

IN THE SPOTLIGHT

The Potential of Circulating Tumor Cells as a Liquid Biopsy to Guide Therapy in Prostate Cancer

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Summary: Miyamoto and colleagues present data that prostate-specific antigen/prostate-specific membrane antigen (PSA/PSMA)-based measurements of androgen receptor (AR) signaling in circulating tumor cells (CTC) enable real-time quantitative monitoring of intratumoral AR signaling. This finding indicates that measuring AR signaling within CTCs may help to guide therapy in metastatic prostate cancer and highlights the use of CTCs as liquid biopsy. *Cancer Discov*; 2(11); 974-5. ©2012 AACR.

Commentary on Miyamoto et al., p. 995 (6).

Early during the formation and growth of a primary tumor (e.g., breast, colon, or prostate cancer), cells are shed into the blood vessels. These circulating tumor cells (CTC) can be detected via different technologies based on their physical and biologic properties. Detection and molecular characterization of these CTCs are among the most active areas of translational cancer research, with more than 400 clinical studies including CTCs as a new biomarker and considering such blood analyses as a real-time liquid biopsy. Aims of research on CTCs include (i) estimation of the risk for metastatic relapse or metastatic progression (prognostic information), (ii) stratification and real-time monitoring of therapies (predictive information), (iii) identification of therapeutic targets and resistance mechanisms, and (iv) understanding metastatic progression in patients with cancer (1, 2). The clinical relevance of CTCs in prostate cancer has been recently reviewed (3). In general, several studies have indicated that CTC counts predict the prognosis of patients with metastatic prostate cancer, whereas the relevance in patients at earlier stages remains to be determined. However, CTCs are not part of the routine clinical management yet. Recently, Danila and colleagues (4) have outlined necessary steps to qualify specific CTC tests for medical decision making in clinical practice or drug development.

The detection of CTCs in the peripheral blood of patients with cancer holds great promise, and many exciting technologies have been developed over the past years (1, 2).

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However, detecting CTCs remains technically challenging. CTCs occur at very low concentrations of one tumor cell in the background of millions of normal hematopoietic cells. Their identification and characterization require extremely sensitive and specific analytical methods, which are usually a combination of enrichment and detection procedures. CTC enrichment includes a large panel of technologies based on the different properties of CTCs, including physical properties (size, density, electric charges, and deformability) and biologic properties (surface protein expression and viability; refs. 1, 2). After enrichment, the CTC fraction usually still contains a substantial number of leukocytes, and CTCs therefore need to be identified by a well-defined method that can distinguish tumor cells from normal blood cells at the single cell level: mRNA- or protein-based strategies. At present, CTC detection is focused on the development of microfluidic devices called CTC-chips, which can handle very small blood volumes. The first CTC-chip consisted of an array of anti-EpCAM antibody-coated microposts (5) and has been further developed into a herringbone (HB) structure.

In this issue of *Cancer Discovery*, Miyamoto and colleagues (6) have used this ^{HB}CTC-chip to analyze CTCs in patients with advanced prostate cancer. In metastatic prostate cancer, androgen deprivation therapy (ADT) is recommended as a first treatment, and secondary hormone therapies are used to suppress androgen receptor (AR) activation in patients with castration-resistant prostate cancer (CRPC). Using the ^{HB}CTC-chip to capture and detect CTCs, Miyamoto and colleagues (6) show that the activity of the AR pathway may be monitored through an immunocytochemical characterization of CTCs via a new established quantitative immunofluorescent assay based on the expression of the AR-regulated genes encoding prostate-specific antigen (PSA) and prostate-specific membrane antigen (PSMA). PSA and PSMA are upregulated following AR activation and AR suppression, respectively. Moreover, they define 3 different CTC patterns: “AR-off” for PSA⁻/PSMA⁺, “AR-mixed” for PSA⁺/PSMA⁺, and “AR-on” for PSA⁺/PSMA⁻.

