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Plasma genotyping in patients with non-small-cell lung cancer: simplifying or confusing the diagnosis?

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The identification of driver mutations in patients with advanced non-small-cell lung cancer has changed the treatment outcomes for patients with actionable driver mutations. Lack of tissue at diagnosis, however, remains a central obstacle in making optimal treatment decisions in patients with advanced disease. Although the US FDA has approved one plasma-based test for detecting epidermal growth factor receptor mutations in patients with advanced stage disease, sensitivity of these assays remains mediocre, necessitating additional tissue testing and possible delays in patients with negative results. Serial monitoring for response and early detection of acquired resistance to targeted therapies is also possible with cell-free DNA, however the benefit of switching therapy prior to detection of changes on imaging is unknown currently.

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Lung cancer is the leading cause of cancer-related mortality in the USA with an estimated 224,390 new cases and 155,990 deaths anticipated in 2016 [1]. Approximately 85% of patients with lung cancer will have non-small-cell lung cancer (NSCLC), of which adenocarcinoma is the predominant histology. The identification of actionable driver mutations has changed the landscape of treatment options and trajectory of outcomes for a cohort of patients and testing patients at diagnosis and on progression is now considered standard of care.

Lack of tissue at diagnosis remains a central obstacle in making optimal treatment decisions in patients with newly diagnosed stage IV NSCLC [2]. Many patients are diagnosed from fine needle aspirations from minimally invasive approaches such as endobronchial ultrasound or CT-guided percutaneous biopsy limiting tissue availability when compared with quantities acquired from core biopsies or resections during thoracoscopy or mediastinoscopy. Primary lung tumors carry less areas of pure carcinoma (20%) compared with other epithelial tumors which limit cytologic yield and availability for molecular testing [3]. While Malapelle *et al.* showed detecting *epidermal growth factor receptor* (EGFR) mutations from cytology specimens compared with macroscopic histologic specimens was nearly identical, they noted specimen availability and low cellularity as major limiting factors for oncogenic driver detection [4]. In a meta-analysis by Ellis *et al.*, the authors analyzed five Canadian laboratories and their ability to successfully test for EGFR mutations in samples from 2114 lung adenocarcinoma patients. Mutation testing was conducted in 84% of samples. The other 16% were unable to be processed due to low specimen yields [5]. The widespread use of next-generation sequencing and its ability to be tested from nanograms of DNA has overcome some of the issues with limited specimen availability, however poor cellularity remains a real barrier to testing for driver mutations.

KEYWORDS

ALK • cell-free DNA • EGFR
• non-small-cell lung cancer
• targeted therapy

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With the introduction of targeted therapy showing superior outcomes to chemotherapy, the identification of patients with actionable mutations is paramount to making treatment decisions [6,7,8,9,10,11]. For example, *EGFR* mutations have been detected in approximately 10% of North American patients diagnosed with NSCLC. *EGFR* tyrosine kinase inhibitors (TKI's) are approved as first-line therapy in patients with advanced NSCLC. Recent studies have demonstrated improved response rate, progression-free survival (PFS) and overall survival (OS) in patients with *EGFR* mutation positive NSCLC treated with an *EGFR* TKI prior to chemotherapy. The majority of patients treated with an *EGFR* TKI develop resistance to therapy within about 12 months, with approximately 50% of patients acquiring the *T790M* second site mutation in exon 20 [12,13]. Recently the US FDA approved a blood-based assay to evaluate for the presence of *EGFR* mutations in newly diagnosed Stage IV lung adenocarcinoma patients and at the time of resistance post exposure to an *EGFR* TKI. Detecting the precise mechanism of resistance has become clinically relevant because of the availability of next-line options. Osimertinib was approved as an *EGFR*-directed third-generation TKI with efficacy against *T790M* [14]. A recent Phase III trial demonstrated osimertinib was superior to platinum-based chemotherapy in patients with *EGFR* mutation positive NSCLC and *T790M* as their mechanism of acquired resistance to first and second-generation *EGFR* TKIs [15].

