Commentary

Plasma genotyping for *EGFR* T790M in non-small cell lung cancer: ready for clinical practice?

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The outstanding article recently published in the *Journal of Clinical Oncology* by Oxnard and coauthors, about plasma genotyping in advanced non-small cell lung cancer (NSCLC) patients treated with osimertinib in the AURA phase I trial (1), represents an important further step towards the validation of the liquid biopsy in all possible settings and offers the cue for some careful reflections.

In their retrospective analysis, the investigators of the first-in-man study of osimertinib performed the genotyping of cell-free plasma DNA (cfDNA) by using BEAMing, comparing the results about T790M acquired mutation with tumor genotyping performed by Cobas test. The aim was to predict the outcome of osimertinib in advanced NSCLC patients with acquired resistance to conventional epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI) harboring TKI-sensitizing mutations of *EGFR* (2).

From previous literature, we know that the fraction of tumor cfDNA ranges from less than 0.1% to more than 30% of total circulating DNA, depending on tumor type, stage and tumor burden, cellular turnover and ability of tumor cells to penetrate in blood vessels. While a small biopsy could be not representative of both real tumor biology and tumor changes, conversely, the liquid biopsy is rapidly available, reproducible and may represent a favorable solution to monitor tumor changes, including tumor response, progression and the occurrence of secondary resistance to targeted therapies. To date, different assays have been developed for detecting somatic alterations present at low-frequency in cfDNA. Among these, real-time quantitative polymerase chain reaction (PCR), digital PCR (dPCR), BEAMing (beads, emulsion, amplification and magnetics) and next-generation sequencing (NGS) platforms have been investigated and compared. Notably, their respective sensitivity is highly variable, ranging from 15% to 0.01%, with the highest for digital platforms, despite the lack of standardization (3). Different studies analyzed the accuracy and concordance between non-digital and digital techniques in detecting *EGFR* mutations both in plasma and in tumor tissue. For plasma *EGFR* sensitizing mutations, sensitivity and specificity were very high (78–100% and 93–100%, respectively) across non-digital (Cobas and Therascreen) and digital platforms (digital droplet PCR and BEAMing), whereas for plasma T790M mutation, sensitivity and specificity were 73% and 67%, respectively, for non-digital assays versus 81% and 58% for digital platforms (4). More recently, a higher concordance between plasma and tumor tissue by using BEAMing (82% for *EGFR* sensitizing mutations and 73% for T790M) was obtained compared to those of Cobas technology (73% for *EGFR* sensitizing mutation and 64% for T790M) (5).

In the AURA 1 study, sensitivity of plasma T790M detection was 70% and specificity was 69%. The 164 plasma positive patients had a response rate (RR) of 63% (95% CI, 55% to 70%) and a progression free survival (PFS) of 9.7 months (95% CI, 8.3 to 11.1 months), while 102 plasma negative patients had a RR of 46% (95% CI, 36% to 56%) with a PFS of 8.2 months (95% CI, 5.3 to 10.9). These differences did not reach statistical significance.

With tumor genotyping, 173 positive patients confirmed

a RR of 62% (95% CI, 54% to 70%) and a PFS of 9.7 (95% CI, 8.3 to 12.5 months), closely similar to the results of plasma positive group, instead 58 patients tumor negative for T790M had lower RR of 26% (95% CI, 15% to 39%) and PFS of 3.4 (95% CI, 2.1 to 4.3) months. These outcomes were significantly different in the two groups.

We can firstly speculate about the difference evidenced between the two groups of T790M-tissue negative and of T790M-plasma negative patients, hypothesizing an undeniable favorable selection bias in the latter group. Indeed, 45 out of 102 plasma negative cases presented a discordant T790M positivity in the tumor tissue and demonstrated outstanding outcomes in terms of both RR (69%) and PFS (16.5 months). This key point represents an interesting aspect, not clearly stressed in the study; it is, indeed, the better outcome by subgroup, unexpectedly higher compared to those of plasma-and-tissue T790M positive patients (RR 64%, PFS 9.3 months). Considering some preclinical basis and recent clinical evidence in favor of a more indolent clinical course of T790M driven tumors (6,7), a "true" positivity on the tumor, in absence of the likely circulating release of T790M predominant clones at the time of disease progression, could subtend a more indolent disease with good prognostic instead of predictive effects. Conversely, the correspondence between plasma and tissue findings should instead guarantee the most reliable predictive value.

Furthermore, among T790M plasma negative patients, the group of 33 cases with also no sensitive *EGFR* mutations detected had unexpectedly good outcomes, reaching a PFS of 15.2 months (95% CI, 11 to 17.9) and RR of 64% (95% CI, 45% to 79%). As suggested by the authors, it is plausible that in this patients' subgroup plasma genotyping assay could be a non-informative test because of the lack of detectable tumor cfDNA, identifying a population with low tumor burden and less aggressive disease.

