ABT-898 Induces Tumor Regression and Prolongs Survival in a Mouse Model of Epithelial Ovarian Cancer

Nicole Campbell, James Greenaway, Jack Henkin, and Jim Petrik

Abstract
Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy and is often not diagnosed until late stages due to its asymptomatic nature. Women diagnosed with EOC typically undergo surgical debulking followed by chemotherapy; however, disease recurrence often occurs. In this study, we evaluated the ability of the thrombospondin-1 mimetic peptide, ABT-898, to regress established, late-stage tumors in a mouse model of human EOC. Ovarian tumors were induced and ABT-898 treatment was initiated at time points that were representative of late stages of the disease to study tumor regression. ABT-898 induced tumor regression and reduced the morbidity of treated animals compared with controls. Analysis of tumors from ABT-898–treated animals showed reduced abnormal tumor vasculature, decreased expression of the proangiogenic compound VEGF, and reduced tumor tissue hypoxia. ABT-898 treatment initiated at late-stage disease also significantly prolonged disease-free survival compared with control animals. Results from this study show that ABT-898 is capable of regressing established ovarian tumors in an animal model of the disease. As most women are detected at advanced stage EOC, ABT-898 may improve our treatment of ovarian cancer.

Introduction
Epithelial ovarian cancer (EOC) is the most common malignancy of the female reproductive tract and is the most lethal gynecologic cancer. Ovarian cancer is detected at a late clinical stage in more than 80% of the cases partly due to diffuse clinical signs and the lack of effective screening techniques. Current treatment for EOC involves surgical debulking followed by platinum- and taxol-based chemotherapy. Patients typically respond favorably to this treatment regimen; however, the majority of women will experience disease recurrence characterized by chemoresistance. There is a need for improved therapeutic options that will successfully treat late-stage ovarian tumors and prevent chemoresistance, ultimately preventing disease recurrence.

Tumor growth is dependent upon formation of new blood vessels from preexisting vasculature, a process termed angiogenesis. VEGF is a potent proangiogenic growth factor and is highly predictive of poor prognosis in numerous cancer types. In contrast, thrombospondin (TSP)-1 is an endogenous inhibitor of angiogenesis that is often inversely expressed with VEGF and increased TSP-1 expression is associated with a favorable prognosis.

Use of compounds that target angiogenic pathways to inhibit growth and metastasis of tumors have had variable outcomes. Initially, the goal of antiangiogenic agents was to decrease tumor vascularity in an effort to impede nutrient delivery and decrease metastasis, however, numerous studies have suggested that tumor vasculature undergoes normalization, which involves pruning back the abnormal, tortuous tumor vasculature while leaving the mature, healthy parental vessels intact. This results in increased tissue perfusion, which can facilitate increased uptake of cytotoxic agents when used in combination therapy. We have previously reported that the TSP-1 mimetic peptide ABT-510 decreases blood vessel density and increases the proportion of mature blood vessels, allowing for enhanced tissue uptake of paclitaxel and cisplatin. A second generation TSP-1 mimetic peptide, ABT-898, has been generated that has enhanced stability, an increased half-life of 4 to 5 hours, and has minimal side effects.

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effects or toxicities (12, 13). In this study, we used ABT-898 to determine its effects on late-stage epithelial ovarian tumors and its ability to induce tumor regression and prolong disease-free survival.

Materials and Methods

Reagents and cell lines
We evaluated the effect of the TSP-1 mimetic peptide ABT-898 (Abbott Labs) on murine and human endothelial and ovarian epithelial cells. Murine microvascular endothelial cells (mEC) were obtained from American Type Culture Collection (ATCC) and were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) with 10% FBS and 1% ABAM, and human umbilical vein endothelial cells (HUVEC) were cultured in F-12K (ATCC) supplemented with 0.1 mg/mL heparin (Sigma), 0.03 mg/mL ECGS (Sigma), 10% FBS, and 1% ABAM. The following epithelial cell lines were also cultured with the appropriate media: ID8 cells (DMEM), normal human ovarian surface epithelial cells (NOSE; generously donated by Dr. Jinsong Liu (MD Anderson Cancer Center, Houston, TX)), OVCAR (RPMI with 20% FBS) and SKOV3 (McCoy’s 5A, supplemented with 10% FBS and 1% ABAM). All cell lines were immediately frozen after acquisition. Once thawed, cells were tested for morphology and growth; absence of Mycoplasma was confirmed, and the cells were used within 3 months. Phenotype of endothelial and epithelial cells was confirmed with expression of cell-specific markers with immunocytochemistry and Western blotting.

