

# Advancing Precision Medicine for Prostate Cancer Through Genomics

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## ABSTRACT

Prostate cancer is the most common type of cancer in men and the second leading cause of cancer death in men in the United States. The recent surge of high-throughput sequencing of cancer genomes has supported an expanding molecular classification of prostate cancer. Translation of these basic science studies into clinically valuable biomarkers for diagnosis and prognosis and biomarkers that are predictive for therapy is critical to the development of precision medicine in prostate cancer. We review potential applications aimed at improving screening specificity in prostate cancer and differentiating aggressive versus indolent prostate cancers. Furthermore, we review predictive biomarker candidates involving *ETS* gene rearrangements, *PTEN* inactivation, and androgen receptor signaling. These and other putative biomarkers may signify aberrant oncogene pathway activation and provide a rationale for matching patients with molecularly targeted therapies in clinical trials. Lastly, we advocate innovations for clinical trial design to incorporate tumor biopsy and molecular characterization to develop biomarkers and understand mechanisms of resistance.

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## INTRODUCTION

Prostate cancer is the most common nonskin cancer and the second leading cause of cancer death in men in the United States.<sup>1,2</sup> Although there has been significant progress in the treatment of prostate cancer, with the approval of three new therapies for metastatic prostate cancer<sup>3</sup> this year, several challenges persist such as a means to match patients with targeted therapies and the implementation of rational combination therapies. The Institute of Medicine recently critiqued the cooperative clinical trial groups in oncology and recommended innovative trial design through the incorporation of predictive biomarker stratification for patient selection.<sup>4</sup> A molecular classification of cancer has the potential benefits of improving response, minimizing the time and adverse effects of treating patients with ineffective therapies, and reducing the sample size needed to show efficacy. High-throughput sequencing technologies have accelerated the molecular characterization of prostate cancer and positioned opportunities for development of precision medicine for therapeutic decision making in this disease. Here we examine the current data on molecular alterations in prostate cancer, the progress in translating these findings into the clinic, and the challenges that lay ahead for translational genomics in prostate cancer.

Genomic results have the potential to be translated clinically as diagnostic, prognostic, or predictive biomarkers. Diagnostic biomarkers facilitate obtaining an accurate cancer diagnosis as part of screening or confirmatory testing. Prognostic biomarkers provide data on risk of disease progression or morbidity and thereby help determine which patients need additional treatment, such as Gleason score 6 (low risk) versus 8 (high risk) prostate cancer. Predictive biomarkers suggest a course of therapeutic action. Here we provide examples, including early potential of *ETS* gene rearrangements as a diagnostic biomarker, and comment on novel approaches to prognostic biomarker development. Germline line mutations have the potential to be diagnostic, prognostic, or predictive and are discussed in another review in *Journal of Clinical Oncology*. Finally, we focus our attention on an in-depth review of putative predictive biomarkers for molecularly targeted therapies in clinical trials.

## ETS GENE FUSIONS AND URINE TESTING

Gene fusions in prostate cancer were first described in 2005 using a bioinformatics approach that detected outlier transcript expression of genes with microarrays.<sup>5,6</sup> The most common chromosomal rearrangements involve the 5' untranslated region of the androgen-regulated gene *TMPRSS2* and

**Table 1.** Clinically Relevant Genomic Alterations in Prostate Cancer

Gene	Alteration Type	Frequency (%)	Potential Treatment Hypotheses
<i>ETS</i> transcription factors	Rearrangement	50 <sup>7</sup>	Indirect targeting of <i>ETS</i> gene fusions through <i>PARP</i> or <i>DNAPK</i> inhibitors <sup>8</sup>
Androgen receptor	Mutation	50 <sup>9,10</sup>	Androgen synthesis inhibitors, next-generation androgen receptor antagonists
	Amplification	50 <sup>9,10</sup>	
<i>PTEN</i>	Loss	50 <sup>9</sup>	PI3K pathway inhibitors <sup>11</sup>
<i>RB1</i>	Loss	25 to 60 <sup>9</sup>	Role in disease progression and castrate resistance <sup>12</sup> with potential for targeting
<i>PIK3CA</i>	Amplification	10 <sup>10</sup>	PI3K pathway inhibitors <sup>11</sup>
	Mutation	5 <sup>10</sup>	
<i>MYC</i>	Amplification	9 <sup>9</sup>	Potential for targeting <sup>13</sup> Neuroendocrine prostate cancer
		40 <sup>10</sup>	
<i>AURKA</i>	Amplification	5 <sup>10</sup>	Aurora kinase inhibitors <sup>14</sup> ; co-occurs with <i>MYC</i> in 40% of neuroendocrine prostate cancers
<i>AKT</i>	Mutation	1 to 2 <sup>15</sup>	AKT inhibitors
<i>RAF</i>	Rearrangement	1 to 2 <sup>16</sup>	RAF inhibitors
	Mutation		
<i>KRAS</i>	Mutation	1 <sup>9,17,18</sup>	RAF, MEK, or PI3K inhibitors
	Rearrangement		

NOTE. Summarizes alterations in prostate cancer that have a treatment hypothesis currently being explored preclinically or in clinical trials. The most common alterations with a treatment hypothesis involve *ETS* rearrangements, androgen receptor, and *PTEN* loss. With only a limited number of samples assessed, a majority of these alterations are not necessarily mutually exclusive.

