The BATTLE 2 Study: A Biomarker-Integrated Targeted Therapy Study in Previously Treated Patients with advanced Non-Small Cell Lung Cancer

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Supplemental Data

SUBJECT ELEGIBILITY

Inclusion Criteria. The following inclusion criteria must be met for entry into the study. Criteria below in bold print must be met before patient is eligible for biopsy to be performed. Subjects will also be clinically eligible for their specific treatment arm:

1) The subject has a diagnosis of pathologically confirmed NSCLC by tumor biopsy and/or fine-needle aspiration.
2) The subject has a diagnosis of either advanced, incurable stage IIIB or stage IV NSCLC, and failed at least one front-line metastatic NSCLC chemotherapy regimen, or EGFR TKI. (Subjects who have failed adjuvant or locally advanced therapy within 6 months are also eligible to participate in the study).
3) The subject has measurable NSCLC (subjects with active new disease growth in previously irradiated site are eligible).
4) The subject's ECOG performance status is ≤ 2 at study entry.
5) The subject has biopsy accessible tumor.
6) The subject has adequate hematologic function as defined by an absolute neutrophil count (ANC) ≥ 1,500/mm^3, platelet count ≥ 100,000/mm^3, WBC ≥ 3,000/ mm^3, and hemoglobin ≥ 9 g/dL.
7) The subject has adequate hepatic function as defined by a total bilirubin level ≤ 1.5 X the upper limit of normal (ULN) (2.5 X ULN for patients with Gilbert's disease is allowed), and alkaline phosphatase, AST and ALT ≤ 2.5 X the upper limit of normal or </= 5.0 x ULN if liver metastases are present.
8) Serum creatinine clearance >50ml/min, either by Cockcroft-Gault formula or 24-hour urine collection analysis.
9) If subject has brain metastasis, they must have been stable (treated and/or asymptomatic) and off steroids for at least 2 weeks.
10) The subject is ≥ 18 years of age.
11) The subject has signed informed consent.

12) The subject is eligible if disease free from a previously treated malignancy, other than a previous NSCLC, for greater than two years. Subjects with a history of prior basal cell carcinoma of the skin or pre-invasive carcinoma of the cervix are allowed.
13) Women of childbearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) for the duration of the study and for 30 days after the last dose of study drug. Childbearing potential will be defined as women who have had menses within the past 12 months, who have not had tubal ligation, hysterectomy or bilateral oophorectomy. Should a woman become pregnant or suspect that she is pregnant while participating in this study, she should inform her treating physician immediately. The subject, if a man, agrees to use effective contraception or abstinence for the duration of the study and for 3 months after the last dose of study drug.
14) Subject is able to swallow capsules and has no surgical or anatomical condition that will preclude the subject from swallowing and absorbing oral medications on an ongoing basis.

Exclusion Criteria. A subject meeting any of the following criteria is not eligible to participate in this study:

1) The subject has received prior chemotherapy, surgery, or radiotherapy within 3 weeks of initiating study drug, or 4 weeks for bevacizumab or investigational drug or 72 hours for erlotinib or the subject has not recovered (</= Grade 1) from side effects of the prior therapy (localized palliative radiotherapy within 2 weeks is allowed).
2) The subject has undergone prior thoracic or abdominal surgery within 30 days of study entry, excluding prior diagnostic biopsy.
3) The subject has cardiac conditions as follows: uncontrolled hypertension BP > 140/90 despite optimal therapy, uncontrolled angina, ventricular arrhythmias, or congestive heart failure New York Heart Association Class II or above (See NYHA in Appendix V), baseline LVEF ≤ 50%. (See LVEF algorithm in Appendix VII), prior or current cardiomyopathy, atrial fibrillation with heart rate >100 bpm, unstable ischaemic heart disease (MI within 6 months prior to starting treatment, or angina requiring use of nitrates more than once weekly).
4) The subject has neuropathy ≥ Grade 2.

