Enhanced Delivery of SN-38 to Human Tumor Xenografts with an Anti-Trop-2–SN-38 Antibody Conjugate (Sacituzumab Govitecan)


Abstract

Purpose: This study examined the delivery of SN-38 to Trop-2–expressing tumors and assessed the constitutive products in the serum, liver, and small intestine in nude mice bearing human xenografts (Capan-1 or NCI-N87) given a single injection of irinotecan (40 mg/kg; 8282; E-mail: dmg.gscancer@att.net

Experimental Design: At select times, tissues were extracted and concentrations of the products measured by reversed-phase high-performance liquid chromatography (HPLC).

Results: In serum, >98% irinotecan cleared within 5 minutes; peak levels of SN-38 and SN-38G were detected in equal amounts at this time, and no longer detected after 6 to 8 hours. IMMU-132 was detected in the serum over 3 days, and at each interval, >95% of total SN-38 was bound to the antibody. Intact IMMU-132 cleared with a half-life of 14 hours, which closely reflected the in vitro rate of SN-38 released from the conjugate in mouse serum (i.e., 17.5 hours), whereas the IgG portion of the conjugate cleared with a half-life of 67.1 hours. In vitro and in vivo studies disclosed IgG-bound SN-38 was protected from glucuronidation. Area under the curve (AUC) analysis indicated that IMMU-132 delivers 20-fold to as much as 136-fold more SN-38 to tumors than irinotecan, with tumor:blood ratios favoring IMMU-132 by 20- to 40-fold. Intestinal concentrations of SN-38/SN-38G also were 9-fold lower with IMMU-132.

Conclusions: These studies confirm a superior SN-38 tumor delivery by IMMU-132 compared with irinotecan. Clin Cancer Res; 21(22); 5131-8. ©2015 AACR.

Introduction

Antibody–drug conjugates (ADC) represent a new therapy class based on the proposition of their being able to deliver cytotoxic agents more specifically to their intended target with less collateral damage. However, this technology has faced many challenges, but with 5 conjugates gaining FDA approval, each using drugs that are active at picomolar concentrations (so-called ultratoxic drugs), it appeared that the major hurdles facing this technology had been overcome and that a core platform on which similar agents could be developed was available (1–5). Our group departed from the current popular approach, and instead developed an ADC with a more moderately toxic agent, SN-38. SN-38, a topoisomerase I inhibitor, is a highly potent drug with activity in the low nanomolar range, administered in a prodrg form, irinotecan. The pharmacology of irinotecan is well known but complex, limiting its bioavailability. First, only a small portion of irinotecan is converted to SN-38, a process that occurs primarily in the liver, but also in the intestine and plasma, as well as in some tumors (6).

Compounding a poor conversion rate is the fact that a sizable portion of SN-38 is readily converted to less active forms, most notably SN-38G, a glucuronidated product whose concentration is often 4 to 30 times higher than SN-38 (7). Enterohepatic recirculation exposes SN-38G to bacterial enzymes in the intestine that reconvert SN-38G to SN-38, which is the most likely cause of late diarrhea, a common and serious toxicity associated with irinotecan therapy (8). SN-38’s lactone ring is another vulnerable conversion site, with irinotecan and SN-38 generally found in equilibrium between the less active carboxylate and the fully potent lactone ring forms (6).

Our antibody–SN-38 ADC platform emerged empirically by examining a number of different linkers. Selecting the best conjugate relied on efficacy studies in human tumor xenograft/mouse models, with the linker designation CL2 providing the best responses (9, 10). Interestingly, in vitro serum stability studies of the initial 3 candidate conjugates revealed that SN-38 was released at different rates, with one having a half-life of about 10 hours, whereas another was highly stable; yet, the selected CL2-linked conjugate had an intermediate stability in serum of 36 hours. This derivative was later modified to simplify scale-up production for clinical use, with the new derivative designated CL2A (11). Numerous animal studies have shown that IMMU-132 improves therapeutic responses compared with irinotecan, suggesting IMMU-132 delivers more SN-38 to the tumors (11–13). The principal goal of this investigation was to quantify this difference, evaluating concentrations of the constitutive products in tumors.
Translational Relevance