Abbreviation: PI3K, phosphatidylinositol 3-kinase.

members of the *ETS* transcription factor family, *ERG* or *ETV1*. The presence of these gene fusions is essentially 100% specific for prostate cancer. Subsequent studies have confirmed *ETS*-related gene fusions in approximately 50% of prostate-specific antigen (PSA) –screened and 15% to 35% of unscreened prostate cancers<sup>6</sup> (Table 1). The most common gene fusion pair occurs with *TMPRSS2* and *ERG* genes, accounting for approximately 90% of *ETS* gene fusions.<sup>7</sup> Many reports have retrospectively examined the correlation of gene fusions to Gleason score, pathologic stage, and disease-specific survival, but thus far, the data have not been consistent.<sup>6</sup> We suspect that some of these inconsistencies may be explained in part by the varied composition of the cohorts evaluated and differences in method of fusion detection. Nevertheless, the high specificity of fusions in prostate cancer has potential value in diagnostic testing by limiting false positives; this is reviewed elsewhere.<sup>19</sup>

PSA is widely offered for prostate cancer screening; however, it has limitations including false positives and the potential to result in overdiagnosis of indolent prostate cancers. To improve on PSA screening, Tomlins et al<sup>20</sup> developed a multiplex assay combining PSA with urine testing for *TMPRSS2-ERG* fusion transcripts and *PCA3* transcripts (noncoding RNA) to improve decision tools that predict the likelihood of cancer at time of biopsy. This diagnostic tool has the potential to help patients who may have benign prostate problems, such as benign prostate hypertrophy, avoid biopsies. Further prospective study is needed to demonstrate that this diagnostic tool selects for clinically significant or high-grade prostate cancer.

Prognostic molecular biomarkers are needed in prostate cancer to help differentiate aggressive from indolent disease. Several candidates have emerged, but clinical implementation has been limited by assay variation, small cohort size, and flaws in biomarker validation study design.<sup>19,21</sup> In addition, prospective studies in prostate cancer are particularly challenging to complete because of the extended follow-up required to determine risk of relapse. Meanwhile, novel high-throughput approaches such as proteomics and genomics have

the potential to systematically nominate candidates. One example is the emerging role of noncoding RNAs in cancer biology that may contain putative candidates for prognostic or predictive biomarkers.<sup>22</sup>

#### PUTATIVE PREDICTIVE BIOMARKERS IN PROSTATE CANCER

Over the past 5 years, rapidly evolving technology in nucleic acid sequencing has enabled large-scale sequencing projects of cancer genomes and transcriptomes<sup>22,23</sup> with exhaustive identification of copy number alterations, somatic point mutations, structural rearrangements, and gene expression alterations.<sup>24,25</sup> Before next-generation sequencing studies, Taylor et al<sup>17</sup> published an integrative approach of the genomic and transcriptomic landscapes for prostate cancer. This work included arrays for copy number/gene expression and complete exon resequencing for mutations on a sample set of 218 prostate cancers comprising 181 primaries and 37 metastases. Since then, subsequent studies have employed next-generation sequencing strategies to further evaluate prostate cancer genomes. Kumar et al<sup>26</sup> used exome capture and sequencing to characterize xenografts of prostate cancer metastases. Berger et al<sup>27</sup> carried out comprehensive whole-genome sequencing on seven primary prostate cancers. This year, two additional studies performed exome capture and sequencing on 112 primary prostate cancers and 50 castrate-resistant prostate cancer (CRPC) metastases.<sup>9,28</sup>

Together, these and other studies have provided a snapshot of both primary and metastatic prostate cancers. To date, the most common molecular events in prostate cancer (Table 1) involve androgen receptor (AR) mutation or amplification,<sup>29-31</sup> gene rearrangements of *ETS* transcription factors,<sup>5</sup> and loss of phosphatase and tensin homolog (*PTEN*; phosphatidylinositol 3-kinase [PI3K] activation).<sup>32-34</sup> Furthermore, there are aberrant mutually exclusive genes that occur at lower frequency (< 5%), including *AKT*,<sup>15</sup> *PIK3CA*,<sup>17</sup> and *RAS*

