The Combination of the Tyrosine Kinase Receptor Inhibitor SU6668 with Paclitaxel Affects Ascites Formation and Tumor Spread in Ovarian Carcinoma Xenografts Growing Orthotopically\textsuperscript{1}

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ABSTRACT

Purpose: The purpose of this study was to investigate the antitumor activity of SU6668, tyrosine kinase inhibitor of vascular endothelial growth factor receptor 2 (VEGFR2), fibroblast growth factor receptor 1 (FGFR1), and platelet-derived growth factor receptor \(\beta\) (PDGFR\(\beta\)), as single-agent therapy and in combination with paclitaxel on ovarian carcinoma xenograft models transplanted in the peritoneal cavity of nude mice.

Experimental Design: HOC22 and HOC79 ascites-producing human ovarian carcinoma xenografts were transplanted i.p. into nude mice. SU6668 was given p.o. (200 mg/kg, daily) as a single agent or in combination with paclitaxel i.v. (6 mg/kg/dose every other day or 20 mg/kg/dose weekly). Tumor burden was evaluated at the end of the treatment period as ascites volume and tumor cells, VEGF, FGF-2, and PDGF levels in ascites, and involvement of the organ of the peritoneal cavity. Response was evaluated as percentage increment of life span (\%ILS).

Results: SU6668 affected ascites formation and tumor burden in the peritoneal cavity of nude mice bearing HOC22 and HOC79 xenografts. Decreased levels of VEGF and PDGF in ascites paralleled this effect. The overall survival of the mice bearing HOC xenograft (HOC79 less response than HOC22) was significantly increased by the treatment with SU6668. The magnitude of the effects depended on the length of treatment and tumor burden at the beginning of treatment. The combination of SU6668 with paclitaxel significantly prolonged the survival of mice bearing HOC79, compared with single therapies. SU6668-based combination therapy was more effective with paclitaxel given at the optimal dose and schedule (20 mg/kg every 7 days for 3 doses) than at the same total dose but split (6 mg/kg every 2 days for 10 doses). However, a similar outcome was observed when giving high-dose paclitaxel (20 mg/kg every 7 days for 3 doses) in monotherapy or split low-dose paclitaxel (6 mg/kg every 2 days for 10 doses) but in combination with SU6668. The addition of paclitaxel, by either schedule, to SU6668 treatment inhibited tumor spread in the peritoneal organs (omentum, pancreas, and diaphragm) even at low doses of paclitaxel. A greater effect was observed with prolonged treatments.

Conclusions: This study shows that SU6668 in combination with paclitaxel inhibits ovarian carcinoma progression in the peritoneal cavity, by blocking ascites formation and tumor spread. Because an adequate schedule and dose of the combination might be as effective as conventional chemotherapy, this should be considered as a therapeutic alternative. These findings provide a rationale for the clinical evaluation of combination therapies affecting multiple biological targets in this tumor type.

INTRODUCTION

About 70\% of patients with ovarian carcinoma are diagnosed at an advanced stage, when the tumor is already metastatic in the peritoneal cavity. Remission with systemic treatment is often short and patients frequently develop resistance after exposure to drugs (1). Systemic side effects also limit the continuation of therapy.

New chemotherapeutic agents have somewhat improved response rates. The taxane paclitaxel has shown significant activity and is currently used in the treatment of refractory ovarian cancer (2). Despite these advances, conventional chemotherapy regimens have been ineffective in increasing the overall survival or obtaining long-lasting remission (3). Therefore, new paradigms and therapeutic approaches need to be investigated to improve the treatment of this neoplasm.

Tumor angiogenesis is essential for the growth and metastatic spread of several solid tumors. Antiangiogenic therapies have entered the clinical phase, and there is a strong belief that
angiotoxic/angiostatic compounds will become useful in combination with conventional therapies (4, 5). This strategy should provide new opportunities for anticancer therapy that does not exacerbate acute systemic or cumulative toxicity and that inhibits tumor growth or regrowth and metastatic spread. The efficacy of cytotoxic anticancer therapy has been potentiated by the coadministration of antiangiogenic agents in several in vivo experimental models (6, 7).