Sacituzumab govitecan (IMMU-132) is an antibody–drug conjugate composed of a humanized anti-Trop-2 IgG coupled with a high ratio of SN-38 molecules. This conjugate was shown previously to improve therapeutic responses in human cancer xenografts compared with irinotecan, its prodrug. Recently, clinical trials with IMMU-132 have shown objective responses in several types of Trop-2–expressing epithelial cancers. In this report, we examine the advantage of delivering SN-38 with IMMU-132 compared with irinotecan in nude mice bearing 2 human tumor xenografts, as well as assess the concentrations of SN-38 and SN-38G in the serum, liver, and intestine. A significant targeting advantage for IMMU-132 was observed, in terms of the delivery of SN-38 in a fully active form to the tumors, improved tumor:blood ratios, and reduced intestinal uptake that elucidates the lower rate and severity of diarrhea in patients.

Materials and Methods

Reagents

IMMU-132 was prepared from the hRS7 anti-Trop-2 IgG and the CL2A–SN-38 drug carrier, as described previously (11), with hydrophobic interactive chromatography (HIC) and liquid chromatography mass spectroscopy (LC-MS) showing a drug–antibody ratio of 7.6 (13). Conjugates prepared under these conditions have about 16 μg SN-38/mg IgG. The final 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 were purchased from Toronto Research Chemicals.

Study design

Table 1 lists the 3 sets of studies performed for this analysis. 2 in nude mice bearing xenografts of the human pancreatic cancer cell line, Capan-1, and 1 with the human gastric carcinoma cell line, NCI-N87. Capan-1 and NCI-N87 were purchased from the ATCC and were authenticated by short tandem repeat (STR) assay by the ATCC and routinely tested for mycoplasma. Surface Trop-2 expression on Capan-1 and NCI-N87 cells in culture is 157,000 and 247,000 molecules, respectively (12).

Table 1. Examination of SN-38 concentrations in tissues of nude mice bearing two human cancer xenografts

<table>
<thead>
<tr>
<th>Study</th>
<th>Tumor</th>
<th>Intervals (n = 3 animals/interval)</th>
<th>Tissues examined</th>
<th>IMMU-132 (SN-38 equivalents)*</th>
<th>Irinotecan (SN-38 equivalents)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Capan-1</td>
<td>SN-38 and irinotecan: 5 min, 1, 2, 6, and 24 h IMMU-132: 1, 6, 24, 48, 72 h</td>
<td>Tumor, serum, liver, small intestine contents</td>
<td>1.0 mg (16 μg)</td>
<td>773 μg (448 μg)</td>
</tr>
<tr>
<td></td>
<td>Capan-1</td>
<td>SN-38 and irinotecan: 1 and 6 h IMMU-132: 1, 6, and 24 h</td>
<td>Tumor, serum, liver, small and large intestine contents</td>
<td>1.0 mg (16 μg)</td>
<td>808 μg (468 μg)</td>
</tr>
<tr>
<td>3</td>
<td>NCI-N87</td>
<td>SN-38 and irinotecan: 5 min, 1, 2, 6, and 8 h IMMU-132: 1, 6, 24, 48, 72 h</td>
<td>Tumor, serum</td>
<td>1.0 mg (16 μg)</td>
<td>840 μg (486 μg)</td>
</tr>
</tbody>
</table>

*SN-38 equivalents for IMMU-132 based on spectrophotometric determinations of protein and SN-38 concentrations. SN-38 equivalents for irinotecan based on mass, with SN-38 representing approximately 58% of irinotecan’s mass.
PO₄ 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.