**Table 2.** Clinical Trials for Targeted Therapies in Prostate Cancer

Target	Ongoing Trials	Therapies	ClinicalTrials.gov Identifier	Genome-Based Enrichment or Randomization
<i>ETS</i> fusions	Study combining ABT-888, oral PARP inhibitor, with temozolomide in patients with metastatic prostate cancer	ABT-888, PARP inhibitor	NCT01085422	<i>ETS</i> randomization
	Study to assess safety and tolerability of oral CC-115 for patients with advanced solid tumors, non-Hodgkin lymphoma, or multiple myeloma	CC-115, DNAPK, and mTOR inhibitor	NCT01353625	None
	Abiraterone acetate with or without veliparib in treating patients with metastatic prostate cancer	Veliparib, PARP inhibitor	NCT01576172	None
PI3K/mTOR/AKT pathway	Phase II study of BKM120 in men with metastatic CRPC	BKM120, PI3K inhibitor	NCT01385293	None
	Phase I/IIa, first time in human study of GSK2636771 in patients with advanced solid tumors with PTEN deficiency	GSK2636771, PI3K inhibitor	NCT01458067	PTEN deficiency by immunohistochemistry
	Phase II study of the efficacy and safety of AP23573 in patients with taxane-resistant AIPC	Ridaforolimus, mTOR inhibitor	NCT00110188	None
	Temsirolimus to reverse androgen insensitivity for CRPC	Temsirolimus, mTOR inhibitor	NCT01020305	None
Androgen signaling	Determine effect of MDV3100 on androgen signaling pathway in correlation with anti-tumor effects of MDV3100 to identify potential predictors of response or resistance to therapy	MDV3100, second-generation AR antagonist	NCT01091103	None
	Safety and efficacy study of MDV3100 in patients with CRPC who have been previously treated with docetaxel-based chemotherapy (AFFIRM) <sup>46</sup>	MDV3100, second-generation AR antagonist	NCT00974311	None
	Safety, pharmacokinetic and proof-of-concept study of ARN-509 in CRPC	ARN 509, second-generation AR antagonist	NCT01171898	None
Combinations	Bicalutamide with or without MK2206 in treating patients with previously treated prostate cancer	Bicalutamide plus MK2206, AKT inhibitor	NCT01251861	None
	Temsirolimus to reverse androgen insensitivity for CRPC	Temsirolimus (mTOR)	NCT01020305	None
	Study of GDC-0068 or GDC-0980 with abiraterone acetate versus abiraterone acetate in patients with CRPC previously treated with docetaxel chemotherapy	Abiraterone plus GDC-0068 (AKT inhibitor) or GDC-0980 (PI3K/mTOR inhibitor)	NCT01485861	None
Private driver mutations	Abiraterone acetate with or without veliparib in treating patients with metastatic prostate cancer	Vemurafenib, RAF inhibitor	NCT01085422	<i>BRAF</i> subset receives vemurafenib

NOTE. There are multiple ongoing trials involving novel agents targeting *ETS*, PI3K pathway, and AR signaling in prostate cancer. Few studies are beginning to target multiple pathways through combination therapies. Although this table is not comprehensive, it is meant to provide an overview of directions for targeted therapies in prostate cancer.

Abbreviations: AFFIRM, A Study Evaluating the Efficacy and Safety of the Investigational Drug MDV3100; AIPC, androgen-independent prostate cancer; AR, androgen receptor; CRPC, castration-resistant prostate cancer; mTOR, mammalian target of rapamycin; PARP, poly (ADP-ribose) polymerase; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog.

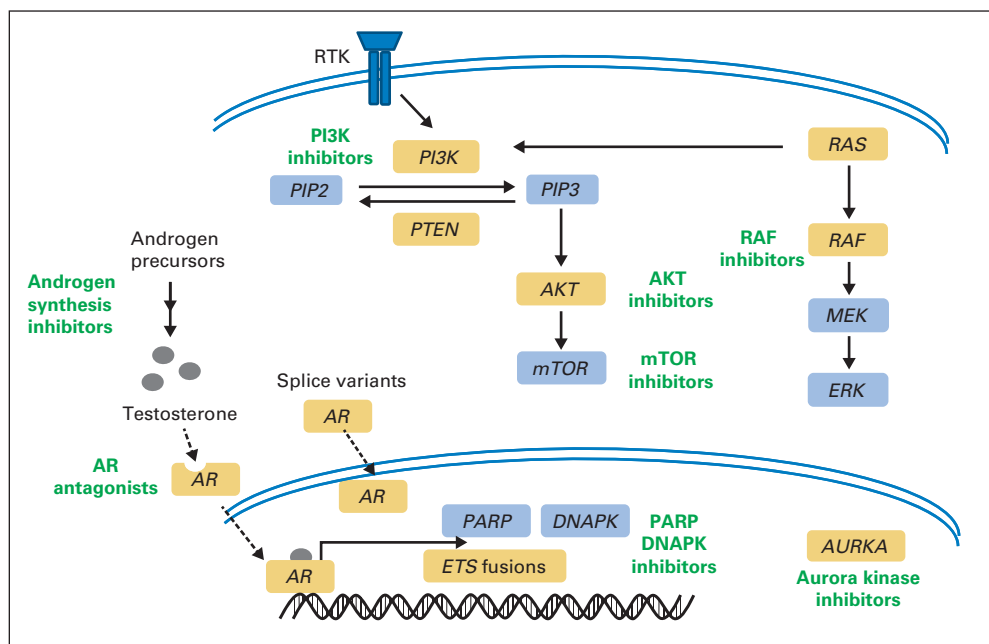
mutations<sup>17</sup> and *BRAF* mutations or fusions.<sup>16,35</sup> The following is a discussion of these potentially actionable genomic alterations.

