Combining ABCG2 Inhibitors with IMMU-132, an Anti–Trop-2 Antibody Conjugate of SN-38, Overcomes Resistance to SN-38 in Breast and Gastric Cancers

Chien-Hsing Chang, Yang Wang, Maria Zalath, Donglin Liu, Thomas M. Cardillo, and David M. Goldenberg

Abstract

Sacituzumab govitecan (IMMU-132), an SN-38–conjugated antibody–drug conjugate, is showing promising therapeutic results in a phase I/II trial of patients with advanced Trop-2–expressing, metastatic, solid cancers. As members of the ATP-binding cassette (ABC) transporters confer chemotherapy resistance by active drug efflux, which is a frequent cause of treatment failure, we explored the use of known inhibitors of ABC transporters for improving the therapeutic efficacy of IMMU-132 by overcoming SN-38 resistance. Two human tumor cell lines made resistant to SN-38, MDA-MB-231-S120 (human breast cancer) and NCI-N87-S120 (human gastric cancer), were established by continuous exposure of the parental cells to stepwise increased concentrations of SN-38 and analyzed by flow cytometry for functional activities of ABCG2 and ABCB1, immunoblotting and qRT-PCR for the expression of ABCG2 at both protein and mRNA levels, and MTS assays for the potency of SN-38 alone or in combination with a modulator of ABC transporters. MDA-MB-231-S120 and NCI-N87-S120 displayed reduced sensitivity to SN-38 in vitro, with IC50 values approximately 50-fold higher than parental MDA-MB-231 and NCI-N87 cells. The increase in drug resistance of both S120 cell populations is associated with the expression of functional ABCG2, but not ABCB1. Importantly, treatment of both S120 sublines with known ABCG2 inhibitors (fumitremorgin C, Ko143, and YHO-13351) restored toxicity of SN-38, and the combination of YHO-13351 with IMMU-132 increased the median survival of mice bearing NCI-N87-S120 xenografts. These results provide a rationale for combination therapy of IMMU-132 and inhibitors of ABC transporters, such as YHO-13351. Mol Cancer Ther; 15(8); 1910–9. ©2016 AACR.

Introduction

Multidrug resistance (MDR) is a common cause of treatment failure in cancer therapy (1–3). Thus, despite the notable success in treating diverse cancers, chemotherapeutics, including antibody–drug conjugates (ADC), lose clinical activity over time, as exemplified by irinotecan (4), doxorubicin (5), paclitaxel (6), cisplatin (7), gemtuzumab ozogamicin (8), inotuzumab ozogamicin (9), and others (1).

In general, the occurrence of drug resistance in cancer cells can be intrinsic or acquired, with each type resulting from a variety of factors (10), such as decreased uptake of soluble drugs, activation of drug-detoxifying systems, modulation or mutation of drug targets, defective apoptosis pathways, and above all, overexpression of one or more efflux pumps of the ATP-binding cassette (ABC) superfamily (11). The human genome comprises a total of 49 genes in the ABC superfamily (12), each assigned to one of seven subfamilies (A through G) based on the order and sequence homology of the transmembrane (TM) domain and the nucleotide-binding folds (NBF). To date, ABCB1 (also known as MDR1 or P-gp), ABCC1 (also known as MRP1), and ABCG2 (also known as BCRP, MXR, or ABC-P) account for most studies on MDR (13–15).

Members of the ABC superfamily are transmembrane proteins, expressed either as a full- or half-transporter. A full-transporter typically contains two transmembrane (TM) domains and two nucleotide-binding folds (NBFs), with the TM domains participating in substrate recognition and translocation across the membrane, while the cytosolic NBFs provide the driving force for transport via hydrolysis of the bound ATP. By contrast, a half-transporter has only one TM domain and one NBF, and must form either homodimers or heterodimers to be functional. A notable example of a full-transporter is ABCB1, whose substrates include vinca alkaloids, anthracyclines, epipodophyllotoxins, taxanes, irinotecan, and SN-38 (1). The five members of the ABCG subfamily are all half-transporters (12), of which ABCG2 has been identified for its role in mediating cellular resistance to SN-38 (16, 17), as well as to tyrosine kinase inhibitors (18).

