Commentary

Harnessing plasma genotyping for precision therapy against lung cancer

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The development of tyrosine kinase inhibitors gefitinib and erlotinib for anti-epidermal growth factor receptor (EGFR) therapy is one of milestones in the history for treatment of lung cancer, a disease that annually causes more than 158,000 deaths in the United States, 610,000 deaths in China, and 1.6 million deaths worldwide (1,2). Activating mutations in the tyrosine kinase domain of the EGFR gene are known to be an oncogenic driver in lung tumorigenesis. It is now clear that non-small cell lung cancer (NSCLC) with EGFR activating mutations represent a distinct biological subtype that is highly sensitive to the treatment with EGFR inhibitors. In fact, the finding that EGFR-mutant lung cancer cells are highly susceptible to the EGFR inhibitors gefitinib (3) and erlotinib (4) has made these two agents the first choice for therapy in patients whose tumors harbor EGFR mutations. Both gefitinib and erlotinib have been reported to significantly improve disease control, objective response rate (ORR), progression-free survival (PFS), and quality of life in patients with EGFR-mutant lung cancer, when compared with conventional chemotherapy (5,6). Approximately 10–17% of lung adenocarcinomas patients in the United States and Europe (7,8) and about 30–65% of lung cancer patients in Asia have EGFR activating mutations (9,10). Deletions in exon 19 and the point mutation L858R in exon 21 are the most common EGFR activating mutations, accounting for about 85% of the EGFR mutations detected in lung cancers (10,11). In the absence of EGFR mutation or gene amplification, there is no significant difference in the responses to the treatment with EGFR inhibitors and conventional chemotherapies (12). Thus, EGFR gene mutation test is mandatory for using EGFR antagonists as the first-line treatment for advanced NSCLC (13). Consequently, genetic profiling of surgical and biopsy samples is routinely performed clinically for lung cancer patients to provide guidance for selection of treatment regimens. A number of DNA sequencing and polymerase chain reaction (PCR) based methods are used clinically to detect EGFR gene mutations in tumor specimens. However, the presence of intratumoral heterogeneity in most cancer patients (14) has imposed a challenge in using the information obtained from analysis of single tiny biopsy samples in clinical practice. Moreover, multiple and serial biopsies are often impractical clinically because of the potential complications of the procedures, including tumor seeding or spreading following percutaneous needle biopsy (15,16).

In a recent article of *Journal of Clinical Oncology*, Oxnard reported results from a retrospective analysis on treatment outcomes with osimertinib (AZD9291) in advanced NSCLC patients based on genotype analysis of patients' plasma and tumor specimens (17). Osimertinib is one of third generation EGFR antagonists that specifically inhibit cancers containing T790M mutations in the EGFR gene (18), which is resistant to the first generation EGFR antagonists' gefitinib and erlotinib. A number of mechanisms of acquired resistance to gefitinib and erlotinib have been reported, nevertheless, the most common cause of the acquired resistance observed clinically is acquisition of the EGFR T790M mutation, which is found in approximately 50% of patients (19). Clinical study revealed that treatment with osimertinib resulted in an ORR of 61% in patients with confirmed EGFR T790M mutation. In

contrast, the patients without EGFR T790M mutation had the response rate of 21% (20). Tumor biopsy was performed to determine the presence EGFR T790M mutation in patients with disease progression after the treatment with erlotinib or gefitinib. This promising result has led to the US Food and Drug Administration (FDA)'s approval of using osimertinib for the treatment of advanced NSCLC with EGFR T790M mutation. The FDA also approved a companion diagnostic test, the cobas EGFR mutation Test v2 which includes T790 mutation analysis in the EGFR gene. Nevertheless, obtaining biopsy specimen for the test is a challenge in clinical practices because of possible risks of biopsy procedure. Thus, results from Oxnard's group may change clinical practice in mutation analysis for precision therapy against lung cancers.

Using BEAMing digital PCR analysis, Oxnard *et al.* found that the sensitivity of plasma genotyping for detection T790M was 70% (17), comparable that reported previously (21). T790M mutation was detected in 18 of 58 (31%) patients with T790M negative tumor. Analysis on clinical outcomes revealed that ORR and median PFS were similar in patients with T790M-positive plasma (ORR, 63%; PFS, 9.7 months) or T790M-positive tumor (ORR, 62%; PFS, 9.7 months). For the patients with T790M-negative plasma, ORR was 46% and median PFS 8.2 months. Among the patients whose plasma genotyping is T790M-negative, favorable outcomes (ORR, 69%; PFS, 16.5 months) were observed when tumor genotyping was positive for T790M, whereas poor outcomes (ORR, 25%; PFS, 2.8 months) were observed when tumor genotyping was negative for T790M. Nevertheless, patients with T790M-positive plasma also responded to the osimertinib treatment differently based on their tumor genotyping results. The ORR and median PFS were higher in those with T790M-positive tumors (ORR, 64%; PFS, 9.3 months) than in those with T790M-negative tumors (ORR, 28%; PFS, 4.2 months). Those result suggested that plasma and tumor genotyping can have complementary roles for T790M mutation test. For approximately 50% NSCLC patients with acquired resistance to the first generation EGFR antagonists, biopsy may be avoided if their plasma genotyping is positive for T790M mutation. However, patients with T790M-negative plasma results may still need a tumor biopsy to determine T790M mutations, which may increase their test cost.

Accumulating evidences have demonstrated that tumor-specific alterations (mutations, translocations, aberrant methylations, and copy-number alterations) can be detected in the plasma cell-free DNA (cfDNA) of cancer patients using digital polymerase chain reaction (dPCR) and next-generation sequencing (NGS) (22). Because blood flows through both primary and metastatic tumors, the genomic profile of cfDNA is expected to constitute a representative readout of collective genomic alterations in all tumors in a patient, including primary and metastatic tumor nodules, thereby circumventing potential problems encountered by conventional biopsy owing to spatial heterogeneity that has been observed in a variety of cancers. For the 18 cases (30%) with T790M genotyping positive in plasma but negative in tumor reported in the study, the presence of T790M mutation in plasma cfDNA was confirmed in 14 cases (78%) by using an alternative assay, suggesting that the discordance might arise from spatial heterogeneity. However, this group of patients had lower ORR than the patients who were T790M positive in both plasma and tumor genotyping. The authors hypothesized that the patients with T790M positive plasma but negative tumor may have T790M positive cancer cells being present as minor clones. Because the half-life of circulating cfDNA is short (~16 minutes) (23), analysis of longitudinal samples can be used to monitor treatment response, disease recurrence, and emergence of treatment-resistant mutations, such reduction of T790M mutant cfDNA in plasma or emergence of osimertinib resistant C797S mutation, as reported by Oxnard's group recently (24). Unfortunately, dynamic changes of T790M mutation in plasma or emergence of C797S mutation were not included this retrospective study, therefore, the conclusion is not available for the putative spatial heterogeneity in this group of patients.

Like other laboratory tests used in clinics, plasma genotyping for precision medicine may have its limitations. Currently, mutational analyses of cfDNA are performed using ddPCR or NGS with small panels of cancer-related genes. One of major challenges in using cfDNA for detecting tumor-specific mutations is that only very limited amount of cfDNA can be isolated from plasma, which is not adequate for ddPCR assays on multiple hot spots of cancer mutations, or for genomic profiling of larger panels of cancer-related genes. Improvement in technologies and standardization of procedures for sample acquisition, processing and analyses will be needed for realization of the potential applications of plasma genotyping in era of precision therapy for cancer.

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Footnote

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