

Molecular Pathways: Targeting Resistance in the Androgen Receptor for Therapeutic Benefit

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Abstract

Androgen receptor signaling is critical in the development and progression of prostate cancer, leading to intensive efforts to elucidate all potential points of inflection for therapeutic intervention. These efforts have revealed new mechanisms of resistance and raise the possibility that known mechanisms may become even more relevant in the context of effective androgen receptor suppression. These mechanisms include tumoral appropriation of alternative androgen sources, alterations in androgen receptor expression, androgen receptor mutations, truncated androgen receptor variants, alterations and cross-talk in recruitment of cofactors to androgen receptor binding sites in the genome, and androgen receptor-driven oncogenic gene fusions. New agents such as enzalutamide, EPI-001, androgen receptor-specific peptidomimetics, novel HSP90 inhibitors, and PARP inhibitors, as well as new approaches to cotargeting the androgen receptor pathway, point to the potential for more complete and durable control of androgen receptor-mediated growth. *Clin Cancer Res*; 20(4); 791–8. ©2013 AACR.

Background

Androgen receptor structure and function in prostate cancer

Prostate cancer is the most common solid tumor and the second most common cause of cancer death in men in the United States, with more than 29,000 men anticipated to have died of metastatic disease in 2013 (1). The androgen receptor is the critical driver of neoplastic prostate progression. Prostate cancer that has spread beyond the reach of definitive local therapy, is treated with androgen deprivation therapy (ADT) to suppress androgen receptor activation.

The human androgen receptor, located on chromosome Xq11–12, is a nuclear receptor transcription factor structurally similar to other steroid hormone receptors. The androgen receptor is divided into distinct functional regions, including the amino-terminal domain (NTD), DNA-binding domain (DBD), hinge region (HR), and the carboxy-terminal ligand-binding domain (LBD; Fig. 1).

The androgen receptor is activated by multiple steroid hormones, primarily testosterone and dihydrotestosterone but also (at lower affinity) by adrenal androgens. Ligand binding releases receptor chaperones such as HSP90 and leads to nuclear translocation and receptor binding to androgen response elements (ARE). DNA binding induces formation

of a signaling complex composed of coactivators and suppressors, which then regulate cell-type-specific signaling.

Androgen receptor signaling normally promotes epithelial differentiation, but in prostate cancer the androgen receptor modulates a broad array of genes regulating cell-cycle, survival and proliferation driving tumor progression (2–5). Advanced prostate cancer is treated with ADT, either as castration monotherapy or as combined therapy with androgen receptor antagonists. ADT induces nearly universal clinical responses; however, currently available agents do not achieve definitive tumor ablation and the majority of cancers become resistant to ADT. This phase of disease represents the lethal phenotype and carries significant morbidity and mortality within months to years. Despite anorchid testosterone blood levels, recapitulation of the intratumoral androgen receptor signaling pathway continues to drive progression and although previously considered "hormone refractory," this phase is more appropriately considered "castration-resistant" prostate cancer (CRPC).

