# Effect of TRA-8 Anti–Death Receptor 5 Antibody in Combination With Chemotherapy in an Ex Vivo Human Ovarian Cancer Model

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**Objectives:** To investigate the cytotoxicity of TRA-8, an antibody that specifically binds death receptor 5, alone and in combination with chemotherapy, using an ex vivo human ovarian cancer model.

**Materials and Methods:** Twenty-six ovarian cancer specimens were obtained during ovarian cancer debulking, and tumor slices were prepared with the Krumdieck tissue slicer. The tumor slices were exposed to varying concentrations of TRA-8, carboplatin/paclitaxel, or the combination of TRA-8 and chemotherapy. Using nonlinear modeling, dose-response curves and $IC_{50}$ values were generated for specimens treated with TRA-8. The additive and synergistic cytotoxic effects of chemotherapy combination with TRA-8 were evaluated in specimens. In addition to adenosine triphosphate viability assays, the treated and untreated slices were assessed by immunohistochemistry to confirm apoptosis induction.

**Results:** Specimens from 13 patients yielded TRA-8–induced $IC_{50}$ values. Of these specimens, 15% were found to be sensitive to TRA-8–induced cytotoxicity at $IC_{50}$ doses less than 500 ng/mL. Specimens from 13 patients underwent combination treatment with TRA-8 and carboplatin/paclitaxel. Of these specimens, 77% exhibited additive cytotoxicity in comparison with those treated with either agent alone, whereas 15% exhibited synergistic cytotoxicity. Immunohistochemical analysis of terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling and cleaved caspase 3 staining demonstrated a dose-dependent increase in apoptosis with the combination treatment.

**Conclusions:** This study demonstrates the efficacy of the death receptor monoclonal antibody TRA-8 in combination with conventional chemotherapy in an ex vivo human ovarian cancer model. This model can be used to assess cytotoxicity of novel agents in combination with chemotherapy in ovarian cancer.

**Key Words:** TRA-8, Death receptor 5, Ovarian cancer, TRAIL, Monoclonal antibody

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Conflict of Interest: Drs Zhou, LoBuglio, and Buchsbaum have intellectual property related to the TRA-8 anti-DR5 antibody.



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Ovarian cancer is a devastating disease that is predicted to result in the deaths of more than 15,000 women in the United States in 2008.[1] The treatment paradigm for advanced-stage ovarian cancer is maximal surgical cytoreduction followed by systemic platinum-based chemotherapy. It has been suggested that a combination of taxane platinum-based chemotherapy regimens administered intravenously or intraperitoneally provides the best survival outcomes.[2] Despite this aggressive treatment program, most patients will develop chemoresistance and cancer recurrence within a few years of initial diagnosis. Multiple chemotherapeutic agents have been used in an attempt to treat patients with recurrence, but response rates are generally low (10%–20%).[3] Additional research is needed to better understand the mechanisms by which ovarian cancer develops

resistance to chemotherapy and to identify novel agents that can be used alone or in conjunction with conventional therapy to improve patient outcomes.

Although multiple factors influence tumor growth and sensitivity to chemotherapy, apoptosis is a key component in the success of cancer therapy. Defective apoptosis leads to unchecked cell growth and has been proposed as one of the primary mechanisms responsible for malignant transformation.[4] Apoptosis may occur as a result of 2 distinct signaling pathways: (1) intrinsic or p53 mitochondrial pathway or (2) extrinsic or death receptor pathway.[4] Cytotoxic chemotherapy acts via the intrinsic pathway, causing genomic instability that results in the stimulation of proapoptotic factors induced by p53 translocation and mitochondrial cytochrome *c*. One proposed mechanism of chemoresistance is a p53 genomic mutation, which may be present in at least 50% of human cancers.[5]