Portions of the homogenates and diluted serum taken from animals given IMM1-132 were subjected first to an acid hydrolysis step that ensured all SN-38 was released from the IMM1-132 conjugate. For this process, 0.15 mL of 6.0 mol/L HCl was added to 0.15 mL of the homogenate or diluted serum spiked with 10-HCPT and then heated to 50°C overnight before neutralizing with 6.0 mol/L NaOH. An equal volume of the precipitating reagent was added, vortexed, and centrifuged. Thus, the previous processing method measures the amount of free SN-38/SN-38G in the IMM1-132 and irinotecan samples, whereas the acid hydrolysis processing isolates the total amount of SN-38/SN-38G specifically in the IMM1-132 sample [designated SN-38 (Total)]. The HPLC analysis method is described in Supplementary Information. AUCs were calculated with GraphPad Prism version 6.0 for Windows (GraphPad Software), using only the trapezoidal area where detectable levels of product were measured in ≥3 consecutive intervals.

Procedures that monitored SN-38 release rates in mouse serum and in vitro glucuronidation are provided in Supplementary Information.

Results

Quantitative HPLC methodological assessments

Before initiating these studies, various aspects of the procedures used for the analysis of tissue samples were assessed. Details are reported in Supplementary Information (Supplementary Fig. S1 and Supplementary Tables S1 and S2). Overall, these studies provided sufficient evidence that the procedures developed for these assessments yielded reliable and reproducible results. The minimum sensitivity of detection in serum was 20 and 110 ng/mL in tissue homogenates.

Of particular importance was the finding that the addition of the precipitating reagent to serum freshly spiked with IMM1-132 recovered only about 7% of the expected amount of SN-38 associated with IMM1-132, indicating that the SN-38 bound to the IgG was not released effectively during the extraction process. Therefore, the acid-hydrolyzing step was introduced, which subsequently showed the expected amount of SN-38 associated with IMM1-132. Furthermore, triplicate samples of serum spiked with SN-38G that were first processed by acid hydrolysis and then extracted found only 3.0% of the SN-38G was converted to SN-38, indicating there was a negligible impact on the determination of SN-38G in the acid-hydrolyzed samples.

Concentrations of products in serum

Although animals given irinotecan in Study 1 examined serum taken over 24 hours, none of the products was detected at this time, and therefore subsequent studies focused on intervals over 6 to 8 h. Measurements of IMM1-132 and its SN-38 products were assayed over 72 hours, with detection by both ELISAs and SN-38 concentrations accounted for 58.7% ± 3.3% ID/mL of the estimated amount of SN-38 equivalents. At each interval examined, free SN-38 levels were less than 5% of the total SN-38 in the serum (Fig. 1B). The concentration of free SN-38 in the 1-hour sample was about 2 times lower than SN-38 levels at this same time in the irinotecan-treated animals. These clearance data are consistent with in vitro studies that indicated 50% of the SN-38 is released from the conjugate in mouse serum every 17.5 hours (Supplementary Fig. S2A). ELISA data also confirmed these findings, with IMM1-132 having the same rate of clearance as the SN-38 (Total), whereas IgG levels were persistently higher (Supplementary Fig. S2B). Pharmacokinetic parameters estimated from the ELISA data show IMM1-132 clearing with a half-life of 14 hours (Supplementary Fig. S2C), reflecting the rate at which SN-38 is released from the conjugate (i.e., 17.5 hours) plus the rate of IgG removal from the blood.

An important difference between irinotecan- and IMM1-132-treated animals was that there were only trace amounts of unbound SN-38 in the serum of animals given IMM1-132, with levels in the 1- and 6-hour samples being 5- to 10-fold less than unbound SN-38; no free SN-38G was detected in the later samples. More importantly, at no time did the acid-hydrolyzed samples have detectable amounts of SN-38G, suggesting that all of the SN-38G generated was likely from the released SN-38, with the SN-38 bound to the conjugate not susceptible to

Figure 1. Clearance of constitutive products in tumor-bearing nude mice given irinotecan or IMM1-132. A, Study 1, Capan-1-bearing mice; irinotecan. B, Study 1; IMM1-132. C, Study 3, NCI-N87-bearing mice; irinotecan. D, Study 3; IMM1-132. AUC estimates are provided in the table for products with 3 or more samples.
glucuronidation, despite the fact that the hydroxyl at the tenth position was available for this modification to occur.