Malignant tumors shed DNA by way of apoptosis, secretion and necrosis. When the tumor burden is relatively high, approximately 50 million cancer cells, plasma-based cfDNA can reliably be detected with some existing technology [16]. This detection typically occurs prior to radiographic detection which occurs when the tumor volume approaches one billion cancer cells. One of the advantages of cfDNA analysis compared with tumor tissue is that it represents the entire molecular milieu of a tumor that can potentially be missed by way of a single biopsy [17,18]. In addition to the issue of tumor heterogeneity, tissue biopsies are limited by access to special operators, tumor location, procedural complications, patient frailty and tissue preservation. Moreover, blood-based testing of cfDNA has the potential for a shorter turnaround time. While all of these attributes make cfDNA seem like an attractive alternative

to tissue biopsy the question remains whether it represents an adequate surrogate or substitute. Numerous studies in current literature have sought to answer this question.

Technology

Before evaluating the studies supporting the use of cfDNA an understanding of the different technologies employed is imperative. Tumor DNA fragments were first noted in plasma 70 years ago, however the clinical utility of cfDNA was not realized until much later after levels were noted to be higher in patients with malignancies compared with healthy controls [19]. In patients with lung cancer this correlation was noted by Yoon *et al.*, who compared plasma cfDNA concentrations between 102 patients with NSCLC and 105 healthy controls and noted a statistically significant difference in median concentrations of cfDNA (22.4 and 10.6 ng/ml for lung cancer and control patients, respectively), even in patients with early stage disease [20]. A meta-analysis by Ai *et al.*, including a total of 22 studies involving 2518 NSCLC patients, indicated patients with the highest tertile of cfDNA concentrations defined as >6 ng/ml had statistically significant shorter median PFS and OS compared with patients with cfDNA levels in low and medium tertiles. The authors also found circulating DNA quantity detected correlated with tumor volume, stage, lymph node involvement and response to therapy [21].

The change in perspective from looking at cfDNA as a purely prognostic biomarker to a predictive one began with the paradigm shift brought on by the discovery of actionable driver mutations. The large majority of assays to assess the presence of these driver mutations in plasma involve PCR and include non-digital (RT-PCR, COBAS, Scorpion-Amplified Refractory Mutation System [ARMS]) and digital (BEAMING and droplet digital) approaches.

RT PCR uses primers directed at target mRNA sequences with the addition of reverse transcriptase to the assay prior to amplification with TAQ polymerases [22]. The Cobas assay involves real-time PCR and is able to identify 42 different mutations in exons 18–21 of the *EGFR* gene. Additionally, it has a quantitative index which provides information about the percentage of mutant DNA present in the sample. Scorpion primers utilize fluorescence to detect PCR products. The Scorpion probe is a hairpin loop configuration which incorporates

both primer and fluorescent probe into the same molecule with the former on the three-prime side and the latter on the five-prime side. An additional component of the molecule is a quencher which also lies on the five-prime end of the molecule and prevents background amplification while the primer binds to the sequence of interest [23]. BEAMING involves purification of the DNA sequence of interest from plasma using conventional PCR. These amplified fragments again undergo emulsion PCR and are covalently bonded to magnetic microbeads. These fragments are then easily separated from the affixed microbeads using another magnet [24]. Droplet digital PCR involves water–oil emulsion that creates nanoliter-sized droplets, each of which serves the same purpose as an individual test tube for PCR-based reactions. The PCR products in each droplet are read in a flow cytometer to quantify the fraction of mutant DNA sequences present [25].

As cited in several cross-platform studies described in the following paragraphs, digital PCR-based plasma assays are thought to have the highest sensitivity (74–100%) for detecting activating EGFR mutations followed by non-digital PCR assays such as Cobas (72–90%) and Scorpion ARMS (43–82%). Sensitivity for detecting *T790M* is lower with each assay, however digital PCR-based assays (71–73%) again demonstrate higher sensitivity than their nondigital counterparts (41–64 and 29% for Cobas and ARMS, respectively). Specificity for detecting EGFR mutations across both digital and nondigital PCR-based assays is excellent and ranges from 93 to 100%.

Surrogate or substitute

The journey to prove adequacy of plasma cfDNA as a surrogate for tumor tissue began 10 years ago. The first study comparing plasma and paired tissue samples by Kimura *et al.* in 2006 included 27 Japanese patients with samples collected prior to gefitinib therapy. Plasma cfDNA was analyzed through the Scorpion ARMS system. Of 11 patients with matched plasma and tumor samples, 72% were concordant. Furthermore, the median PFS was nearly triple in patients with EGFR activating mutations in cfDNA compared with those who did not possess such mutations [26].

