Editorial

Liquid biopsy in non-small cell lung cancer: come of age

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Comment on: Yanagita M, Redig AJ, Paweletz CP, *et al*. A prospective evaluation of circulating tumor cells and cell-free DNA in EGFR mutant non-small cell lung cancer patients treated with erlotinib on a phase II trial. Clin Cancer Res 2016. [Epub ahead of print].

Submitted Aug 26, 2016. Accepted for publication Sep 05, 2016.
doi: 10.21037/tcr.2016.10.40
View this article at: http://dx.doi.org/10.21037/tcr.2016.10.40

With understanding of lung cancer biology and advances in technology, treatment for advanced non-small cell lung cancer (NSCLC) is currently guided according to the genetic abnormalities, including epidermal growth factor receptor (EGFR) mutation, translocation in ALK, ROS1 or RET, B-raf mutation, etc. In the treatment of EGFR-mutated NSCLC, EGFR tyrosine kinase inhibitors (TKIs) are recommended as first-line therapy based on high response rates and longer progression free survival (PFS) compared to platinum-doublet chemotherapy (1-3). Unfortunately, most patients eventually develop acquired resistance to EGFR TKIs after 10–12 months of PFS. Extensive studies of repeated biopsy demonstrated various different resistant mechanisms. Among them, EGFR T790M mutation confers most common resistance mechanism, accounting for 50–60% of patients with EGFR TKI resistance. Other mechanisms include activation of the alternative pathway such as c-met amplification or HER2 amplification and phenotypic change like small cell lung cancer transformation or epithelial-mesenchymal transition (EMT) (4). Recently, several novel targeted agents to overcome T790M mutation have been developed and being actively investigated. These 3rd generation EGFR TKIs such as osimertinib, rociletinib, or olmutinib are associated with robust efficacy in patients harboring T790M mutation (5,6). Furthermore, combination of EGFR TKI with c-met inhibitor also showed promising activity in patients with high c-met amplification. Therefore, to understand the underlying resistance mechanism and to guide optimal treatment, repeated biopsy is essential and considered the gold standard at the time of progression. However, the invasive nature of repeated biopsies makes it difficult to obtain samples from patients especially those with poor performance or inaccessibility due to tumor location, tumor containing blood vessel or air bronchogram, existence of tumor necrosis, or previous radiation site. Tumor heterogeneity is another limitation and a single snapshot study cannot represent the dynamic changes of genetic abnormalities due to evolving nature of tumor progression.

As minimally invasive methods, the circulating cell free DNA (cfDNA) in plasma and circulating tumor cells (CTCs) have been used as surrogates for tumor tissues in detecting genetic alterations (7). In the article that accompanies this editorial, Yanagita *et al*. reported prospective longitudinal monitoring of both CTCs and cfDNA in EGFR mutant NSCLC patients treated with erlotinib accompanied by repeated biopsy (8). Plasma genotyping was performed by droplet digital PCR for EGFR exon 19 deletion, L858R, and T790M and CTCs were isolated by CellSave and analyzed by immunofluorescence for CD45 and pan-cytokeratin. They found that at progression, T790M was identified in 66% (23/35) of tissue biopsies and in 23% (9/39) of cfDNA, whereas CTCs were observed in 47% (18/38) of patients. Intriguingly, CTC analysis at progression identified c-met amplification in 3 samples where tissue analysis could not be performed. Furthermore, T790M was detected in two samples from cfDNA analysis in which rebiopsy was not possible. The authors suggest that cfDNA and CTCs are complementary as non-invasive methods but cfDNA may offer more clinical utility than CTCs for serial monitoring.

