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ORIGINAL ARTICLE

Sorafenib is efficacious and tolerated in combination with cytotoxic or cytostatic agents in preclinical models of human non-small cell lung carcinoma

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Abstract *Purpose*: Sorafenib tosylate (sorafenib, BAY 43-9006, Nexavar®) is a multi-kinase inhibitor that targets tumor cell proliferation and angiogenesis. These studies evaluated the efficacy and tolerability of combinations of sorafenib plus agents used to treat non-small cell lung cancer (NSCLC) using preclinical models of that disease. *Methods*: Intravenous (iv) vinorelbine and interperitoneal (ip) cisplatin were administered intermittently (q4d × 3) in combination with sorafenib administered orally (po) once daily for 9 days starting on the same day as the standard agent. In studies with sorafenib and gefitinib, both agents were administered po daily for 10 days starting on the same day. Treatment in all studies was initiated against established sc tumors, and each study was conducted in duplicate. Efficacy was assessed as the delay in tumor growth to a specified size (TGD). *Results*: Vinorelbine (6.7 mg/kg) and sorafenib (40 mg/kg) produced TGDs of 2.4 and 7.8 days, respectively, in the NCI-H460 NSCLC model. Combination therapy produced a 10.0-day TGD with no increase in toxicity. Combination therapy in the NCI-H23 NSCLC model with the highest evaluated dose levels of sorafenib plus cisplatin was well tolerated and produced TGDs equivalent to those produced by cisplatin alone. Lower dose levels of each agent produced approximately additive TGD's. Combination therapy in the A549 NSCLC model with sorafenib and gefitinib produced TGDs equivalent to that produced by sorafenib alone with no toxicity. Tumor growth in the MDA-MB-231 mammary tumor model, that contains mutations in signal transduction proteins downstream of the EGF receptor (the target of gefitinib) was also inhibited by sorafenib, but not by gefitinib. *Conclusion*: Concurrent administration of sorafenib and vinorelbine, cisplatin or gefitinib was at least as efficacious as the individual agents alone and was well tolerated. These results support the inclusion of sorafenib in clinical trials in NSCLC employing combinations of both cytotoxic and cytostatic agents.

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Introduction

Sorafenib is a novel, multi-kinase inhibitor that targets both tumor proliferation and tumor angiogenesis [45] and has recently been approved for the treatment of advanced renal cell cancer (RCC). The molecular targets of sorafenib include several key signal transduction proteins CRAF, BRAF (both wild-type and the V600E mutant), c-KIT, FLT-3, RET [10] vascular endothelial growth factor receptor-2 (VEGFR-2), VEGFR-3, and platelet-derived growth factor receptor-β (PDGFR-β). RAF kinase is a serine/threonine protein kinase, which acts downstream of Ras to relay proliferative and survival signals to the cell nucleus in a broad spectrum of human tumors. In addition,

CRAF is a major regulator of endothelial cell survival [2]. The VEGF and PDGF receptors are central to tumor angiogenesis [7, 14], and PDGFR may also play a role in patients with chronic myeloproliferative cancers [5]. FLT-3 is important in acute myelogenous leukemia [36], and c-KIT plays an integral role in gastrointestinal stromal tumors [19] and BRAF and RET are important drivers of thyroid cancer [35]. It has also been reported that sorafenib induces apoptosis in multiple human tumor cell lines [46] through the inhibition of the translation and downregulation of myeloid cell leukemia-1 (Mcl-1), a Bcl-2 family member. A recent report by Rahmani et al. [34] describes a MEK-independent effect of sorafenib on the inhibition of eIF4E phosphorylation in leukemia cells and suggests a possible linkage between eIF4E and translational control of Mcl-1. Sorafenib-induced apoptosis in the absence of caspase activation has also been reported by Panka et al. [30]. They showed that in melanoma cells, sorafenib-induced apoptosis was largely mediated through the nuclear translocation of apoptosis-inducing factor (AIF). Therefore, sorafenib is expected to affect tumor growth by directly inhibiting tumor cell proliferation and promoting apoptosis in a variety of tumor types as well as inhibiting tumor angiogenesis, leading to tumor stasis with occasional tumor regressions.

We have reported previously that sorafenib is effective in preclinical models of a broad spectrum of tumors [25, 45]. The clinical evaluation of sorafenib includes assessment of its combinability with the current standard of care for multiple cancers. In the case of NSCLC, one drug that constitutes the current standard of care is cisplatin, a DNA alkylating agent [8]. The cytotoxic effect of cisplatin is attributed to inhibition of DNA synthesis. Vinorelbine, a semisynthetic vinca alkaloid that inhibits mitotic microtubule polymerization, is another agent approved for first-line therapy of patients with advanced NSCLC. It is believed to exert its anti-tumor effect by binding to ß-tubulin and inhibiting microtubule assembly, causing dissolution of mitotic spindles and, ultimately, cell-cycle arrest in metaphase of tumor cell division [17].

Gefitinib is a small-molecule inhibitor of the kinase activity of the epidermal growth factor receptor (EGFR) [42]. Studies of its mechanism of action indicate that gefitinib is an ATP-competitive inhibitor of EGFR, and blocks autophosphorylation of the receptor when the receptor is stimulated by binding epidermal growth factor (EGF) or transforming growth factor alpha (TGFα).

In this report, we describe preclinical in vivo evaluations of sorafenib combined with each of these agents in human tumor xenograft models. Models employed for combination chemotherapy evaluations were measurably sensitive to, but not cured by, either agent when administered alone. The commonly used clinical schedule of administration of vinorelbine and cisplatin consist of intermittent dosing on a schedule of once every 3 weeks. This schedule was modeled in our preclinical studies as an intermittent therapy consisting of bolus dosing once every 4 days for a total of three treatments (q4d × 3). Gefitinib is administered clinically on a daily basis, and this schedule was also used in our studies. These studies evaluated the tolerability and anti-tumor efficacy of continuous daily sorafenib treatment starting the same day as treatment with vinorelbine, cisplatin or gefitinib.