Another early study assessed 35 patients with advanced stage lung adenocarcinoma and detected *exon 19* deletions and *L858R* in 17 and

26% of pretherapy plasma samples by using droplet digital PCR. When cfDNA results were compared with results from tumor tissue, plasma demonstrated sensitivity and specificity of 92 and 100%, respectively. More than only demonstrating concordance several other observations were gleaned from the study analysis. From five patients with EGFR mutations who had cfDNA samples available pre- and post-initiation of TKI therapy, four out of five showed marked drops in copy numbers of mutant DNA. Three of these four patients had partial responses whereas one had a complete response. The one patient with persistent EGFR mutant copy numbers present in plasma had increased tumor growth [27].

Douillard *et al.* retrospectively assessed plasma and tumor tissue from the IFUM trial using Scorpion ARMS. The IFUM study included 1060 Caucasian patients with locally advanced or metastatic lung adenocarcinoma who were screened for the presence of activating EGFR mutations and subsequently treated with gefitinib if positive [28]. In their retrospective analysis 652 plasma and tumor tissue samples were matched with 94% concordance. CfDNA demonstrated 65.7% sensitivity and 99.8% specificity for mutational detection. Overall response rate for patients with EGFR mutant positive tumor tissue was 77% compared with 69% with EGFR mutant positive cfDNA. The authors concluded that while EGFR mutation status can be detected accurately by cfDNA, a negative plasma assay does not rule out the possibility of false negatives and tissue biopsy should be obtained in this scenario [29].

Mok *et al.* retrospectively assessed patient samples from the FAST-ACT 2 trial by using the Cobas EGFR assay. The FAST-ACT 2 trial was a Phase III study where Stage IIIB and IV NSCLC patients were randomized in a 1:1 fashion to gemcitabine and platinum chemotherapy alone or gemcitabine, platinum and erlotinib [30]. A total of 241 tumor samples and 427 plasma samples from 451 enrolled patients were available for analysis. Forty percent of tissue samples and 32% of plasma samples were positive for activating EGFR mutations. The sensitivity of the plasma assay was 76% and the specificity was 96% for these activating mutations. Median PFS and OS of patients with plasma positive EGFR mutations treated with combination TKI + chemotherapy was 13.8 months and 32.4 months compared with 6.1 months and 19.0 months for patients treated with chemotherapy alone. There was no

statistically significant difference in PFS and OS between the two arms in *EGFR* wild-type patients leading investigators to conclude that cfDNA is highly specific for *EGFR* mutations and that peripheral *EGFR* mutant positivity was predictive of response to therapy with an *EGFR* TKI [31].

Sacher *et al.* conducted a study to prospectively validate droplet digital PCR in the detection of *EGFR* mutations from plasma in 180 patients with advanced NSCLC. Sensitivity of the plasma PCR assay was 82% for exon 19 deletions, 74% for *L858R* and 77% for *T790M*. Sensitivity for mutations was higher in patients with sites of multiple metastases, in particular those with disease in liver or bone. Positive predictive value of the assay was 100% for *deletion 19* and *L858R* and 79% for *T790M*. The authors postulated specificity for *T790M* was lower than for activating mutations due to increased intratumor heterogeneity for the second site mutation. This is consistent with findings from several studies where *T790M* plasma-positive but tissue-negative patients respond to third-generation TKIs [32].

A meta-analysis from Luo *et al.* included 20 studies where plasma cfDNA and tumor tissue were compared in patients with *EGFR* mutant lung adenocarcinomas. 19 of the 20 included studies were conducted in Asia and the most frequent assay used to analyze patient samples was Scorpion ARMS. Compared with tumor tissue, the pooled sensitivity and specificity of cfDNA for *EGFR* mutation status was 67 and 94%, respectively. Importantly the authors noted several of the other higher sensitivity assays such as HPLC and digital PCR were less represented and thus actual sensitivity may have been underestimated [33].

The ASSESS trial evaluated the concordance between cfDNA and tumor biopsy in 1188 European and Japanese patients with treatment naive metastatic and locally advanced NSCLC. A number of plasma-based cfDNA assays were utilized with Therascreen, Cobas and Cycleave being the most common. Concordance between plasma and tissue was 89% with cfDNA displaying 46% sensitivity and 97% specificity for detecting *EGFR* mutations. The aggregate sensitivity of cfDNA was thought to be lower for this study when compared with sensitivities cited in other analyses due to less consistent use of high sensitivity plasma assays such as Therascreen and Cobas [34].

