Editorial

EV-based EGFR genotyping in bronchoalveolar lavage fluid—a step forward from blood?

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The efficacy of osimertinib aiming at T790M-mediated EGFR-TKI resistance was proven in a large, randomized, phase III trial (AURA 3 trial) leading to becoming the standard treatment in T790M-positive cases following the 1st-line EGFR-TKI failure (1,2). However, the demonstration of T790M is mandatory to use this drug, which poses a clinical problem related with re-biopsy. Sometimes, the procurement of adequate tissue through re-biopsy is very difficult or even unable to do according to the location and size of tumor (3,4). The poor performance status of several patients is another obstacle for repeated tissue biopsy. In these cases, the less invasive liquid biopsy with cell-free DNA (cfDNA) in blood which had been already approved for the clinical application can be a reasonable alternative (5). Nonetheless, the analysis of tissue sample is preferentially recommended because of the high false negative rate of the plasma cfDNA T790M testing at present despite its definite advantages and future promising perspectives (1).

The composition of EVs which are released by exocytosis from tumor cells may reflect that of parent cells. They contain various kind of components such as proteins, lipids, RNAs and even double-stranded DNA (6). In addition, because of their stability which is secured by protecting envelope, EVs can be more reliable sources than plasma cfDNA for analysis of genetic materials. In fact, the combined use of RNA in EVs and cfDNA increased the sensitivity for EGFR mutation detection in plasma, especially in the subgroup of M0/M1a disease with low levels of cfDNA (7). As for T790M of which allele frequency is lower than that of sensitizing EGFR mutations such as 19 deletion and L858R resulting in the low detection rate, an analysis of pooled AURA extension and AURA2 studies showed 61% of sensitivity by the cobas EGFR Mutation Test v2 using cfDNA (8) while a test using RNA/DNA in EVs and cfDNA resulted in 92% of sensitivity (9).

Hur *et al.* ventured to use EV DNA in bronchoalveolar lavage fluid (BALF) rather than in plasma for the detection of EGFR mutation (10). It seems to be a clever attempt because BALF would have much more tumor-derived EVs than plasma considering the vicinity to tumor itself although the bronchoscopic approach is required to retrieve the BALF. The average sensitivity and specificity of BALF EV-based EGFR genotyping were 76% and 87%, respectively, while the sensitivity improved according to the increased stage. Surprisingly, BALF EV-based EGFR typing found all tissue-proven EGFR mutant cases (n=31) and detected 6 additional mutant cases in stage IV. The concordance rate was 79% in stage I, 100% in stage II, 74% in stage III, and 92% in stage IV. As TNM stage advanced, especially in the presence of metastasis, concordance rate significantly increased ($P<0.05$).

These are very impressive results which might be contributed by the superiority of EV DNA to cfDNA and BALF to plasma. Authors exhibited that BALF EV-based

EGFR typing could find 11 more EGFR mutation-positive patients compared to the conventional tissue/cytology-based test. However, we don't know whether these patients truly have EGFR mutation or not because the results about drug response are not provided in this study. Nevertheless, as we recognize that false negative results can be obtained in cases of the low tumor cell proportion in tissue/cytology samples, this BALF EV-based test has the potential to find some of those missing patients by conventional approach. For the clinical application in near future, it should be proven by more confirmatory studies as well as expanding studies to demonstrate the performance for detection of T790M along with the clinical response to osimertinib.

Furthermore, it is noteworthy that BALF EV EGFR testing in stage I lung cancer showed the relatively high positive predictive value of 85.7% despite the low sensitivity of 35.3%. Physicians often face the difficulty in making a decision how to manage the slowly growing ground-glass nodule (GGN) with low to intermediate risk of cancer because the tissue diagnosis of GGN is very difficult in most cases. According to the study by Kobayashi *et al.*, three fourths of resected GGNs were positive for EGFR, KRAS, ALK or HER2 mutations (11). Therefore, considering the high positive predictive value of BALF EV genotyping, the detection of mutation by this method may be helpful to direct treatment to the surgical resection rather than the watchful follow-up. The low sensitivity could be improved by the use of techniques with higher sensitivity such as next-generation sequencing and droplet digital PCR.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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