In epithelial ovarian tumors, the correlation between angiogenesis and clinical pathological factors is controversial. However, relationships between tumor vascularization and malignancy have been shown for ovarian carcinoma (8, 9), and the expression of cytokines and growth factors has been associated with its progression and prognosis (10, 11).

Angiogenesis is induced by numerous angiogenic growth factors (12), with VEGF the most commonly associated with an angiogenic phenotype in most tumor types (13, 14). High levels of VEGF are produced in the ascitic fluid of ovarian carcinoma patients, and the association between ascites volume and VEGF levels has been reported in experimental models (15–18). The biological activity of VEGF relies on VEGFRs that are selectively expressed on endothelial cells. The direct role of VEGF and its receptors, mainly VEGFR-2 (Flk-1/KDR), in angiogenesis is well documented (13, 19). VEGFR kinase inhibitors have been reported to suppress both the primary tumor and metastases. Several of them have entered clinical trials (20).

Other growth factors, such as FGF-2 and PDGF, have been associated with the growth and vascularization of solid tumors (21–23). Their activity in concert with VEGF has been reported (24). However, FGF is also mitogenic for tumor cells, and its receptors are expressed in a variety of tumor as well as endothelial cells (25, 26). Similarly, PDGF and its receptors have been implicated in the proliferation of numerous tumor types (27, 28). PDGFR is also expressed on stroma cells and is required for the growth of pericytes that support new vessel formation (29). High levels of FGF-2 and PDGF have been reported in advanced epithelial ovarian cancers (30–32). The signaling cascade generated by these three ligands and their receptors is complex, directly and indirectly affecting tumor angiogenesis and growth (20, 33).

SU6668 is a small-molecule synthetic inhibitor of the TK activity of VEGFR-2, PDGFR and FGFR (34). It affects tumor vascularization and the growth of different types of xenograft (34, 35). The molecule is being tested as a single agent in early clinical trials (33). The purpose of this study was to investigate whether the addition of SU6668 to conventional therapy with paclitaxel improved survival in a model of ovarian carcinoma xenograft growing in the peritoneal cavity of nude mice. The second aim of the study was to investigate the effect of these inhibitors, which affect multiple TKRs, on ascites formation and metastatic spread of ovarian carcinoma.

MATERIALS AND METHODS

Animals. Female NCr-mu/mu mice were obtained from the animal production colony of the National Cancer Institute (NCI-BTB-DTP), Frederick Cancer Research and Development Center (Frederick, MD). Mice were used at 6- to 8-weeks of age and had a mean body weight of 21 ± 2 g. The mice were housed in filtered-air laminar-flow cabinets and were manipulated using aseptic procedures. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (Decreto Legislativo No. 116, Gazzetta Ufficiale (G.U.), Suppl. 40, Feb. 18, 1992; Circolare No. 8, G.U., July, 1994) and international laws and policies (European Economic Community Council Directive 86/609, Official Journal Legislation 358, 1, Dec. 12, 1987; Guide for the Care and Use of Laboratory Animals, United States National Research Council, 1996).

HOC Xenografts. HOC79 and HOC22 xenografts were established from patient ascites and maintained i.p. in nude mice as described previously (36, 37). These xenografts grow in the peritoneal cavity of nude mice producing ascites and solid lesions on abdominal organs, primarily the diaphragm, omentum, and pancreas. Both HOC xenograft models secrete high levels of VEGF in ascites.