### Common Genomic Events

***ETS* gene fusions.** Because of a prevalence of 50% of prostate cancers, a targeted therapy for *ETS* gene fusion products could affect thousands of patients with the disease. Currently, there are no therapies that directly target this gene fusion in clinical development. Like other gene fusions involving transcription factors, drug development has been hindered. Nonetheless, there are promising approaches emerging such as synthetic lethality screens and blocking specific protein-protein interactions of *ETS* transcription factors.<sup>36</sup> Meanwhile, there are preclinical data that provide a rationale for targeting components of the transcription factor complex associated with *ETS* transcription factors through poly (ADP-ribose) polymerase (PARP)

or *DNAPK* inhibition.<sup>8,37</sup> Additionally, recent work by Knudsen et al<sup>38</sup> has implicated PARP activity downstream of AR signaling, providing further rationale for dual AR and PARP inhibition. There are several clinical trials for metastatic CRPC evaluating PARP and *DNAPK* inhibitors and combined PARP and AR blockade, where knowledge of *ETS* fusion status will be a critical correlative element to the study (Table 2).

**AR.** The discovery that hormones regulate prostate cancer growth in the 1940s led to the first effective systemic therapies for prostate cancer and resulted in a Nobel Prize for Charles Huggins.<sup>39</sup> In the 1990s, focused efforts on AR revealed escape mechanisms from androgen blockade through either mutation or amplification of *AR*.<sup>29,30</sup> Additionally, these resistance mechanisms corroborate the mounting realization that androgen signaling remains important despite castrate levels of testosterone. Focus on persistent androgen



**Fig 1.** Pathway-guided treatment in prostate cancer. This diagram highlights pathways for targeting in prostate cancer, including the phosphatidylinositol 3-kinase (PI3K) pathway, *ETS* rearrangements, and androgen signaling. Green indicates putative treatment hypotheses for relevant pathways. Each of these pathways can be driven by genomic aberrations, such as point mutations, copy number alterations, and rearrangements. The diagram also highlights less common driving mutations involving *RAS*, *RAF*, and *AKT* oncogenes. Furthermore, there is potential for cooperation between pathways because these are not necessarily mutually exclusive. Genes highlighted in gold have known genomic alterations. AR, androgen receptor; mTOR, mammalian target of rapamycin; PARP, poly (ADP-ribose) polymerase; RTK, receptor tyrosine kinase.

signaling, including intratumoral testosterone production, has garnered support for the next generation of androgen synthesis inhibitors and AR antagonists.<sup>40-42</sup> Abiraterone is a selective inhibitor of androgen synthesis through blockade of CYP17 used in the adrenal glands and testes, and within prostate tumors. In a randomized controlled trial against placebo, abiraterone resulted in improved overall and progression-free survival.<sup>43</sup> Similarly, the next generation of anti-AR agents, including MDV3100<sup>44-46</sup> and ARN 509,<sup>47,48</sup> has shown promising activity in men with metastatic CRPC.

Current data on prostate cancer genomics suggest more than 50% of metastatic CRPCs display *AR* amplification or mutation<sup>9</sup> (Table 1). As the next generation of targeted therapies for androgen signaling are developed, genomic sequencing strategies may be useful in determining potential resistance mechanisms. Still unknown is the efficacy of combination therapy targeting both androgen synthesis and receptor.

In addition to mutations and amplifications involving *AR*, Dehm et al<sup>49</sup> have described alternatively multiple spliced isoforms of *AR* that result in constitutively active androgen signaling. These variants generally function through loss of the C-terminal ligand-binding domain, preserving the N-terminal domain that contains the DNA binding and transactivation domains. Although multiple isoforms already have been revealed, unbiased next-generation sequencing approaches have identified additional isoforms and may yet be valuable for understanding other potential mechanisms of resistance.<sup>50,51</sup> The frequency of *AR* splicing variants for patients with metastatic CRPC is not known. Thus, the relative significance of splicing and whether it occurs concurrently with other mechanisms of resistance, such as mutation or amplification, has not been clarified. This is complicated further by evidence suggesting genomic events such as cryptic splice sites or deletions may influence splicing patterns. Although there are currently no targeted therapies that prevent *AR* splicing, there are efforts to inhibit *AR* signaling by blocking the effects of the N-terminal domain that contains the DNA binding domain.<sup>52</sup> The interplay of *AR* splice variants and next-generation antiandrogen therapies can be

studied through comprehensive genomic and transcriptome data for patients with metastatic CRPC participating in clinical trials.

