Published in final edited form as:

Clin Cancer Res. 2013 September 1; 19(17): . doi:10.1158/1078-0432.CCR-13-0708.

Phase II Study of Bevacizumab in Combination with Sorafenib in Recurrent Glioblastoma (N0776): A North Central Cancer Treatment Group Trial¹

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Abstract

Purpose—We hypothesized that vertical blockade of VEGF signaling by combining bevacizumab with sorafenib in recurrent glioblastoma (rGBM) patients would result in a synergistic therapeutic effect. We also investigated whether VEGF, VEGFR2, and HIF-1 α single nucleotide polymorphisms (SNPs), circulating biomarkers of angiogenesis and Magnetic Resonance (MR) imaging markers, such as apparent diffusion coefficient (ADC), correlated with treatment efficacy and/or toxicity.

Patients/Methods—Patients received bevacizumab (5 mg/kg every 2 weeks) with sorafenib (200 mg bid, weekly, days 1-5) (Group A), but due to toxicity the starting sorafenib dose was subsequently modified to 200 mg qd (Group B).

Results—54 patients were enrolled: 19 patients in Group A and 35 in Group B. Objective response rate was 18.5% with median duration of 6.7 mo (range 0.5-24.1 mo). Six-month progression free survival (PFS6) was 20.4% (11/54), and median OS was 5.6 months (95% CI 4.7 – 8.2); outcome was similar between the two dose groups. We identified single nucleotide

¹This study was conducted as a collaborative trial of the North Central Cancer Treatment Group and Mayo Clinic and was supported in part by Public Health Service grants CA-25224, CA-37404, CA-35090, CA-63848, CA-35269, CA-52352, CA-60276, CA-37417, CA-35267, CA-35103, CA-35431, and CA-63849 from the National Cancer Institute, Department of Health and Human Services. The content is solely the responsibility of the authors and does not necessarily represent the views of the National Cancer Institute or the National Institute of Health.

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Conflicts of Interest: Dr. Jan Buckner has a consultant relationship to disclose with Genentech which is uncompensated. Dr. Patrick Flynn has been paid directly honoraria from Genentech and Bayer.

polymorphisms in the VEGF and VEGFR2 promoter regions which were associated with PFS6 ($p<0.022$). Among molecular markers of angiogenesis, a higher $\log_2$ baseline level of stromal cell derived factor-1 was associated with PFS6 success ($p=0.04$). The circulating endothelial cell $\log_2$ -fold decreased during treatment with subsequent increase at disease progression ($p=0.022$). Imaging analysis demonstrated a trend associating ADC-L with poor outcome.

Conclusions—The bevacizumab/sorafenib combination did not improve outcome of recurrent GBM patients versus historic bevacizumab treated controls. Biologic markers of response and resistance to bevacizumab in gliomas were identified which merit prospective validation.

Keywords

bevacizumab; sorafenib; recurrent glioblastoma

Introduction

Glioblastoma (GBM) is the most common glioma histology and has a dismal prognosis with a median survival of 16 to 18 months, despite multimodality treatment (1). When GBM recurs, available treatment options have a limited impact on outcome.

Glioblastoma is characterized by a microenvironment of intense angiogenesis, contributing to tumor growth and progression (2-4). Inhibition of angiogenesis ligand (e.g., vascular endothelial growth factor; VEGF) or receptor (e.g., VEGFR) signaling in recurrent glioma patients has resulted in clinical benefit (5-7). The anti-VEGF antibody bevacizumab (Avastin) received FDA accelerated approval in 2009 for treatment of recurrent GBM on the basis of sustained imaging responses. Despite PFS prolongation, bevacizumab's impact on overall survival remains undefined. Novel approaches to build on bevacizumab efficacy, possibly exploiting multiple targets in the angiogenesis cascade or preventing the development of resistance to bevacizumab warrant further investigation.

Sorafenib is a small molecule inhibiting the kinase activity of Raf, VEGFR2 (the main VEGF receptor in GBM), VEGFR3, c-kit, and PDGFR- β . Both VEGFR2 and PDGFR- β play a key role in driving angiogenesis, and aberrant activation of Ras signaling is a common finding in glioblastoma (8). Sorafenib has shown preclinical and modest early clinical activity against GBM (9); the single agent maximum tolerated dose for patients not on enzyme inducing anticonvulsants was 600 mg bid.

