IN THE SPOTLIGHT

Digital Circulating Tumor Cell Analyses for Prostate Cancer Precision Oncology

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Summary: In this issue of Cancer Discovery, Miyamoto and colleagues adapted their microfluidic CTC-iChip isolation platform with a digital RNA-PCR readout for eight prostate-specific transcripts and two assays for the androgen receptor mRNA splice variant ARV7 and the TMPRSS2-ERG translocation transcript. In patients with metastatic castration-resistant prostate cancer at initiating abiraterone therapy in a first-line setting, the resulting RNA-based digital circulating tumor cell signatures identified patients with a shorter overall survival, and in patients with clinically localized disease, the signatures identified those with seminal vesicle invasion and pelvic lymph node involvement. Cancer Discov; 8(3); 269–71. ©2018 AACR.

See related article by Miyamoto et al., p. 288 (7).

Precision medicine attempts to understand disease at a deeper level with the aim of improving the ability to stratify patients by risk and to develop more targeted therapies. In oncology, molecular analyses of tumor material, which are often done by genetic analyses, are essential to realize precision medicine. Further aims of precision oncology include the longitudinal tracking of tumors to measure response to applied interventions and to identify biomarkers and mechanisms of drug resistance (1).

In men, the most common malignancy is prostate cancer, which represents a major cause of cancer-related deaths. For the management of these patients, there are two scenarios that urgently need reliable prognostic information and biomarkers for realizing precision oncology. The first scenario includes patients with metastatic castrate-resistant prostate cancer (mCRPC). Novel agents targeting the androgen receptor (AR) pathway, such as abiraterone or enzalutamide, have drastically changed outcome by slowing disease progression and improving survival (2). However, development of resistance is inevitable and means to predict progression-free survival are currently lacking. The propensity of metastatic prostate cancer to spread to the bone hampers options to acquire tissue for repeated genetic analyses. The second scenario includes patients with localized disease for whom the diagnosis usually depends on systematic prostate biopsy by transperineal or transrectal access routes to obtain up to 12 tissue samples, which is associated with side effects such as bleeding, infections, or acute urinary retention. The selection of the primary treatment option, that is, expectant management, surgery, or radiation, represents a challenge (2). Hence, both scenarios raise the question whether noninvasive means can be used to assess disease status and to predict treatment efficacy.

To this end, “liquid biopsies,” tumor-derived components detectable in the peripheral blood of patients, that is, circulating tumor cells (CTC), circulating tumor DNA (ctDNA), or extracellular vesicles, are increasingly being used for clinical applications, such as early cancer detection, disease staging, monitoring for recurrence, prognostication, and selection of therapy (3). In the field of urologic oncology, CTCs have been widely studied, and quantification of CTCs, that is, establishing the number of CTCs in the peripheral blood, correlates with disease outcome (4). However, detailed molecular analyses of CTCs may provide much more information than a simple enumeration. For example, they provide unique opportunities to study AR transcript variants, such as ARV7, a truncated splice variant with no ligand-binding domain, which has recently been associated with a higher likelihood of primary resistance to androgen signaling inhibitors (5). Furthermore, single-cell RNA sequencing of prostate CTCs demonstrated substantial heterogeneity of AR splice variants (6).