The extrinsic pathway is activated by a transmembrane protein known as tumor necrosis factor–related apoptosis-inducing ligand (TRAIL). Death receptors 4 and 5 (DR4 and DR5) initiate the extrinsic pathway when bound by TRAIL, ultimately resulting in activation of executioner caspases and apoptosis.[6] Many human cancer cell lines, including ovarian cancer, express DR4 and DR5 and are susceptible to TRAIL-induced apoptosis.[7] Tumor necrosis factor–related apoptosis-inducing ligand is a promising agent for cancer therapy because it causes apoptosis independently from chemotherapy[7]; however, there has been some concern that human hepatocytes may undergo cell death when exposed to certain TRAIL reagents.[8] In addition, TRAIL may bind to nonfunctional decoy receptors, thus interfering with apoptosis.[9] Therefore, TRA-8, an agonistic monoclonal antibody specific to DR5, was developed for cancer therapy.[10] TRA-8 does not induce apoptosis in human hepatocytes and has shown appreciable activity in multiple cancers including breast, cervix, and ovary.[11] We have previously shown that TRA-8 has a single-agent activity against primary human ovarian cancer.[11] In this current study, we sought to investigate the cytotoxicity of TRA-8 in combination with carboplatin/paclitaxel in a novel ex vivo human ovarian cancer model.

## MATERIALS AND METHODS

### Specimen Collection and Processing

An institutional review board approval was obtained at The University of Alabama at Birmingham, and patients with suspected advanced-stage ovarian cancer were identified for the study. At the time of initial cytoreductive surgery, patients with large tumor volume were selected, and cancer specimens were collected less than 30 minutes after surgical resection. Patient demographic data were recorded at the time of surgery. Tumor histology was confirmed by a gynecologic pathologist, and all primary ovarian cancer cell types were included for analysis except low malignant potential ovarian tumors. Patients with recurrent disease or prior treatment with chemotherapy were excluded.

Metastatic disease in the omentum was selected for tissue slicing, and specimens were placed in chilled complete culture media (Dulbecco's Modified Eagle's Medium [DMEM] with 4.5 g/L of glucose, L-glutamine, sodium pyruvate, 10% fetal bovine serum, 1% penicillin/streptomycin, 2.5 μg/mL amphotericin B, and 50 μg/mL gentamycin). The tumor was transported to a room with a temperature of 4°C, and a coring device (Alabama Research and Development, Munford, Ala) was used to create 5-mm cylindrical tissue cores from the omental specimen. The Krumdieck tissue slicer sequentially cut the cores as previously described.[11] Briefly, the slicer device was filled with chilled sterile media, and a reciprocating blade was used to create 300-μm thick slices at 30 revolutions per minute. Slices were carefully inspected for tissue quality, and those that appeared irregular or necrotic were discarded. The remaining slices were placed in 24-well plates with 1.5 mL of complete Dulbecco's Modified Eagle's Medium (DMEM) media and were incubated at 37°C with 5% $CO_2$ and atmospheric oxygen for 48 hours.

### Treatment Conditions

Once the tumor slices were placed in 24-well plates in complete media, slices were treated alone or in combination with increasing doses of TRA-8 (30, 100, 300, 600, and 1000 ng/mL), carboplatin (10, 30, and 50 μM), and paclitaxel (200, 600, and 1000 nM). Slices were also treated with the combination TRA-8 plus carboplatin/paclitaxel. Three doses were chosen to provide a range of cytotoxicity based on results from preliminary experiments (data not shown). The ratio of carboplatin to paclitaxel was kept constant to allow for an accurate analysis of synergism. The slices were treated in replicates of 6 and compared with untreated controls for every experiment. In slices that received combination therapy, carboplatin/paclitaxel was given for 24 hours followed by TRA-8. Once 48 hours of incubation were completed, slices were sonicated for 15 seconds in a 50:50 mixture of complete media and ATPLite mammalian cell lysis solution (PerkinElmer Inc, Waltham, Mass). The sonicated solution was added to an equal volume of lyophilized substrate buffer in aliquots of 4, and adenosine triphosphate (ATP) levels for each slice were determined via ATP-dependent light emissions in counts per second. Mean ATP levels were derived for each slice. A ratio of treated slices to untreated controls was calculated to estimate the amount of tumor killed by treatment.

### Immunohistochemistry

Treated and untreated tumor slices from several specimens were assessed by immunohistochemistry (IHC) and compared with representative tumor slices fixed immediately after slicing. The methods used for bright-field immunohistochemical analysis have been previously described.[12] Specifically, the tumor slices were mounted on Bond-Rite sialinized slides and soaked in Tris buffer.

