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## Evaluation of KRAS mutations, angiogenic biomarkers and DCE-MRI in patients with advanced non-small cell lung cancer receiving sorafenib

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### Abstract

**Purpose**—Sorafenib, a multikinase inhibitor targeting Raf and VEGFR has shown activity in unselected patients with NSCLC. At present there are no validated biomarkers indicative of sorafenib activity.

**Experimental Design**—Patients received sorafenib 400mg bid daily to determine activity and tolerability and to measure its biological effects. KRAS mutation status (N=34), angiogenesis markers (VEGF, bFGF, FLT-1, PLGF-1) and imaging with DCE-MRI to determine early changes in tumor vascular characteristics were evaluated. Three parameters  $K^{trans}$ ,  $K_{ep}$  and  $v_e$ , were measured by DCE-MRI at baseline and day 14 of cycle 1. Cytokine analysis was performed on days 0, 14, 28 and 54.

**Results**—37 pts with previously treated stage IV NSCLC were enrolled in this single center phase II trial. In 34 evaluable patients, 2 had partial responses, and 20 had stable disease for 3-17 months, a disease control rate of 65%. The median progression-free survival (PFS) was 3.4 months and median overall survival (OS) was 11.6 months. Toxicity was consistent with the known side effects of sorafenib. KRAS (32%) and EGFR mutations (22%), showed no correlation with response, PFS or OS.  $K_{ep}$ , was significant in predicting an improvement in OS ( $p=0.035$ ) and PFS ( $p=0.029$ ). Cytokine analysis demonstrated an improved OS for bFGF day 0  $<6$  vs  $>6$  pg/ml ( $p=0.042$ ) whereas a PFS benefit was seen with bFGF at day 28  $<6$  vs  $>6$  ( $p=0.028$ ).

**Conclusions**—KRAS and EGFR mutational status showed no correlation with response, PFS or OS. Radiological and cytokine changes may act as biomarkers indicative of early angiogenesis inhibition.

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## Introduction

Treatment outcomes for advanced non small cell lung cancer (NSCLC) have been limited by the empiric administration of cytotoxic chemotherapy (1). Small molecule tyrosine kinase inhibitors have demonstrated single agent activity in a wide variety of solid tumors including NSCLC (2), however, the lack of validated predictive factors for many of these targeted treatments remains problematic. Recent evidence has highlighted the importance of individualizing therapy based on certain molecular characteristics (EGFR mutations and EML4-ALK translocations) (3-5). KRAS mutations are present in approximately 30% of NSCLC and are responsible for the proliferation signaling of the RAS/RAF/MEK/ERK pathway and indicate a poor prognosis and poor response to EGFR inhibitors (6,7). Therapeutic targeting of the Ras pathway has so far been unsuccessful. RAF serine-threonine kinases are the principal effectors of RAS and are considered an important target for cancer therapy. Sorafenib is a multikinase inhibitor that inhibits C-RAF and B-RAF; VEGFRs 1, 2 and 3, and PDGF  $\beta$ ; Flt3; RET; and c-KIT(8-11). Sorafenib has shown activity in preclinical models of NSCLC and several phase I (9,12-15) and phase II trials (16,17). To date there is limited data with regards to sorafenib sensitivity amongst NSCLC patients with different KRAS mutational status.

Vascular endothelial growth factor (VEGF) is up-regulated in many tumors(18). Unfortunately, there are no validated biomarkers in clinical practice available to predict those patients who will gain clinical benefit, or that can be used to monitor therapeutic response. Increases in VEGF and decreases in soluble VEGF receptors have been reported in phase I and phase II trials involving different VEGFR tyrosine kinase inhibitors (TKIs) and may represent a class effect (16,19-23). Correlating these angiogenic cytokine changes with clinical benefit has however been difficult. The goal of this phase II trial was to determine if sorafenib is active in NSCLC and to determine the impact of K-Ras mutational status. Several correlative studies were performed during treatment to measure biological and clinical effects of sorafenib.

