Synthetic Lethality Exploitation by an Anti–Trop-2-SN-38 Antibody–Drug Conjugate, IMMU-132, Plus PARP Inhibitors in BRCA1/2-wild-type Triple-Negative Breast Cancer

Thomas M. Cardillo, Robert M. Sharkey, Diane L. Rossi, Roberto Arrojo, Ali A. Mostafa, and David M. Goldenberg

Abstract

Purpose: Both PARP inhibitors (PARPi) and sacituzumab govitac (IMMU-132) are currently under clinical evaluation in triple-negative breast cancer (TNBC). We sought to investigate the combined DNA-damaging effects of the topoisomerase I (Topo I)–inhibitory activity of IMMU-132 with PARPi disruption of DNA repair in TNBC.

Experimental Design: In vitro, human TNBC cell lines were incubated with IMMU-132 and various PARPi (olaparib, rucaparib, or talazoparib) to determine the effect on growth, double-stranded DNA (dsDNA) breaks, and cell-cycle arrest. Mice bearing BRCA1/2-mutated or wild-type human TNBC tumor xenografts were treated with the combination of IMMU-132 and PARPi (olaparib or talazoparib). Study survival endpoint was tumor progression to >1.0 cm³ and tolerability assessed by hematologic changes.

Results: Combining IMMU-132 in TNBC with all three different PARPi results in synergistic growth inhibition, increased dsDNA breaks, and accumulation of cells in the S-phase of the cell cycle, regardless of BRCA1/2 status. A combination of IMMU-132 plus olaparib or talazoparib produces significantly improved antitumor effects and delay in time-to-tumor progression compared with monotherapy in mice bearing BRCA1/2-mutated HCC1806 TNBC tumors. Furthermore, in mice bearing BRCA1/2-wild-type tumors (MDA-MB-468 or MDA-MB-231), the combination of IMMU-132 plus olaparib imparts a significant antitumor effect and survival benefit above that achieved with monotherapy. Most importantly, this combination was well tolerated, with no substantial changes in hematologic parameters.

Conclusions: These data demonstrate the added benefit of combining Topo I inhibition mediated by IMMU-132 with synthetic lethality provided by PARPi in TNBC, regardless of BRCA1/2 status, thus supporting the rationale for such a combination clinically. Clin Cancer Res; 23(13); 3405–15. ©2017 AACR.
agents as ionizing radiation or platinum-based therapeutics, while these repair pathways are being blocked with PARPi (12, 13). In addition to agents that directly interact with DNA to cause breaks, agents that inhibit topoisomerase I (Topo I), including irinotecan, have been shown to synergize with PARPi to deter the growth of a range of human tumor cell lines, including those of lung, ovarian, colon, and breast cancers (14, 15). These efforts demonstrate that by combining a DNA-damaging agent with an HRR synthetic lethality–based therapy, improved antitumor effects can be achieved compared with single modalities.

Saciutzumab govitecan (IMMU-132) is an antibody–drug conjugate (ADC) composed of the active metabolite of irinotecan, SN-38, conjugated to an anti–Trop-2 antibody (drug:Ab ratio = 7:6; ref. 16). Preclinically, IMMU-132 has significant efficacy across a broad range of Trop-2–positive human cancer xenograft models, including non–small cell lung cancer (NSCLC), pancreatic, colon, gastric cancers, and TNBC (16–18). In both gastric and TNBC tumor cells, IMMU-132 specifically mediated dsDNA breaks in Trop-2–expressing cells (16, 17). In a current clinical trial (ClinicalTrials.gov, NCT01631552), IMMU-132 has achieved objective responses against a range of solid tumors, including small-cell lung carcinoma (19), NSCLC (20), and TNBC (21). In TNBC patients, with a median of 18 weeks old, were purchased from Taconic Farms. Xenografts were established by harvesting cells from tissue culture and mixing 1:1 with Matrigel, such that each mouse received a total of 1 x 10^7 cells s.c. in the right flank. Tumor volume was determined by measurements in two dimensions using calipers, with volumes defined as: V = l x w^2/2, where l is the longest dimension of the tumor and w the shortest. Mice were randomized into treatment groups, and therapy began when tumor volumes were approximately 0.3 cm^3. Treatment regimens, dosages, and number of animals in each experiment are described in the Results section and in the figure legends. Asynchronous cells were used for cell-cycle analysis, as described in Supplementary Data.

Materials and Methods
Cell lines, ADCs, and PARPi
All human cancer cell lines were purchased from the American Type Culture Collection (ATCC). Each cell line was maintained according to the recommendations of the ATCC, routinely tested for mycoplasma using MycoAlert Mycoplasma Detection Kit (Lonza), and authenticated by short-tandem repeat assay by the ATCC. Cells were in culture less than 6 months when employed in experiments. IMMU-132 and a control ADC (anti-CD20 hA20-SN-38) were prepared at Immunomedics, Inc., as described previously (18). PARPi (olaparib, talazoparib, and rucaparib) were purchased and dissolved in DMSO according to the manufacturer’s recommendations (Selleck Chemicals).

