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Editorials

Molecular Characterization of Circulating Tumor Cells: Holy Grail for Personalized Cancer Treatment?

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The classic biopsy approach does not allow monitoring of primary tumor evolution over time, and sampling of metastatic sites is not always possible for practical reasons. Through a simple blood draw, circulating tumor cells (CTCs)[2] can serve as a "liquid biopsy" and an early marker of response to systemic therapy, whereas molecular characterization of CTCs has strong potential to be translated to individualized targeted treatments *(1–3)*. It has been shown through numerous studies that CTC detection and enumeration in the blood of cancer patients is associated with poor outcome, and a number of clinical trials are now assessing their clinical importance *(4)*. However, CTCs are very rare and make up a small minority of cells circulating in blood, so their molecular analysis beyond enumeration is technically very challenging *(1, 2)*. Discovering the complete picture of CTC biology will enable the dream of precision medicine and personalized therapy to be realized, by facilitating the targeting of specific cell subtypes. This is not an easy task, since CTCs are not only rare but heterogeneous at the same time, even within the same patient *(5, 6)*.

In this issue of *Clinical Chemistry*, 2 different European groups present some very interesting data on the potential utility of CTC molecular characterization in the clinic *(7, 8)*. Although they use completely different experimental approaches, and although they focus on different cancer types, both groups show clearly that understanding CTC biology could be a key issue in favor of cancer patients.

Neves et al. have developed a work flow to efficiently purify single CTCs from clinical samples captured through the FDA-cleared CellSearch™ system *(7)*. This system is automated and widely used in clinical laboratories for CTC enumeration, since it gives clinically important information at the metastatic setting for breast, colorectal, and prostate cancer *(9)*. However, the CellSearch system has some limitations because it is a closed system designed to detect and enumerate $EpCAM^+/CK^+/CD45^-/DAPI^+$ CTCs (EpCAM = epithelial cell adhesion molecule, CK = cytokeratins, DAPI = 4′,6-diamidino-2-phenylindole). CellSearch is not designed to enable downstream molecular characterization of CTCs, beyond an extra fluorescent channel that can be used for evaluation of the expression of an extra marker in CTCs, e.g., human epidermal growth factor receptor 2 (HER2). Moreover, CTCs captured in CellSearch cartridges are contaminated with a high background of hematopoietic cells. Neves et al. now show that flow sorting can provide a fast, automatable, and effective way to isolate individual CTCs from CellSearch cartridges that is superior to micromanipulation, having the major advantage of direct use of the standard fluorescence staining of the CellSearch system. To achieve this, the authors have used a special device (MoFlo XDP) that accurately isolates single cells from CellSearch cartridges and deposits them as single events for further analysis. In this way, by use of the widely used CellSearch cartridges, this protocol enables further comprehensive genetic analysis on the single-cell level and can provide insights into the genomic alterations of CTCs. The authors used single cell array–based comparative genomic hybridization to screen the whole genome of single CTCs for copy number variations. Moreover, DNA amplified from single CTCs isolated by use of this protocol was analyzed for the presence of mutations in *PIK3CA* (phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit α)[3] and *TP53* (tumor protein p53) genes. The results of this study confirm CTC heterogeneity even within the same patient, since differences in copy number alterations between individual CTCs were clearly seen in all cases. The proposed protocol can be used for an affordable and systematic isolation of single CTCs from clinical samples after using CellSearch. Downstream analysis by use of this approach will facil-

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[2] Nonstandard abbreviations: CTC, circulating tumor cell; EpCAM, epithelial cell adhesion molecule; CK, cytokeratins; DAPI, 4′,6-diamidino-2-phenylindole; HER2, human epidermal growth factor receptor 2; FACS, fluorescence-activated cell sorting; ERCC1, excision repair cross-complementation group 1; MUC1, mucin 1, cell surface associated; CA, cancer antigen.

[3] Human genes: *PIK3CA,* phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit α; *TP53,* tumor protein p53.

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itate further molecular characterization of rare CTCs, help achieve a better understanding of their biology, and serve as a basis for their molecular screening in the clinical setting.

