Evolution of the Liquid Biopsy in Metastatic Prostate Cancer

Jose G. Moreno and Leonard G. Gomella

We reviewed the literature for the biologic, prognostic, and predictive significance of circulating prostate cancer tumor cells (CTCs), and circulating tumor DNA in the blood of metastatic castration resistant prostate cancer patients. CTCs demonstrate robust prognostic value independent of PSA in predicting overall survival. The CTC androgen receptor variant receptor 7 phenotype predicts resistance to androgen receptor synthesis inhibitors and sensitivity to taxane based chemotherapy in metastatic castration resistant prostate cancer patients who are candidates for second line therapy. Research is rapidly pivoting toward circulating tumor DNA analysis because the approach is sensitive, prognostic, cost effective, and it can elucidate mechanisms of systemic therapy. UROLOGY 132: 1−9, 2019. © 2019 Elsevier Inc.

A liquid biopsy (LB) is broadly defined by the National Cancer Institute as a “test done on a sample of blood to look for cancer cells from a tumor that are circulating in the blood or for pieces of DNA from tumor cells that are in the blood.” Similar to a contemporary tissue biopsy, a LB can inform about disease burden, prognosis, response to targeted therapy, and elucidate changes in cancer cell signaling which drive therapy resistance. A tissue biopsy reflects tumor biology at a single site at a single time point in the context of organ microenvironment, while a LB allows probing of multisite clonal variants in real-time without invasive tissue sampling.

In 1869, tumor cells were identified in the blood in a patient with overwhelming metastatic disease by light microscopy. However, it was not until 130 years later that minimal metastatic tumor burden was first identified by the molecular detection of prostate specific antigen (PSA) expressing cells from the circulation. These early molecular techniques used reverse transcriptase polymerase chain reaction (RT-PCR) to detect circulating prostate cells. These early studies demonstrated that PSA-expressing cells in the circulation were more often detected in metastatic prostate cancer compared to localized disease. Further prognostic PSA RT-PCR validation was hampered by lack of quantitation, false positives, instability of RNA in blood, and perhaps an initial focus on locally advanced disease. Research then moved toward immunofluorescent detection of circulating tumor cells (CTCs) which could be quantified with an assay which eventually would be prognostically validated.

While serum PSA has proven itself as a critically important marker in localized prostate cancer, its prognostic utility in mCRPC is not as discerning. Recognized limitations of PSA and imaging in the assessment of mCRPC include: the need to wait 3 months after specific treatments (due to PSA release from apoptotic cells), flare-phenomena, PSA steroidal modulation, and interobserver variability of imaging interpretation. Prognostic and predictive CTC assays have now been developed for mCRPC which are commercially available, and may assist with second line androgen receptor (AR) signaling inhibitors (ARSIs) selection. Coincident with this paper submission, there are over 1578 prostate CTC PubMed citations. Research efforts are now pivoting toward circulating tumor DNA (ctDNA) analysis as a tool to characterize metastatic tumor response to systemic therapy with 262 prostate ctDNA PubMed citations.

This review focuses on the prognostic utility of CTCs, predictive value of the AR splice variant 7 (AR-V7), CTC phenotype, and emerging powerful and sensitive ctDNA assays.

BIOLOGY OF CTCs, AND CT DNA

CTCs represent tumor cells shed into the bloodstream from primary or metastatic tumor sites. Individual and/or clusters of CTCs may invade and metastasize by losing their epithelial phenotype and gaining mesenchymal features with greater plasticity and invasive capacity in a process known as epithelial-mesenchymal cell transition (EMT). This is relevant because the canonical definition of CTCs includes epithelial cell adhesion molecule (EpCAM) surface positivity, nuclear staining, and cyto-keratin in the cytoplasm of CTCs. Antibodies directed at EpCAM may fail to detect EMT carcinoma cells.
transiting the circulation. More recent CTC detection systems can detect CTCs with EMT morphology, which may allow broader CTC enumeration and analysis. CTC interactions with immune cells, and platelets may also modulate the levels of CTCs in the blood stream.

