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A Phase II, Open-Label Study of Ramucirumab in Combination with Paclitaxel and Carboplatin as First-Line Therapy in Patients with Stage IIIB/IV Non–Small-Cell Lung Cancer

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Introduction: The objective of this study was to determine whether the addition of ramucirumab to first-line paclitaxel–carboplatin chemotherapy in patients with advanced non–small-cell lung cancer (NSCLC) resulted in a 6-month progression-free survival (PFS) rate that compares favorably with the historic rate for bevacizumab combined with paclitaxel–carboplatin in this patient population.

Methods: In this phase II, single-arm, open-label, multicenter study, 40 patients with advanced NSCLC received ramucirumab (10 mg/kg intravenous [IV]) followed by paclitaxel (200 mg/m^2 IV) and carboplatin area under the curve = 6 on day 1 every 21 days as first-line therapy. Therapy continued for up to six cycles. Patients not experiencing withdrawal criteria may have continued ramucirumab monotherapy every 3 weeks. The primary endpoint was PFS at 6 months, with 80% power to detect a 6-month PFS rate of at least 55%.

Results: The 6-month PFS rate was 59.0% and the objective response rate was 55.0%. The most common treatment-related adverse events were fatigue, peripheral neuropathy, nausea, epistaxis, and myalgia. Single-nucleotide polymorphism (SNP) rs2981582 on the *FGFR-2* gene had significant associations with improved overall survival, PFS, and best overall response (p values without multiplicity adjustment were 0.0059, 0.0429, and 0.0392, respectively).

Conclusion: Ramucirumab in combination with paclitaxel–carboplatin resulted in a 6-month PFS rate and safety profile that compared favorably with the historical control. In addition, no deaths were associated with this treatment. Furthermore, we describe an association of SNP on *FGFR-2* gene with survival and response. These findings warrant further clinical investigation in patients with NSCLC.

Key Words: Angiogenesis, Lung cancer, Ramucirumab, Paclitaxel, Carboplatin.

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Angiogenesis, the formation of new capillaries and blood vessels, is a tightly controlled, multistep process that is a component of normal human physiology, including development of the embryonic vasculature, wound healing, and tissue repair. Pathologic angiogenesis contributes to tumor growth and metastasis, and other human diseases such as diabetic retinopathy, rheumatoid arthritis, and psoriasis.[1–3] The importance of vascular endothelial growth factor (VEGF) and vascular endothelial growth factor receptor-2 (VEGFR-2) in angiogenesis and tumor growth has been demonstrated in a variety of animal models, in which disabling the function of the VEGF/VEGFR-2 pathway inhibited new blood vessel formation and tumor growth.[4–7]

VEGF and VEGFR-2 are overexpressed in the majority of human cancers, including carcinomas of the gastrointestinal

tract, pancreas, breast, cervix, bladder, ovary, uterus, endometrium, kidney, and lung.[6] VEGFR-2 expression and tumor microvessel density have been associated with poor prognosis, advanced disease, increased risk of metastasis and recurrence, and lower progression-free survival (PFS) in multiple types of cancers, including non–small-cell lung cancer (NSCLC).[2,6,7]

Therapeutic agents that interfere with the function of VEGF and its receptors may be efficacious antitumor therapy. Antitumor effects have been demonstrated by disabling the function of the VEGFR-2 signaling pathway using anti-VEGF antibodies and small molecule VEGFR-2 tyrosine kinase inhibitors (TKIs) in a variety of animal models.

The addition of antiangiogenic therapy (anti-VEGF: bevacizumab) to cytotoxic chemotherapy in treatment-naïve patients with advanced NSCLC in Eastern Cooperative Oncology Group (ECOG) Study Trial-E4599 was shown to improve survival.[8]

Ramucirumab is a human immunoglobulin G, subclass 1 monoclonal antibody that specifically binds to the extracellular domain of VEGFR-2 with high affinity.[9] This antibody blocks the binding of the VEGF ligand to VEGFR-2, inhibits VEGF-stimulated activation of both VEGFR-2 and p44/p42 MAP kinases, and neutralizes VEGF-induced endothelial cells' proliferation and migration.[9]

Two completed phase I studies of ramucirumab have evaluated pharmacokinetics and demonstrated safety and tolerability at clinically relevant doses, with preliminary evidence of clinical efficacy.[10,11] In these studies, 62 patients with advanced cancer received ramucirumab either weekly, every other week, or every third week at doses ranging from 2 to 20 mg/kg. Objective antitumor activity and antiangiogenic effects were observed over a wide range of dose levels, suggesting that ramucirumab may have a favorable therapeutic index in treating malignancies amenable to VEGFR-2 inhibition. Furthermore, ramucirumab has been approved by the US Food and Drug Administration in gastric cancer following progression on initial chemotherapy based on the positive results of a phase III study.[12]

This phase II trial was conducted to assess whether the addition of ramucirumab to combination therapy with paclitaxel and carboplatin results in favorable PFS in patients with advanced NSCLC compared with historical controls.

