Effects of SU101 in Combination with Cytotoxic Agents on the Growth of Subcutaneous Tumor Xenografts

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ABSTRACT

SU101 (leflunomide, N-[4-(trifluoromethyl)-phenyl] 5-methylisoxazole-4-carboxamide), an inhibitor of platelet-derived growth factor receptor signaling, has shown promising clinical activity in Phase I and II studies. Currently, SU101 in combination with cytotoxic agents is in late-stage clinical development for the treatment of cancers. In previous reports, efficacy in vivo versus varied tumor xenografts was observed. As part of the preclinical development of SU101 as a cancer therapy, the combination of SU101 with cytotoxic agents was studied in athymic mice bearing small, established, s.c. human tumor cell xenografts of glioblastoma (SF763T cells), lung (Calu-6 cells), or head and neck (KB cells) origin. In the SF763T model, the combination of SU101 with carmustine resulted in a statistically significant growth inhibition of 74% compared with the vehicle control; this combination was more effective than either agent alone. In the Calu-6 model, the combination of SU101, cisplatin, and etoposide resulted in a growth inhibition of 75% that was statistically greater than that of the vehicle-treated control group and groups treated with one or two agents. In the KB model, the combination of SU101, 5-fluorouracil, and cisplatin resulted in a statistically significant growth inhibition of 69% compared with the vehicle control. Treatment with one or two agents did not significantly inhibit growth in this model. Importantly, in addition to enhanced efficacy resulting from combination therapies, the combination treatments tested were well tolerated, as evidenced by lack of mortality. These data suggest that SU101 in combination with cytotoxic agents may provide clinical benefit and warrant further clinical investigation.

INTRODUCTION

SU101 (N-[4-(trifluoromethyl)-phenyl] 5-methylisoxazole-4-carboxamide, also known as leflunomide; Fig. 1) inhibits PDGF receptor-mediated signal transduction including tyrosine phosphorylation, DNA synthesis, cell cycle progression, and cellular proliferation (1, 2). In contrast, its primary metabolite, SU0020 (also known as A77 1726; Fig. 1), an open-ring structure with the same molecular formula and weight as the parent compound (3), inhibits pyrimidine biosynthesis, which is considered to be the primary mechanism of the anti-inflammatory and immune-modulating activities of leflunomide. Leflunomide (Arava) has been approved recently in Europe and in the United States for the treatment of rheumatoid arthritis (3).

In this report, we investigate the anticancer effects of SU101 exerted through its inhibition of PDGF receptor signaling. Ablative signaling of PDGF, a major mitogen for fibroblasts, smooth muscle cells, and glial cells (4), and its receptors has been implicated in the proliferation of numerous tumor types including glioma (5, 6), anaplastic astrocytoma (7, 8) and lung (9–12), prostate (13–16), head and neck (17, 18) and breast (19, 20) tumors. Unlike other receptor tyrosine kinases such as HER-2, in which overexpression is observed in human tumors (21), PDGF receptors are frequently coexpressed with ligand (5, 7–9, 11, 13, 14), producing an autocrine loop that continuously stimulates cell growth. PDGF receptors are also expressed on stromal cells (20, 22), and paracrine growth stimulation of stromal cells in tumors by PDGF has been observed (23). Furthermore, the PDGF receptor is required for the growth of pericytes, small cells that support new microvessel formation (24, 25). Neovascularization is required for tumor growth beyond a minimum volume (26); hence, disrupting pericyte growth may inhibit tumor growth. Because PDGF and its receptors play a role in a wide variety of tumor types and in several cell types within tumors, SU101 may be a useful therapeutic for many cancers. Indeed, SU101 has been shown to be a cytostatic agent with low toxicity in numerous in vivo animal models (2).

SU101 is currently undergoing late-stage clinical development for the treatment of cancer including glioblastoma multiforme, anaplastic astrocytoma, and prostate cancer (27–31). Phase I trials in patients with advanced solid tumors demonstrated that SU101 was well tolerated as a 24-h continuous i.v. infusion at doses of up to 443 mg/m2 for 4 consecutive weeks every 6 weeks. In addition, one partial response (after 11 courses of treatment) was demonstrated in a patient with an anaplastic astrocytoma (32). Because of the known effects of SU101 on PDGF receptor signaling, immunohistochemical studies to examine expression of the PDGF α- and β-receptors were conducted on tumor biopsies from patients in this clinical study.
SU101 and Chemotherapy Combinations

The results showed PDGF α- and β-receptor staining in the majority (15 of 19) of malignant neoplasms studied. The high incidence of neoplasms that stained positive for PDGF receptors indicates that autocrine and/or paracrine stimulation by PDGF may be a more common mechanism of irregular tumor proliferation among neoplasms of diverse origin than was previously appreciated (32).