“Naked” ctDNA consists of cell free strands of DNA that are approximately 180-200 bp in size. These tumor-derived fragments of DNA should not be confused with more abundant sources of cell free nonmalignant DNA (cfDNA) which include: fetal DNA in expecting mothers, after intense exercise, and disease states such as end-stage renal failure, stroke, myocardial infarction, following surgery, and trauma. DNA isolated from single or cluster of CTCs is also not the same as ctDNA. A cancer patient’s ctDNA can be released passively from cells due to apoptosis and necrosis from CTCs, primary tumor, and/or metastatic sites. Secondly, ctDNA may be actively secreted through release of extracellular vesicles—a postulated mechanism of cell to cell communication driving tumorigenesis. Quantifiable ctDNA load has been shown to correlate with tumor burden in mCRPC. The amount of ctDNA as a fraction of cfDNA may range from 0.01% to more than 90%.

Prostate cancer mutational drivers have been detected in 89% of metastatic tissues sites in lymph node, bone, liver, and other sites. Mutations identified in these tissue metastatic sites include the following signaling pathways: AR (62.7%), PI3K (49%), DNA repair genes (28.7%), cyclin-dependent kinase (7%), and WNT. Moreover, ctDNA genomic changes mirror mutations found in metastatic tissue sites. DNA mutations within pooled CTCs (as opposed to naked ctDNA) correlate with genomic alterations from matched tissue. Whole exome DNA sequence of pooled CTCs from 2 metastatic prostate cancer patients demonstrated that 51 of 73 mutations (70%) of the CTC DNA mutations were found in the matched tissue. Emerging evidence supports the concept that both ctDNA and circulating DNA within CTCs recapitulates the biology of cancer within the tissues. Advantages of ctDNA analysis include noninvasively revealing tumor genetic evolution from multiple metastatic sites, potentially allowing more effective timely targeted therapy. Interpreting ctDNA variants requires careful analysis because somatic mutations can be found in healthy patients due to clonal hematopoiesis which can cause age-related somatic ctDNA mutations in genes including TP53.

CTC ANALYSIS

CTC Isolation and Enumeration

A comprehensive survey of the lab techniques used to isolate and enumerate CTCs is beyond the scope of this review, and are described elsewhere. A preliminary step in many CTC assays often involves an enrichment step to concentrate CTCs from whole blood. These concentration methods are coupled with a detection stage using PCR, multiparameter flow cytometry, or immunofluorescent cell imaging. Laborious Ficoll-paque gradient enrichment, coupled with PSA RT-PCR, was used to detect CTCs in prostate cancer, but it was limited by lack of PCR quantitation and issues with mRNA degradation.

The most widely published CTC enumeration system is the automated CellSearch System which was originally developed by Immunicon (Immunicon, Huntington, PA), Janssen/Johnson and Johnson (Janssen, Horsham, PA), and more recently by Menarini Biosystems (Menarini Silicon Biosystems, Huntington, PA). This platform immunomagnetically enriches epithelial cells by targeting EpCAM with antibodies bound to ferrofluid nanoparticles which are then placed in a magnetic field. These concentrated CTCs, still contaminated by leukocytes, are immunofluorescently stained for cytokeratins nuclear DAPI, and the leukocyte cell surface marker CD45. Automated digital microscopy detect CTCs canonically defined as EpCAM+CK+DAPI+CD45. A limitation of CellSearch is that it may fail to detect EMT cells transitioning the circulation or neuroendocrine cells without the epithelial phenotype.

Microfluidic systems isolate CTCs based on their larger size, lower elasticity, and dielectric properties that allow greater manipulation of the unfixed specimen. One such platform, CTC-iChip (Massachusetts General Hospital, MA), works by negative depletion and can isolate CTCs down to a single cell and allow subsequent whole genome mRNA expression analysis. Negative depletion works by efficiently capturing hematopoietic cells which enables tumor CTC epitope independent isolation. High sensitivity of this assay allowed for analysis of CTCs from patients with localized disease. A practical limitation of microfluidic systems is that they are not formally approved for clinical commercial use although Parsortrix (ANGLE, Surrey, United Kingdom) is being evaluated by the FDA for approval for CTC enumeration in breast cancer (personal communication).