Materials and methods

Tumor lines and reagents

MDA-MB-231 human mammary adenocarcinoma cells (obtained from the National Cancer Institute) were maintained in DMEM (GIBCO) supplemented with 1% L-glutamine (GIBCO), 1% HEPES buffer (GIBCO), and 10% heat-inactivated fetal bovine serum (FBS) (JRH Biosciences). A549 and NCI-H460 human non-small cell lung carcinomas were obtained from the American Type Tissue Culture Collection Repository and were maintained in RPMI 1640 media (GIBCO) supplemented with 10% heat-inactivated FBS. The NCI-H23 model was obtained from the Tumor Repository of the National Cancer Institute and was established and maintained as a serial in vivo passage of subcutaneous (s.c.) fragments of 3 mm^2 implanted in the flank using a 13-gauge trocar. The passage was regenerated every 3–4 weeks.

The chemical name of sorafenib is [*N*-(3-trifluoromethyl-4-chlorophenyl)-*N*′-(4-[2-methylcarbamoyl pyridin-4-yl]oxyphenyl)urea]. The tosylate salt form of sorafenib was formulated as previously described [45]. The indicated dose levels of sorafenib in these studies represent the dose of the tosylate salt form. Cisplatin (Bedford laboratories) was supplied as a 50 mg/ml solution and was diluted to the desired concentration with saline immediately prior to administration. Vinorelbine (GlaxoWellcome) was supplied as a 10 mg/ml solution, and was diluted with D5W (5% dextrose) to the desired concentration immediately before use. Gefitinib (Albany Medical Research) was formulated for dosing as a suspension using 50 mM sodium lactate pH 4.0. Dosing solutions were prepared by further dilution with 0.9% saline for injection. All dosing

solutions were light protected at room temperature, and were used the day they were prepared.

Tumor xenograft experiments

Female NCr-*nu/nu* mice (Taconic Farms) were used as hosts for all xenograft models. MDA-MB-231, A549, and NCI-H460 tumors were generated by harvesting cells from mid-log phase cultures. Cells were then pelleted and resuspended in HBSS (GIBCO) to a final cell count of 3–5 $\times 10^7$ cells/ml. A volume of 0.1 ml of the cell suspension was injected subcutaneously into the right flank of each mouse. Treatment was initiated when all mice in each experiment had established tumors from 75 to 150 mg in weight. Sorafenib was administered 4 h prior to other agents on days when the schedules of the individual agents coincided. The general health of mice was monitored and mortality was recorded daily. Tumor dimensions and body weights were recorded twice weekly starting with the first day of treatment. Animals were euthanized according to Bayer or Southern Research Institute IACUC guidelines. Treatments resulting in greater than 20% lethality and/or 20% net body weight loss associated with a moribund condition were considered 'toxic'.

Tumor weights were calculated using the equation $(l \times w^2)/2$, where l and w represent the largest and smallest dimensions collected at each measurement. Anti-tumor efficacy was evaluated by the incidence of durable complete regressions (CR), partial regressions (PR), and tumor growth delay (TGD). Complete regression was defined as a reduction in tumor size to below the limit of palpation; PR was a reduction in tumor size of > 50% of baseline values but still palpable. A minimum duration of 5 days was required for a CR or PR to be considered durable. Tumor growth delay was expressed as (T–C), where T and C represent the median times for tumors in the treated (T) and untreated control (C) groups to attain the evaluation size for that experiment [23, 37].

Sorafenib plus vinorelbine was evaluated in the NCI-H460 human NSCLC model. Vinorelbine was administered iv on a q4d × 3 schedule at dose levels of 10.0 or 6.7 mg/kg alone or in combination with sorafenib administered po on a qd × 9 schedule at 80 or 40 mg/kg, with both treatments starting on the same day. Sorafenib was administered in the morning of each day. Vinorelbine was administered in the afternoon (approximately 3–5 h after sorafenib) to assure that sorafenib was at or near its maximum exposure when vinorelbine was administered. An evaluation endpoint of three tumor mass doublings, attained by untreated control tumors in 7.4 and 7.1 days in replicate experiments, was used to assess anti-tumor efficacy.

Sorafenib plus cisplatin was evaluated using the NCI-H23 human NSCLC model. Cisplatin was administered ip on a q4d × 3 schedule at dose levels of 5.4 or 3.6 mg/kg alone or in combination with sorafenib administered po daily for 9 days (qd × 9) at 80 or 40 mg/kg. The timing of administration of these agents was the same as described for the combination of sorafenib plus vinorelbine. An evaluation endpoint of two tumor mass doublings, attained by untreated control tumors in 9.2 and 14.5 days in replicate experiments, was used to assess anti-tumor efficacy.

The efficacy of sorafenib and gefitinib administered as single agents was investigated in the MDA-MB-231 human mammary tumor model and A549 human NSCLC model. In both experiments, each agent was administered po on a qd × 10 schedule. The A549 NSCLC model was subsequently selected for the evaluation of the efficacy of the combination of sorafenib plus gefitinib.

Gefitinib has previously been reported to have an approximate maximum tolerated dose level of 200 mg/kg when administered as a single agent on a daily administration schedule. However, when co-administered with either paclitaxel, doxorubicin, oxaliplatin or topotecan, the gefitinib dose was reduced to 150 mg/kg [40]. Therefore, in the experiments reported here, gefitinib was evaluated at dose levels of 150 and 75 mg/kg. Sorafenib was administered at dose levels of 80 and 40 mg/kg. An evaluation endpoint of one tumor mass doubling, attained by untreated control tumors in 5.2 and 7.6 days in replicate experiments, was used to assess anti-tumor efficacy.

Statistical analysis

The effect of single agent treatments on tumor growth delays were statistically analyzed with One-way ANOVA with individual group comparisons evaluated by Bonferroni's Multiple Comparison test. Drug interactions in groups treated with sorafenib and one of the standard chemotherapy agents were evaluated with a Two-way ANOVA. In both tests, a P value of < 0.05 was considered significant.

Results

Combination chemotherapy of sorafenib and vinorelbine

The effects of therapy with 80 mg/kg sorafenib and the maximum tolerated dose of vinorelbine on the growth

of NCI-H460 NSCLC xenografts either as single agents or in combination are illustrated in Fig. 1. The tolerability and efficacy of all treatments evaluated in this study are summarized in Table 1, Experiments 1 and 2.