Clinical-Translational Advances

Mechanisms of resistance to androgen receptor pathway inhibition

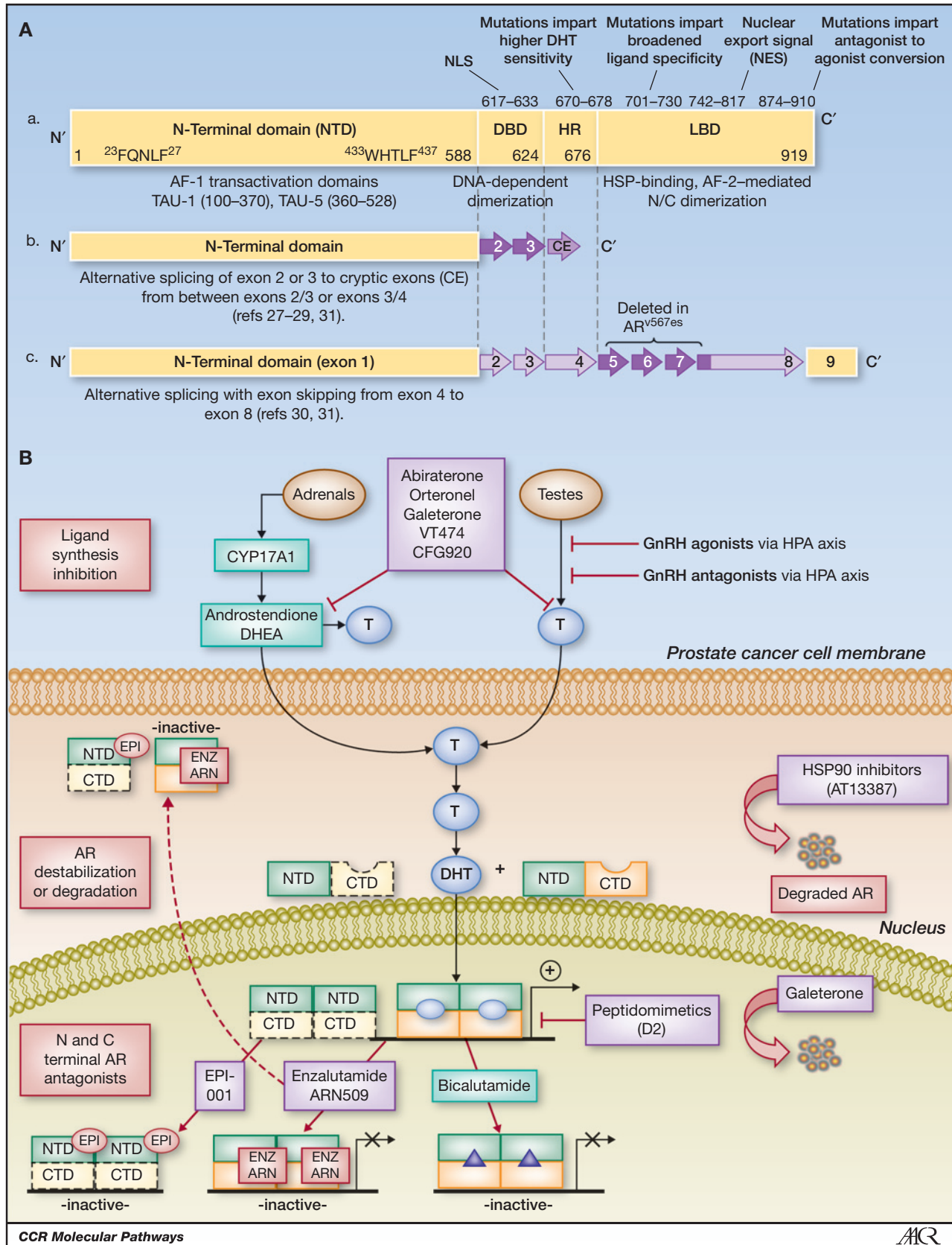
Adaptive responses to ADT include tumoral appropriation of alternative androgen sources, alterations in androgen receptor expression, structural alterations in the androgen receptor, including mutation and truncated androgen receptor variants, alterations in cofactor recruitment, and androgen receptor activation via cross-talk with signal transduction pathways (6). These ligand and androgen receptor-related alterations have been validated as important targets in CRPC based on the clinical efficacy of new agents designed to target them.

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Tumor androgen levels in metastases from castrated patients exceed tissue androgen levels in primary prostate tumors from untreated patients (7). Potential nongonadal sources of intratumor androgens include circulating adrenal androgens, as well as *de novo* or intracrine synthesis of androgens within prostate cancer cells (7–9). Abiraterone is a selective irreversible inhibitor of the steroidogenic enzyme CYP17 and suppresses serum and tissue androgen levels more effectively than standard ADT (10–12). Abiraterone in chemotherapy-naïve and following docetaxel treatment in patients with CRPC provided survival and quality-of-life benefits, leading to U.S. Food and Drug Administration (FDA) approval in both settings (13, 14) and supporting the importance of inhibiting nongonadal androgen sources in CRPC.

