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

Cons: Can liquid biopsy replace tissue biopsy?—the US experience

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Introduction

Development of new genotype-directed therapies has shifted a paradigm of treatment for advanced lung cancer patients. Currently, only tyrosin kinase inhibitors (TKIs) for *EGFR* mutations, *ALK* or *ROS1* rearrangements are clinically available in the US, but multiple agents to target other mutations are in the pipeline. Thus, the National Comprehensive Cancer Network (NCCN) guidelines for non-small cell lung cancer (NSCLC) recommend at least *EGFR* mutation and *ALK* testing, and strongly endorse broader molecular profiling that include five additional genes (*ERBB2*, *BRAF*, *MET*, *ROS1* and *RET*) to guide multiple matched therapies for patients with metastatic disease (1). However, only small biopsies or fine needle aspiration are available for both histologic diagnosis/subtyping and genetic testing in the majority of advanced stage patients, and the tissue often becomes insufficient for genomic analysis after initial histology diagnosis ± stains for subtyping (2). This limitation of tissue biopsy is elucidated by a study reporting that up to 30% of patients at a community-based academic center did not undergo guideline-recommended molecular testing, despite an institutional reflex testing policy (3).

Inter- and intra-tumoral heterogeneity may also limit the tissue-based genotyping, in particular when mechanisms for resistance to TKIs need to be evaluated. To decide on the next course of action at the time of resistance development, a patient is encouraged to undergo re-biopsy to obtain tissue for additional molecular profiling. In patients with multiple metastases, however, mechanisms of resistance may be heterogeneous, and selection of a single site for biopsy may not provide a representative profile of the predominant resistance mechanism (4). Given that lung cancer patients with recurrence have generally poor performance status that likely limits the role of interventional procedures, it is not realistic to obtain multiple biopsies from different sites. As a result, our understanding of the mechanisms of lung cancer progression and relapse is limited in most cases.

While challenges in obtaining adequate tumor tissue and issues of heterogeneity continue to hamper tissue molecular profiling, minimally invasive technologies to capture genomic contents of tumor in fluids, combined with sensitive genotyping assays, have become available. Thus, the potential role of "liquid biopsy", as an alternative for tissue biopsy, has been evaluated.

Liquid biopsy platforms

Liquid biopsy consists of minimally invasive technologies to capture circulating biomarkers such as circulating tumor cells (CTCs) and nucleic acids including cell-free RNA, micro-

RNA (miRNA), and circulating cell-free DNA (cfDNA), a subset of which may represent circulating tumor DNA (ctDNA). Notably, cell-free nucleic acids can be detected not only in blood but also in other bodily fluids, including urine and saliva (5). Among the circulating biomarkers, CTCs, if detected, can be used not only for DNA-based analysis but also for RNA and protein-based profiling and may elucidate the heterogeneity of this circulating population in ways that indirect molecular approaches cannot (6). However, they are exceedingly rare (occurring on average at a frequency of 1 in 100 million cells) (7), and detection rates of CTCs in NSCLC are generally low despite the advances in cell capture technologies (8). Small noncoding RNAs, including miRNAs, are stabilized by processing proteins in the circulation, while cell-free RNA is subject to rapid degradation in the circulation. The miRNAs can also be quantified using qRT-PCR, thus circulating miRNAs are considered attractive biomarkers (9). In fact, multiple studies have reported the potential utility of circulating miRNAs to diagnose and screen NSCLC as well as to predict prognosis or response to treatment (8). Unfortunately, however, many of these studies have used different sets of markers and thresholds for positivity, thus circulating miRNAs do not appear to have immediate clinical effects.

Conversely, cfDNA may contribute to clinical decision-making. Many groups have investigated genotyping of cfDNA as an alternative to tumor tissue genotyping (8). Circulating cell-free tumor DNA (ctDNA) consists mainly of 166 base pair double-strand DNA fragments resulting from apoptosis and necrosis leading to release of nuclear DNA into the circulation (10). These fragments have a short half-life in circulation, ranging from 15 minutes to several hours, due to rapid hepatic and renal clearance (11), thus ctDNA reflects a real-time genomic signature of the tumor. Defined oncogenic mutations are used to detect ctDNA, and various assays have been employed for detection of mutant sequences in cfDNA, including Scorpion amplified refractory mutation system (ARMS), allele-specific quantitative PCR, PCR with peptide nucleic acid clamps, massively parallel sequencing, and droplet digital PCR (8). Importantly, these assays must be optimized for high sensitivity to detect tumor-cell derived DNA, since levels of ctDNA (as little as 0.01% of cfDNA) may be significantly lower than those of DNA directly isolated from cancer tissue (12). Conversely, many groups have focused more on improving specificity of assays that will make the assay useful as a screening tool. In fact, the recent meta-analysis of 20 studies evaluating *EGFR* mutation status in plasma has reported the overall sensitivity (67.4%) and specificity (93.5%) of genotyping using cfDNA compared to tissue genotyping, with more recent studies showing 96–100% specificities. Of note, the majority of the studies included in the analysis used single-gene assays (13).

