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### Liquid Biopsy: Circulating Tumour Cells and Circulating Tumour DNA



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#### Markers for Therapeutic Progress in Cancer

Liquid biopsy in the oncology field is an emerging noninvasive diagnostic technique, which enables tracking of the course of the disease at various points in time. This diagnostic test refers to the genetic analysis of either circulating tumour cells (CTCs) or circulating tumour DNA (ctDNA) in order to predict drug response and monitor therapy. Although still in the validation stage, liquid biopsy for cancer diagnostics potentially has advantages over the genetic analysis of excisional biopsies from primary tumours.

Blood-borne dissemination of tumour cells to sites that are distant from the primary lesion (such as bone marrow, bone, brain, lung or liver) is a major determinant of patient prognosis. Liquid biopsy of CTCs captures metastatic cells released from the primary tumour into the circulation (van de Stolpe et al. 2011; Pantel & Alix-Panabières 2013).

In metastatic disease, it has been established that cancerous cells in primary tumours differ greatly in their genomic characteristics from cancerous cells in distant metastatic sites (Eccles & Welch 2007; Pantel & Alix-Panabières 2013). This is because cancerous cells become de-differentiated, enabling them to mutate rapidly. Following this they lose the ability to respond to growth control checkpoints, and they gain survival advantages through alterations in adhesion and invasion properties unique to the metastatic niche. They also resist apoptosis as well as anoikis or the inability to survive outside the normal anatomic location. Each metastatic niche has its own microenvironment, and cancer cells respond to cues in the microenvironment by mutating (Eccles & Welch 2007). Therefore genetic analysis of biopsies alone from the resected primary tumour may not yield sufficient information for the decision on effective cancer treatment.

Targeting metastatic disease will therefore require understanding of the genomes of potential initiator cells as well as metastatic cells. Outgrowth of these cells into overt metastases would need to be treated with therapies directed specifically toward the corresponding genetic subsets of these initiator cells. Liquid biopsy of CTCs, which reflects the total of CTCs from primary as well as different metastatic sites, can be used to evaluate minimal residual disease, yields genetic information on acquired mutations and is therefore, a most promising technique for enabling decisions on therapeutic regimens. Additionally, this technique yields new information on the pathobiology of metastases and guides the development of novel therapeutics, all of which will inevitably improve clinical management of metastatic disease (Murtaza et al. 2013). Since CTCs in the peripheral blood are both rare and genetically heterogeneous, great efforts are underway to improve and standardise isolation and characterisation techniques (van de Stolpe et al. 2011; Alix-Panabières et al. 2012). CTCs are isolated based on physical properties, including size, deformity and electric charge as well as biological properties, including positive selection for the cell surface marker, EpCAM (epithelial cell adhesion molecule) and negative selection for CD45 to exclude leukocytes using conjugated magnetic beads isolation techniques. As discussed in van de Stolpe et al. 2011 and Alix-Panabières et al. 2012, CTCs do not always "obey" the current definition of CTCs, and other markers of CTCs are also under investigation, including stem cell markers and markers of epithelial-mesenchymal transition (EMT). Assays that target specific cancer mRNAs are also being evaluated for isolation of CTCs for downstream molecular and genetic analysis.

Even in the absence of disseminated disease (metastases) decisions regarding tumour therapy are greatly complicated by marked genetic heterogeneity of tumour tissues. Genotyping of individual tumour biopsies, therefore, often does not allow for a general decision on effective clinical treatment. Furthermore, surgically-obtained biopsies are invasive, often pose anatomical limitations, and repetitive biopsies for monitoring response to therapy are unfeasible. Dynamic genetic changes also occur during tumour progression and the development of drug resistance. The development of liquid biopsy of blood samples for ctDNA has been greatly aided by improvements in DNA sequencing, PCR-based digital approaches and genomics techniques. All apoptotic and necrotic cells release cell-free DNA into the bloodstream, but since cancerous cells have higher cellular turnover than most normal cell types, circulating cell-free DNA is increased in cancer.