CRPC tumors also respond to ADT by upregulating androgen receptor expression. Although 20% to 30% of CRPC tumors demonstrate amplification of the androgen receptor locus, other means include increased transcription rates or stabilization of mRNA or protein (15). Increased androgen receptor expression contributes to prostate cancer growth by compensating for castrate androgen levels, and in prostate cancer models was both necessary and sufficient to induce tumor growth (16). Novel androgen receptor antagonists have, accordingly, demonstrated encouraging clinical efficacy in CRPC. Enzalutamide (formerly MDV3100) is a competitive androgen receptor antagonist that binds androgen receptor with 5- to 8-fold greater affinity than other antiandrogens, decreases androgen receptor nuclear translocation, and reduces chromatin occupancy at canonical AREs (17). Unlike bicalutamide, enzalutamide did not demonstrate agonist potential *in vitro*, and retained activity against androgen receptor mutations associated with bicalutamide resistance (W741C). In a phase III randomized study (AFFIRM), enzalutamide improved overall survival by 37% compared with placebo in men with CRPC previously treated with docetaxel (18), confirming that therapy directly targeting the androgen receptor provided clinical benefit and confirming the relevance of this mechanism of resistance in CRPC (19). A phase III study of enzalutamide (PREVAIL, NCT01212991) in docetaxel-naïve men has completed accrual with interim results pending.

Insights into mechanisms of resistance mediated by androgen receptor signaling led to rapid completion of phase III studies culminating with FDA approval of abiraterone and enzalutamide. These observations fundamen-

tally changed the landscape of CRPC, credentialing the androgen receptor signaling axis as the most relevant target in CRPC, and emphasizing that novel approaches to cotargeting the androgen receptor and the networks on which it depends is an important focus moving forward.

Androgen receptor adaptations in CRPC—novel insights and biology

Androgen receptor mutations. Under the pressure of androgen deprivation and androgen receptor antagonism, the androgen receptor is susceptible to both somatic mutation and aberrant transcription. Specific mechanisms of resistance have been associated with mutations in specific regions of the receptor, including broadening of ligand specificity and conversion of antagonists to agonists (Fig. 1; refs. 20 and 21). *In vitro* selection with enzalutamide has revealed a new mutation (F876L), which mediates conversion of enzalutamide to an androgen receptor agonist (22–24), while maintaining sensitivity to first generation agents (e.g., bicalutamide). The frequency of androgen receptor mutation in CRPC tumors treated with luteinizing hormone-releasing hormone (LHRH) agonist and first-generation androgen receptor antagonists is low (8–25%; ref. 25). However, the frequency of these mutations may become a more significant mechanism of resistance in context of more complete suppression of androgen receptor signaling.

Androgen receptor splice variants. An alternative response to ADT is induction of androgen receptor variants (ARV) with deletion of the LBD resulting in ligand-independent constitutive activity (refs. 26–31; Fig. 1). ARVs homo- and heterodimerize with full length androgen receptor, promoting nuclear localization and increasing androgen receptor signaling in absence of ligand (30). Variants most commonly identified in CRPC tumors, ARV7 and ARV567, have both unique and overlapping transcriptional programs compared with wild-type androgen receptor (32). Although ARVs lacking the LBD clearly drive ligand-independent growth when evaluated in prostate cancer models, the specific role of ARVs in prostate cancer development and progression is still under debate. That ARV7 is found in normal prostate epithelium and associates with a shorter time to recurrence after prostatectomy, suggests variants play a role in prostate cancer pathogenesis (28, 29). Transgenic expression of ARV567 in prostate epithelium leads to adenocarcinoma by 50 weeks, also consistent with a potential role for ARVs in mediating prostate carcinogenesis (S. Plymate, in preparation). High levels of ARV7 and ARV567