A new CTC assay known as the AR-V7 LB (Epic Sciences, San Diego, CA) relies on rapid high-content imaging of all nucleated blood cells, and it bypasses enrichment altogether. This CTC platform employs a “no cell left behind” strategy skirting EpCAM positivity by using cell size as a key CTC detection characteristic. Red blood cells are lysed and all nucleated cells are plated and analyzed on custom glass slides for standard immunofluorescent staining and detailed morphologic interpretation of the remaining CTCs. This platform counts EpCAM positive and EpCAM negative CTCs that exhibit the EMT phenotype. This strategy has been used to detect the AR-V7 truncated AR in CTCs.

CTCs are Prognostic in mCRPC

CTC enumeration with CellSearch in mCRPC patients has been shown to correlate with poor outcomes independent of PSA in several studies (Table 1). In a prospective study of 231 mCRPC patients, baseline CTC
<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>Population Characteristics</th>
<th>Patients With ≥5 CTCs</th>
<th>Statistically Significant CTC Endpoint</th>
<th>CTC vs PSA Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>De Bono et al (2008)¹⁷</td>
<td>231</td>
<td>Starting new line of cytotoxic therapy; ECOG 0-2</td>
<td>57%</td>
<td>CTC ≥ 5 worse prognosis at: baseline, 2-5, 6-8, 9-12, and 13-20 weeks; conversions to positive and negative significant</td>
<td>CTC predicted OS better than PSA at all time points; CTC reduction week 9-12 had better ROC-AUC (receiver operator characteristics-area under curve) characteristics than PSA 30% reduction</td>
</tr>
<tr>
<td>Scher et al (2009)²⁰</td>
<td>156</td>
<td>Starting first line cytotoxic therapy; ECOG 0-1</td>
<td>54%</td>
<td>CTC independent of discrete cut-off value significant</td>
<td>CTC as a continuous variable more predictive of survival than PSA at baseline and during follow-up; LDH at baseline more predictive than CTC</td>
</tr>
<tr>
<td>Goldkorn et al (2014)²¹</td>
<td>263</td>
<td>Starting cytotoxic first line and astrasentan second line; ECOG 0-1</td>
<td>51%</td>
<td>CTC ≥ 5 prognostic at baseline, and most prognostic at 3 weeks</td>
<td>CTC as a continuous variable at baseline had higher ROC-AUC than PSA</td>
</tr>
<tr>
<td>Scher et al (2015)²²</td>
<td>711</td>
<td>Abiraterone vs prednisone alone with prior docetaxel; ECOG 0-2</td>
<td>48%</td>
<td>CTC ≥ 5 prognostic at 12 weeks; CTC and LDH are a surrogate for OS at 12 weeks</td>
<td>CTC predicted OS at 12 weeks better than PSA 30%, and PSA 50% decrease. CTC plus PSA combination inferior to PSA alone in predicting OS at 12 weeks</td>
</tr>
<tr>
<td>Heller (2018)²³</td>
<td>6081</td>
<td>Starting abiraterone/prednisone or placebo/prednisone or enzalutamide or orteronel/ prednisone or cabozantinib or prednisone</td>
<td>51%</td>
<td>CTC nonzero at baseline and zero at 13 weeks and CTC ≥ 5 conversion to ≤4 at 13 weeks highest predictor of OS</td>
<td>CTC conversions had highest level of discriminatory power superior to PSA decreases of 30%, 50%, and 70%</td>
</tr>
<tr>
<td>Lorente (2018)²⁴</td>
<td>511</td>
<td>Abiraterone vs prednisone alone with prior docetaxel; ECOG 0-2</td>
<td>Not applicable</td>
<td>Increasing CTCs with baseline &lt;5 during 1st 12 weeks independent predict OS</td>
<td>CTC progression more predictive of PSA at baseline, but comparison to PSA progression was not reported</td>
</tr>
</tbody>
</table>
count was prognostic of overall survival (OS) and progression-free survival (PFS) with a categorical cut point of favorable (<5 CTCs per 7.5 mL whole blood) vs unfavorable (≥5 CTCs per 7.5 mL whole blood) that was independent of PSA.23 Moreover, conversions of CTC count from unfavorable to favorable or vice versa during treatment was associated with a poorer prognosis at 2 to 5 weeks after treatment.20 By contrast, a 30% or 50% decline in PSA was prognostic only after 6 to 8 weeks of therapy.17 The FDA approved the CellSearch CTC enumeration system in 2008 for use in mCRPC.25 These patients were starting a new cytotoxic chemotherapy regimen in patients with heavier disease burden. The Prostate Cancer Clinical Trials Working Group III has advocated incorporating CTC enumeration in mCRPC clinical trials.6 A limitation of CTCs at baseline in mCRPC is that the sensitivity of detecting ≥5 CTCs/7.5 mL of blood ranges between 48% and 57% (Table 1). However, in a prospective study of 5660 mCRPC cases treated with abiraterone, prednisone, enzalutamide, orteronel, or cabozantanib, 8 CTC response points were evaluated and all were prognostic.23 The most prognostic endpoint was a ≥1 CTC at baseline that went to zero cell count at 13 weeks occurring in 71% of patients.23 Conversely, CTC conversion from zero to detectable CTCs was a very poor prognostic indicator.23 Both CTC conversion measures at baseline and at 13 weeks had the highest discriminatory power for OS that was higher than PSA decreases of 30%, 50%, and 70%.23 The study included patients with minimal disease burden. CTC enumeration in combination with traditional biomarkers (LDH, PSA, hemoglobin, alkaline phosphatase, or albumin) was prospectively evaluated in a 711 mCRPC patients receiving abiraterone. The most useful combination was CTC and LDH at 12 weeks after treatment which was associated with a 2% vs 46% 2 year survival if CTCs ≥ 5/7.5mL blood plus LDH > 250 IU/L was the end-point.24 Although CTCs are a robust independent prognostic biomarker, the predictive value of CTC AR-V7 status may be even more clinically useful by impacting therapeutic decision making as outlined in the AR-V7 section.