The apparent protection of SN-38 from glucuronidation while bound to the conjugate was investigated further using an in vitro glucuronidation assay. HPLC analysis of the nonhydrolyzed SN-38 sample found 47% of the recovered products had been converted to SN-38G (recovery = 70%; Supplementary Table S4). For the IMMU-132 samples, there were 16.1 nmol/L of SN-38 plus 7.1 nmol/L SN-38G, which based on an initial SN-38 concentration of 425.3 nmol/L, the combined products represent a release of 5.5% of the antibody-bound SN-38. This indicates that the reaction conditions did not appreciably release SN-38 from the conjugate. The acid-hydrolyzed IMMU-132 samples contained a total of 452.6 nmol/L of SN-38 and 4.5 nmol/L of SN-38G, indicating that only 1% of the total recovered product was converted to the SN-38G form (recovery = 107%); however, since the amount of SN-38G in the acid-hydrolyzed sample was less than in nonhydrolyzed samples, these results reveal there was no detectable SN-38G derived from the SN-38 bound to the conjugate, thereby confirming the in vivo data that SN-38 bound to IMMU-132 is protected from glucuronidation.

In Study 2, irinotecan and IMMU-132 were given to nude mice bearing larger sized Capan-1, examining 2 of the same intervals for irinotecan (1 and 6 hours) and 3 intervals (1, 6, and 24 hours) for IMMU-132 (Supplementary Fig. S3). Concentrations of all products in the serum generally were similar in these 2 studies, but for irinotecan (1 and 6 hours) and 3 intervals (1, 6, and 24 hours) in the IMMU-132–treated animals, 0.149 ± 0.035 g, and in the IMMU-132–treated animals, 0.149 ± 0.046 g. Study 2 used Capan-1 tumors that were approximately twice the size as the first study to determine whether this impacted tumor localization (6 irinotecan-treated animals, 0.365 ± 0.085 g; 9 IMMU-132–treated animals, 0.342 ± 0.125 g).

Concentrations of all products in the Capan-1 tumors from the irinotecan-treated animals were similar between the 2 studies (Fig. 2A). For example, irinotecan levels at 1 hour in the first and second studies averaged 12.34 ± 5.23 μg/g (1.6 ± 0.7% ID/g) and 11.08 ± 0.14 μg/g (1.4 ± 0.02% ID/g), respectively, with SN-38 concentrations of 0.19 ± 0.05 and 0.21 ± 0.01 μg/g, respectively. Whereas in Study 1, where SN-38 was detectable in the tumors only through 2 hours, in Study 2, several of the 6-hour tumor samples had detectable SN-38 or SN-38G. AUC determinations for Study 1 alone and using data combined from both studies, which allowed SN-38 AUCs to be extrapolated over 6 hours, found that only about 5% of the irinotecan in the tumor was converted to SN-38 + SN-38G (Table 2), with about half being in the form of SN-38G, whereas in the serum, concentrations of...
Enhanced Tumor Targeting of SN-38 with Sacituzumab Govitecan

Table 2. Concentrations of products over time (AUC) in mice bearing Capan-1 or NCI-N87 tumors administered irinotecan or IMMU-132

<table>
<thead>
<tr>
<th>Tissue</th>
<th>SN-38</th>
<th>irinotecan-treated</th>
<th>SN-38G</th>
<th>irinotecan</th>
<th>IMMU-132-treated</th>
<th>SN-38 (Total)</th>
<th>SN-38 deliver ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Capan-1</td>
<td>Study 1 only</td>
<td>0.40</td>
<td>1.08</td>
<td>48.37</td>
<td>54.25</td>
<td>135.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Study 1 + 2</td>
<td>1.09</td>
<td>0.6</td>
<td>46.73</td>
<td>49.02</td>
<td>45.0</td>
</tr>
<tr>
<td></td>
<td>NCI-N87</td>
<td></td>
<td>2.10</td>
<td>0.03</td>
<td>45.69</td>
<td>43.88</td>
<td>20.8</td>
</tr>
</tbody>
</table>