With the molecular mechanisms of intrinsic and acquired drug resistance in cancer increasingly being delineated, multiple approaches to circumvent MDR have emerged. For example, the sensitivity of inotuzumab ozogamicin in ABCB1–expressing sublines of Daudi and Raji lymphomas could be restored effectively...
with PSC-833 (8), a second-generation modifier of ABCB1 (19). The use of a hydrophilic linker for conjugating DM1 to antibodies also enabled such ADCs to evade ABCB1-mediated resistance (20), presumably due to the generation of a cytotoxic metabolite that was better retained by the ABCB1-expressing cells. In addition, targeting detoxifying enzymes, such as glutathione S-transferase, with intracellularly activated produgs was found to be promising (21). However, the strong rationale of using inhibitors of ABC transporters to overcome MDR has met little success in clinical trials, which could be in part due to both imperfect inhibitors and inadequate study design, and is being addressed by developing newer agents with greater substrate specificity, higher potency, lower toxicity, and improved pharmacokinetic properties.

Sacituzumab govitecan, hereafter referred to as IMMU-132 (Supplementary Fig. S1), is a Trop-2–targeting ADC of SN-38, the active metabolite of irinotecan. IMMU-132 departs from most ADCs in its use of a moderately, not ultratoxic drug, its high drug-to-antibody ratio (DAR) without impairing target affinity and pharmacokinetics, and its selection of a pH-sensitive, cleavable linker to confer cytotoxicity to both tumor and bystander cells (22–24). This novel ADC is currently in clinical trials for patients with advanced triple-negative breast cancer (25), uterine cervical cancer (26), and other solid cancers. As these patients were all heavily pretreated with chemotherapy, the presence of acquired resistance with the expression of MDR genes is highly likely, which may affect the therapeutic outcome of IMMU-132. In this study, we explored the use of known inhibitors of ABC transporters for improving the therapeutic efficacy of IMMU-132 by overcoming SN-38 resistance.

Materials and Methods

Cell lines and cultures

Human cancer cell lines (MDA-MB-231, breast; NCI-N87, stomach; A549, lung; HCT15, colon) were purchased from the ATCC with authentication by short tandem repeat profiling. Each cell line was maintained according to the recommendations of ATCC and routinely tested for mycoplasma using MycoAlert Mycoplasma Detection Kit (Lonza). The two lines of ATCC and routinely tested for mycoplasma using MycoAlert Mycoplasma Detection Kit (Lonza). The two

Working solutions of IMMU-132 (at a concentration of 2,500 nmol/L in SN-38 equivalents) and SN-38 at 2,500 nmol/L were prepared from respective stock solutions in sterile media. From these working solutions, serial 5-fold dilutions were made in sterile media to yield final concentrations between 500 and 0.0064 nmol/L in test wells. Plates were incubated at 37°C in a humidified chamber with 5% CO₂ for 96 hours, after which 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium was added and returned to the incubator for 4 hours. The S120 clones were maintained in medium containing 120 nmol/L of SN-38 but cultured in drug-free medium for 4 months. The S120 clones were maintained in medium containing 120 nmol/L of SN-38 but cultured in drug-free medium for 4 months.

Antibodies, IMMU-132, and reagents

Polyclonal rabbit anti-ABC2 antibody (#4477) was purchased from Cell Signaling Technology and murine anti-γH2AX-AF488 (05-636-AF488) from EMD Millipore. Pheophorbide A (PhA), fumitremorgin C (FTC), Ko143, YHO-13351, doxorubicin, paclitaxel, rhodamine 123, and verapamil were obtained from Sigma. SN-38, purchased from Biddle Sawyer Pharma, was diluted to 1 mmol/L in DMSO and stored in aliquots at −20°C. Irinotecan-HCl injection was bought from Areva Phar-
in 4% formalin for 15 minutes, then washed and permeabilized in 0.15% Triton X-100 in PBS for 15 minutes. After washing twice with 1% BSA-PBS, the cells were incubated with murine anti-
γH2AX-AF488 for 45 minutes at 4°C. The signal intensity of
γH2AX was measured by flow cytometry using a FACSCalibur
(BD Biosciences).