In vitro combination cytotoxicity assays, Western blotting, and cell-cycle analysis
Cytotoxicity studies were conducted as described previously (18) and are presented in Supplementary Data. Cell lysates were prepared, and immunoblotting for phospho-H2A.X, poly(ADP-ribose) (PAR), PARP, FACCID2, BARD1, Rad51, ERCC1, and β-actin was done as described in Supplementary Data. Concentrations, timing, and primary antibodies are shown in the figure legends. Asynchronous cells were used for cell-cycle analysis, as described in Supplementary Data.

In vivo therapeutic studies
All animal studies were approved by Rutgers School of Biomedical and Health Sciences Institutional Animal Care and Use Committee. NCr female athymic nude (nu/nu) mice, 4 to 8 weeks old, were purchased from Taconic Farms. Xenografts were established by harvesting cells from tissue culture and mixing 1:1 with Matrigel, such that each mouse received a total of 1 x 10^7 cells s.c. in the right flank. Tumor volume was determined by measurements in two dimensions using calipers, with volumes defined as: V = l x w^2/2, where l is the longest dimension of the tumor and w the shortest. Mice were randomized into treatment groups, and therapy began when tumor volumes were approximately 0.3 cm^3. Treatment regimens, dosages, and number of animals in each experiment are described in the Results section and in the figure legends. The lyophilized IMMU-132 and control ADC (anti-CD20, hA20-CL2A-SN-38) were reconstituted and diluted as required in sterile saline. Olaparib was diluted in 2-hydroxy-propyl-β-cyclodextran/PBS (10% w/v) and administered i.p. to the mice. Talazoparib was diluted in 10% DMSO/5% Kolliphor HS15/85% PBS and administer p.o. to the animals.

Mice were deemed to have succumbed to disease progression and euthanized once tumors grew to greater than 1.0 cm^3 in size. A partial response is defined as shrinking the tumor >30% from initial size. Stable disease is when the tumor volume remains between 70% and 120% of initial size. Time-to-tumor progression (TTP) was determined as time when tumor grew more than 20% from its nadir.

Hematologic toxicity of combined IMMU-132 and olaparib was assessed in female BALB/c mice, as described in Supplementary Data. Dosages and timing are described in the Results section and in the figure legends.
Synergy and Efficacy of Combined IMMU-132 Plus PARPi in TNBC

Statistical analysis of in vivo data

A critical-Z test was performed on the survival data of treatment and control groups with \( P \leq 0.05 \) for any mouse deemed an outlier. Such mice were removed from further statistical analysis and are noted in the Results section. Statistical analysis of tumor growth was based on AUC. 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 statistical analysis of growth curves. A two-tailed \( t \) test was used to assess statistical significance between the various treatment groups and controls, except for the saline control, where a one-tailed \( t \) test was used in the comparison. Survival studies were analyzed using Kaplan–Meier plots (log-rank analysis), using the Prism GraphPad Software (v6.05) package (Advanced Graphics Software, Inc.). Significance was set at \( P \leq 0.05 \).

Results

Changes in expression of various HRR proteins mediated by IMMU-132

It had previously been found that upregulation of several different proteins involved in HRR upon DNA damage (FANC2, BARD1, Rad51, and ERCC1) plays an important role in resistance to DNA-damaging treatment in TNBC, particularly in BRC1/2–wild-type tumor cells (22–24). To determine the effect IMMU-132–mediated DNA damage has on these other repair proteins, BRC1/2–wild-type TNBC cell lines (MDA-MB-231, MDA-MB-468, and BT-20) were exposed to various concentrations of IMMU-132 (25, 50, and 100 nmol/L SN-38 equivalents) for 24 hours. In two of the cell lines (MDA-MB-231 and BT-20), levels of all four HRR proteins examined were elevated, suggesting that the cells were activating these DNA-repair pathways in response to IMMU-132–mediated damage (Supplementary Fig. S1). In a BRC1/2-defective cell line (HCC1806), levels of these proteins remained unchanged in response to IMMU-132 exposure. Interestingly, MDA-MB-468 (BRC1/2–wild-type, PTEN defective), FANC2, Rad51, and ERCC1 levels dropped relative to basal levels upon IMMU-132 exposure. These data suggest that HCC1806 and MDA-MB-468 TNBC tumors would be the most sensitive to the combination of IMMU-132 and PARPi, because they do not activate these HRR repair pathways.