PATIENTS AND METHODS

Eligibility Criteria

Patients greater than or equal to 18 years with ECOG performance status (PS) of 0 to 1 and histologically or cytologically confirmed, measurable, stage IIIB or IV NSCLC were eligible. American Joint Committee on Cancer (AJCC) sixth edition was used for staging derivation. Eligibility criteria included adequate hepatic, renal, hematologic, and coagulation function. Patients with untreated central nervous system metastases were excluded. Other exclusion criteria included prior systemic chemotherapy for stage IIIB/IV NSCLC, prior systemic chemotherapy or radiation therapy for stage I–IIIA NSCLC less than 1 year before the first dose of study medication, prior bevacizumab therapy, evidence of major blood vessel invasion or encasement by cancer, uncontrolled thrombotic or hemorrhagic disorders, serious nonhealing wounds, or grade 3 to 4 gastrointestinal bleeding within 3 months before study entry.

Study Design

This was a phase II, single-arm, multicenter, open-label study of combination therapy of ramucirumab with paclitaxel and carboplatin in patients with advanced NSCLC conducted at eight centers in the United States. The primary objective of the study was to evaluate the 6-month PFS rate. Secondary objectives included PFS, overall survival (OS), objective response rate (ORR), safety/tolerability, and the pharmacokinetic profile of ramucirumab, in addition to an exploratory analysis of potential biomarkers in tumor tissue and their potential association with clinical outcomes.

Each center's institutional review board or ethics committee approved the protocol. This study was conducted in accordance with good clinical practices and International Conference on Harmonisation (ICH) guidelines as implemented in the European Union and Japanese guidelines, and any other regional/national requirements for clinical trials, as applicable. Patients provided written informed consent before undergoing study procedures or receiving study treatment.

Treatment and Dose Adjustments

Patients received ramucirumab 10 mg/kg via IV infusion over 1 hour on day 1 of each 21-day cycle (i.e., every 3 weeks). Study combination treatment continued for up to six cycles (each cycle consisting of 1 infusion of each medication on day 1 of a 3-week period), or until there was evidence of disease progression or intolerable toxicity. In the absence of any withdrawal criteria, patients completing combination therapy could continue to receive ramucirumab monotherapy every 3 weeks, provided there was ongoing evidence of clinical benefit.

Patients received paclitaxel 200 mg/m^2 via IV infusion over 3 hours on day 1 following the ramucirumab infusion. Before each infusion of paclitaxel, patients were premedicated with an oral steroid (such as dexamethasone 20 mg administered 6 and 12 hours before paclitaxel [or IV equivalent]), an antihistamine (H1 antagonist), and an antiemetic (such as cimetidine [300 mg IV]). Patients received carboplatin on day 1 after paclitaxel as an IV infusion over 30 minutes. The target area under the curve (AUC) for carboplatin treatment was AUC = 6, unless the dose was modified. The creatinine clearance used to calculate the carboplatin dose was estimated based on serum creatinine, using the modified Calvert formula. Antiemetics (a 5-HT3 receptor antagonist or equivalent) were given in conjunction with carboplatin and therapy; corticosteroids were given prophylactically before carboplatin administration and continued for 72 hours.

Doses were modified for ramucirumab-related infusion reactions, hypertension, thrombotic events, and proteinuria; paclitaxel hypersensitivity reactions; and carboplatin treatment-related events. In addition, doses of paclitaxel and/or carboplatin were reduced (by 20–25%) or held in the presence of certain hematologic and nonhematologic toxicities. If a second episode of certain events occurred or if the event resolved, chemotherapy was readministered at a reduced dose (30–50%). If the dose of chemotherapy was reduced, subsequent dose increases were not permitted. Events causing ramucirumab discontinuation were occurrence of greater than

2 g/24 hours proteinuria, or a protein level that did not return to less than 2 g/24 hours within 2 weeks; grade 3/4 hypertension, bleeding or hemorrhagic event, arterial thrombotic, or venous thrombotic event considered by the investigator to be life-threatening or symptomatic and not adequately treated, or infusion reaction; grade 4 pulmonary embolism; or hemoptysis that exceeded the severity grade present at study entry. A grade greater than or equal to 1 central nervous system hemorrhage required all three drugs to be discontinued. If ramucirumab was permanently discontinued, then therapy with the other study agents continued and the patient remained on study; if carboplatin or paclitaxel was permanently discontinued, all study treatments were discontinued and the patient was discontinued from the study.

Safety and Efficacy Assessments

Evaluations done at baseline and each cycle included physical examination, vital signs, ECOG PS, and hematology and chemistry profiles. Adverse events (AEs) were categorized and graded at each cycle according to National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 3.0.

