

# Mutation analyses of circulating tumor cells, a real-time “Liquid biopsy” molecular study for patients with cancer

## Editorial

In cancer management, biomarkers are used for numerous purposes, such as predicting patient survival, identifying patients who are more likely to benefit from a treatment, and monitoring disease progression or therapeutic efficacy. The identification of biomarkers helps to ensure that patients receive the best therapeutic strategies, thereby avoiding unnecessary treatments and associated toxicities. At present, treatment decisions in the metastatic setting are based on analysis of earlier primary tumor samples or metastatic sites. However, biopsies from primary tumors or metastatic sites are not always feasible, and most important, mutation status of the primary and metastatic lesions can change during the course of disease and therapy. The natural evolution of the tumor itself and the specific therapies to which the patient has been exposed contribute to these changes. Circulating tumor cells (CTCs) isolated from the blood of patients with metastatic carcinoma provide a source of tumor cells and can be potential replacements for such repeatable tumor biopsies.

CTCs have been detected in a majority of epithelial cancers, including those from prostate, colorectal, and breast. CTCs are tumor cells shed from either the primary tumor or its metastases and can thus be regarded as “liquid biopsies” of metastasizing cells. Little is known about the timing of CTC release from primary tumors, their heterogeneity, or their functional properties. Although their exact composition is unknown, a fraction of these cells are thought to be viable metastatic precursors capable of initiating a clonal metastatic lesion. Several studies suggest that the characterization of the mutation status of CTCs could serve as a marker of micrometastatic tumor loads associated with a patient’s prognosis and accurately predict the effectiveness of therapy in several cancers.<sup>1-3</sup> Thus, CTC detection and mutational analysis has enormous potential for metastases prediction, monitoring response of patients to therapy, and early prediction of relapse.

For example, in colorectal cancer, mutations in driver genes (APC, KRAS, or PIK3CA) found in the primary tumor and metastasis were also detected in matched CTCs from patients, and interestingly, some CTCs showed amplification of CDK8, a potential new therapeutic target for CDK inhibitors.<sup>4</sup> Recently, the analysis of hundreds of CTCs obtained from patients with colorectal cancer revealed high intra-patient and inter-patient heterogeneity of KRAS.<sup>5,6</sup> CTCs with mutated KRAS will escape anti-EGFR therapy, and their early detection may help to guide therapy in individual patients. In breast cancer, resistance to HER2-targeting therapies is a key issue, and activation of the PI3K pathway by mutations in the PIK3CA gene plays a key role in this process; studies on pools of CTCs showed that 15.9% of patients with metastatic breast cancer had mutations in the PIK3CA gene.<sup>7</sup> More information about the occurrence of these activating mutations in the context of therapeutic interventions is needed.

Establishing the role of a diagnostic test in the medical setting is not straightforward, and for CTCs, the situation is complicated

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by the broad range of devices currently in use for their isolation. CTCs are found in frequencies on the order of 1-10 CTC per mL of whole blood in patients with metastatic disease, which contains a few million white blood cells and a billion red blood cells. Because of their low frequency, enrichment is needed to increase sensitivity to an acceptable level. CTC assays usually start with an enrichment step that increases the concentration of CTCs by several log units and enables an easier detection of single tumor cells. Furthermore, the only FDA-approved CTC enumeration test, Cell Search, relies on the detection of the surface epithelial cell adhesion molecule, EpCAM, but it is unclear whether all CTCs from epithelial tumors express EpCAM. It is well known that during the epithelial to mesenchymal transition, the expression of epithelial markers on CTCs, such as EpCAM and cytokeratin (CK), may be down-regulated and become undetectable. Although EpCAM might be down-regulated during EMT, the current view is that tumor cells with a partial EMT (or “intermediate phenotype” between epithelial and mesenchymal) are the most aggressive subclones.<sup>8</sup> However, CTCs, when detected, may represent a genomic snapshot of the current disease, and therefore can be used as “liquid tumor biopsies” to study mutations and monitor disease progression or therapeutic efficacy.

The need for improved CTC isolation and characterization methods to enable clinical applications is a current limitation in the analysis of CTCs. However, it was demonstrated that the analysis of cancer-related mutations in CTCs is feasible in a hospital-based clinical laboratory. Future clinical trials focused on interventional studies are needed in order to demonstrate the clinical utility of CTCs.

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## Conflicts of interest

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