Editorial

Circulating tumor DNA analysis in patients with EGFR mutant lung cancer

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Comment on: Wang Z, Cheng Y, An T, *et al.* Detection of EGFR mutations in plasma circulating tumour DNA as a selection criterion for first-line gefitinib treatment in patients with advanced lung adenocarcinoma (BENEFIT): a phase 2, single-arm, multicentre clinical trial. Lancet Respir Med 2018;6:681-90.

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Genotype-oriented treatment has led to dramatic clinical improvements for patients with non-small cell lung cancer (NSCLC) harboring a targetable oncogenic driver (1). The identification of several targetable oncogenes and improved knowledge of resistance mechanisms provide rationale for obtaining clinical samples to assess tumor biology and the evolution of drug resistance (2).

Fragments of DNA that are shed into the blood by tumor cells can be detected as circulating tumor DNA (ctDNA) (3). With appropriate controls and caveats, ctDNA can be tumor specific and provide molecular information about fragmented DNAs from tumor cells and their specific mutations. Genotyping of blood ctDNA avoids some of the issues plaguing invasive tumor biopsies, and ctDNA assays can sample tumor DNA arising from multiple tumor subclones and metastatic sites; by contrast, tissue-based DNA analysis is typically performed on an individual tumor sample (4). Quantitative and qualitative analyses of ctDNA provide real-time evaluation for diagnostic and prognostic assessments (5). Therefore, ctDNA appears to be a highly promising biomarker assay for the detection of actionable mutations, response to therapy, and emerging drug resistance mechanisms in NSCLC (6).

Multiple studies have assessed the validity of ctDNA assays in NSCLC (7-9). In general, PCR-based assays for detecting oncogenic drivers show high diagnostic specificity. The average of specificities for lung cancer canonical driver mutations was 96% (95% CI: 83–99), and average of sensitivities was 66% (95% CI: 63–69) across five prior studies that used tissue-based genotyping as the comparator (5-9). To demonstrate the relevance of using ctDNA analysis as a clinical test, prospective clinical trials must demonstrate the clinical utility of ctDNA profiling. Though no trials have showed this utility as a stand-alone diagnostic test, one assay based on ctDNA PCR for detecting *EGFR* mutations in NSCLC patients is approved in the United States and Europe at present. The increasing number of potential genetic drivers in advanced-stage NSCLC prompted interest in developing next-generation sequencing (NGS)-based multiplex ctDNA assays for the detection of a wide range of genomic events (10,11). Since detecting subclonal variants by high-sensitivity ctDNA assays may predict for non-responders to targeted therapies, alternative treatment strategies could be devised for patients whose cancer harbors multiple co-occurring mutations.

Another possibility for ctDNA analysis is to quantitatively assess drug effects by measurement of ctDNA serially during treatment(s). Correlations between ctDNA changes and treatment effects have been demonstrated in several NSCLC studies (5,12,13). In addition, some studies show that ctDNA analysis can identify the onset of drug resistance mutations months before conventional radiographic imaging, offering the potential opportunity to augment or alter therapy before clinical progression with the opportunity for enhanced clinical outcomes (14-16).

The article by Wang and colleagues entitled "*Detection*

of EGFR mutations in plasma circulating tumour DNA as a selection criterion for first-line gefitinib treatment in patients with advanced lung adenocarcinoma (BENEFIT): a phase 2, single-arm, multicentre clinical trial" is an important single-arm prospective study that delivers three important messages for clinical practice.

First, this study shows the effectiveness of *EGFR* mutation detection in ctDNA to identify patients for first-line gefitinib treatment. This BENEFIT trial provides initial results supporting the feasibility of using plasma ctDNA-based *EGFR* mutation analysis prospectively in the first-line EGFR-TKI treatment setting. In the trial, the proportion of patients achieving an objective response was 72.1% (95% CI: 65.0–78.5) and median progression-free survival (PFS) was 9.5 months (95% CI: 9.07–11.04). These results are comparable to the tissue-based detection and therapy assignment in clinical trials of first-line gefitinib, such as NEJ002 (PFS: 10.8 months) and WJTOG 3405 (9.2 months) in Asia (17,18). This result provides support for the clinical use of ctDNA assays for tumor genotyping, compared to conventional tissue biopsy. Cases in which the *EGFR* variant is not detected by ctDNA profiling but identified by tissue profiling are relatively frequent (30%; 78 of 260 patients in this trial); thus, undetected ctDNA assay results should be confirmed by tissue biopsy. On the other hand, tissue negative and ctDNA positive patients for EGFR mutation occurred in only 4.2% (8 of 190 patients) of patients in this study and median PFS was notably shorter (6.0 months) among this group.

The low sensitivity of *EGFR* mutation genotyping by ctDNA profiling could be a bottleneck. The authors mentioned that low sensitivity is due to definition of positive *EGFR* mutation by digital droplet PCR assays and transporting samples from study centers to the central laboratory at room temperature. However, these problems are possible in clinical practice. Therefore, simpler and more efficient techniques for handling ctDNA at the hospital site may be needed. If these issues are improved and a biomarker from liquid biopsies is also established for immune check point inhibitor therapy, liquid biopsy would likely become the first choice for NSCLC biopsy in the clinic.