**TABLE 1.** Patient demographics

| | N = 26 | % |
|---|---|---|
| Age, yr | | |
| Median (SD) | 66 (10) | |
| Range | 48–85 | |
| Race | | |
| White | 19 | 73 |
| African American | 7 | 27 |
| Histology | | |
| Papillary serous | 17 | 65 |
| Endometrioid | 4 | 15 |
| Carcinosarcoma | 3 | 12 |
| Adenocarcinoma NOS | 2 | 8 |
| Stage | | |
| IIC | 1 | 4 |
| IIIC | 23 | 88 |
| IV | 2 | 8 |
| Debulking status | | |
| Optimal | 16 | 62 |
| Suboptimal | 10 | 38 |

**TABLE 2.** Mean cytotoxicity of TRA-8 monotherapy on human ovarian cancer slices

| TRA-8 (ng/mL) | % Control | SE |
|---|---|---|
| Control | 100.0 | 3.5 |
| 30 | 82.5 | 3.7 |
| 100 | 83.2 | 3.8 |
| 300 | 68.7 | 2.8 |
| 600 | 61.8 | 2.8 |
| 1000 | 60.2 | 3.3 |

Mean viability values (expressed as percent of untreated control) were calculated for TRA-8 monotherapy in 11 patients. Increased cytotoxicity was seen at the highest doses of TRA-8 (1000 ng/mL).

Paraffin sections were cut at 5-μm thick and mounted on the sialinized slides. In a similar fashion as reported by Estes et al,[11] the tumor slices and the paraffin sections were evaluated with the terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) assay, Ki-67 staining, and cleaved caspase 3 expression. The tumor cells were classified by one of the coauthors (W.G.) with respect to the percentage of cells determined at each staining intensity from 0 to +4 as previously described.[12]

**TABLE 3.** Carboplatin/paclitaxel combination treatment of tumor specimens from 20 patients with ovarian cancer

| Carboplatin (μM) / Paclitaxel (nM) / Patient | 10 / 200 Mean (SE) | 30 / 600 Mean (SE) | 50 / 1000 Mean (SE) |
|---|---|---|---|
| 1 | 65.5 (4.5) | 48.1 (4.4) | 50.7 (7.3) |
| 2 | 116.5 (21.1) | 80.2 (17.4) | 47.3 (9.1) |
| 3 | 67.8 (14.1) | 98.6 (13.9) | 45.1 (4.5) |
| 4 | 22.5 (3.2) | 34.7 (4.4) | 26.8 (4.3) |
| 5 | 75.1 (3.7) | 68.0 (2.4) | 45.6 (5.1) |
| 6 | 83.9 (12.7) | 58.8 (4.1) | 40.5 (2.4) |
| 7 | 85.7 (15.2) | 57.3 (7.0) | 45.8 (1.4) |
| 8 | 61.9 (0.9) | 46.8 (4.2) | 38.0 (2.8) |
| 9 | 65.8 (11.0) | 84.3 (10.9) | 75.6 (2.5) |
| 12 | 69.6 (13.6) | 79.3 (17.8) | 26.5 (6.8) |
| 13 | 103.0 (12.1) | 58.8 (9.4) | 71.1 (12.2) |
| 14 | 59.8 (3.5) | 94.3 (11.2) | 74.5 (20.9) |
| 15 | 71.5 (8.0) | 59.5 (8.4) | 48.1 (6.7) |
| 16 | 105.9 (2.5) | 80.0 (13.1) | 69.2 (1.8) |
| 17 | 91.5 (6.6) | 101.0 (7.8) | 57.9 (3.7) |
| 18 | 89.8 (3.6) | 90.1 (3.1) | 82.2 (7.2) |
| 19 | 78.1 (4.3) | 76.5 (8.5) | 69.0 (6.7) |
| 20 | 100.9 (4.9) | 98.6 (10.8) | 85.8 (5.4) |
| 21 | 92.7 (9.2) | 71.8 (7.5) | 48.0 (4.4) |
| 22 | 81.9 (14.2) | 83.0 (5.3) | 51.5 (2.5) |
| Mean | 79.5 (3.0) | 73.5 (3.5) | 55.0 (3.3) |

A mean cytotoxicity (expressed as percent of untreated control) of 20.5% was seen at the lowest treatment dose (10 μM of carboplatin/200 nM of paclitaxel) compared with 45.0% cytotoxicity at the highest dose (50 μM of carboplatin/1000 nM of paclitaxel). Carboplatin 50 μM/paclitaxel 1000 nM produced significantly greater cytotoxicity ($P < 0.05$) from the other 2 dosages using the Tukey multiple comparison test.

