EGFR mutation detection in ctDNA from NSCLC patient plasma: A cross-platform comparison of leading technologies to support the clinical development of AZD9291

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ABSTRACT

Objectives: To assess the ability of different technology platforms to detect epidermal growth factor receptor (EGFR) mutations, including T790M, from circulating tumor DNA (ctDNA) in advanced non-small cell lung cancer (NSCLC) patients.

Materials and methods: A comparison of multiple platforms for detecting EGFR mutations in plasma ctDNA was undertaken. Plasma samples were collected from patients entering the ongoing AURA trial (NCT01802632), investigating the safety, tolerability, and efficacy of AZD9291 in patients with EGFR-sensitizing mutation-positive NSCLC. Plasma was collected prior to AZD9291 dosing but following clinical progression on a previous EGFR-tyrosine kinase inhibitor (TKI). Extracted ctDNA was analyzed using two non-digital platforms (cobas® EGFR Mutation Test and therascreen® EGFR amplification refractory mutation system assay) and two digital platforms (Dropet DigitalTM PCR and BEAMing digital PCR [dPCR]).

Results: Preliminary assessment (38 samples) was conducted using all four platforms. For EGFR-TKI sensitizing mutations, high sensitivity (78–100%) and specificity (93–100%) were observed using tissue as a non-reference standard. For the T790M mutation, the digital platforms outperformed the non-digital platforms. Subsequent assessment using 72 additional baseline plasma samples was conducted using the cobas® EGFR Mutation Test and BEAMing dPCR. The two platforms demonstrated high sensitivity (82–87%) and specificity (97%) for EGFR-sensitizing mutations. For the T790M mutation, the sensitivity and specificity were 73% and 67%, respectively, with the cobas® EGFR Mutation Test, and 81% and 58%, respectively, with BEAMing dPCR. Concordance between the platforms was >90%, showing that multiple platforms are capable of sensitive and specific detection of EGFR-TKI-sensitizing mutations from NSCLC patient plasma.

Conclusion: The cobas® EGFR Mutation Test and BEAMing dPCR demonstrate a high sensitivity for T790M mutation detection. Genomic heterogeneity of T790M-mediated resistance may explain the reduced specificity observed with plasma-based detection of T790M mutations versus tissue. These data support the use of both platforms in the AZD9291 clinical development program.

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Abbreviations: ARMS, amplification refractory mutation system; BEAM, beads, emulsions, amplification, and magnetics; CR, complete response; ctDNA, circulating tumor DNA; dPCR, digital polymerase chain reaction; ddPCR, Droplet Digital PCR; EGFR, epidermal growth factor receptor; M1a/M0, disease confined to the thoracic cavity; M1b, extra-thoracic metastatic disease; NSCLC, non-small cell lung cancer; PCR, polymerase chain reaction; PR, partial response; SD, stable disease; TKI, tyrosine kinase inhibitor.

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http://dx.doi.org/10.1016/j.lungcan.2015.10.004
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1. Introduction

Nearly all patients with non-small cell lung cancer (NSCLC) who initially respond to epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI) therapy develop resistance. In such patients, it is now recommended to obtain a biopsy in order to characterize the mechanism of resistance. However, obtaining sufficient tissue for mutation analysis in patients with advanced disease is challenging, as invasive interventions may be ineffective and unsafe. Moreover, detection of disease-relevant mutations from the biopsy of a single tumor lesion may not be reflective of the patient’s complete disease burden, especially in heterogeneous cancers [1,2].

In recent years, circulating tumor DNA (ctDNA) has emerged as a specific and sensitive blood-based biomarker for detection of EGFR mutations. T790M is the most common mechanism of resistance to first-line EGFR-TKIs, detectable in nearly 60% of tissue biopsies taken after resistance develops [3,4]. Although the mechanism by which ctDNA is released into the bloodstream is unclear [5], it is thought to be related to physiological events related to the tumor cells, including apoptosis and necrosis [6]. Several studies have demonstrated that mutations, including the EGFR T790M mutation, detected in plasma ctDNA are highly concordant with those detected in tumor tissue in patients [7–12], indicating that ctDNA as a liquid biopsy is a feasible and minimally invasive alternative to tissue biopsy. Other clinical applications for ctDNA in this setting include molecular assessment of patients at diagnosis [11], serial (real-time) monitoring of patients for the development of resistance mutations (which is not practical/possible with repeat biopsies) [11,13], and for the clinical management of patients [14]. Importantly, because ctDNA analysis does not involve formaldehyde fixation, there is reduced frequency of a false positive result due to deamination [15]. Although several methodologies are available for mutation analysis from ctDNA, including digital polymerase chain reaction (dPCR), mutant-enriched PCR, and peptide nucleic acid-locked nucleic acid PCR [16], there is a paucity of widely accepted and approved methods for ctDNA analysis.