CTC CHARACTERIZATION

Translational research has evolved beyond CTC enumeration to downstream analysis of DNA, RNA, and protein profile from enriched CTC pools or as single captured cells. As a result of the high prevalence of TMPRSS2-ERG fusion in prostate cancer, it was characterized via CTC RT-PCR in a 41 mCRPC patients receiving abiraterone trial, and no significance was found.26 In a prospective PTEN concordance study of 48 mCRPC patients, concordance was demonstrated between tumor tissue PTEN gene status and PTEN loss identified by FISH in CTCs with the Epic Science platform.27 This is important because a postulated pathway of androgen resistance may involve crosstalk PTEN/PI3K/AKT and AR pathways.28 PTEN loss in tumor tissue may predict response to Akt inhibition when ipataertib is combined with abiraterone.29 Other studies have focused on CTC phenotype markers exhibiting the EMT and neuroendocrine smaller noncytokeratin positive cell morphology, which may signal resistance to ARSIs and potential sensitivity to chemotherapy.29 Similarly, high CTC heterogeneity may be a better predictor of survival on taxanes such as docetaxel vs ARSIs.30

CTC AR-V7 Status and Prediction of Response to ARSIs and Taxanes

CTC AR-V7 translational research has now evolved into a clinically available assay. AR-V7 is a truncated AR mRNA splice variant that lacks the androgen ligand binding domain.31 A positive AR-V7 anomaly leads to a constitutively activated AR complex.31 Detection of CTC AR-V7 mRNA transcript in mCRPC patients was first described by qRT-PCR performed on EpCAM immuno-magnetically captured cells using the AdnaTest platform (Quiagen, Hilden, Germany).31 In another mCRPC study, the identification of AR-V7 expression was associated with a worse clinical outcome independent of the number of prior lines of hormonal therapy.32 This assay demonstrated dynamic changes in a longitudinal study with multiple systemic agents.33 A conversion to positive was associated with both taxanes, and ARSIs, but a reversion to negative occurred only in the taxane treated men.34

A second-generation AR-V7 detection platform was developed by Epic Sciences (Epic Science, San Diego, CA) which involves immunofluorescent CTC staining available through Genomic Health (Redwood City, CA) under the name Oncotype DX AR-V7 Nucleus Detect Test. The assay is performed by Epic Sciences, but marketed by Genomic Health.19 The assay can detect single cell resolution of nuclear AR-V7 protein in multiple CTC subtypes including EpCAM and CK negative cells, which was not possible with the CellSearch system. In the initial study of 191 evaluable patients, nuclear AR-V7 positive CTCs were found in 18% of mCRPC patients, including 3% first-line, 18% second-line, and 31% of third or greater line therapy.19 Nuclear AR-V7 CTC protein expression was associated with superior survival on taxane therapy over ARSI therapy. In a follow-up multi-institutional validation study of 142 mCRPC patients, nuclear-localized AR-V7 provided individual patient benefit in selecting taxane or ARSI inhibitor therapy.34