Reverse transcription quantitative real-time PCR

Total RNA was extracted using RNeasy Mini Kit (Qiagen) and
reverse transcribed to complementary DNA using SuperScript
IV First-Strand Synthesis System (Life Technologies). qPCR was
performed for each sample in triplicate on a Bio-Rad CFX96
Real-Time System using the TaqMan Master Mix II, all procured from Life Technologies. The mRNA level of ABCG2 was normalized to that of GAPDH and expressed as
2−ΔΔCt, where ΔΔCt = [Ct (ABCG2) − Ct (GAPDH)], and Ct (threshold cycle) is the number of PCR cycle corresponding
to the intersection between an amplification curve and a threshold line determined for a target gene.

In vivo therapy studies

NCI female athymic nude (nu/nu) mice, 4 weeks old, were purchased from Taconic Farms. NCI-N87 and NCI-N87-S120
tumor xenografts were established by harvesting cells from tissue
culture, making a 1:1 cell suspension in Matrigel (BD Biosciences), and injecting each mouse with a total of 1 × 106 cells
subcutaneously in the right flank. Mice tumor (TV) was determined by measurements in two dimensions using calipers, with
dimensions defined as: L × W/2, where L is the longest dimension of the tumor and W the shortest. Mice were randomized into treat-
ment groups of 9 to 10, and therapy begun when tumor volumes were approximately 0.25 cm3. Mice bearing NCI-N87-S120
tumors were treated with irinotecan (40 mg/kg i.v., every other
day for 5 times) or IMMU-132 (0.5 mg i.v. twice weekly for four
weeks). YHO-13351 (0.6 mg i.v.) was administered at the same
time the therapy started and again at 4 hours posttherapy. For the IMMU-132 + YHO-13351 combination group, a third injection of
YHO-13351 was administered 24 hours post-IMMU-132
administration. Control mice received each agent alone. For
YHO-13351 control mice, they were injected on the same sched-
ule as when combined with irinotecan. Another group of mice
bearing parental NCI-N87 tumors served as a control for ef-
ciency of ABCG2, in both parental cells, the intracellular levels
of ABCG2 were approximately 0.25 cm3. Mice bearing parental NCI-N87 tumors were treated with irinotecan (40 mg/kg i.v., every other
day for 5 times) or IMMU-132 (0.5 mg i.v. twice weekly for four
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YHO-13351 control mice, they were injected on the same sched-
ule as when combined with irinotecan. Another group of mice
bearing parental NCI-N87 tumors served as a control for ef-
ciency of IMMU-132 and irinotecan in tumors lacking the ABCG2 pump. The lyophilized IMMU-132 was reconstituted and diluted in
sterile saline as required. Irinotecan-HCl injection was diluted in sterile saline and the final dose based on body weight (40 mg/kg). Mice were euthanized and deemed to have succumbed to disease
once tumors grew to >1.0 cm3 in size.

Statistical analysis

Statistical analysis for the tumor growth data was based on AUC
and survival time. Profiles of individual tumor growth were
obtained through linear curve modeling. An f test was employed to
determine equality of variance between groups prior to statis-
tical analysis of growth curves. A two-tailed t test was used to assess
statistical signiﬁcance between groups. As a consequence of incompleteness of some of the growth curves due to deaths,
statistical comparisons of AUC were only performed up to the
time at which the ﬁrst animal within a group was sacriﬁced. Log-
rank analysis to compare the Kaplan–Meier survival curves of two
groups was performed with GraphPad Prism V6.05. Signiﬁcance
was set at P ≤ 0.05.