## Statistical Analysis

Cell viability was measured using a ratio of ATP levels for treated slices to untreated controls (percent control). The comparisons of cell viability among different treatment combinations and dosages were evaluated using analysis of variance tests with log (percent control) as an outcome variable. The pairwise differences were analyzed using the Tukey multiple comparison test. $Log_{10}$ $IC_{50}$ values for TRA-8 monotherapy, defined as the $log_{10}$ of the TRA-8 concentration producing 50% reduction in ATP levels (in counts per second) compared with the untreated tumor slices, were determined for each patient. For data quality control purposes, the slices with extreme coefficients of variation and outliers more than 2 SDs from the mean were excluded from the analyses (range, 0–3). Data analysis was also performed with outliers included, and this did not significantly alter the results.

To estimate the $IC_{50}$ values for TRA-8, the 4-parameter logistic equation ($y$ = Min + [Max-Min] / $[1+ x/\beta]^{\alpha}$) was used, where $y$ is percent control, $x$ is $log_{10}$ (concentration), the parameter $\beta$ represents $IC_{50,}$ $\alpha$ is used to scale concentration for proper transformation, and Min and Max represent the minimum and maximum cell viability. Nonlinear regression procedure in SAS version 9.1 (SAS Institute Inc, Cary, NC) was used for representation.

The cytotoxicity data were evaluated to determine whether the combination cytotoxic effects of TRA-8 and carboplatin/paclitaxel were synergistic, additive, or antagonistic. The dose-response relationships for the treatment combinations were modeled using a second-order response surface model with linear, quadratic, and interaction terms for the treatment concentrations. The outcome variable was the logarithm of percent control (34 and 35). When multiple drugs with different mechanisms of action were studied in combination, an

**TABLE 4.** Effect of carboplatin/paclitaxel and TRA-8 of 11 patients with ovarian cancer tumor specimens

| Patient | TRA-8 (100 ng/mL) | Carboplatin/ Paclitaxel (30 μM/600 nM) | TRA-8 Plus Carboplatin/Paclitaxel (100 ng/mL Plus 30 μM/600 nM) |
|---|---|---|---|
| 12 | 118.7 (23.0) | 79.3 (17.8) | 46.3 (14.7) |
| 13 | 65.8 (5.9) | 58.8 (9.4) | 21.1 (2.5) |
| 14 | 68.6 (5.1) | 94.3 (11.2) | 38.2 (2.7) |
| 15 | 87.8 (7.7) | 59.5 (8.4) | 63.7 (3.2) |
| 16 | 78.2 (6.2) | 80.0 (13.1) | 67.6 (4.9) |
| 17 | 75.9 (3.3) | 101.0 (7.8) | 53.7 (5.2) |
| 18 | 86.7 (4.3) | 90.1 (3.1) | 80.8 (1.7) |
| 19 | 81.3 (6.6) | 76.5 (8.5) | 64.4 (4.7) |
| 20 | 86.8 (8.5) | 98.6 (12.7) | 73.3 (3.4) |
| 21 | 71.7 (1.4) | 71.8 (7.4) | 57.2 (9.2) |
| 22 | 93.5 (24.7) | 83.0 (5.3) | 76.1 (13.5) |
| Mean (SE) | 83.2 (3.8) | 81.2 (3.6) | 58.4 (3.2) |

The mean cytotoxicity of combination treatment (expressed as percent control) at a moderate dose of TRA-8 (100 ng/mL) and carboplatin/paclitaxel (30 μM/600 nM) was 41.6%, more than 20% cytotoxicity than either treatment alone. Combination treatment produced significantly greater cytotoxicity ($P < 0.05$) compared with the other 2 groups using the Tukey multiple comparison test. No significant difference was found between TRA-8 monotherapy and carboplatin/paclitaxel.