In a separate Phase II study, SU101 treatment of patients with advanced ovarian cancer also resulted in one partial response lasting for more than 22 months (27). A Phase II study of SU101 in patients with PSA-positive prostate cancer has also been conducted. Thirty-eight of 44 patients were evaluable for PSA responses with one complete response (undetectable on more than two occasions) and two partial responses [>50% decrease in PSA (28)]. These clinical data demonstrated that SU101 administered as a single agent can delay the progression of metastatic prostate cancer in some patients. Overall, early clinical results indicated that SU101 may be useful for treatment of a variety of tumor types.

Although SU101 administered as a single agent has shown promising clinical activity to date, it will likely be used in combination with cytotoxic agents. To this end, ongoing clinical trials are designed to investigate the activity of SU101 in combination with chemotherapeutic agents (29, 31). In general, the use of combination therapies allows greater efficacy than is achievable with single-agent treatment (33). Additionally, toxicity may be reduced by administering a lower dose of each agent than is required for single-agent therapy. Overall, the potential advantages of combination therapy include maximal cell kill within the range of toxicity tolerated by the patient, a broader range of coverage of resistant cells in a heterogeneous tumor population, and prevention or slowing the development of tumor cells in patients resistant to chemotherapy.

A precedent for the combination of a receptor tyrosine kinase inhibitor with a cytotoxic agent has been established with monoclonal antibodies against HER-2/neu. In xenograft models in athymic mice, the combination of an anti-HER-2 antibody and CDDP was found to be significantly more efficacious than either agent alone (34). Furthermore, a humanized monoclonal anti-HER-2 antibody has been used for the treatment of human breast cancer in combination with CDDP, resulting in objective response rates higher than those demonstrated with CDDP alone (35, 36). In addition, proof of principle has been shown for the combination of epidermal growth factor receptor inhibitors and cytotoxic agents both in vitro and in vivo xenograft model systems (37–40).

To determine whether SU101 could enhance the efficacy of cytotoxic agents in preclinical studies, combination studies were conducted in a broad range of tumor types including glioblastoma, lung, and head and neck tumor xenograft models in athymic mice. The chemotherapeutic agents chosen for these studies included agents used as either a single agent or in combination with other cytotoxic chemotherapeutic agents against these types of cancers. BCNU, an alkylating agent that inhibits DNA, RNA, and protein synthesis, was first used in the 1950s for the treatment of malignant glioma and still remains the most effective chemotherapeutic agent for this disease (41, 42). In this study, a range of doses of both BCNU and SU101 was administered to determine whether combination treatment with lower doses could achieve the same efficacy as higher doses of the single agents. CDDP, an intra- and interstrand DNA cross-linker, has been used in combination for the treatment of lung cancer (43–45) and as both a single agent and in combination for the treatment of head and neck tumors (46–48). VP-16, which blocks DNA synthesis through inhibition of topoisomerase II, has been used in combination with other agents for the treatment of lung cancer (43–45). Similarly, 5-FU, an antimetabolite DNA synthesis inhibitor, has been used in combination for the treatment of head and neck tumors (49, 50). In the present studies with CDDP, VP-16, and 5-FU, the administered doses of these cytotoxic agents and SU101 were less than optimal for maximum efficacy. This study design is commonly used for preclinical drug evaluation to determine whether combination treatment with agents at nontoxic doses is efficacious (51–53). Results of these studies indicate that combinations with SU101 and several cytotoxic agents are more efficacious than the agents alone, with no observable toxicity.

MATERIALS AND METHODS

SU101. SU101 (leflunomide) is 5-methyl-N-[4-(trifluoromethyl)phenyl]isoxazole-4-carboxamide. The synthesis was based on a procedure published previously (54). For the study using SF763T glioma cells, SU101 was administered in a polyethylene glycol MW300-based formulation designated PBTE:D5W. For the studies using Calu-6 and KB tumor cells, SU101 was administered in DMSO.