The majority of cfDNA is derived from apoptotic or necrotic tumor cells that release their fragmented DNA into the circulation. The isolation of cfDNA remains challenge due to high degree of fragmentation and its low

concentration in the blood. However, highly sensitive and specific molecular methods such as ddPCR or BEAMing have been developed to detect genetic alterations including single gene mutation or even whole genome sequencing in cfDNA which can guide personalized treatment decisions (9). A phase IV single arm study of gefitinib as first-line therapy in Caucasian EGFR mutation positive NSCLC patients along with preplanned exploratory analysis of EGFR mutations in paired tumor and plasma samples demonstrated that cfDNA analysis can reliably detect EGFR mutation status of the tumor, suggesting that cfDNA analysis can be considered for mutation analysis if repeated biopsy is unavailable or inaccessible (10). Based on these data, cfDNA test for sensitizing EGFR mutation in NSCLC has been approved in Europe. Recently, a pooled analysis of 20 eligible studies reported 67% of sensitivity and 93% of specificity for detection of sensitizing EGFR mutation in cfDNA (11). cfDNA also offers the potential for longitudinal monitoring for the development of resistance mutations such as T790M. Several studies demonstrated EGFR T790M can be non-invasively monitored in cfDNA during the course of EGFR TKIs treatment and this resistance mutation can be detected even earlier than radiological progression (12). Also, the efficacy of osimertinib or rociletinib has been observed in plasma genotyped T790M positive patients (13,14). Recently, FDA approved Cobas EGFR mutation test using plasma specimens as a companion diagnostic test. This is the first liquid biopsy test approved for use by FDA.

As another source of circulating biomarkers, CTCs have been introduced 10 years before. Given the rarity and lack of consensus on expression markers to identity CTCs, significant challenges still remain in CTC detection even though various methods have been developed for isolation and characterization of CTCs (15). Epithelial cell adhesion molecule (EpCAM) based enrichment is most commonly used. CTC-chip is another method using microfluidic-based platform which can separate CTCs from peripheral blood samples without processing samples. Compared to cfDNA, CTCs have several advantages. First of all, enumeration, isolation and visualization are possible. Several small studies reported that serial CTC counts might be useful in predicting the response to treatment. Also isolated CTCs confer further molecular characterization such as mutation, mRNA, or whole exome sequencing in single cell level (16). However, it still remains challenge that there is no standardized method for detecting CTCs. Moreover, the number of CTCs in NSCLC patients is much lower than those of small cell lung cancer patients.

This single center prospective study analyzed cfDNA and CTCs quite extensively and the results are quite intriguing, but several issues should be considered. First of all, at baseline, 37% of patients did not show either CTCs or cfDNA and only 41% (17/41) of patients had detectable cfDNA. At the time of progression, 42% of patients had no detectable CTCs or cfDNA and only 25% (9/36) of patients had detectable cfDNA. Given that advanced stage of NSCLC patients were enrolled in this study, cfDNA detection rate is quite low compared to the results of previous studies. One explanation discussed by the author was attributed to the use of heparinized tubes which might be associated with potential interference with ddPCR. Furthermore, T790M mutation was noted in 66% (23/35) of patients in repeated biopsy. But T790M mutation was identified only in 18% (7/39) of samples suggesting lower sensitivity than previous reports. The reason for the low sensitivity has not been determined yet. About 60% of patients had no detectable CTCs at progression, suggesting potential possibility of loss of epithelial marker due to epithelial to mesenchymal transition. It does not seem to be correlated between CTCs and cfDNA detection in this study. It appears that cfDNA is more associated with prediction of PFS than CTCs. The author pointed out the limitation of non-invasive method to detect small cell lung cancer transformation either by cfDNA or CTCs, where further improvement of technology should be warranted.

It is clear that the analysis of CTCs and cfDNA has paved new way as liquid biopsy diagnostics and the growing body of evidences suggests that these two methods are complementary. In particular, the analysis of cfDNA and CTCs for detection of genetic abnormalities to guide treatment and to monitor resistance to targeted therapies in NSCLC should be incorporated in daily clinical practice. More standardization of clinical assays and clinical validation should be needed.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Shaohua Cui (Department of Pulmonary Medicine, Shanghai Chest Hospital, Shanghai Jiao Tong University, Shanghai, China).

Conflicts of Interest: The author has completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2016.10.40). The author has no conflicts of interest to declare.

Ethical Statement: The author is 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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Cite this article as: Ahn MJ. Liquid biopsy in non-small cell lung cancer: come of age. Transl Cancer Res 2016;5(Suppl 4):S629-S631. doi: 10.21037/tcr.2016.10.40