# Liquid biopsy in lung cancer: present and future

In lung cancer, as well as in other malignancies, the so-called "liquid biopsy" is quickly moving from research into clinical practice. Although it has not yet reached its full potential, the "liquid biopsy" is no longer a promise but a reality that is allowing a better monitoring and treatment selection of cancer patients in many hospitals and oncology departments. We can already envision a day when "liquid biopsy" tests will be as common and useful as imaging techniques and "standard" biopsies, offering invaluable prognostic and predictive information. The objective of this special issue of *Translational Lung Cancer Research* (*TLCR*) is to offer an overview of this exciting area, with a particular emphasis on the clinical utility of the so-called "liquid biopsy" and the diversity of applications, methodologies and information that can be derived from it.

"Liquid biopsy" refers to non-invasive tests done in blood samples (or other body fluids) from cancer patients in order to detect materials originated in the tumor. Although the term is almost universally used, many pathologists argue that is not correct. In their opinion, the so-called "liquid biopsies" are not true biopsies. These pathologists are probably right. A true biopsy is usually performed by a surgeon or a pneumologist and involves extraction of tissues or sample cells for examination by a pathologist under a microscope, commonly after some kind of fixation and staining. "Liquid biopsies" are not obtained by surgeons, involve extraction of blood and not of solid tissues, pathologists only occasionally intervene and fixation or staining are equally rare. To make things worse, the "liquid" part in the term "liquid biopsy" can also be misleading. The materials originated in the tumor that are to be detected in such a "biopsy" are never liquids. Sometimes they are cells or fragments of cells, such as circulating-tumor cells (CTCs), exosomes or tumor-educated platelets; sometimes they are nucleic acids dissolved in the blood, such as circulating tumor DNA or RNA (ctDNA, ctRNA).

The differences between a "real" and a "liquid" biopsy or "liquid sample", as the pathologists would probably prefer to call them, explain the advantages of the latter. "Liquid" biopsies will never replace real biopsies, which are irreplaceable sources of information that cannot be obtained by any other means. However, they are already offering all sorts of additional data that could not be obtained in any other way. In patients who cannot be biopsied, or where biopsies do not have enough tissue, "liquid biopsy" offers a mean to perform genetic testing for targeted therapy. Also, in patients with advanced disease, it is not feasible to obtain biopsies of every metastatic site. But blood reaches both the primary tumor and the metastases, and materials coming from all can be found in a "liquid biopsy". Finally, unlike "real" biopsies, blood can be repeatedly obtained and used to monitor the course of the disease, including early detection of response and relapse or emergence of resistance to a particular therapy.

In the first article of this special issue, *Liquid biopsy based biomarkers in non-small cell lung cancer for diagnosis and treatment monitoring*, Pérez-Callejo *et al*. review the methodologies available for the isolation and analysis of ctDNA, exosomes, tumor-educated platelets and CTCs, describe the unique information that can be derived from each of these materials and discuss their clinical applications in lung cancer. Their conclusion is clear; liquid biopsies, particularly CTCs and cfDNA, will guide treatment decision, improve the outcome for lung cancer patients and allow early diagnosis of tumors that are not yet visible on imaging.

CTCs and ctDNA are the two materials most commonly analyzed in lung cancer "liquid biopsies". Which one should be preferred is a burning issue that is discussed by Calabuig-Fariñas *et al*. in their article *Circulating tumor cells versus circulating tumor DNA in lung cancer. Which one will win?* Their answer is that this will be a war with two victors, since CTCs and ctDNA will play complementary roles based on their relative strengths and limitations. They consider that ctDNA will be the material chosen for the analysis of mutations, copy number aberrations and DNA methylation changes, while CTCs, which provide the opportunity to study whole cells, will allow DNA, RNA, and protein-based molecular profiling, in addition to *in vivo* studies.

If CTCs and ctDNA are already a reality in the clinical practice, exosomes constitute one of the most promising areas of research. In this special issue, they are reviewed in the article *Exosomes as a source of genetic material in non-small cell lung cancer: a truly Pandora's box*. There, Reclusa *et al*. prove the accuracy of this definition. The stability of the exosomes in the blood and their similarity to the cells of origin, make it possible to detect in them all types of biomarkers as well as the "instructions" for migration and aggressiveness of the tumor and information on druggable targets. In view of this, once methodologies are properly standardized, the authors conclude that exosome analysis will be quickly incorporated in the clinical practice.