Chemotherapeutic Agents. CDDP (Platinol-AQ; 1 mg/ml), VP-16 (VePesid; 2 mg/ml), and BCNU (BiCNU) were obtained from Bristol-Myers Squibb (Princeton, NJ). 5-FU (Fluoracil Injection; 2 mg/ml) was obtained from Roche Laboratories (Nutley, NJ). All chemotherapeutic agents were prepared in their respective clinical formulations.
Tumor Cell Lines and Cell Culture. Cell culture media, glutamine, and FBS were purchased from Life Technologies, Inc. (Gaithersburg, MD). SF763T cells were established in cell culture at SUGEN as described previously (2) from resected s.c. xenografts derived from SF763 human glioblastoma cells obtained from Dr. Michael Berens (The Barrow Neurological Institute, Phoenix, AZ). SF763T cells were cultured in MEM supplemented with 10% FBS, 2 mM glutamine, 1 mM sodium pyruvate, and nonessential amino acids. Calu-6 human lung tumor cells (ATCC HTB-56) and KB human head and neck tumor cells (ATCC CCL-17) were obtained from the American Type Culture Collection [Manassas, VA (KB cells have been shown to contain several chromosomal markers for HeLa cells, indicating that they may have been contaminated with HeLa cells)]. Both of these cell lines were grown in MEM with Earle’s balanced salt solution and nonessential amino acids supplemented with 10% FBS.

Tumor cells to be implanted (described below) were harvested from cell culture flasks using 0.05% Trypsin-EDTA solution and collected by centrifugation at 450 for 10 min. Cell pellets were resuspended in sterile PBS or serum-free medium to a suitable concentration for implantation.

s.c. Xenograft Models in Athymic Mice. The study using SF763T glioblastoma cells was contracted with the SRI (Birmingham, AL). Female athymic NCr-nu mice from Taconic Farms, Inc. (Germantown, NY) were used for this study. Female athymic CD1 nu/nu mice obtained from Charles River Laboratories (Wilmington, MA) were used for the human tumor xenograft studies with the Calu-6 and KB cell lines. All animals were maintained under clean room conditions and received sterile rodent chow and water ad libitum. SF763T, Calu-6, and KB tumor cells were implanted s.c. into the hind flank of mice. In all studies, tumors were allowed to establish themselves for 7–10 days before drug treatment was initiated.

Drug Treatment and Tumor Measurement. Animals with established tumors (approximately 100–150 mm³) were randomized into treatment groups of 6–12 mice each. The day that treatment commenced was designated day 1. For the study conducted at SRI with the SF763T model, a range of doses of BCNU and SU101 were administered, as indicated in Table 1. Tumors were measured every 3–4 days using calipers, and tumor weights were determined by assuming unit density and calculating the volume of a ellipsoid with the formula (length × width³)/2. For the studies conducted at University of California Los Angeles with the Calu-6 and KB models, suboptimal doses of CDDP, VP-16, 5-FU, and SU101 were administered, as indicated in Tables 2 and 3, to determine whether the combination treatments could increase the efficacy of low, nontoxic doses of each compound. Tumors were measured with calipers every 3–4 days, and volumes were calculated as the product of length × width × height. For all studies, Ps were calculated using the two-tailed Student’s t test.

RESULTS

SF763T Tumor Xenografts. SU101 is in clinical development for treatment of glioblastoma; thus, a glioblastoma tumor xenograft model was used to evaluate the efficacy of SU101 in combination with BCNU, the standard chemotherapeutic for this disease. The SF763T model was chosen because PDGF receptors are expressed on the cells in culture and in the resulting tumors, and SU101 was previously shown to inhibit growth of these tumors (2). Various doses of SU101 and BCNU were administered alone or in combination to athymic mice bearing s.c. SF763T xenografts. The treatment regimen and tumor inhibition results on day 22 are presented in Table 1. Dose-dependent inhibition of tumor growth was observed after daily administration of SU101 alone at doses of 5, 10, and 20 mg/kg or administration of BCNU at 12, 18, and 27 mg/kg on days 1, 5, and 9. Treatment with 12 mg/kg BCNU did not result in statistically significant tumor inhibition (33% inhibition; P > 0.05). However, this dose of BCNU in combination with daily administration of SU101 at 10 mg/kg resulted in an inhibition of tumor growth of 63% that was more effective than either agent administered alone (Table 1; Fig. 2A) and was statistically different from the vehicle control and SU101 treatment. This combination was not statistically different from BCNU administered at 12 mg/kg as measured on day 22; however, statistical differences were observed on day 15 (P = 0.01). The combination of 18 mg/kg BCNU with 10 mg/kg SU101 resulted in a