## Patients and Methods

### Patients

Thirty seven patients were enrolled between 2005 – 2009 onto the trial, which had been reviewed and approved by the National Cancer Institute's Institutional Review Board. Verbal and written consent was obtained from all patients. Enrollment criteria included age older than 18 years, an Eastern Cooperative Oncology Group (ECOG) performance status of 0-1, a life expectancy greater than 3 months and histologic or cytologic confirmation of recurrent or progressive advanced NSCLC, and only one line of prior chemotherapy. A protocol amendment in February 2008 allowed enrollment of patients who had received greater than one standard or investigational treatment regimen. Suitable candidates required adequate bone marrow, liver and renal function, measurable disease (RECIST 1.0) and to be at least 28 days since any prior radiation or major surgery. Patients with symptomatic brain metastases were excluded unless they had treatment for their brain metastases and had stable disease for at least 3 months without steroids. Originally patients with squamous cell carcinoma were allowed on study but a second protocol amendment excluded these patients due to side-effects seen in the ESCAPE trial(24). The study followed the current guidelines of the International Conference on Harmonization for good clinical practice and the Declaration of Helsinki.

## Study Design

The primary objective of this single arm study was to determine the response rate (RR) and toxicity of sorafenib administered at a dose of 400 po mg BID daily in a 28 day cycle, in relapsed or recurrent NSCLC patients. Secondary objectives included a correlation of the patient's response to treatment with KRAS mutation status and with changes in angiogenic cytokines, an evaluation of the application of DCE-MRI to determine early changes in tumor vascularity during treatment, and an evaluation of time to progression and overall survival. Treatment continued until objective evidence of tumor progression or intolerable drug-related side effects. Adverse events were defined by the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 3.0. Two dose reductions were allowed for clinically significant toxicities attributed to sorafenib while on study: dose level -1 was 200mg BID and dose level -2 was 200mg OD. Doses were not re-escalated once toxicities had recovered. Drug could be held a maximum of 3 weeks prior to discontinuation from study.

## Imaging Studies - DCE-MRI

Dynamic contrast enhanced magnetic resonance imaging (DCE MRI) was used to evaluate changes in vascularity at baseline and at day 14 (+/- 3 days) of cycle 1. DCE MRI was performed with a 1.5-T MR system (Philips Achieva, The Best, The Netherlands) using a dedicated receive-only six-channel phased array coil (see supplemental text). Data from DCE MRI were analyzed using a 2-compartment (Kety) model, also known as the general kinetic model (GKM)(25, 26). The GKM model analysis was performed with an IDL-based (Interactive Data Language; Research Systems Inc., Boulder, CO, USA) research tool (Cine Tool, GE Healthcare, USA). Manual region-of-interest measurements were obtained from each slice of the target lesion. The GKM model produces three parameters:  $k_{ep}$  the reverse contrast transfer rate,  $K^{trans}$ , the forward contrast transfer rate and  $v_e$  the extravascular fraction. Baseline and follow up after treatment cycles  $k_{ep}$ ,  $K^{trans}$ , and  $v_e$  values were obtained.

## Cytokine analysis

Serial plasma samples were collected from all patients in an EDTA-containing vacutainer at pretreatment (baseline – day 0), and on day 14, 28 and 54. After centrifugation, the samples were aliquoted, immediately frozen, and stored at -80°C. Plasma analysis performed included evaluation of VEGF, placental-derived growth factor (PLGF), basic fibroblast growth factor (bFGF), and VEGF receptor 1 (sVEGFR1 or FLT-1) using multiplex array plates from Meso-Scale Discovery (Gaithersburg, MD). The concentrations of the cytokines were determined with recombinant standards and expressed as pg/ml.

## KRAS/EGFR/BRAF Mutation Identification

Five to ten 5-micron sections from formalin-fixed, paraffin-embedded (FFPE) tissue sections were de-paraffinized by standard methods. Macro-dissection was performed on selected specimens to obtain at least 10% tumor cell content for DNA isolation, as necessary. DNA isolation was carried out with the QIAmp FFPE DNA Kit on an automated QIAcube instrument (Qiagen, Germantown, MD). Targeted analysis of KRAS codons 12, 13, and 61 was performed using pyrosequencing technology on a PyroMark Q24 instrument (Qiagen) and the PyroMark Q24 KRAS v2.0 kit (Qiagen), as described by Ogino et al(27). Targeted analysis for EGFR mutations involving exon 20 codon 790, exon 21 codons 858/861/863 was performed using pyrosequencing, while exon 19 deletions were assessed by capillary electrophoresis using a Genetic Analyzer 3130xl (Applied Biosystems), as described by Pan et al(28). Primers for the EGFR pyrosequencing reactions were designed in our laboratory and are available on request. For BRAF V600 mutation detection, the

extracted DNA was subjected to an initial PCR using a single primer set (sequence available on request) encompassing codon V600. Pyrosequencing was carried out on a Qiagen PyroMark Q24 system (Valencia, CA).