interaction term that represents the product of the single agents was used.[13] If the interaction term was not significant ($P \geq 0.05$), the relationship between TRA-8 and carboplatin/paclitaxel was considered additive. In other words, only the single agents contributed to the cytotoxicity observed without any additional cytotoxic effect from the interaction term. The interaction term was significant if $P < 0.05$. If the significant interaction term was negative, the combination effect was defined as synergistic; that is, the combination of carboplatin/paclitaxel and TRA-8 caused greater than additive cytotoxicity. The combination effect was considered antagonistic if the significant interaction term was positive.

## RESULTS

### Patient Characteristics

Ovarian cancer specimens were collected and assayed from 26 patients undergoing primary cytoreductive surgery at The University of Alabama at Birmingham from August 2006 to April 2008. Specimens from 15 patients were treated with carboplatin/paclitaxel, and 11 were treated with TRA-8, carboplatin/paclitaxel, and combination. The patients' median age was 66 years (range, 48–85 years). Most patients were white (73%), stage III disease (88%), and papillary serous histologic subtype (65%; Table 1).

### Cytotoxic Tissue Slice Assay

Mean cytotoxicity values (expressed as percent of untreated control) were calculated for TRA-8 monotherapy in specimens of 11 patients (Table 2). On average, 17.5% cytotoxicity was observed at the lowest dose (30 ng/mL) compared with 39.8% cytotoxicity at the highest dose of TRA-8 (1000 ng/mL). The tumor specimens ranged from very sensitive to TRA-8 ($IC_{50}$, 2.9 ng/mL) to TRA-8 resistant ($IC_{50}$ >500 ng/mL). An $IC_{50}$ curve for TRA-8 was generated from 13 patient specimens. Overall, 2 (15.4%) of the 13 specimens were sensitive to TRA-8 monotherapy, defined as an $IC_{50}$ < 500 ng/mL. In TRA-8–resistant specimens, tumor kill of 10% to 30% was demonstrated.

Tumors in 20 patients were treated with combination carboplatin/paclitaxel using 3 standardized doses of 10, 30, and 50 μM of carboplatin and 200, 600, and 1000 nM of paclitaxel, respectively. The cytotoxicity results of patients treated with carboplatin/paclitaxel are displayed in Table 3. A mean cytotoxicity of 20.5% was seen at the lowest treatment dose (10 μM of carboplatin/200 nM of paclitaxel) compared with 45% cytotoxicity at the highest dose (50 μM of carboplatin/1000 nM of paclitaxel). As anticipated, sensitivity to carboplatin/paclitaxel treatment was variable, but cell kill was observed in all treated tumor slices compared with untreated controls. A statistically significant difference was noted between low and high doses ($P < 0.001$) and mid and high doses ($P < 0.001$) of carboplatin/paclitaxel. There was no significant difference between low and mid doses of carboplatin/paclitaxel ($P = 0.21$).

Doses of TRA-8 from 10 to 1000 ng/mL were combined with a full range of doses of carboplatin/paclitaxel in the specimens from 13 patients to determine if the combination treatment resulted in synergistic or additive cytotoxicity. Synergistic cytotoxicity was seen in specimens from 2 patients (15.4%); additive cytotoxicity was seen in specimens from 10 patients (76.9%); and antagonistic cytotoxicity was seen in one patient's specimen (7.7%).