Dose Finding and Drug Treatment. SU6668 [(Z)-3-[2,4-dimethyl-5-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid] was provided by SUGEN Inc. (South San Francisco, CA). SU6668 was formulated as 50 mg/ml in an aqueous-base cremophor vehicle [16.92% 1N sodium hydroxide solution, 24.6% cremophor EL (polyoxyethylene castor oil), 1.56% benzyl alcohol, 35.14% PEG 400, 16.79% deionized water] and was stored in the dark at −20°C in ready-to-use aliquots. Each aliquot was thawed at the beginning of the cycle and kept at 4°C for the full-cycle treatment. SU6668 administered p.o. has shown dose-dependent inhibition of xenograft models, with 200 mg/kg being the most, nontoxic, efficacious dose (34). Accordingly, dose-finding studies on our model of HOC 79 survival showed significant activity at doses of 200 mg/kg, but only a modest effect with 50 mg/kg. On this basis, the dose of 200 mg/kg (100 μl p.o.), daily in cycles of five injections per week was chosen for our studies.

Paclitaxel (provided by Indena S.p.A., Milan, Italy) was dissolved in 50% cremophor EL and 50% ethanol and was further diluted in 5% glucose; it was injected i.v. at the dose of 6 mg/kg every other day or 20 mg/kg once a week, as specified in the “Results.” The vehicle (defined as “vehicle-SU6668” and “vehicle-paclitaxel”) were administered by the same schedule and route as were the active compounds.

For the combination studies, the doses and schedules of paclitaxel were chosen on the basis of dose-finding studies on the I9A xenograft model (38) transplanted s.c. into nude mice. In this model, paclitaxel at different doses and schedules was administered continuously until tumor reached 1.5 g. Twenty mg/kg (one-third of the MTD) weekly and 6 mg/kg (one-tenth of the MTD) every other day in combination with SU6668 (200 mg/kg) were selected as the most active schedules administrable for a prolonged period with no signs of toxicity. Evaluation of Treatment. HOC22 and HOC79 xenografts were injected i.p. as a cell suspension into 10–12 nude mice...
mice (10 × 10⁶ cells/animal) for each experimental group. When indicated, four additional mice per group were given injections to determine tumor burden at the beginning or end of the treatment period (see “Necropsy . . .” below). Treatment was started 7 days after tumor transplantation unless otherwise specified and continued for 3, 5, or 7 weeks as detailed in the “Results.” Mice were monitored twice a week for body weight loss and tumor formation in the peritoneal cavity (abdominal distension) and were euthanized when they became moribund, the day of death being considered the limit of survival. At autopsy, the peritoneal cavity was macroscopically examined to ascertain the presence of tumor deposits. Results are plotted as the percentage survival against days after tumor transplant. The %ILS was calculated as 100 × [(median survival day of treated group − median survival day of control group) / median survival day of control group]. Differences in survival were analyzed by the log-rank test (7). Data are representative of two different experiments.

Necropsy and Evaluation of Tumor Burden. At the beginning and end of the treatment period, four nude mice per group were euthanized by carbon dioxide inhalation and were necropsied to establish the tumor burden. Ascites was harvested as described above, was processed as described in detail elsewhere (39). Briefly, the supernatant after centrifugation was frozen and maintained at −80°C until analysis. VEGF, PDGF, and FGF-2 were measured using a commercial ELISA kit (Human, Quantikine; R&D Systems, Minneapolis, MN), according to the manufacturer’s instructions. The sensitivity of the assay was 9 pg/ml, 1.7 pg/ml, and 3 pg/ml for the three growth factors, respectively. Each sample was analyzed in duplicate.

RESULTS

Response of HOC Xenograft to SU6668. Two HOC xenografts, HOC22 and HOC79, were implanted in the peritoneal cavity of female nude mice, and treatment with SU6668 started on day 7 when tumor involvement of the omentum and pancreas, with ascites was already present (7). Three weekly cycles of SU6668 increased the survival time for mice bearing HOC79 (%ILS = 23% and 29%, in two independent experiments) and HOC22 (%ILS = 67% and 81% in two independent experiments). The HOC79 xenograft, showing a low response to
Table 1 Effect of SU6668 in combination with paclitaxel (PTX) on tumor burden in the peritoneal cavity of nude mice bearing HOC79