In vitro synergistic growth inhibition of TNBC when IMMU-132 is combined with various PARPi

Changes in IC\textsubscript{50} values for IMMU-132 when combined with various PARPi were determined in human TNBC cell lines with BRC1/2 mutations (HCC38 and HCC1806) and those with wild-type BRC1/2 (MDA-MB-468, MDA-MB-231, and BT-20). Isobolograms for IMMU-132 were clearly enhanced when combined with olaparib, rucaparib, or talazoparib, demonstrating a synergistic interaction in all four cell lines (Supplementary Fig. S2). IC\textsubscript{50} values for IMMU-132 alone were 1.9 to 82.5 nmol/L, whereas olaparib and rucaparib IC\textsubscript{50} values were 5.4 to 256.7 nmol/L (Table 1). Although talazoparib was more potent (4.7 to 322 nmol/L) than either olaparib or rucaparib, IC\textsubscript{50} values for IMMU-132 indicated it was >2-fold more potent than talazoparib. Calculated combinatorial index (CI) values demonstrate that even when combined with IC\textsubscript{50} concentrations, an additive effect was achievable (i.e., CI = 1.0), but this was further enhanced to synergy (i.e., CI < 1.0) when as little as IC\textsubscript{20} concentrations were used (Table 1). Importantly, this synergy occurred in both BRC1/2–mutated and –wild-type cell lines, despite the upregulation of HRR proteins shown in MDA-MB-231 and BT-20 in response to IMMU-132–mediated DNA damage.

Table 1. CI values for IMMU-132 plus olaparib, rucaparib, or talazoparib in four TNBC cell lines

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Single-agent IC\textsubscript{50} values (mean ± SD)</th>
<th>Cl values</th>
<th>Cl values</th>
<th>Cl values</th>
<th>Cl values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IMMU-132 (nmol/L)</td>
<td>Olaparib (μmol/L)</td>
<td>Rucaparib (μmol/L)</td>
<td>Talazoparib (μmol/L)</td>
<td>IMMU-132</td>
</tr>
<tr>
<td>HCC1806</td>
<td>1.9 ± 0.4</td>
<td>7.3 ± 1.4</td>
<td>5.4 ± 0.3</td>
<td>4.7 ± 1.0</td>
<td>0.81</td>
</tr>
<tr>
<td>HCC38</td>
<td>3.4 ± 0.5</td>
<td>28.5 ± 18.7</td>
<td>16.9 ± 0.7</td>
<td>104.9 ± 44.7</td>
<td>0.46</td>
</tr>
<tr>
<td>MDA-MB-468</td>
<td>3.5 ± 0.4</td>
<td>9.8 ± 1.0</td>
<td>10.3 ± 0.8</td>
<td>322.3 ± 27.7</td>
<td>0.87</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>7.5 ± 1.2</td>
<td>27.7 ± 0.0</td>
<td>7.1 ± 1.0</td>
<td>36.3 ± 8.0</td>
<td>0.89</td>
</tr>
<tr>
<td>BT-20</td>
<td>82.5 ± 22.5</td>
<td>256.7 ± 40.4</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.91</td>
</tr>
</tbody>
</table>

NOTE: CI values determined as described in Materials and Methods. IC\textsubscript{10}, IC\textsubscript{20}, and IC\textsubscript{30}, CI values determined when IMMU-132 or PARPi used at concentrations calculated to cause 10%, 20%, and 30% growth inhibition when used alone, respectively. Abbreviation: n.d., not done.
Figure 1.
Western blot assessment of effects on PAR, PARP, and dsDNA breaks mediated by IMMU-132 plus PARPi in TNBC tumor lines. Cells were plated overnight in 6-well plates before the addition of chemotherapeutics. After a 24-hour incubation, cells were harvested and cell lysates resolved and transferred for Western analysis as described in Materials and Methods. PAR and FL-PARP levels were determined on the same gel. Assessment of dsDNA breaks (p-H2A.X) was calculated as ratios relative to untreated control (Unt) normalized to β-actin protein loading control (Δp-H2A.X).

A, All four TNBC cell lines (HCC38, HCC1806, MDA-MB-468, and MDA-MB-231) were exposed to olaparib (Olap), IMMU-132, or the combination of both. IMMU-132 concentrations are expressed in terms of SN-38 equivalents.