The evaluation endpoint of three tumor mass doublings was attained in Experiment 1 in 7.4 days. Vinorelbine administered at 10.0 mg/kg as a single agent was in excess of the maximum tolerated dose in this study, as evidenced by excessive weight loss of 25.4%. The 6.7 mg/kg dose was tolerated, resulting in a 19.2% weight loss and a 2.4-day TGD (not significant), with no PRs or CRs. Sorafenib was well tolerated as a single agent, resulting in minor weight loss (9.6–10%) and no lethality at either 80 or 40 mg/kg. The observed weight loss in the sorafenib-treated groups was similar to the 7.7% weight loss observed in the vehicle-treated control animals. Sorafenib produced TGDs of 8.3 and 7.8 days ($P < 0.001$ for both treatments) at dose levels of 80 and 40 mg/kg, respectively. Thus sorafenib produced TGDs approximately equivalent to the duration of therapy.

Concurrent administration of sorafenib and vinorelbine at tolerated dose levels produced TGDs that were

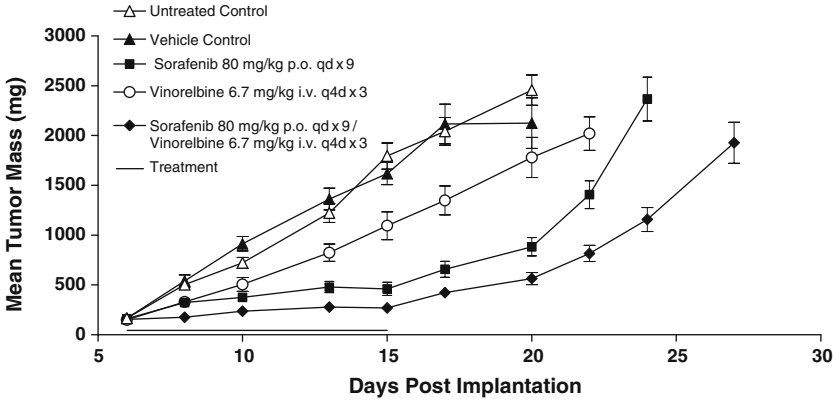

Fig. 1 Effects of the maximum tolerated doses of sorafenib and vinorelbine, administered alone or in combination, against NCI-H460 human non-small cell lung cancer (NSCLC) tumor xenografts. Female NCr-*nu/nu* mice ($n = 10$/group) were implanted sc with NCI-H460 tumor cells and treatments were initiated when all mice had tumors of 75–150 mg. Vinorelbine was administered iv on a q4d × 3 schedule at 6.7 mg/kg, either alone (*open circle*) or with (*filled diamond*) sorafenib administered po on a qd × 9 schedule at 80 mg/kg (*filled square*). Changes in mean tumor weight were recorded over time and are expressed as mean ± SEM

Table 1 Antitumor effect of sorafenib in combination with vinorelbine against established NCI-H460 human NSCLC xenografts

Sorafenib dose[a] (mg/kg)	Vinorelbine dose[b] (mg/kg)	Experiment 1					Experiment 2				
		Lethality	Maximum net weight loss[c] (%)	CR	PR	TGD[d] (days)	Lethality	Maximum net weight loss[c] (%)	CR	PR	TGD[d] (days)
0	0	0/10	7.7	0/10	0/10	−0.4	0/10	2.7	0/10	0/10	0
80	0	0/10	9.6	0/10	0/10	8.3[e]	0/10	6.6	0/10	0/10	10.1[e]
40	0	0/10	10.0	0/10	0/10	7.8[e]	0/10	4.6	0/10	0/10	8.0[e]
0	10	0/10	25.4	0/10	0/10	4.1[f]	0/10	16.0	0/10	0/10	5.7[f]
0	6.7	0/10	19.2	0/10	0/10	2.4	0/10	16.0	0/10	0/10	3.3
80	10	4/10	Toxic				4/10	Toxic			
40	10	0/10	14.1	0/10	1/10	10.0	0/10	11.6	0/10	1/10	10.8
80	6.7	0/10	14.8	0/10	0/10	11.1	2/10	15.0	0/10	0/10	9.1
40	6.7	0/10	11.4	0/10	0/10	10.0	0/10	7.9	0/10	0/10	12.3

[a] Sorafenib was administered po on a qd × 9 schedule starting on Day 6 after tumor implantation in each Experiment when all animals had established tumors measuring 75–150 mg

[b] Vinorelbine was administered iv on a q4d × 3 schedule also starting on Day 6 after tumor implantation in each Experiment

[c] Maximum net weight loss expressed as a percent of the initial body weight on the day treatment was initiated minus the tumor weight

[d] TGD determined as the difference in the median time for the treated and control groups to attain three tumor mass doublings from the start of therapy. The control untreated tumors attained the evaluation endpoint in 7.4 days in Experiment 1 and 7.1 days in Experiment 2

[e] Tumor growth delay significantly increased ($P < 0.001$ by One-Way ANOVA) relative to the Control Group

[f] Tumor growth delay significantly increased ($P < 0.01$ by One-Way ANOVA) relative to the Control Group

not significantly different from those produced by sorafenib administered alone. Toxicity associated with the combination therapy was equivalent to that produced by vinorelbine alone. Addition of 80 mg/kg sorafenib to 10.0 mg/kg vinorelbine produced 40% lethality. This is consistent with the excessive weight loss produced by vinorelbine alone at the same dose level. The combination of 80 mg/kg sorafenib with 6.7 mg/kg vinorelbine resulted in a 14.8% weight loss and a TGD of 11.1 days, with no lethality. Combination of 40 mg/kg sorafenib with 10.0 or 6.7 mg/kg vinorelbine resulted in weight loss of 14.1 and 11.4% and TGDs of 10.0 and 10.0 days, respectively, with no lethality. Relative to the weight loss produced by vinorelbine alone at the same dose levels (25.4 and 19.2% for dose levels of 10.0 and 6.7 mg/kg), both combination regimens produced less weight loss. Thus, the addition of sorafenib to the vinorelbine regimen was well tolerated and there was no adverse interaction with respect to anti-tumor efficacy.