Thress *et al.* conducted a cross-platform analysis of the different assays to detect *EGFR* mutations in cfDNA from patients enrolled in the AURA trial, a Phase I/II trial of osimertinib. Samples were analyzed prior to treatment with osimertinib but after documented clinical progression on a first- or second-generation *EGFR* TKI. Thirty-eight plasma samples were analyzed by digital (droplet digital and BEAMING) and nondigital (Cobas and Therascreen) platforms and compared with tumor tissue. The platforms with the greatest sensitivity and specificity for detecting both activating *EGFR* mutations along with *T790M* were Cobas and BEAMING; the latter displayed the highest sensitivity for detecting *T790M*. Concordance between the two assays was 90, 93 and 90% for *L858R*, *exon 19* deletions and *T790M*, respectively. Clinical response rate was identical between patients with *T790M* detected in plasma and tumor tissue and approached 60% [35].

Karlovich *et al.* also compared the Cobas and BEAMING plasma assays with tumor tissue from 110 patients enrolled on the Tiger-X trial with rociletinib, a third-generation *EGFR* TKI that is no longer in clinical development. *EGFR* sensitizing mutations were detected in plasma with a sensitivity of 73% while the sensitivity for *T790M* was 64% through the Cobas assay. *EGFR* activating mutations were detected in plasma with a sensitivity of 82% while *T790M* was captured in 73% of patients through the BEAMING assay. Thirty-five patient samples were analyzed by both assays and demonstrated 86% concordance for activating *EGFR* mutations and 83% for *T790M* [36]. Results from this study were updated by Wakelee *et al.* at ASCO 2016 and included 417 patients at the time of completion. The response rate to rociletinib was assessed based on *T790M* positivity in tumor tissue, plasma and urine. Overall response rates were 39.4, 33.6 and 40.7%, respectively [37].

Oxnard *et al.* assessed the correlation between plasma and tumor tissue retrospectively in the first in-human study of osimertinib. *EGFR* mutant patients who had progressed on first-line TKI therapy and were found to have *T790M* by tissue biopsy, were assessed for the presence of the resistance mutation peripherally through the *EGFR* Cobas V2 assay. There was 92% concordance between cfDNA and tumor tissue and the plasma assay was 72% sensitive and 96% specific. Patients with *T790M* detected

peripherally (>0.06% AF) had nearly identical overall response rates and PFS when compared with patients with *T790M* detected by tumor tissue (ORR 62 and 63%, respectively; identical PFS of 9.7 months). Most impressively, this study suggested a positive peripheral plasma test for *T790M* was enough to treat patients with osimertinib. A negative peripheral cfDNA result was not definitive as 47 patients who were *T790M* negative in plasma were positive for the mutation on tumor biopsy. The authors concluded in the face of a negative plasma test, tissue diagnosis is still warranted [38].

Serial monitoring

Several studies have looked beyond concordance and assessed whether *EGFR* mutant positive cfDNA is predictive of response to TKI therapy and whether plasma mutant copy numbers vary with treatment. In the previously mentioned TIGER-X trial a small subgroup of 24 patients were assessed for changes in *EGFR* mutational levels with plasma assessments prior to, during and after receiving rociletinib. Patients who experienced a partial response all had a marked drop in their *EGFR* mutant levels to <10 molecules/ml by day 21. The patients who experienced progressive disease did not have a significant difference in their *EGFR* mutant levels between days 1 and 21. Riedeger *et al.* evaluated a cohort of 16 advanced stage lung adenocarcinoma patients who underwent serial plasma sampling while on TKI therapy. Therapeutic responses were observed in cfDNA within 26 h of starting the TKI. Long periods of stable *EGFR* mutant cfDNA levels suggested continued response to therapy whereas steady increase in mutant levels preceded future progression. Progression in plasma occurred as early as 4 months prior to clinical progression [39].

Digital droplet PCR was utilized in a longitudinal assessment of patients with advanced stage lung adenocarcinoma and activating *EGFR* mutations from the Korean lung cancer consortium. The cfDNA assay was 74% sensitive and 100% specific for *EGFR* mutations compared with tissue. Among 54 patients who had serial samples collected, 40 had exon 19 deletions or L858R mutations detected in plasma. Out of these 40 patients, 34 had *EGFR* mutant copy numbers reduced to undetectable levels during treatment. Interestingly there was a statistically significant difference in PFS and OS between

the patients who achieved undetectable plasma *EGFR* mutant levels and those who did not (10.1 vs 6.3 months and 23.7 vs 11.2 months, respectively). Forty-nine patients had cfDNA samples available at the time of progression and 14 developed *T790M*. Eight of the 14 patients had *T790M* detected in plasma 2 to 12 months prior to radiographic progression while 6 had the mutation detected at the time of progression. This was the first study to perform CT scans serially along with cfDNA monitoring to provide a radiographic correlate to mutant plasma copy numbers [40]. The utility of switching patients based on copy number increases in *EGFR* mutant cfDNA rather than imaging progression has not been validated.