### Statistical Considerations

The study was conducted using a phase II optimal design in order to determine if sorafenib was able to be associated with a response rate (PR + CR) which could rule out 5% ( $p=0.05$ ) in favor of a more desirable 20% response rate ( $p=0.20$ ). Using  $\alpha=0.10$  (probability of accepting a poor drug) and  $\beta=0.10$  (probability of rejecting a good drug), initially 12 patients were to be enrolled onto the trial. If zero of the 12 patients responded, then accrual would end while if 1 or more of the first 12 patients had a CR or a PR, then accrual would continue until a total of 37 patients with measurable disease had been enrolled. If 4 or more of 37 had a response, then this would warrant further investigation in a subsequent trial. The associations between KRAS and EGFR mutations and response (PR+SD vs. PD) were determined by a Fisher's exact test. Comparisons of continuous parameters were made using an exact Wilcoxon rank sum test. The difference from baseline to days 14, 28, and 54 was determined by a Wilcoxon signed rank test. Survival and progression free survival analyses were initially performed using univariate Cox models to screen for the association between outcome and values of a continuous parameter less than or greater than the median value. For the cases in which a trend was identified, the p-value was confirmed by a log-rank test p-value. In view of the exploratory nature of these analyses, any p-values reported have not been adjusted for multiple comparisons, and the results of these analyses are considered hypothesis generating.

## Results

### Patient Characteristics

Baseline demographic data and disease characteristics are listed in table 1. All patients had received prior therapy with 21 patients (57%) having had at least 2 prior regimens. Seventeen patients (46%) had received only one previous regimen prior to study enrollment. In total 43% had received prior EGFR tyrosine kinase inhibitors and 40% had prior bevacizumab.

### Efficacy

All patients had progressive disease at the time of enrollment. The median duration of treatment was approximately 3 months (97 days, range, 12 to 517 days). Three patients were considered not evaluable for response, one patient withdrew from study after 4 weeks and the 2 others due to a lack of target lesions. In total, two partial responses (6%) were seen (Figure 1). The first patient had metastatic squamous cell carcinoma and had received only one previous regimen of carboplatin and paclitaxel for 6 cycles. His tumor was wildtype for KRAS, EGFR and BRAF. The second patient had metastatic adenocarcinoma to the brain and adrenal gland. He had received one previous chemotherapy regimen of carboplatin, gemcitabine and bevacizumab prior to commencing sorafenib. His tumor was wildtype for KRAS and BRAF and not enough tissue was available for EGFR analysis.

Median progression free survival (PFS) for evaluable patients was 3.4 months and median overall survival (OS) was 11.6 months (Figure 2). The disease control rate was 65% with 2 PRs (6%) and 20 SD (59%). In patients who had received only one previous therapy 2 PRs, and 7 SD were seen. In an evaluation of all 37 patients, stable disease greater than 3 months (3 cycles) ranging from 3 to 17 months was documented in 20 patients (54%) with a median duration of 5.4 months. One patient had clinical disease progression and died 3 weeks after sorafenib was discontinued. There were no differences in terms of RR, PFS or OS

depending on whether patients had one or multiple lines of prior therapy or between squamous cell and adenocarcinomas.

### KRAS and EGFR mutations

Table 2 summarizes the characteristics of patients with either wildtype or KRAS/EGFR. Three patients out of 37 did not have tissue available for KRAS analysis. KRAS mutations occurred in 11/34 patients (32%). Fourteen patients (38%) did not have evaluable tissue to perform EGFR mutation analysis. Of the remaining 23 patients there were 5 (22%) subjects with an activating EGFR mutation. There was reciprocal exclusion of EGFR and KRAS mutations, as reported by others (29). Interestingly 3 (8%) never smoking patients with adenocarcinomas demonstrated KRAS mutations. There was no correlation between the mutational status of KRAS or EGFR and RR, PFS or OS. The DCR observed in KRAS mutant and KRAS wildtype patients was 60% and 71% respectively ( $p=0.69$ ). The DCR observed in EGFR mutant and EGFR wildtype patients was 40% and 69% respectively ( $p=0.33$ ).