As described by Diaz and Bardelli (2014), current efforts in the development of ctDNA liquid biopsy are towards improving the ability to discriminate ctDNA from normal cell-free DNA. Mutations are present in ctDNA and not in the DNA of normal cells, and liquid biopsy of ctDNA is being designed to specifically detect point mutations in multiple genes of interest. This technique would allow for better characterisation of mutations that arise in cancer cells in response to treatment during development of metastases and development of drug resistance (Murtaza et al. 2013; Dawson et al. 2013). Additional genetic alterations that can be determined in ctDNA are epigenetic alterations, microRNAs and loss of heterozygosity (Schwarzenbach et al., 2013). As with liquid biopsy of CTCs, liquid biopsy of ctDNA reflects the ctDNA from the primary tumour site as well as all metastatic niches. Since the half-life of ctDNA is two hours or less, liquid biopsy of ctDNA can provide rapid, real-time and repetitive assessment of the effectiveness of treatment.

The development of drug resistance is currently inevitable with single-targeted therapy. In a recent study, Diaz et al. used ctDNA liquid biopsy to monitor the emergence of drug-resistant clones during the course of treatment with panitumumab, an anti-EGFR therapy for colorectal cancer

(Diaz et al. 2012). Colorectal tumours initially lacking KRAS mutations (wild type for KRAS) initially responded to targeted therapy with EGRF blockade, but resistance to the treatment developed in parallel with the appearance of KRAS mutations in the sera detected using ctDNA liquid biopsy. Using mathematical modelling approaches, the researchers came to the conclusion that mutation-harboring clones were present at low levels prior to initiation of treatment. During treatment, wild type cells were killed allowing the expansion of mutation-bearing cells. This study using serial ctDNA liquid biopsies provided a profound insight into molecular and genetic evolution of the development of drug resistance: "The time to recurrence is simply the interval required for the subclone to repopulate the lesion". Combination therapy targeting multiple mutations will allow the remission period to last longer.

A study by Murtaza et al. also used liquid biopsy of ctDNA to study acquired resistance to cancer therapy (Murtaza et al. 2013). They used exome sequencing of serial plasma samples from patients with advanced breast, ovarian and lung cancer to track, over the period of two years, the evolution of genetic changes in metastatic cancers in response to therapy, findings that validate the liquid biopsy ctDNA technique.

It is likely that a combination liquid biopsy using analyses of CTCs as well as ctDNA will emerge in clinical practice. This is discussed in a recent review (Pantel & Alix-Panabières 2013). Liquid biopsy of CTCs and ctDNA will undoubtedly be developed to further define the wider range of mutations occurring in breast cancer compared to cancers such as colorectal and pancreatic cancers, which seem to have mutations in a more limited number of genes. Greater liquid biopsy development is needed for cancers existing behind the blood brain barrier, which shed little to no ctDNA into the bloodstream (reviewed in Siravegna and Bardelli). Unfortunately, these are the types of cancers from which surgical biopsies are difficult to obtain. Another point to consider is cancers in which metastases occur not through the blood-borne route but through lymphatics or across body cavities (as in ovarian carcinomas), between the endothelium and along neurons (as in pancreatic carcinomas) (Eccles & Welch, 2007). In these cases, liquid biopsy of ctDNA rather than CTCs may be more useful, because ctDNA is released into the bloodstream from all cells.

## **Conclusion**

Despite the enormous potential in the field of oncology for the use of liquid biopsy of circulating tumour cells (CTCs) and circulating tumour DNA (ctDNA), and despite the rapid development and use of tools for comprehensive tumour genome analysis, liquid biopsy cannot yet replace gold standard diagnostic techniques in clinical application. Harmonisation of procedures will be needed to create clinical standards validating liquid biopsy as a clinically relevant biomarker in cancer therapy. This calls for a decent multicentre approach in clinical trials of liquid biopsy.

## **Key Points**

- Cancer cells are released into the circulation.
- Cancer cells release DNA into the circulation.
- Circulating cancer cells and DNA reflect specific tumour genomes.
- Liquid biopsy allows noninvasive, genomespecific therapeutic decisions.
- Clinical validation of liquid biopsy in oncology calls for additional multicentre trials.

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