Editorial

Liquid biopsy in the practice of neo-oncology

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Selecting the optimal platform for molecular diagnostic profiling is a crucial step in the management of patients with advanced lung adenocarcinomas. With an ever-expanding number of targetable oncogenic drivers, there was an evolution of molecular diagnostic platforms. Molecular testing previously followed a one-driver-one-test approach, with the use of Sanger sequencing, reverse transcription polymerase chain reaction (RT-PCR), fluorescence in situ hybridization (FISH) or Immunohistochemistry. Multiplex PCR-based platforms were developed to simultaneously interrogate multiple oncogenes, however these assays only detect the expression of selected known hotspot mutations and oncogenes and do not have the ability to discover new or additional drug targets (1,2). Next-generation sequencing (NGS), also known as massively parallel sequencing, represents an effective way to capture a large amount of genomic information about a cancer (3). In addition to known hotspot oncogenic mutations or gene rearrangements in non-small cell lung cancer (NSCLC), NGS has also identified genetic abnormalities that are previous known in other cancer types as well and uncovered many novel genetic abnormalities without knowledge of their biologic functions (2). The use of NGS has also resulted in the identification of new actionable genomic alterations as well as uncovered false negative tests in close to two thirds of lung adenocarcinomas that previously tested "negative" for known alterations by multiple conventional non-NGS tests (1,4-6).

Genotyping tumor tissue in search of actionable genetic alterations has become routine practice in clinical oncology. Although sampling tumor tissue has significant inherent limitations. First, it has been estimated that approximately 25% of lung carcinoma patients have no tissue available. In addition, tumor tissue is subject to selection bias resulting from tumor heterogeneity. A single tumor biopsy may not be able to represent the comprehensive genetic landscape of the disease. Furthermore, tumor tissue is a single snapshot in time, the molecular profile of the tumor significantly changes following treatment with targeted agents (7,8). Molecular monitoring of the disease over time is necessary in order to identify mechanisms of resistance and to adapt the therapy to the new molecular landscape (9). Finally, tissue biopsies are an uncomfortable, invasive procedure for patients, can be difficult to obtain, and can cause clinical complications (7,8).

Fragmented DNA is found in circulation in the cell-free component of whole blood. It can be released into the bloodstream by apoptosis or necrosis of cells, or actively by cells (10). The ability to detect and analyze circulating cell-free DNA (cfDNA) allows us to perform molecular profiling of the tumor by isolating circulating tumor DNA (ctDNA) from peripheral blood, a possibility also known as liquid biopsy. Accessing ctDNA from peripheral blood has clear advantages. It is minimally invasive and allows dynamic monitoring of molecular changes in the tumor rather than relying on a static time point. It contains genetic defects identical to those of the tumors themselves, thus able to provide information regarding point mutations, rearrangements, amplifications and even aneuploidy (7). Tumor heterogeneity is both a challenge for liquid biopsies and the reason they can be more useful than tissue biopsies. Initially, mutations with low allele fraction owing to only being present in a subset of tumor cells may be missed by liquid biopsies, however, the ability for minimally invasive samples to be sequenced repeatedly over time will allow for faster recognition of

known resistance mutations. Furthermore, liquid biopsies may be useful in monitoring tumor burden and could potentially detect minimal residual disease after surgery or therapy with curative intent (3). The use of liquid biopsy has become very relevant in the field of lung cancer, where 2nd resistance to EGFR (epidermal growth factor receptor) therapy is developed. Allocating the EGFR T790M mutation allows an efficient therapy with an FDA and EMEA approved 3rd generation TKI (tyrosine kinase inhibitor) named osimertinib upon its excellent efficacy in this cohort of patients with ORR (overall response rate) of 61% and PFS (progression-free survival) of 9.6 months (11). However, diagnosing the progression event and allocating the resistance mechanism requires clinical, imaging and molecular efforts.

Chabon *et al*. (12) reported recently in Nature communications their interesting experience with ctDNA as a reflection to tumor heterogeneity. CAPP-Seq ctDNA analysis was used to study resistance mechanisms in 43 T790M-mutant NSCLC patients after receiving first-line treatment with the third-generation EGFR inhibitor rociletinib. Frequent intra-patient heterogeneity was observed with multiple resistance mechanisms in 46% of patients. Recurrent resistance mechanisms to rociletinib involved MET, EGFR, PIK3CA, ERRB2, KRAS and RB1. EGFR C797S, which arises in ~33% of patients treated with another third-generation EGFR TKI, osimertinib, was observed in only one patient, suggesting that the dominant mechanisms of resistance between them differ. Increased MET copy number was the most frequent resistance mechanism, contrary to preclinical studies that have suggested that resistance to third-generation EGFR TKIs would primarily involve additional mutations in EGFR itself. A novel EGFR L798I mutation and emergence of activating KRAS mutations were described. Patients with multiple pre-existing resistance mechanisms experienced inferior responses and significantly shorter progression-free survival (PFS). Similarly, erlotinib-resistant xenografts treated with rociletinib developed MET amplification that could have been overcome with the MET inhibitor crizotinib. These findings suggested that targeting EGFR T790M alone will sub-optimally treat patients whose tumors display multiple resistance mechanisms. Chabon *et al.* have raised the assumption that ctDNA analysis-based strategies for combining drugs with different patterns of resistance mechanisms are needed, in order to provide patients with the greatest clinical benefit. For example, sequencing osimertinib after rociletinib could potentially allow T790M-mutant patients to remain on an EGFR TKI longer, by delaying the emergence of C797S. Unfortunately, the clinical development of rociletinib has been stopped by Clovis Oncology. Still, 3rd acquired resistance (e.g., to osimertinib) or non-T790M mutation mechanisms are anticipated (9).

The current treatment approach for patients whose disease has progressed on first-line EGFR TKI therapy, includes osimertinib, which was recently granted accelerated approval by the FDA for EGFR T790M mutant patients, continuation of first-generation EGFR TKIs or platinum-based doublet chemotherapy for eligible patients. A combination of afatinib and the EGFR-targeting antibody cetuximab may be considered as well (13). Other combination therapies are being investigated, based on the categories of resistance mechanisms: secondary mutations in EGFR; bypass or alternative activation; and histological and phenotypic transformation. One combination strategy is targeting horizontal pathways through a combination of an EGFR TKI with inhibitor to a bypass signaling pathway, but results are preliminary and immature (14). Some of the trials that are taking place include a combination of the MET inhibitor capmatinib (INC280) with gefitinib (NCT01610336) and erlotinib (NCT02468661), a combination of the PI3K inhibitor buparlisib (BKM120) with gefitinib (NCT01570296), a combination of the TORC1/2 inhibitor INK128 with osimertinib (NCT02503722) (15) and a combination of the JAK1 inhibitor INCB39110 with Osimertinib (16). It is noteworthy that third-generation EGFR TKIs are covalent-binding and mutant-selective, therefore have the advantage of sparing wild-type EGFR and can potentially increase the therapeutic window and overcome overlapping toxicities (14,17). The role of liquid biopsies, as a potential primary diagnostic tool in this therapeutic approach, has been vastly discussed. ctDNA analysis could allow a dynamic non-invasive monitoring of resistance mutations during treatment and could overcome the diagnostic limitations of tumor heterogeneity (14,15,17).

Despite the many benefits of this therapeutic approach, one must keep in mind the significant financial implications of its implementation. The costs of NGS of liquid biopsies are high and the need for repeated liquid biopsies may limit its usage among a large part of the community. Furthermore, the costs of targeted therapies are still very high, and the striving for combination therapies will most likely raise the cost of treatment. The question is, can patients afford it?

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Footnote

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