Overall, the 31% of discordance between tumor negative and plasma positive T790M should be surely handled with care. Despite the temptation of classifying as "true" false positive 18 of 58 cases, the likely heterogeneity of resistance mutations (that could be not present in the rebiopsy sample) and the ultra-sensitivity of the alternative plasma assays (droplet digital PCR, ddPCR) used to confirm the discordant cases, these are both elements that lay the ground for doubt about too many certainties. Of 18 discordant cases, 14 were confirmed using alternative tests with variable sensitivity (1% for Cobas, $<10^{-6}$ for ddPCR) compared to BEAMing. This finding suggests that plasma detection of *EGFR* resistance mutations might be clinically more relevant than tissue testing. The four "false positive" cases detected by the high sensitivity of the ddPCR should lead to carefully consider the empty clinical meaning of detecting, with a technique of much higher sensitivity than those of BEAMing, few fragments of tumor DNA occasionally released in the plasma by minority clones.

One of the certainties confirmed by this study is represented by the reliability of the plasma genotyping for T790M as predictive factor beyond all the previous considerations, with similar outcomes of the plasma positive patients to those of the tumor positive cases. Furthermore, in the T790M plasma positive patients with lack of tissue for tumor testing or discordant tumor results, we could also consider the potential role of calculating the relative T790M allelic fraction (AF) in plasma (*EGFR*-mutant cfDNA relative to wild-type *EGFR* cfDNA). AF was indeed higher in 108 patients with plasma T790M positive tumors than in 16 patients with T790M negative tumors (P=0.0047), and moreover a relative AF >10% was associated with a greater depth of response compared to those with AF <10%, despite no significant association was seen overall. In this light, we should consider that an actual quantification of the mutation AF, despite being difficult to obtain with real-time PCR based methods such as Cobas, can be conversely achieved with BEAMing, ddPCR and NGS platforms, which imply a much higher throughput than the previous techniques.

The subsequent obvious consideration is suggested by the worst outcome of patients with plasma-and-tissue T790M negative, which had a RR of 25% and a PFS of 2.8 months. The gap from this clinical results to those extremely favorable of patients with plasma negative but tumor positive T790M (RR 69%, PFS 16.5 months) makes undoubtedly mandatory the rebiopsy in all cases of plasma negative genotyping before excluding therapy with osimertinib.

Notwithstanding the now validated plasma genotyping of *EGFR* sensitizing mutations (L858R and exon 19 deletions), with a specificity of 97–98% in this same study population, we are undoubtedly alerted about the potential error from its exclusive clinical application also in the field of T790M resistance mutation. Nevertheless, the importance of including this feasible assessment in clinical practice is currently crucial, especially in the light of the recently published amazing results of the phase III AURA 3 randomized clinical trial, demonstrating the wide superiority of osimertinib over standard chemotherapy after

progression to first line EGFR-TKI in T790M positive patients (PFS of 8.5 *vs.* 4.2 months; HR 0.42; 95% CI, 0.29–0.61) (8). Considering the remarkable benefit from osimertinib in this population and its confirmation as the new standard of care in this setting, the selection criteria must be clearly defined and validated to reproduce the study results also in clinical practice. The reported PFS values, in fact, are referred to the population with T790M positive status both on tumor and plasma analyses. High rates of false negative plasma testing were reported in this trial (sensitivity was only 51%), thus recommending the analysis of a biopsy sample in all patients progressive to first line EGFR-TKI and with plasma T790M negative result (notably, a positive tumor sample was required for enrollment in the study, making not possible to address clinical outcomes of patients with potential false positive results on plasma samples) (8).

Also from the AURA 2 phase II study, included in a pooled analysis by Jenkins *et al.*, data indicated that approximately 60% of patients with T790M positive NSCLC could have avoided an invasive biopsy by use of a plasma test, despite once again alerting about the false negative results (9,10).

Thus, while the feasibility of T790M plasma genotyping seem to be consolidated and undoubtedly shared, its utility can be considered as diriment for the therapeutic choice only in positive cases, whilst a negative result must lead to a new biopsy. Moreover, with an expected outcome of 28% RR and 4.2 months PFS from osimertinib per plasma positive/tumor negative population of Oxnard's study, the phantom of the "false negative" cases on tissue should be thrown out by the awareness that with either the new drug or chemotherapy this subgroup will probably reach the same outcome in terms of effectiveness. In fact, the control arm of AURA 3 trial demonstrated an RR of 31% and a PFS of 4.2 months, suggesting that also in patients with tumor sample unavailable, therapy with osimertinib should maybe be considered as the better tolerated option (8).

From a strictly laboratory point of view we must be aware that the only tests currently available for diagnostic plasma evaluation of *EGFR* mutations are based on real time PCR. Beaming, ddPCR and NGS based tests are still currently for research use only and once approved for diagnostic use would be available only in selected reference centers. Waiting for the approval of new diagnostic tests and given the actual clinical need of widespread availability of plasma EGFR mutation testing, real time PCR based tests are still to be considered the clinical-laboratory standard of practice. We eventually agree with the final recommendation by the authors in support of the use of both plasma- and tissue-based assays and we can state that, to be ready for clinical practice, the clinician could be confident in the positive predictive value of a T790M positive plasma genotyping for treatment with osimertinib, preferably avoiding ultra-sensitive assays, but should instead remember the extremely lower negative predictive power of a T790M negative plasma test, proposing the rebiopsy for negative cases.

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Footnote

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