The rationale for testing the bevacizumab/sorafenib combination in recurrent glioblastoma was its potential to block angiogenesis at the ligand and receptor level simultaneously, thus creating therapeutic synergy. Two previously completed phase I trials had established the phase II dose of this regimen (10, 11). Objective responses were observed in heavily pretreated solid tumors including ovarian and renal cell carcinoma patients.

The goal of this phase II trial was to assess the clinical activity of the bevacizumab/sorafenib combination in GBM, as measured by six-month progression free survival (PFS6), and to evaluate its safety and adverse effects in this patient population. We also sought to examine the relationship between genetic polymorphisms and circulating biomarkers of vascular response and clinical outcome, to assess the potential utility of MR imaging markers in predicting outcome, and to assess the impact of treatment on patients' quality of life.

Methods

Patient Eligibility

Eligible patients were ≥ 18 years of age and had histologic confirmation of grade 4 astrocytoma at initial diagnosis or recurrence. They were required to be on a stable dose of corticosteroids or no corticosteroids for ≥ 1 week before baseline imaging, to have had their last chemotherapy treatment ≥ 4 weeks before study entry (≥ 6 weeks for nitrosoureas) and to be ≥ 12 weeks from completion of RT. Patients were also required to have an ECOG performance score of 0 to 2; and adequate hematologic, hepatic and renal function. All patients were required to sign the respective IRB approved consent form prior to enrollment.

Exclusion criteria included inadequately controlled hypertension, prior anti-angiogenic therapy, more than one chemotherapy regimen for progressive disease, evidence of bleeding diathesis, coagulopathy or therapeutic anticoagulation with warfarin, history of myocardial infarction or unstable angina ≥ 6 months prior to registration, surgery ≥ 28 days before registration, history of stroke or transient ischemic attack ≥ 6 months prior to registration or evidence of CNS hemorrhage on baseline CT or MRI. Patients requiring enzyme inducing anti-epileptics drugs for seizure control were not eligible, given their impact on sorafenib metabolism.

Study treatment

Patients initially received sorafenib 200 mg bid for 5/7 days per week and bevacizumab 5 mg/kg IV every two weeks (cycle length = 14 days). Sorafenib dose escalation to 200 mg bid (7/7 days) was allowed if no grade 3 or 4 toxicity was observed in cycle 1. After the trial met a prespecified interim toxicity stopping rule following accrual of the first 19 patients, the protocol was amended to decrease the starting dose of sorafenib to 200 mg daily while keeping the bevacizumab dose unaltered.

Definition of response

Neuroimaging with magnetic resonance imaging was performed at baseline, before the third treatment cycle, and every fourth cycle thereafter. RANO criteria were used to determine response and progression (12). Scans were centrally reviewed at completion of the study by a neuroradiologist (TK). In order to calculate the apparent diffusion coefficient (ADC), regions of interest were manually traced around all contrast-enhancing tumors, excluding areas of necrosis and hemorrhage, on baseline scans and on follow-up scans through two months' time using Analyze software (Biomedical Imaging Resource, Mayo Clinic, Rochester, MN). ADC statistics were calculated for these regions of interest, employing an ADC histogram approach, in 31 patients (13).

Evaluation of Quality of Life

Study patients completed the FACT-Br (Functional Assessment for Cancer Therapy – Brain) at baseline and every other cycle.

Correlative analysis

a. Analysis of circulating endothelial cells (CECs)—Analysis of CECs was performed at baseline, Cycle 1 Day 3 ($\pm$ 1 day), prior to treatment Cycle 2, prior to treatment Cycles 3, 5, 7, 9, 11 and 13 and at disease progression, withdrawal or removal. Whole blood samples were collected in Vacutainer EDTA tubes, refrigerated and shipped the same day as collected by Priority Overnight Service with a cold pack to the reference library. CECs were counted as previously described (14, 15); analysis was performed ≥ 48 hours from sample collection.

b. Analysis of circulating biomarkers of angiogenesis—Measurement of angiogenic proteins in plasma was performed at baseline, Cycle 1 Day 3 (± 1 day), prior to treatment cycle 2, prior to treatment Cycle 3, and prior to treatment cycles 5, 7, 9, 11 and 13. Blood was collected in EDTA-containing vacutainers, processed into plasma aliquots and frozen at -80°C . Frozen plasma samples were shipped overnight in dry ice to the NCCTG Biospecimen Bank. To reduce inter-assay variability, plasma samples were delivered to the reference laboratory for analyses after the last patient specimen was received. Plasma levels of bFGF, SDF-1 α , HGF, soluble c-kit, Ang-2 and PlGF were determined using ELISA (R&D Systems) as per the manufacturer's instructions.