The potential of single cell sorting by fluorescence-activated cell sorting (FACS) and whole-genome amplification of CTCs after CellSearch was first described by Swennenhuis et al. *(10)*. A main limitation of both these approaches is that only EpCAM-positive epithelial cells can be isolated and analyzed because this is the isolation system used by CellSearch, whereas an invasive phenotype of CTCs that undergo epithelial mesenchymal transition is not analyzed *(11)*.

Also in this issue of *Clinical Chemistry*, the clinical utility of CTC molecular characterization is extended to the most recognized clinical challenge for ovarian cancer, the detection of platinum-resistant disease *(8)*. Kuhlmann et al. evaluated the presence of CTCs positive for excision repair cross-complementation group 1 (ERCC1) in the blood of ovarian cancer patients as a predictive biomarker for platinum resistance. In this approach, which is based on the *Adna*Test^{TM} platform for CTC detection, CTCs are isolated through immunomagnetic enrichment on the basis of 2 epithelial epitopes, EpCAM (an isolation strategy that is common to the CellSearch approach) and MUC1 (mucin 1, cell surface associated) and are further analyzed downstream by multiplex RT-PCR, targeting EpCAM, MUC1, cancer antigen (CA)-125, and ERCC1 transcripts. According to the results presented in this study, in patients with ovarian cancer, ERCC1^{+} CTCs constituted an independent predictor for overall and progression-free survival. A striking finding of this study was that the presence of ERCC1^{+} CTCs at primary diagnosis was an independent predictor of platinum resistance, whereas ERCC1 expression in corresponding primary tissues did not predict platinum resistance and was clinically noninformative. The expression of ERCC1 in CTCs can be used as a novel noninvasive blood-based biomarker for stratifying response to platinum-based chemotherapy at primary diagnosis and for guiding individualized therapy decisions. This is the first time that this marker has been evaluated in CTCs of ovarian cancer patients. However, due to the limited number of patients, this study is of a rather explorative character, and its encouraging results need to be validated in larger patient cohorts.

Molecular characterization of CTCs can provide important information for the identification of therapeutic targets and resistance mechanisms in these cells, as well as for the stratification of patients and real-time monitoring of systemic therapies *(1, 2)*, especially when a combination of single-cell analysis with next-generation sequencing technologies is used *(12, 13)*. Moreover, the isolation, ex vivo culture, and characterization of CTCs may provide an opportunity to noninvasively monitor the changing patterns of drug susceptibility in individual patients as their tumors acquire new mutations. In a very recent proof-of-concept study, Yu et al. established CTC cultures from patients with estrogen receptor–positive breast cancer *(14)*. Drug sensitivity testing of these CTC lines with multiple mutations revealed potential new therapeutic targets. This strategy may help identify the best therapies for individual cancer patients over the course of their disease.

Beyond CTCs, recently discovered technologies enable the extraction of molecular information on the primary tumor by analyzing in detail tumor-derived genetic material from circulating tumor DNA, circulating miRNAs, and exosomes *(15)*. All these liquid biopsy approaches have a high potential to give detailed information on tumor genome evolution over time, through simple blood draws that can be used for serial monitoring of patients. However, CTC analysis, compared with circulating cell-free DNA and exosomes as a liquid biopsy approach, has a clear advantage; it addresses viable cells that are not simply carriers of individual tumor biomarkers, but also represent live tumor cell entities that can give information on emerging tumor subclones with altered mutational and drug sensitivity profiles that can be targeted by specific drugs. From this point of view, CTC molecular characterization can offer an essential component of personalized cancer treatment. I strongly believe that in the near future CTC molecular characterization will enable treatment decisions to be based on evolving tumor molecular profiles and drug sensitivity patterns of these cells. In this context, these 2 papers in this issue of *Clinical Chemistry* are pointing in the right direction.

Several key issues remain to be addressed before liquid biopsy approaches can be implemented into routine clinical practice. Preanalytical issues, blood collection systems, and analysis steps should be carefully standardized and subjected to thorough internal and external quality control systems. Moreover, validation of clinical utility of CTC analysis assays through defined endpoints, such as overall survival, in multicenter clinical trials is absolutely necessary before their implementation in clinical practice.

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