In early 2019, AR-V7 was further validated by 2 independent CTC blood based assays in the PROPHECY trial which demonstrated that a positive AR-V7 predicted worse PFS and OS with abiraterone and enzalutamide.35 This prospective, multicenter, blinded trial compared 2 CTC assays: Johns Hopkins RT-PCR (RNA) to Epic (protein) AR-V7 assay in 118 mCRPC patients which showed 82% agreement.35 Overall, patients had a positive AR-V7 result in 10%-24% at baseline in the Epic and Hopkins cohorts, respectively. Both assays respectively had a 0% to 11% PSA or a 0%-6% soft tissue response to
ARSIs. Both assays signal relative resistance to ARSIs, and greater sensitivity to chemotherapy. Interestingly, Halabi risk score, and CellSearch CTC count were not associated with OS after adjustment for AR-V7 status, but risk score was associated with PFS and OS in the Hopkins negative group.

**ctDNA Analysis**

**ctDNA Detection Platforms**

ctDNA analysis translational research is evolving toward “total naked ctDNA” detection as opposed to more complex and very costly analysis of DNA sequences specifically isolated within single, cluster or groups of CTCs. Detection of ctDNA is based on the premise that ctDNA somatic tumor mutations exist which are not found in the sequence of normal DNA within the same individual. Summarizing the numerous complex sequencing technologies is beyond the scope of this review, but they can be broadly classified as nondigital (Sanger sequencing), digital (Pyrosequencing), and next-generation sequencing (NGS). A prerequisite of NGS is that sequences of uncharted DNA which is much more costly. The advantage of NGS is that it is an approximately 10-fold higher in sensitivity compared to pyrosequencing from whole genome sequencing which explores denovo sequences of normal DNA within the same individual. Targeted sequencing has been performed by several different companies and is beyond the scope of this review, but they can be broadly classified as nondigital (Sanger sequencing), digital (Pyrosequencing), and next-generation sequencing (NGS). A prerequisite of NGS is that sequences of uncharted DNA which is much more costly. The advantage of NGS is that it is an approximately 10-fold higher in sensitivity compared to pyrosequencing because it targets specific DNA sequences.

Since ctDNA represents less than 1% of total circulating cfDNA, the Sanger sequencing and pyrosequencing methods are not feasible as they can only detect ctDNA in patients with heavy cancer burdens. Emerging exquisitely sensitive NGS kits capable of simultaneously analyzing mutations, insertions, deletions, amplifications, and fusions in up 73 genes have been developed. The 2 most common commercially available LB ctDNA kits are by Guardant 360 (Guardant Health, Redwood City, CA) and Foundation-ACT (Foundation Medicine, Cambridge, MA). These kits probe ctDNA genes involved in 4 of the mutational groups in metastatic prostate cancer namely: AR related, PI3K pathway, DNA repair pathway, and cyclin-dependent (CDK).

**ctDNA and Prognosis in mCRPC**

The level of ctDNA detection in mCRPC varies from 43% to 82% depending on the mutated tumor genes being studied, disease burden, and sequencing platform utilized (Table 2). In a recent study, increasing fractional loads of ctDNA correlated with poor outcomes. A ctDNA% >30 was strongly related to poor therapy response even after adjustment for clinical prognostic factors. This suggests that ctDNA% reflects aspects of tumor biology or disease volume that are not captured by typical clinical parameters including: PSA, hemoglobin, LDH, metastatic sites, ECOG, and age. Other cancers which show a correlation between ctDNA load and progression include: melanoma, ovarian, breast, and colon cancers.