c. VEGF, VEGFR2 and HIF-1 α SNP analysis—The following 14 SNPs were analyzed: VEGF rs699947, rs1005230, rs833061, rs1570360, rs2010963, rs25648, rs3025039, and rs10434; VEGFR2 rs2071559, rs2305948, rs1870377 and rs2219471; and HIF-1 α rs11549465 and rs11549467. DNA was extracted at baseline from whole blood collected in 10 mL EDTA tubes. Genotyping was performed in the Genotyping Shared Resource, Mayo Clinic, Rochester, MN, using TaqMan® Drug Metabolism Genotyping Assays (Applied Biosystems, Foster City, CA) or direct sequencing.

Statistical design

This was a one stage phase II, three outcome design (16). Fifty three patients provided 90% power to detect a six-month progression free survival (PFS6) difference of 30% vs. 45%, with an alpha error of 0.10. Survival and time to progression curves were compared via the logrank test; Cox proportional hazard models were used to assess the relationship between time-to-event endpoints and outcome.

Information on prescribed corticosteroid amounts for each cycle was collected and expressed as dexamethasone equivalent doses. The amount of change from baseline was calculated as fold change (dose received during the cycle divided by the baseline dose). Changes from baseline to cycles 1-12 were analyzed via t-test; linear mixed effects models were used to analyze the linear cycle to cycle slope.

For the quality of life data analysis, patients were considered distressed if the score on a FACT-Br subscale or total was ≤ 50 . Absolute change from baseline values was calculated and changes over time were evaluated using t-tests and repeated measures linear mixed effect regression models.

To analyze the SNP data, Fisher's Exact tests were used to determine genotype association with the primary endpoint (PFS6) or the most common grade 3+ adverse events. Adjustments for multiple testing were made using the false discovery rate as per Benjamini (17). Kaplan-Meier and Cox proportional hazard models were used to compare outcome between genotype subgroups.

Unequal variance two sample and paired t-tests were performed to compare values between patient outcome groups and serial CEC measurements within a patient. Linear regression models were used to assess associations between circulating biomarkers of angiogenesis and patient outcomes.

For analysis of MR imaging data, two-mixture generalized lambda distributions were used to model the apparent diffusion coefficient (ADC) histogram data, as described by Pope et al (13, 18).

Results

Patient characteristics

Patient characteristics are summarized in table 1; 54 patients were accrued on the study, 19 in the starting dose level, (Group A, sorafenib 200 mg bid for 5/7 days, bevacizumab 5 mg/kg every 2 weeks) and 35 patients in the amended dose schedule (Group B, sorafenib 200 mg qd, bevacizumab 5 mg/kg every two weeks).

Efficacy analysis

The study did not meet its primary endpoint. Progression free survival (PFS) at 6 months for all study patients was 20.4% (11/54 patients) with a median progression free survival of 2.9 mo (95% CI: 2.3-3.6) and median overall survival 5.6 mo (95% CI: 4.7-8.2). Efficacy outcomes according to sorafenib dose are summarized in table 2. There was no significant difference in PFS or overall survival between the two sorafenib dose cohorts. Objective response rate for all study patients was 18.5% (10/54), with median duration of 6.8 mo. Overall response rate appeared to be higher at the highest sorafenib dose cohort, but the difference did not reach statistical significance. Analysis of ADC histograms (13, 18, 19), showed a trend suggesting an association between low ADC-L (ADC of the lower curve: mean ADC of the lower distribution) and poor outcome ($p=0.088$); there was no association between baseline ADC or ADC changes from baseline to week 4 and outcome, however.

Toxicity

Toxicity was graded according to the National Cancer Institute Common Terminology Criteria version 3.0. Following accrual of the first 19 patients, the trial met the prespecified interim toxicity stopping rule with 3/19 patients experiencing grade 4 non-hematologic toxicity and a 42% treatment discontinuation rate. Decrease of the starting sorafenib dose resulted in improved treatment tolerance. Overall, most common grade 3/4 toxicities were fatigue (13/54, 24%), hypertension (8/54, 15%), and hypophosphatemia (7/54, 13%) (Fig 1). Supplementary table 1 summarizes the most common grade 3/4 toxicities per dose group. Sorafenib dose significantly impacted treatment tolerance: 26.3% (5/19 patients) went off study due to toxicity and 15.7% (3/19) due to treatment refusal in group A versus 14.3% (5/35 patients) and 5.7% (2/35) respectively in group B.