<table>
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<th>Treatment combination</th>
<th>Dose (mg/kg)</th>
<th>Regimen (days dosed)</th>
<th>Inhibition (%)</th>
<th>P</th>
<th>P relative to specific treatment groups</th>
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<td>NS</td>
<td>Vehicle</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>1, 5, and 9</td>
<td>36</td>
<td>0.03</td>
<td>Vehicle</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1, 5, and 9</td>
<td>57</td>
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<td>1, 5, and 9</td>
<td>0.006</td>
<td>BCNU (18 mg/kg)</td>
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Table 1 Inhibition of s.c. growth of SF763T tumor xenografts

SF763T cells were implanted into athymic mice as described in “Materials and Methods.” Treatment groups (n = 6 animals/group) received SU101 by i.p. administration or BCNU by i.v. administration as indicated. Tumor weights were determined, and the percent inhibition was assessed on day 22, and compared with the indicated treatment groups. NS, not significant, P > 0.05.
Table 2  Inhibition of s.c. growth of Calu-6 tumor xenografts

Calu-6 cells were implanted into athymic mice as described in “Materials and Methods.” Treatment groups (n = 10 animals/group) received i.p. administration of the compounds indicated. Control animals received equivalent volumes of vehicle (DMSO) according to the regimen shown for each experiment. Tumor volumes were measured, and the percent inhibition was compared with the indicated treatment group on the final day of each experiment (experiment 1, day 20; experiment 2, day 29). NS, not significant, P > 0.05.

<table>
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<tr>
<th>Treatment combination</th>
<th>Dose (mg/kg)</th>
<th>Regimen (days dosed)</th>
<th>Inhibition (%)</th>
<th>P relative to specific treatment groups:</th>
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<td>1–20 (2×/week)</td>
<td>17</td>
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<td>33</td>
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</tr>
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<td>53</td>
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<tr>
<td>CDDP</td>
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<td>2</td>
<td>0.006</td>
<td>SU101 alone</td>
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<td><strong>Experiment 2</strong></td>
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</tr>
<tr>
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<td>1–20 (2×/week)</td>
<td>33</td>
<td>0.007 Vehicle</td>
</tr>
<tr>
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<td>2</td>
<td>30</td>
<td>0.049 Vehicle</td>
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<td>4, 7, and 10</td>
<td>20</td>
<td>NS Vehicle</td>
</tr>
<tr>
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<td>75</td>
<td>&lt;0.0001 Vehicle SU101 alone</td>
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<td>CDDP</td>
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<td></td>
<td>0.005</td>
<td>VP-16 alone</td>
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<tr>
<td>VP-16</td>
<td>10</td>
<td>4, 7, and 10</td>
<td>0.0004</td>
<td>CDDP/VP-16</td>
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Table 3  Inhibition of s.c. growth of KB tumor xenografts

KB cells were implanted into athymic mice as described in “Materials and Methods.” Treatment groups (n = 9 animals/group) received i.p. administration of the compounds indicated. Control animals received equivalent volumes of vehicle (DMSO) according to the regimen shown for each experiment. Tumor volumes were measured, and the percent inhibition (assessed on day 29) was compared with the indicated treatment groups. NS, not significant, P > 0.05.

<table>
<thead>
<tr>
<th>Treatment combination</th>
<th>Dose (mg/kg)</th>
<th>Regimen (days dosed)</th>
<th>Inhibition (%)</th>
<th>P relative to specific treatment groups:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SU101</strong></td>
<td>5</td>
<td>1–29 (2×/week)</td>
<td>29</td>
<td>NS Vehicle</td>
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<td>CDDP</td>
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<td>2 and 25</td>
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<td>SU101</td>
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<td>1–29 (2×/week)</td>
<td>69</td>
<td>0.001 Vehicle</td>
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<td>CDDP</td>
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<td>2 and 25</td>
<td>0.005</td>
<td>SU101 alone</td>
</tr>
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<td>0.0003</td>
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<td>5-FU</td>
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<td>3, 5, 26, and 29</td>
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statistically significant 74% inhibition of tumor growth (Table 1; Fig. 2A). This treatment combination was more effective than either agent administered alone. After cessation of treatment with BCNU on day 9 and cessation of treatment with SU101 on day 21, tumor growth was monitored for an additional 14 days (Fig. 2B). During this observation period, tumor growth continued to be inhibited by the combination of 12 mg/kg BCNU and 10 mg/kg SU101 until day 29 (as determined by the statistical difference from the vehicle-treated control animals) and by the combination of 18 mg/kg BCNU and 10 mg/kg SU101 until day 32. Overall, the combinations of BCNU (12 or 18 mg/kg) with SU101 (10 mg/kg) were additive. One animal from the 12 mg/kg BCNU plus SU101 treatment group died, demonstrating a low mortality rate for these treatment combinations.