HOC79 was transplanted i.p. into nude mice, and treatments started 7 days later. Mice were treated with SU6668, paclitaxel, SU6668 + paclitaxel, or vehicles as in Fig. 4. At the end of the treatment (day 26) mice were sacrificed to estimate the tumor burden as volume of ascites and number of cells (n = 4). The degree of tumor involvement of omentum, pancreas, and diaphragm was histologically determined as described in "Material and Methods."a

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<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>SU6668</th>
<th>PTX 6b</th>
<th>PTX 6 + SU6668</th>
<th>PTX 20</th>
<th>PTX 20 + SU6668</th>
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<tr>
<td><strong>Ascites fluid</strong></td>
<td>6.3 (2.6–7.8)</td>
<td>2.1 (1.9–2.1)</td>
<td>2.7 (2.2–2.9)</td>
<td>2.2 (0–2.5)</td>
<td>2.2 (0–2.4)</td>
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<td>(ml)</td>
<td>(6–574)</td>
<td>(52–135)</td>
<td>(13.5–210)</td>
<td>(1–25)</td>
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<td><strong>Tumor burden</strong></td>
<td>397 (210–480)</td>
<td>90 (52–135)</td>
<td>67 (13.5–210)</td>
<td>18 (1–25)</td>
<td>13 (1–42)</td>
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<td><strong>Omentum/pancreas involvement</strong></td>
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<td>25%</td>
<td>75%</td>
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a PTX 6, 6 mg PTX/kg; PTX 20, 20 mg PTX/kg; n.e., not evaluable.

SU6668, as a single-agent treatment, was chosen for additional studies, described as follows.

Analysis of the tumor burden at the beginning and end of treatment (days 7 and 25) of representative mice bearing HOC79 (four per group) showed that SU6668 limited tumor growth in the peritoneal cavity (Fig. 1). The ascitic fluid (Fig. 1A) in mice treated with SU6668 accumulated less as treatment proceeded (2.2 ± 0.04 ml at the beginning of treatment on day 7, and 3 ± 0.05 ml at the end of treatment on day 25) compared with vehicle-treated mice (6.6 ± 2 ml on day 25). The tumor burden in the ascites (43 × 10⁶ ± 20 cells, on day 7) was inhibited in mice receiving SU6668 (92 × 10⁶ ± 9 cells), compared with vehicle (165 × 10⁶ ± 31 cells) on day 25 (Fig. 1B). Accordingly, VEGF levels paralleled tumor growth in vehicle-treated mice (1,450 pg ± 350 on day 7, and 10,145 ± 1,939 pg on day 25) and were lower in SU6668-treated mice (6,574 ± 3,300 pg at the end of the treatment, day 25; Fig. 1C).

PDGF levels in vehicle-treated mice were 8.2 ± 4.6 pg on day 7, and 73 ± 30 pg on day 25, and were lower in SU6668-treated mice (32.3 ± 16 pg at the end of the treatment, day 25; Fig. 1D). No significant levels of PGE-2 were detected in the ascitic fluid.

The tumor burden, the level of growth factors, and the survival time of mice that were given the oral vehicle (vehicle-SU6668) were no different from untreated mice (data not shown).

Response to SU6668 Depends on the Treatment Schedule. HOC79 is a fast-growing tumor that causes hemorrhagic ascites and tumor deposits to the omentum, pancreas, and diaphragm (see results in Table 1). The antitumor activity of SU6668 was tested against early-stage (beginning of treatment 3 days after tumor implantation) and advanced-stage tumor (beginning of treatment 21 days after tumor implantation; Fig. 2A). Cytohistological results showed that, 3 days after HOC79 injection, all of the mice presented tumor cells in the peritoneal lavage and microscopic tumor deposit in the omentum and pancreas. Treatment with SU6668 that was started at this time significantly improved the survival of mice bearing HOC79 (MST, 45 days; range, 40–49 days; %ILS, 45%) compared with vehicle-treated mice (MST, 31 days; range, 27–33 days). In contrast, treatment when the tumor was advanced did not affect the course of the tumor (MST, 35 days; range, 29–47 days; %ILS, 9%) compared with mice receiving vehicle at the same time (MST, 32 days; range, 30–34 days). In this group, only a few mice were able to complete the three cycles of treatment, and at necropsy, all of the mice had the same tumor burden as vehicle-treated mice.