 

The following five articles of this special issue describe areas of particular clinical interest regarding ctDNA analysis. In the first of the series, *Methylation analyses in liquid biopsy*, Lissa and Robles review the exciting field of DNA methylation, which has emerged as a promising marker for detection, prognosis and follow-up of tumor dynamics. They summarize the investigational applications of methylated ctDNA in lung cancer, the technologies used and the challenges that befall the implementation of methylated ctDNA into the clinical setting. Taken all together, they foresee an increasing number of methylation-based biomarkers in lung cancer that will lead to the development of companion diagnostic kits on ctDNA, ready to be implemented in the routine clinical practice.

Next-generation sequencing (NGS), which allows to simultaneously detect multiple alterations in many genes, is revolutionizing genetic testing. Although initially developed to be used in nucleic acids derived from cells or tissues, many efforts are being devoted in order to make NGS possible in liquid-biopsy derived materials, particularly ctDNA. These efforts and the challenges ahead are reviewed by Malapelle *et al*. in the article *Next Generation Sequencing techniques in liquid biopsy: focus on Non Small Cell Lung Cancer patients*. Although liquid biopsy NGS requires a careful validation of the whole process including blood collection, ctDNA extraction, library preparation, sequencing and variant calling, the authors present evidence that NGS can be successfully applied to ctDNA analysis and has the potential to become a new gold standard technique for mutational testing in NSCLC patients.

Mutations in the *EGFR* gene are the most common druggable alterations in advanced NSCLC and they are used in the clinical setting to select patients for treatment with EGFR tyrosine kinase inhibitors (TKIs). In addition, detection of *EGFR* mutations in serial liquid biopsies can be extremely helpful to follow the course of the disease, as Mayo-de-Las-Casas *et al*. describe in their article *Usefulness of circulating free DNA (cfDNA) for monitoring EGFR mutations in advanced NSCLC patients: a case report*. They present a patient with a long follow-up, where the evolution of *EGFR* mutations in blood and cerebrospinal fluid mirrored the evolution of the disease, including early response, relapse with emergence of T790M-associated resistance and appearance of brain metastases.

Mutations in the *KRAS* gene are common in NSCLC, particularly in smokers, and its prognostic and predictive role, both in "true" and "liquid" biopsies is controversial. This controversy, and the current state-of-the-art of *KRAS* mutation analyses using non-invasive approaches is review by Garzón *et al*. In their article *KRAS mutations in the cfDNA of NSCLC patients* they conclude that, although large prospective studies are required to draw definitive answers about the prognostic or predictive value of *KRAS* mutations in ctDNA, they can already be used to monitor the course of the disease in *KRAS* mutated patients.

Most of studies on ctDNA and other liquid biopsy-derived materials in lung cancer have been performed in advanced-stage patients. The analysis of ctDNA and detection of genetic alterations in early-stage patients presents additional challenges, which are summarized by Pérez-Ramírez *et al*. in their review *Liquid biopsy in early stage lung cancer*. Although the low concentration of ctDNA in the blood of early-stage NSCLC patients has hampered its use, the authors are confident that more specific and sensitive techniques will soon enable the use of cfDNA for routine NSCLC diagnosis and monitoring tumor burden, as well as for identifying hidden residual disease after surgery or tumor biomarkers.

Last but not least, Aguado *et al*. review an area often overshadowed by cfDNA analyses but that has the potential to become the new, great step forward in liquid biopsy. In their article *Fusion gene and splice variant analyses in liquid biopsies of lung cancer patients*, they offer an overview of how RNA derived from plasma, platelets and CTCs can be used to detect actionable alterations in advanced NSCLC patients, such as fusion involving *ALK*, *ROS1*, *RET* and *NTRK* genes or *MET* splicing variants. RNA offers the best available choice for accurate detection of these alterations in blood, which will represent a significant advance for treatment selection at the time of the diagnosis, as well as for monitoring treatment outcome and predicting disease progression in patients carrying these alterations.

 

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