B, HCC1806 cells exposed to rucaparib (Ruc) and IMMU-132 or to (C) talazoparib (Tala) and IMMU-132.
Rucaparib and talazoparib were used at 10- and 100-fold lower concentrations, respectively, when combined with IMMU-132 in HCC1806 (Fig. 1B and C, respectively). Likewise, the amount of IMMU-132 required to demonstrate increases in dsDNA breaks, when combined with these two PARPi, was lower (0.1, 1, and 3 nmol/L SN-38 equivalents). Rucaparib alone resulted in a 1.6-fold increase in dsDNA breaks, whereas 0.1 nmol/L SN-38 equivalents of IMMU-132 (2 ng/mL of the ADC) resulted in a 2.2-fold increase. Together, IMMU-132 plus rucaparib further increased the amount of dsDNA breaks 3.9-fold, which is 77% higher than either single agent. Talazoparib and IMMU-132 combined demonstrated a dose-response with the amount of dsDNA breaks increasing by 78% at the lowest IMMU-132 concentration to 216% at the highest concentration. These data demonstrate that three different PARPi, when combined with IMMU-132, result in increased DNA damage in the form of double-stranded breaks above that observed with single-agent treatments, which is consistent with the in vitro cytotoxicity assays demonstrating synergistic growth inhibition when IMMU-132 was combined with these PARPi.

Changes in cell cycle mediated by IMMU-132 plus olaparib

Cell-cycle changes were assessed in all four TNBC cell lines when IMMU-132 and olaparib were incubated with asynchronous cells for 24 hours (Supplementary Fig. S3). Greater than 80% of the cells in the untreated groups were in the G1-phase. Cells began to accumulate in S-phase when exposed to either olaparib or IMMU-132 alone. However, the combination demonstrated an even greater percentage of cells in S-phase, with a concomitant decrease in the percentage of cells in G1-phase and little change in G2–M. For both BRCA1/2-mutated cells and BRCA1/2–wild-type MDA-MB-231, the combination of IMMU-132 and olaparib resulted in a greater than 2-fold increase in the percentage of cells in the S-phase relative to each agent alone. MDA-MB-468 demonstrated the least increase in the percentage of cells in S-phase relative to single-agent treatment, but this was due primarily to the greater effect each agent alone had, with more than 32% and 50% of the cells in S-phase upon treatment with olaparib or IMMU-132 alone, respectively. Even so, the combination increased the number of cells in S-phase by 12% over IMMU-132 alone. Similar results were observed when IMMU-132 was combined with rucaparib (1 μmol/L) or talazoparib (0.2 μmol/L), in which the combination of IMMU-132 plus either PARPi increased the percentage of cells in S-phase for all four cell lines, relative to single-agent treatment (Supplementary Tables S1 and S2, respectively).

Improved efficacy of IMMU-132 in mice bearing TNBC tumors when combined with olaparib or talazoparib

Therapeutic efficacy of combining IMMU-132 with olaparib was tested in HCC1806 and MDA-MB-468 tumor xenografts (Fig. 2). Importantly, in both experiments, the dose and schedule of IMMU-132 administered to the animals were chosen to give only a modest therapeutic effect in order to detect a potential interaction between IMMU-132 and olaparib.

In mice bearing HCC1806 tumors (Fig. 2A), the combination therapy resulted in a significant antitumor effect when compared with all other treatment groups (P < 0.0017; AUC; Supplementary Table S3). In this group, all of the mice exhibited a partial response, with 30% being tumor-free (i.e., complete response) when the experiment ended on therapy day 108. Mean TTP for this group was 36.9 ± 8.1 days (Table 2). In contrast, tumors in mice given IMMU-132 or olaparib monotherapy progressed with mean TTP’s of 17.6 ± 3.9 and 9.1 ± 4.8 days, respectively. Between the two monotherapy groups, IMMU-132 significantly delayed tumor progression when compared with olaparib therapy (P = 0.0009).

Overall, the combination had a significantly greater delay in TTP when compared with either single-agent therapy group (P < 0.0001). Of note, this combination proved to be well-tolerated by the mice, with no appreciable loss in body weight (Supplementary Fig. S4A). Even mice treated with 4-fold more IMMU-132 (500 μg twice weekly x 4 weeks) combined with olaparib demonstrated no significant loss in weight (Supplementary Fig. S4B).

In mice bearing MDA-MB-468 tumors, the combination of IMMU-132 plus olaparib had a significant antitumor effect when compared with all other treatment groups, including IMMU-132 monotherapy (Fig. 2B; P < 0.0040; AUC; Supplementary Table S4). In terms of response rate, 30% of mice treated with only IMMU-132 exhibited a partial response, as did 20% of the animals treated with olaparib (Table 2). In contrast, all of the mice in the combination group exhibited a partial response, with a TTP of 55.2 ± 6.5 days, which was significantly better than all the other groups (P < 0.0004) except IMMU-132 alone, which approached significance at a TTP of 45.1 ± 13.6 days (P = 0.0604). As in the HCC1806 tumor model, the mice tolerated the combination of IMMU-132 and olaparib without loss of body weight (Supplementary Fig. S4C).

