**SUPPLEMENTARY METHODS**

***EGFR* mutation analysis**

***cobas****® testing*

The **cobas**® EGFR tissue test has been analytically validated for performance characteristics such as sensitivity, specificity, and reproducibility. It can identify 41 different mutations in the *EGFR* gene including G719X, L858R, S768I, 5 insertions in exon 20, and 29 deletions in exon 19. The total DNA input used for each test was 150 ng. Analysis and result reporting was fully automated. The **cobas**® EGFR plasma test detects the same mutations as the **cobas**® EGFR tissue test and additionally identifies L861Q. The total DNA input used for each test was 75 uL from a 100 uL eluate. Analysis of results was performed with the EGFR Plasma Analysis Package Software (Roche Molecular Systems, Inc., in development). Investigators conducting the **cobas**® testing were blinded to the BEAMing results.

*BEAMing*

BEAMing uses digital PCR followed by flow cytometry: A pre-amplification step is conducted with a high-fidelity DNA polymerase. An aliquot of the pre-amplified product is then used to perform single molecule PCR on magnetic beads in water-in-oil emulsions. Beads with mutant products are distinguished from wild-type using allele-specific, fluorescently labeled probes and the bead populations are counted by flow cytometry.

For the present study, primers and probes were designed to interrogate the T790M and L858R substitution mutations in *EGFR*, as well as several of the most common deletions in exon 19 including E746\_A750del [COSM6223 and 6225], E746\_S752>V, L747\_A750>P, L747\_T751del, and L747\_P753>S, which account for approximately 90% of all exon 19 deletions (1). Prior to the study, validation was performed to establish the performance characteristics of the assays. The Limit of Detection of all *EGFR* assays was determined to be 0.02% (the fraction of mutant beads relative to wild-type beads). The total number of genome equivalents (GE) of DNA isolated from plasma was quantified with a modified LINE-1 quantitative real-time PCR assay, as described previously (2). Mutant plasma copy numbers were obtained by multiplying the percentage of mutant beads by the number of GE. The investigators who performed the BEAMing analysis were blinded to the **cobas**® tissue and plasma results.

**Statistical Analysis**

For the concordance analysis, positive percent agreement and negative percent agreement are used instead of the corresponding terms sensitivity and specificity to indicate that the tumor result is not a gold standard comparator due to known tumor heterogeneity of the T790M resistance mutation.

**References**

1. Chung KP, Wu SG, Wu JY, Yang JC, Yu CJ, Wei PF, et al. Clinical outcomes in non-small cell lung cancers harboring different exon 19 deletions in EGFR. Clin Cancer Res. 2012;18:3470-7.

2. Rago C, Huso DL, Diehl F, Karim B, Liu G, Papadopoulos N, et al. Serial assessment of human tumor burdens in mice by the analysis of circulating DNA. Cancer Res. 2007;67:9364-70.