NOTE: AUCs are expressed as (µg/g·h). In study 1, SN-38 AUC derived from 5 minutes to 2 hours; SN-38G and irinotecan from 5 minutes to 6 hours. AUC from 5 minutes to 6 hours for irinotecan-treated mice combining Studies 1 and 2. For IMMU-132-treated animals, SN-38 (Total) AUC are derived from 1 to 72 hours.

Concentrations of SN-38 estimated the conversion rate to be about 25%. In the NCI-N87 tumors taken from the SN-38G-treated animals (average weight = 0.31 ± 0.08 g, n = 15), peak levels of irinotecan remaining at about 10 µg/g (~1.2% ID/g) for the first 2 hours before decreasing to 1.69 ± 0.34 µg/g at 6 hours (Fig. 2B). SN-38 concentrations were at their highest level at 5 minutes (0.35 ± 0.08 µg/g, representing 0.07 ± 0.02% ID/g of the total SN-38 equivalents in the administered irinotecan dose) and remained relatively constant over the 8-hour sampling period at a level of about 0.25 µg/g. SN-38G was initially 40% lower than the SN-38 levels and was undetectable at 6 and 8 hours. While the absolute levels of SN-38 in the NCI-N87 tumors overall were not substantially different from Capan-1, the sustained detection of SN-38 in the NCI-N87 tumors over 8 hours improved the AUC by nearly 2-fold (Table 2).

In the IMMU-132–treated animals, the only product detected in the tumors at any interval was SN-38 (Fig. 2C and D). Concentrations in the Capan-1 tumors peaked at 6 hours in both studies, averaging 1.88 ± 0.57 µg/g and 1.26 ± 0.36 µg/g (11.8 ± 3.6 and 7.9 ± 2.3% ID/g) in Study 1 and 2, respectively. Overall, a comparison of Capan-1 tumor uptake in the 2 studies again found generally similar values, with a tendency for the smaller tumors in Study 1 to have slightly higher concentrations on a per gram basis (Fig. 2D). Comparing the AUC for SN-38 content in the tumors from the first study for irinotecan- and IMMU-132–treated animals, the data suggest as much as 136- to 11.5-fold higher concentrations of SN-38 could be delivered to the liver homogenates (animals given IMMU-132) than the liver homogenates from animals given irinotecan (0.4% ± 0.1% of administered SN-38 equivalents). SN-38G was not detected in the acid-hydrolyzed or nonhydrolyzed liver homogenates from animals given IMMU-132. In Study 2, concentrations in the various products were somewhat higher, but the patterns of uptake and clearance for each of the products in both the irinotecan- and IMMU-132–treated animals were similar (Supplementary Fig. S4C).

Animals given irinotecan had detectable products in the small intestine even at 5 minutes after injection, peaking at 1 hour (Fig. 3). Combining data from Studies 1 and 2, irinotecan levels at 1 hour were 90.61 ± 23.18 µg (~11% ID; AUC = 345.7 µg·h). The AUC for SN-38G in the small intestinal contents exceeded SN-38 AUC by nearly 18-fold (149.9 µg·h vs. 8.2 µg·h, respectively).

With IMMU-132, SN-38 concentrations were similar in the acid-hydrolyzed and nonhydrolyzed samples, indicating that the intact conjugate was not transported from the liver to the intestine. For example, in a 6-hour fecal sample, unbound SN-38 levels were 0.14 ± 0.04 versus 0.23 ± 0.07 µg for the nonhydrolyzed and hydrolyzed samples, respectively (2-tailed paired t test; P = 0.053). The AUC for SN-38G was about 4-fold higher than SN-38 (14.3 µg·h·g⁻¹ vs. 3.3 µg·h·g⁻¹). However, the combined AUC for SN-38 and SN-38G in the IMMU-132–treated animals was still 9-fold lower than the combined AUC in the irinotecan-treated animals. This difference is even greater if the irinotecan AUC is added to the total amount of product in the intestine, as a portion of it could convert to SN-38, resulting in toxicity.