5) The subject is pregnant (confirmed by serum β-HCG if applicable) or is breastfeeding. In the event of inconclusive pregnancy test results, the attending physician will have final determination of pregnancy status.
6) Subjects will be excluded for other concurrent severe and/or uncontrolled medical disease which could compromise participation in the study (i.e., uncontrolled diabetes, severe infection requiring active treatment, severe malnutrition, chronic severe liver or renal disease).
7) Refractory nausea and vomiting, chronic gastrointestinal diseases (e.g. inflammatory bowel disease), or significant bowel resection that would preclude adequate absorption.
8) Subjects with poorly controlled diabetes (HbA1c >8%) are excluded.
9) Subjects whose tumor harbors the EML4-ALK fusion gene are excluded unless the patient has failed treatment with Anaplastic Lymphoma Kinase (ALK) inhibitor.
10) Subjects are excluded if they have QTc prolongation >450 msec (Bazett's Formula) for males or >470 ms for females on screening or other factors that increase the risk of QT prolongation or arrhythmic events (*e.g.*, heart failure, hypokalemia, family history of long QT interval syndrome) including heart failure that meets New York Heart Association (NYHA) class II or above or require use of a concomitant medication that can prolong the QT interval. (See Appendix VIII)
11) Subjects who have abnormal K+ or Mg++ levels will be excluded if these levels cannot be corrected to within normal range with adequate supportive treatment prior to study drug initiation.
12) Subjects whose tumor harbors an EGFR mutation are excluded unless the subject failed treatment with EGFR TKIs in which case the subject can be randomized to Arms 2, 3, and 4.

Drug Specific Eligibility Criteria based on Treatment Arms

1) Subjects are excluded from the erlotinib monotherapy arm if they have progressed on prior EGFR TKI therapy; from the AKT inhibitor arm(s) if they have received prior AKT inhibitor therapy; from the MEK inhibitor arm if they have received prior MEK inhibitor therapy; and from Sorafenib arm if they have previously received the drug or have prior history of clinically significant hemoptysis or bleeding diathesis as per principal investigator judgment.

TISSUE BIOPSY AND BIOMARKER METHODOLOGY

Tumor tissue biopsy. Tissue biopsies (FNA and CNB) were performed at baseline and at the end of Cycle 2 (optional) while participating in the study. In addition, archival diagnostic tissue samples (optional) were also collected for biomarker analysis. Tissue could be obtained via image-guided core biopsy or other core biopsy methods.

Image-guided core biopsy. Study subjects underwent image-guided core biopsy after being fasting in regard to solid food for a minimum of six hours and fasting for oral medications and small amounts of liquids for two hours prior to the procedure. The participant was monitored with continuous electro-cardiographic, respiratory, and oximetric monitoring, with intermittent blood pressure monitoring. Approximately 4-5 core biopsies of the tumor were performed. Specimens were used to analyze biomarkers.

Biomarker Methodology

To evaluate molecular biomarkers using the formalin-fixed paraffin-embedded (FFPE) CNB tissue specimens, 13 5-μm histology sections were obtained, as follows: 1) H&E histology analysis (n=1 section); 2) DNA extraction for mutation analyses (*EGFR*, *KRAS*; n=1 or 2 sections); 3) FISH analysis (*ALK* break-apart FISH; n=2 sections); and 4) Sequenom™ mutation analysis of 9 genes. The first fresh core was assigned to gene expression profiling and the second fresh core for DNA targeted NGS[11]. All specimens were assigned an identification number linked to the clinical trial identification number for subsequent processing in the laboratory. Certification of the presence of adequate tumor tissue in the FFPE tissue specimens by histologic examination was performed within 24 to 48 hours, and analysis of the 3 markers required for patient randomization, namely *EGFR*, *ALK* and *KRAS* was performed, completed, and reported, in most cases, within 14 days..

Microdissection and DNA extraction. Malignant tumor cells were manually microdissected from 4 sequential 5-μm-thick H&E- stained FFPE histology sections. DNA was extracted using 25 μL of Pico Pure TM DNA Extraction solution (Arcturus) containing proteinase K and

incubated at 65°C for 24 hours. Subsequently, proteinase K inactivation was performed by heating samples at 95°C for 10 minutes.

Mutation analysis. Mutations of *EGFR* (exons 18–21), *KRAS* (exons 1, codons 12 and 13; and exon 2, codon 61), were studied using DNA extracted from micro- dissected FFPE tumor cells. The DNA sequences were PCR amplified using primers as previously described (1) Each PCR amplification was performed in 30 μL of volume containing 2 μL of DNA (approximately 100 ng of genomic DNA), 0.3 of μM for- ward and reverse primers, and 15 μL of HotStarTaq (1.5 units of DNA polymerase) Master Mix (Qiagen) for 40 cycles at 94°C for 30 seconds, for 30 seconds at the primer pairs' annealing tempera- ture (Supplementary Table S1), and at 72°C for 45 seconds, followed by 7 minutes of extension at 72°C. All PCR products were directly sequenced using Applied Biosystems PRISM dye termina- tor cycle sequencing method (Perkin-Elmer). All sequence variants were confirmed by independent PCR amplifications from at least 2 independent DNA extractions, and sequenced in both directions.