Results

Establishment of SN-38-resistant cell lines

Table 1 summarizes the IC50 values of the parental cell lines
(MDA-MB-231 and NCI-N87) and their SN-38-resistant coun-
terparts (MDA-MB-231-S120 and NCI-N87-S120), as deter-
mined for SN-38, doxorubicin and paclitaxel. Compared with the parental cells, both S120 cells are about 50-fold more
resistant to SN-38 and relatively not cross-resistant to either
doxorubicin or paclitaxel. NCI-N87-S120 cells cultured for 3
weeks or longer in SN-38-free medium gradually restored
their sensitivity to SN-38, resulting in a revertant cell line
(NCI-N87-S120-REV) with reduced resistance to SN-38 (IC50 =
50 mmol/L) over a period of 4 months.

Overexpression of functional ABCG2 in the S120 cell lines

The presence of ABCG2 in the S120 cells, but little, if any, in
the parental cells is shown by Western blot analysis (Fig. 1A)
and corroborated by qRT-PCR, which indicate that virtually no
mRNA transcripts of ABCG2 could be detected in MDA-MB-231
and NCI-N87 cells (Supplementary Table S1). On the other
hand, the mRNA levels of ABCG2 relative to GAPDH in
MDA-MB-231-S120 and NCI-N87-S120 are calculated to be
27,408-fold and 167-fold higher than those in MDA-MB-231
and NCI-N87, respectively (Fig. 1B). The expression of ABCG2
was also confirmed in samples obtained from NCI-N87-S120,
but not NCI-N87, xenografts (Fig. 1C).

That ABCG2 is functionally active in the two S120 cell popula-
tions, but absent in the parental cells, is evident from the four
histograms shown in Fig. 2 for PhA, which is a fluorescent
substrate of ABCG2. In both parental cell lines, the intracellular levels of PhA, as measured by the median fluorescence intensity (MFI), are relatively high and remain practically the same with or without
FTC, a potent mycotoxin (Supplementary Fig. S2) initially iden-
tiﬁed for its effective reversal of resistance to mitoxantrone,
doxorubicin, and topotecan in a multidrug-selected cell line. In

Table 1. Sensitivity of the parental and SN-38-resistant cell lines to selected drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>NCI-N87</th>
<th>NCI-N87-S120</th>
<th>RF</th>
<th>MDA-MB-231</th>
<th>MDA-MB-231-S120</th>
<th>RF</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN-38</td>
<td>4.3 ± 0.7</td>
<td>21 ± 39</td>
<td>49.1</td>
<td>4.8 ± 1.7</td>
<td>248 ± 79</td>
<td>51.7</td>
</tr>
<tr>
<td>SN-38 + YHO-13351</td>
<td>3.9 ± 0.5</td>
<td>6.4 ± 1.6</td>
<td>1.6</td>
<td>2.4</td>
<td>16 ± 11</td>
<td>6.7</td>
</tr>
<tr>
<td>SN-38 + Ko43</td>
<td>1.3 ± 0.4</td>
<td>1.3 ± 0.4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>7.9 ± 0.5</td>
<td>3.6 ± 0.6</td>
<td>2.3</td>
<td>10.9 ± 0.6</td>
<td>4.31 ± 4.6</td>
<td>4.0</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>32.2 ± 2.7</td>
<td>7.46 ± 3.3</td>
<td>2.3</td>
<td>10.9 ± 0.6</td>
<td>4.31 ± 4.6</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not determined.

*RF is the resistant factor obtained as the IC50 of SN-38.

*The nontoxic concentrations used for YHO-13351 and Ko43 are 2 and 1 μmol/L, respectively.
levels with a comparable fold-increase with that of SN-38 (Supplementary Table S2). In contrast, in either S120 cells, a high level of intracellular PhA similar to that in the parental cells could only be observed with the addition of FTC, whereas the omission of FTC resulted in a greater than 95% reduction of intracellular PhA. Similar results were obtained in human lung cancer A549 cells, known to express functional ABCG2 (28). In separate studies with rhodamine 123 and verapamil as the substrate and the inhibitor for ABCB1, respectively, activity of ABCB1 was detected in human colorectal cancer HCT15 cells, which served as a positive control for expressing ABCB1 (29), but not in either NCI-N87-S120 or NCI-N87 (Supplementary Table S2).