**Supplementary Table S1. Clinical characteristics of patients in the present analysis**

|  |  |  |
| --- | --- | --- |
|  | **Observational Study (n=80)** | **CO-1686 Phase 1 (n = 94)e** |
| **Characteristics** |  |  |
| **Age (y)** |  |  |
| **Median** | **61**  | **61** |
| **Range** | **27-83** | **29-83** |
| **Sex** |  |  |
| **Female** | **56 (70.0%)** | **72 (76.6%)** |
| **Male** | **24 (30.0%)** | **21 (22.3%)** |
| **Race** |  |  |
| **Asian** | **42 (52.5%)** | **15 (16.0%)** |
| **Black/African American** | **3 (3.8%)** | **2 (2.1%)** |
| **White** | **35 (43.7%)** | **71 (75.5%)** |
| **Other** |  | **4 (4.3%)** |
| **Missing** | **0** | **2 (2.1%)** |
| **Histology (at diagnosis)** |  |  |
| **Adenocarcinoma** | **68 (85.0%)** | **83 (88.3%)** |
| **Other a** | **12 (15.0%)** | **11 (11.7%)** |
| **ECOG at study entry** |  |  |
| **0** | ***Not available*** | **23 (24.5%)** |
| **1** |  | **70 (74.5%)** |
| **Number of previous therapies** |  |  |
| **0** | **37 (46.2%)** | **0 (0%)** |
| **1** | **20 (25.0%)** | **12 (12.8%)** |
| **2** | **12 (15.0%)** | **15 (16.0%)** |
| **3** |  **5 (6.2%)** | **14 (14.9%)** |
| **4** |  **3 (3.8%)** | **18 (19.1%)** |
| **5+** |  **3 (3.8%)** | **34 (36.2%)** |
| **Biopsy methodb** |  |  |
| **Tissue samplesc** **(Fraction with valid genotyping)**  | **28 (28/28 valid)**  | **66 (55/66 valid)** |
| **Cytology samplesd****(Fraction with valid genotyping)** | **19 (16/19 valid)** | **8 (7/8 valid)** |
| **Unknown/ Data not available****(Fraction with valid genotyping)** | **4 (4/4 valid)** | **20 (10/20 valid)** |
| **a Other histology subtypes for the observational study: large cell carcinoma (2); Not Otherwise Specified (2); Bronchoalveolar carcinoma (1); NSCLC (4); metastatic papillary malignant neoplasm (1); unspecified/unconfirmed (2)** **Other histology subtypes for the Phase 1 study: Bronchoalveolar carcinoma (4); large cell carcinoma (1); squamous (1); metastatic carcinoma (2); non mucinous carcinoma (1); missing (2)****b Biopsy material was not provided for 29 patients in the Observational Study.****c Tissue samples included CNB, resection, excisional biopsy and bronchoscopy.****d Cytology samples included FNA, PE, and BAL.****e At the time of data analysis, one patient had missing status for “sex”, two patients had missing status for “race”, one patient had missing status for ECOG score, and one patient had missing status for “Number of previous therapies” in the ph1 clinical database.** |

**Supplementary Table S2. Plasma/tissue concordance results for del19 and L858R mutations**

 **cobas® Tumor Test**

 **Mutation Status**

 **Mutation Mutation Total**

  **Positive Negative**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  **cobas® Plasma Test****Del19** |  |  |  |  |
|  Mutation Positive | 29 |  0 | 29 |  |
|  Mutation Negative | 12 | 54 | 66 |  |
| Total | 41 | 54 | 95 |  |
| **L858R** |  |  |  |  |
|  Mutation Positive | 21 | 0 | 21 |  |
|  Mutation Negative | 6 | 68 | 5 |  |
| Total | 27 | 68 | 95 |  |

**Del19 (tumor as reference) 95% CI**

 PPA: 71% (29/41) [54%, 84%]

 NPA: 100% (54/54) [93%, 100%]

 OPA: 87% (83/95) [79%, 93%]

**L858R (tumor as reference) 95% CI**

PPA: 78% (21/27) [58%, 91%]

 NPA: 100% (68/68) [95%, 100%]

 OPA: 94% (89/95) [87%, 98%]

**Comparison between Del19 and L858R (Fisher’s Exact Test)**

PPA: p = 0.58

 NPA: p = 1.00

 OPA: p = 0.21

CI = Confidence Interval

**Supplementary Table S3. *EGFR* mutations identified in tissue and plasma by the cobas® EGFR mutation test**

|  |
| --- |
| **Tumor *EGFR* Mutation** |
| **Plasma *EGFR* Mutation** |  | Ex19 del only | L858R only | Ex20 ins only | L858R and Ex20 ins | S768I and G719X | L858R, S768I and T790M | L861Q, G719X and T790M | Ex19 del and T790M | L858R and T790M | T790M | Negative |
| Ex19 del only | **18** | 0 | 0 | 0 | 0 | 0 | 0 | **1** | 0 | 0 | 0 |
| L858R only | 0 | **8** | 0 | 0 | 0 | 0 | 0 | 0 | **1** | 0 | 0 |
| Ex20 ins only | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| L858R and Ex20 ins | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| S768I and G719X | 0 | 0 | 0 | 0 | **1** | 0 | 0 | 0 | 0 | 0 | 0 |
| L858R, S768I and T790M | 0 | 0 | 0 | 0 | 0 | **1** | 0 | 0 | 0 | 0 | 0 |
| L861Q, G719X and T790M | 0 | 0 | 0 | 0 | 0 | 0 | **1** | 0 | 0 | 0 | 0 |
| Ex19 del and T790M | **1** | 0 | 0 | 0 | 0 | 0 | 0 | **9** | 0 | 0 | 0 |
| L858R and T790M | 0 | **1** | 0 | 0 | 0 | 0 | 0 | 0 | **10** | 0 | 0 |
| T790M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Negative | **6** | **2** | **1** | **1** | 0 | 0 | 0 | **6** | **3** | **1** | **23** |
|  | **Totals** | **25** | **11** | **1** | **1** | **1** | **1** | **1** | **16** | **14** | **1** | **23** |