Second, the authors show dynamic alterations of *EGFR* mutations in plasma samples during treatment. At week 8 of gefitinib treatment, 147 (88%) of 167 patients had clearance of *EGFR* mutations in ctDNA and 20 (12%) had persistent *EGFR* mutations detectable. Median PFS was greater in patients with clearance of the *EGFR* mutations at week 8 compared with those who had persistent *EGFR* mutations detected [11.0 months (95% CI: 9.43–12.85) *vs.* 2.1 months (1.81–3.65); HR: 0.14, 95% CI: 0.08–0.23; P<0.0001]. This is an important message because this offers an opportunity to document tumor progression early by more frequent or earlier radiographic imaging when the *EGFR* mutation clearance is not seen. One unanswered question is whether we should consider combination therapies for patients with druggable co-occurring mutations at baseline or emerging early during EGFR inhibitor treatment among these patients with persistent *EGFR* mutations detected by ctDNA analysis (19).

Further consideration will be needed for the patients whose sample detected *EGFR* T790M mutation during treatment. The use of osimertinib in patients with *EGFR* T790M (occurring at resistance to first-generation EGFR-TKIs) by ctDNA analysis is an accepted clinical approach (20). In 69 patients with an acquired *EGFR* T790M mutation identified by digital droplet PCR analysis during gefitinib treatment, the median time to *EGFR* T790M emergence was 7.6 months (95% CI: 6.0–10.0) in this trial. The median time from *EGFR* T790M emergence to disease progression was 2.0 months (95% CI: 2.0–4.9). No evidence supports altering therapy at the time of ctDNA-based detected of *EGFR* or other gene mutations before progressive disease is observed by conventional assessment; and certain findings indicate that ctDNA changes are not always concordant with radiological imaging with respect to tumor response. We do not know whether early intervention by osimertinib treatment based on ctDNA *EGFR* T790M positivity will improve clinical outcome. Since *EGFR* T790M is an actionable target, additional evidence to guide the clinical sequence of different classes of EGFR-TKIs is needed.

Third, the authors also performed ctDNA-based NGS analysis at baseline. They separated the 179 patients into three subgroups according to the additional genetic aberrations detected. The subgroups consist of one with only *EGFR* mutations (n=58), one with *EGFR* mutations and other mutations in tumor suppressor genes (*TP53*, *RB1*, or *PTEN*) (n=97), and one with additional oncogenic alterations (*MET*, *ERBB2*, *KRAS*, *BRAF*, *RET*, or *ROS1*) besides *EGFR* mutations (n=24). Median PFS was 13.2 months (95% CI: 11.5–15.0), 9.3 months (7.6–11.0), and 4.7 months (1.9–9.3) for the three subgroups, respectively (only *EGFR* mutations *vs EGFR* mutations and tumor-suppressor mutations, HR: 1.78, 95% CI: 1.23–2.58; P=0.002; only *EGFR* mutations *vs. EGFR* mutations and multiple driver mutations, HR: 2.66, 95% CI: 1.58–4.49; P=0.0003). Surprisingly, analysis of 20 patients who had

persistent *EGFR* mutations detected at week 8 showed that 90% of them harbored coexisting alterations in tumor-suppressor genes, oncogenic drivers, or both at baseline. These data could account partly for why 20–30% of patients with EGFR TKI sensitizing mutations had no or inferior response to first-line EGFR-TKI therapy (17,18,21,22). This may suggest that evaluating intra-tumor and inter-tumor co-occurring gene mutations is important before initiating EGFR-TKI treatment. Indeed, mutations in *TP53* and *PTEN* are reported to contribute to resistance to EGFR-TKI treatment in *EGFR*-mutant NSCLC (23,24).

Our group previously reported on ctDNA analysis prior to osimertinib among 41 patients. Primary resistance to osimertinib was associated with concurrent oncogenic alterations in *MET*, *NF1*, *CDK4*, *CCNE*, *CDK6*, *PIK3CA* and *APC* (25). Pathway level alterations in cell cycle genes were associated with poor response to osimertinib. Decreased PFS to subsequent osimertinib treatment was also associated the presence of concurrent cell cycle gene alterations. This study suggests a function for MAPK, PI3K, and WNT pathway alterations in driving primary osimertinib resistance and identifies certain cell cycle gene alterations as a biomarker of non-response to osimertinib treatment.

Based on these two independent datasets, genetic co-alterations may co-promote cancer progression and targeted therapy resistance. We suggest that not only tumor-suppressor genes but also cell cycle genes should be examined before initiating EGFR-TKI treatment. Additional basic research should be done to determine which alterations are likely to contribute functionally to resistance and how best to therapeutically co-target such co-occurring alterations to prevent, or overcome the associated drug resistance.

In summary, Wang and colleagues provide an important step forward supporting the utility of *EGFR* mutation detection based on ctDNA to assess the likelihood of response to first-line EGFR-TKI treatment, with implications for clinical diagnostic and therapeutic decision making. Their use of dynamic measurement of *EGFR* mutation status and profiling of co-occurring gene alterations at baseline provide considerable support for prospectively testing novel strategies of EGFR-TKI sequencing and early detection and therapeutic intervention against co-alterations driving drug resistance to forestall the evolution of therapy resistance.

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Footnote

Conflicts of Interest: TG Bivona is a consultant or advisory board member to Revolution Medicines, Array Biopharma, Novartis, AstraZeneca and receives research support from Revolution Medicines and Novartis. S Nanjo receives research support from AstraZeneca.

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