The activity of ABCG2 in the two S120–resistant cell lines also was demonstrated by comparing the levels of DNA double-strand breaks (DSB) induced by SN-38 with those in the parental cells, as measured by a flow cytometric assay for quantification of γH2AX, whose signal intensities directly correspond to the number of DSBs formed (30). As shown in Fig. 3A, upon treatment with 250 nmol/L of SN-38, the levels of γH2AX rose steadily in the parental, but not the resistant S120 cells, culminating after 3 hours, in an increase over the untreated controls that was about 2-fold for MDA-MB-231 and about 4-fold for NCI-N87 (Fig. 3B). ABCG2 is implicated in preventing the increase of γH2AX in the S120 cells treated with SN-38, as the addition of both FTC (10 μmol/L) and SN-38 (250 nmol/L) to MDA-MB-231-S120 could elevate γH2AX levels with a comparable fold-increase with that of SN-38–treated MDA-MB-231 (Fig. 3C). Similar results were obtained with NCI-N87-S120 when treated with SN-38 or IMMU-132 in the presence of FTC (Fig. 3D).

Sensitizing S120 cells to SN-38 with selected ABCG2 inhibitors

The effect of two known ABCG2 inhibitors, Ko143 (31) and YHO-13351 (32), on reversing the resistance of S120 cells to SN-38 was examined in vitro at a concentration not affecting the growth of either the parental or the S120 cells. As shown in Fig. 4A–C of the representative dose–response curves, and in Table 1 of the pertaining IC50 values, the addition of either ABCG2 inhibitor, while conferring little impact on the sensitivity of parental cells to SN-38, reduced the IC50 of SN-38 by more than 90% in both resistant S120 cell lines. Limited studies also were done for other ABCG2 inhibitors, such as FTC (33), cyclosporine A (34), and GF120918 (35), with results showing comparable or less potency (data not shown). Noting the reported instability of Ko143 in rat serum (36), YHO-13351 was selected over Ko143 for in vivo evaluation.

Improved efficacy of IMMU-132 when combined with YHO-13351 in SN-38–resistant NCI-N87-S120 tumors

NCI-N87-S120 tumors grew slower in the mice than did parental NCI-N87 (Fig. 5A; P = 0.005, AUC), with the median survival for untreated animals more than 2-fold longer for the mice bearing NCI-N87-S120 (P = 0.0006 vs. NCI-N87). Although treatment with IMMU-132 or irinotecan provided no significant survival benefit to mice bearing NCI-N87-S120 tumors (Fig. 5B), both of these therapies resulted in a greater than 2-fold increase in survival in mice bearing NCI-N87-S120 tumors (P < 0.0001; Fig. 5C). However, when IMMU-132 therapy was combined with YHO-13351 in mice bearing SN-38–resistant NCI-N87-S120, a significant 64% improvement in survival was achieved in comparison with untreated animals (P = 0.0852). As the in vitro assay showed there was no difference between parental cell lines treated with SN-38 alone or in combination with YHO-13351, and the tumor xenograft samples of NCI-N87 were absent of ABCG2 by Western blot analysis (Fig. 1C), a combination of IMMU-132 and YHO-13351 was not examined in mice bearing NCI-N87.

Discussion

IMMU-132 is a first-in-class ADC made by conjugating the moderately toxic drug, SN-38 (nanomolar potency), with a partially stable linker, site specifically and at a high DAR of 7.6, to a humanized antibody against Trop-2 expressed in many solid cancers. Preclinical studies have demonstrated that IMMU-132, in comparison with irinotecan, protects the lgG-bound SN-38 from glucuronidation, delivers much more SN-38 (20- to 136-fold higher) to tumor xenografts, resulting in
improved pharmacokinetics and pharmacodynamics (37). As such, IMMU-132 provides a paradigm change that contrasts the prevailing approach of conjugating a low level of an ultratoxic payload (picomolar potency) with a stable linker to an antibody capable of internalization upon target engagement (38, 39). Importantly, the ongoing phase II studies with IMMU-132 as a single agent in patients with metastatic triple-negative breast cancer (mTNBC) who had received a median of 5 (range = 2 to 12) prior lines of therapy have shown an interim objective response rate of 31% by RECIST 1.1 in 58 evaluable patients (40), thus extending the results obtained in phase I trials, which indicated IMMU-132 had acceptable toxicity and encouraging therapeutic activity in patients with difficult-to-treat solid cancers (25). Promising initial results have also been reported for IMMU-132 administered to patients with platinum-resistant urothelial carcinoma (26).