The effects observed using these dose levels of each agent were confirmed in an independent experiment (Table 1, Experiment 2). Control tumor growth was equivalent to that in the previous experiment with the evaluation point of three tumor mass doublings being reached in 7.1 days. Weight loss associated with tumor growth was less in this experiment (2.7% compared with 7.7% previously) and the toxicity associated with the vinorelbine treatment was also reduced (the 10 mg/kg dose level of vinorelbine produced only a 16% weight loss compared with 25.4% in the previous experiment). As in the previous experiment, sorafenib produced TGDs approximately equivalent to the duration of therapy and vinorelbine produced a marginal anti-tumor efficacy ($P < 0.01$) at the 10 mg/kg dose level. The combination of the highest dose levels of sorafenib and vinorelbine produced the same level of toxicity seen in the first experiment and combinations of lower dose levels were tolerated with TGDs that were not significantly different from those produced by sorafenib alone at the same dose level.

Combination chemotherapy of NCI-H23 xenografts with sorafenib and cisplatin

The effect of therapy with sorafenib and cisplatin at their optimum dose levels either alone or in combination against established NCI-H23 xenografts is shown in Fig. 2. The tolerability and efficacy of all treatments evaluated are summarized in Table 2, Experiments 1 and 2.

The evaluation endpoint used in Experiment 1, two tumor mass doublings, was attained in 9.2 days. Cisplatin as a single agent produced dose-dependent tumor growth delays of 29.1 and 12.1 days at dose levels of 5.4 and 3.6 mg/kg, respectively. Due to the variability in control tumor growth in this model, only the TGD produced by the 5.4 mg/kg dose level of cisplatin was

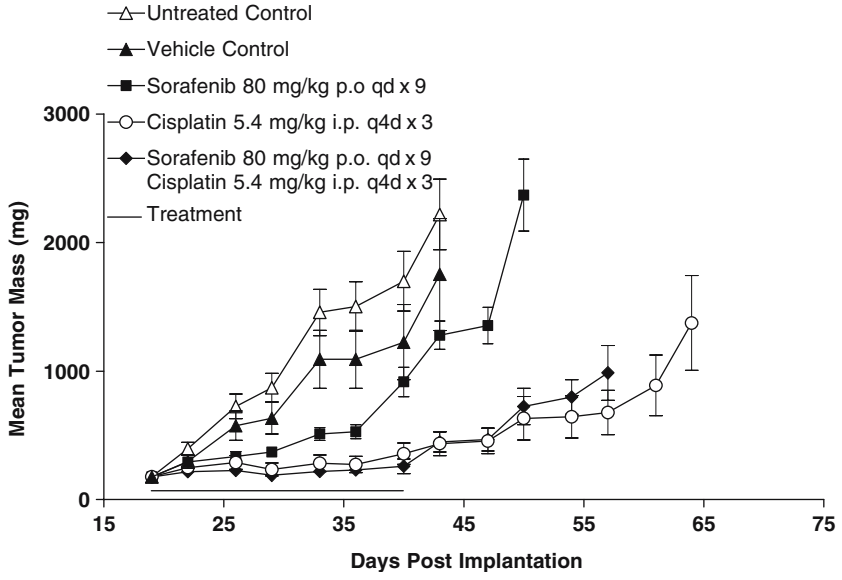

Fig. 2 Effects of the maximum tolerated doses of sorafenib and cisplatin, administered alone or in combination, against NCI-H23 NSCLC tumor xenografts. Female NCr-*nu/nu* mice (n = 10/group) were implanted sc with NCI-H23 tumor fragments and treatments were initiated when all mice had tumors of 126–221 mg. Cisplatin was administered ip on a q4d × 3 schedule at 5.4 mg/kg (*open circle*), either alone or in combination (*filled diamond*) with sorafenib administered po for 9 days (qd × 9) at 80 mg/kg (*filled square*). Changes in mean tumor weight were recorded over time and are expressed as mean ± SEM

Table 2 Antitumor effect of sorafenib in combination with cisplatin against established NCI-H23 human NSCLC xenografts

Sorafenib dose[a] (mg/kg)	Cisplatin dose[b] (mg/kg)	Experiment 1					Experiment 2				
		Lethality	Maximum net weight loss[c] (%)	CR	PR	TGD[d] (days)	Lethality	Maximum net weight loss[c] (%)	CR	PR	TGD[d] (days)
0	0	0/10	2.0	2/10	0/10	0.1	2/10	2.0	0/10	0/10	1.4
80	0	0/10	3.1	0/10	0/10	10.4	1/10	3.1	0/10	0/10	9.7
40	0	0/10	3.7	0/10	0/10	8.7	0/10	3.7	0/10	0/10	5.3
0	5.4	0/10	2.3	2/10	1/10	29.1[e]	0/10	2.3	0/10	0/10	21.4[e]
0	3.6	0/10	1.8	0/10	0/10	12.1	2/10	1.8	0/10	0/10	10.9
80	5.4	0/10	9.7	0/10	0/10	23.1	1/10	9.7	4/10	0/10	27.5
40	5.4	0/10	6.0	2/10	0/10	35.9	0/10	6.0	3/10	0/10	26.8[f]
80	3.6	0/10	5.3	1/10	0/10	12.4	0/10	5.3	1/10	0/10	20.7
40	3.6	0/10	3.1	2/10	0/10	21.0[f]	0/10	3.1	3/10	0/10	34.1[f]

[a] Sorafenib was administered po on a qd × 9 schedule starting on Day 19 after tumor implantation when all animals had established tumors measuring 126–245 mg (Experiment 1) or starting on Day 18 (Experiment 2) when all animals had tumors measuring 88–294 mg

[b] Cisplatin was administered ip on a q4d × 3 schedule also starting on the first day of treatment in each Experiment

[c] Maximum net weight loss expressed as a percent of the initial body weight on the day treatment was initiated minus the tumor weight

[d] TGD determined as the difference in the median time for the treated and control groups to attain two tumor mass doublings from the start of therapy. The control untreated tumors attained the evaluation endpoint in 9.2 days in Experiment 1 and 14.5 days in Experiment 2

[e] Tumor growth delay significantly increased ($P < 0.01$ by One-Way ANOVA) relative to the Control Group

[f] Tumor growth delay demonstrates a significant interaction in the combination treatment ($P < 0.05$ by Two-Way ANOVA) relative to treatment with the individual agents at the same dose levels used in the combination

statistically significant ($P < 0.01$). Weight loss associated with cisplatin treatment was 1.8–2.3% with no drug-related lethality. Sorafenib produced tumor growth delays of 10.4 days at 80 mg/kg and 8.7 days at 40 mg/kg. Weight loss associated with sorafenib administration was 3.1–3.7%. Therefore, as in the H460 experiments reported above, sorafenib produced TGDs approximately equivalent to the duration of therapy.