Patients were evaluated for response according to Response Evaluation Criteria in Solid Tumors v1.0 guidelines using serial tumor assessments at baseline and every 6 weeks (i.e., every two cycles) from the start of the first dose of study medication. Imaging studies included computed tomography scan or magnetic resonance imaging of the chest and upper abdomen (including both adrenal glands).

Pharmacokinetic Assessments

During cycles 1 to 6, blood samples were collected from all patients before infusion and 1 hour after the conclusion of the infusion. The samples were analyzed for ramucirumab using a validated Enzyme Linked Immunosorbent Assay method (Intertek Pharmaceutical Services, San Diego, CA). These data were summarized using Phoenix WinNonlin 6.3. For the purposes of data summaries, all values below the quantifiable lower limit of the assay were treated as missing.

Serum Biomarker Assays

Serum samples were obtained from patients for analysis of potential biomarkers. The following analytes were assayed on samples collected at baseline using assay kits from Mesoscale Discovery (MSD): *HGF*, *TNF-alpha*, *IL1-beta, IL-6, IL-8, Thrombomodulin, E-Selectin, P-Selectin, sICAM3, KDR, PIGF, Tie-2, VEGF-A, VEGF-C, VEGF-D, bFGF,* and *sFLT-1*. The various analytes were measured using non-GLP quantitative sandwich electrochemiluminescence kits manufactured by MSD.

Single-Nucleotide Polymorphisms

In total, 21 individual single-nucleotide polymorphisms (SNPs) were assessed for germline mutations in whole blood samples from the following genes (with number of SNPs per gene in parentheses): *VEGFR-2* (3), *NOS3* (1), *VEGF-A* (5), *NRP1* (1), *ICAM1* (2), *HIF1a* (1), *NOS1* (eNOS) (1), *SDF-1a* (1), *IL-8* (1), *CXCR1* (1), *FCGR2a* (1), *FCGR3A* (1), *FGFR-2* (1), and *HIF1* (1).

Copy Number Variant-Fluorescence In Situ Hybridization Assay

The following genes were assessed for copy number variants (CNVs) in formalin-fixed, paraffin-embedded (FFPE) tissue sections using the fluorescence in situ hybridization (FISH) assay: *FGFR-1, VEGF-A, VEGFR-1,* and *VEGFR-2*. Signals were enumerated in at least 50 interphase nuclei per specimen. For each gene, several patient-level summaries were reported, including the mean copy number per cell (continuous measurement), mean score (ordinal measure derived from the mean), and percentage score (based on the percentage of cells displaying less than or equal to 2, 3, or greater than or equal to 4 copies of the gene signal).

Data and Statistical Analyses

The sample size was calculated based on the historical data from a randomized phase III study of bevacizumab in combination with paclitaxel and carboplatin as first-line therapy demonstrating a 6-month PFS rate of 55% in first-line NSCLC patients. A 6-month PFS rate of at least 55% was considered acceptable, whereas a 6-month PFS rate of 35% or lower was of no interest. A 6-month PFS rate between these outcomes was equivocal. This sample size provided 80% power to demonstrate the assumed 6-month PFS rate at a 5% significance level. Based on this, the current study was designed to enroll approximately 40 patients.

Efficacy and safety analyses were performed on all patients who provided informed consent and received any quantity of study medication using SAS version 8.2 or later (SAS Institute, Cary, NC), or comparable software.

For exploratory analyses involving correlation of biomarkers with efficacy (OS, PFS, and best overall response [BOR]), biomarkers were used as covariates in statistical models as follows. Cox proportional hazard models and logistic regression models were used for time-to-event and binary efficacy outcomes, respectively.[13] For serum biomarkers, if greater than 20% of the samples were below the limits of quantitation, then the biomarkers were treated as binary covariates dichotomized at the upper/lower limit of quantitation. Otherwise, they were treated separately as (1) continuous covariates and (2) continuous covariates, treated as binary with automatic cut-point selection using applicable maximal χ^2 technique.[14] Since most patients in the intent-to-treat population were non-Hispanic whites, for correlative analyses of SNPs with clinical outcomes, the analysis population was restricted to non-Hispanic white patients who had SNPs assayed. For serum biomarkers and CNV data, the analysis populations consisted of all patients who had the assay types performed. Standard quality control filtering methods were applied to SNPs before testing for association with clinical outcomes.