***PTEN* and *PI3K* pathway.** The *PTEN* gene is a tumor suppressor that encodes a protein phosphatase recurrently mutated in cancer. *PTEN* activity removes a phosphate from phosphoinositides at the plasma membrane (Fig 1) and negatively regulates the PI3K–AKT–mammalian target of rapamycin pathway. Thus, either loss or inactivation of *PTEN* genes leads to PI3K pathway activation. Somatic loss of *PTEN* occurs in multiple cancer types and provides a mechanistic rationale for PI3K pathway inhibitors.<sup>53</sup> Through in vivo models, heterozygous loss of *PTEN* cooperates with other oncogenes to promote growth of prostate tumors.<sup>54</sup>

Genomic evidence of *PTEN* loss in prostate cancer through point mutation, deletion, or rearrangement has been observed in at least 50% of metastatic CRPCs<sup>9,27</sup> (Table 1). However, this does not take into account other mechanisms of functional *PTEN* inactivation such as epigenetic or post-translational regulation. For example, Berger et al<sup>27</sup> identified novel gene rearrangements involving the *PTEN*-interacting gene *MAGI2*, predicting that loss or mutation of *MAGI2* would mimic functional *PTEN* inactivation. Certainly, there are other means to activate components of the PI3K–AKT pathway. Interestingly, recent studies have demonstrated a relationship for *ERG* fusions and androgen signaling with *PTEN* loss. A transgenic mouse model of *ERG* fusions was crossed into a heterozygous *PTEN* background and resulted in accelerated prostate tumor progression.<sup>34</sup> This concurs with genomic data showing that *PTEN* loss and *ETS* fusions are not mutually exclusive events. With respect to androgen signaling, Carver et al<sup>34</sup> demonstrated inhibition of either androgen or PI3K signaling resulted in reciprocal activation of the other pathway. This work has provided a rationale for dual inhibition of *AR* and PI3K signaling in clinical trials.

***Retinoblastoma.*** Retinoblastoma (*RBI*) is a tumor suppressor gene with somatic alterations in multiple cancers. *RBI* has multiple cellular functions; with respect to cell cycle, it represses transcription of genes involved in DNA synthesis and mitosis. There are multiple

means to somatically inactivate the RB1 pathway, including *RB1* mutation or deletion, *cyclinD1* expression, and *p16INK4* mutation or loss. In prostate cancer, RB1 inactivation occurs through genomic deletion of *RB1* in approximately up to 20% to 60% of cancers.<sup>9,28,55</sup> Sharma et al<sup>12</sup> demonstrated that RB1 inactivation resulted in enhanced AR expression dependent on the E2F1 transcription factor. Currently, researchers are actively exploring indirect targeting of RB1 inactivation through MDM2 and HDAC inhibitors.<sup>56</sup>

***c-MYC.*** The *MYC* oncogene is dysregulated in multiple cancers, including prostate cancer.<sup>57</sup> When a *MYC* transgene was overexpressed in mice, all developed intraepithelial neoplasia and eventually invasive prostate cancer.<sup>58</sup> Because of its expression in CRPC, researchers explored *MYC* as a potential mechanism of androgen independence, and in vitro *MYC* expression conferred AR-independent growth.<sup>59</sup> Similarly, because of its co-occurrence with PI3K activation, Clegg et al<sup>60</sup> generated mice with constitutive AKT1 and *MYC* activity and found accelerated development of invasive prostate cancer. Furthermore, although prostatic growth in *AKT1* mice is sensitive to mammalian target of rapamycin inhibition, dual *AKT1/MYC* mice were less sensitive. Recent sequencing studies demonstrated that *MYC* is amplified in 10% of metastatic CRPCs.<sup>9</sup> However, in a subset of prostate cancers with neuroendocrine differentiation, Beltran et al<sup>10</sup> found that *MYC* amplification is more common (40%) and co-occurs with amplification of aurora kinase A (*AURKA*). *AURKA* is a kinase that regulates cell-cycle progression and is a putative target for cancer with several AURK inhibitors in clinical trials.<sup>14</sup> Beltran et al demonstrated that *MYC* and *AURKA*-coamplified prostate cancer was sensitive to AURKA inhibitors in vitro and in vivo, thereby providing a rationale for trials in this molecular and histologic subset. Thus, targeting *MYC* is an active area of research and especially relevant to prostate cancer because of its cooperation with androgen independence, PI3K pathway, and aurora kinase activation.

