

Editor's note:

In the era of personalized medicine, a critical appraisal new developments and controversies are essential in order to derived tailored approaches. In addition to its educative aspect, we expect these discussions to help younger researchers to refine their own research strategies.

Controversies on Lung Cancer: Pros and Cons

Pros: Can tissue biopsy be replaced by liquid biopsy?

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Rationale for tissue versus liquid biopsies

In the past decade, 'personalized' or 'stratified' management based on the molecular features of tumors of patients with advanced non-small-cell lung cancer (NSCLC) has entered routine clinical practice. Identification on tumor tissue of predictive biomarkers of response to targeted treatments is now mandatory for optimal patient care, but faces several biological and technological challenges. First, comprehensive characterization of multiple tumor specimens obtained from the same patient illustrated that intratumor heterogeneity exists between different regions in the same tumor (spatial heterogeneity), as well as between the primary tumor and local or distant recurrences in the same patient (temporal heterogeneity) (1). Moreover, recent studies have characterized the dynamic changes of tumor features over time with the emergence of treatment-resistant subclones that were present at a minor frequency in the primary tumor (2). Thus, inter- and intratumor heterogeneity poses a pivotal challenge to guide clinical decision-making in lung oncology as biopsies may be inaccurate in capturing the complete genomic landscape of a patient's NSCLC (2). Second, the complete 'picture' of the tumor is often limited by the tumor accessibility because of the increased rate of clinical complications associated with the invasive procedures

necessary to obtain tissue at the time of initial diagnosis as well as throughout the course of disease treatment (3). The poor performance status of many advanced NSCLC patients may also limit the role of uncomfortable interventional biopsy procedures (3). Moreover, a significant barrier to biomarker testing is the availability of an adequate amount of tissue (e.g., tumor cellularity and size of the specimen) due to increasing diagnostic demands and declining amounts of tissue delivered per patient. Up to 80% of NSCLC patients with advanced disease will only have tissue from small biopsies or cytology, limiting the ability to perform additional tests, and as many as 31% of patients do not have accessible tissue (4). Even when tissue can be collected, preservation methods such as formalin fixation can display high levels of C > T/G > A transitions in the 1–25% allele frequency range, potentially leading to false positive results for molecular assays (4). Although this has improved for individual gene mutations, there are still limitations for next generation sequencing (NGS) analyses. Finally, tissues biopsies also increase the cost of patient care and the turnaround time for getting results can be sometimes longer than those expected by the physician for patient treatment. In light of these limitations on the use of tissue biopsies, new ways to observe tumor genetics and tumor dynamics have evolved.

The concept of liquid biopsy

Although tumor tissue is still the gold standard source for clinical molecular analyses, cancer-derived material circulating in the bloodstream has become an appealing alternative showing promise to overcome some of the challenges described above. Thus, “liquid biopsy” is the term coined to describe such diagnostic procedures performed on cancer-derived material captured in a blood sample.

There are several sources of tumor material that can be assessed by liquid biopsy: cell-free or complexed nucleic acids including circulating cell-free DNA (cfDNA), of which a subset represent circulating tumor DNA (ctDNA), cell-free RNA (cfRNA), and circulating tumor cells (CTCs) (5). cfDNA is composed of small fragments of DNA that are not associated with cells or cell fragments, originating from apoptotic and necrotic tumor cells but also from normal cells that are released into the bloodstream (6). Defined oncogenic mutations are used to infer the presence of ctDNA (6). cfDNA can be detected in other bodily fluids, including urine, saliva or cerebrospinal fluid (5). CTCs represent intact, viable non-hematological cells with malignant features that can be isolated from blood (5). CTCs are released into the bloodstream during hematogenous spread of the cancer and present as single cells or clusters. Tumor cells actively release into the bloodstream several species of cfRNAs, enriched in exosomes that strongly resist RNases in the blood, including circulating non-coding RNAs (e.g., microRNAs, small nucleolar RNAs, PIWI-interacting RNAs, and long non-coding RNAs). Notably, platelets can also sequester free nucleic acids and thus may serve as an alternative source of cfRNA released by tumor cells (7).

Interestingly, tumors containing ~50 million malignant cells release sufficient DNA for the detection of ctDNA in blood (8). In contrast, positron emission tomography—computed tomography imaging generally detects tumors measuring no less than 7 to 10 mm in size and containing ~1 billion cells (3). Recent advances in cell capture devices, plasma isolation techniques, and highly sensitive sequencing-based methods have made the introduction of the liquid biopsy of NSCLC into the clinic financially and technically feasible (3,6).