In general, usage of the genotypic model was not feasible for SNPs due to low cell counts for the rare homozygote group. However, the two genes of primary interest (i.e., *VEGF-A* and *VEGFR-2*) had SNPs with reasonable representation within

each genotype group. Hence, SNPs in *VEGF-A* or *VEGFR-2* that passed quality control were tested under the genotypic model (ternary variables). All SNPs (including those in *VEGF-A* and *VEGFR-2*) were also modeled as presence versus absence of the minor allele (binary variables). All analyses relating CNV-FISH to clinical endpoints were done using both the patients' mean signals and the patients' percentage score. No multiplicity correction was applied other than in the use of maximal χ^2 and in the correlative analyses of SNPs (this was done within individual endpoints using the method to control false discovery rate and *q* values were reported along with the individual *p* values).[14,15]

RESULTS

Patient Characteristics and Treatment

The first patient was enrolled in January 2009 and the last patient completed treatment in January 2012. Of 41 patients enrolled, 40 received study treatment and were included in all analyses. Table 1 lists baseline demographics and disease characteristics. The median age was 59.5 years (range, 35–78 years). The majority of patients were female (25 patients, 62.5%), white (34 patients, 85.0%), had an ECOG PS of 1 (25 patients, 62.5%), tumor histology of adenocarcinoma (34 patients, 85.0%), and stage IV disease (33 patients, 82.5%) at baseline. Lungs (39 patients, 97.5%) and lymph nodes (24 patients, 60.0%) were the most common sites of metastasis. Smoking status and tumor *EGFR* or *KRAS* mutation status were not collected on this study.

TABLE 1. Key Demographics and Characteristics at Baseline

Parameter	Ramucirumab + Paclitaxel + Carboplatin, *n* = 40
Sex, *n* (%)	
Male	15 (37.5)
Female	25 (62.5)
Race, *n* (%)	
Asian	2 (5.0)
Black or African American	4 (10.0)
White	34 (85.0)
Ethnicity, *n* (%)	
Hispanic or Latino	3 (7.5)
Non-Hispanic or Latino	37 (92.5)
Age, years	
Mean (SD)	59.1 (10.22)
Median	59.5
Min–Max	35–78
ECOG PS, *n* (%)	
0	15 (37.5)
1	25 (62.5)
Current staging at enrollment, *n* (%)	
IIIB	6 (15.0)
IV	33 (82.5)
Missing[a]	1 (2.5)
Histology/cytology, *n* (%)	
Adenocarcinoma	34 (85.0)
Large cell	2 (5.0)
Squamous	1 (2.5)
Other histology	3 (7.5)
Site of metastatic disease, *n* (%)	
Lung	39 (97.5)
Lymph node	24 (60.0)
Pleura	18 (45.0)
Bone	15 (37.5)
Previous therapy, *n* (%)	
Chemotherapy	5 (12.5)
Hormonal therapy	1 (2.5)
Immunotherapy	2 (5.0)
Radiotherapy	13 (32.5)
Previous surgery, *n* (%)	34 (85.0)

[a]The baseline staging for this patient was IIIB.
ECOG PS, Eastern Cooperative Oncology Group performance status; Min, minimum; Max, maximum; SD, standard deviation.

Dose Administration

Thirty-two patients received all doses of ramucirumab at greater than or equal to 90% of the planned 10-mg/kg dose level. The median duration of ramucirumab therapy was 24.4 weeks (range, 3.0–95.1) and the median number of ramucirumab infusions was 8.0 (range, 1–31). The mean cumulative ramucirumab dose was 94.51 mg/kg (standard deviation [SD] = 63.630 mg/kg, range, 10.2–320.3 mg/kg) and the mean relative dose intensity was 94.9% (SD = 7.6%, range, 65.9–105.0%). Supplemental Figure 1 (Supplemental Digital Content, http://links.lww.com/JTO/A640) presents the duration of study drug exposure. A total of 26 patients (65.0%) received ramucirumab monotherapy subsequent to discontinuation of paclitaxel and carboplatin (13 discontinued due to AEs). There were no ramucirumab dose reductions; seven patients had dose omissions.

The median duration of paclitaxel therapy was 18.0 weeks (range, 6.0–22.1) and the median number of paclitaxel infusions was 6.0 (range, 2–6). The mean cumulative paclitaxel dose was 926.06 mg/m^2 (SD = 293.144 mg/m^2, range, 224.7–1300.0 mg/m^2) and the mean relative dose intensity was 88.9% (SD = 13.4%, range, 56.2–106.6%). Twelve patients had paclitaxel dose reductions and 12 patients had dose omissions.

The median duration of carboplatin therapy was 18.0 weeks (range, 6.0–22.1) and the median number of carboplatin infusions was 6.0 (range, 2–6). The mean cumulative carboplatin dose was 3363.47 mg (SD = 1225.492 mg, range, 958.0–5601.0 mg) and the mean relative dose intensity was 93.0% (SD = 17.9%, range, 49.2–133.4%). Nine patients had carboplatin dose reductions and 10 patients had dose omissions.