**ctDNA Genomic Mutations and Recapitulation of mCRPC Mutational Drivers**

Concordance between ctDNA genomic alterations and matched metastatic tissue biopsy analysis was found in a prospective study of 202 treatment-naïve mCRPC patients who were about to start abiraterone and enzalutamide. NGS targeted 72 clinically relevant genes, and 75% of free cfDNA samples had significant evaluable amounts of ctDNA (greater than 2%). Detected alterations included AR amplifications (64.5%), SPOP mutations (8.8%), and inactivation of tumor suppressor genes (TP53, PTEN, RB1, APC, CDKN1B, BRCA2, and PIK3R1). Furthermore, unique alterations were found solely in ctDNA fractions suggesting that a LB may more accurately reflect clonal disease over a traditional single biopsy site tumor sample.

**ctDNA Changes With ARSIs and Chemotherapy**

In a temporal ctDNA study of 65 mCRPC patients receiving enzalutamide, targeted sequencing was possible in 119/122 specimens. Detection of AR mutations and/or copy and RB1 loss were associated with primary resistance. Clinically actionable DNA repair and PIK3 alterations were also found. In the 202 patient ARSI naïve mCRPC trial, mutations in BRCA2 and ATM were strongly associated with poor clinical outcome independent of clinical prognostic factors and ctDNA load. Alterations in TP53 predicted rapid resistance. Unlike other similar studies, however, AR amplification was not a significant predictor. Yet, AR gene structural rearrangements were found in several patients with primary resistance. Beyond targeting androgen signaling in mCRPC, a recent strategy has emerged to focus on acquired DNA repair defects involving BRCA1 and 2, ATM, and PALB2. Presence of germline mutations in these genes is associated with a strong increased risk of prostate cancer. Acquired mutations were found in higher frequencies in mCRPC who failed multiple therapies. These tumors with DNA repair defects are susceptible to poly (adenosine diphosphate ribose) polymerase (Poly adenosine diphosphate ribose) inhibitor-based chemotherapy. In a fifty patient Poly adenosine diphosphate ribose trial, 94% of patients had evaluable cfDNA. A ≥50% reduction in ctDNA was associated with improved OS, as well as PFS at 8 weeks after olaparib therapy. Moreover, response to therapy was associated with reduction in somatic mutations or clearing of the ctDNA mutations. Resistance to therapy was reflected in ctDNA by reverting BRCA2 and PALB2 mutations and this could be used to predict futile therapy.

A new management dimension that ctDNA sequencing may add to clinical care is the identification of the rare patient who may benefit from targeted therapy outside of the usual clinical guideline. For example, ctDNA mutations may help identify the rare less than 1 percent of
<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>Population Characteristic</th>
<th>Percent With ctDNA</th>
<th>Technique Sequencing</th>
<th>Clinical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Romanel (2015)</td>
<td>97</td>
<td>Undergoing ARSI or cytotoxic therapy</td>
<td>82%</td>
<td>Next-Gen Seq</td>
<td>AR copy gain or T878A or L702H AR mutation (45%) had higher risk of abiraterone resistance on multivariate analysis</td>
</tr>
<tr>
<td>Annala (2017)</td>
<td>319</td>
<td>Undergoing ARSI</td>
<td>Not available</td>
<td>Targeted Seq</td>
<td>Combined germline and cfDNA BRCA2 had worse PFS on ARSI and similarly with docetaxel suggesting that this genomic data could be used to select patients for PARP therapy</td>
</tr>
<tr>
<td>Goodall (2017)</td>
<td>49</td>
<td>Undergoing PARP therapy</td>
<td>Not available</td>
<td>Targeted and whole exome Seq (WES)</td>
<td>Decrease cfDNA associated with worse outcome on multivariate analysis. All somatic tumor mutations were present in cfDNA. Reverting germline and somatic BRCA2 and PALB2 mutations observed as mechanism of resistance</td>
</tr>
<tr>
<td>Wyatt (2016)</td>
<td>65</td>
<td>Undergoing enzalutamide</td>
<td>43%</td>
<td>Targeted and deep Seq</td>
<td>Aberrations in cfDNA AR amplification and mutations plus PI3K pathway alterations found as mechanisms of resistance to enzalutamide</td>
</tr>
<tr>
<td>Conteduca (2017)</td>
<td>265</td>
<td>Undergoing ARSI therapy</td>
<td>Not available</td>
<td>ddPCR</td>
<td>Plasma AR status using ddPCR predicted worse outcomes on abiraterone and enzalutamide</td>
</tr>
<tr>
<td>Wyatt (2017)</td>
<td>45</td>
<td>Undergoing ARSI therapy</td>
<td>65.6%</td>
<td>Targeted and whole exome Seq (WES)</td>
<td>Majority of patients cfDNA driver mutations matched metastatic somatic tissue mutations suggesting that cfDNA can guide mCRPC treatment</td>
</tr>
<tr>
<td>Belic (2018)</td>
<td>148</td>
<td>Undergoing ARSI therapy</td>
<td>64%</td>
<td>Targeted and whole exome Seq (WES)</td>
<td>cfDNA levels correlated with PSA, bone metastasis and LDH. AR amplification was inconsistently associated with progression on ARS inhibitors</td>
</tr>
</tbody>
</table>
patients who may respond to immune to checkpoint blockade. In a recent 1346 prostate cancer case series in which patients underwent somatic tumor tissue sequencing of the microsatellite instability (MSI) or dMMR (DNA mismatch repair) phenotype, 11 patients were selected based on a positive result by PCR or immunohistochemistry and treated with anti-PD-1 immunotherapy with pembrolizumab therapy. Six patients (54%) had a greater than 50% PSA response with 4 durable radio-graphic responses.47 LB studies which evaluate concor-dance between somatic tissue MSI-high/mismatch repair deficient sequences vs ctDNA sequences have not yet been reported. A ctDNA or CTC approach to detecting the MSI/dMMR phenotype is relevant because this phenotype may not evolve until later in the mCRPC disease course.47