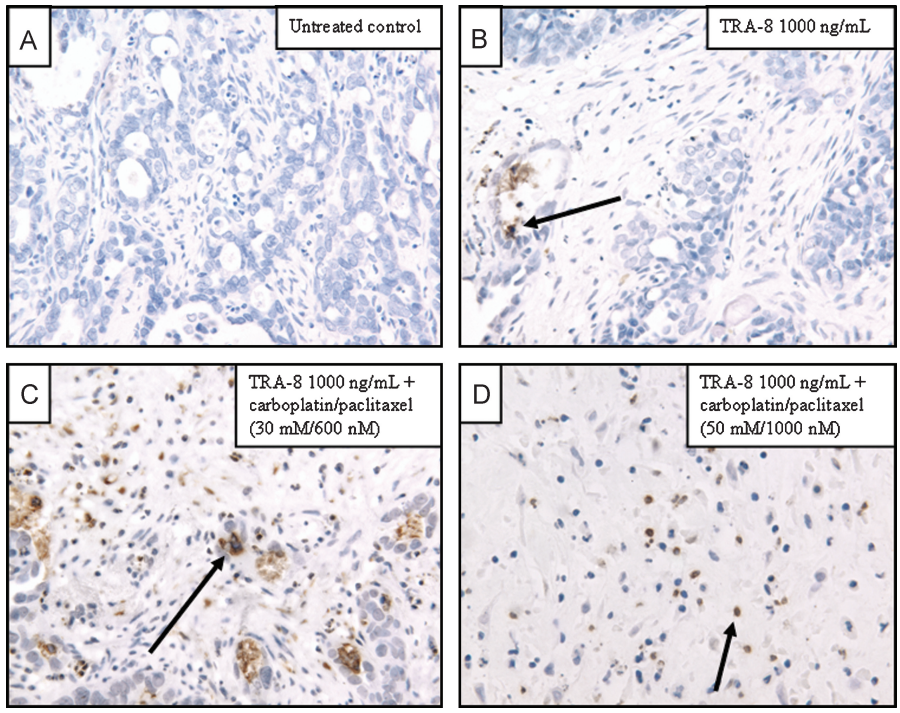


**FIGURE 1.** An IHC analysis of treated and untreated slices at 48 hours demonstrated increased apoptosis induction with combination treatment of TRA-8 and carboplatin/paclitaxel via the TUNEL assay. A, Untreated controls had less than 1% apoptosis, versus (D) 95% apoptosis for high-dose combination treatment as graded by one of the coauthors (W.G.). Assigned scores were the average of 2 separate blinded assessments. B, TRA-8 monotherapy demonstrated up to 8% TUNEL staining and (C) intermediate degrees of apoptosis (15%–30%) were seen at lower combination treatment conditions. Note that most nonstaining cells in D are lymphoid and polynuclear cells; most epithelial cells underwent apoptosis when exposed to combination treatment. Arrows denote apoptotic bodies.

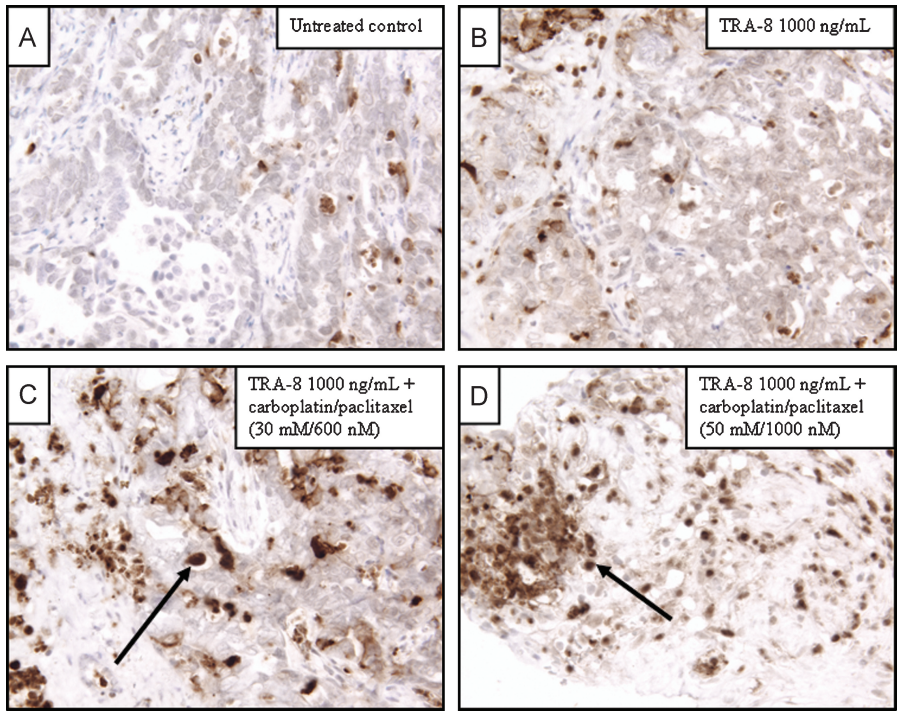


**FIGURE 2.** An IHC analysis of treated and untreated slices at 48 hours demonstrated increased cleaved caspase 3 activation with combination treatment of TRA-8 and carboplatin/paclitaxel (as denoted by arrows). Cleaved caspase 3 expression ranged from 30% in untreated controls (A) to 95% at the highest dose combination (D).