**Calu-6 Tumor Xenografts.** Lung cancer is typically treated with combination therapy (44); therefore, studies of SU101 in combination with other agents in a lung xenograft model were conducted. Although PDGF β-receptor was not detected in tumor cells of Calu-6 xenografts, we have previously shown that SU101 administered daily beginning 1 day after tumor cell implantation inhibited the growth of Calu-6 cells in athymic mice (2). Thus, this model was chosen for studies of SU101, CDDP, and VP-16 administered as single agents or in combination. For each compound, suboptimal doses were administered to determine whether combination treatment significantly enhanced efficacy. The treatment regimen and tumor inhibition results for two separate experiments, experiments 1 and 2, are shown in Table 2. The tumor growth curves for the individual treatment groups from both experiments are shown in Figs. 3 and 4, respectively. In the first experiment, which was designed to test a combination of SU101 and CDDP, statistically significant growth inhibition was observed with CDDP alone (33%; P = 0.014) compared with the vehicle control (Fig. 3). SU101 administered alone twice weekly did not show a statistically significant inhibition of tumor growth (17%) compared with the vehicle control. However, the combination of CDDP and SU101 resulted in a greater inhibition of tumor growth (53%) than the vehicle control and was also more effective than either compound administered as a single agent (Table 2). A second experiment was conducted to examine the effects of SU101 and CDDP in combination with a third agent, VP-16. The results (Table 2; Fig. 4) demonstrate that SU101 and
CDDP, administered as single agents, produced a statistically significant inhibition of tumor growth of 33% \( (P = 0.008) \) and 30% \( (P = 0.05) \), respectively, compared with the vehicle control. In contrast, VP-16 administered alone was not effective; however, in combination with CDDP, a statistically significant 39% \( (P = 0.001) \) inhibition of tumor growth compared with vehicle control was observed. The most effective treatment for reducing the growth of Calu-6 lung tumor xenografts was the combination of SU101, CDDP, and VP-16 (Table 2). The 75% inhibition of tumor growth was statistically different from that

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**Fig. 2** Inhibition of s.c. growth of SF763T tumor xenografts. SF763T glioma cells \( (1 \times 10^7 \text{ cells/mouse}) \) were implanted s.c. into the hind flank of athymic mice. Tumors were allowed to establish themselves to an average mass of 115 mg before the initiation of treatment (designated day 1). Treatment groups \( (n = 6 \text{ animals/group}) \) are defined in Table 1. SU101 treatment was stopped on day 21, and the last dose of BCNU was administered on day 9. A shows the results through day 22, at which time the percentage of inhibition indicated in Table 1 was calculated. B shows the results through the end of the experiment. Data plotted are the mean tumor mass ± SE.
of the vehicle control and all other drug treatment groups. No mortality was observed in any treatment group from either experiment with the Calu-6 model. These results indicate that SU101 may enhance the effect of standard chemotherapy for the treatment of lung cancer.

