Editorial

Liquid biopsy for T790M mutation detection: A ray of hope?

Lung cancer continues to be a menace to the managing clinician in spite of several advances in the diagnostics and therapeutics of this dreaded disease. Since the recognition of epidermal growth factor receptor (EGFR) exon 19 and 21 mutations in the pathogenesis of non-small cell lung cancer (NSCLC) as driver mutation, substantial progress has been made in terms of diagnosis as well as management protocols for nonsquamous NSCLC. EGFR mutation-directed therapy has made a significant difference in progression-free survival, and it has now become a routine clinical practice to test for EGFR mutations in tissue biopsy samples of these patients using immunohistochemistry. EGFR mutation testing can be performed on cytology as well as histology samples. In recent years, the use of endobronchial ultrasound-guided transbronchial needle aspirate sample is being evaluated and has been found promising as a specimen for tumor mutational analysis.[1] First-line EGFR mutation-directed therapy in the form of erlotinib or gefitinib has been the therapy of choice for years now. Unfortunately, however, disease progression on first-line tyrosine kinase inhibitor (TKI)-based therapy is a rule rather than the exception. The reasons for this progression include the development of new mutations conferring resistance to TKI (T790M on exon 20, in upto 50% cases), MET amplification (5%–20% cases), and transformation of histology.[2-4]

Detection of these new mutations is important and usually requires rebiopsy of the tumor, which in many instances may not be feasible. A significant number of individuals on TKI therapy have progression and development of new lesions in inaccessible locations such as the brain or vertebral column (while primary tumor may show response), thus making rebiopsy nearly impossible. The poor performance status of these patients also makes rebiopsy difficult. In fact, it is estimated that up to 40% of relapsed patients with NSCLC may be unable to provide a tumor tissue sample suitable for molecular analysis. Hence, extensive search is ongoing for a relatively noninvasive, sensitive, and reliable marker as a surrogate for tissue for mutation testing. The concept of mutation testing in blood or blood products is termed as a liquid biopsy. Thus, there is a growing utility of rapid plasma genotyping or "liquid biopsies" which employs circulating tumor DNA (ctDNA) in peripheral blood for molecular profiling. This is based on the fact that solid tumors often shed their genetic material in the blood and the cell-free ctDNA can be obtained using relatively less invasive means.[5] The reliability of serum as a surrogate specimen for tissue in detecting EGFR mutations has been under investigation for many years, with several uncertainties still persisting. In addition, whether EGFR mutations detected in serum have the same prognostic value as tissue EGFR mutations remains unresolved. In the past few years, several studies have attempted to address the above issues with variable results. Five systematic reviews/meta-analyses have been conducted to determine the consistency and diagnostic ability of serum/plasma for detecting EGFR mutations.[6-10] The results of these show that blood may be a promising alternative to tissue for detecting EGFR mutations in NSCLC, especially after disease progression on first-line TKIs, giving a high specificity (88%–97%) and diagnostic odds ratio (23.9–38.3) along with moderate sensitivity and concordance with respect to tissue. All these meta-analysis, however, reported limitations in analysis due to significant heterogeneity between studies, use of different techniques for mutation detection, and relative small sample sizes in most included studies.

This issue of *Lung India* has an important study by Merinda and Wulandari for testing ctDNA to look for T790M mutation among patients who progressed on initial TKI therapy.[11] The current study has shown a T790M mutation in 18 out of 39 patients who progressed on gefitinib or erlotinib. Since there was no comparison with tissue T790M mutation testing, the sensitivity or specificity of the test cannot be deciphered from these data. As known from previous studies, the false-positive rates of ctDNA testing for T790M mutations are generally low, and it is also an acceptable method as recommended by the College of American pathologists.[12] This testing has intermediate sensitivity while high specificity, meaning thereby a negative test does not rule out the presence of T790M mutation as a cause of resistance to TKI therapy.[13,14] In such patients, a negative liquid biopsy should be reconfirmed by tissue testing. It is also essential to keep in mind that the acquired T790M mutations are often subclonal. A ctDNA sample can show the original sensitizing EGFR mutation (e.g. L858R) and still have a false-negative test for T790M, requiring a tissue or cytology sample for confirmation. In the current study, out of 39 samples tested for T790M mutation, 17 had the original sensitizing mutation detection as well. The benefit of including the original sensitizing mutation (e.g. exon 19 deletion, L858R) in this assay helps to confirm that tumor DNA is being shed into the circulation, although this may not be practical for less common sensitizing mutations. Detection of the original sensitizing mutation in plasma acts as an internal control for plasma testing; hence, a plasma sample with no detectable sensitizing mutation or a T790M mutation can be considered uninformative and should be followed by tissue biopsy.[15] Because of the high specificity of ctDNA, a positive T790M finding in ctDNA is deemed to be

equivalent to a tissue biopsy and thus can be used to guide therapy with osimertinib.[15] However, the current study has not provided any follow-up data and further treatment of these patients, so it does not shed light on this aspect.

The authors have provided data regarding the absence of difference in the baseline characteristics, as well as the drug used in initial treatment (gefitinib or erlotinib), of patients with T790M mutations as compared to those without it, as detected by ctDNA. This finding may somewhat be misleading, keeping in view the fact that no gold standard tissue T790M testing results were available for these patients. The droplet digital PCR method used in this study has higher sensitivity as compared to other conventional means.[16] This method can detect cases where the T790M allele is present in only a small proportion of tumor cells. Importantly, T790M allele frequency does not appear to influence response rate, indicating that even patients with subclonal T790M-positive tumors may benefit from second-line treatment with osimertinib.

In addition, a liquid biopsy may help to identify patients with EGFR T790M mutation who would not otherwise be eligible to receive osimertinib because of a false-negative tissue biopsy result, caused by the fact that biopsied site may not be representative of all metastatic sites. Liquid biopsy, in this sense, represents the tumor burden as a whole rather than a part of it. Thus, digital droplet PCR can determine the absolute quantity of mutant EGFR levels in plasma samples and can also be used to monitor treatment response or disease progression serially.

The successful use of liquid biopsy for clinical decision-making has led us to an era of immense diagnostic and therapeutic possibilities. Despite several limitations, the current study adds to our understanding of the subject. It is imperative that further robust studies with standardized techniques and follow-up data are designed to provide clinically meaningful results.

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