***ALK* gene fusion using FISH.** For *ALK*, gene copy number per cell was analyzed as follows: Unstained 4-μm sections of FFPE tumor tissue were used for dual-color FISH assays using the LSI *ALK* (anaplastic lymphoma kinase) Dual Color, Break-Apart Rearrangement Probe (Abbott Molecular). This probe set includes a 250-kb DNA fragment telomeric to *ALK* (3′ end) labeled in SpectrumOrange and a 300-kb fragment centromeric to *ALK* (5′ end) labeled in SpectrumGreen. The hybridization essay was performed according to a protocol previously described[10] (2). Signals were enumerated in at least 50 tumor nuclei per core using an epifluorescence microscope with single interference filters sets for green (FITC), red (Texas red), and blue (4′,6-diamidino-2-phenylindole) as well as dual (red/green) and triple (blue, red, green) band-pass filters.

DNA targeted next generation of sequencing (NGS) and data processing. Massively parallel sequencing of all coding exons from 287 cancer-related genes and selected introns from 19 genes was based on the FoundationOne™ test (Foundation Medicine®) and was performed as previously described [33]. Briefly, ≥50ng of tumor DNA extracted from FFPE tumor biopsy was used for sequencing library construction and hybridization-based targeted capture of 4557 exons

and 47 introns corresponding to known cancer-related genes, prior to 49x49 paired-end sequencing on the Illumina HiSeq2000 platform to >500x mean coverage. Subsequent analysis of DNA sequence data was performed according to an in-house pipeline that facilitates accurate identification of base substitutions, short insertions/deletions (indels), focal amplifications, bi-allelic deletions and specific gene fusions.

Figure legend

Online Figure 1. (A) Progression-free survival for all patients (B) Overall survival for all patients. (C) Progression-free survival by KRAS mutation status for all patients (log-rank test *P*=0.89) (D) Overall survival by KRAS mutation status for all patients (log-rank test *P*=0.56)

Online Figure 2. Progression-free survival among epithelial and mesenchymal tumors by arm. (A) Arm1 (log rank test, *P*=0.49), (B) Arm 2 (*P*=0.99) (C) Arm 3 (*P*=0.028) Progression-free survival was superior for patients with mesenchymal tumors. (D) Arm 4. (*P*=0.20). E;epithelial tumors, M: mesenchymal tumors.

Online Figure 3: Overall survival among epithelial and mesenchymal tumors by arm. (A) Arm1 (log rank test, *P*=0.99), (B) Arm 2 (*P*=0.72), (C) Arm 3 *(P*=0.18), (D). Arm 4 (*P*=0.014). Among patients treated with sorafenib overall survival was superior for patients with mesenchymal tumors. E; epithelial tumors, M: mesenchymal tumors

Online Figure 4. EMT signature among patients with *KRAS* mutations. (A).Overall survival was superior for patients with mesenchymal tumors (*P*=0.02). (B) Overall survival among patients with *KRAS* wild type treated with sorafenib (*P*=0.40). (C)Among patients with *KRAS* mutations treated with sorafenib , overall survival was superior for patients with mesenchymal tumors (*P*=0.01) . E;epithelial tumors, M: mesenchymal tumors.

Online Figure 5. Progression-free survival among KRAS wild-type and mutated patients by arm. (A) Arm1 (log rank test, *P*=0.33) (B) Arm 2 (*P*=0.07) (C) Arm 3 (*P*=0.31) (D) Arm 4. (*P*=0.60).

Online Figure 6: Overall survival among KRAS wild-type and mutated patients by arm. (A) Arm1 (log rank test, *P*=0.02) (B) Arm 2 (*P*=0.96), (C) Arm 3 *(P*=0.69), (D) Arm 4 (*P*=0.06).

