Therapeutic Targeting of the Endothelin A Receptor in Human Ovarian Carcinoma

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ABSTRACT

The endothelin A receptor (ETₐR) autocrine pathway is overexpressed in many malignancies, including ovarian carcinoma. In this tumor, engagement of ETₐR triggers tumor growth, survival, neoangiogenesis, and invasion. To evaluate whether ETₐR represents a new target in cancer treatment, we examine in vitro and in vivo the effect of the selective ETₐR antagonist ABT-627 (atrasentan), a small p.o. bioavailable molecule, in mono- and combination therapy with taxane. ABT-627 effectively inhibits cell proliferation, vascular endothelial growth factor (VEGF) secretion of ovarian carcinoma cell lines, and primary cultures. ETₐR blockade also results in the sensitization to paclitaxel-induced apoptosis. In ovarian carcinoma xenografts, in which the ET-1/ETₐR autocrine pathway is overexpressed, tumor growth was significantly inhibited in ABT-627-treated mice compared with control. The therapeutic efficacy of ABT-627 was associated with a significant reduction in microvessel density, expression of VEGF, and matrix metalloproteinase-2, and increased the percentage of apoptotic tumor cells. Combined treatment of ABT-627 with paclitaxel produced additive antitumor, apoptotic, and antiangiogenic effects. These findings demonstrate that the small molecule ABT-627 is a candidate for clinical testing as an antitumor agent in ovarian cancer patients, especially in combination with taxane therapy. Interruption of ETₐR signaling therefore, represents, a promising therapeutic strategy in ovarian carcinoma.

INTRODUCTION

Ovarian cancer is the leading cause of gynecologic cancer-related deaths. About 26,500 women are diagnosed yearly with an overall 5-year survival rate of only 47%. Despite recent advances in cytoreductive surgery and combination chemotherapy, improvement in long-term survival of these patients has been slight (1, 2).

The ET¹ family is composed of three isopeptides, ET-1, -2, -3, which are potent mitogens for several human tumors including carcinoma of the prostate (3, 4), ovary (5), colon (6), cervix (7), breast (8), endometrium (9), as well as melanoma (10) and Kaposi’s sarcoma (11). ETs and their receptors have been implicated in cancer progression through autocrine and paracrine pathways.

ET-1, which is the most common circulating form of ETs, is produced by many epithelial tumors. The peptide signals through two G protein-coupled receptors, ETₐR and ETₐR, that have different affinities for ETs (12). The ETₐR receptor (ETₐR) binds the three peptide isotypes with equal affinity. In contrast, ETₐR binds ET-1 with higher affinity than the other isofoms (13). The ET-1/ETₐR autocrine pathway has a key role in the development and the progression of prostatic, ovarian, and cervical cancers (14).

We have previously demonstrated that ET-1 and the ETₐR are overexpressed in primary and metastatic ovarian carcinomas, as compared with normal ovaries (5). In ovarian tumor cells, ET-1 acts as an autocrine growth factor selectively through the ETₐR (15). Ligand binding to the receptor results in activation of a pertussis toxin-insensitive G protein that stimulates phospholipase C activity and increases intracellular Ca²⁺ levels, activation of protein kinase C, MAPK and p125 focal adhesion kinase phosphorylation (13). Among downstream events after ETₐR activation in ovarian carcinoma, ET-1 causes epidermal growth factor receptor transactivation, which is partly responsible for MAPK activation, suggesting that the coexistence of ET-1 and epidermal growth factor autocrine circuits in these tumor cells could enhance their growth potential (16).

Neovascularization is an early and critical event in ovarian cancer progression (17, 18). In this regard, we previously demonstrated that elevated expression of ET-1 and its cognate receptor was significantly associated with MVD and VEGF expression (19). ET-1 modulates various stages of neovascularization, including endothelial cell proliferation, migration, invasion, protease production, and tube formation, and stimulates neovascularization in vivo (20, 21). Furthermore, activation of ETₐR by ET-1 stimulates VEGF production by increasing levels of the transcription factor HIF-1α, a critical regulator of tumor growth and angiogenesis (22).