Mouse model
Mice were purchased from Charles River Laboratories and maintained in accordance with the Canadian Council on Animal Care. To evaluate the preclinical efficacy of ABT-898, we used an orthotopic, syngeneic mouse model of EOC described previously (14). Briefly, transformed murine ovarian surface epithelial cells from C57b/6 mice of EOC described previously (14). Briefly, transformed murine ovarian surface epithelial cells from C57b/6 mice (ID8; 1.0 x 10^6) were injected directly under the ovarian bursa of syngeneic mice. In this model, 60 days posttumor induction, mice form primary ovarian masses and by 90 days posttumor induction, there are large ovarian tumors, numerous secondary peritoneal lesions, and abdominal ascites. The ability of ABT-898 to induce regression of established epithelial ovarian tumors was assessed in our animal model. Tumors were allowed to develop for 60 or 80 days posttumor induction, at which time treatment was initiated with i.p. once daily injections of ABT-898 (25 mg/kg) or D5W vehicle control (200 µL). ABT-898 has not shown any toxicity or immune response in this syngeneic mouse model. Mice were euthanized 90 days posttumor induction, which corresponded to 30 days of treatment in the 60-day posttumor induction group and 10 days of treatment in the 80-day posttumor induction group. Primary tumors were collected and peritoneal tumors were assessed for metastatic spread on the basis of a lesions scoring system in which mice were categorized as having no observable abdominal lesions, 1 to 2 lesions, between 3 to 10 lesions, or greater than 10 lesions as a way to quantify the extent of abdominal disease, as we have done previously (8).

Survival
In a second cohort of mice, daily treatments with ABT-898 were initiated at 60 or 80 days posttumor induction (n = 12 animals per group) and animals continued to receive treatment until they became moribund, which was assessed on the basis of noticeable ascites and an increased weight gain of 20% of their pretumor induction body weight. All animals were euthanized at 150 days posttumor induction and those that were free of morbidity (had not developed ascites fluid) were recorded accordingly. The ovaries of these animals were assessed histologically to ensure that the surgical injection of ID8 cells was successful and created the presence of a focal necrotic region from which regressed tumors had originated.

In vitro endothelial and epithelial cell death
ABT-898–induced apoptosis in endothelial and epithelial cells was evaluated with a terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay (Roche) and by immunofluorescence staining for caspase-3. Endothelial and ovarian tumor cells were cultured in serum-free media alone or with ABT-898 (50 nmol/L) or vehicle control (5% dextrose in PBS) for 24 hours and were then fixed in 10% buffered formalin for 1 hour and permeabilized with 0.2% Triton-X for 5 minutes. For detection of apoptosis, cells were washed with PBS and incubated with the TUNEL reaction mixture (label solution and enzyme solution) for 60 minutes at 37°C in the dark. Cells were rinsed with PBS; nuclei were stained with 4’,6-diamidino-2-phenylindole (DAPI), and coverslips were mounted onto slides with Prolong Gold antifade (Invitrogen) and allowed to dry overnight. Negative and positive controls were generated according to the kit instructions. All slides were visualized using an Olympus BX-61 fluorescent microscope, and quantification of the percent immunopositive cells was done by a blinded individual using integrated morphometry software (Metamorph). For fluorescence detection of caspase-3, cells were incubated in the presence of a rabbit anti-human caspase-3 antibody (Cell Signaling; 1:200 dilution) overnight at 4°C, then rinsed and incubated with rabbit Alexa Fluor 488 secondary antibody (Molecular Probes; 1:100 dilution) for 2 hours at room temperature. Brightfield and fluorescence images were captured with an Olympus phase contrast fluorescence microscope and overlayed using integrated morphometry software (Metamorph).