Zheng *et al.* assessed 124 patients with activating *EGFR* mutations who had progressed on first-line *EGFR* TKI therapy. Twenty-five patients had matched plasma and tumor tissue postprogression with plasma analysis through digital droplet PCR. Concordance for *T790M* between the two was 88% with digital droplet PCR displaying a sensitivity of 81% and specificity of 100%. Seventy-six patients had pre-progression plasma available and *T790M* was detected in 46%, on average 2 months before any evidence of clinical progression [41]. No therapeutic changes were made based on the detection of *T790M* peripherally.

Beyond EGFR

ALK (anaplastic lymphoma kinase)fusions are detected in 7% of North American patients with advanced stage NSCLC. Crizotinib is currently approved as first-line treatment for patients with Stage IV NSCLC positive for the *ALK* rearrangement. While approximately 60% of crizotinib-treated patients respond initially, the majority of patients experience disease progression [42]. Currently ceritinib and alectinib are approved at the time of progression with activity dependent on the mechanism of acquired resistance [43,44]. The challenge with designing PCR assays in the *ALK* setting is primer design as the break point regions within the *ALK* gene occur within large intron islands [45]. Capture-based deep sequencing offers a new potential option for screening for the presence of the fusion gene and its secondary mutations.

In a recent study targeted deep sequencing from cfDNA was performed in 24 patients with IHC and FISH confirmed *ALK* positive Stage IV NSCLC and 36 controls. Sensitivity

for detecting the *ALK* rearrangement was 79% while specificity was 100%. Seven patients also underwent longitudinal cfDNA monitoring and correlative imaging studies with CT scans. In all seven patients rising levels of cfDNA and *ALK* allelic fraction percentage preceded or accompanied radiographic progression. In one of the seven patients, the secondary *ALK* mutation L1196M was noted in cfDNA [46]. Next-generation sequencing was performed at baseline and on study from plasma in patients enrolled on a Phase I trial with ensartinib, a second-generation *ALK* inhibitor. These results were compared with tissue results (FISH/IHC) in treatment-naïve patients and patients with acquired resistance to crizotinib and revealed 74% concordance. Plasma and tissue genotyping was available on 27 patients enrolled on study of which 26 were confirmed to be *ALK* positive via FISH. In 16 patients who had the *ALK* rearrangement present in plasma, all responded to therapy while nine plasma samples were not evaluable. Serial plasma sequencing demonstrated a decrease in *ALK* copy numbers in patients who were responding and increase in copy numbers in those who were progressing [47].

Conclusion

Blood-based testing is a reality in the diagnostic armamentarium for patients with advanced NSCLC. CfDNA has gone from merely a prognostic biomarker in advanced stage NSCLC patients to a predictive one in driver mutation positive patients. Sensitivity of cfDNA assays for detecting *EGFR* and *ALK*, while improving with new PCR and deep sequencing technologies, remains suboptimal with significant inter-assay variability. High specificity of the assays, however, makes them excellent screening tools. Tissue biopsy remains the standard of care in the cfDNA-negative setting and there is a continued need for technological refinement of the assays to improve sensitivity. Plasma-based monitoring of driver mutation positive NSCLC patients has shed new insight into the dynamic changes in mutant DNA copy numbers once therapy is initiated and raised the possibility of detecting resistance prior to radiographic progression. The utility of switching therapy based on cfDNA changes without radiographic correlates remains to be seen.