High levels of ET-1 were detected in the majority of ascitic fluids of ovarian cancer patients and were significantly correlated with VEGF asitic concentrations, suggesting that ET-1 enhances the secretion of extracellular matrix-degrading proteases. Thus, ET-1 acting through the ETₐR consistently induces the activity of two families of metastasis-related proteases, MMPs and the urokinase type plasminogen activator system. Interestingly, we found that the addition of a specific ETₐR antagonist blocked ET-1-induced migration and invasion of ovarian carcinoma cells (23).

ET-1 acts as an antipotic factor, suggesting that the peptide may also modulate cell survival pathways (24, 25). This is further supported by the demonstration that ET-1 is effective in inhibiting PAC-induced apoptosis, and that an ETₐR antagonist completely blocks the ET-1-induced survival effect. Engagement of the ETₐR by ET-1 triggers activation of antiapoptotic signaling through Bcl-2-dependent and phosphatidylinositol 3-kinase-mediated Akt pathway (26).

In view of the above findings, the ETₐR has been proposed as a potential target for anticancer therapy (27). The recent identification of low-molecular-weight compounds that inhibit ligand-induced activation of the ETₐR now offers the possibility of testing this therapeutic approach in a clinical setting. Among various ETₐR antagonists, ABT-627 (atrasentan) is a p.o. bioavailable ETₐR antagonist that potently (Kᵢ = 34 pm) and selectively binds to the ETₐR, blocking signal transduction pathways implicated in cancer cell proliferation and in other host-dependent processes that promote cancer growth (28).

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3 Abbreviations used are: ET, endothelin; ETₐR, ET A receptor; MAPK, mitogen-activated protein kinase; VEGF, vascular endothelial growth factor; HIF-1, hypoxic inducible factor-1; MMP, matrix metalloproteinase; MVD, microvessel density; PAC, paclitaxel; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP biotin nick end labeling; Ab, antibody.
In the present study, we tested *in vitro* and *in vivo* the antiprolif-erative, antiangiogenic, and prosapoptotic activities of ABT-627 on human ovarian carcinoma, in which the ET$_{A}$R is frequently over-expressed and the ET-1/ET$_{A}$R autocrine pathway is biologically active. A large body of experimental and clinical evidence has shown that the cytotoxic activity of certain compounds, such as taxanes, can be enhanced by combination with agents that block growth factor receptors involved in antiapoptotic signaling pathways (29, 30). We, therefore, determined whether ABT-627 has a cooperative effect with PAC, which is currently used in the treatment of human ovarian carcinoma.

**MATERIALS AND METHODS**

**Materials.** Clinical grade ABT-627 (atrasentan) was provided by Abbott Laboratories (Abbott Park, IL), and PAC was provided by Bristol Myers Italy.

**Primary cell cultures and cell lines.** Two primary ovarian carcinoma cells (PMOV1 and PMOV2) were derived from ascitic fluids that were freshly obtained after informed consent from two untreated patients bearing serous ovarian carcinoma (stage III) at the Regina Elena Cancer Institute. Briefly, cells were harvested by centrifugation at 200 g for 5 min at room temperature, resuspended in Dulbecco’s PBS, and then centrifuged through F1, cell-Histopaque 1077 (Sigma, St. Louis, MO). Interface cells were washed in culture medium, and 5 × 10$^{6}$ viable cells were seeded in-75 cm$^{2}$ culture flasks. All of the experiments herein reported were conducted between the first and second in vitro passag. The purity of primary cultures was assessed by immunophenotyping with a panel of monoclonal Abs recognizing ovarian tumor-associated antigens, such as MOV18, keratin 7, and CA 125 (kindly provided by Professor M. I. Colnaghi, Istituto Nazionale Tumori, Milan, Italy). The human ovarian carcinoma cell line OVCA 433 and HEY cell lines were generous gifts from Prof. Giovanni Scambia (Catholic University School of Medicine, Rome, Italy). OVCA 433 was established from ascites obtained from patient with advanced serous ovarian adenocarcinoma (31). The HEY cell line was derived from a xenograft of a peritoneal deposit of a cystoadenocarcinoma of the ovary (32). Cervical carcinoma-derived cell line C33A, purchased from American Type Culture Collection, is human papillomavirus negative, does not produce ET-1, and was used as a reference (20). The VEGF (147) Ab is a rabbit polyclonal IgG raised against a NH$_2$-terminal epitope (1-140) common to all splice variants of VEGF. For ET$_{A}$R, an Ab was raised against a decapeptide (DNPYSTGKL) of its extracellular NH$_2$-terminal domain, and for ET$_{B}$R an Ab was raised against a peptide (CGLSRIGGEFFGDPRP) of its NH$_2$-terminal domain. Vessel count was performed by two independent observers on a ×200 field, according to the criteria of Weidner et al. (33). To quantify apoptosis, TUNEL assay was performed using a commercially available *in situ* apoptosis detection kit (Boeringer Mannheim). For the quantification of total TUNEL expression, the number of apoptotic cells was counted in 10 randomly selected fields (∼200) as a percentage of total cells using an immunofluorescence microscopy.