Immunohistochemistry
Immunohistochemistry was done to quantify expression of the endothelial cell marker CD31 and a marker of hypoxia, carbonic anhydrase. At 90 days posttumor induction,
induction, following 30 days of treatment with ABT-898, ovarian tissue was harvested from animals and immediately formalin fixed. Five micrometer sections were cut and mounted onto slide, deparaffinized, rehydrated, and incubated with 1% hydrogen peroxide for 10 minutes at room temperature. Antigen retrieval was conducted by immersing sections in 10 mmol/L citrate buffer at 90°C for 12 minutes. Tissues were blocked for 10 minutes at room temperature with 5% normal serum and incubated with anti-CD31 (BD Biosciences Pharmingen) or carbonic anhydrase (Abcam) antibodies overnight at 4°C in a humidity chamber. Slides were incubated with anti-mouse biotinylated secondary antibody (Sigma-Aldrich) for 2 hours at room temperature followed by ExtrAvidin (Sigma-Aldrich) for 1 hour at room temperature. Antigens were visualized using DAB (Sigma-Aldrich) and counterstained with Carazzi’s hematoxylin. After mounting, slides were imaged by a blinded individual using brightfield microscopy.

**Evaluation of microvessel density and vessel maturity**

Microvessel density was evaluated in tissue sections that were immunostained for CD31 as mentioned above. Images were obtained at ×200 magnification and 4 fields of view per section were used. Microvessel density (number of vessels per field and blood vessel area) was quantified using Metamorph integrated morphometry software (Molecular Devices). To evaluate vessel maturity, a marker for mature vessels was colocalized with endothelial cells using immunofluorescence in tumor sections. Immunostaining was done with CD31 and alpha smooth muscle actin (α-SMA), a pericyte marker. Briefly, slides were processed as mentioned above and the CD31 antibody was incubated for 1 hour at room temperature. Tumor sections were washed with PBS and antimouse secondary antibody conjugated to Alexa Fluor 594 (Invitrogen) was applied for 1 hour at room temperature. Slides were then incubated for 1 hour at room temperature with anti-SMA primary antibody (Fitzgerald Industries International) and secondary antibody conjugated to Alexa Fluor 488 (Invitrogen) was applied for 1 hour. Tissues were counterstained with DAPI and mounted using Prolong Gold (Invitrogen). SMA-positive vessels indicated mature blood vessels and they were quantified as a percentage of total CD31-positive vessels.

**In vivo determination of epithelial and vascular endothelial cell apoptosis**

Following treatment with DSW or ABT-898 in vivo from 60 days posttumor induction, tissues were collected at 90 days posttumor induction and processed for immunofluorescence and apoptosis. For determination of epithelial tumor cell apoptosis, tissues from DSW- or ABT-898–treated animals were analyzed using the TUNEL assay according to manufacturer’s instructions (Roche). For evaluation of vascular endothelial cell apoptosis, 2 adjacent tissue sections were placed on each slide, and 1 was immunofluorescently stained for CD31, whereas the other was probed for α-SMA as above. Following incubation with Alexa Fluor 594–conjugated secondary antibodies, tissues were rinsed and subjected to TUNEL analysis as described above. Following the TUNEL procedure, images of CD31, α-SMA, and TUNEL were collected, and images of adjacent sections were oriented and overlaid using image processing software. This approach allowed us to determine the incidence of apoptosis in the endothelium of vessels with and without pericyte coverage.