Efficacy

The PFS rate at 6 months derived from Kaplan–Meier analysis was 59.0% (95% confidence interval [CI]: 41.3–72.9%). A total of 15 patients (37.5%) had disease progression within 6 months from the start of study treatment. The

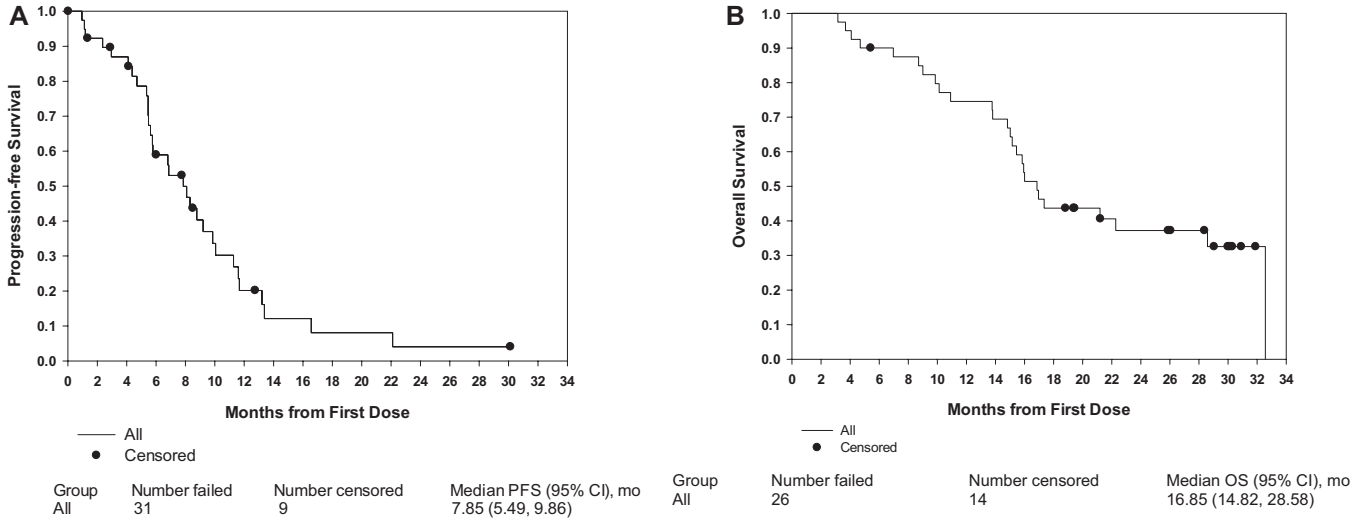


FIGURE 1. Kaplan–Meier estimates for PFS (*A*) and OS (*B*). CI, confidence interval; mo, months; OS, overall survival; PFS, progression-free survival.

median Kaplan–Meier estimate of PFS was 7.85 months (95% CI: 5.49–9.86 months; Fig. 1*A*). The median OS time was 16.85 months (95% CI: 14.82–28.58 months; Fig. 1*B*). Of the 40 patients treated, 26 had died at the time of data cutoff date (January 6, 2012). The ORR was 55.0% (95% CI: 38.5–70.7%; Table 2). One patient (2.5%) had a complete response (CR) and 21 patients (52.5%) had a partial response (PR). The disease control rate (CR + PR + stable disease) was 90.0% (95% CI: 76.3–97.2%). Fig. 2 shows a waterfall plot of best overall percent change from baseline of target lesion measurements for treated patients.

Safety

A total of 34 patients (85.0%) experienced treatment-emergent adverse events (TEAEs) considered related to ramucirumab. Most treatment-related TEAEs were CTCAE grade 2 or 3. The most common treatment-related TEAEs (of all grades) were fatigue (21 patients, 52.5%), peripheral neuropathy (13 patients, 32.5%), nausea (11 patients, 27.5%), and epistaxis and myalgia (9 patients each, 22.5%). Ten patients (25.0%) experienced a grade 3 treatment-related TEAE and five patients (12.5%) experienced a grade 4 treatment-related TEAE. Neutropenia (four patients, 10.0%), thrombocytopenia and fatigue (three patients each, 7.5%), and peripheral neuropathy (two patients, 5.0%) were the most frequently reported grade 3 treatment-related TEAEs (Table 3). No grade 3 or above hemoptysis was reported in this study. Febrile neutropenia and pulmonary embolism (two patients each, 5.0%) and neutropenia and thrombocytopenia (one patient each, 2.5%) were the only grade 4 TEAEs related to treatment (Table 3).