Figure 1. A, schematic of the full-length androgen receptor (a) and exon structure of major splice variants [ARV7 (b) and ARV567 (c)]. Domains of the androgen receptor include the amino (N) terminal domain, the DBD, the HR, and the carboxy (C) terminal LBD. DHT, dihydrotestosterone. Mutations that occur in specific regions are indicated. Adapted from Green et al. (68) with permission from Elsevier. B, ADVANCES: *inhibition of androgen receptor ligand production*: inhibition of CYP17A by abiraterone, orteronel, galeterone, VT474, or CFG920 suppresses testicular, adrenal, and tumoral androgen synthesis. Galeterone can suppress CYP17A, antagonize androgens, and reduce androgen receptor levels. Abiraterone at high doses can inhibit androgen receptor and HSD3B. *Androgen receptor degradation*: androgen receptor can be destabilized through inhibition of HSP90 binding. *Androgen receptor–cofactor association*: peptidomimetics (e.g., D2) can disrupt androgen receptor interaction with cofactors, decreasing androgen receptor–driven gene transcription. *Androgen receptor antagonism*: enzalutamide and ARN509 block ligand/receptor interactions at the LBD and reduce androgen receptor–DNA association and androgen receptor nuclear accumulation. EPI-001 blocks androgen receptor NTD–coactivator association and nuclear accumulation and is active against truncated androgen receptor variants lacking the C-terminal domain (CTD). Adapted from Knudsen and Scher (69).

were associated with shorter survival in patients with CRPC and bone metastases, consistent with a role in tumor progression (26, 28, 29). Notably, a subgroup of bone metastases demonstrated nearly equivalent protein levels of full-length and truncated androgen receptor variants by Western blot. Rapid induction of ARVs following castration may itself facilitate prostate tumor survival, or may serve a bridging role until induction of additional tumor growth mechanisms (33). Alternatively, castration may promote outgrowth of ligand-independent tumor cell clones in which ARV expression is mediated by genomic rearrangement of androgen receptor (34).

Androgen receptor nuclear transport. Androgen receptor nuclear localization occurs via binding to the microtubule-based dynein motor [via a binding site in exon 4 of the canonical nuclear localization signal (NLS), ref. 35]. While the primary mechanism of taxane activity in CRPC is microtubule stabilization and disruption of cellular division, taxanes also inhibit microtubule-mediated nuclear androgen receptor transit, and cytoplasmic sequestration of androgen receptor in circulating tumor cells correlated with clinical response to taxane therapy (36). Importantly, LBD-deleted ARVs may demonstrate differential sensitivity to taxane-mediated nuclear exclusion. Although the nuclear transit of ARV567 (which retains exon 4) is taxane sensitive, the NLS of AR-V7 is located in the cryptic exon and is unique from that of full length androgen receptor (37). Accordingly, ARV7 is not susceptible to taxane-mediated nuclear exclusion (38). Thus, androgen receptor antagonists targeting the NTD may enhance taxane efficacy in ARV-expressing tumors. This may be of particular relevance in abiraterone and enzalutamide-resistant tumors, as preclinical data demonstrate that androgen receptor variants may contribute to resistance to these agents, and emerging clinical data suggests these patients have diminished sensitivity to docetaxel (39).

Nuclear receptor superfamily participation in androgen receptor signaling. Under selection pressure, the androgen receptor can broaden ligand specificity to include ligands of the closely related steroid receptor superfamily (e.g., progesterone, cortisol). Conversely, members of the nuclear receptor superfamily may be able to maintain androgen receptor signaling in androgen-deprived environments by inducing a cistrome (the genome-wide locations of a transcription factor's binding sites), which closely resembles the androgen receptor cistrome. The glucocorticoid receptor has been shown to share response elements with androgen receptor in multiple gene targets, and activates a transcriptional program that largely overlaps with that induced by androgen receptor (40). The pioneering transcription factor FOXA1 regulates differential binding of glucocorticoid receptor or androgen receptor to these targets, potentially functioning as a critical regulator of glucocorticoid receptor function in prostate cancer (40). Recently published studies also suggest that GR may play an important role in resistance to the androgen receptor antagonist enzalutamide (41). Cells selected *in vitro* for enzalutamide resistance were interrogated with cDNA array analysis and revealed

dramatically upregulated GR levels compared with parental cells. Knockdown of GR in the enzalutamide-resistant cells partially abrogated resistance to enzalutamide. Additional confirmation of this effect in human specimens is anticipated. An analysis of AFFIRM, the phase III study of enzalutamide in postdocetaxel patients, suggested use of glucocorticoids was associated with inferior survival (independent of other known prognostic factors) and may be driving an adverse biology (42). A similar analysis in COU-301, a phase III study of abiraterone in the same patient population, suggested that glucocorticoids were used in patients with greater comorbidity and worse prognosis disease, potentially explaining the inferior outcomes (43). Whether GR biology drives progression in CRPC will require additional analysis of clinical samples and the impact of glucocorticoid use in patients with CRPC.