Concurrent combination therapy with cisplatin and sorafenib was well tolerated. Body weight loss was 3.1–9.7%, which was not significantly greater than that produced by either individual therapy and there was no lethality. The tumor growth delay produced by the combination of 5.4 mg/kg cisplatin with either 80 mg/kg sorafenib (23.1 days) or 40 mg/kg (35.9 days) was similar to that produced by cisplatin alone at the same dose level (29.1 days).

The tumor growth delay of 3.6 mg/kg of cisplatin plus 80 mg/kg of sorafenib (12.4 days) did not differ from that of cisplatin alone at the same dose level (12.1 days). However, the tumor growth delay produced by the addition of 40 mg/kg sorafenib (21.0 days) was greater than that produced by either single agent alone ($P < 0.05$).

Thus, at the optimal dose of cisplatin of 5.4 mg/kg, the addition of sorafenib did not alter the anti-tumor efficacy of cisplatin. At lower doses of both cisplatin and sorafenib, the combination therapy produced a TGD approximately equivalent to the sum of the TGDs of the individual therapies in the combination. Overall, sorafenib did not adversely affect the anti-tumor efficacy of cisplatin at either of the dose levels tested.

The effects observed using these dose levels of each agent were confirmed in an independent experiment (Table 2, Experiment 2). Control tumor growth was slightly slower in this experiment with the evaluation endpoint of two tumor mass doublings not reached until 14.5 days as compared with 9.2 days in the previous experiment. There was also sporadic lethality in this study that was not associated with body weight loss and did not appear to be related to drug administration (2/10 mice died during the treatment period in the vehicle-treated group). It is possible this lethality was due to technician error during dosing. However, the TGDs associated with the single agent therapies in the study were consistent with those observed previously. The combination treatments also produced similar TGDs to those observed in the first experiment but with a larger number of complete regressions which may reflect the poorer tumor growth in this experiment. It is noteworthy that the positive interaction observed between the low dose levels of sorafenib (40 mg/kg) plus cisplatin (3.6 mg/kg) was confirmed in this study ($P < 0.05$).

Single agent efficacy of sorafenib and gefitinib

The goal of these experiments was to identify an appropriate model to explore combination chemotherapy with sorafenib plus gefitinib. The anti-tumor

efficacy of treatment with these agents alone against the MDA-MB-231 human mammary tumor and A549 human NSCLC models is summarized in Table 3, and illustrated in Figs. 3 and 4. Both sorafenib and gefitinib were well tolerated in both models, with no lethality and no significant body weight loss.

The evaluation endpoint used to assess efficacy in both models was time to two tumor mass doublings. Untreated control tumors attained that size in 9.2 days in the MDA-MB-231 study, and in 17.6 days in the A549 study. Gefitinib was inactive against the MDA-MB-231 model, with a TGD of 0.5 days. However, sorafenib was highly efficacious in this model, and produced a TGD of > 25 days ($P < 0.001$), with 50–60% durable tumor regressions (CRs plus PRs). Based on the high sensitivity of this model to sorafenib, coupled with a lack of sensitivity to gefitinib, MDA-MB-231 was not chosen for studies of combination chemotherapy with these agents.

Gefitinib produced a TGD of 10.5 days ($P < 0.05$) and sorafenib produced a TGD of > 40 days ($P < 0.001$) at both the 80 and 40 mg/kg dose levels in the A549 study. Neither gefitinib nor sorafenib led to tumor regressions as single agents in this study. The A549 model was selected for subsequent combination chemotherapy studies on the basis of measurable antitumor efficacy of each agent with no tumor regressions.

Table 3 Effects of sorafenib and gefitinib against MDA-MB-231 and A549 tumors

Test article	Dose (mg/kg)	MDA-MB-231 mammary tumor			A549 NSCLC tumor		
		Maximum weight loss [% (day)]	TGD[a] (days)	Regressions[b]	Maximum weight loss [% (day)]	TGD[a] (days)	Regressions[b]
Control	Untreated	0	N/A	0/10	0	N/A	0/10
Gefitinib	0	0	0.5	0/10	0	3.5	0/10
Gefitinib	150	1.7 (9)	0.5	0/10	3.5 (24)	10.5[c]	0/10
Sorafenib	0	1.1 (9)	0	0/10	0	–3.5	0/10
Sorafenib	40	2.0 (9)	> 25[d]	6/10	1.7 (21)	> 40[d]	0/10
Sorafenib	80	4.4 (16)	> 25[d]	4/8	8.4 (24)	> 40[d]	0/10

[a] TGD: determined as the difference in the median time for the treated and control groups to attain two tumor mass doublings from the start of therapy. Mean tumor weight in the MDA-MB-231 study was 100 mg at the start of therapy on Day 6, and was 90 mg in the A549 study at the start of therapy on Day 14. The median time for the control tumors to reach two mass doublings was 9.2 days in the MDA-MB-231 study, and 17.6 days in the A549 study

[b] Regressions represents the sum of complete and partial regressions (CR + PR)

[c] Tumor growth delay significantly increased ($P < 0.05$ by One-Way ANOVA) relative to the Control Group

[d] Tumor growth delay significantly increased ($P < 0.001$ by One-Way ANOVA) relative to the Control Group