Future perspective

With multiple agents approved and in clinical development, treatment approaches for patients with *EGFR* and *ALK* mutant positive NSCLC continues to evolve and may help define the approach for all patients with driver mutations. The opportunity for dynamic monitoring to assess when and how resistance occurs remains an active area of investigation but the utility of switching therapy prior to radiographic changes remains unknown. While a biopsy for tissue diagnosis and molecular testing remains standard of care, limitations in capturing tumor heterogeneity and lack of operators to perform the procedures remains a challenge. Moreover, it is not always feasible to obtain repeat tissue biopsies from patients at the time of diagnosis or progression leaving blood-based testing as a potential therapeutic strategy. As the sensitivity of cfDNA assays continue to improve plasma may become the gold standard for detecting oncogenic driver mutations. With time, serial cfDNA analysis may prompt physicians when to perform imaging, limiting patient exposure to unnecessary testing. In such a scenario, copy numbers of the mutant sequences would be monitored to reveal which patients were responding to therapy and which patients would need a change in therapeutic course. The detection of resistance mutations such as *T790M* or *L1196M* to clinically significant copy numbers would prompt initiation of specific next lines of therapy potentially even prior to radiographic progression. However, retrospective data demonstrating a survival advantage would be needed prior to such strategies being employed. For now, blood-based testing provides an option for patients reluctant to undergo more than one biopsy at the time of diagnosis or after progression in patients on targeted therapy. The caveat remains that a negative test, especially in never or light smokers, is not a substitute for a negative tissue diagnosis.

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EXECUTIVE SUMMARY

- *EGFR* and *ALK* are the most common actionable oncogenic drivers in non-small-cell lung cancer (NSCLC) adenocarcinoma patients. Others include *ROS1*, *BRAF* and *MET*.
- The primary mechanism of resistance to first-generation *EGFR* tyrosine kinase inhibitors (TKIs) is the acquisition of the second site T790M mutation. Resistance to *ALK* inhibitors is less uniform, however *L1196M* is one of the more frequently encountered resistance mutations.
- The existence of multiple lines of *EGFR* and *ALK* inhibitors has made the timely determination of when clinical resistance occurs important. Cell-free DNA (cfDNA) represents an alternative to tissue biopsy in this space given its rapid turn-around time, lack of invasiveness and ability to represent complete molecular tumor heterogeneity.

Technology

- CfDNA was initially thought to be purely prognostic in NSCLC patients. The ability to detect oncogenic drivers peripherally transformed it into a predictive biomarker.
- Most of the current cfDNA assays to detect *EGFR* mutations are PCR-based and can be classified into digital (BEAMING, droplet) and nondigital (RT-PCR, Cobas, Scorpion-Amplification Refractory Mutation System) categories.
- Digital PCR-based assays appear to demonstrate the highest sensitivity and specificity for detecting *EGFR* activating mutations and T790M from plasma compared with nondigital counterpart assays.

Surrogate or substitute

- Osimertinib has now been approved as a third-line TKI with specific activity against *EGFR* T790M along with a companion diagnostic cfDNA assay (*EGFR* Cobas V2). Given the high specificity of the plasma assay a positive test for T790M is sufficient to initiate therapy with the medication. A negative test, however, still warrants tissue biopsy.
- In several studies patients with T790M positive cfDNA but negative tumor tissue show response to third-generation *EGFR* TKI. This suggests incomplete capture of tumor heterogeneity may be a limitation of considering tissue as the diagnostic gold standard.

Serial monitoring

- In trials where *EGFR* mutant patients have undergone cfDNA monitoring before initiating first-line therapy and at time of progression, dynamic changes in mutant copy number and emergence of acquired resistance have been noted in peripheral blood. This raises an intriguing possibility where serial monitoring of cfDNA may be able to demonstrate response to current therapy and preclinical progression.
- Making changes to therapy based on copy number changes in cfDNA has yet to be validated.

Beyond EGFR

- PCR-based cfDNA assays for detecting *ALK* gene rearrangements are more challenging given the limitations of designing primers with target breakpoint regions submerged amid intron islands. Deep sequencing from plasma may represent the answer for detecting *ALK* rearrangements, and initial studies utilizing this technology suggest similar sensitivity and specificity to PCR-based peripheral *EGFR* assays when compared with tissue biopsy.
- In trials where *ALK* rearranged patients have undergone cfDNA monitoring while on first- and second-generation *ALK* inhibitors, rising *ALK* copy numbers and allelic fraction percentages preceded objective progression.

Conclusion

- The high specificity of cfDNA assays makes them outstanding screening tests for detecting *EGFR* and *ALK* mutations.
- Sensitivity of cfDNA assays for detecting oncogenic drivers is still not optimal (<85% in most studies) and is even lower for detecting second site mutations (<75% for T790M). Given this limitation a negative plasma test still mandates the need for tissue biopsy.
- Monitoring copy numbers of *EGFR* and *ALK* along with the emergence of secondary mutations in cfDNA of NSCLC patients on targeted inhibitors raises the possibility whether resistance can be detected prior to radiographic or clinical progression. This is yet to be validated in studies.

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