### Plasma Biomarker Analysis

All 37 patients are evaluable for analysis. Thirty two patients (86%) had plasma drawn at baseline whereas only 14 samples (38%) were available for day 54 analysis. Increases in VEGF and decreases in VEGFR1 from baseline to day 54 were detected. Increases in plasma PLGF levels were also seen at these time-points (supplemental table 1). The 4 cytokines were each divided at their respective median values and evaluated for their association with overall survival and progression free survival. Four of the parameters evaluated demonstrated an association with improved OS and PFS by having univariate two-tailed  $p$ -values  $<0.10$  (supplemental table 2 and 3). Of these parameters, bFGF at baseline  $<6$  vs.  $>6$  was significant for OS ( $p=0.042$ ) and similar levels on day 28 were significant for PFS ( $p=0.028$ ) (Figure 3). The difference between day 28-day 0 PLGF was significant for OS ( $<11$  v  $>12$ ),  $p=0.0027$  (supplemental Figure 2). Cytokine analysis did not predict response to sorafenib.

### DCE MRI

Twenty-six patients (70%) had DCE-MRI scans at baseline and on day 14 ( $\pm 3$  days) of cycle 1. Target lesions were localized in the lung (20 patients), adrenal gland (2 patients), liver (1 patient), mediastinum (1 patient), chest wall (1 patient) and neck (1 patient). Decreases in either  $K_{ep}$  or  $K^{trans}$  were seen in twenty-one patients (81%), whereas an increase was observed in five patients (19%) (Supplemental Figure 1).  $K_{ep}$ ,  $K^{trans}$  and  $v_e$  measurements at day 0, day 14 and the difference between the day 14 and the day 0 measurements (day14-day0) were each divided at their respective median values and evaluated for their association with RR, PFS and OS. In a univariate exploration the  $K_{ep}$  difference of  $< -0.15$  vs.  $> -0.14$  was statistically significant, with an improved OS ( $p=0.035$ ) and PFS ( $p=0.029$ ) for those patients demonstrating a  $K_{ep}$  difference of  $< -0.15$  compared to patients with a  $K_{ep}$  difference of  $> -0.14$  (Figure 3). Non significant differences were associated with  $K^{trans}$  and  $v_e$ . DCE-MRI did not help predict response to sorafenib.

### Safety

The toxicities of sorafenib have been well documented and similar side effects to those previously described were seen in this study (Table 3). In total, 16 patients tolerated the full dose of 400mg po bid but 21 patients needed a dose reduction (57%). Of these, 11 patients required one dose reduction and 10 required a second reduction to 200mg po OD. All patients who were reduced to dose level -2 tolerated sorafenib at this dosage. Twelve

patients (32%) required a dose reduction during cycle one. The most frequent grade 3 drug-related AEs were: hypertension (16%), hand/foot syndrome (14%), dyspnea (14%), and hypophosphatemia (14%). One patient developed a squamous cell carcinoma of the skin while on study which was treated by excision and discontinuation of drug. A second patient developed a keratoacanthoma, which was removed and observed while continuing on drug. The most common reason for treatment discontinuation was disease progression (n = 33; 94%). To date 26 patients have died, 7 (27%) died within 60 days from coming off study. There was one death while on study secondary to clinical progression.

## Discussion

In this trial sorafenib demonstrated activity with a response rate of 6%, a DCR of 65%, a PFS of 3.4 months, and an OS of 11.6 months. Two other phase II clinical trials have reported activity of sorafenib in heavily pretreated patients with NSCLC with an OS of 6.7 months, median PFS of 2.7 months and stable disease in 59% (16) and in ECOG 2501 a DCR of 47% and a median PFS of 3.6 months (17). There is little known of the possible differences in the sensitivity of NSCLC to sorafenib according to KRAS mutational status. Furthermore the identification of biomarkers predictive of treatment outcomes for anti-VEGF therapies has so far met with limited success. In the BATTLE trial the overall DCR for patients treated with sorafenib was 58%. Subset analyses showed DCR of 61% in patients with KRAS mutations vs 56% KRAS wildtype but only a 23% DCR in patients with an EGFR mutation compared to 64% in patients without an EGFR mutation ( $p=0.048$ )(30). Here we report a DCR of 60% in patients with KRAS mutations vs 71% in KRAS wildtype ( $p=0.69$ ) and a DCR of 40% in EGFR mutations vs 69% in patients without an EGFR mutation ( $p=0.33$ ). Smit et al observed 3 PRs and a median PFS of 3 months (95% CI: 2.2-3.8 months) in 10 patients with previously treated advanced NSCLC all harboring KRAS mutations(31).