Given the ever-growing number of targetable genetic alterations with matched targeted agents, the performance of massively parallel sequencing [next-generation sequencing (NGS)] platforms that enable broader genetic profiling has been evaluated using cfDNA, and some studies have shown similar sensitivities and specificities of NGS as compared to single-gene assays (14,15). Some NGS platforms are designed to detect not only driver and resistant mutations, but also rearrangements, insertions and amplifications using cfDNA with similar sensitivities and specificities and a reasonable turnaround time (15). The capability of testing a broad range of genetic alterations in a single blood sample and near perfect specificities of these targeted plasma NGS assays will likely increase their utility in the screening setting.

Clinical application of liquid biopsy in the US

Given the recent FDA approval of a third-generation EGFR inhibitor, osimertinib, for patients with T790M-positive *EGFR* mutant lung cancer, an evaluation of mechanisms for acquired resistance to first-generation EGFR TKIs has never been more important. A T790M-positive clone, however, comprises only a subset of *EGFR*-mutated tumor cells, and several series have described T790M results varying over multiple post-progression biopsy specimens (4). Thus, genotyping of cfDNA appears to be complementary to tissue genotyping, since it can be performed repeatedly during treatment with TKIs, and it may be able to identify a subpopulation missed in a single biopsy specimen (16). Furthermore, response rates to osimertinib are reportedly similar whether T790M is detected in tissue samples or in plasma (17). Pharmacodynamic monitoring of *EGFR* or T790M in cfDNA, however, has not been a part of routine practice yet, since it remains unclear whether a therapeutic decision based on the emergence of a subclone before clinical or radiographic progression will improve patient outcomes (8).

Some academic centers in the US have implemented a plasma assay for selected genes/hot spots in routine clinical molecular testing for advanced NSCLC patients (per personal communication with Dr. Lynette Sholl). In addition, some of the Clinical Laboratory Improvement

Amendments (CLIA) certified laboratories/companies in the US offer plasma-based molecular testing that can be reimbursable. Digital Sequencing™ (Guardant360, www.guardanthealth.com/guardant360/) is one such example (14). At our institution, we prioritize tissue-based genotyping, given the not perfect sensitivity of plasma-based genotyping, but we have also been evaluating both liquid and the corresponding tissue biopsies, in particular in patients who developed resistance to TKIs, in order to gain insight on the concordance of these two types of specimens/assays. As already discussed, however, there are a significant number of patients who do not have enough tissue for molecular testing and/or do not have a biopsy-amenable lesion and/or don't want to go through a repeat biopsy. In those cases, we only use Digital Sequencing™ for molecular testing.

Can liquid biopsy replaces tissue biopsy?

As we discussed, liquid biopsy has screening as well as complementary roles in clinical management of advanced NSCLC patients and may replace tissue biopsy to some extent in the future. However, it seems less likely that liquid biopsy will completely replace tissue biopsy sometime soon. There are several reasons for that. Most importantly, the diagnosis and subtyping of lung cancer need to be established based on histology. Plasma genotyping assays are more sensitive in patients with extrathoracic metastases, and the possibility of detecting a mutation in cfDNA of early stage patients is significantly lower (18). Furthermore, even if a mutation is detected in plasma, there is no guarantee that it is coming from a presumed lung cancer. Similarly, while the presence of mutations involving specific genes such as *EGFR* and *KRAS* usually indicates non-squamous NSCLC, given the relatively low prevalence of such alterations in non-squamous NSCLC, their absence does not specify a histologic subtype. For the initial diagnosis and histologic subtyping, circulating miRNAs may become useful in the future, however (19). In addition, histologic transformation to small cell carcinoma, which has been reported in approximately 5–7% of EGFR TKI resistant cases (20), may not be detected by the currently available liquid biopsy platforms. Thus, liquid biopsy will not be able to replace tissue biopsy until technologies to capture miRNA and CTCs as well as cfDNA further advance, genotyping assays become more sensitive, and our understanding of histology specific molecular alterations significantly improves.

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

Related to: Ilié M, Hofman P. Pros: Can tissue biopsy be replaced by liquid biopsy? Transl Lung Cancer Res 2016;5:420-3.
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