**Expression of VEGF**

Protein levels of proangiogenic VEGF was determined in vitro and in vivo using Western blot analysis. In vitro, ID8 cells were cultured in the presence of 50 mmol/L ABT-898 in serum-free DMEM, supplemented with 1% antibiotic/antimycotic (Gibco) for 24 hours. For in vivo experiments, tumor tissue was collected at 90 days posttumor induction from mice treated with ABT-898 for 10 days (80-day posttumor induction group) or 30 days (60-day posttumor induction group) and was flash frozen. Cells were lysed and tumor tissues were homogenized in radioimmunoprecipitation assay buffer with protease and phosphatase inhibitors, and protein concentrations were determined using a DC protein assay (Bio-Rad Laboratories). All Western blots were done using an XCell II Blot Module System (Invitrogen). Samples (20 μg of total protein) were reduced and subjected to SDS-PAGE. Proteins were transferred to polyvinylidene difluoride membranes (Bio-Rad Laboratories) and blocked at room temperature for 1 hour in 5% skim milk with TBST. Membranes were probed for overnight at 4°C with primary antibody against VEGF (Santa Cruz). Following washes with TBST, membranes were incubated for 1 hour at room temperature with antirabbit IgG horseradish peroxidase–linked secondary antibodies (Cell Signaling Technology, Inc.). Expression of VEGF was detected using Western Lightning Chemiluminescence Reagent Plus (PerkinElmer BioSignal, Inc.) and visualized using medical X-ray film (Konica Minolta Medical Imaging Inc.). Computer assisted densitometry was done using AlphaEase FC software (Alphalnotech) and results were quantified and reported as integrated densitometry values relative to β-actin.

**Statistical analysis**

All in vitro experiments contained 3 replicates and all in vivo experiments consisted of at least 6 animals per group. Results from cell apoptosis, ovarian tumor weight, blood vessel density, and maturity were evaluated by
Results

**ABT-898 induces apoptosis in endothelial and epithelial cells and significantly reduces the size of epithelial ovarian tumors**

In this study, animals were allowed to develop epithelial ovarian tumors for 60 or 80 days, which replicates late-stage human disease, before being treated with ABT-898. We evaluated the apoptotic inducing effect of ABT-898 on cells comprising the vascular and epithelial compartments of ovarian tumors. Various endothelial and epithelial cell lines that were treated with ABT-898 in culture for 24 hours exhibited a significant increase in cell death compared with untreated controls (Fig. 1A and B). In human and murine endothelial and epithelial cancer cells, treatment with 50 nmol/L ABT-898 for 24 hours resulted in approximately a 5-fold increase in apoptosis. Cancer cells showed an increase in expression of caspase-3 following treatment with the peptide (Fig. 1C).

Animals that received daily i.p. injections of ABT-898 for 30 days (60 days posttumor induction) or 10 days (80 days posttumor induction) had a significant reduction in tumor size compared with the size of the tumors at the start of treatment and in comparison with the vehicle (D5W) treatment controls also collected at 90 days posttumor induction (Fig. 2).

**ABT-898 reduces the number of peritoneal lesions and prevents formation of ascites fluid**

The number of peritoneal lesions and the presence of ascites fluid were determined in animals following treatment of ABT-898 as described above (n = 6 mice per treatment group). Four of 7 animals that received treatment at 80 days posttumor induction had less than 2 visible lesions in their peritoneal cavity compared with 1 of 6 animals that exhibited a similar frequency of lesion formation in the D5W control group (Fig. 3). Examination of the 60-day posttumor induction group revealed that treatment of ABT-898 significantly lowered the abdominal or extraovarian lesion formation compared with controls. Five of 6 animals had 0 to 2 detectable metastatic lesions and 3 had no lesions whereas among controls, 5 of 6 animals had more than 2 lesions and none of the controls was lesion free. All 6 animals that received the vehicle control developed ascites fluid during the study; however, treatment with ABT-898 reduced the number of animals that developed ascites. In the 80-day posttumor induction group, 2 of 6 animals had no ascites, a significant reduction compared with controls. Most interestingly, there was a complete absence of ascites in mice whose treatment began at 60 days posttumor induction (30 days of ABT-898 treatment; Fig. 3).
ABT-898 remodels the vasculature and reduces tumor tissue hypoxia

The effect of ABT-898 on tissue hypoxia and tumor vasculature was evaluated in vivo. ABT-898 treatment reduced the levels of tumor tissue hypoxia compared with controls, as indicated by the proportion of tissue positive for carbonic anhydrase (Fig. 4A). Blood vessel maturity was measured as the percentage of blood vessels associated with α-SMA-positive pericytes. Immunofluorescence colocalization of CD31 and α-SMA showed that in late-stage disease, ABT-898 increased the proportion of mature vessels compared with vehicle-treated controls (Fig. 4B). ABT-898 treatment resulted in a significant decrease in tumor vessel area and blood vessel density. Treatment for 30 days resulted in statistically significant reduction in vessel area compared with both controls (P < 0.05) and 10-day treatment (P < 0.05; Fig. 4C).