AZD9291 is a highly selective, irreversible EGFR-TKI that targets both EGFR-TKI-sensitizing mutations (e.g., exon 19 deletion and L858R) and the T790M resistance mutation [17]. AZD9291 has shown promising clinical activity in the ongoing phase I AURA trial (clinicaltrials.gov identifier: NCT01802632) in patients with EGFR-TKI-resistant advanced NSCLC [18]. To date, patients have been selected for AURA trials using tissue testing for the T790M resistance mutation with the cobas® EGFR Mutation Test (Roche Molecular Systems, Inc., Pleasanton, CA, USA), the companion diagnostic developed for AZD9291. However, as part of the clinical development process for AZD9291, alternative selection strategies are also being evaluated, namely EGFR mutation testing using plasma ctDNA. To this end, a comparison was undertaken of multiple platforms for the detection of EGFR mutations in plasma ctDNA to identify the most appropriate technology for use during AZD9291 clinical development.

2. Methods

2.1. Patients

Plasma samples were collected from patients enrolled in the ongoing phase I AURA study. In brief, AURA (NCT01802632) is an open-label, multicenter study designed to assess the safety, tolerability, and efficacy of AZD9291 in patients with EGFR mutation-positive NSCLC who have progressed following prior therapy with an EGFR-TKI agent [18]. All patients had measurable disease at baseline, with radiological documentation of disease progression while receiving continuous treatment with an EGFR-TKI. Patients were required to have EGFRm-positive NSCLC, confirmed by either the presence of a tumor harboring an EGFR mutation known to be associated with EGFR-TKI sensitivity, or evidence of a clinical benefit with an EGFR-TKI, followed by systemic objective progression (according to Response Evaluation Criteria in Solid Tumors or World Health Organization criteria) while on continuous EGFR-TKI treatment as per Jackman criteria [19].

2.2. Ethics statement

The study was conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonisation/Good Clinical Practice, applicable regulatory requirements, and AstraZeneca’s policy on bioethics. All patients provide written informed consent before any study-specific procedures, sampling, and analyses.

The AURA protocol was reviewed and approved by Institutional Review Boards at each trial site before patient enrollment.

2.3. Plasma sample collection and DNA extraction

Plasma samples were collected from patients enrolled into AURA, following progression on a previous EGFR-TKI, but prior to dosing with AZD9291; samples were taken from patients in both dose escalation and dose expansion cohorts. Plasma samples from identical timepoints were pooled per patient and 2 ml (where available) were assigned for each platform assessment; samples were stored at −80°C and were tested in a blinded fashion.

Patient-matched tumor material (formalin-fixed and paraffin-embedded) was available for 80% of plasma samples to enable ctDNA-tumor concordance testing. Tumor tissue originated from pre-study biopsies collected following progression on the last line of therapy. Tumor tissue genotyping was conducted in one of three central laboratories using a standardized cobas® EGFR Mutation Test assay (Roche Molecular Systems, Inc.), but with a protocol modification that allowed the testing laboratories to use more slides than stated in the package insert if it was thought to be deemed necessary. In the dose expansion cohorts, T790M mutation status was identified either locally or centrally; all local results were confirmed by a mandatory retrospective centralized tissue result for each patient; in the dose escalation cohorts, a central tissue test was not required but a local EGFRm tissue mutation result was available for most patients.

The sensitivity of the plasma assays was calculated as follows: 100% × true positives/(true positives + false negatives), where true positives and false negatives were defined according to the tissue-based test. The specificity of the plasma assays was calculated as follows: 100% × true negatives/(false positives + true negatives), where true negatives and false positives were defined according to the tissue-based test [20]. Overall concordance between each plasma assay and the tissue-based test was also calculated.