Comparing CTC and ctDNA Platforms in a Clinical Framework

Investigative steps needed to bring a LB test to the bedside have been outlined. The Institute of Medicine has adopted 3 semantic terms that characterize the necessary steps needed to take a laboratory blood test from bench to bedside: analytic validity, clinical validity, and clinical utility. Analytic validity refers to the ability of an assay to detect and measure with statistical significance the presence of a biomarker of interest accurately, reproducibly, and reliably. Inherent to analytic validity is the requirement of having standardized specimen handling, processing, and storage. Analytic validity includes measures of sensitivity, specificity, and accuracy. Clinical validity refers to the ability of an assay to divide, with statistical significance, 1 population into 2 or more groups on the basis of outcomes such as progression free or OS. Clinical utility refers to the ability of an assay to demonstrate, with statistical significance, improvement in treatment or management compared to not using the assay.

Both the CellSearch CTC, and Epic Science CTC AR-V7 assays have undergone analytic validity, clinical validation, and clinical utility studies in mCRPC. CellSearch uses a CellSavetube, and Epic Science uses a Streck tube. CellSearch is a prognostic CTC marker that prognosticates survival in mCRPC in treatment naïve and in patients with second, and third lines of therapy. Utility of CellSearch depends on the endpoints utilized, and the most robust are the CTC conversions from either ≥1 to 0 or ≥5 to ≤4 CTCs at 13 weeks which occur in approximately 70% of cases.23 As noted CellSearch is FDA approval and is covered by some private insurers.25 Epic Science AR-V7 CTC is a predictive assay that predicts response to ARSIs vs taxanes in mCRPC patients who are already on ARSIs or who have received taxane therapy. Approximately 15% of cases will test positive to for AR-V7.21,32 Genomic Health offers the AR-V7 test under the name Oncotype DX AR-V7 Nucleus Detect Test in the United States. It received coverage by CMS to be used on mCRPC patients already on ARSI therapy including apalutamide.46 Clinically validated ctDNA LB assays are not yet available in prostate cancer. However, the FDA has approved 5 LB ctDNA tests in lung and colon cancer.36 In nonsmall cell lung cancer serial LB can rapidly reveal a resistant T790M epidermal growth factor receptor (EGFR) mutation which can evolve after first, and second generation EGFR targeted therapy. A T790M genotype can predict response to osimertinib even in patients with brain metastasis.36 Hence, a LB biopsy can rapidly and noninvasively detect evolution of resistant disease allowing effective tar-geted therapy selection. Similarly, metastatic colon cancer patients with RAS (KRAS, NRAS, BRAF) mutations may predict targeted therapy response.36 LB ctDNA EGFR testing of nonsmall cell lung cancer is now included in the NCCN guidelines. However, the NCCN guidelines have not yet included CTC, AV-R7, or ctDNA LB testing in prostate cancer.