Miyamoto and colleagues have studied the clinical relevance of this AR characterization in CTCs in 2 patient cohorts: (i) patients with untreated metastatic prostate cancer and (ii) patients with CRPC. Indeed, the initiation of ADT in

treatment-naïve patients with metastatic prostate cancer with detectable CTCs led to a switch of the majority of CTCs from “AR-on” to “AR-off” signal, preceding the disappearance of CTCs. In marked contrast, patients with CRPC before the initiation of abiraterone showed a strong heterogeneity in AR activity among CTCs: an abundance of both “AR-off” and “AR-mixed” CTCs and only a few “AR-on” CTCs. Interestingly, (i) the presence at baseline of more than 10% “AR-mixed” CTCs was associated with decreased overall survival, and (ii) the increase of “AR-on” CTCs despite abiraterone acetate therapy was correlated with reduced overall survival. This study showed that the PSA/PSMA-based AR signaling assay in CTCs enables real-time quantitative monitoring of intratumoral AR signaling. Measurement of treatment-induced signaling responses within CTCs may help to predict and guide therapy in metastatic prostate cancer. However, these preliminary results need to be validated in large prospective clinical studies of patients with prostate cancer undergoing second-line hormonal therapy.

The current study has implications beyond the treatment of prostate cancer because it successfully shows the proof-of-principle of the use of CTCs as liquid biopsy. Currently, the choice of a targeted therapy for an individual patient is made upon analysis of the primary tumor for the expression and/or genomic status of a specific molecular target. However, recent studies indicate that primary carcinomas (in particular, prostate cancer) show a marked inpatient heterogeneity in regard to genotypic and phenotypic characteristics and tumor cells may change during the extended time period between primary tumor resection and metastatic relapse due to parallel progression and/or natural selection of the fittest clones (7). Thus, the direct analysis of metastatic cells may provide important additional information for stratification of patients to expensive therapies with considerable side effects. However, biopsies of metastases carry strong limitations and risks, whereas blood sampling is an easy and safe method. Moreover, metastases from different sites are genetically heterogeneous, and CTCs have the advantage that they may represent the entire spectrum of distant metastases (2). However, the small number of CTCs captured by current technologies may pose a severe limitation to the liquid biopsy concept. Increasing the yield of CTCs by a substantial increase in the blood volume analyzed for CTCs to 1.5 L is now feasible using a new *in vivo* CTC capture device (8). Whether this technology is able to allow the same downstream molecular analyses as the chip technology is the subject of current investigations.

Another limitation of the HB chip assay used by Miyamoto and colleagues (6) is the fact that EpCAM antibodies were used for CTC capture (9). An important drawback of EpCAM-based enrichment is that mesenchymal-like cancer cells that underwent an epithelial-mesenchymal transition (EMT) are missed because epithelial markers such as EpCAM are downregulated during EMT (10). Recent work suggests that EMT might particularly affect tumor cells with stem cell-like properties (11), thereby indicating that EpCAM-positive CTCs may not have metastasis-initiating capacities. Thus, an aggressive and clinically relevant subset of cancer cells might be missed using EpCAM-based CTC assays for liquid biopsy. However, most genes upregulated in cancer cells during EMT (e.g., vimentin) are also expressed in the surrounding normal

blood cells. There is an ongoing search for markers expressed on (semi)-mesenchymal CTCs but absent on normal blood cells. Future versions of CTC assays need to implement these markers as capture antibodies to achieve an optimal representation of the biologically distinct subsets of CTCs.

Disclosure of Potential Conflicts of Interest

K. Pantel has a commercial research grant from Veridex and Roche, receives honoraria for service on the speakers' bureau for Veridex, Roche, and Novartis, and serves as a consultant/advisory board member of Veridex, Alere, and Gilupi. C. Alix-Panabières receives honoraria for service on the speakers' bureau for Sanofi, Roche, and Veridex, is a consultant/advisory board member of Janssen, Veridex, and Sanofi, and receives commercial research support from Veridex and Roche.

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