg

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