2.4. Mutation detection

Extracted ctDNA was tested for three common EGFR mutations (exon 19 deletion, L858R, and T790M) using multiple platforms. This involved non-digital detection using the cobas® EGFR Mutation Test and the therascreen™ EGFR amplification refractory mutation system (ARMS) assay (Qiagen, Venlo, The Netherlands) and digital detection using the highly sensitive and quantitative Droplet Digital PCR (ddPCR™; Bio-Rad/MolecularMD, Hercules, CA, USA) and beads, emulsions, amplification, and magnets (BEAMing dPCR technique (Sysmex Inostics, Inc., Mundelein, IL, USA)).
ctDNA, circulating tumor DNA; ddPCR, Droplet Digital PCR; M1a/M0, disease confined to the thoracic cavity; M1b, extra-thoracic metastatic disease; NSCLC, non-small cell lung cancer.

Table 1

<table>
<thead>
<tr>
<th>Exon 19 deletion</th>
<th>cobas® EGFR Mutation Test</th>
<th>therascreen® ddPCR™</th>
<th>BEAMing ddPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>86%</td>
<td>82%</td>
<td>93%</td>
</tr>
<tr>
<td>(24/28)</td>
<td>(23/28)</td>
<td>(26/28)</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>(10/10)</td>
<td>(10/10)</td>
<td>(10/10)</td>
<td></td>
</tr>
<tr>
<td>Concordance</td>
<td>89%</td>
<td>87%</td>
<td>95%</td>
</tr>
<tr>
<td>(28/28)</td>
<td>(28/28)</td>
<td>(26/28)</td>
<td></td>
</tr>
</tbody>
</table>

For the therascreen® assay, DNA was extracted from plasma as described in the QiAamp Circulating Nucleic Acid Handbook, second edition (QIAGEN) using the protocol, purification of circulating nucleic acids from 1 ml, 2 ml, or 3 ml plasma. Modifications of the protocol and specific details are as follows: samples were centrifuged at 3000 rpm for 2 min and the supernatant was transferred to a clean tube, prior to extraction (step 3); a brief centrifuge spin was performed after the 60°C incubation (step 4); 55 µl buffer AVE was added at the elution step 15; DNA was stored at −80°C. Prior to mutation testing, all samples were assessed for total amplifiable DNA and subsequently processed for the detection of EGFR mutations and data analysis using the therascreen® EGFR RQqPCR Kit Handbook version 1 (QIAGEN); a minor protocol modification was that 16 samples per run were tested.

Testing on the ddPCR™ and BEAMing ddPCR platforms was performed at MolecularMD (Cambridge, MA, USA) and Sysmex Inostics (Hamburg, Germany), respectively.

3. Results

3.1. Initial platform comparison (n = 38)

A preliminary assessment using all four platforms was performed with 38 plasma samples. High sensitivity and specificity, using a tissue test result as a non-reference standard, was observed with the cobas® EGFR Mutation Test, the therascreen® EGFR ARMS assay, and the BEAMing ddPCR for EGFR-sensitizing mutations (Table 1). The same was true with the ddPCR™ for the L858R EGFR mutation. No sensitivity or specificity data were available for the exon 19 deletion mutation for the ddPCR™ (Table 1) as it was only designed to detect a minority of known exon 19 deletions.

Digital platforms appeared to detect a higher percentage of T790M mutations, compared with non-digital platforms (Table 1). In the single case where ddPCR™ detected a positive T790M mutation that was not detected in the tissue sample, BEAMing ddPCR also classified this sample as T790M positive.

T790M mutations were more readily detected in the plasma of patients with extra-thoracic metastatic disease (M1b) than patients with disease confined to the thoracic cavity (M1a/M0) (P < 0.01; Fig. 1).
Table 2
Performance of two different plasma assays relative to a tissue test result as a non-reference standard for detection of epidermal growth factor receptor mutations from circulating tumor DNA in a set of 72 plasma samples.

<table>
<thead>
<tr>
<th>Exon</th>
<th>cobas® EGFR Mutation Test</th>
<th>BEAMing dPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 deletion</td>
<td>82% (23/28)</td>
<td>82% (23/28)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>97% (30/31)</td>
<td>97% (30/31)</td>
</tr>
<tr>
<td>Specificity</td>
<td>87% (20/23)</td>
<td>87% (20/23)</td>
</tr>
<tr>
<td>L858R Sensitivity</td>
<td>97% (35/36)</td>
<td>97% (35/36)</td>
</tr>
<tr>
<td>Specficity</td>
<td>73% (30/41)</td>
<td>81% (33/41)</td>
</tr>
<tr>
<td>T790M Sensitivity</td>
<td>67% (16/24)</td>
<td>58% (14/24)</td>
</tr>
</tbody>
</table>

BEAMing, beads, emulsions, amplification, and magnets; dPCR, digital polymerase chain reaction; EGFR, epidermal growth factor receptor.