Given the ability of IMMU-132 and olaparib to improve efficacy significantly compared with single-agent therapy in MDA-MB-468 tumors, which carry wild-type BRCA1/2, another therapy study utilized mice bearing MDA-MB-231 tumors that likewise are wild-type for BRCA1/2, but demonstrated synergy when IMMU-132 and PARPi were combined in vitro. MDA-MB-231 tumors progressed very rapidly, reaching experimental endpoints (i.e., tumor volume >1.0 cm3) within 14 days of starting therapy. Given this rapid disease progression, antitumor effects are represented as the ability to provide survival benefit and not tumor regressions. As noted in the previous two therapy experiments, olaparib alone had no antitumor effects in mice bearing MDA-MB-231 tumors, with median survival no different than saline control mice (Fig. 2C). Not unexpectedly, IMMU-132 alone did not significantly improve the overall survival of the treated animals, since it was shown previously to be resistant to this therapy (16). However, when mice were treated with the combination of IMMU-132 plus olaparib, there was a significant increase in median survival from 14 days for saline control animals to 21 days for those mice treated with the combination (P = 0.0005). Likewise, treatment with olaparib also resulted in a significant survival benefit when combined with the nonspecific control ADC [median survival time (MST) = 17 days; P = 0.0257], suggesting that the olaparib does sensitize this resistant tumor to SN-38. These results show, unexpectedly, that even in tumors resistant to PARPi and IMMU-132 monotherapies, the combination is able to overcome this resistance and impart a survival benefit when compared with saline control animals. Because talazoparib’s potency was greater than olaparib in vitro, both alone and when combined with IMMU-132, mice bearing HCC1806 tumors were treated with the combination of IMMU-132 and talazoparib (Fig. 2D). Whereas mice in the olaparib experiments were treated with 50 mg/kg, talazoparib-treated animals were given only 0.33 mg/kg. As observed in the olaparib experiments, IMMU-132 plus talazoparib had a significant antitumor effect when compared with all other treatments, including
Figure 2.
Therapeutic efficacy of IMMU-132 plus PARPi in BRCA1/2-deficient and −wild-type TNBC tumor xenograft disease models. Tumors were established, and disease endpoints are described in Materials and Methods. All the ADCs and controls were administered in the amounts indicated (green arrows, PARPi injections; purple arrows, ADC injections). A, Mice bearing HCC1806 tumors (N = 9–10) were injected with IMMU-132 weekly for 4 weeks and with olaparib daily (Monday through Friday) for 4 weeks. B, MDA-MB-468 tumor–bearing mice (N = 9–10) were injected weekly with IMMU-132 for 2 weeks with 1 week off before repeating. Likewise, olaparib was administered daily (Monday to Friday) for 2 weeks with 1 week off before repeating. C, Mice bearing MDA-MB-231 tumors (N = 10) were treated with IMMU-132 and olaparib under the same schedule as the HCC1806 mice. D, Mice bearing HCC1806 tumors (N = 9–10) were treated with IMMU-132 weekly for 4 weeks and talazoparib daily (Monday to Friday) for 4 weeks. All IMMU-132 and control ADC injections were administered as i.v. Olaparib was administered i.p. and talazoparib p.o.
monotherapy with IMMU-132 or talazoparib (P < 0.0088, AUC). Mean TTP was 21.8 ± 9.6 days, which was >2.8-fold longer than any of the other treatment groups (P < 0.0021; Table 3). Similar to the combinations with olaparib, IMMU-132 plus talazoparib did not result in any observable toxicity in the mice (Supplementary Fig. S4D).

Hematologic tolerability of IMMU-132 plus olaparib in naïve BALB/c mice

Clinical studies indicate that there is no off-tumor, on-target toxicities in patients (19), and therefore an experiment was performed to determine if the combination of a PARP inhibitor with IMMU-132’s SN-38 would have increased toxicities at therapeutically active doses. Because normal tissues of mice are not targeted by the anti–Trop-2 antibody comprising IMMU-132, toxicity studies were conducted in mice to determine possible off-target toxicities at doses of IMMU-132 and olaparib shown to have therapeutic activity when combined in tumor-bearing mice (i.e., 250 μg IMMU-132 plus 50 mg/kg olaparib). The schedule used mimics the clinical regimen of IMMU-132 (i.e., weekly for 2 weeks with 1 week off per cycle) with olaparib administered also over a 2-week period. This same dose schedule does demonstrate antitumor activity over this 2-week period, as shown in the efficacy studies described above (Fig. 2A and B). Naïve BALB/c mice were administered a 2-week course of IMMU-132 plus olaparib therapy to ascertain any possible myelotoxicity. On day 15 (i.e., 7 days after the last IMMU-132 injection and 3 days after last dose of olaparib), there was no evidence of hematologic toxicity in any of the mice (Fig. 3). In particular, the combination did not result in any significant drop in total white blood cell (WBC) or lymphocyte counts. Most importantly, there was no evidence of neutropenia or thrombocytopenia in any of these mice. Only mice treated with olaparib monotherapy demonstrated a significant drop in lymphocytes relative to vehicle control animals (P = 0.0481), although the mean (3.92 ± 1.31 K/μL) remained within the normal range (3.8 to 8.9 K/μL). After a further 7 days (i.e., day 22), the assessment of toxicity was determined in the remaining mice. At this time-point, all three treatments showed a significant drop in WBC and lymphocyte counts relative to control animals (P < 0.0012). However, most of these values still remained within an acceptable range, with the lowest values found in those mice treated with the combination, which fell just below the lower end of the normal range (mean lymphocyte count of 3.62 ± 0.54 K/μL vs. 7.04 ± 1.17 K/μL for control, P = 0.003). These results indicate that the combination of IMMU-132 and olaparib, which has been shown to elicit antitumor effects in TNBC tumor-bearing mice, is not associated with any significant myelotoxicity, and suggests a potentially high therapeutic window.