Fig. 2B shows that the prolonged treatment, i.e., for seven cycles, further improved the survival of mice bearing HOC79 (MST, 53 days; range, 52–61 days; %ILS, 81%) compared with mice treated for only three cycles (MST, 45 days; range, 40–49 days; %ILS, 45%). Vehicle-treated mice had a MST of 29 days (range, 28–31 days) and 31 days (range, 27–33 days) after 7 and 3 cycles, respectively, of treatment (Fig. 2B). SU6668, 200 mg/kg/injection, was well tolerated for the prolonged treatment, with no obvious side effects. However, in this case also, all of the animals had progressive disease, and their death by tumor was confirmed at necropsy.

Effect of SU6668 in Combination with Paclitaxel on HOC79 Xenografts. To see whether the response to the compound could be improved, we tested it in combination with paclitaxel (Fig. 3). HOC79 is a xenograft only partially responsive to paclitaxel, and tumors progress under paclitaxel treatment (37). Five-week therapy with paclitaxel increased the survival of mice (MST, 92 days; range, 66–140 days; %ILS, 206%) compared with vehicle-treated mice (MST, 30 days; range, 27–34 days). In this study, the 5-week treatment with SU6668 also significantly increased the survival of mice (MST, 50 days; range, 42–50 days; %ILS, 66%). Paclitaxel in combination with SU6668 significantly prolonged the survival of mice bearing HOC79 (MST, 154 days; range, 140–192 days; %ILS, 413%), and it was significantly more potent than paclitaxel as a single agent. The combination with paclitaxel did not cause significant body weight loss. Moreover, clinical signs, gross lesions, and histological hepatic findings, indicative of toxic effect, were not observed in animals necropsied at the end of the treatment.

Effect of Different Schedules of Paclitaxel in Combination with SU6668 on the Survival and Tumor Burden in Mice Bearing HOC79 Xenografts. HOC79, implanted i.p., formed tumors in all of the mice, with a median survival of 42 days (range, 37–43 days; Fig. 4). To define the optimal treatment condition of paclitaxel in combination with SU6668, two different schedules of paclitaxel 20 mg/kg (optimal dose) every...
7 days for 3 doses and 6 mg/kg (minimal active dose) every 2 days for 10 doses, both for a total of 60 mg/kg (MTD) were used (Fig. 4; Table 1). HOC79 was sensitive to both schedules, 20 mg/kg, every 7 days for 3 doses being more active (MST, 82 days; range, 71–138 days; %ILS, 95%) than 6 mg/kg every 2 days for 10 doses (MST, 65 days; range, 57–86 days; %ILS, 54%). SU6668 alone, given for only three weeks, although less active than paclitaxel, also increased the survival of nude mice (MST, 52 days; range, 48–55 days; %ILS, 23%). Combined, the two drugs significantly prolonged survival of mice bearing HOC79 (MST, 80 days; range, 72–97 days; %ILS, 90%, and MST, 127 days; range, 83–152 days; %ILS, 200%, with 6 and 20 mg/kg/dose, respectively). As shown in Fig. 4, both paclitaxel schedules significantly boosted the antitumor effect of SU6668. Interestingly, the same increase in survival was obtained with the high doses of paclitaxel (20 mg/kg/dose) given as a single agent (%ILS, 95%) and with low doses of paclitaxel (6 mg/kg/dose) but combined with SU6668 (%ILS, 90%).