Quality of Life and Steroid Use Assessment

All 54 patients had FACT-Br questionnaires completed at baseline. Forty-six patients had at least 1 questionnaire, 31 patients had at least 2 questionnaires and 20 patients had at least 3 questionnaires completed during treatment. Patients who were distressed at baseline did not have an increased hazard rate for PFS or OS. Two of the subscales (Physical Well Being and Social/Family Well Being) decreased from baseline to completion of cycles 2 ($p = 0.054$ and 0.016 , respectively) and 4 ($p=0.016$ and $p=0.055$, respectively), as well as in the linear mixed effect model ($p = 0.0001$ and $p=0.026$, respectively). Overall, these data indicate that treatment with bevacizumab/sorafenib did not improve the quality of life in recurrent GBM patients, possibly due to regimen associated toxicity.

There was a 40% reduction in steroid use during cycle 1 and 30% dose reduction in cycle 2; these changes approached but did not reach statistical significance ($p=0.057$ and $p=0.0052$ respectively). The linear mixed effects model results similarly indicate strong evidence of a per cycle decrease from the baseline dose levels of corticosteroids, with an average 15.4% dose decrease per cycle slope ($p\text{-value}=1.15 \times 10^{11}$).

Correlative analysis

a. Circulating endothelial cell (CEC)—Circulating endothelial cell analysis was performed in 49/54 patients. Median baseline CEC were 83.3 (mean 136; range 6.5 to 594 cells/mL). There was no correlation between baseline CEC values and PFS6 ($p=0.19$). The CEC $\log_2$ -fold change from baseline decreased during treatment reaching significance at cycles 2, 7, 9 and 11 ($p=0.03$, 0.047, 0.004, and 0.005 respectively, Figure 2A); there was subsequent significant increase in patients who were removed from the study due to progression as compared to other reasons ($p=0.022$, t-test, Figure 2b).

b. Circulating biomarkers of angiogenesis—The circulating biomarkers Ang-2, bFGF, HGF, PlGF, SDF-1 α and soluble c-kit were measured at baseline and during treatment (see methods). A larger $\log_2$ baseline level of SDF-1 α and a $\log_2$ -fold, change in Ang-2 or soluble c-kit at cycle 1 day 3 were associated with PFS6 success ($p=0.04$, $p=0.036$, and 0.027, respectively).

c. Analysis of VEGF, VEGFR2, and HIF-1 α single nucleotide polymorphisms (SNPs)—SNP analysis was performed in all 54 patients. This analysis and genotype frequencies are summarized in Supplementary table 2; SNP relationships with toxicity and outcome are summarized in table 3. In summary, PFS6 success was altered in recurrent GBM patients with mutant alleles in the VEGF promoter: PFS6 success increased for mutant rs699947 and rs833061 and PFS6 success decreased for mutant rs1005230 and rs1570360, and VEGFR2 promoter: PFS6 success increased for heterozygous rs2071559. Furthermore, increased incidence of grade 3 fatigue and hypertension was observed in patients with heterozygous alleles in the VEGF promoter (rs1005230, rs699947, and rs833061), and decreased incidence of grade 3 fatigue in patients with heterozygous alleles in the VEGF 3'UTR (rs10434). There was no association between the SNPs analyzed and sorafenib induced hand-foot syndrome. Our data represents the first evidence that VEGF and VEGFR2 genetic polymorphisms could predict outcome in GBM patients treated with bevacizumab.

Discussion

We showed that the sorafenib/bevacizumab combination has clinical activity in recurrent GBM, but at the previously recommended phase II dose of sorafenib (200 mg bid, weekly, days 1-5) and bevacizumab (5 mg/kg every two weeks) is associated with toxicity, necessitating a decrease in sorafenib starting dose. Response rate, PFS6, and overall survival outcome in patients treated with this combination appear inferior as compared to previously reported outcomes with single agent bevacizumab in prospective or retrospective series (6, 7, 20). This could be reflective of the patient population in our study, treated in community-based NCCTG centers (21), as compared to tertiary academic centers in which previously published outcome data were based. For example, our patients had worse performance scores and a higher percentage was on corticosteroids at study entry as compared to other trials (6, 7). Further supporting this possibility, the PFS6 of single agent bevacizumab treated patients in the recently reported European BELOB (22) and Australian CABARET trials (23) ranged from 16% to 24%, consistent with the results of our study. It is unlikely that the inferior outcome in our study can be attributed to the lower bevacizumab dose (5 mg/kg q 2 wk), as compared to the standard dose of 10 mg/kg q 2 wk, approved for GBM treatment. A bevacizumab dose of 5 mg/kg was employed in the first proof of principle demonstration of bevacizumab activity in recurrent glioma patients (24) and has been subsequently used in other patient series (25, 26) with comparable outcomes, consistent with data in other malignancies such as colorectal cancer (27). Nevertheless, the negative impact