ABT-898 reduces expression of VEGF in vitro and in vivo

ID8 cells treated with 50 mmol/L ABT-898 for 24 hours expressed lower levels of VEGF compared with the untreated controls (Fig. 4D). Protein was collected from whole tumor tissue homogenates from mice who received ABT-898 for 10 days (80 days posttumor induction) and 30 days (60 days posttumor induction) showed a significant reduction in the number of secondary lesions compared with D5W-treated controls. A significant reduction was also observed between the 60- and 80-day posttumor induction groups (A). All of the animals treated with the vehicle control (D5W) had developed ascites fluid at 90 days posttumor induction. There was a significant reduction in the number of mice with ascites following treatment with ABT-898. Approximately 40% of mice treated for 10 days (80 days posttumor induction) did not present with ascites fluid. Animals treated at 60 days posttumor induction did not show any signs of ascites fluid production, showing the ability of ABT-898 to inhibit its formation (B). n = 6 animals per group; in A, *, P < 0.05 compared with D5W control; in B, bars with different symbols are statistically different, P < 0.05.
ABT-898 induces apoptosis in endothelial cells of immature but not mature blood vessels

Double-labeling immunofluorescence, coupled with TUNEL analysis allowed us to view endothelial cell apoptosis in immature (without pericyte coverage) and mature (pericyte covered) tumor vessels. ABT-898 induced apoptosis predominantly in vessels that were void of pericytes, as indicated by a lack of α-SMA staining (Fig. 5). Endothelial cells from mature, pericyte-covered vessels were typically void of apoptosis (Fig. 5).

Figure 4. Treatment with ABT-898 reduces tumor tissue hypoxia, alters the vascular profile, and decreases VEGF protein levels in vitro and in vivo. Ovaries harvested from mice treated with ABT-898 for 30 days (ABT-898 60 d posttumor induction group) were immunostained for carbonic anhydrase to evaluate tumor hypoxia (A). There was a significant reduction in the percent of immunopositive staining in tumors from animals that were treated daily with ABT-898 compared with controls (B). Colocalization experiments conducted with CD31 (red) and SMA (green) revealed that following treatment with ABT-898, there was an increase in the proportion of pericyte-covered mature blood vessels at 60 and 80 days posttumor induction (C). n = 6 animals per group. Bars, A, 150 μm; B, 120 μm. Ovaries were collected from animals treated with ABT-898 for 30 (60 days posttumor induction) and 10 (80 days posttumor induction) days and immunohistochemical analysis investigated vasculature parameters. Treatment with ABT-898 significantly (P < 0.05) reduced the blood vessel density (top) and tumor vessel area (bottom) at both time points. A further significant reduction in tumor vessel area was noted in the 60-day posttumor induction group compared with 80 days posttumor induction (C). n = 6 animals per group; bars with different symbols are statistically different, P < 0.05. ABT-898 reduced VEGF protein levels in vitro and in vivo. ID8 cells were either untreated (Cntrl) or treated with 50 nmol/L ABT-898 for 24 hours in serum-free media (D). Ovarian tumor tissue was collected at 90 days posttumor induction from untreated mice (Cntrl) or mice in which ABT-898 (25 mg/kg) treatment was initiated at 60 or 80 days posttumor induction (D). In vitro experiments were conducted in triplicate and repeated 3 times, whereas in vivo experiments included 6 mice per treatment group. The above blots depict an average representative from each group. PTI, posttumor induction.
ABT-898 prolongs survival in a mouse model of epithelial ovarian cancer

