

# Real-time liquid biopsy: circulating tumor cells versus circulating tumor DNA

Catherine Alix-Panabières<sup>1,2</sup>, Klaus Pantel<sup>3</sup>

<sup>1</sup>University Medical Centre, Saint-Eloi Hospital, Institute of Research in Biotherapy, Department of Cell and Tissue Biopathology of Tumors, Laboratory of Rare Human Circulating Cells, Montpellier, France; <sup>2</sup>University Institute of Clinical Research UM1-EA2415-Epidemiology, Biostatistics & Public Health, Montpellier, France; <sup>3</sup>Department of Tumor Biology, Centre of Experimental Medicine, University Medical Centre Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany

Correspondence to: Prof. Dr. Klaus Pantel, M.D, Ph.D. Institute of Tumor Biology, University Medical Center Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany. Email: pantel@uke.uni-hamburg.de.

**Abstract:** Liquid biopsy is a new diagnostic concept, i.e., analysis of therapeutic targets and drug resistance-conferring gene mutations on circulating tumor cells and cell-free circulating tumor DNA released into the peripheral blood from metastatic deposits. Here, we discuss current challenges and future perspectives of the liquid biopsy concept in clinical oncology. We postulate that this concept will contribute to a better understanding and clinical management of drug resistance in cancer patients.

**Keywords:** Cancer; blood; biomarkers; therapy; circulating tumor cells (CTCs); circulating tumor DNA (ctDNA)



Submitted May 16, 2013. Accepted for publication Jun 04, 2013.

doi: 10.3978/j.issn.2305-5839.2013.06.02

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There is increasing evidence that distant metastases can harbor unique genomic alterations different from the original primary tumor, and the direct analysis of metastatic cells will, therefore, reveal important information for systemic cancer therapy targeting metastatic disease (1). However, biopsy of overt metastases is an invasive procedure limited to certain locations and not easily acceptable in the clinic. Moreover, recent work has shown that different metastatic sites harbour different genomic aberrations (2) and biopsy of one or two accessible metastases may not be representative.

An alternative approach is the analysis of blood samples for circulating tumor cells (CTCs) or cell-free circulating tumor DNA (ctDNA), which can be performed repeatedly and might allow real-time monitoring of cancer therapies in individual patients. The peripheral blood is a pool of cells and/or DNA derived from the primary tumor and different metastatic sites and may, therefore, provide a comprehensive real-time picture of the whole tumor burden in an individual patient.

Recently, Dawson *et al.* used targeted or whole-genome

sequencing to identify somatic genomic alterations and designed personalized assays to quantify ctDNA in serially collected plasma samples (3). ctDNA was detected in 29 of the 30 (97%) metastatic breast cancer patients in whom somatic genomic alterations were identified. Dawson *et al.* claimed that ctDNA levels showed a greater dynamic range and greater correlation with changes in tumor burden than did CTCs. However, they have used the EpCAM-based CellSearch® system with its known sensitivity problems for CTCs that underwent an epithelial-mesenchymal transition (4). Interestingly, Dawson *et al.* could show a good correlation between ctDNA and CTC levels in patients with higher CTC counts, which is consistent with other reports in prostate and colon cancer (5,6).

Elevated concentrations of cell-free ctDNA fragments have been found in blood plasma and serum of cancer patients. ctDNA fragments mainly originate from apoptotic or necrotic tumor cells which discharge their DNA into the blood circulation. With the development of next generation sequencing technologies, the field of ctDNA analysis—which originally started more than

20 years ago (7)—has revived and focused on genomic aberrations relevant to targeted therapy in patients with metastatic cancer (e.g., k-ras mutations for EGFR inhibition in colorectal cancer) (8).

Although the analysis of plasma samples (ctDNA) appears to be convenient, pre-analytical conditions for ctDNA analysis must be standardized. For example, normal DNA from dying blood cells after blood collection will contaminate the specimens and dilute ctDNA. Immediate plasma separation, storage and shipment on dry ice make multicenter trials more complicated. Besides these technical considerations, the key question regarding the biology and clinical relevance of ctDNA analyses is why cell-free DNA mainly released from dying tumor cells should give important information on resistant clones? Possible hypotheses that need to be tested in future studies are that resistant viable tumor cells may release ctDNA and/or that a fraction of these cells might undergo apoptosis and release fragmented ctDNA into the blood. In contrast, analyses of CTCs allow an in-depth assessment of viable metastatic tumour cells at various levels (DNA, RNA, proteins) (9,10) and functionally (*in vitro/in vivo*) (11), which may contribute to the identification of metastases-initiator cells (12), these cells are the prime targets of anti-metastatic therapies and they may have developed special resistance mechanisms.

The term “*liquid biopsy*” originally introduced for the analysis of CTCs (1) is also used now for ctDNA analysis. The current definition of a biopsy is, however, ‘*the removal of cells or tissues for examination by a pathologist*’ (National Cancer Institute at the National Institutes of Health). While this term is suitable for CTCs, it appears somewhat misleading when applied to ctDNA, a fragmented cell component released mainly by dying tumor cells.

In conclusion, promising proof-of-principle data in small cohorts of metastatic patients with high ctDNA amounts have been published by Dawson *et al.* (3). But larger prospective trials are needed to demonstrate clinical utility. Future studies need to show whether ctDNA (or CTCs) detected in blood are representative of all relevant metastatic cell clones located at different sites and whether the information obtained by molecular analyses of ctDNA (or CTCs) might contribute to an improved clinical outcome of cancer patients.

## Acknowledgements

*Disclosure:* The authors declare no conflict of interest.

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**Cite this article as:** Alix-Panabières C, Pantel K. Real-time liquid biopsy: circulating tumor cells *versus* circulating tumor DNA. *Ann Transl Med* 2013;1(2):18. doi: 10.3978/j.issn.2305-5839.2013.06.02