3.2. Subsequent platform comparison (n = 72)
A subsequent assessment using two of the original four platforms was performed with a set of distinct baseline plasma samples from 72 additional, non-overlapping patients on the AURA trial. Of the digital platforms, BEAMing PCR was selected for further testing as ddPCR™ did not allow a full assessment of EGFR mutations. Of the non-digital platforms, the cobas® EGFR Mutation Test was selected for further testing as the therascreen™ EGFR ARMS-PCR had a lower sensitivity observed for T790M. Subsequent testing using the cobas® EGFR Mutation Test was conducted at Roche Molecular Systems, Inc. while BEAMing PCR testing continued to be done at Sysmex Inostics.

As seen in the initial comparison, high sensitivity and specificity were observed with the cobas® EGFR Mutation Test and BEAMing dPCR for EGFR-TKI-sensitizing mutations (Table 2). Both the cobas® EGFR Mutation Test and BEAMing dPCR demonstrated good sensitivity for T790M mutation detection (Table 2). As observed in the initial comparison, BEAMing dPCR showed high sensitivity (81%) but a lower specificity (58%). The sensitivity of the cobas® EGFR Mutation Test was markedly improved compared with the initial comparison (Table 2).

Strong overall concordance was demonstrated between the cobas® EGFR Mutation Test and BEAMing dPCR for detection of EGFR mutations from ctDNA (Table 3).

An analysis of the 20 discordant T790M results was undertaken. There were four ‘false’ plasma negative results detected by the cobas® EGFR Mutation Test; all four were called positive by BEAMing, but three of these were very near the lower limit of detection (0.020% mutant) (Table 4). There were seven cases where both plasma assays did not detect any T790M mutation despite a positive result in the patient matched tumor tissue. Finally, there were nine ‘false’ plasma positives detected by BEAMing dPCR; seven of these were also scored positive by the cobas® EGFR Mutation Test (Table 4).

Table 4
Discordant results with two different plasma assays for detection of the EGFR T790M mutation from circulating tumor DNA.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Tissue</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Positive</td>
<td>Positive (0.021%)</td>
</tr>
<tr>
<td>2</td>
<td>Positive</td>
<td>Positive (0.048%)</td>
</tr>
<tr>
<td>3</td>
<td>Positive</td>
<td>Positive (0.064%)</td>
</tr>
<tr>
<td>4</td>
<td>Positive</td>
<td>Positive (0.202%)</td>
</tr>
<tr>
<td>5</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>7</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>8</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>9</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>10</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>11</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>12</td>
<td>Positive</td>
<td>Negative (0.026%)</td>
</tr>
<tr>
<td>13</td>
<td>Negative</td>
<td>Positive (0.027%)</td>
</tr>
<tr>
<td>14</td>
<td>Positive</td>
<td>Positive (0.054%)</td>
</tr>
<tr>
<td>15</td>
<td>Negative</td>
<td>Positive (0.08%)</td>
</tr>
<tr>
<td>16</td>
<td>Negative</td>
<td>Positive (0.283%)</td>
</tr>
<tr>
<td>17</td>
<td>Negative</td>
<td>Positive (0.340%)</td>
</tr>
<tr>
<td>18</td>
<td>Negative</td>
<td>Positive (0.344%)</td>
</tr>
<tr>
<td>19</td>
<td>Negative</td>
<td>Positive (0.491%)</td>
</tr>
<tr>
<td>20</td>
<td>Negative</td>
<td>Positive (1.11%)</td>
</tr>
</tbody>
</table>

BEAMing, beads, emulsions, amplification, and magnets; dPCR, digital polymerase chain reaction; EGFR, epidermal growth factor receptor.

A preliminary assessment of clinical response to AZD9291 as a function of the T790M resistance mutation detected at baseline (i.e., prior to initiation of AZD9291) but following the development of resistance to first-line EGFR-TKI) is shown in Fig. 2. The clinical response rate in patients positive for the T790M mutation in plasma was almost identical to that for patients positive for the T790M mutation in tissue (59% vs. 61%).