**KB Tumor Xenografts.** CDDP has been used for combination treatment of head and neck tumors (49, 50) as well as lung cancer. Thus, the antitumor efficacy of SU101 administered alone or in combination with CDDP and 5-FU was examined in athymic mice bearing KB tumor xenografts. SU101 had not been tested previously in this model. KB cells growing in culture do not express detectable PDGF β-receptor, but tumor xenografts have not been evaluated for expression of this receptor (2). The KB model was studied to determine the breadth of tumor types that may be inhibited by combinations of SU101 with cytotoxic agents. The treatment regimen and tumor inhibition results from the current study are shown in Table 3. The tumor growth curves for the individual treatment groups through day 29 (the last day of treatment) are shown in Fig. 5. As in the Calu-6 studies, suboptimal doses of SU101, CDDP, and 5-FU were administered. Agents administered alone did not significantly inhibit tumor growth by day 29 (SU101, 29% inhibition, \( P > 0.05 \); CDDP, 38% inhibition, \( P > 0.05 \); 5-FU was not effective as a single agent in this model (separate study; data not shown). In addition, SU101 administered with either CDDP or 5-FU as well as the combination of CDDP and 5-FU did not significantly inhibit xenograft growth (Table 3; Fig. 5). However, the combination of SU101, 5-FU, and CDDP resulted in a statistically significant, 69% growth inhibition on day 29 as compared with the vehicle control and all other treatment groups (Table 3). Animals treated with the combination of SU101, 5-FU, and CDDP were monitored for an additional 21 days after the cessation of treatment. On day 50, these animals showed a statistically significant tumor inhibition of 69% as compared with the vehicle control (\( P = 0.0004 \)). These data demonstrate that the combination of SU101, CDDP, and 5-FU was significantly more effective than any agent administered alone or in combination with one other agent. Additionally, the reduction in tumor volume resulting from the combination treatment (SU101, CDDP, and 5-FU) persisted for 21 days after the cessation of treatment. No mortality was observed in any treatment group. As in the glioblastoma and lung xenograft models, results with this tumor model indicate that SU101 administered in combination with cytotoxic chemotherapeutic agents may increase tumor growth inhibition without increased toxicity.

**DISCUSSION**

Aberrant signaling of PDGF has been proposed to play a role in many human cancers (55–57), suggesting that the PDGF receptor pathway is a therapeutic target for cancer treatment. SU101 (Fig. 1) has previously been shown to inhibit PDGF signaling (1, 2), leading to a decrease in the proliferation of human tumor cells including those of glioma, ovarian, melanoma, prostate, and lung origin. In contrast to the parent compound, the open ring metabolite of SU101, SU0020 (also known as A77 1726; Fig. 1), is a potent inhibitor of \( \text{de novo} \) pyrimidine biosynthesis (58). The metabolite inhibits the growth of various cell types in culture, and this inhibition can be reversed by the addition of uridine (1, 58). SU0020 is formed rapidly after the administration of SU101 to animals (59) and patients (32). Thus, it is possible that the inhibition of pyrimidine biosynthesis by the metabolite may contribute to the antitumor activity of SU101, although uridine did not reverse the efficacy in xenograft tumor models (1).

SU101 is in late-stage clinical trials for the treatment of cancer (27–31). It is likely that SU101 will be used clinically in
combination with other agents. In this report, several human tumor cell lines in established s.c. xenograft models were used to study the antitumor effects of SU101 in combination with cytotoxic chemotherapeutic agents. Athymic mice bearing s.c. human tumor xenografts have been used as models to help predict the activity of new combinations of chemotherapeutic agents in the treatment of human cancers (60).

SU101 is undergoing Phase III clinical evaluation for treatment of glioblastoma. Since the 1950s, glioblastoma has been treated with BCNU, an alkylating agent. However, over several decades, there has been no real improvement in the survival of these patients (41, 42). Combination therapy with another agent such as SU101 may be warranted to improve treatment for patients with glioblastoma. We have previously shown that SU101 inhibits the growth of SF763T, a human glioblastoma tumor cell line, in vitro and in vivo, via the inhibition of PDGF receptor signaling (2). Thus, this model was used to determine whether the use of SU101 and BCNU, agents with very different mechanisms of action, could significantly improve the inhibition of glioma tumor cell growth in vivo. SU101 or BCNU administered alone resulted in statistically significant, dose-dependent reductions in tumor growth compared with the vehicle control group. Additionally, combining SU101 at 10 mg/kg with BCNU at 18 mg/kg produced a more pronounced, statistically significant, 74% inhibition of tumor growth, and this treatment combination was more effective than either agent administered alone. Overall, many of the combinations of BCNU and SU101 were additive. The increase in efficacy of suboptimal doses of BCNU by combination treatment with SU101 may result in a less toxic treatment for patients with glioblastoma. A Phase III clinical study of SU101 in combination with BCNU for the treatment of patients with recurrent malignant glioma is ongoing (31). Of the 18 patients enrolled in this study, one patient has shown a partial response by magnetic resonance imaging. Further data evaluations are ongoing.