Androgen receptor-driven ETS fusion genes. The most common mutation in prostate cancer is fusion of the androgen receptor-regulated protease TMPRSS2 with members of ETS family of transcription factors (44). The most common androgen receptor-driven fusion, TMPRSS2:ERG, induces high-level expression of ERG, which associates endogenously with PARP1 (which ribosylates proteins involved in DNA mismatch repair; ref. 45), and both ERG-mediated transcriptional activity and cell invasion require PARP1 activity (46). Targeting of PARP in tumors deficient in other components of DNA repair such as BRCA1/BRCA2 induces significant clinical responses, a result of presumed "synthetic lethality" resulting from complete abrogation of DNA repair (47). Notably, ETS transcription factors, including ERG, drive DNA double-strand break formation, and concurrent inhibition of PARP in ERG-overexpressing cells further increases DNA damage. This finding raises the potential that cotargeting the androgen receptor pathway with inhibitors of PARP in ERG fusion positive prostate tumors may reconstitute synthetic lethality, even in tumors that are BRCA1/2 mutation negative (46).

Clinical Implications

Clinically, resistance to first- and second-generation androgen receptor-targeting agents is universally associated with reactivation of androgen receptor signaling, as manifested by a rising prostate-specific antigen (PSA). Induction of resistance to abiraterone and enzalutamide in preclinical models also remains largely dependent on androgen receptor signaling, either via induction of steroidogenesis, or upregulated expression of androgen receptor and truncated ARVs, and complete abrogation of androgen receptor function has yet to be realized (48–50). Leveraging previously unexplored biology, exploring rational combinations earlier in the course of disease, and improving efficacy of steroidogenesis inhibitors and androgen receptor antagonists all have potential to improve outcomes (51). One example of novel targeting includes inhibiting the androgen receptor NTD, which has resisted drug targeting because of difficulty crystallizing its highly disordered structure. Targeting the NTD with novel agents carries the potential to

Table 1. Actively recruiting clinical trials with androgen receptor targeting and cotargeting in CRPC (clinicaltrials.gov)

Target	Drugs	Design	NCT#
Androgen receptor and CYP17	Enzalutamide and abiraterone	Phase I/II	NCT01650194
Androgen receptor and CYP17	Galeterone	Phase II	NCT01709734
Androgen receptor and CYP17	ARN-509 and abiraterone	Phase Ib	NCT01792687
HSP90 and CYP17	AT13387 and abiraterone	Phase I/II	NCT01685268
Androgen receptor and VEGFR	Enzalutamide and tivozanib	Phase II single arm	NCT01885949
Androgen receptor and CYP17	CFG920	Phase I/II	NCT01647789
Androgen receptor and PARP	Velaparib and abiraterone	Phase II	NCT01576172

address constitutively active androgen receptor (driven by mutation or splice variants affecting the LBD), as well as androgen receptor amplification. Screens of libraries for NTD-binding agents identified EPI-001, a nonsteroidal compound that binds the NTD and inhibits the growth of castration-sensitive cell lines (52, 53). Multiple analogs of EPI-001 have been developed with greater antitumor efficacy in both sensitive and castration-resistant xenograft models, including those driven by ARV expression (52).

Alternative approaches include concurrent targeting of androgen receptor and steroidogenesis production. Drawing from experience in treating infectious diseases such as HIV and tuberculosis, combining effective agents with complementary mechanisms of action may provide more durable disease control through rapid diminution of the cellular pool available to undergo mutation in response to treatment stress (54). This provides rationale for testing combinations of abiraterone and second-generation androgen receptor antagonists such as enzalutamide and ARN-509 in multiple stages of prostate cancer, from metastatic CRPC to treatment naïve, high-risk localized disease (Table 1). ARN-509 is structurally similar to enzalutamide, with similar mechanism of action and activity *in vitro*, but superior activity in xenograft experiments (55). Phase I/II studies of ARN-509 as a single agent have been recently completed with results pending.