Discussion

Exploitation of defects to a cell’s HRR pathways has gained widespread interest as a means to induce synthetic lethality in various human cancers, including TNBC (1, 2). Of particular interest are those drugs that target PARP (26, 27). Although some clinical studies have suggested that use of PARPi can provide meaningful responses, other trials have shown no significant improvements in clinical outcome (7, 10). Given these mixed results, new approaches seek to combine PARPi with other DNA-damaging agents, such as platinum-based drugs, microtubule inhibitors, or irinotecan (14, 22, 28–31). Here, we examined the effect of combining several PARPi (olaparib, rucaparib, and talazoparib) with our Topo I–inhibiting ADC, anti–Trop-2/SN-38-ADC, and IMMU-132. All three PARPi synergized with IMMU-132 to inhibit the growth of four human TNBC cell lines. This occurred independently of BRCA1/2 status, as evidenced by the synergistic growth inhibition in vitro of two cell lines with wild-type BRCA1/2. IMMU-132 combined with these PARPi increased the dsDNA breaks above that observed with each single agent, and the accumulation of cells in the S-phase of the cell cycle. Most importantly, in mice bearing either a BRCA1/2-mutated TNBC tumor (HCC1806) or ones with wild-type BRCA1/2 (MDA-MB-468 and MDA-MB-231), the combination of IMMU-132 plus a

| Table 2. TTP for TNBC tumor-bearing mice treated with IMMU-132 plus olaparib |
|-----------------------------|---------|------------------|---------------|------------------|---------|
| Treatment                  | N       | TTP (days)       | IMMU-132 vs. controls (P value) | Combination vs. controls (P value) | N       |
| IMMU-132 + Olaparib        | 10      | 218 ± 9.6        | N.A.          | N.A.             | 10      |
| Control ADC + Olaparib     | 10      | 6.8 ± 5.4        | 0.6979        | 0.0004           |         |
| IMMU-132                   | 9       | 7.9 ± 6.6        | N.A.          | 0.0002           | 9       |
| Olaparib                   | 10      | 5.9 ± 5.9        | 0.5099        | 0.0005           |         |
| Control ADC                | 10      | 4.6 ± 2.1        | 0.1866        | 0.0003           |         |
| Saline                     | 10      | 4.2 ± 1.9        | 0.0709        | 0.0001           |         |

Abbreviations: N, number of mice per group; N.A., not applicable; TTP, time-to-tumor progression for mice not tumor-free as defined in Materials and Methods; % PR, percentage of mice that exhibited a positive response to treatment.

| Table 3. TTP for HCC1806 tumor-bearing mice treated with IMMU-132 plus talazoparib |
|-----------------------------|---------|------------------|---------------|
| Treatment                  | N       | TTP (days)       | IMMU-132 vs. control (P value) |
| IMMU-132 + Talazoparib     | 10      | 218 ± 9.6        | N.A.          |
| Control ADC + Talazoparib  | 10      | 6.8 ± 5.4        | 0.6979        |
| IMMU-132                   | 9       | 7.9 ± 6.6        | N.A.          |
| Talazoparib                | 9       | 5.9 ± 5.9        | 0.5099        |
| Control ADC                | 10      | 4.6 ± 2.1        | 0.1866        |
| Saline                     | 10      | 4.2 ± 1.9        | 0.0709        |

Abbreviations: N, number of mice per groups; N.A., not applicable; TTP, TTP as defined in Materials and Methods.
PARPi resulted in significant antitumor effects above those observed with monotherapy. These results clearly illustrate the potential clinical benefit that may be derived by combining IMMU-132 with a PARPi in TNBC.