In the current study, NCI-N87-S120 and MDA-MB-231-S120, the two sublines made resistant to SN-38, were shown to be 50-fold less responsive to SN-38 than their parental cells. The sensitivity of NCI-N87 to SN-38 could be restored to within 5-fold of NCI-N87 when propagated in vitro without SN-38 after a period of 3 weeks or longer, suggesting a nongenetic origin of such acquired resistance, which may or may not be clinically relevant. The presence of ABCG2 in the two S120 sublines, but not their parents, was supported by several lines of evidence, including the demonstration of active efflux of PhA; the detection of expressed protein by Western blotting, which was corroborated with qRT-PCR of mRNA; the increased accumulation of SN-38 by FTC using gH2AX as a surrogate marker; and the reverted resistance by YHO-13351 or Ko143. Of note, both S120 sublines were found not to carry ABCB1 and neither phenotype was cross-resistant to doxorubicin or paclitaxel, similar to a previous observation (41) that the ABCG2-expressing, SN-38-resistant human colorectal HCT116-SN50 cancer subline showed no significant cross-resistance to doxorubicin (as well as to 5-fluorouracil and oxaliplatin).

**Figure 2.** Functional assay of ABCG2. Both parental and S120 cells were incubated with 1 μmol/L PHA alone (blue) or together with 10 μmol/L FTC (orange) to generate the PhA efflux histogram. The MFI pertaining to each signal was provided in the histograms. In both parental cells, which do not express ABCG2, the intracellular levels of PhA were high and remained practically the same with or without FTC. In the S120 cells, the active efflux by ABCG2 resulted in much lower levels of PhA, which was restored with the addition of FTC. MDA-MB-231 (left upper panel), MDA-MB-231-S120 (right upper panel), NCI-N87 (left lower panel), NCI-N87-S120 (right lower panel).

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Figure 3.
γH2AX assay for DSB by flow cytometry. Cells were treated or not treated with SN-38 (250 nmol/L) for 3 hours, and the levels of γH2AX were monitored hourly by flow cytometry and shown as MFI in a bar diagram (A) or as percentage of untreated (B). The effect of FTC (10 μmol/L) to increase the formation of DSB/γH2AX was shown for MDA-MB-231-S120 treated with SN-38 (C) and for NCI-N87-S120 treated for either SN-38 or IMMU-132 (D).
Figure 4.
Dose-response curves of parental and S120 cells treated with different concentrations of SN-38 in the absence and presence of YHO-13351 or Ko143. Reversal of SN-38 resistance by YHO-13351 was shown for MDA-MB-231-S120 (A), NCI-N87-S120 (B), and by Ko143 for NCI-N87-S120 (C).
Figure 5.
Efficacy of IMMU-132 in mice bearing SN-38-resistant NCI-N87-S120 gastric carcinoma xenograft. **A,** mean tumor growth curves for NCI-N87 and NCI-N87-S120 xenografts. **B,** mice bearing NCI-N87-S120 SN-38-resistant human gastric tumors were treated with IMMU-132, irinotecan, YHO-13351, or combinations as indicated on the graph and described in Materials and Methods. **C,** mice bearing parental NCI-N87 tumors treated with IMMU-132 or irinotecan at the same dose and schedule as used in NCI-N87-S120 tumor-bearing animals. In the survival curves of B and C, the starting day of therapy (when tumor volumes reached \( \approx 0.25 \text{ cm}^3 \)) was marked as day 0. Mice were euthanized once tumors grew to \( \approx 1.0 \text{ cm}^3 \) in size.
In other human cancer clones selected for resistance to SN-38 via continuous exposure of parental cell lines to the drug in culture, overexpression of ABCG2 has also been reported for the sublines generated from MCF-7 (42, 43), MDA-MB-231 (43), the small-cell lung carcinoma PC-6 (17), the non–small cell lung adenocarcinoma H23 (44), and the cervical carcinoma HeLa (45). Cross-resistance of these sublines to doxorubicin varied somewhat, with the resistance ratio (IC50, in resistant subline divided by IC50 in the parental cell) being less than 1.3 for the sublines of PC-6 (17), 2.5 for the sublines of HeLa (45), and about 7.0 for the subline of H23 (44). Whereas cross-resistance to doxorubicin was not determined for the SN-38–resistant sublines derived from MCF-7 (42, 43) or MDA-MB-231 (43), these sublines remained sensitive to vincristine (42), cisplatin (42, 43), and docetaxel (43).