FUTURE DIRECTIONS

Preliminary studies suggest that ctDNA may be more sen-sitive than CTC analysis in detecting disease (Tables 1 and 2). A caveat is that sensitivities between the ctDNA studies vary widely depending on the platform utilized (Table 2). Interestingly, a direct comparison between Guardant 360 vs PlasmaSELECT (Personal Genome Diagnostics, Inc, Baltimore, MD) NGS assays in the blood of 40 mCRPC patients demonstrated 16 patients (40%) without genomic congruence.48 In another study, a direct comparison the Foundation Medicine vs Guardant NGS assay in the tissue of 8 patients with other cancers from a community practice demonstrated only 22% concordance between the 2 assays.49 This suggests that large, multicenter blinded validation concordance ctDNA studies are needed. ASRs move earlier into the prostate cancer disease state, prompt detection of targetable resistance may allow for effective personalized LB guided treatment earlier in a patients disease course.

CONCLUSIONS

Parallel to therapeutic advances in mCRPC, significant developments are evolving in the LB arena beyond PSA which may assist in management of this heterogeneous disease. Components of commercially available LB assays include CTC count, CTC AV-R7 characterization, and in a translational research setting ctDNA analysis (Table 3). Clinical CTC enumeration validation studies show robust survival prognostication of CTC superior to PSA in mCRPC. PSA, however, remains informative in minimal metastatic castrate resistant disease because it is more sensitive. The AR-V7 LB test may be clinically use-ful in selecting ARSI vs taxane chemotherapy in patients progressing on first line therapy who are being considered for second line ARSI vs taxane chemotherapy. The ability to detect resistance with peripheral blood ctDNA analysis is perhaps the most exciting development in mCRPC research. Moreover, ctDNA analysis and phenotypic
characterization of shed tumor will continue to advance our understanding of the mechanisms of tumorigenesis.

Acknowledgment. Authors wish to thank: Ms. Charlotte Moreno for proof reading and reference management, Dr. Patricia Gomella, Dr. Dolores Shupp-Byrne, and Urology reviewers for thoughtful review of the manuscript, Dr. Mel Pilar Espallat, Dr. Denis Smirnov, Dr. Thomas Shibu for discussions, Ms Barbara Devine for submission assistance, Mr. Jonathan Trilleras for thoughtful review of the manuscript, Dr. Mel Pilar Espallat, Moreno for proof reading and reference management, Dr. Patricia Gomella, Dr. Dolores Shupp-Byrne, and Urology reviewers for thoughtful review of the manuscript, Dr. Mel Pilar Espallat, Dr. Denis Smirnov, Dr. Thomas Shibu for discussions, Ms Barbara Devine for submission assistance, Mr. Jonathan Trilleras for literature searches, and Debbie Moreno for enduring support.

Table 3. Comparison of commercially available LB clinical assays in mCRPC

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>CellSearch* (7.5mL)</th>
<th>Oncotype DX AR-V7† (8mL)</th>
<th>Guardant 360‡ (10mL)</th>
</tr>
</thead>
</table>
| Cell count at baseline, and changes at 13 weeks after therapy | Yes
| slide fixation, immunofluorescent detection | Predicts response to 2nd line ARSI or Taxane therapy | Potentially predict response to PARP inhibitor or immunotherapy; translation research |
| Clinical significance                         | Prognostic of survival CTC ≥5 median OS 11.5 mo or changes during initial 13 wk from baseline are prognostic |
| Tube CellSaveTM (7.5mL)                       | Select public and private M1CRPC at baseline or starting 1st or later line therapy |
| Medicare coverage                             | Streck (8mL)        | Streck (10mL)            | Not yet defined |
| Appropriate patients to test                  | Yes M1CRPC patients on ARSI or Taxane therapy |

* CellSearch Menarini Silicon Biosystems, Huntington, Pennsylvania.
† Oncotype DX AR-V7 Genomic Health, Redwood City, California.
‡ Guardant 360.
§ FoundationOne Foundation Medicine, Cambridge, Massachusetts.
¶ PlasmaSELECT Personal Genome Diagnostics, Baltimore, Maryland.

References
2. Ashworth TR. A case of cancer in which cells similar to those in the tumors were seen in the blood after death. Aust Med J. 1869;14:146–147.