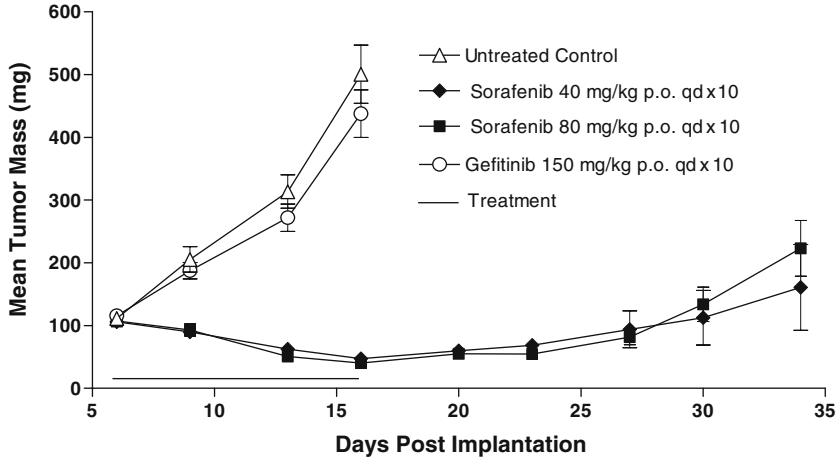

Fig. 3 Effects of sorafenib or gefitinib against MDA-MB-231 human mammary tumor xenografts. Female NCr-*nu/nu* mice (n = 10/group) were implanted sc with MDA-MB-231 tumor cells and treatment was initiated when all mice had tumors of 75–150 mg. Both gefitinib (150 mg/kg, *open circle*) and sorafenib (80, *filled square* or 40, *filled diamond* mg/kg) were administered po on a qd × 10 schedule. Changes in mean tumor weight were recorded over time and are expressed as mean ± SEM

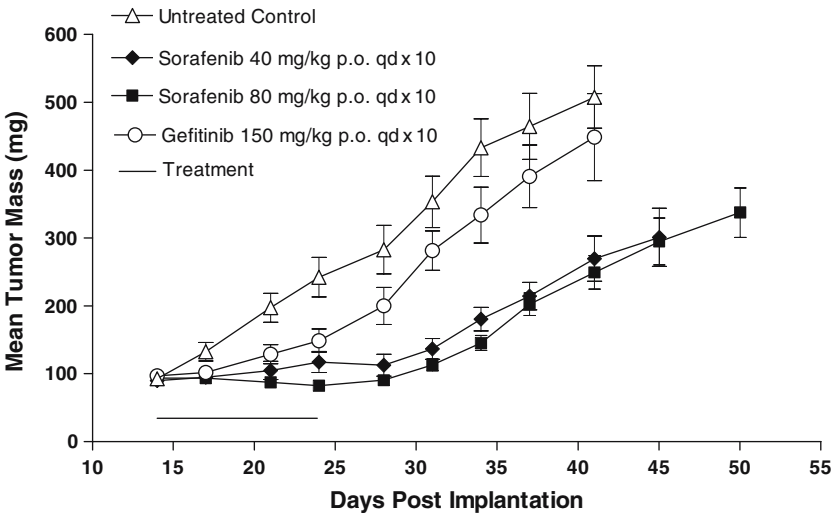

Fig. 4 Effects of sorafenib or gefitinib against A549 human NSCLC tumor xenografts. Female NCr-*nu/nu* mice ($n = 10$/group) were implanted sc with A549 tumor cells and treatment was initiated when all mice had tumors of 75–150 mg. Both gefitinib (150 mg/kg, *open circle*) and sorafenib (80, *filled square* or 40 *filled diamond* mg/kg) were administered po on a qd × 10 schedule. Changes in mean tumor weight were recorded over time and are expressed as mean ± SEM

Combination chemotherapy of sorafenib and gefitinib

The effect of therapy with sorafenib and gefitinib at their optimum dose levels either alone or in combination against established A549 xenografts is illustrated in Fig. 5, and the tolerability and efficacy of all treatments are summarized in Table 4, Experiments 1 and 2.

The evaluation endpoint for Experiment 1, one tumor mass doubling, was attained in 5.2 days. Consistent with the previous study in this model, gefitinib and sorafenib were well tolerated as single agents with minimal weight loss (1.9–5.8%) and no lethality. The antitumor efficacy of gefitinib was not dose-dependent (TGDs of 11.6 and 11.0 days at 75 and 150 mg/kg, respectively). These TGDs were not statistically significant. However, sorafenib produced significant TGDs of 19.9 and 25.0 days at dose levels of 40 and 80 mg/kg, respectively ($P < 0.001$ for each treatment).

The anti-tumor efficacy of the combination was similar to that of the most efficacious agent in the combination, sorafenib. Sorafenib at 80 mg/kg plus gefitinib at 150 mg/kg produced a TGD of 27.0 days with three tumor regressions (CRs plus PRs). This combination of the highest dose levels of each compound resulted in a 16.1% weight loss with no lethality. The combination of a reduced dose level of either agent (75 mg/kg gefitinib plus 80 mg/kg sorafenib, or 150 mg/kg gefitinib plus 40 mg/kg sorafenib) produced TGDs of 23.2 and 22.2 days, respectively, with two tumor regressions each, and with slightly less weight loss (approximately 11%). Combination therapy at the lower dose level of both agents (40 mg/kg sorafenib plus 75 mg/kg gefitinib) resulted in a weight loss of 6% and a TGD of 19.6 days and one tumor regression. The TGDs of each combination therapy group were not significantly different from the TGD produced by sorafenib alone at the same dose level.

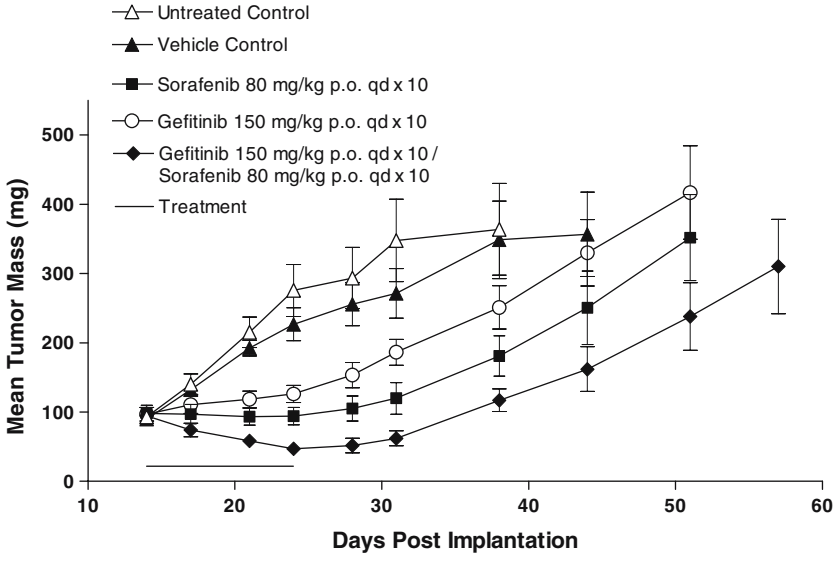

Fig. 5 Effects of the maximum tested doses of sorafenib and gefitinib, administered alone or in combination against A549 human NSCLC tumor xenografts. NCr-*nu/nu* mice ($n = 10$/group) were implanted sc with A549 tumor cells and treatments were initiated when all mice had tumors of 75–150 mg. Both gefitinib (150 mg/kg, *open circle*) and sorafenib (80 mg/kg, *filled square*) were administered po on a qd × 10 schedule either alone or in combination (*filled diamond*). Changes in mean tumor weight were recorded over time and are expressed as mean ± SEM

