Detection of Androgen Receptor Mutations in Circulating Tumor Cells: Highlights of the Long Road to Clinical Qualification

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There is evidence that androgen receptor (AR)4 gene function not only is necessary for the growth and differentiation of the healthy prostate gland but also may contribute critically to treatment failure in progressive stages of castration-resistant prostate cancer (CRPC)5 (1–3). The mechanisms include AR overexpression, AR gene amplification, an increase in the levels of the androgen synthetic machinery leading to increased intratumoral androgens, ligand-independent activation, and gain-of-function mutations in the gene itself (2). The clinical importance of these findings has been validated in trials of the novel androgen receptor antagonist MDV3100, which was developed in a cell-based screen for activity in cells with overexpressed AR, and of abiraterone acetate, a 17,20 lyase inhibitor that blocks androgen synthesis in the testis, the adrenals, and the tumor (3, 4).

The development and validation of reliable, reproducible protocols to accurately assess functional status, frequency, and functional relevance of mutations in AR could then be critical for the successful development and clinical implementation of novel targeted strategies for patients with advanced stages of prostate cancer, particularly those with progressive CRPC. Although a recent study on the integrative genomic profiling of human prostate cancer was limited by the interrogation of only a modest number of genes and patient samples, the reported data strongly suggested that the overall rate of mutations may be low in prostate cancer (5). In particular, mutations were infrequent among common, broadly mutated oncogenes, such as PIK3CA (phosphoinositide-3-kinase, catalytic, alpha polypeptide), KRAS (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog), and BRAF (v-raf murine sarcoma viral oncogene homolog B1). Importantly, however, the study reported a higher frequency of AR mutations. Alterations in AR, including mutations, gene amplification, and overexpression, were common in the metastatic tumor samples analyzed but were notably absent in tumor samples representing untreated localized disease (5). Assessing the frequency of specific molecular changes in CRPC, however, is limited by the general unavailability of metastatic tumor samples for profiling, as well as by a lack of analytically valid assays for measurement.

Circulating tumor cells (CTCs) isolated from blood have been hypothesized to be capable of fulfilling the unmet need for tumor tissue for molecular profiling for biomarkers that predict sensitivity to treatment. Biomarker qualification requires analytically valid assays that can then be studied in prospective clinical trials designed for a specific context of use. A variety of CTC assays are available and under study, but at present only 1, CellSearch® (Veridex/Johnson & Johnson), has undergone full analytical validation for enumeration (6) and been shown in prospective trials to provide prognostic information at baseline and after treatment in patients with breast, colorectal, or prostate cancer (7–9). The US Food and Drug Administration (FDA) clearance is as an “aid to monitoring” disease in conjunction with other measures. The assay is not cleared for the assessment of predictive markers.

A limitation of CellSearch is that CTCs are not detected in patients with clinically localized disease or in patients who are in the clinical state of increasing prostate-specific antigen (10, 11). To address this issue, we measured the mRNA copy number in 2 AR-regulated prostate-specific genes [KLK3 (kallikrein-related peptidase 3) and KLK2 (kallikrein-related peptidase 2)] (11) in blood prepared with the PAXgene Blood RNA System (PreAnalytiX). A direct comparison of the 2 assays revealed highly concordant results that were significantly associated with the presence of skeletal metastases and with overall survival. Combining these measures, however, did not seem to contribute importantly to enhancing the predictive accuracy

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4 Human genes: AR, androgen receptor; PIK3CA, phosphoinositide-3-kinase, catalytic, alpha polypeptide; KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; BRAF, v-raf murine sarcoma viral oncogene homolog B1; KLK3, kallikrein-related peptidase 3; KLK2, kallikrein-related peptidase 2.
5 Nonstandard abbreviations: CRPC, castration-resistant prostate cancer; CTC, circulating tumor cell; FDA, US Food and Drug Administration.
above that of the CTC counts obtained with the FDA-cleared CellSearch assay (10, 11).

Marketed along with the CellSearch Circulating Tumor Cell Kit for CTC enumeration is the CellSearch Tumor Cell Kit for molecular analysis (although the FDA has not cleared the latter). In this issue of the Journal, Jiang et al. (12) used the Circulating Tumor Cell and Profile kits with the AllPrep DNA/RNA Micro Kit (Qiagen) to amplify AR exons 3–8 by the PCR. The amplicons were subsequently subjected to digestion with the SURVEYOR endonuclease and fractionation by WAVE® denaturing HPLC (Transgenomic). Assays for mutations were postulated to be more sensitive and specific than assays for the presence of nonmutated tissue-specific genes. The authors used KRAS mutations as the target and obtained results with direct resequencing that suggested the possibility of detecting mutations in the target gene in mixtures containing as little as 2.5% of the target in the enriched CTC sample (12). They subsequently detected 27 AR mutations (19 missense mutations, 2 silent mutations, 5 deletions, and 1 insertion) in CTC samples from 20 (57%) of 35 informative patients with CRPC. Whereas other investigators had previously reported many alterations as somatic mutations in tissue samples from metastatic lesions, Jiang et al. also discovered novel AR mutations of putative functional significance (12). The estimated frequency of AR mutations among metastatic prostate cancer patients reported by Jiang et al. is strikingly similar to that of Taylor et al. (5), who used a very different approach to address this issue.

To confirm these findings prospectively first requires a focus on the analytical techniques used and then documentation of the patient population studied. The first concern is the large number of cycles used in the nested PCR protocol, which in theory could detect a single copy of the gene target. The second concern is that the limits of detection for AR mutations were not studied directly, but their determination will ultimately be required for analytical validation of this approach. Additional concerns include the detection of AR mutations in patient samples with low or 0 CTC counts, as well as the low correlation between the CTC count and the frequency of mutated AR genes in each sample. The latter concern may be explained by the difference in the biomarker reported, because the CellSearch Profile Kit measures the total number of epithelial cell adhesion molecule–positive cells captured by the immunomagnetic bead separation, not the fraction of cells that meet the strict FDA-cleared definition of a CTC, which only includes epithelial cell adhesion molecule–positive cells that are intact with a 4′,6-diamidino-2-phenylindole–positive nucleus surrounded by cytoplasm, cytokeratin positive, and CD45 negative.

To determine whether an alteration in the AR gene represents a somatic change requires concurrent analysis of DNA from nonpathologic lymphocytes, as reported by Taylor et al. (5). Jiang et al. did not perform this assessment in their study (12), nor did they evaluate age-matched, healthy, nonsymptomatic male volunteers as potential negative controls. Recognizing that the alterations present in late-stage CRPC reflect both the intrinsic biology of the tumor and the specific therapies the tumor has been exposed to and ultimately progressed from will require the evaluation of a sufficient number of patients in representative discrete clinical cohorts before any results can be considered for evaluation in prospective trials. For the latter issue, an understanding of the functional significance of specific AR mutations and the association of the presence of a lesion with a clinical outcome will be required to better enable the design of a prospective trial that assesses their role as predictive biomarkers.

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