Topo I is an enzyme utilized by the cell to allow unwinding of the DNA strand during transcription and replication (32). PARP-1, the most abundant of the PARP proteins, has been found to colocalize with Topo I throughout the cell cycle. However, upon DNA damage, PARP-1 dissociates from Topo I, resulting in reduced activity of this enzyme (33). We hypothesized that by combining the Topo I inhibitor carried by IMMU-132 (i.e., SN-38) with a PARPi, there would be an accumulation of dsDNA breaks due to the inability of the remaining HRR pathways in the cell to repair this damage with high fidelity, resulting in apoptosis and cell death. In addition, in cells lacking functional BRCA1/2 or are otherwise deficient in HRR, the more error-prone, nonhomologous end-joining (NHEJ) pathway is utilized, further compromising the cell toward irreparable DNA damage and apoptosis (34). Others have found that in both BRCA1–wild-type and -mutated TNBC cell lines, the combination of CPT-11 (the produg of SN-38) and a PARPi could result in synergistic growth inhibition in vitro (22). Here, too, we demonstrated that IMMU-132, when combined with olaparib, rucaparib, or talazoparib, mediates synergistic tumor cell growth inhibition and increased dsDNA breaks in both BRCA1/2-mutated and –wild-type cell lines. These data confirm the ability of IMMU-132–mediated inhibition of Topo I, when combined with PARPi, to synergize growth inhibition in human TNBC cells regardless of BRCA1/2 mutational status.

HRR pathways are more active during the late S–G2-phase of the cell cycle, whereas the more error-prone NHEJ repair pathway is most evident throughout the S-phase (35). Cells with HRR defects, as well as those exposed to PARPi, will arrest at G2–M-phase (23, 36). Although we found that PARPi (olaparib, rucaparib, and talazoparib) and IMMU-132 exposure resulted in small increases in the number of cells in the G2–M-phase of the cell cycle, it was also evident that there was greater accumulation in the S-phase. Most importantly, the combination demonstrated an even greater percentage of cells in S-phase, with a concomitant decrease in the percentage of cells in G0- and little change in the G2–M-phase. Interestingly, the interaction of PARPi-1 and Topo I was found to be most concentrated during the S-phase (33). Our data are consistent with this and suggest that when using a PARPi
combined with IMMU-132, we are disrupting this PARP-1/Top 1 interaction, resulting in accumulation in the S-phase and thus toward the more error-prone NHEJ repair pathway that predomi-
nates during this phase of the cell cycle.

Combining IMMU-132 therapy with a PARPi (olaparib or talazoparib) in mice bearing both BRCA1/2-mutated and –wild-type tumors demonstrated significant antitumor effects greater than that observed with monotherapy, thus extending our in vitro observations to in vivo TNBC models. Likewise, others have demonstrated improved efficacy when a PARPi is combined with a DNA-damaging chemotherapeutic in mice bearing human TNBC xenografts (22, 30). In one example, CPT-11 was used in combination with veliparib to improve the therapeutic outcome significantly in mice bearing s.c. MX-1 TNBC tumors (22). Similar to our experience, the PARPi alone had no effect on tumor growth, whereas the combination of CPT-11 and veliparib demonstrated a significantly enhanced antitumor effect. It should be noted that CPT-11 is more effectively converted to its active SN-38 form in mice than in humans, and therefore similar findings may not be reproduc-
ible in the clinical setting (37). However, by using IMMU-132 with its SN-38 payload attached, we are not dependent on the ef-
ciency of prodrug-to-drug conversion by the patient, and therefore the antitumor effects we observed with the combination of IMMU-132 plus PARPi are more clinically relevant.

Approximately 80% of breast cancer patients carrying heredi-
tary BRCA1 mutations present with TNBC (38). Although BRCA1/ 2 expression is an important marker in TNBC, is it not the only one. Many other BRCA1/2–wild-type TNBCs occur as sporadic tumors that share traits with germline BRCA–mutated tumors and are termed to have BRCAness (39, 40). For example, analysis of germline mutations in 158 TNBC patients demonstrated that in addition to BRCA1 mutations (17% of patients), mutations of the CHEK2 checkpoint kinase 2 gene, nibrin NBN gene, and ATM genes also were found (3.9%), and are themselves involved in various aspects of the HRR pathway (41–43). Overall, the evi-
dence suggests that besides BRCA1/2 status, many other genes contribute to the BRCAness of TNBC. In accordance with these observations, our ability to synergize and demonstrate signifi-
cantly improved efficacy in the MDA-MB-468 tumor could be explained by the PTEN mutation it carries and its loss of function. Loss of PTEN expression results in Rad51 dissociation from DNA replication forks, and subsequent destabilization and stalled replication. Cells lacking PTEN have de cient HRR functions, likely due to reduced Rad51 recruitment to the replicating DNA, culminating in loss of fidelity during DNA synthesis (44). As with BRCA1/2 mutations, cells lacking PTEN function are prone to HRR deficiencies and, subsequently, have been shown to be sensitive to PARPi (45, 46). Our results demonstrate that IMMU-132 combined with a PARPi will signi cantly improve ef cacy in both traditional BRCA1/2-defective TNBC tumors and in tumors dem-

strating BRCAness in the form of de ciencies in other proteins involved in HRR pathways (e.g., PTEN).