Table 4 Antitumor effect of sorafenib in combination with gefitinib against A549 human NSCLC xenografts

Sorafenib dose[a] (mg/kg)	Gefitinib dose[a] (mg/kg)	Experiment 1					Experiment 2				
		Lethality	Maximum net weight loss[b] (%)	CR	PR	TGD[c] (days)	Lethality	Maximum net weight loss[b] (%)	CR	PR	TGD[c] (days)
0	0	0/10	1.5	1/10	0/10	−0.6	0/10	1.1	0/10	0/10	−0.6
80	0	0/10	5.8	0/10	0/10	25.0[d]	0/10	0.5	2/10	2/10	27.2 [d]
40	0	0/10	5.2	1/10	0/10	19.9[d]	0/10	None	0/10	0/10	10.4 [d]
0	150	0/10	5.4	0/10	0/10	11.0	0/10	None	0/10	0/10	10.2[e]
0	75	0/10	1.9	0/10	0/10	11.6	0/10	None	1/10	0/10	8.7[f]
80	150	0/10	16.1	0/10	3/10	27.0	0/10	10.3	6/10	3/10	27.1
40	150	0/10	10.7	1/10	1/10	22.2	0/10	6.2	1/10	8/10	19.5
80	75	0/10	11.4	0/10	2/10	23.2	0/10	7.0	6/10	2/10	38.2
40	75	0/10	6.0	1/10	0/10	19.6	0/10	3.7	4/10	4/10	29.1

[a] Sorafenib and gefitinib were administered po on a qd × 10 schedule starting on Day 14 after tumor implantation when all animals had established tumors measuring 75–150 mg in each Experiment

[b] Maximum net weight loss expressed as a percent of the initial body weight on the day treatment was initiated minus the tumor weight

[c] TGD determined as the difference in the median time for the treated and control groups to attain one tumor mass doubling from the start of therapy. The control untreated tumors attained the evaluation endpoint in 5.2 days in experiment 1 and 7.6 days in Experiment 2

[d] Tumor growth delay significantly increased ($P < 0.001$ by One-Way ANOVA) relative to the Control Group

[e] Tumor growth delay significantly increased ($P < 0.01$ by One-Way ANOVA) relative to the Control Group

[f] Tumor growth delay significantly increased ($P < 0.05$ by One-Way ANOVA) relative to the Control Group

The effects observed using these dose levels of each agent were confirmed in an independent experiment (Table 4, Experiment 2). Control tumor growth was similar to that in the previous experiment with the evaluation endpoint of one tumor mass doubling being attained in 7.6 days. All treatments were again tolerated with minimal weight loss and no lethality. Sorafenib and gefitinib produced significant TGDs as single agents. The combination therapies produced TGDs that were not significantly different from those produced by sorafenib at the same dose level but were associated with a greater incidence of tumor regressions in this experiment.

Discussion

The clinical development of sorafenib includes investigation of the efficacy and safety of combination of sorafenib with multiple cytotoxic and cytostatic therapies for a variety of cancers. Two common cytotoxic chemotherapy agents used to treat NSCLC are vinorelbine and cisplatin, both of which are administered on intermittent dosing schedules. The most aggressive combination chemotherapy schedule that is anticipated in the clinic would employ daily administration of sorafenib throughout the entire treatment period encompassing the intermittent administration of vinorelbine and cisplatin. This treatment schedule was modeled in our preclinical studies as bolus dosing of either cytotoxic agent, q4d × 3, starting on the same day as once-daily treatment with sorafenib (concurrent therapy). Although this most aggressive combination therapy schedule was expected to have the greatest potential for toxic interactions, it was well tolerated in these studies using the NCI-H23 and NCI-H460 NSCLC models, with no lethality or significant increase in weight loss over that produced by the cytotoxic agents alone. Furthermore, the addition of sorafenib to cisplatin therapy either had no adverse impact on the anti-tumor efficacy of the cytotoxic agent (high dose of cisplatin) or the combination was approximately additive with the TGD of the combination equivalent to the sum of the TGDs of the individual therapies in the combination (low dose of cisplatin). The combination of sorafenib plus vinorelbine had no adverse impact on the anti-tumor efficacy of sorafenib (the more efficacious agent in that combination).

These results suggest that sorafenib may not have altered the exposure of cytotoxic agents evaluated. Each cytotoxic agent was administered at or near its maximum tolerated dose. Any increase in exposure produced by co-administration of sorafenib would have been expected to increase the toxicity of the combination. Likewise, given the steep dose-response curves for these agents, any sorafenib-mediated reduction in their exposure would have been expected to diminish the anti-tumor efficacy of the combination relative to that produced by the cytotoxic agent alone. It is also possible that the additional anti-tumor efficacy contributed to a given

combination by sorafenib could have masked effects of sorafenib on the pharmacokinetics of the cytotoxic agent. Although sorafenib has not been combined with cisplatin in man, it has been combined with a related agent, oxaliplatin, with no detectable pharmacokinetic interaction [21]. Sorafenib has not yet been combined with vinorelbine in man. Therefore, the pharmacokinetic interactions of these agents should be determined in any future clinical studies combining these agents.

Our observations are consistent with those previously reported [16, 26, 32] that therapy of human tumor xenografts in athymic mice with a CRAF-directed antisense oligonucleotide produced additive anti-tumor efficacy when administered in combination with cytotoxic chemotherapy. In those studies, the cytotoxic agents evaluated included cisplatin, mitomycin C, doxorubicin, mitoxantrone, epirubicin, paclitaxel, docetaxel, and gemcitabine.