To investigate the effects of these combination therapies on the growth of HOC79, we did an interim necropsy of four mice per group at the end of treatment (day 26). Results are summarized in Table 1. Vehicle-treated mice had more ascitic fluid (median, 5.8 ml) and more tumor cells (median, 397 × 10^6 cells) than mice treated with either SU6668 (ascites 2.1 ml and cells 90 × 10^6) or paclitaxel alone (6 and 20 mg/kg/injection: ascites 2.6 and 2.3 ml, respectively, and cells 67 × 10^6 and 13 × 10^6, respectively; Table 1). The blockade of ascitic fluid accumulation in the groups given the combination of paclitaxel with SU6668 was paralleled by a decrease in tumor cells. Only a few cells were detectable in the peritoneal lavage from mice treated with the combination at 6 mg/kg/dose (18 × 10^6), and almost no cells were detectable in those treated with the combination at 20 mg/kg (Table 1). The level of VEGF and PDGF in ascites paralleled the tumor burden (data not shown). Ascites and organs were collected for cytological and histopathological analysis (see description that follows and Table 1).
Cytological and Histological Analysis. In cytological specimens from untreated mice, the neoplastic cells formed clusters, often in a papillary configuration. Single, usually larger neoplastic cells could also be seen. In all cases, there were few inflammatory cells. Similar findings were observed in samples from mice treated with SU6668 alone. In animals treated with paclitaxel alone and with the combination of paclitaxel plus SU6668, the neoplastic cells were more anaplastic, showing marked anisokaryosis and frequent, atypical, mitotic figures.

On day 26 at the end of treatment, vehicle-treated mice had widespread tumor implants on the omentum, pancreas, and diaphragm (Fig. 5). No metastases were detected to organs outside the peritoneal cavity, and the tumor did not invade the host tissue to which they were adherent. The pattern of tumor dissemination did not change along with the treatments. Animals treated with SU6668 alone had metastatic deposits similar to untreated controls (Table 1). A dose-schedule-related effect was observed, with greater activity in animals given paclitaxel 20 mg/kg for 3 injections than in those given 6 mg/kg for 10 injections. However, in animals treated with the combination of paclitaxel plus SU6668, the reduction of metastatic deposits occurred with both schedules. The reduction of tumor involvement in mice treated with the high dose of paclitaxel and with the combination of paclitaxel plus SU6668 was particularly evident in the diaphragm and was also evident in the diaphragm when the low dose of paclitaxel was used (Table 1 and Fig. 5). These findings suggest that the combination treatment affects tumor dissemination in the peritoneal cavity.

DISCUSSION

The inhibition of intracellular growth-factor-signal transduction pathways offers a promising strategy for pharmacological intervention (40, 41). Several low-molecular-weight compounds that target ligand-induced activation of growth factor receptor TK activity are now being investigated as inhibitors of angiogenesis (20, 42–45). SU6668 is an orally active compound with a broad spectrum of TKR activity, selectively blocking endothelial cell proliferation more than tumor cell proliferation (34). It has shown antitumor activity against a range of preclinical models (34, 35) and is currently in early clinical trials. The present study shows the antitumor effect of SU6668, alone or with paclitaxel, on tumor growth, ascites production, and metastatic spread in an orthotopic model of HOC transplanted into nude mice.

SU6668 given orally inhibited the growth of HOC79 and HOC22 ovarian carcinoma xenografts in the peritoneal cavity of nude mice, leading to a significant increase time of survival. Different sensitivity was observed in the two models, the HOC22 being more responsive than HOC79. However, the magnitude of the response depended on the stage of the disease and the length of the treatment. In fact, the survival of mice bearing the HOC79 xenograft was greater with prolonged treatments and starting with a low tumor burden (Fig. 2). The HOC79 model was selected for studies in combination with chemotherapy because it showed a moderate response to SU6668 and also to paclitaxel as single agents (Fig. 3).