ABT-898 treatment caused a significant reduction in the size of ovarian tumors (Fig. 2). To determine whether this treatment had an impact on survival, we continued treatment and assessed for signs of morbidity. Treatment began at 60 or 80 days posttumor induction and continued until animals became moribund, which was defined as abdominal distension due to ascites fluid accumulation. Animals whose treatment began at 80 and 60 days posttumor induction had a significantly longer survival compared with controls. Mice that received ABT-898 at 80 days had a mean survival of 108 days posttumor induction compared with 98 days for controls. Treatment that started at 60 days posttumor induction resulted in the animals living on average 130 days compared with 94 days posttumor induction. The experiment was terminated at 150 days posttumor induction and all animals were euthanized independent of any signs of morbidity. Approximately 27% of mice with treatment started at 80 days and 46% of mice starting at 60 days posttumor induction had no sign of disease such as abdominal distension, coat condition deterioration, or lethargy and at 150 days posttumor induction had no grossly observable ovarian tumors at euthanasia (Fig. 6A and B).

Long-term treatment of ABT-898 significantly reduces the number of peritoneal lesions

Mice involved in the survival study were scored for the presence of peritoneal lesions at the time of necropsy. Mice, in which treatment was initiated at either 60 or 80 days posttumor induction, continued to receive treatment until they became moribund (or until the experiment was terminated at 150 days posttumor induction). Animals that received treatment had significantly fewer lesions when sacrificed compared with untreated controls. The 80 days posttumor induction group had a reduction in the number of lesions, whereas the 60 days posttumor induction group had greater than 50%, with no signs of peritoneal disease (Fig. 6C and D).

Discussion

We have shown that the TSP-1 mimetic, ABT-898, can induce regression of advanced-stage EOC and that it induces apoptosis of tumor cells and endothelial cells of immature tumor blood vessels. Although several studies have targeted the VEGF family in ovarian cancer (15–18), another effective approach for the treatment of various cancers are peptides derived from the TSR antiangiogenic domain of TSP-1 (19). Numerous studies have shown the antiangiogenic and antitumor effects of 3TSR (20–22) and ABT-510 (11, 23, 24) and have shown safety and efficacy in animal and human studies (12, 13, 25, 26). We have previously evaluated ABT-510 in the treatment of EOC and reported decreased tumor burden as well as an increase in the uptake of the chemotherapy drugs cisplatin and paclitaxel through vascular normalization (8). Due to the relatively asymptomatic nature of EOC, approximately two-thirds of women are not diagnosed until advanced stages (stage III, IV; ref. 27), at which time disease has spread throughout the peritoneum and the 5-year survival rate is only approximately 37% (28, 29). In our animal model, we can replicate late-stage disease before beginning treatment, which mimics the current clinical situation. In this study, we evaluated the ability of ABT-898 to regress established ovarian tumors and...
prolong disease-free survival. ABT-898 induced apoptosis in both vascular endothelial and tumor cells, resulting in reduced tumor size and decreased tumor microvessel density. This combined antiangiogenic and antitumor effect may explain the potent regression of advanced tumors following relatively short treatment periods of 10 or 30 days. Recently, ABT-898 has been shown to have a similar combined antiangiogenic and proapoptotic influence on ovarian cells, in which it inhibits follicular angiogenesis and concomitantly induces atresia of antral follicles (30). Antiangiogenic therapies may act to prune tumor vessels, removing the immature and abnormal tumor vessels resulting in vessel normalization and an increased perfusion pressure of the residual tumor tissue (31, 32). In our study, ABT-898 specifically induced endothelial cell death in immature tumor vessels that were not associated with pericytes, and following treatment, the remodeled blood vessels were more functional with enhanced vascular supply. Vessel pruning by ABT-898 is supported by other studies that have shown a proapoptotic effect of TSP-1 on endothelial cells in vitro and in vivo (20, 33–35). By specifically targeting immature tumor vessels, ABT-898 seems to have limited impact on established vasculature and may explain why we have not seen changes in the vasculature in other peritoneal organs that are exposed to this TSP-1 mimetic. ABT-898 also exerts a proapoptotic effect on the epithelial cell compartment, which likely contributed to the significant decrease in tumor mass seen at both the 60- and 80-day posttumor induction groups. TSP-1 has also been shown by us and others to induce apoptosis in nonendothelial leukemias and ovarian cancer cells, which may provide an important contribution to its antitumor activity in addition to its antiangiogenic effects (8, 11, 36, 37). Tumor vasculature exhibits inefficient flow causing numerous hypoxic areas with limited blood supply (38, 39) resulting in suboptimal drug delivery that can facilitate drug resistance, allowing the tumor to persist and grow (40, 41). By increasing vascular supply to a smaller tumor, ABT-898 may increase the delivery and uptake of chemotherapy drugs, enhancing their effectiveness and reducing drug resistance.