Inhibition of RAF kinase activity might have been expected to increase the cytotoxic activity of vinorelbine through modulation of the apoptotic response of tumor cells through inhibition of the ERK-mediated phosphorylation of the BH3 proteins, Bad and Bim [13, 22] or through downregulation of Mcl-1[34, 46]. Although these potential interactions were not evaluated in our studies, our results demonstrate that the net anti-tumor effect of the combination chemotherapy was approximately additive without increasing the toxicity of vinorelbine due to concurrent treatment with sorafenib.

The current results combining sorafenib with cisplatin are in contrast to those predicted by Heim et al. [18] who reported that sorafenib inhibited the formation of DNA adducts and reduced the potency of platinum compounds in colorectal carcinoma cells in culture. There was an expectation that sorafenib would therefore reduce the efficacy of cisplatin in vivo. The observation that there was no reduction of cisplatin efficacy in vivo may be a consequence of the use of a NSCLC model in this study while Heim et al. [18] employed colorectal cell lines. In addition, because of the relatively low levels of DNA adducts that could be detected in vitro, both agents were applied at concentrations that were much higher than clinically observed plasma levels. For example, this effect of sorafenib was observed at an exposure of 24 μM, whereas the Cmax at the recommended clinical dose reaches only 10 μM [6]. The exposure attained in the current studies was not measured as the animals were retained for tumor growth delay measurements after the end of treatment. In separate studies, sorafenib attains a C_{max} of approximately 40 μM under the conditions used here (data not shown). Since sorafenib is efficacious at a C_{max} of 10 μM in man, the levels required for efficacy in the mouse can not be viewed as predictive of the exposures required for efficacy in man.

Sorafenib inhibits VEGFR-2, VEGFR-3, PDGFR-ß, FLT-3, RET and c-KIT in addition to RAF kinases. It may have been expected that sorafenib would abrogate the anti-tumor efficacy of directly cytotoxic agents either by reducing the fraction of proliferating tumor cells available for these agents to target or by restricting delivery of cytotoxic agents to the tumor through inhibition of angiogenesis or vascular permeability. However, a number of anti-angiogenic therapies have been reported to be combinable with standard chemotherapy in preclinical models. TNP-470 has been shown to be combinable with 5-flurouracil [28]. Thrombospondin-1 inhibition could be combined with irinotecan [3]. The MMP inhibitor, AG3340, could be combined with carboplatin or paclitaxel [38]. In addition to general anti-angiogenic therapies, treatments targeting some of the same molecular mechanisms involved in the action of sorafenib have also been shown to be combinable with standard chemotherapy. Anti-VEGF antibody therapy has been combined with gemcitabine therapy in a preclinical model of pancreatic cancer [9], with carboplatin in a preclinical model of ovarian cancer [43], and with paclitaxel in preclinical models of prostate cancer [15, 42]. Anti-VEGF antibody therapy has also been shown to enhance the anti-tumor efficacy of metronomic chemotherapy with a variety of agents [20]. Clinically, anti-VEGF antibody therapy in combination with carboplatin/paclitaxel therapy has demonstrated an increase in survival time [41].

The current observations regarding the combinability of sorafenib with cytotoxic chemotherapy are also consistent with those previously reported for another inhibitor of VEGFR, PDGFR and c-KIT. SU11248 has been reported to be combinable with docetaxel, 5-FU, and doxorubicin in the MX-1 mammary tumor model [1]. However, the findings with SU11248 could not be viewed as predictive of those with sorafenib due to the different spectrum of kinases inhibited by the two molecules.

The data reported here demonstrate that the MDA-MB-231 mammary tumor model was highly sensitive to sorafenib but not to gefitinib. Gefitinib is an inhibitor of the tyrosine kinase activity of EGFR. Inhibition of EGFR signaling by gefitinib has been associated with AKT-dependent reduction of VEGF production in squamous cell carcinoma cell lines [33]. Gefitinib also reduces the translation of HIF1-α in these experiments. However, the activity of gefitinib is limited to a subset

of tumors expressing certain EGFR mutations [24, 29, 31]. Sorafenib also has the potential to inhibit EGFR signaling further down the signaling cascade, at the level of RAF kinase. The MDA-MB-231 cell line overexpresses EGFR [12] but also expresses activating mutations of both K-RAS and BRAF. The observation that sorafenib is effective against the MDA-MB-231 model, whereas gefitinib is not, can be understood in the context of these multiple disruptions of the Ras/Raf pathway, which supersede EGFR signaling. This differential efficacy of sorafenib and gefitinib demonstrates that tumors that proliferate in response to signal transduction perturbations beyond the level of the growth factor receptor can still be growth inhibited by a signal transduction inhibitor.

Sorafenib also demonstrated anti-tumor efficacy against the A549 NSCLC tumor line to a degree similar to that of gefitinib. This is consistent with previous reports of the broad spectrum of anti-tumor efficacy of Sorafenib [25, 44, 45]. The anti-tumor efficacy produced by gefitinib in these experiments was consistent with the efficacy reported previously: a dose level of 50–75 mg/kg inhibited A549 tumor growth by approximately 50% during the treatment period [40]. Combination of sorafenib and gefitinib led to anti-tumor efficacy similar to that achieved with sorafenib alone. Concurrent administration of sorafenib did not abrogate the efficacy of gefitinib in this model. Overall, these results indicate that these two therapies are combinable. This finding is consistent with the previous reports that gefitinib is combinable with several cytotoxic chemotherapies [11, 27, 40]. It has also been reported that the combination of gefitinib with VEGFR-2 inhibition was more efficacious that of either single agent in the DU-145 human prostate tumor model [39]. However, a more potent inhibitor of VEGFR-2 has also been shown to be more efficacious than gefitinib in the A549 model used in these studies [4]. This is the first demonstration of the combinability of two signal transduction inhibitors acting in the same signal transduction pathway.

In conclusion, the present results suggest that concurrent administration of sorafenib had no adverse impact on the anti-tumor efficacy of cisplatin, vinorelbine or gefitinib, without compromising tolerability in human tumor xenograft models. Sorafenib also demonstrated anti-tumor efficacy against a breast tumor model carrying both K-RAS and BRAF mutations which was insensitive to gefitinib. These promising findings with sorafenib in combination treatment in human tumor xenograft models support the inclusion of sorafenib in clinical studies of combination chemotherapy with these agents.

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