The usefulness of SU101 for the treatment of lung cancer was also evaluated. Calu-6 cells, an anaplastic carcinoma of lung origin, have been used routinely as a model of both in vitro and in vivo lung cancer (61, 62), and SU101 was previously shown to be efficacious in this model (2). Lung cancer patients have been treated with CDDP, a DNA cross-linker, and VP-16, a topoisomerase II inhibitor, both as single agents and in combination with other cytotoxic chemotherapeutic agents (43–45). Only limited success has been achieved in humans with single-agent or multiagent chemotherapy in non-small cell lung cancer (44). In contrast, our experiments with SU101 in combination with a suboptimal dose of CDDP and with both CDDP and VP-16 demonstrated significant enhancement of growth inhibition of the Calu-6 xenografts. The antitumor effects of SU101 administration with CDDP or with CDDP and VP-16 were additive without causing toxicity because no mortality was observed. These studies suggest that combinations of suboptimal doses of SU101 and chemotherapeutic agents that act by different mechanisms of action may be effective in humans for the treatment of lung cancer. The possibility of using lower doses of chemotherapeutic agents by coadministering SU101 may also reduce toxicity.

As with lung cancer, patients with squamous cell carcinoma of the head and neck generally have only limited benefit from current chemotherapeutic treatment (48). The response rate with CDDP and 5-FU combinations in humans has been reported to be 60%; however, there is no indication that any consistent improvement in survival exists over that of patients receiving only local treatment (49, 50). Thus, SU101 was evaluated in the KB model of head and neck cancer to determine whether it could enhance the efficacy of CDDP and 5-FU. Although KB cells in culture do not express detectable PDGF β-receptor (2), this model has been extensively reported in the literature (for examples, see Refs. 63 and 64); hence, it was chosen for combination studies. In our experiments, the combination of suboptimal doses of SU101 and chemotherapeutic agents that act by different mechanisms of action may be effective in humans for the treatment of lung cancer.

Overall, the studies presented here indicate that SU101 enhanced the effects of suboptimal doses of cytotoxic compounds without increasing toxicity, suggesting the potential use of SU101 in combination with cytotoxic agents in human clinical trials. Because SU101 inhibits tumor cell proliferation by blocking PDGF receptor signaling (2), it is of particular interest to use SU101 in combination in the treatment of tumors that exhibit a PDGF-dependent proliferative response. A broad range of tumor types have been found to express PDGF receptors (5–20). Furthermore, PDGF and its receptors are known to be involved in the growth of tumor stromal tissue (20, 22). The role of PDGF and its receptors in tumor stromal development was demonstrated in an animal model by expressing PDGF in WM9.
human melanoma cells (23). Implantation of the PDGF-expressing cells into athymic mice resulted in the formation of xenograft tumors with connective tissue septa and abundant vasculature, but tumors resulting from implantation of the parental cells that do not express PDGF did not contain connective tissue and had few vessels. These results suggest that inhibition of PDGF signaling may block the formation of tumor stroma, although the composition of (65) and requirement for stroma (23, 66) are not the same in all tumor xenograft models.

PDGF also promotes the growth of new microvasculature through stimulation of pericytes, small cells that surround and stabilize microvessels (24, 25). Neovascularization is required for tumor growth beyond a minimum volume (26); thus, disrupting pericyte growth may inhibit tumor growth. As with stroma, the degree of vascularization varies between tumor xenograft models. The efficacy of SU101 in a particular model may depend on PDGF receptor expression in the tumor cells, stromal cells, and/or pericytes. Indeed, SU101 was shown to inhibit the growth of 13 of 16 in vivo tumor models tested, with inhibition ranging from 53–95% (2). This included three models in which the PDGF β-receptor was not detected in the tumor cells of the xenografts by immunohistochemistry with an antibody against the human PDGF β-receptor. Expression of the PDGF α-receptor in the tumor cells and expression of either PDGF receptor on the murine stromal tissue were not evaluated in these studies. Because PDGF and its receptors play a role in a wide variety of tumor types and in several cell types within tumors, SU101 may be a useful therapeutic agent for many cancers in combination with cytotoxic agents and as a single agent. The efficacy of combinations of SU101 and cytotoxic agents was demonstrated in the current studies in the Calu-6 and KB xenograft tumor models, although neither tumor cell type expresses detectable PDGF β-receptors. Thus, these types of combinations of agents may offer another approach that will lead to new strategies for clinical intervention for the treatment of human cancer.

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Effects of SU101 in Combination with Cytotoxic Agents on the Growth of Subcutaneous Tumor Xenografts

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