**Western Blot Analysis.** Total cell lysates were obtained from homogenized HEY tumor specimens. The protein extracts were resolved by 7.5% SDS-PAGE and probed with an anti-VEGF polyclonal Ab (Santa Cruz Biotechnology). Immunoreactive proteins were visualized by enhanced chemoluminescence (Amersham International) according to the manufacturer’s instructions.

**Statistical Analysis.** Statistical evaluations of data were made by the two-sided Student’s test with Bonferroni corrections. Time course of tumor growth was compared across the treatment groups with the use of two-way ANOVA, with group and time as the variables.

**RESULTS**

**Inhibition of Ovarian Carcinoma Cell Proliferation and Potentiation of PAC-induced Apoptosis by ET$_{A}$R Blockade.** To evaluate the effect of ABT-627 on the proliferation of various ovarian carcinoma cells, we used two primary cultures (PMOV1 and PMOV2) and two established cell lines (HEY and OVCA 433). All of these cells express functional ET$_{A}$R, ranging from ∼20,000 (PMOV1 and PMOV2) to 35,600 (HEY) and 43,600 (OVCA 433) ET$_{A}$-binding sites/cell, and secrete high levels of ET-1. We treated these ovarian cancer cells with different concentrations of ABT-627 for 72 h and measured the effect of ABT-627 on cell viability. Treatment
with ABT-627 at doses ranging between 0.01 and 2 μM determined a dose-dependent inhibition of spontaneous growth rate with a comparable IC₅₀ of ~1 μM in all of the tested cell lines. The inhibitory effect of the ABT-627 was absent in C33A cells, a cervical cell line not expressing ET₄R (7, 35), indicating that only ET₄R-expressing cells respond to growth inhibition by ABT-627. To determine whether there was a time-dependent effect of the ABT-627 treatment on the growth inhibition, primary cell cultures and cell lines were incubated for up to 5 days in the absence or presence of ET₄R (ABT-627, 1 μM) and ET₄R (BQ-788, 1 μM) antagonists. In all of the ovarian carcinoma cells, spontaneous growth was significantly inhibited in the presence of ABT-627. The addition of the ET₄R antagonist did not affect the basal growth rate of the cells, even in PMOV1 cells, which coexpressed mRNA for ET₄ and ET₆ receptors (34). These studies demonstrate that endogenous ET-1 acts as an autocrine modulator of ovarian carcinoma cell proliferation only through ET₄R, which was selectively inhibited by ABT-627 (Fig. 1).

To determine whether the antiproliferative effect of ABT-627 resulted in the induction of programmed cell death, we evaluated the percentage of dying cells in ABT-627-treated and control cultures. As shown in Fig. 2, ABT-627 (1 μM) treatment increased the percentage of apoptotic HEY and OVCA 433 ovarian cancer cells after 48 h of treatment (P ≤ 0.05). In these cells, activation of ET₄R by ET-1 resulted in the induction of programmed cell death, we evaluated the potential combined proapoptotic effect of treatment with ET₄R antagonist and PAC. HEY and OVCA 433 cells were incubated with 60 nm PAC alone or in combination with ABT-627 (1 μM). As expected, the addition of ABT-627 significantly increased PAC-induced apoptosis (P ≤ 0.0001) in both cell lines (Fig. 2). These results established that ET₄R-activated autocrine survival pathways were affected by treatment with ABT-627.