Online Table 1. 8-week disease control status by patient characteristics

Variable	Level	No 8-wk DC	With 8-wk DC	Total	*P*-value
Age group	49 or less	6 (35.3%)	11 (64.7%)	17	0.26
	50-59	30 (47.6%)	33 (52.4%)	63	
	60-69	32 (44.4%)	40 (55.6%)	72	
	70+	21 (61.8%)	13 (38.2%)	34	
Gender	Female	52 (51.0%)	50 (49.0%)	102	0.38
	Male	37 (44.0%)	47 (56.0%)	84	
White race	White	78 (47.6%)	86 (52.4%)	164	0.99
	Others	11 (50.0%)	11 (50.0%)	22	
Smoking status	Current	11 (36.7%)	19 (63.3%)	30	0.37
	Former	56 (48.7%)	59 (51.3%)	115	
	Never	22 (53.7%)	19 (46.3%)	41	
KRAS	Mut	24 (46.2%)	28 (53.8%)	52	0.87
	WT	65 (48.5%)	69 (51.5%)	134	
Prior erlotinib therapy	No	53 (46.1%)	62 (53.9%)	115	0.55
	Yes	36 (50.7%)	35 (49.3%)	71	
ECOG PS	0	13 (76.5%)	4 (23.5%)	17	0.03
	1	66 (46.8%)	75 (53.2%)	141	
	2	10 (35.7%)	18 (64.3%)	28	
# of prior therapies	1	16 (57.1%)	12 (42.9%)	28	0.54
	2	19 (44.2%)	24 (55.8%)	43	
	3	21 (47.7%)	23 (52.3%)	44	
	4	15 (40.5%)	22 (59.5%)	37	
	5	6 (40.0%)	9 (60.0%)	15	
	6+	12 (63.2%)	7 (36.8%)	19	
Histology	Adeno	67 (48.6%)	71 (51.4%)	138	0.49
	Squamous	17 (51.5%)	16 (48.5%)	33	
	Others	5 (33.3%)	10 (66.7%)	15	

Online Table 2. Multivariable logistic regression model for 8-wk DC

Parameter		DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept		1	-0.1306	1.3279	0.0097	0.9216
Treatment	Arm1 vs. Arm4	1	-0.9939	0.6036	2.7118	0.0996
Treatment	Arm2 vs. Arm4	1	0.0844	0.4515	0.0350	0.8516
Treatment	Arm3 vs. Arm4	1	0.1264	0.3757	0.1132	0.7366
Age		1	0.0249	0.0183	1.8545	0.1733
# of prior therapies		1	-0.0155	0.0944	0.0269	0.8698
ECOG PS		1	-0.9752	0.3568	7.4722	0.0063
Gender	Female vs. male	1	0.3741	0.3313	1.2749	0.2589
White race	Others vs. white	1	0.3355	0.5101	0.4325	0.5108
Smoking status	Current vs. never	1	-0.3665	0.5401	0.4603	0.4975
Smoking status	Former vs. never	1	-0.1517	0.4025	0.1420	0.7063
KRAS	Mut+ vs. wt	1	-0.0368	0.3770	0.0095	0.9223
Histology	Adeno vs. Squamous	1	-0.4978	0.4736	1.1048	0.2932
Histology	Others vs. Squamous	1	-0.9224	0.7069	1.7028	0.1919

Online Table 3. Multivariable Cox regression model for PFS

Parameter		DF	Parameter Estimate	Standard Error	Chi-Square	Pr > Chi Sq	Hazard Ratio
Treatment	Arm1 vs. Arm4	1	0.31394	0.27129	1.3392	0.2472	1.369
Treatment	Arm2 vs. Arm4	1	-0.29079	0.22995	1.5991	0.2060	0.748
Treatment	Arm3 vs. Arm4	1	-0.01551	0.18878	0.0068	0.9345	0.985
Age		1	0.00353	0.00866	0.1662	0.6835	1.004
# of prior therapies		1	-0.00179	0.04456	0.0016	0.9680	0.998
ECOG PS		1	0.30330	0.16810	3.2556	0.0712	1.354

Parameter		DF	Parameter Estimate	Standard Error	Chi-Square	Pr > Chi Sq	Hazard Ratio
Gender	Female vs. male	1	-0.05392	0.16625	0.1052	0.7457	0.948
White race	Others vs. white	1	-0.10142	0.23228	0.1906	0.6624	0.904
Smoking status	Current vs. never	1	0.02192	0.26569	0.0068	0.9343	1.022
Smoking status	Former vs. never	1	0.0005402	0.19987	0.0000	0.9978	1.001
KRAS	Mut+ vs. wt	1	0.06432	0.19181	0.1125	0.7374	1.066
Histology	Adeno vs. Squamous	1	-0.00622	0.22234	0.0008	0.9777	0.994
Histology	Others vs. Squamous	1	0.38767	0.30683	1.5964	0.2064	1.474