Although we and others have established ABCG2 as a key player in reducing SN-38 sensitivity of various SN-38–resistant cancer sublines, the potential involvement of DNA topoisomerase I (Top1), to which SN-38 specifically binds and acts as an inhibitor, in the SN-38 resistance mechanism of such cancer cells, is less defined and remains a focus of continuous research, with the current knowledge pointing to Top1 mutation (46, 47) and degradation (48) as the two main roles of Top1 underlying the molecular mechanism of resistance to camptothecin in general and SN-38 in particular.

When cultured in vitro, SN-38–resistant sublines of MCF-7 or MDA-MB-231 had longer doubling times than their parental cells (43). Thus, it is not surprising that NCI-N87–S120 xenografts grew significantly slower in the mice than NCI-N87, which is consistent with the notion that drug-resistant tumor sublines selected in vitro frequently manifest less aggressive properties than their drug-sensitive parental cell lines (49). Nevertheless, in vitro studies show that the NCI-N87–S120 xenograft retained ABCG2 expression and was resistant to IMMU-132, yet its growth could be significantly subdued by IMMU-132 in combination with YH0–13351. A parallel study shows the parental xenograft was responsive to IMMU-132 or irinotecan, but with a shorter median survival time. Together, these in vivo results suggest that suitable inhibitors that are tolerated well by the host animals can overcome ABC resistance and that the resistant tumor lines can become appreciably responsive to IMMU-132 and to a lesser extent to irinotecan. We are pursuing further work to address the feasibility of preclinical testing for such drug resistance as a predictive bioassay (43, 44) to select patients who should receive ABC-blocking therapy with IMMU-132. Meanwhile, we are examining the suitability of clinically tested tyrosine kinase inhibitors, some of which interfere with the functions of ABC transporters at nontoxic levels (50), to enhance the potency of IMMU-132 in cancer cells that are intrinsically or made resistant to SN-38.

Disclosure of Potential Conflicts of Interest

All authors are current employees of Immunomedics, Inc., and have stocks or stock options of Immunomedics, Inc. No other potential conflicts of interest were disclosed by the authors.

Authors’ Contributions

Conception and design: C.-H. Chang, T.M. Cardillo, D.M. Goldenberg

Development of methodology: C.-H. Chang, D. Liu, T.M. Cardillo

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Y. Wang, M. Zalath, D. Liu, T.M. Cardillo

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.-H. Chang, Y. Wang, M. Zalath, D. Liu, T.M. Cardillo

Writing, review, and/or revision of the manuscript: C.-H. Chang, D. Liu, T.M. Cardillo, D.M. Goldenberg

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T.M. Cardillo, D.M. Goldenberg

Study supervision: C.-H. Chang, T.M. Cardillo, D.M. Goldenberg

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References


5. Miller RL, Bukowski RM, Budd GT, Purvis J, Weick JK, Sheppard K, et al. Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Y. Wang, M. Zalath, D. Liu, T.M. Cardillo


Overcoming SN-38-ADC Drug Resistance


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