Effect of ET₄R Antagonist on VEGF Production. ET₄R overexpression can promote tumor development through its stimulatory action on cancer cell growth. However, ET₄R may also regulate angiogenesis by promoting the tumor production of VEGF (19, 22). Endogenous levels of the angiogenic factor, VEGF, were measured by ELISA in the conditioned medium of HEY cells incubated in serum-free medium for 24 h. Untreated HEY cells secreted ~375 pg/VEGF/10⁶ cells/24 h. Treatment with either ABT-627 (1 μM) or PAC (60 nm) alone caused a ~45% inhibition of VEGF secretion (P ≤ 0.001, compared with control). The combination of ABT-627 with PAC exerted a marked inhibitory effect, reaching almost 60% reduction of VEGF secretion to 150 pg/10⁶ cells/24 h (P < 0.001; Fig. 3).

Inhibition of Growth of Human HEY Ovarian Carcinoma in Nude Mice. The potential antitumor effect of ABT-627 in vivo was assessed in murine tumor xenografts. Human ovarian carcinoma cells HEY, which overexpress ET₄R and secrete high levels of ET-1 (34), were grown as s.c. tumors in nude mice. Seven days later, when well-established HEY xenografts were palpable with a tumor size of ~0.25 cm³, mice were randomized into treatment and vehicle control groups of 10 animals each. The treated mice were given injections i.p. for 21 days with two different concentrations of ABT-627, 2 mg/kg/day and 10 mg/kg/day. Treatment with ABT-627 produced a 65% inhibition of HEY tumor growth on day 40 after tumor injection with either low (2 mg/kg/day) or high (10 mg/kg/day) doses (P < 0.001 compared with control; Fig. 4A). ABT-627 treatment was
generally well tolerated with no detectable signs of acute or delayed toxicity, even at the highest ABT-627 dose (10 mg/kg/day). Tumor growth suppression by treatment with 2 mg/kg/day ABT-627 was comparable with that achieved by treatment with PAC (20 mg/kg i.v. given three times, once a day on days 1, 5, and 9). Immunohistochemical analysis using specific anti-ET-1, anti-ET A R Abs demonstrated that tumor xenografts expressed detectable levels of ET-1 and ET A R (data not shown). These results are consistent with the concept that ABT-627 suppresses the growth of ETA R-expressing tumors in nude mice. The comparison of time course of tumor-growth curves by two-way ANOVA with group and time as variables showed that the group-by-time interaction for tumor growth was statistically significant ($P < 0.0001$; Fig. 4).

Furthermore, the tumor growth inhibition obtained with ABT-627 persisted for up to 4 weeks after the termination of treatment. We next evaluated whether the cooperative proapoptotic effect of ABT-627 and PAC that was observed in vitro could also be obtained in vivo. For combined treatment, a 2-mg/kg/day dose of ABT-627 was selected because it induced a 65% inhibition of tumor growth, was well tolerated, and corresponded to that used in human clinical trials (28). This dose of ABT-627 was given i.p. for 21 days in combination with three i.v. administrations of PAC (20 mg/kg) given three times, once a day on days 1, 5, and 9. More marked tumor growth inhibition (90% of controls) was elicited by combined treatment with ABT-627 and PAC ($P < 0.0001$; Fig. 5). HEY tumor xenografts, freshly excised on day 40 after tumor cell injection, were analyzed for tumor growth inhibition and immunohistochemically. As shown in Table 1, the combined treatment was highly effective with no histological evidence of HEY tumors in 4 of 10 mice. The dual treatment at the dose and schedule tested were well tolerated, as judged by the absence of weight loss or other signs of acute or delayed toxicity. We, therefore, by maintaining the treated animal cohort up to 66 days, determined the duration for which bioactivity persisted with combined PAC and ABT-627 treatment. As compared with control tumor xenografts, the growth...
delay in established tumors persisted for up to 4 weeks after the termination of treatment with ABT-627 combined with PAC (Fig. 5).

Effect of ABT-627 Treatment on Angiogenesis and Apoptosis in Vivo. Because HEY cells express ET<sub>AR</sub> and various autocrine and paracrine angiogenesis-related factors including ET-1, VEGF, and MMP-2, we evaluated the expression of these factors in vivo after ABT-627 treatment at a lower dosage (2 mg/kg/day). Immunohistochemical evaluation of the expression of VEGF, performed on HEY tumors on day 40 after tumor cell injection, revealed a marked reduction (45%) in the percentage of VEGF-positive HEY cells in ABT-627-treated mice (P < 0.0001; Table 1; Fig. 6). Tumor-induced vascularization, which was quantified as MVD using Ab against CD31, was directly proportional to the expression of VEGF. There was a parallel reduction in MVD in tumors after treatment with ABT-627 (45% inhibition compared with control tumors; P < 0.0001; Fig. 6; Table 1).