Table 3. Determination of tumor:serum AUC ratios

<table>
<thead>
<tr>
<th></th>
<th>Capan-1 (study 1 + 2)</th>
<th>NCI-N87</th>
<th>Tumor/serum AUC ratio Capan-1</th>
<th>NCI-N87</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irinotecan</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SN-38</td>
<td>3.27</td>
<td>1.09</td>
<td>2.11</td>
<td>0.33</td>
</tr>
<tr>
<td>SN-38G</td>
<td>4.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SN-38 (Total)</td>
<td>27.48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMMU-132</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free SN-38</td>
<td>3.87</td>
<td>49.02</td>
<td>45.88</td>
<td>12.7</td>
</tr>
<tr>
<td>Free SN-38G</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SN-38 (Total)</td>
<td>357.21</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: AUCs, expressed as µg/g·h were averaged from all 3 studies, with values derived from 5 minutes to 8 hours for irinotecan-treated animals or from 1 to 72 hours for IMMU-132-treated animals.

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Irinotecan-treated animals also had high levels of irinotecan and SN-38 in the large intestine, with SN-38 levels being nearly 20-fold higher at 6 hours than in animals given IMMU-132. SN-38G levels in animals given irinotecan were much lower than measured in the small intestine, likely reflecting the known conversion that occurs as a result of bacterial enzymes (refs. 8, 17–19; Supplementary Table S5).

Discussion

SN-38 is a highly potent drug but delivery by irinotecan is compromised by (i) rapid clearance from the blood, (ii) poor conversion rate, and (iii) a rapid conversion to the inactive SN-38G form. Thus, we developed a new type of ADC that uses SN-38, expecting that this ADC would improve the pharmacokinetics of SN-38 and also provide a mechanism for selective tumor retention via the targeting antibody. However, several other advantages were found. First, SN-38 bound to the antibody is in an inactive state, and therefore the intact conjugate in the serum would not pose a toxicity concern. Second, others had indicated that by coupling an agent to the 20th position, the lactone ring’s integrity is preserved (20). Conversion of the lactone ring to the less toxic carboxylate form accounts for as much as 30%–40% of the total SN-38 after a 90-minute irinotecan infusion, and this increases to as much as two thirds at equilibrium (6). While we have not assessed lactone ring stability with IMMU-132, coupling to the 20th position should preserve lactone ring integrity. Finally, in vitro and in vivo studies found that SN-38 bound to the conjugate is not susceptible to glucuronidation, which also significantly reduces the potency of SN-38, with SN-38G concentrations in serum being 4 to as much as 30 times higher than SN-38 with irinotecan therapy (7). Thus, IMMU-132 retains SN-38 in its fully active form as it circulates in the serum. The IMMU-132 fraction that localizes in the tumor will have the opportunity to be internalized by the action of antibody binding to Trop-2, but because the antibody would hold the conjugate in the tumor for a sustained period, SN-38 also can be released locally, where its ability to be transported quickly across membranes would represent another path for internalization. SN-38 released from the conjugate elsewhere in the body, we anticipate, will behave in the same manner as SN-38 released from irinotecan.

Although the more recently approved ultratoxic ADCs use linkers that bind the drug stably while in serum, as premature release of those agents would increase toxicity and reduce their therapeutic window, our in vivo experience revealed the optimal therapeutic activity for an SN-38 ADC required a linker that neither held the drug stably nor released it too early (9, 10, 21). This conjugate system also permits the site-specific coupling of nearly 8 molecules of SN-38 per IgG without affecting the physiochemical nature, immunoreactivity, or the pharmacokinetics of the conjugate compared with unconjugated IgG (12). Conjugates using ultratoxic agents are generally substituted with about half this amount of drug mainly because higher substitution levels were found to alter pharmacokinetics and reduce the therapeutic index (22, 23).