**Supplementary Table S4. Concordance between tumor and BEAMing plasma *EGFR* status**

 **cobas® Tumor Test**

 **Mutation Status**

 **Mutation Mutation Inadequate Total**

  **Positive Negative Tissue**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  **BEAMing plasma****Activating Mutation** |  |  |  |  |  |
|  Mutation Positive |  49a | 1 | 9 | 59 |  |
|  Mutation Negative | 11 | 2 | 5 | 18 |  |
| Total | 60 | 3 | 14 | 77 |  |
| **T790M** |  |  |  |  |  |
|  Mutation Positive | 33 | 9 | 9 | 51 |  |
|  Mutation Negative | 12 | 9 | 5 | 26 |  |
| Total | 45 | 18 | 14 | 77 |  |

**Activating mutations (tumor as reference) 95% CI**

 Positive Percent Agreement: 82% (49/60) [70%, 90%]

 Negative Percent Agreement: 67% (2/3) [9%, 99%]

 Overall Percent Agreement: 81% (51/63) [69%, 90%]

**T790M (tumor as reference) 95% CI**

Positive Percent Agreement: 73% (33/45) [58%, 85%]

 Negative Percent Agreement: 50% (9/18) [26%, 74%]

 Overall Percent Agreement: 67% (42/63) [54%, 78%]

a **cobas**® tumor test results were unavailable for two patients: one tested by *therascreen*® EGFR test and one tested by SNaPshot

CI = Confidence Interval

**Supplementary Table S5. Overall concordance between cobas® and BEAMing EGFR mutation tests (n = 35: 34 patients from the Phase 1 study and 1 patient from the Observational study)**



* **ND = not detected**
* **a local investigator *EGFR* test result; tumor submitted for cobas® test was unevaluable**
* **b S768I was also detected but was not assessed by BEAMing**
* **Orange shading represents discordant cases between plasma BEAMing and plasma cobas® test results**

**Supplementary Table S6. Platform Comparison of T790M tests in a sample set enriched for low copy plasma cases**

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* **ND = Not Detected**
* **Orange shading represents T790M tumor-positive cases where T790M was not detected in plasma by any platform.**

**Supplementary Table S7. *EGFR* mutation detection by cobas® plasma test and NSCLC disease classification (n = 72)**

 ***EGFR*** **Mutation Disease Patients Subset with Percentage P valueb**

 **classification with mutationa mutation in plasma**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Activating Mutations | M1a/M0 | 24 | 10 | 42% |  |
|   | M1b | 51 | 46 | 90% | <0.001 |
|   |  |  |  |  |  |
| T790M | M1a/M0 | 14 | 2 |  14% |  |
|  | M1b | 22 | 21 |  95% | <0.001 |

a Includes patients with an *EGFR* mutation detected in tissue only, plasma only, or both tissue and plasma. Three

patients had two activating mutations and each was counted as a separate mutation.

b Fisher’s exact test used for comparisons

**Supplementary Figure S1**



**Supplementary Figure S1.** Tumor burden is a weak predictor of ability to detect *EGFR* mutations in plasma. A, the sum of the target lesions by RECIST for patients with no detectable *EGFR* activating mutations in plasma compared to those with mutations detected in plasma. B, the same analysis as performed in (A) for T790M. The bars in the graphs represent median target lesion values, and the p values were derived from a Mann-Whitney test.

 **Supplementary Figure S2**



**Supplementary Figure S2.** T790M to activating mutation ratio in plasma is associated with depth of response to rociletinib. T790M to activating mutation ratios are expressed as a percentage. SLD is the sum of the longest diameters of target lesions as defined by RECIST 1.1 criteria. The Spearman correlation coefficient is *r*. Activating mutation levels in plasma were required to be at least 10 molecules/mL for inclusion in the analysis.No relationship was found between clinical response and the absolute plasma copy number of either T790M or the activating mutation (not shown).