of the sorafenib/bevacizumab combination therapy on tumor biology cannot completely be excluded.

In preclinical studies, sorafenib has resulted in antglioma activity *in vitro* and *in vivo* in orthotopic GBM models (9). Notwithstanding, this has only translated into modest single agent activity in recurrent glioma patients (28). The rationale for the sorafenib/bevacizumab combination strategy in this study was the vertical inhibition of the VEGF/VEGFR axis, a key driver in glioma angiogenesis by combining bevacizumab mediated VEGF ligand blockade with sorafenib mediated receptor blockade. Our clinical results do not support the efficacy of this strategy as compared to single agent bevacizumab, likely due to the limited sorafenib penetration to the CNS, its modest single agent efficacy even in higher doses (2) and the toxicity of the combination. In general, and despite the potential therapeutic promise, combining anti-VEGFR tyrosine kinases with bevacizumab has been challenging and associated with significant toxicity frequently necessitating significant dose reductions as compared to single agent doses (11, 29, 30). Of note, sorafenib/bevacizumab combination doses previously reported to be well tolerated in other solid tumors such as ovarian cancer and renal cell carcinoma (11, 29, 30) still resulted in unacceptable toxicity in glioma patients treated in our study. Combination of other more potent and specific VEGFR tyrosine kinase inhibitors such as sunitinib with bevacizumab has also been shown to be unsafe with long-term follow up revealing the development of microangiopathic hemolytic anemia, renal insufficiency and neurologic toxicity (31). These data indicate that vertical blockade of the VEGF/VEGFR axis in GBM can be toxic when currently available agents are employed and alternative strategies should be considered in order to build on bevacizumab efficacy.

Because antiangiogenic drugs interact with non-malignant endothelial cells and the tumor microenvironment, the genetic background of the patient may play a major role determining the efficacy of these drugs spanning across different tumor types. Along these lines, the analysis of VEGF, VEGFR2 and HIF-1 α SNPs as predictors of outcome in our trial was intriguing. Specifically, increased PFS6 successes were observed in GBM patients with mutant alleles in the VEGF promoter (rs699947 and rs833061) and VEGFR2 heterozygous promoter (rs2071559). It is of note that the VEGFA polymorphism rs699947 has been associated with overall survival in metastatic breast cancer patients receiving paclitaxel/bevacizumab and the VEGFR2 polymorphism rs833061 was associated with progression-free survival and overall survival in colorectal cancer patients treated with first-line FOLFIRI with bevacizumab (32-34). The pharmacogenomics data generated in our trial represents the first attempt to associate genotypic difference with outcome in glioma patients treated with bevacizumab; we are in the process of further validating them in the ongoing Alliance trials N0872 and N1174. If confirmed, they could facilitate selection of patients who may benefit from treatment with bevacizumab in the recurrent disease setting. These polymorphisms could also have an impact on benefit realized from bevacizumab use in the upfront setting.

There was no correlation between baseline circulating endothelial cell counts or other circulating markers of angiogenesis such as Ang-2, bFGF, PlGF, SDF-1 α and PFS6 in recurrent GBM patients treated with bevacizumab/sorafenib; this could have been impacted by the observed toxicity which resulted in early treatment discontinuation in 14.3%/26.3% of the patients (groups A and B respectively) and influenced by the lower bevacizumab dose employed in our study. Nevertheless, monitoring the differences in CEC log₂-fold change from baseline during this treatment combination may predict progression and potentially provides insights to mechanisms of bevacizumab resistance. Circulating endothelial cells express high levels of the endoglin receptor CD105 and the re-emergence of these cells in association with progression, as in our trial, supports the hypothesis that these cells might mediate the development of secondary resistance. We are currently exploiting strategies

combining bevacizumab with the anti-CD105 antibody TRC105 in order to block this potential escape mechanism: a randomized phase II trial of this combination is ongoing (Alliance N1174).