Focusing on moderate doses of TRA-8 and carboplatin/paclitaxel, 11 tumor specimens were treated with 100 ng/mL of TRA-8 alone, carboplatin 30 μM/paclitaxel 600 nM, and the combination of TRA-8 and chemotherapy. The mean cytotoxicity of the 11 tumor specimens treated with 100 ng/mL of TRA-8 at was 16.8%. Cytotoxicity produced by TRA-8 at 100 ng/mL varied from no cytotoxicity in patient number 12 to 34.2% in patient specimen number 13 (Table 4). The mean cytotoxicity of the specimens treated with the carboplatin/paclitaxel combination was 18.8%. In contrast, a mean cytotoxicity of 41.6% was achieved with the combination of TRA-8 and carboplatin/paclitaxel, compared with 16.8% for TRA-8 alone and 18.8% for carboplatin/paclitaxel alone (Table 4). The greater cytotoxicity achieved with combination treatment was statistically significant compared with TRA-8 alone ($P < 0.05$) and carboplatin/paclitaxel alone ($P < 0.05$) using the Tukey multiple comparison test.

## Immunohistochemical Analysis

Available tumor slices underwent IHC analysis via TUNEL assay, Ki-67 staining, and cleaved caspase 3 expression. Increased TUNEL staining was observed in a dose-response fashion (Fig. 1). In the untreated slices, less than 1% TUNEL staining was noted. The slices treated with TRA-8 monotherapy demonstrated 1% to 8% TUNEL staining, and the slices treated with carboplatin/paclitaxel had 3% to 10% staining. The combination of TRA-8 and carboplatin/paclitaxel produced the most apoptosis, with 95% staining observed at the highest doses (TRA-8, 1000 ng/mL + carboplatin, 50 μM/paclitaxel, 1000 nM; Fig. 1). Intermediate amounts of apoptosis (15%–30%) were seen at lower combination treatment conditions. Similarly, increased cleaved caspase 3 staining was observed in a dose-response fashion (Fig. 2). Cleaved caspase 3 expression ranged from 30% in untreated controls to 95% at the highest dose combination. A significant change in cellular proliferation as evidenced by decreased Ki-67 staining was not observed (data not shown).

## DISCUSSION

Because of high recurrence rates and poor long-term survival in patients with ovarian cancer, novel treatment strategies are being investigated. Increasing the dose intensity of systemic chemotherapy causes debilitating toxicity while providing negligible improvements in survival. For example, in a phase III trial by the Gynecology Oncology Group, 485 patients with epithelial ovarian cancer were assigned to receive either standard-dose chemotherapy or a 2-fold increased-dose regimen.[14] There was no survival difference between the 2 groups, and the dose-intense group had significantly more toxicity. It is clear that alternate strategies must be pursued to augment the action of systemic chemotherapy in patients with epithelial ovarian cancer. Monoclonal antibodies have the potential to overcome cancer resistance and increase the effectiveness of traditional chemotherapy at standard doses.

Numerous monoclonal antibodies have been studied in the context of ovarian cancer including bevacizumab (anti–vascular endothelial growth factor), cetuximab (anti–epidermal growth factor receptor), trastuzumab (anti–human epidermal growth factor receptor-2), and oregovomab (anti–cancer antigen 125).[15] Targeting the transmembrane TRAIL death receptors with an agonistic antibody offers an attractive therapeutic approach. Tumor necrosis factor–related apoptosis-inducing ligand death receptors tend to be preferentially expressed in cancer cells and induce apoptosis via the extrinsic pathway independent of chemotherapy.[6,7] Studies have suggested that TRAIL has the potential to overcome chemoresistance in ovarian cancer cells. Several investigators have treated resistant epithelial ovarian cancer cell lines with chemotherapy, TRAIL, or both.[16] The combination of chemotherapy and TRAIL