In addition to these factors, the delivery advantage of IMMU-132 can be highlighted in several other ways. First, a single dose of 40 mg/kg of irinotecan (~0.8 mg) administered in this study carried about 30-fold more SN-38 equivalents than IMMU-132 administered at a single 20 mg/kg dose (1.0 mg IMMU-132 containing 16 µg SN-38), yet by AUC comparisons, IMMU-132 delivered from 20-fold to as much as 136-fold more SN-38 into the tumor than irinotecan. Comparing the AUCs of SN-38 in the tumor to AUCs of free SN-38 in the serum also favored IMMU-132 by 20- to 40-fold. The single dose of irinotecan given to the mice, when converted to a human equivalent dose, is 3.25 mg/kg or 126 mg/m² for a 70 kg/1.8 m² patient, which is 2.8-fold lower than the recommend 350 mg/m² for a single dose of irinotecan given once every 3 weeks. In contrast, the human equivalent dose of IMMU-132 is 4.1 mg/kg, yet the recommended dose level of IMMU-132 for phase II clinical trials is 10 mg/kg weekly × 2 every 3 weeks or about 5-fold higher equivalent dose than the dose given to mice. Thus, the tumor AUC for irinotecan and IMMU-132 were adjusted to a human equivalent dose given weekly × 2 every 3 weeks, the IMMU-132 treatment would deliver as much as 40 to 220 times the amount of SN-38 as the irinotecan dose. However, this difference might be even higher in patients, as (i) humans are at least 5-fold less efficient than mice in converting irinotecan to SN-38 and therefore more SN-38 was available in the mice examined than would be in humans; (ii) humans generate much more inactive SN-38G than SN-38 (mice had equal amounts of SN-38G and SN-38, whereas humans have at least 4-fold more SN-38G than SN-38; ref. 7), which might be expected to alter the balance of SN-38 and SN-38G in the tumors between mice and humans; and (iii) although not measured in this study, a sizable portion of SN-38 derived from irinotecan is in the inactive carboxylate form, whereas SN-38 delivered to the tumor by IMMU-132 would be expected to be released in the active lactone form due to linker coupling to the 20th position on the lactone ring (20).

Both the Capan-1 human pancreatic and the NCI-N87 human gastric cancer cell lines express relatively high levels of Trop-2 (12), and in vivo studies have shown that both respond better to the IMMU-132 treatment than irinotecan (11, 12). Peak concentrations of SN-38 in these tumors occurred between 1 and 6 hours, and despite the 1.6-fold higher expression of Trop-2 in NCI-N87, tumor uptake of IMMU-132 was similar to the Capan-1 tumors. Levels of SN-38 were found in the tumor of animals given IMMU-132, and as the topoisomerase I activity of SN-38 is enhanced in S- phase cells, maintaining an SN-38 presence for 3 days compared with 8 hours would be another advantage. Because resistance to irinotecan therapy can occur for a variety of reasons (24–27), it is uncertain whether the ability to enhance SN-38 delivery with this ADC will affect resistance in a clinically meaningful manner. We
are currently in the process of developing SN-38–resistant cell lines that should provide some insights. Other agents designed to improve the bioavailability of SN-38 have been reported; 2 of these have been examined clinically. EZN-2208 was a PEG-conjugated SN-38, which couples a branched PEG moiety to the lactone ring of SN-38 and stabilized the lactone ring, but its linkage chemistry appeared to have about 12-minute half-life in human serum (28). While providing improved responses in animal models, the agent failed to provide an indication of clinical benefit (29). Eritinotecan pegol is a PEG conjugate composed of a 4-arm, branched PEG, with each arm harboring irinotecan, which has been reported to extend the half-life of SN-38 to about 50 hours (30–33). Preclinical studies in rats showed eritinotecan pegol serum concentrations decreasing from about 700 to 30 µg/mL over 12 hours, with low levels (~30 ng/mL) of both irinotecan and SN-38 detected within the first few minutes; SN-38 concentrations then remained between 10 and 20 ng/mL over 14 days (31). Comparing 3 doses of eritinotecan pegol or irinotecan (days 0, 4, and 8) found that SN-38 concentrations in HT-29 tumor xenografts were 300-fold higher than with irinotecan (31), but SN-38 concentrations in the tumor were still about 17-fold lower than eritinotecan pegol, suggesting about 5% conversion rate at the tumor site. SN-38G concentrations were not reported in this preclinical study, but clinical results have indicated substantially higher levels of SN-38G than SN-38. Promising antitumor responses have been reported for eritinotecan pegol clinically, with dose-limiting toxicity being severe diarrhea that is not manifested until a median of 63 days from the start of therapy (32, 33). Clinical studies with IMMU-132 found dose-limiting neutropenia occurring within 1 week of the start of treatment, but the incidence of severe diarrhea is greatly reduced. Diarrhea from irinotecan therapy can occur as a result of intestinal transport of irinotecan, SN-38, and SN-38G, with enterohepatic recirculation of SN-38G thought to be the primary cause of late diarrhea. With IMMU-132, free SN-38 concentrations in the intestine were as much as 20 times lower in mice. Because SN-38G levels in the serum are also very low with IMMU-132, we suspect that this reduces the pool of drug available for enterohepatic recirculation. These 2 factors likely combine to explain the lower incidence and severity of diarrhea observed clinically with IMMU-132 than other agents delivering SN-38 (34–37).