Figure 6. ABT-898 prolongs disease-free survival and reduces the number of secondary lesions. In a separate cohort of mice, animals continued to receive daily i.p. injections of ABT-898 until they became moribund. Animals treated at 80 days posttumor induction had a significant increase in survival compared with D5W controls. The mean survival in this group was 108 days compared with 98 days for the controls. At 150 days posttumor induction, 27% of the mice did not exhibit signs of disease (A). There was also an overall increase in survival in animals treated at 60 days posttumor induction. These mice were disease free for 130 days on average compared with 94 days posttumor induction for the controls. At the duration of the study, 46% of the mice in this group did not display signs of disease (B). n = 15 animals per group. Animals that commenced treatment at 80 days posttumor induction had a significant reduction in the number of secondary lesions compared with controls. On average, treated mice had a score of 2 to 10 lesions compared with more than 10 lesions that the controls exhibited (C). There was also a significant reduction in the number of secondary lesions in the 60-day posttumor induction group. More than 50% of treated mice did not have any lesions compared with controls that had around 10 lesions (D), n = 15 animals per group; *, P < 0.05.
During ovarian tumor growth, tumor cells typically begin to shed from the surface, a process known as transcoelomic metastasis (42). These cells will then seed throughout the peritoneal cavity and attach to the abdominal wall, the surfaces of peritoneal organs, and mesenteries. The cells will coalesce and form well-differentiated secondary tumors with recruitment of an independent blood supply. The proangiogenic drive from the primary tumor, along with the multiple secondary peritoneal lesions, often present/generate significantly increased expression of proangiogenic VEGF (43–45). In addition to its proangiogenic effect, VEGF is a potent stimulator of vessel permeability (46, 47). It is thought that increased tumor vessel permeability contributes to fluid extravasation in primary and secondary ovarian tumors, and, ultimately, the accumulation of abdominal ascites. In our study, ABT-898 reduced VEGF protein levels in ID8 cells cultured in vitro and in collected tumor tissue, which would contribute to the reduced blood vessel density and increased tumor cell apoptosis observed in this model.

Mice that were treated with ABT-898 at 60 and 80 days posttumor induction had a significant reduction in the number of secondary lesions compared with controls. The reduced number of peritoneal lesions was also associated with reduced ascites following treatment. Most notable was the fact that the animals in the 60-day posttumor induction group had a complete absence of ascites fluid. Treatment with ABT-898 significantly prolonged disease-free survival in both groups and a number of animals were completely void of any signs of disease at 150 days. Of the animals that did present with morbidity before 150 days, survival may have been enhanced by periodic draining of abdominal ascites or a combinational therapy including chemotherapy drugs such as cisplatin or taxol, which are procedures carried out clinically.

Despite some of the mice in the 60-day posttumor induction treatment group having to be euthanized early due to accumulation of ascites fluid, more than half of these animals did not display peritoneal lesions. It seems that ABT-898 treatment in these animals eliminated the peritoneal lesions, but ascites were still being generated from the residual primary tumor. In these mice, treatment was encouraging as most women succumb to EOC due to peritoneal disease rather than primary tumor burden.

Currently, the standard of care for patients with EOC involves cytoreductive surgery followed by chemotherapy. Over the past few decades, optimization of this treatment regime has resulted in a slight overall increase in survival (2); however, the long-term survival rate remains low. Results from this study suggest that ABT-898 may be beneficial in the treatment of late-stage EOC and may improve survival for women diagnosed with this disease.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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