4. Discussion
Several technologies have been described for the detection of EGFR mutations using plasma ctDNA. In the current study, four such technologies were compared, with the aim of identifying robust platform(s) to support the ongoing AZD9291 clinical development program. The study was conducted in two parts: (i) initial comparison of four platforms using a small set of plasma samples; (ii) subsequent comparison of two platforms using a larger set of additional plasma samples. All samples (plasma and patient-matched tumor tissue) originated from patients enrolled in the ongoing phase I AURA trial. The cross-platform comparison showed that the cobas® EGFR Mutation Test and BEAMing dPCR had highly concordant results, with high sensitivity (73–81%) for the detection of the T790M mutation; good specificity was also achieved with these two platforms (58–67%). The objective response rate (ORR) to AZD9291 was almost identical in patients positive for the T790M mutation in plasma and in tissue (both assessed using the cobas® EGFR Muta-
tion Test). Collectively, these results show that the cobas® EGFR Mutation Test and BEAMing dPCR have the potential to identify patients harboring T790M mutations from plasma ctDNA.

Four platforms were evaluated in the current study. The two non-digital platforms comprised the cobas® EGFR Mutation Test and the therascreen® EGFR ARMS assay; both of these testing methods arise from established tissue tests that have been adapted for low-DNA-input plasma samples. The cobas® EGFR Mutation Test and the therascreen® EGFR RQ PCR test detect 41 [21] and 21 [22] mutations, respectively, in exons 18–21 of the EGFR gene; importantly, both tests detect the T790 M mutation. The two digital platforms were the BioRad ddPCR™ and BEAMing dPCR. Digital PCR methods are purportedly more sensitive than other assays for mutant sequence detection [23] and are fully quantitative, making them suitable for quantification of longitudinal plasma samples, which may allow for monitoring of disease/mutation evolution over time. Both BEAMing and ddPCR were specifically included as they have been used to profile plasma ctDNA for a variety of cancer-related mutations [11,23–27]. Although the ddPCR™ generated comparable T790M results to BEAMing in the initial comparison of all four platforms, its ability to detect only a minority of known exon 19 deletions led to its exclusion from the subsequent platform comparison.

To the best of the authors’ knowledge, this is the first comparison of the cobas® EGFR Mutation Test and BEAMing dPCR to be published. The results support the potential use of both platforms for the detection of EGFR mutations from patient plasma samples. The cobas® EGFR Mutation Test showed high sensitivity for the T790M mutation, and had comparable sensitivity to BEAMing dPCR. An improvement in sensitivity was observed with the cobas® EGFR Mutation Test between the initial and subsequent comparisons. The difference in sensitivity between the two datasets may be a result of the greater sample size in the subsequent comparison and optimization of the assay by Roche between comparisons, resulting in the identification of more T790M mutations. As a complementary approach, because of its quantitative nature, BEAMing dPCR may allow for a more detailed assessment of T790M allelic levels and potential correlation with clinical response. Such an analysis with a large patient cohort from AURA is now ongoing and will be reported elsewhere. BEAMing dPCR may also be useful for dynamic monitoring of longitudinal samples where a binary outcome (i.e., simply the presence or absence of a signal) is not appropriate.

The T790M resistance mutation was more frequently detected in the plasma of patients with metastatic versus locally advanced disease. This has been observed in a similar study [11], and suggests that tumor bulk and metastatic status may impact the presence of plasma-mutant EGFR and should be further validated in a clinical setting [11].

AZD9291 is an orally administered EGFR-TKI that is highly potent against EGFR-TKI-sensitizing mutations and the T790M resistance mutation, but with a margin of selectivity against wild-type EGFR activity [17,28]. While currently approved EGFR-TKIs, including gefitinib and erlotinib, are effective in the treatment of NSCLC, nearly all patients who respond to these treatments will eventually develop acquired resistance, and approximately 60% of EGFR-TKI-resistant tumors will carry a T790M mutation in exon 20 [17,28,29]. Treatment strategies in patients with acquired resistance are limited at present; although irreversible EGFR-TKIs such as afatinib and dacomitinib have been developed, their clinical utility is limited in the T790M-resistant setting, likely owing to dose-limiting toxicities associated with non-selective inhibition of wild-type EGFR [17,28,30,31]. The safety, tolerability, and efficacy of AZD9291 is currently being investigated in the ongoing AURA program [18]. Preliminary phase I data show that AZD9291 demonstrates promising clinical activity in patients with centrally confirmed T790M mutation-positive disease (confirmed ORR of 61% across all doses) [18]. In the current study, the clinical ORR (complete and partial) was almost identical between patients positive for the T790M mutation in plasma ctDNA and by tissue genotyping, suggesting that plasma detection T790M mutation may be a suitable alternative to tissue biopsies.