### Uncommon and Private Genomic Events

**PIK3CA.** Activating mutations in *PIK3CA* were described after exon resequencing an initial panel of colorectal cancer tissues and later were found across multiple cancer histologic subtypes, including breast and ovarian cancers.<sup>61</sup> Functional characterization of *PIK3CA* hotspot mutations revealed downstream activation of PI3K pathway components, including AKT phosphorylation, and phenotypes, including cell proliferation.<sup>62</sup> Its role as a driving oncogene and prevalence across multiple cancer subtypes<sup>53</sup> have led to the development of PI3K inhibitors. Although some trials are focusing on disease groups, select trials are including molecular enrichment based on activating *PIK3CA* mutations for patients with any cancer histology.

In prostate cancer, *PIK3CA* mutations occur at a frequency of up to 5% and copy number gains of up to 10%<sup>10,63</sup> (Fig 1). In contrast, as mentioned earlier, the loss of *PTEN* is more common in prostate cancer, reaching 50%. As PI3K inhibitors are developed in prostate cancer, an important question will be whether there is a clinical or functional difference between subsets defined by *PIK3CA* mutations versus *PTEN* loss. Furthermore, one can speculate that inhibition of PI3K in *PIK3CA*-mutant prostate cancer may drive reciprocal androgen signaling, and such molecular subsets could potentially benefit from dual blockade.

**AKT.** AKT is a serine/threonine protein kinase and is a component of the PI3K pathway (Fig 1). Carpten et al<sup>64</sup> discovered recurrent activating mutations in *AKT1* after resequencing the coding regions

for *AKT1*, *AKT2*, and *AKT3* genes in breast, colorectal, and ovarian cancer tissues. Rather than mutations in the catalytic kinase domain, however, they identified a mutation, corresponding to an E17K amino acid substitution, in the pleckstrin homology domain affecting protein localization. The mutation resulted in constitutive *AKT1* activation and in vitro and in vivo transforming properties. Boormans et al<sup>15</sup> sequenced *AKT1* in 188 prostate cancer specimens and identified the activating E17K mutation in 1.4% of cases. Because this is a rare occurrence in prostate cancer, recent genome and exome sequencing studies have not reported this mutation.

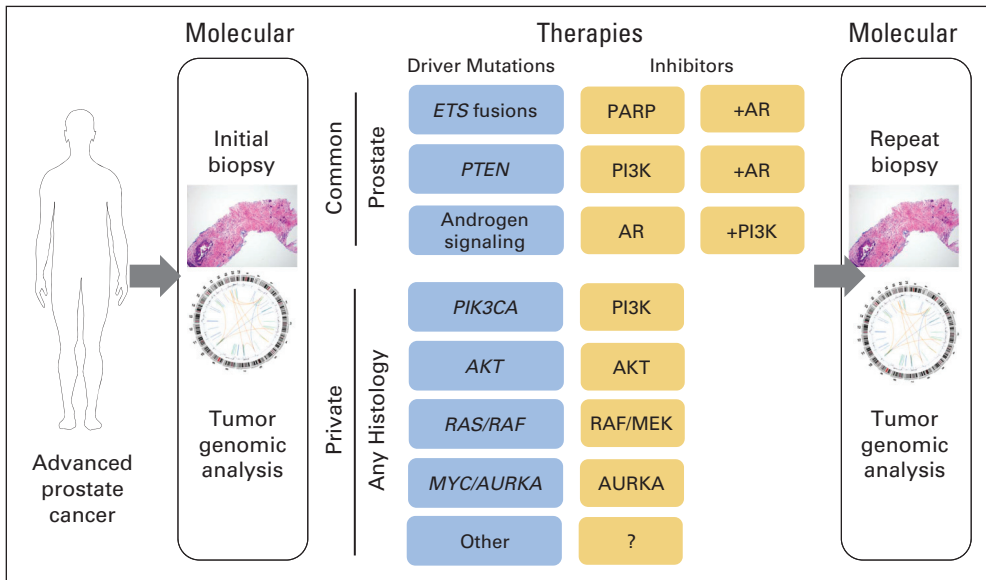
**RAS-RAF pathway.** *BRAF* encodes a serine/threonine protein kinase involved in the RAS signaling cascade. Davies et al<sup>65</sup> hypothesized that additional oncogenic kinase mutations might exist in downstream components of the RAS pathway. They sequenced exons of multiple RAS pathway genes in a panel of cancer cell lines and identified a recurrent mutation in the kinase domain of *BRAF*. An activating mutation, V600E, was recurrent in melanoma and other cancer subtypes and resulted in constitutive activation of the RAF kinase (Fig 1). In addition to the successful application of RAF and MEK inhibitors in *BRAF*-mutant melanoma,<sup>2,66</sup> multiple inhibitors are in clinical development pipelines for other *BRAF*-mutant cancers.