Currently, no direct RAS inhibitor has proven clinically effective and agents such as sorafenib that bypass RAS and inhibit effector molecules downstream of the mutant GTPase (e.g. RAF) are being evaluated. Preclinical data have suggested that sorafenib inhibits cell growth by inducing G<sub>1</sub> arrest in NSCLC cell lines independent of KRAS genotype(32). Here we report that sorafenib inhibited the growth of NSCLC in a manner independent of KRAS mutational status with no differences in RR, PFS or OS being detected between patients with KRAS wildtype or mutant tumors. The inhibitory effect of sorafenib in KRAS wildtype tumors was also independent of whether there was a mutant EGFR gene present or not. Preclinical studies have indicated that sorafenib blocks the ERK signaling pathway only in wild type KRAS tumors and it inhibits NSCLC cell growth by targeting B-RAF in cells with wild-type KRAS and C-RAF in cells with mutant KRAS(32,33). These results need to be validated in clinical trials and were not assessed in this study.

It has been suggested that for each class of drug it may be possible to identify certain cytokine changes that occur during treatment that may serve as pharmacodynamic or efficacy markers. In this trial we noted an increase in baseline VEGF levels and a decrease in sVEGFR-1 which have been reported in other studies and likely represents a class effect. The predictive role of pretreatment VEGF levels in patients with NSCLC and who are treated with antiangiogenic therapy remains controversial with some suggestion that bioavailable, rather than circulating VEGF may provide the most predictive value(34-37). Germline polymorphisms in VEGF are also being evaluated as a means to help predict patients likely to respond to sorafenib(38). In an exploratory analysis of 4 plasma cytokines, we found distinct patterns of cytokine changes that may act as predictors of response to sorafenib. BFGF levels at day 0 and day 28 showed significance in terms of an OS ( $p=0.042$ ) and PFS benefit ( $p=0.028$ ) and may act as prognostic and predictive biomarkers

(Figure 3). These correlative studies are considered hypothesis generating and may form the basis for future trials.

Functional and molecular imaging may also be used for pre-therapy molecular phenotyping and may prove effective as pharmacodynamic predictive biomarkers. DCE-MRI is non-invasive and is sensitive to tumor perfusion parameters such as vascular volume, vascular permeability and flow. Typically, aggressive tumors are characterized by a rapid enhancement followed by a subsequent rapid wash-out period(26,39). In this study,  $K_{ep}$  demonstrated a significant predictive value for OS ( $p=0.035$ ) and PFS ( $p=0.029$ ).  $K_{ep}$  is considered more robust than the other parameters since it is not as dependent on the T1 values of the tissue or  $V_e$ (26). Although  $K^{trans}$  and  $K_{ep}$  are correlated with each other, it is not surprising that one parameter might better predict OS and PFS than another. For instance,  $K^{trans}$  can be influenced by changes in  $v_e$  values after treatment, variability in image noise, T1 measurement and is very sensitive to patient motion. In this study most of the target lesions were localized to the lungs, adrenal glands and liver all of which are subject to significant motion.

In conclusion, this trial demonstrates that sorafenib provides clinical benefit for patients with heavily pretreated advanced NSCLC irrespective of their KRAS mutational status. Although this study is limited by the relatively small sample size and varied population it is indicative of the population most often seen in thoracic oncology clinics whereby a wide array of histologies and prior therapies are encountered. Establishing predictive biomarkers for anti-angiogenics remains a significant challenge as discovery and validation will have to be tailored to the known mechanisms of action of a certain agent in a certain disease, and will require standardization of biomarker assays amongst protocols. Challenges to overcome include establishing adequate criteria to measure response and the need for spatially resolved dynamic biomarkers to meet the heterogeneous and dynamic nature of cancer. Preliminary biomarker data are emerging but mostly from single arm trials making it difficult to ascertain whether the marker is prognostic or predictive. A combination of vascular permeability imaging and circulating factors measured at various time points may yield a 'composite biomarker' to make robust predictions. Ultimately these data will have to be tested and validated in large, well-designed, prospective clinical trials.