Activation of ET<sub>AR</sub> in HEY cells leads to up-regulation of MMP secretion and activation, as well as promotion of cell invasion (23). In this context, we evaluated by immunohistochemistry whether ET<sub>AR</sub>...
blockade inhibits MMP-2 expression. ABT-627 treatment significantly reduced the percentage of HEY cells positive for MMP-2 (67% inhibition compared with control; \( P \leq 0.0001 \); Fig. 6; Table 1).

On the basis of the knowledge that ABT-627 induces apoptosis by blocking the ET-1/ET\(_A\)R antiproliferative pathway, we also evaluated the effect of such treatment on the induction of apoptosis in HEY xenografts, by using the in \textit{sit}u TUNEL method. A significant increase in the percentage of TUNEL-positive cells was found in HEY tumors treated with ABT-627 (\( P \leq 0.001 \); Fig. 6; Table 1).

Finally, because ABT-627 treatment significantly enhances the antitumor activity of the cytotoxic drug PAC, which has been shown to affect tumor neovascularization \textit{in vivo} (36), we tested whether its therapeutic efficacy was directly correlated with a decrease in proangiogenic molecules (VEGF, MMP-2), in MVD, and with an increase in apoptosis of tumor cells. Under our experimental conditions, PAC treatment caused a reduction in MVD, VEGF, and MMP-2 expression, and an increase in the tumor apoptotic index similar to that induced by ABT-627 treatment. Almost complete inhibition of VEGF, MMP-2 expression, and tumor neovascularization, and an increase in apoptosis, were observed after combined treatment of ABT-627 (2 mg/kg/day) with PAC (20 mg/kg/day; \( P \leq 0.0001 \); Fig. 6; Table 1). HEY tumor xenografts, freshly excised on day 40 after tumor cell injection, were also analyzed for VEGF expression by Western blots (Fig. 7). We observed a marked reduction of VEGF expression in animals treated with ABT-627 or PAC. Approximately 70% inhibition was obtained when ABT-627 was combined with PAC.

DISCUSSION

The ET\(_A\)R autocrine pathway contributes to ovarian cancer progression by inducing cell proliferation, survival, angiogenesis, and metastatic spread (5, 26, 19, 23). These results suggest that the pharmacological inhibition of the ET\(_A\)R signaling pathway may improve cancer treatment (37). A promising approach in this context has been the development of small molecules capable of inhibiting the binding of the endogenous ligand to the ET\(_A\)R. Among these novel compounds, ABT-627 is a potent and p.o. bioavailable antagonist that reverses or blocks the ET-1-mediated effects \textit{in vitro} and \textit{in vivo} (27).

In view of this, the therapeutic potential of ABT-627 was evaluated by analyzing its activity on cell proliferation, neovascularization, induction of apoptosis, and tumor growth inhibition in ovarian carcinoma cells with a functional ET\(_A\)R-driven autocrine pathway. ABT-627 treatment inhibited cell proliferation and increased programmed cell death in primary cultures and ovarian carcinoma cell lines. Furthermore, ABT-627 treatment with no associated toxicity displayed antitumor activity \textit{in vivo} against established HEY cancer xenografts in nude mice. The extent of tumor inhibition was similar to that obtained using the cytotoxic drug PAC. Of major interest, enhancement of this antitumor activity was observed when mice were treated with ABT-627 in combination with PAC. The combination treatment produced complete clinical and cytological tumor regression in 4 of 10 mice on day 40 after tumor injection. Furthermore, in the remaining animals, this schedule caused almost complete suppression of tumor growth.