SU6668 affects multiple TKRs, FGFR1, PDGFR, and VEGFR-2, and may, therefore, influence tumor growth in vivo through several mechanisms including the inhibition of endothelial cell proliferation and/or survival, as well as the inhibition of stroma and tumor cell proliferation. SU6668 did not affect the proliferation of the HOC cells cultured in vitro (data not shown). According to previous reports (34), under the same experimental conditions, it affects the proliferation of endothelial cells (IC50 = 8 × 10⁻⁷ M and 3 × 10⁻⁵ M when stimulated by VEGF or FGF-2, respectively). Furthermore, a cycle of treatment with SU6668 at 200 mg/kg p.o. daily (same dose used in the trials herein described) significantly inhibited the angiogenic response in the plug of Matrigel (46) implanted s.c. (data not shown). Recently, it has been suggested that SU6668, by inhibiting multiple growth factor receptors, affects endothelial cells directly by preventing their survival and, indirectly, by decreasing pericyte coverage (35, 47). On the other hand, one of us (J. M. C.) has shown that the inhibition of VEGFR-2 and PDGFRβ induced by SU6668 in vivo was associated with rapid
vessel killing in tumors, leading to broad and potent antitumor effects (48). The HOC xenografts used in this study also express high levels of PDGF. We have shown that the release of PDGF and VEGF in ascites are both decreased in SU6668-treated mice, and this paralleled the inhibition of tumor burden in the peritoneal cavity. Although the precise roles of these growth factors in the regulation of ovarian cancer growth and progression is still unclear, the main effect of SU6668 as single-agent therapy in our model was related to ascites formation.

VEGF, also known as vascular permeability factor (VPF), is a potent stimulator of vascular permeability and is thought to play a major role in the development of malignant ascites (49). The correlation between angiogenesis and ascites production and VEGF/VPF has been reported (18). Ascites formation has been inhibited in experimental models using function-blocking monoclonal antibodies, which block access of VEGF produced by the tumor to its receptors (18, 50). The inhibition of malignant ascites of a HOC xenograft model by an inhibitor of the VEGFR TKs has also been reported (44). Interestingly, in that study, the outcome of treatment depended on the expression of VEGF/VPF by the tumor (44). Indeed, our ovarian carcinoma xenografts express high levels of VEGF and produce a large amount of ascites during tumor progression.

Although these data suggest a role for VEGF in the growth and ascites formation of ovarian carcinoma, the mechanism by which VEGF regulates ascites production remains to be clarified. Our data indicate that SU6668 can help control ascites production. Daily doses of SU6668 completely prevented ascites production, although it started again when treatment was stopped. This indicates the need for a prolonged/continuous treatment with these kind of inhibitors. Prolonged treatment with SU6668 did in fact improve the survival time of mice (Fig. 2). However, this treatment alone was not sufficient to prevent tumor spread in the peritoneal cavity.

An emerging concept in tumor angiogenesis-based therapy is the use of biological-based therapies, including angiogenesis inhibitors combined with cytotoxic agents (6). The administration of standard cytotoxic anticancer therapies with angiogenesis inhibitors that have different mechanisms of action has shown efficacy in several experimental systems in vivo (7). We found that the combination of the TKR inhibitor SU6668 with paclitaxel boosted the latter’s anticancer activity, leading to a significant increase in survival.

SU6688 was given at the most efficacious dose (34). We have guided the schedule of paclitaxel in our preliminary dose-finding studies. Twenty mg/kg was the maximal dose administrable for several weeks without sign of toxicity. Six mg/kg was one-tenth the MTD of paclitaxel, yet was still detectable in the tumor tissue 24 h after its administration. Our data show that, although the combination of SU6668 with paclitaxel was always more efficacious than the monotherapy, the outcome on the survival of mice bearing HOC 79 was not long lasting. It is possible that the optimal dose/schedule was not achieved in our model and that a more complex, multiple-target therapy needs to be applied to obtain complete response (6, 51).

It is not well understood how angiogenesis inhibitors interfere with the activity of cytotoxic drugs. Higher levels of cytotoxic drugs, including paclitaxel, were described in the tumors of animals treated with the two angiogenesis inhibitors TNP-470 and minocycline (52). Recently it has been shown that the inhibition of PDGF signaling in tumor stroma enhanced the antitumor activity of paclitaxel, and this was associated with an increase of paclitaxel uptake in tumors (53). Preliminary results indicate that the tissue distribution of paclitaxel was not affected by pretreatment with SU6668 (data not shown). The tumor and tissue distribution of paclitaxel in mice pretreated with SU6668 for prolonged periods warrants further investigation.

