**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**

# Rebuttal from Dr. Mino-Kenudson

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Submitted Jul 25, 2016. Accepted for publication Aug 01, 2016.
doi: 10.21037/tlcr.2016.08.02
**View this article at:** http://dx.doi.org/10.21037/tlcr.2016.08.02

First of all, I would like to congratulate Drs. Ilie and Hofman for their excellent discussion in support of the possibility of liquid biopsy to replace tissue biopsy. As they accurately pointed out, if validated for routine clinical use, liquid biopsy would overcome the following issues inherent to tissue biopsy: (I) inability of capturing the complete genomic landscape of the non-small cell lung cancer (NSCLC) patient that is attributed to inter- and intratumoral heterogeneity and/or often limited tumor accessibility due in part to poor performance status of the advanced NSCLC patient limiting the role of interventional procedures (1); (II) insufficient quantity and quality of tissue hampering molecular testing, in particular next generation sequencing (NGS) (2); (III) potential false positive results due to high levels of artificial C>T/G>A transitions induced by preservation methods such as formalin fixation (3); (IV) high cost of biopsy/FNA procedure and turn-around-time longer than expected.

As for the intra- and intertumoral heterogeneity issue, growing evidence suggests that cell-free tumor DNA (ctDNA) represents a molecular proxy of the overall disease. However, it remains to be formally proven that multiple metastatic sites located in different organs equally shed ctDNA, since apoptosis, among multiple mechanisms, likely produces the majority of ctDNA in circulation, and passive release of ctDNA from apoptotic or necrotic cells depends on various conditions including the location, size and vascularity of the tumor (4). Similarly, the insufficient quantity of DNA for molecular testing is an issue associated with not only small biopsy and FNA specimens but also ctDNA. Circulating DNA derived of the tumor varies greatly from <0.01% to >90% (5) and the amount of ctDNA is related to the tumor burden (5,6). Technically, the sensitivity of plasma genotyping assays is limited to 0.01%, and if the fraction of ctDNA in a sample is at or below 0.01%, it is considered negative for ctDNA (5,7). Thus, advanced stage tumors with low-level micrometastatic disease as well as early stage tumors that have lower numbers of ctDNA fragments (8) may have false negative results. In fact, while approximately 30% of small tissue samples are insufficient for molecular testing (2,9), the overall sensitivity of genotyping using cfDNA compared to tissue genotyping is approximately 70–75% (10,11). Given that liquid biopsy can be performed repeatedly during the entire disease course, which is also useful for an early detection of residual tumor and/or resistant clones, and it may be able to identify a subpopulation missed in a single tissue biopsy, genotyping of cfDNA could be complementary to tissue genotyping, but the imperfect sensitivity with the currently available assays appears to prevent liquid biopsy from replacing tissue biopsy.

Another issue associated with plasma genotyping is

its inability to confidently diagnose and subtype lung cancer. As previously discussed, the lower number of ctDNA fragments in early stage tumors may be below the sensitivity of genotyping assays (5,7,8). Given that the recent advance in histology-directed therapy, the differentiation of squamous cell carcinoma from non-squamous cell carcinoma, in particular adenocarcinoma is of paramount importance. However, the low prevalence of molecular alterations associated with squamous cell carcinoma or adenocarcinoma hampers subtyping of lung cancer by plasma genotyping. Conversely, other elements of liquid biopsy, namely circulating micro-RNA (miRNA) and circulating tumor cells (CTCs), may play a role in diagnosis, subtyping and prognostic stratification of lung cancer. Multiple studies have reported on the potential utility of miRNAs to diagnose NSCLC (12), as well as to differentiate squamous cell carcinoma from adenocarcinoma in both tissue and plasma-based assays (13-15). The levels of CTCs have been shown to correlate with an advanced stage and patient outcomes in various stages of NSCLC (16), as well as response to therapy (17). In addition, CTCs could be used for fluorescence in situ hybridization and/or immunohistochemistry to evaluate biomarkers such as *ALK* rearrangements (17). Unfortunately, however, CTCs in NSCLC suffer from relatively low detection rates despite the recent improvements in CTC-capture technology, and there are no standard panels of miRNA markers or cutoffs for positivity that are applicable in routine practice (12).

In summary, minimally invasive liquid biopsy does have several advantages that complement tissue biopsy and holds promise in personalized therapy of NSCLC patients. However, there remain challenges/issues that need to be resolved before liquid biopsy can be fully implemented in routine clinical practice.

## Acknowledgements

None.

## Footnote

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

*Comment on:* 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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**Cite this article as:** Mino-Kenudson M. Rebuttal from Dr. Mino-Kenudson. Transl Lung Cancer Res 2016;5(4):430-432. doi: 10.21037/tlcr.2016.08.02