With regard to prostate cancer, genomic studies of prostate cancer in North America have demonstrated canonical *BRAF* and *KRAS* mutations of approximately 1% to 2%.<sup>9,28</sup> In addition to point mutations, activating gene fusions involving *BRAF* have been described in 1% to 2% of prostate cancers, as detected by transcriptome sequencing.<sup>16</sup> These gene rearrangements result in the expression and constitutive activity of a RAF kinase that in vitro has transforming activity and is sensitive to RAF inhibitors. Similarly, gene fusions involving *KRAS* fusions were also identified in 1% of metastatic CRPCs and were sensitive to in vitro knockdown experiments.<sup>18</sup> Thus, this represents a potential treatment hypothesis for patients with such gene fusions. Furthermore, it underscores the increasing complexity of potential driving mutations that may be relatively rare and the potential variety of mutation classes that may be possible (point mutations *v* rearrangements).

### NOVEL GENOMIC ABERRATIONS IN PROSTATE CANCER

Recent sequencing studies in prostate cancer have uncovered a number of novel genomic alterations that have potential for clinical translation. In two studies of primary and metastatic prostate cancers, high-throughput sequencing revealed recurrent copy number alterations and point mutations.<sup>9,28</sup> Here we highlight alterations in several genes: *SPOP* (13%), *FOXA1* (3% to 4%), *AURKA* (40% of neuroendocrine prostate cancers), *MED12* (4% to 5%), *MAGI-2* (discussed previously), and *CHD1*; each is of particular biologic and potential clinical interest.

*SPOP* encodes a subunit of the ubiquitin ligase complex and was highlighted by Barbieri et al<sup>28</sup> in their study of primary prostate cancers through exome sequencing. They observed a mutation frequency of 15%; other prostate cohorts ranged from 6% to 15%. Interestingly, *SPOP* mutations predominantly affected the substrate-binding region of the protein. Furthermore, they observed that *SPOP* mutations were mutually exclusive from *ETS* rearrangements, implying that these are separate events in prostate tumorigenesis. Although in vitro transfection of *SPOP* mutants did not affect cell growth or viability, cells



**Fig 2.** Translating genomics for prostate cancer trials. This diagram outlines a path to genomics-driven trials in prostate cancer. Patients with advanced prostate cancer would undergo a fresh tumor biopsy for assessment of their current disease and molecular stratification to trials, including random assignment within those groups. Because of the prevalence of phosphatidylinositide 3-kinase (PI3K) pathway activation, *ETS* rearrangements, and androgen signaling, genomic enrichment of patients for these common disease subsets may follow traditional trial structures including combination treatments. However, for rare or private molecular disease subsets, these patients may be better suited to studies based on molecular aberrations and that include multiple histologies. For all studies, repeat biopsy for genomic assessment will be valuable for evaluating mechanisms of resistance, including tumor subclone selection, including tumor subclone selection. AR, androgen receptor; PARP, poly (ADP-ribose) polymerase.

displayed increased invasion. Taken together, *SPOP*-mutant prostate cancer may represent a novel molecular subset and has potential for therapeutic applications after further experimental biology.

*FOXA1* encodes forkhead box A1, a transcription factor that has been shown to be a coregulator of AR.<sup>67</sup> Identified point mutations involve the forkhead domain in DNA binding. *FOXA1* mutants promoted tumor growth in xenografts while negatively affecting androgen signaling in vitro.

*AURKA*, as discussed earlier, encodes a kinase involved in cell-cycle regulation and is readily being targeted in clinical trials for cancer. Beltran et al<sup>10</sup> identified co-occurring amplifications of *MYC* and *AURKA* in prostate cancer with neuroendocrine differentiation and demonstrated sensitivity to aurora kinase inhibition. These data provide a therapeutic rationale for exploring aurora kinase inhibitor in *AURKA*-amplified prostate cancer (Fig 2) and reflect an excellent example of the rapid translation of basic science discoveries through sequencing approaches into clinical trials.

*MED12* is a subunit of a mediator complex and cyclin-dependent kinase 8, which is recurrently mutated in uterine leiomyomas. The mutation observed in prostate cancer was recurrent at one position in five patient cases and could conceivably affect cyclin-dependent kinase function.

*CHD1* is a helicase DNA-binding protein involved in chromatin remodeling found to be homozygously deleted in 8% to 10% of prostate cancer array and next-generation sequencing studies.<sup>9,28,68,69</sup>

Knockdown experiments of *CHD1* in nontumorigenic prostate epithelial cells resulted in increased invasiveness but were not transforming.<sup>68</sup> Interestingly, *CHD1* loss was associated with additional homozygous gene deletions. Furthermore, *CHD1* loss seemed to be mutually exclusive from *ETS* rearrangements in the cohorts tested.

It is clear that additional experimental biology will be needed to delineate the roles of these novel findings. However, it is clear they represent an expanding molecular classification of prostate cancer through genomic technologies and promising opportunities for clinical translation.