Because not all TNBC tumors exhibit BRCAness, we evalu-
ated the ability of IMMU-132 plus a PARPi to inhibit tumor growth in such tumor types. MDA-MB-231 carries no mutations to PTEN, BRCA1/2, or other known HHR proteins, and as such is expected to be resistant to PARPi treatment (22, 46). Previous in-vivo studies demonstrated that mice bearing MDA-MB-231 tumor xenografts were resistant to IMMU-132 therapy (16). One reason for this resistance may be over-expression of Rad51 in MDA-MB-231 (47). Cells exposed to SN-38 and olaparib have been found to increase Rad51 recruitment in the HRR pathway (48). In fact, we found that IMMU-132 exposure does result in an increase in several different proteins involved in HRR, including Rad51, BARD1, FANCD2, and ERCC1 in BRCA1/2–wild-type cell lines. Of note was the observation that while these proteins are upregulated in some cell lines (MDA-
MB-231 and BT-20), they are actually downregulated in MDA-
MB-468, which may account for its sensitivity to both IMMU-
132 monotherapy and the combination therapy. At the same time, it is likely that this increased expression of these HRR proteins in MDA-MB-231 allows for a more efficient repair of damaged DNA caused by IMMU-132 than would otherwise occur in cells that had low expression. A second possible reason for MDA-MB-231 resistance may be that it has low expression of Trop-2 on its surface, which could limit how much SN-38 can be delivered to the tumor by IMMU-132 (17). However, when IMMU-132 and its SN-38 payload were combined with olaparib, we achieved a signi cant survival benefi c in MDA-MB-231 tumor–bearing animals. Moreover, although the amount of SN-38 delivered by IMMU-132 alone may be insuf cient to retard tumor growth, enough must accrete in the tumor so that when combined with olaparib, we are able to slow tumor progression enough to provide a survival benefi t. We are currently studying various MDA-MB-231 clones that were trans-
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Synergy and Efficacy of Combined IMMU-132 Plus PARPi in TNBC
cancer patients will achieve improved therapeutic responses without unmanageable toxicity.

In summary, through the utilization of PARP inhibition to target TNBC and create synthetic lethality combined with Topo I inhibition mediated by IMMU-132, a synergistic growth–inhibitory outcome was achieved in human TNBC tumor lines, regardless of BRCA1/2 status. Although BRCAAness is an important element in this combined effect, it is not limiting. Indeed, PARP inhibitors are under investigation in a variety of cancers involving breast, ovarian, pancreatic, NSCLC, gastric cancer, glioblastoma, melanoma, and others. Our results indicate that the combination of PARP and IMMU-132 could broaden the range of tumors that are usually treated with the former. It is interesting that IMMU-132 does target all of these cancer types (17), which may make this combination a rational approach. However, combination studies with PARP have been problematic because of hematologic toxicity, thus requiring dosing modifications. These studies definitively here have shown tolerability for the combination in terms of hematologic parameters and animal body weight, but the ultimate clinical experience will of course be critical. Given the promising results obtained thus far with IMMU-132 in patients with TNBC, the logical next step is to combine this therapy with PARP to further improve clinical outcome.

Disclosure of Potential Conflicts of Interest

R.M. Sharkey holds ownership interest (including patents) in, and is a consultant/advisory board member for, Immunomedics, Inc. D.M. Goldenberg holds ownership interest (including patents) in Immunomedics, Inc. No potential conflicts of interests were disclosed by the other authors.

Authors' Contributions

Conception and design: T.M. Cardillo, R.M. Sharkey, D.L. Rossi, D.M. Goldenberg

Development of methodology: D.L. Rossi

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T.M. Cardillo, D.L. Rossi, A.A. Mostafa

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T.M. Cardillo, R.M. Sharkey, D.L. Rossi, R. Arrojo, A.A. Mostafa, D.M. Goldenberg

Writing, review, and/or revision of the manuscript: T.M. Cardillo, R.M. Sharkey, D.L. Rossi, D.M. Goldenberg

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

Study supervision: T.M. Cardillo, D.M. Goldenberg

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References


Synthetic Lethality Exploitation by an Anti–Trop-2-SN-38 Antibody–Drug Conjugate, IMMU-132, Plus PARP Inhibitors in BRCA1/2–wild-type Triple-Negative Breast Cancer

Thomas M. Cardillo, Robert M. Sharkey, Diane L. Rossi, et al.