Online Table 3. Multivariable Cox regression model for OS

Parameter		DF	Parameter Estimate	Standard Error	Chi-Square	Pr > Chi Sq	Hazard Ratio
Treatment	Arm1 vs. Arm4	1	-0.28645	0.30769	0.8667	0.3519	0.751
Treatment	Arm2 vs. Arm4	1	-0.26790	0.23494	1.3003	0.2542	0.765
Treatment	Arm3 vs. Arm4	1	0.04332	0.20463	0.0448	0.8323	1.044
Age		1	0.00843	0.00925	0.8315	0.3618	1.008
# of prior therapies		1	0.0003273	0.04963	0.0000	0.9947	1.000
ECOG PS		1	0.65636	0.19043	11.8799	0.0006	1.928
Gender	Female vs. male	1	-0.21257	0.17609	1.4572	0.2274	0.809
White race	Others vs. white	1	-0.03934	0.25668	0.0235	0.8782	0.961
Smoking status	Current vs. never	1	0.51291	0.28095	3.3329	0.0679	1.670
Smoking status	Former vs. never	1	0.12453	0.20679	0.3626	0.5470	1.133
KRAS	Mut+ vs. wt	1	-0.12759	0.20239	0.3975	0.5284	0.880

Parameter		DF	Parameter Estimate	Standard Error	Chi-Square	Pr > Chi Sq	Hazard Ratio
Histology	Adeno vs. Squamous	1	0.06150	0.23942	0.0660	0.7973	1.063
Histology	Others vs. Squamous	1	-0.18236	0.33362	0.2988	0.5846	0.833

Online Table 4. Summary of 8 week disease control rate (DCR) by *KRAS* mutation status

Arm	DCR	*KRAS* WT	*KRAS* Mut	Total	*P*-value
Overall	8wkDC	65(49%)	24(46%)	89(48%)	0.87
	No8wkDC	69(51%)	28(54%)	97(52%)	
Arm 1	8wkDC	5(36%)	1(20%)	6(32%)	0.99
	No8wkDC	9(60%)	4(80%)	13(65%)	
Arm 2	8wkDC	16(57%)	2(25%)	18(50%)	0.23
	No8wkDC	12(43%)	6(75%)	18(50%)	
Arm 3	8wkDC	24(49%)	13(62%)	37(53%)	0.43
	No8wkDC	25(51%)	8(38%)	33(47%)	
Arm 4	8wkDC	20(47%)	8(44%)	28(46%)	0.99
	No8wkDC	23(53%)	10(56%)	33(54%)	

Online Table 5 . Testing interaction between *KRAS* mutation and erlotinib-containing therapy with respect to PFS

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > Chi Sq
Erlotinib-containing therapy	1	-0.28486	0.18205	2.4483	0.1177

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > Chi Sq
KRAS mutation	1	-0.20690	0.19304	1.1488	0.2838
Interaction	1	0.91670	0.37312	6.0361	0.0140

Online Table 5.Testing interaction between Kras mutation and erlotinib-containing therapy with respect to OS

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > Chi Sq
Erlotinib-containing therapy	1	-0.42548	0.19693	4.6680	0.0307
KRAS mutation	1	-0.31968	0.20939	2.3310	0.1268
Interaction	1	0.66623	0.39289	2.8755	0.0899

Online Table 6. Relationship between EMT score and 8-week DC

Variable	DC8wk	NObs	# of missing	Mean +/- Std, Median (Min, Max)	*P*-value
EMT score	8-wk DC	89	29	0.06 +/- 0.64, 0.02 (-1.1, 1.8)	0.72
	No 8-wk DC	97	27	0.07 +/- 0.84, -0.1 (-1.7, 2.2)	

Online Table 7. Relationship between KRAS mutation (G12C, G12D, G12V) and 8-week DC

DC8wk	KRAS mutation			
	G12C	G12D	G12V	Total
8-wk DC	6 (46%)	7 (58%)	4 (44%)	17
No 8-wk DC	7 (54%)	5 (42%)	5 (56%)	17
Total	13	12	9	34
Not evaluable	0	1	1	2

Fisher's *P*-value = 0.83

Online Table 7. Relationship between *KRAS* mutation (G12C, G12D, G12V) and PFS

KRAS	Total	Failed	Censored	Percent Censored
G12C	13	12	1	8%
G12D	13	13	0	0%
G12V	10	9	1	10%
Total	36	34	2	6%

Log-rank *P*-value = 0.28

Online Table 7. Relationship between *KRAS* mutation (G12C, G12D, G12V) and OS

KRAS	Total	Failed	Censored	Percent Censored
G12C	13	11	2	15%
G12D	13	11	2	15%
G12V	10	7	3	30%
Total	36	29	7	19%

Log-rank *P*-value = 0.98