Concentrations of the intact conjugate in the serum, as monitored by an anti-SN-38 capture antibody probe ELISA or by total SN-38 using HPLC, showed IMMU-132 cleared at a rate that was slightly faster than SN-38 released from the conjugate when held in serum in vitro. This was expected, as the IgG clearance would contribute to the overall rate in vivo. ELISA monitoring of IgG in the serum found that it clears more slowly, staying in the circulation for a longer period. Similar results are being found in patient samples (34–37), where (i) the IgG component clears more slowly than the conjugate, (ii) >95% of the total SN-38 in the serum is bound to the IgG, (iii) the amount of free SN-38G in the serum is only a fraction of the free SN-38, and (iv) there is no evidence of glucuronidation of SN-38 bound to IgG. Analysis of serum samples taken from patients given 8 to 10 mg/kg of IMMU-132 on days 1 and 8 of 21-day cycles has found free SN-38 concentrations at levels of about 100 ng/mL 30 minutes after the end of a 2- to 3-h infusion, whereas the total SN-38 levels average about 4,000 ng/mL (34). These levels decrease steadily, being undetectable within 2 to 3 days; SN-38G levels in sera never exceeded SN-38. Thus, we suspect the levels of free SN-38 in the serum of patients receiving IMMU-132 therapy, which are similar to the peak levels of SN-38 seen in patients given irinotecan therapy (18), contribute to the dose-limiting neutropenia in patients given IMMU-132, whereas the significantly lower levels of SN-38G with IMMU-132 reduce the incidence of severe diarrhea.

In conclusion, this ADC platform departs from the current practice using stably linked ultratoxic agents (subnanomolar), using instead a moderately toxic agent (low nanomolar) that is released in serum. IMMU-132 was found to deliver much higher levels of SN-38 to tumors than irinotecan and, importantly, all of the SN-38 delivered to the tumor by IMMU-132 is released in its most potent form. In relationship to gastrointestinal toxicity, the much lower amounts of SN-38G in the serum and significantly lower amounts of SN-38/SN38G in the intestine with IMMU-132 are expected to reduce the risk for severe diarrhea in patients, which is confirmed in clinical studies (34–38). Thus, IMMU-132 is an ADC that appears to have unique properties that combine to make an effective therapeutic agent in the solid cancers tested.

Disclosure of Potential Conflicts of Interest


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References


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