#### Statement of Translational Relevance

KRAS mutations are present in approximately 30% of NSCLC and indicate a poor prognosis and a poor response to EGFR inhibitors. Currently, no direct RAS inhibitor has proven clinically effective and agents such as sorafenib that bypass RAS and inhibit effector molecules downstream of the mutant GTPase (e.g. RAF) are being evaluated. To our knowledge this paper is the first completed study of the direct impact of RAS mutations in NSCLC patients treated with sorafenib. The most striking observation from this report is that sorafenib inhibited the growth of NSCLC in a manner independent of KRAS mutational status with no differences in RR, PFS or OS being detected between patients with KRAS wildtype or mutant tumors. The inhibitory effect of sorafenib in KRAS wildtype tumors was also independent of whether there was a mutant EGFR gene present or not.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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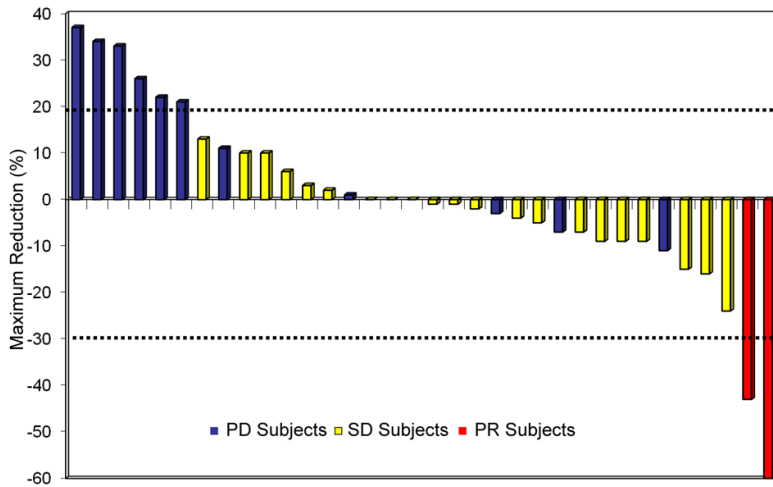
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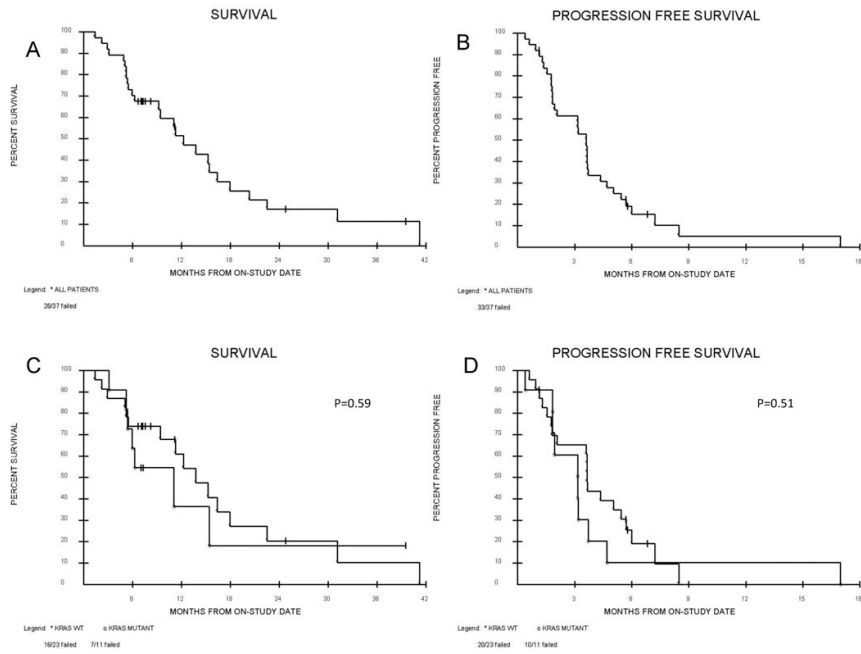