Advantages of liquid biopsies in lung cancer

A liquid biopsy can in principle provide the same genetic information as a tissue biopsy, which is necessary to interrogate key companion diagnostics (6). The liquid

biopsy approach holds clear advantages over “traditional” tissue biopsies. It is a source of fresh tumor-derived material, unhampered by preservatives. Sampling the blood is minimally invasive and avoids the complications of biopsies. Recent large studies comparing the performance of cfDNA analysis to tissue biopsy in NSCLC demonstrated the clinical value of the liquid biopsy approach (9,10). This positive result led to the approval of use of cfDNA analysis for *EGFR* mutation analysis for IRESSA® (in patients where a tumor sample was not evaluable), making it the first *EGFR* tyrosine kinase inhibitor (TKI) for which cfDNA testing is included in the label. Currently, a liquid biopsy provides an alternative sample type in routine clinical practice when tumor sampling is unavailable, inappropriate or difficult to obtain. Furthermore, less invasive sampling for genomic characterization of ctDNA or CTCs allows repeatable assessing of the clonal dynamics throughout the course of therapy and early identification of therapeutic resistance drivers. Resistance to *EGFR*-TKIs invariably develops, and in ~60% of patients resistance is mediated by selection for clones harboring a secondary mutation, p.T790M in the *EGFR* gene, to which the efficacy of third-generation *EGFR*-TKI has been recently demonstrated (11). The presence of p.T790M needs to be confirmed with re-biopsy at relapse and frequently this could be limiting in NSCLC patients. However, the feasibility of resistance monitoring by plasma DNA sequencing has been recently proved in *EGFR* mutated NSCLC (12). Moreover, the use of complementary liquid biopsy approaches such as cfDNA and CTCs may provide the most complete assessment of each patient’s resistance, which still needs to be validated in predicting response to T790M-targeted inhibitors (13). Moreover, sequential treatments using drugs that inhibit specific molecular targets and, in parallel, tracking the emergence of resistance-associated genetic aberrations would allow the oncologist to anticipate the most effective subsequent treatments (14). In principle, this knowledge could be used to provide early initiation of alternate therapies before relapse is detected by clinical or radiological examination (15). With regard to the tumor heterogeneity, molecular characterization of ctDNA or CTCs is an attractive alternative to tissue biopsies, as a liquid biopsy is likely to better reflect the global (primary and metastatic sites) molecular status of the patient (15). Finally, only a small amount of blood is required for a liquid biopsy, generally 10 mL of whole blood. For molecular assays on cfDNA the protocols including centrifugation, extraction and analysis of sequences are straightforward and standardized, and are

becoming more and more often automated. The possibility of rapidly repeating the analysis is reassuring, notably when the results are unclear or initial technical problems due to incorrect procedures or defective management of the pre-analytical steps occurred. Notably, plasma-based testing has a significantly shorter turnaround time (16).

While the area of predictive biomarkers is the most advanced example of the use of liquid biopsies in lung clinical oncology, alternative uses have been recently described such as for the identification of diagnostic, prognostic, and predisposition biomarkers of lung cancer, although the transfer into routine practice has not yet been made. For instance, the levels of CTCs correlate with an advanced stage and have been shown to be a poor prognostic factor in both surgically resectable and advanced stage NSCLC or after chemotherapy (17). Moreover, recent improvements in CTC-capture technology allowed correlation between the CTC number and response to therapy, and provided an opportunity for non-invasive fluorescence *in situ* hybridization or immunocytochemistry studies of CTCs in relationship to *ALK*-targeted treatment in NSCLC (18). In addition, Nilsson *et al.* were the first to detect *ALK* rearrangements in the platelets of patients with NSCLC (7). Furthermore, the capture of viable CTCs recently enabled CTC-derived xenograft models (CDX) for study of drug sensitivity at baseline and at progression from NSCLC patients (19). The CDX approach contrasts with the majority of tissue-derived xenografts generated from resected tissue during surgery with curative intent. CDX can be generated from patients with little deviation from routine practice (19). Finally, a proof-of-principle study demonstrated that CTCs can be detected in patients with chronic obstructive pulmonary disease without clinically detectable lung cancer. As a consequence of early detection by blood screening, early surgical removal of the tumor decreased the risk of tumor recurrence (20).

In conclusion, it is evident that a liquid biopsy holds several advantages over a tissue biopsy in providing minimally invasive personalized treatment and improving the follow-up of NSCLC patients in the clinical setting. However, validation is still needed to enable the enlargement of the potential applications of liquid biopsies before widespread use in routine clinical practice in lung oncology.

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Footnote

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