In our study, SU6668 was combined with paclitaxel at an optimal dose and schedule, or with the same total dose but split. The first schedule was more efficacious. The interim analysis of tumor spread at the end of the combination treatment once again indicated that SU6668 as single-agent therapy mainly inhibited ascites formation. The addition of
paclitaxel affected tumor spread and growth in the peritoneal organs. We have previously shown that paclitaxel can act as an antiangiogenic agent at noncytotoxic concentrations (46) and that it affects ovarian carcinoma motility (38). Whether these effects are attributable to its cytotoxic activity, prevention of ovarian carcinoma cell spread, or inhibition of tumor angiogenesis, remains to be shown. However, an important observation has emerged from this analysis. Paclitaxel given as a single agent at low doses (6 mg/kg dose) only marginally affected tumor burden in ascites and did not affect the growth of the solid tumor in peritoneal organs. Interestingly, the combination of low doses of paclitaxel with SU6668 was as efficacious on tumor burden and on mice survival as the high dose of paclitaxel (20 mg/kg/dose) as single-agent therapy (Fig. 4; Table 1). If combinations like this are as effective as conventional chemotherapy and better tolerated, they should be considered as therapeutic alternatives.

Because chemotherapeutics also affect endothelial cell functions and act as antiangiogenic/antivascular agents (54, 55), it has been proposed that rescheduling the administration of cytotoxic drugs to provide subtherapeutic, more constant doses may prevent the ability of endothelial cells to repair damage and, hence, minimize tumor recovery (56). The antivascular effect of low-dose chemotherapy is selectively enhanced in newly formed vessels by the addition of the angiogenesis inhibitor (57, 58). Although these findings are in favor of combining lower doses of a cytotoxic agent with angiogenic inhibitors, more studies with additional schedule/doses and prolonged treatments, paralleled by pharmacokinetics and pharmacodynamics, are still needed to define dose/schedule and optimize treatments.

Whatever the target of the two agents, this study shows the benefit of the combination with paclitaxel even at low doses. Ascitic fluid is an ideal environment for tumor cell survival and replication, enabling them to spread in the peritoneal cavity and to widely colonize the peritoneal surface. SU6668 may have reduced vascular permeability, leading to a decrease in ascites fluid and, to a lesser extent, to a decrease in tumor burden, but it has also reduced the spreading of tumor cells that implant on peritoneal surfaces. On the other hand, paclitaxel kills tumor cells, thus also reducing the production of tumor-associated growth factors, such as VEGF and PDGF, and eventually affecting tumor spread itself. The result is that the two treatments combined are active against the progression of ovarian carcinoma in the peritoneal cavity. The low dose of the cytotoxic drug allows a long treatment without evident side effects. This is worth considering because an antiangiogenic-like agent must be given for a long time to be active. Prolonged treatment with such drugs, which can be administered p.o. for lengthy periods, can give long-term control of local cancer growth (ascites) and metastatic spread.

The present study shows that the combination of SU6668 with paclitaxel resulted in a better outcome for HOC-bearing mice. We observed no toxic effects at the doses administered, even with long treatment. SU6668 had a significant effect on ascites formation, whereas paclitaxel had more effect on tumor spread, and these effects can be improved by the combination. This tumor model offers a prototype system for studying the antitumor activity of this class of inhibitors in combination with cytotoxic agents with different mechanisms of action and, ultimately, for defining the optimal schedule/dosing for clinical trials.
SU6668 Potentiates Paclitaxel in Ovarian Carcinoma

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The Combination of the Tyrosine Kinase Receptor Inhibitor SU6668 with Paclitaxel Affects Ascites Formation and Tumor Spread in Ovarian Carcinoma Xenografts Growing Orthotopically

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