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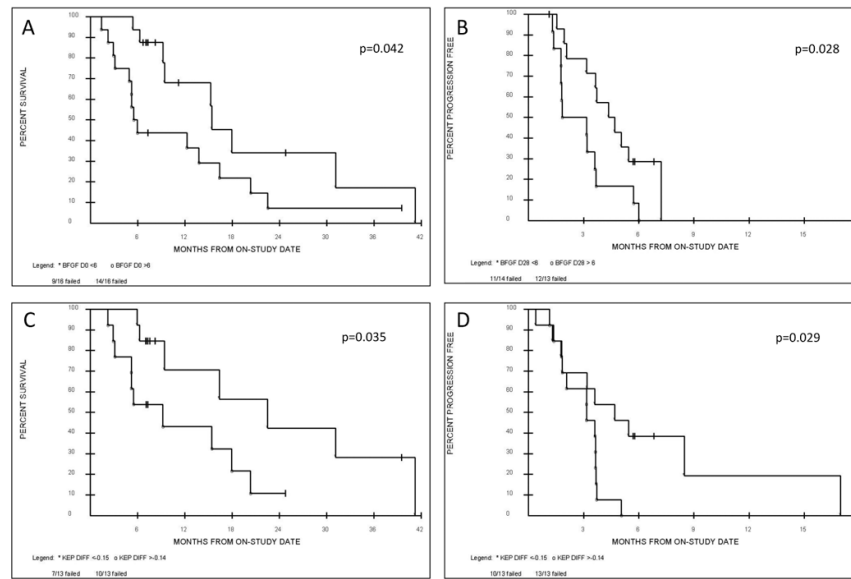
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**Figure 1. Maximum percent reduction or best response of target lesions in patients (N=34) with available post baseline tumor measurements**  
 PD, progressive disease; SD, stable disease; PR, partial responses. Two PRs measuring 60% and 43% and 20 patients (59%) with stable disease with a median duration of 5.4 months. Tumor shrinkage (1% to 24%) was seen in 12 patients with stable disease and tumor growth (2% to 16%) was seen in the remaining 8 patients. In total 8 patients (24%) had stable disease  $\geq$  6 months, with 3 patients on study for 6 months, 1 for 7 months, 2 for 8 months, 1 for 10 months and 1 patient for 17 months respectively. Progressive disease as best response was seen in 12 patients (35%), with 3 patients considered not evaluable.



**Figure 2. Overall survival (OS) and Progression free survival (PFS)**  
 Kaplan meier curves for (A) OS (B) PFS (C) OS according to KRAS wildtype or mutation (D) PFS according to KRAS wildtype or mutation. Median OS for all patients on study is 11.6 months. Median OS for KRAS wildtype is 13.2 months and 7.2 months for KRAS mutant ( $p=0.59$ ). Median PFS for all patients on study is 3.4 months. Median PFS for KRAS wildtype is 3.6 months and 2.6 months for KRAS mutant.



### Figure 3. Cytokine and DCE-MRI analysis

Exploratory analysis demonstrating potential trends towards an association with OS and PFS by having univariate two-tailed p-values  $<0.10$  included basic fibroblast growth factor (bFGF) and the reverse constant transfer rate (Kep) as measured via DCE-MRI. (A) Patients with baseline (day 0) bFGF levels  $<6$  pg/ml had an improved OS compared to patients with higher baseline bFGF levels  $>6$  pg/ml. (B) Patients with lower bFGF levels  $<6$  pg/ml as measured post cycle 1 (day 28) had an improved PFS than patients with higher day 28 bFGF levels  $>6$  pg/ml, indicating a potential prognostic and predictive role for bFGF. Similarly Kep as measured by DCE-MRI may act as a radiological biomarker. Patients demonstrating a Kep difference of  $<-0.15$  (the difference between the day 14 and the day 0 measurements i.e. day14-day0) compared to patients with a Kep difference of  $>-0.14$  showed (C) an overall survival benefit and (D) a progression free survival benefit.