### CLINICAL DEVELOPMENT OF TARGETED THERAPIES

Clinical translation of prostate genomic data for patients is already under way, with clinical trials addressing androgen signaling, PI3K pathway activation, and *ETS* family rearrangements (Table 2). Trials for PARP inhibitors in prostate cancer are targeting sensitivity through *ETS* rearrangements and expression, and some trials are being enriched for patients with *ETS* rearrangements. With emerging data on effective second-generation AR antagonists, the question becomes whether androgen signaling has been fully shut down at time of disease progression, and what the mechanism of resistance is. In addition, promising preclinical work is ongoing for agents that target *ETS* fusions and the N terminus of AR. Because of the frequency of *ETS* family and AR aberrations in prostate cancer, these potential treatments could be widely applicable to a majority of patients with advanced prostate cancer.

Moving forward, the challenges for translating current achievements in prostate cancer genomics will be determining optimal combination treatments and addressing patients who have private or rare driving mutations. For example, the preclinical rationale for combined PI3K pathway and androgen signaling inhibition has spurred trials to address these hypotheses (Table 2). However, the majority of trials do not routinely incorporate molecular eligibility or stratification, such as identification of patients with *PTEN* or PI3K pathway aberrations. Integrating molecular analysis of tumor biopsies before and after therapy (Fig 2) will help develop and prove predictive biomarkers for those patients most likely to derive benefit. This can be done for all patients through retrospective analysis or prospectively for biomarker-enriched patients.<sup>70</sup> Moreover, biopsy-based molecular analysis will provide the tissue data needed to understand emerging resistance to these investigational therapies and provide a rationale for the next therapy or combination approach.

For patients with private or relatively rare aberrations (1% to 2%), completing clinical trials for these pathways will be severely limited in terms of geographic availability to patients and cost to cancer centers to

maintain trials despite few patients enrolled. Additionally, trials will be problematic to complete within a timeframe acceptable to pharmaceutical companies if performed within a single-disease cohort. As a solution to challenges for these molecular subsets, we advocate approaching patients with pathway-based eligibility and open enrollment for any histology of metastatic cancer (Fig 2). Although such studies would have heterogeneous patient populations that could confound the trials, we consider these to be exploratory or compassionate studies.

To facilitate the molecular stratification of patients with these rare or private driving mutations, we and others have developed clinical tumor sequencing studies that include real-time next-generation sequencing from fresh biopsies of metastatic disease.<sup>71-73</sup> Tumor characterization includes a combination of whole-genome or -exome and transcriptome sequencing, review of results through a multidisciplinary sequencing tumor board, and return of clinically actionable results. The Stand Up to Cancer program and the Prostate Cancer Foundation recently funded two multicenter studies to implement a clinical tumor sequencing program for patients with metastatic CRPC and melanoma. These studies will facilitate the concept of prediction medicine through molecular stratification in trials, identify novel molecular subsets, and facilitate understanding of resistance mechanisms to targeted therapies.

## FUTURE DIRECTIONS AND CHALLENGES

Although application of prostate cancer genomic data is being translated into clinical trials, approaches employing high-throughput sequencing technologies have important limitations, including tissue biopsies, tumor microenvironment, epigenetics, and heterogeneity. Compared with other epithelial cancers, prostate cancer presents unique challenges for tumor biopsy assessments because a significant fraction of patients with metastatic prostate cancer have bone-only metastasis. This places limitations on tissue acquisition and handling for sequencing approaches. Up to half of bone biopsies yield poor or inadequate tumor tissue for sequencing evaluation. Although these sequencing strategies focus on cancer cell populations, they may miss significant interactions with the host, including tumor microenvironment and immune system.<sup>74</sup> Similarly, prostate cancer, like other cancers, displays heterogeneity as has been seen in multifocal disease in primary prostate tissues. A key question will be whether multiple metastatic disease sites display such heterogeneity. Although they are likely to have dominant clones with driving mutations, it is unclear whether treatment decisions and responses will be affected by heteroge-

neity through emerging resistance. Thus, we anticipate that future advances in sequencing, such as single-cell sequencing, may facilitate microenvironment characterization. Whereas clinical implementation of high-throughput sequencing has focused on DNA and RNA level aberrations, little has been implemented with respect to epigenetic profiling strategies. However, the number of predictive epigenetic aberrations and matching therapies is limited. As the compendium of epigenetic predictive biomarkers and matching therapies grows and the cost of sequencing technologies continues to decrease, we anticipate incorporation of epigenetic profiling into both clinical application and basic research.

Despite these limitations, we look forward to the next decade as genomic technologies are clinically applied for advanced prostate cancer. The extensive molecular characterization of prostate cancer has positioned this disease for the development of clinical trials for personalized oncology. Translating genomics into trials will innovative trial design and facilitate early application of cutting-edge basic science for patients with prostate cancer.

## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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