The same rationale underlies testing of single agents with activity against multiple sites of the androgen receptor signaling axis. Galeterone (TOK-001/VN-124-1) was identified through library screening for combination inhibitors of CYP17 and androgen receptor (56). *In vitro* it suppresses androgen receptor levels, providing a separate potential avenue for inhibiting ARV or mutant androgen receptor-driven tumors (57, 58). A phase I study in chemotherapy-naïve CRPC demonstrated 50% PSA reduction in 22% of patients, with a 50% decline at the highest dose level. The maximally tolerated dose was not reached, with the most common side effects being fatigue, liver function test abnormalities, pruritus, nausea, and diarrhea. A phase II study (ARMOR2) is currently ongoing in multiple cohorts of patients with CRPC (Table 1). Interestingly, abiraterone has also been found to exhibit weak antagonism against both wild type and various mutant androgen receptors,

including those activated by exogenous glucocorticoids (T877A) and bicalutamide (W741C) (59), providing a potential rationale for dose escalation.

Another strategy is targeting androgen receptor–coregulator interactions. A recent study reports a peptidomimetic (small organic molecules without a peptide backbone) that mimics the LXXLL motif found in androgen receptor coregulators. The compound, D2, disrupted binding of androgen receptor to proteins such as PELP1 (which plays a scaffolding role for assembly of the androgen receptor transcriptional complex), prevented androgen-induced nuclear translocation comparable to that obtained with enzalutamide, and inhibited growth of androgen receptor positive prostate cancer cells *in vitro* and *in vivo* (60).

Agents targeting suppression of the androgen receptor protein, such as inhibitors of the androgen receptor chaperone HSP90, carry potential to completely abrogate androgen receptor signaling, independent of tissue ligand levels, androgen receptor mutation, or ARV structure (61, 62). Next generation HSP90 inhibitors show activity in preclinical models and have significant advantages over agents such as geldenamycin, including improved potency and lack of requirement for activation by enzymes such as diaphorase, which are not highly expressed in prostate tissue (63). Phase I studies with newer agents show promising activity against heavily pretreated CRPC, and phase II studies as monotherapy and in combination with abiraterone in patients with abiraterone-refractory CRPC are ongoing (Table 1).

Conclusions

Perhaps the most significant challenge to defining optimal sequencing and combinations of next generation of agents is the heterogeneity of CRPC. At inception of CRPC, molecular profiling of the androgen receptor axis reveals specific patterns of androgen receptor pathway components. Some tumors are positive for steroidogenic machinery in conjunction with upregulated androgen receptor suggesting modulation by ligand, others show no evidence for steroidogenic potential but only upregulation of androgen receptor, suggesting modulation by

androgen receptor itself, and a smaller number lack any signature for upregulation of ligand or receptor (64, 65). Efforts to characterize molecular phenotype of metastatic prostate cancers, such as the SU2C/AACR/PCF sequencing project (66) will be critical to defining which pathways are relevant for targeting in abiraterone and enzalutamide refractory cancers. In addition, correlating tissue biopsy with a common platform for analyzing circulating tumor cells and cell-free DNA, as means of noninvasively sampling relevant biology, will await adoption of an assay that simultaneously detects and efficiently collects samples for analysis (67). Using tumor biopsy to enrich patient populations, as is being utilized in the study of PARP inhibition in TMPRSS2:ERG-positive and -negative tumors (NCT01576172; Table 1), will be critical to moving novel combination therapies to the patients who need them as rapidly as possible (54).

Disclosure of Potential Conflicts of Interest

S.R. Plymate serves as a consultant/advisory board member for ESSA. B. Montgomery has received commercial research grants from Janssen, Med-

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Authors' Contributions

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Development of methodology: B. Montgomery

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S.R. Plymate, B. Montgomery

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): E.A. Mostaghel, S.R. Plymate, B. Montgomery

Writing, review, and/or revision of the manuscript: E.A. Mostaghel, S.R. Plymate, B. Montgomery

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): B. Montgomery

Study supervision: B. Montgomery

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