**Table 1**  
**Clinical Characteristics of Patients (N=37)**

Characteristic		Number of Patients (%)
Sex	Female	18 (49%)
	Male	19 (51%)
Histology	Adenocarcinoma	22 (60%)
	Adenocarcinoma with BAC features	9 (24%)
	Squamous cell carcinoma	3 (8%)
	Poorly differentiated carcinoma	2 (5%)
	Large cell neuroendocrine	1 (3%)
Ethnicity	White	25 (68%)
	African American	5 (13%)
	Asian	4 (11%)
	Hispanic/Latino	3 (8%)
ECOG performance status	PS 1	5 (14%)
	PS 0	32 (86%)
Age	Median age	61 yrs
	range	30-85 yrs
No. of prior chemotherapy and targeted regimens	0	0 (0%)
	1	17 (46%)
	2	4 (11%)
	3	8 (22%)
	4	3 (8%)
	5	3 (8%)
	6	2 (5%)
Previous therapy	platinum (Cisplatin/Carboplatin)	33 (89%)
	Taxane (Docetaxel/Paclitaxel)	29 (78%)
	Erlotinib/Gefitinib	16 (43%)
	Bevacizumab	15 (40%)
	Pemetrexed	13 (35%)

Table 2

## KRAS and EGFR mutation status

Patient	Sex (M/F)	Ethnicity	Smoking Status	Histology	KRAS	EGFR
1	F	White	Never	Adenocarcinoma	N/A	N/A
2	M	White	Smoker	Adeno-BAC	WT	WT
3	F	Asian	Never	Adenocarcinoma	G12C	WT
4	M	White	Smoker	Squamous cell	WT	WT
5	M	White	Smoker	Squamous cell	WT	N/A
6	M	Hispanic	Smoker	Adenocarcinoma	WT	WT
7	M	White	Smoker	Adenocarcinoma	G13D	WT
8	M	White	Smoker	Adeno-BAC	G12C	WT
9	F	Hispanic	Smoker	NSCLC	WT	N/A
10	M	White	Smoker	Large cell	WT	WT
11	M	White	Never	Adenocarcinoma	WT	N/A
12	F	Black	Never	Adenocarcinoma	WT	L858R
13	F	White	Smoker	Squamous cell	WT	WT
14	M	White	Smoker	Adenocarcinoma	WT	N/A
15	M	Asian	Smoker	Adenocarcinoma	N/A	N/A
16	F	White	Smoker	Adeno-BAC	WT	WT
17	M	Black	Smoker	Poorly differentiated	WT	N/A
18	F	White	Never	Adenocarcinoma	G12D	N/A
19	F	White	Smoker	Adeno-BAC	G12V	WT
20	M	White	Smoker	Adeno-BAC	WT	WT
21	M	Black	Smoker	Adeno-BAC	WT	N/A
22	M	White	Smoker	Adenocarcinoma	Q61H	WT
23	F	Asian	Never	Adenocarcinoma	WT	Ex 19del15
24	F	White	Smoker	Adenocarcinoma	WT	N/A
25	M	White	Never	Adeno-BAC	G12C	WT
26	M	White	Smoker	Adenocarcinoma	N/A	N/A
27	F	Asian	Never	Adenocarcinoma	WT	Ex 19del15

Patient	Sex (M/F)	Ethnicity	Smoking Status	Histology	KRAS	EGFR
28	F	White	Smoker	Adeno-BAC	G12D	WT
29	F	White	Smoker	Adenocarcinoma	G12C	WT
30	F	Hispanic	Never	Adenocarcinoma	WT	N/A
31	M	White	Smoker	Adenocarcinoma	WT	L858R
32	M	Black	Never	Adenocarcinoma	WT	Ex19del18
33	F	Black	Never	Adenocarcinoma	WT	N/A
34	F	White	Smoker	Adenocarcinoma	WT	WT
35	F	White	Smoker	Adenocarcinoma	G12C	N/A
36	F	White	Smoker	Poorly differentiated	WT	WT
37	F	White	Smoker	Adeno-BAC	G12C	WT

Adeno-BAC: Adenocarcinoma with bronchioloalveolar features; WT, wildtype; N/A, not available



**Toxicity****Table 3**

Adverse Event	Any Grade		Grade 3-4	
	Number	%	Number	%
Palmar-plantar erythrodysesthesia	22	59	6	16
Rash/desquamation	20	54	2	5
Hypertension	16	43	6	16
Diarrhea	14	38	1	3
Fatigue	11	30	3	8
Anorexia	9	24	0	0
Nausea	8	22	1	3
Dry skin	7	19	0	0
Mucositis	7	19	1	3
Pruritus	7	19	1	3