resulted in synergistic tumor apoptosis compared with either treatment alone. These studies provide in vitro evidence that the combination of TRAIL and cytotoxic chemotherapy results in significantly more apoptosis than chemotherapy alone, especially in cells that are chemoresistant or TRAIL resistant. It is hypothesized that the chemotherapeutic drugs up-regulate the death receptors in TRAIL-resistant cells. In an immunohistochemical study, Arts et al[17] found that DR5 expression was up-regulated in most ovarian cancer specimens treated with cytotoxic chemotherapy, whereas there were no changes in DR4, TRAIL, or the inhibitory decoy receptors.

Earlier studies of the anti-DR5 antibody TRA-8 have also demonstrated cytotoxicity in various cancer cell lines and xenografts.[10,18] Our group has taken a unique approach to further study TRA-8 by using the Krumdieck tissue slicer to process and evaluate fresh tumors from patients.[11] This ex vivo technique offers several advantages, including duplication of the 3-dimensional tumor microenvironment without serial passaging of cells. The slices are heterogeneous and contain various degrees of tumor, stroma, fat, and blood vessels. Although this results in a degree of variation between slices, it is a better representation of the heterogeneity in patient tumors than cell cultures or xenografts. In the future, this technique may be used to determine if a patient is sensitive to TRA-8 before initiating therapy.

Our group has previously reported that most human ovarian cancer specimens were sensitive to TRA-8 monotherapy.[11] In the current study, we sought to evaluate paclitaxel and carboplatin in this ex vivo model and the combination of chemotherapy and TRA-8. We found that 15% of patients were sensitive to TRA-8 monotherapy, which was considerably less than those of our previous study. This is likely explained by our modifications in treatment conditions used for combination therapy. Because prior studies[19] have demonstrated that chemotherapy pretreatment may sensitize death receptors and improve anti–death receptor binding, the slices receiving combination treatment were treated with carboplatin/paclitaxel for 24 hours before adding TRA-8. To achieve uniform conditions between TRA-8 monotherapy and combination treatment, the slices treated with TRA-8 alone were first incubated in complete media for 24 hours. In our original study,[11] the slices were treated with TRA-8 monotherapy after the initial plating of tumor, and this may account for the lower incidence of TRA-8 sensitivity in the current study.

We found that increasing doses of paclitaxel and carboplatin caused significant cell death in most of the ovarian cancer specimens. More importantly, the combination of a moderate dose of TRA-8 plus paclitaxel and carboplatin produced more than 20% cytotoxicity than either treatment alone. Most of the tumor specimens treated with TRA-8 and chemotherapy experienced either additive or synergistic cytotoxicity. Furthermore, IHC demonstrated that increased apoptosis-induced DNA nicking and cleaved caspase 3 activation occurred with the combination of TRA-8 plus paclitaxel/carboplatin in a dose-response fashion.

This is the first study to our knowledge that demonstrates the efficacy of a death receptor monoclonal antibody in combination with conventional chemotherapy in an ovarian cancer preclinical model. These findings are clinically relevant as multiple studies have shown that dose-intense chemotherapy increases toxicity with minimal improvement in survival. Furthermore, the addition of monoclonal antibodies to standard chemotherapy regimens is currently being evaluated in several clinical trials.

In summary, the anti-DR5 monoclonal antibody TRA-8 is a promising agent for ovarian cancer therapy. This study contributes to the mounting evidence that combining a death receptor monoclonal antibody with cytotoxic chemotherapy significantly enhances the apoptosis of ovarian cancer cells. A phase I clinical trial evaluating humanized TRA-8 in patients with recurrent or refractory cancers has recently been completed.[20] A phase II trial studying humanized TRA-8 plus gemcitabine in patients with pancreatic cancer is currently accruing patients. Future studies will continue to exploit the apoptotic pathway to overcome chemoresistance and focus on the effectiveness of humanized TRA-8 in combination with standard chemotherapy in patients with advanced ovarian cancer.

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