Eight patients (20.0%) experienced serious adverse events (SAEs) considered related to treatment. Febrile neutropenia (grade 4) was reported by two patients (5.0%); the remaining related SAEs were reported by one patient each. Thirteen patients discontinued ramucirumab treatment due to AEs. The most frequently reported AEs (two patients each, 5%) leading to discontinuation were infusion-related reaction (not related to ramucirumab), neutropenia (one incident was

TABLE 2. Best Overall Response

	Ramucirumab + Paclitaxel + Carboplatin, *n* = 40
Best response, *n* (%)	
CR	1 (2.5)
PR	21 (52.5)
SD	14 (35.0)
Progressive disease	3 (7.5)
Not evaluable[a]	1 (2.5)
Objective response rate (CR + PR), %	55.0
95% CI (exact)	(38.5–70.7)
Disease control rate (CR + PR + SD), %	90.0
95% CI (exact)	(76.3–97.2)

[a]Not evaluable due to missing scans.

CI, confidence interval; CR, complete response; PR, partial response; SD, stable disease.

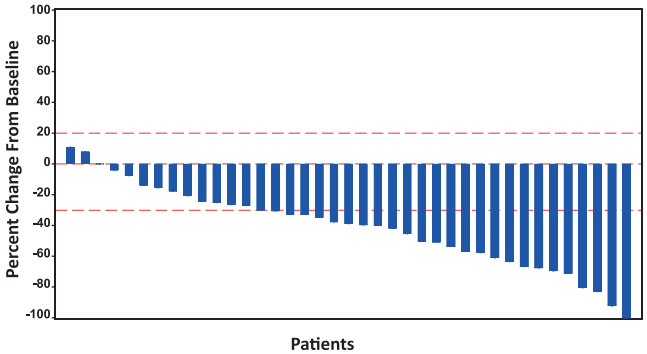


FIGURE 2. Waterfall plot of best overall percent change from baseline in target lesion measurement (*n* = 39).

TABLE 3. Adverse Events Possibly, Probably, or Definitely Related to Treatment

	Ramucirumab + Paclitaxel + Carboplatin, *n* = 40			
Event[a]	All Grades, *n* (%)	Grade 3, *n* (%)	Grade 4, *n* (%)	Grade 5, *n* (%)
Thrombocytopenia	8 (20.0)	3 (7.5)	1 (2.5)	0
Neutropenia	6 (15.0)	4 (10.0)	1 (2.5)	0
Febrile neutropenia	3 (7.5)	1 (2.5)	2 (5.0)	0
Anemia	2 (5.0)	1 (2.5)	0	0
Constipation	5 (12.5)	1 (2.5)	0	0
Fatigue	21 (52.5)	3 (7.5)	0	0
Anorexia	7 (17.5)	1 (2.5)	0	0
Peripheral neuropathy	13 (32.5)	2 (5.0)	0	0
Dyspnea on exertion	2 (5.0)	1 (2.5)	0	0
Pulmonary embolism	2 (5.0)	0	2 (5.0)	0
Hypertension	5 (12.5)	1 (2.5)	0	0

[a]Includes those events graded 3, 4, or 5, with their respective all grades total. At each level of summarization, a patient is counted once according to the TEAE with worst grade. One patient is counted for each TEAE when several events are reported for the same patient.

TEAE, treatment-emergent adverse event.

not related to ramucirumab), and fatigue. The majority of AEs leading to ramucirumab discontinuation occurred within the first six cycles of treatment. No patients died during the study or within 30 days of the last dose of study medication.

Pharmacokinetics

Ramucirumab concentration–time data were available for 39 NSCLC patients. Concentrations at 1-hour postend of infusion appeared to be stabilized after the third infusion, with geometric mean (coefficient of variation [CV%]) values ranging from 365 μg/ml (21%) to 394 μg/ml (31%) between cycle 4 and cycle 6. Greater interindividual variability was observed for trough concentrations relative to concentrations obtained at 1-hour postend of infusion. The geometric means (CV%) of trough concentration ranged from 36.8 μg/ml (100%) to 73.6 μg/ml (60%) between cycle 4 and cycle 6.

Biomarker Analysis

To ensure that a relatively homogenous group of patients was analyzed for mutational differences with respect to the clinical outcomes of interest, the correlative analyses of SNPs with clinical outcomes were restricted to the non-Hispanic white patients who had SNPs assayed. Different translational research populations were considered for each assay platform: SNPs (restricted to non-Hispanic whites [n = 22]), serum biomarkers (n = 23), and FISH-CNV (n = 23). The SNP results from one patient in the translational research population were excluded from the analysis due to failing the quality control criteria, reducing this group to 21 patients. Notably, SNP rs2981582 on the *FGFR-2* gene had significant associations (without multiplicity adjustment) with all three clinical outcomes (Figs. 3*A–C*); unadjusted p values for tests of association with OS, PFS, and BOR were, respectively, 0.0059, 0.0429, and 0.0392 and false discovery rate q values for tests of association of that SNP with OS, PFS, and BOR were, respectively, 0.1000, 0.4838, and 0.3580.