Overall, the concordance between the BEAMing dPCR and the cobas® EGFR Mutation Test was 90% for the T790M mutation. This high rate of agreement using two distinct assay platforms run in a blinded fashion builds confidence in the overall plasma result. Indeed, analysis of the 20 discordant results between plasma and

![Fig. 2. Clinical response to AZD9291 according to EGFR T790M mutation at baseline (data cut-off date December 2014). CR, complete response; EGFR, epidermal growth factor receptor; PR, partial response; SD, stable disease.](image-url)
tissue from the 72-patient set reveals that the BEAMing dPCR and cobas® EGFR Mutation Test outcomes were comparable in 14/20 cases. Furthermore, all six of those instances where the plasma tests disagreed arose in cases where the T790M allelic fraction was very low (<0.2%) and perhaps below the detection limit of the cobas® EGFR Mutation Test plasma assay. Taken together, these data suggest that tumor heterogeneity, and not technological limitations alone, may explain the tissue–plasma discordance and the ‘reduced’ T790M specificity observed with the plasma assays. T790M tumor heterogeneity has been reported of late, with different tumor biopsies within the same patient demonstrating differing EGFR mutational profiles [1, 2]. In all, these data suggest that plasma ctDNA may be more precise and informative than tissue as the blood mirrors the entire tumor burden [2]; additionally, a single biopsy is often taken from a non-progressing lesion. However, heterogeneous tumors may harbor additional resistance mechanisms and thus ultimately result in lower clinical response rates. Supporting this concept, in patients with plasma positive but tumor negative for T790M, the clinical ORR was 38% (three/eight patients) and the disease control rate was 75% (six/eight patients). These data appear more reflective of the clinical responses observed in patients tumor negative for T790M and thus suggest the potential for tumor heterogeneity, albeit with the caveat of involving a small number of patient samples.

Plasma ctDNA has potential in the management of patients with NSCLC in routine clinical practice, particularly considering that DNA extraction and mutation detection using a commercially available and approved test could be performed in as little as 1 day [14, 32], allowing for real-time disease testing. That said, robust validation in prospective clinical trials [16, 33], such as that planned in the AURA program, is required.

In conclusion, multiple platforms are capable of sensitive and specific detection of EGFR-TKI-sensitizing mutations from ctDNA in patients with NSCLC. The cobas® EGFR Mutation Test assay and BEAMing dPCR showed highly concordant T790M resistance mutation detection, with 70–80% sensitivity, using a tissue test result as a non-reference standard, for genotyping. Genomic heterogeneity of T790M-mediated resistance may account for the reduced specificity observed with plasma–versus tissue-based detection. The rate of clinical response to AZD9291 was almost identical in patients positive for the T790M mutation in plasma and in tissue, indicating that plasma detection may be a suitable alternative to tissue genotyping in patients with NSCLC.

Collectively, the results support the potential use of both platforms in the AZD9291 clinical development program.

Conflicts of interest
All authors are employees of, and hold shares in, AstraZeneca.

Role of the funding source
AstraZeneca funded this research, including the experimental design, the collection, analysis and interpretation of data, and the writing of the report. Authors (all employees of AstraZeneca) were responsible for the decision to submit the article for publication.

Author contributions
KST: designed study, analyzed data, interpreted data; RB: performed the experiments, read and approved final manuscript; THC: primarily analysis of therascreen™ data; SD and SJ: study conception/design and data interpretation; HB: data acquisition (tumor) and joint contributor to data interpretation of the 38 sample set; TH: clinical study lead, clinical sample and data acquisition, read and approved final manuscript; MC: designed and supervised clinical study, interpreted data, read and approved manuscript; JCB: contributed to design of the study, study interpretation of data, and manuscript preparation.

Acknowledgments
The study was funded by AstraZeneca. Medical writing services were provided by Kerry Acheson, Ph.D., of iMed Comms, Macclesfield, UK, and were funded by AstraZeneca. The authors thank Wei Wen, Sean Chien, and Ha Bich Tran (Roche Molecular Systems, Inc.) for generating the cobas® EGFR Mutation Test data as well as the investigators and patients who participated in the AURA study.

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