In addition to genotyping and measurement of cellular and molecular markers of angiogenesis, we did investigate imaging markers as possible predictors of response to treatment. In contrast to previously reported data on the predictive value of the apparent diffusion coefficient histogram analysis (18, 19), baseline ADC and change from baseline at week 4 were not associated with outcomes. In agreement with these same previous studies, however, our study did demonstrate a trend associating a low ADC-L with poor outcome ($p=0.088$). The fact that this association did not reach statistical significance could be due to the smaller sample size in our study.

In summary, the combination of bevacizumab and sorafenib does not appear to have increased activity versus single agent bevacizumab in recurrent GBM patients and this combination is associated with increased toxicity. However, a number of interesting biologic observations including pharmacogenomic analysis and analysis of circulating endothelial cells in this study merits further prospective evaluation and can be used to build future combinatorial strategies in recurrent GBM patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Statement of Translational Relevance

This phase II trial of bevacizumab in combination with sorafenib tests the concept of vertical blockade of VEGF signaling in recurrent glioblastoma by combining ligand and receptor inhibition. Bevacizumab is an approved agent for treatment of glioblastoma; its clinical benefit is short-lived, however, in the majority of patients. Identifying predictive markers of response and understanding mechanisms of resistance can contribute to the development of more effective bevacizumab-based regimens. In this trial we performed extensive correlative analysis to investigate single nucleotide polymorphisms that could predict clinical benefit, as well as circulating biomarkers of angiogenesis, including circulating endothelial cells, and MR imaging markers. To our knowledge, this is the first trial of bevacizumab in recurrent GBM to prospectively report on angiogenesis biomarkers during treatment course and assess genotype associations with outcome. Our findings and mechanistic insights are currently being validated in ongoing randomized phase II trials in recurrent GBM patients.

Figure 1.
Most commonly observed treatment-related toxicities.

CEC Changes

Figure 2.

(A) Circulating endothelial cells expressed as log₂-fold change from baseline decreased during treatment reaching significance at cycles 2, 7, 9 and 11, and increased prior to progression (B).

Table 1
Patient characteristics

All Patients			Group A	Group B
			N = 19	N = 35
Age	Median (range)	55 (25-76)	54 (25 – 68)	55 (28 – 76)
PS	0	15 (28%)	3 (16%)	12 (63%)
	1	31 (57%)	12 (63%)	19 (54%)
	2	8 (15%)	4 (21%)	4 (12%)
Steroids	Yes	36 (67%)	15 (79%)	21 (60%)
No. of chemo regimens for recurrent disease				
1		48 (89%)	14 (74%)	34 (97%)
0		6 (11%)	5 (26%)	1 (3%)

Table 2
Efficacy Outcomes

	Group A (N=19)	Group B (N=35)
Median Follow-up (range)	5.6 mo (0.76 – 27.7)	5.6 (1.4 – 22.7)
Response	6 (32%)	4 (11%)
Stable Disease	12 (63%) median duration 1.4 mo	22 (63%) median duration 2.0 mo
PFS6	5 (26%)	6 (17%)
PFS Median (95% CI)	3.61 mo (1.84, 5.95)	2.66 mo (2.07, 3.25)
OS Median (95% CI)	5.58 mo (4.11, 8.48)	5.62 mo (3.22, 8.67)

Table 3
SNP Relationships with Outcome and Toxicity

SNP (n=54)	6 month PFS (p value ^I)	Fatigue (p value ^I)	Hypertension (p value ^I)	Skin reaction (p value ^I)
rs699947	0.011 *	0.022 *	0.006 *	0.085
rs1005230	0.011 *	0.022 *	0.006 *	0.085
rs833061	0.013 *	0.014 *	0.010 *	0.071
rs1570360	0.004 *	0.072	0.153	0.227
rs2010963	0.055	0.114	0.233	0.084
rs25648	0.145	0.065	0.154	0.103
rs3025039	0.324	0.286	0.322	0.215
rs10434	0.100	0.025 *	0.123	0.166
rs2305948	0.324	0.286	0.341	0.429
rs2071559	0.025 *	0.052	0.085	0.168
rs1870377	0.098	0.093	0.152	0.055
rs2219471	0.107	0.082	0.188	0.268
rs11549465	0.157	0.438	0.516	0.670

^I Fisher's Exact Test p-value

* statistical significance

False Discovery Rate for the 12 p-values < 0.05 is 11% (method of Benjamini and Hochberg)