High *IL-6* serum levels had significant associations with both decreased PFS and OS (p values from maximal χ^2 analyses of 0.0038 and 0.0029, respectively), but not with BOR (p = 0.2883) (data not shown).

Correlative analyses of CNV-FISH data with clinical endpoints did not yield any significant (5% level) findings.

DISCUSSION

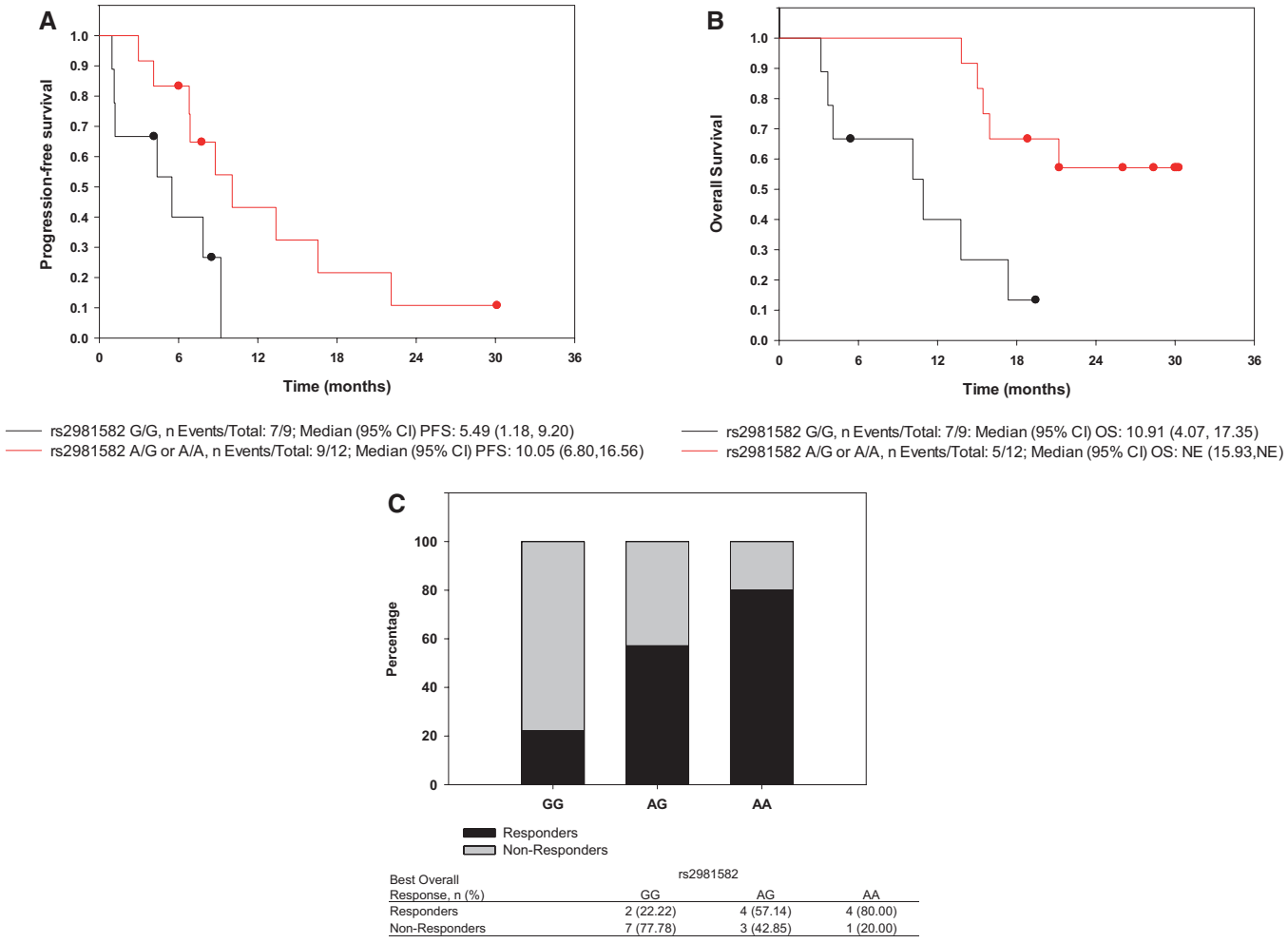
The ECOG 4599 study demonstrated that the combination of bevacizumab with paclitaxel-carboplatin chemotherapy resulted in a survival benefit (median OS of 12.3 months in the bevacizumab-containing arm compared with 10.3 months in the chemotherapy-alone arm).[8] However, there were more treatment-related deaths in the bevacizumab-containing arm. In this single-arm phase II study, the addition of ramucirumab to a standard, platinum-based chemotherapy regimen resulted in comparable PFS with promising OS and acceptable safety. The 6-month PFS rate was 59.0%, with a median PFS of 7.85 months, a median ORR of 55.0%, and a clinical benefit rate (CR + PR + stable disease) of 90.0%. The median OS was 16.85 months. The effects of ramucirumab on survival and PFS were consistent with the ECOG 4599 study, in which the addition of bevacizumab improved median PFS to 6.2 months, compared with 4.5 months with chemotherapy alone.[8]

Ramucirumab was administered every 3 weeks as a 10 mg/kg IV infusion. Exposure appeared to be stabilized after the third infusion. Medium to large interindividual variability was observed for trough concentrations.