In this issue of Cancer Discovery, Miyamoto and colleagues developed their CTC-based pool of technologies further to study CTCs as prognostic biomarkers in patients with prostate cancer (7). Because CTCs are rare in the peripheral blood and frequently account for merely one in a billion nucleated cells (8), a key step for molecular CTC analyses is enrichment and isolation of these cells. To this end, Miyamoto and colleagues employed their microfluidic (CTC-iChip) technology to deplete hematopoietic cells and to enrich CTCs (Fig. 1). The CTC enrichment was followed by RNA-based digital droplet PCR (ddPCR) and a digital RNA-based scoring system, which the same group developed earlier for the detection of CTCs in patients with hepatocellular carcinoma (9). In the first step, they curated prostate tissue–specific transcripts not detectable in normal blood cells. This resulted in a set of eight genes suitable for developing an RNA-based signature, including four androgen-responsive transcripts [KLK3 (PSA), KLK2, TMPRSS2, and AGR2], two androgen-repressed
A novel sensitive strategy to analyze CTCs in men with prostate cancer is based on two technologies: first, microfluidic cell enrichment (CTC-iChip), and second, RNA-based digital droplet-PCR, including evaluation using sophisticated bioinformatics tools. The CTC-iChip depletes hematopoietic cells from blood samples, resulting in a relatively enriched CTC population. After RNA extraction and preparation of cDNA, RNA-based ddPCR assays for eight prostate-specific transcripts, which are not expressed in normal hematopoietic cells, are classified with a multiclass support vector machine. For each gene, a weight was generated on the basis of their expression, and CTCM and FTC scores were calculated for patients with metastatic and localized diseases, respectively. For patients with metastatic disease, two additional assays for the AR mRNA splice variant ARV7 and the TMPRSS2-ERG translocation transcript expression were developed. In patients with mCRPC and at initiating abiraterone therapy in a first-line setting, both the FTC score and the ARV7 assay were predictive for shorter overall survival, whereas the TMPRSS2-ERG assay was not. In patients with clinically localized disease, the FTC score was predictive for seminal vesicle invasion and pelvic lymph node involvement.

Figure 1. A novel sensitive strategy to analyze CTCs in men with prostate cancer is based on two technologies: first, microfluidic cell enrichment (CTC-iChip), and second, RNA-based digital droplet-PCR, including evaluation using sophisticated bioinformatics tools. The CTC-iChip depletes hematopoietic cells from blood samples, resulting in a relatively enriched CTC population. After RNA extraction and preparation of cDNA, RNA-based ddPCR assays for eight prostate-specific transcripts, which are not expressed in normal hematopoietic cells, are classified with a multiclass support vector machine. For each gene, a weight was generated on the basis of their expression, and CTCM and FTC scores were calculated for patients with metastatic and localized diseases, respectively. For patients with metastatic disease, two additional assays for the AR mRNA splice variant ARV7 and the TMPRSS2-ERG translocation transcript expression were developed. In patients with mCRPC and at initiating abiraterone therapy in a first-line setting, both the FTC score and the ARV7 assay were predictive for shorter overall survival, whereas the TMPRSS2-ERG assay was not. In patients with clinically localized disease, the FTC score was predictive for seminal vesicle invasion and pelvic lymph node involvement.
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REFERENCES


Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

for localized disease (CTC_L score). This CTC_L score applied preoperatively was strongly associated with seminal vesicle invasion and pelvic lymph node involvement, indicating early prostate cancer dissemination.

The work by Miyamoto and colleagues significantly extends options of CTC analyses and offers solutions to eminent problems in the management of patients with prostate cancer. Furthermore, the intriguing data of HOXB13 expression illustrate that digital CTC signatures may also provide insight into the biology of cancer cell dissemination. The LNCaP cell spike-in experiments suggest that transcripts from a single cell may be detectable, but the exact specificity and sensitivity of the CTC scores will have to be determined in larger clinical trials. A particular challenge will be whether this approach may allow distinguishing between localized low-grade prostate cancers, which are often harmless, and high-grade prostate cancers, which usually require treatment. Moreover, due to the low case numbers in this study, the value of the digital CTC signatures will have to be confirmed in larger studies, ideally in prospective multicenter clinical trials. The success of such larger studies will depend on the availability of the CTC-iChip, which is not offered on a commercial basis and which is available only in the laboratory of the authors. CTCs have evolved to be the preferred liquid biopsy component for the analysis of AR splice variants such as ARV7. However, somatic copy-number alterations, gene fusions, and point mutations can also be determined by ctDNA analyses (10), which do not require sophisticated equipment such as the CTC-iChip. It will be interesting to see which liquid biopsy approach will eventually be most broadly used in clinical applications, in particular for the detection of minimal residual disease. In summary, there is no doubt that liquid biopsies will evolve to be an indispensable tool in precision oncology, and this study is a big step forward in improving management for the most frequent tumor in men, that is, prostate cancer.
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