Elevated expression of ET-1 and its receptors have been shown to be significantly associated with increased vascularization, as assessed by MVD and VEGF production (19). These effects are attributable to the ability of ET-1, acting through the ET\(_A\)R, to stimulate VEGF production by increasing HIF-1\(\alpha\) to an extent comparable with that induced by hypoxia (22). Because increased HIF-1\(\alpha\) activity may also influence tumor progression independent of its regulation of VEGF expression (38), the inhibition of HIF-1\(\alpha\) could have therapeutic activity especially in those malignancies, such as ovarian tumors, in which HIF-1 overexpression correlates with mortality (39). Furthermore, ET\(_A\)R activation by ET-1 promotes ovarian carcinoma cell migration and invasion by up-regulating the secretion and activation of multiple tumor proteinases (23). Because the above pleiotropic activities of ET-1 are inhibited \textit{in vitro} by ET\(_A\)R antagonists, we determined whether treatment with ABT-627 that resulted in tumor growth inhibition was associated \textit{in vivo} with the inhibition of the expression of factors that are relevant for tumor progression, including invasion, angiogenesis, and metastasis. Immunohistochemical analysis of the tumor xenografts, indeed, demonstrated that ABT-627 treatment \textit{per se} is capable of producing a significant decrease in VEGF expression and MVD. The selective ET\(_A\)R antagonist, therefore, appears to exert an \textit{in vivo} antitumor effect that may be attributable to direct blockade of the ET\(_A\)R-dependent mitogenic pathway and, at least in part, to the inhibition of VEGF secretion that sustains the proliferation of tumor blood vessels.

Tumors of ABT-627-treated animals exhibited also a significant decrease in MMP-2 expression, which is a critical mediator of invasiveness in ovarian carcinoma (40). Although other proteases are likely to be involved, the inhibition of MMP-2 activity is likely to represent a relevant mechanism by which ABT-627 could inhibit tumor invasiveness.

Recent findings have shown that in tumor vascularization, "vasculogenic mimicry" (i.e., the presence of vascular channels, cord, and sinuses that are formed by tumor cells and that lack endothelial cell lining) and mosaic vessels that are lined by endothelial and tumor cells) may occur (41). This is also the case for ovarian tumors, because invasive ovarian cancer cells have been shown to form microvascular channels (42). In high-stage, high-grade ovarian cancers, 7–10% of channels containing RBCs were lined by tumor cells. Interestingly, the invasive ovarian cancer cells that are capable of generating tubular networks \textit{in vitro} expressed both MMPs and ET\(_A\)R.\(^4\) Tumor cells displaying a molecular vasculogenic phenotype may serve as an important indicator of tumor plasticity in a growing tumor mass, requiring an aggressive treatment regimen. Therefore, the antiangiogenic effect of ABT-627 treatment in our model might also be explained by its interference with the formation of microvascular channels lined by tumor cells overexpressing ET\(_A\)R and MMP-2, in the absence of endothelial cells expressing ET\(_B\)R (43–45).

The enhancement of antitumor activity was also accompanied by a significant decrease in the expression of VEGF and MMP-2 and, hence, a decrease in mean MVD and an increase in apoptotic tumor cells (TUNEL +). ET-1 acts through ET\(_A\)R as a survival factor by protecting HEY and OVCA 433 cells against PAC-induced apoptosis via activation of antiapoptotic signaling pathways (26). Because impairment of apoptotic pathways is a major molecular mechanism leading to chemoresistance (46), ET\(_A\)R blockade by ABT-627 leading to the sensitization of tumor cells to PAC-induced apoptosis could produce an additive therapeutic effect. This, indeed, seems to occur \textit{in vivo} after combined treatment with ABT-627 and PAC, because the inhibition of tumor growth achieved with this protocol correlates with the highest tumor apoptotic index.

Preliminary results of two independent Phase II studies performed in

\[\text{VEGF}_{165}\]

\[\beta\text{-actin}\]

\[\text{ABT}^+\ C \ ABT \ PAC \ PAC\]

\(^4\) Unpublished observations.

Fig. 7. Effects of the different treatments on VEGF expression in tissue specimens. VEGF protein levels were evaluated in tissue lysates by Western blotting.
prostate cancer-bearing patients have shown the feasibility of oral administration of ABT-627 for prolonged periods with no major toxic effects at doses that provided steady-state plasma level within the biologically effective concentrations demonstrated in the present study (28, 47–49).

In conclusion, these findings demonstrate the antitumor activity of ABT-627 and provide a rationale for the clinical evaluation of this molecule, alone and in combination with cytotoxic drugs such as taxanes in patients with ovarian tumors and, potentially, in other epithelial tumors that overexpress functional ET_{A}, ET_{B} (50).

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REFERENCES


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