There were no unexpected toxicities, and AEs were within the acceptable range when compared with the ECOG 4599 study.[8] A total of 34 patients experienced TEAEs considered related to study medication, with the majority of events being CTCAE grade 2 or 3. Eight patients (20.0%) experienced SAEs considered related to treatment. No grade 3 or above hemoptysis was reported in this study. Whereas most AEs are similar to those observed with bevacizumab,[8] it is notable that within this study, no patients died during the study or within 30 days of the last dose of study medication.

Recently, genomic analyses have led to advances in the treatment of lung adenocarcinoma and improvements in patients' outcomes. Examples include the use of EGFR inhibitors, such as gefitinib and erlotinib for lung adenocarcinoma bearing *EGFR* mutation, and ALK inhibitors, such as crizotinib for *ALK* translocations.[16–19] Other approaches include studies of cytokine expression profiles and association with clinical outcomes in NSCLC. Similar to our findings, Koh and colleagues found that prognosis for patients with increased *IL-6* expression was worse than for patients with lower *IL-6* expression.[20]

In our biomarker analysis, SNP rs2981582 on the *FGFR-2* gene had significant associations (without multiplicity adjustment) with all three clinical outcomes. *FGFR-2* amplification, mutations, and germline SNP have been associated with clinical outcomes in several human cancers, including gastric, endometrial, and breast cancer.[21] For example, *FGFR-2* has been identified as a breast cancer susceptibility gene and SNPs located in the second intron of *FGFR-2*



Best Overall Response, n (%)	GG	AG	AA
Responders	2 (22.22)	4 (57.14)	4 (80.00)
Non-Responders	7 (77.78)	3 (42.85)	1 (20.00)

FIGURE 3. Biomarker analysis and association with clinical outcomes: association of SNP rs2981582 G/G and A/G or A/A with PFS (*A*), OS (*B*), and best overall response rate (*C*) in treated non-Hispanic Caucasian patients in the translational research population with SNP data ($n = 21$). The median and upper ends of the 95% CI for OS in the patients with A/G or A/A genotype were not estimable. CI, confidence interval; NE, nonestimable; OS, overall survival; PFS, progression-free survival; SNP, single-nucleotide polymorphism.

were found to correlate with an increased risk of developing breast cancer. SNP rs2981582 in *FGFR-2* was shown to be associated with breast cancer risk for *BRCA1* and *2* carriers with ER-positive breast cancer.[22] Meyer and colleagues[23] have shown that SNP rs2981582 is associated with higher level of *FGFR-2* transcription, both in cell lines and in tumors. The SNP is located in the transcription factor binding sites (C/EBPb and Oct-1/Runx2) and could affect the binding efficiency of these factors. Genome-wide analysis of ER binding sites has revealed three potential ER binding sites within the *FGFR-2* gene,[24] and ER and Oct-1/Runx2 may cooperate to increase gene expression. The disease-associated genotype of SNP rs2981582 in *FGFR-2* may also depend on the signaling potential of *FGFR-2* in ER-positive cells. One mechanism by which elevated levels of *FGFR-2* may contribute to the establishment of an autocrine signaling loop is by reducing the cell's propensity to undergo apoptosis.[25] To our knowledge, this has not yet been shown in NSCLC. Mouse models have confirmed that fibroblast growth factor (FGF) signaling has oncogenic potential, including roles in angiogenesis, but have also importantly demonstrated that FGF signaling can have tumor suppressive function.[21] Since the biological effect (if any) of this intronic SNP in *FGFR-2* is not clear, it is not possible to speculate on why it appears associated with clinical benefit within this study. In addition, as the study was not randomized, it is not possible to attribute any effect to prognostic as opposed to potential predictive associations. Finally, if predictive, it is not possible to ascribe the effect to any specific component of the three-drug combination given to these patients. The association with clinical benefit was consistent across three separate measures of clinical benefit (OS, PFS, and response), albeit without strict control for multiple testing; hence, this biomarker is a potential candidate for further exploration in the future. In addition, a lack of information of the *EGFR* mutation status and smoking status limits conclusions for this study population. However,

ramucirumab appeared to be well tolerated with encouraging signs of efficacy, and with a potential biomarker to investigate in additional detail, further development of this compound in front-line NSCLC should be considered.

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