New Approaches to Targeting the Androgen Receptor Pathway in Prostate Cancer

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Abstract: The androgen signaling axis has been the main therapeutic target in the management of advanced prostate cancer for several decades. Over the past years, significant advances have been made in terms of a better understanding the androgen receptor (AR) pathway and mechanisms of castration resistance, along with the development of more potent AR-targeted therapies. New drugs, such as abiraterone, enzalutamide, apalutamide, and darolutamide, have been approved for castration-resistant prostate cancer and also have demonstrated an overall survival benefit in the castration-sensitive state. Despite these major advances, the majority of patients eventually present with disease progression and a rise in prostate-specific antigen, reflecting a continuous dependence of disease on the AR pathway. In this setting, a number of AR-related mechanisms of resistance have been described, and novel strategies to overcome them are an important unmet need. In this manuscript, we review the most promising strategies to target the AR pathway in prostate cancer, including bromodomain and extraterminal (BET)/bromodomain inhibitors, CREB-binding protein/p300 inhibitors, N-terminal domain inhibitors, proteolysis-targeting chimeras, and AR-targeting vaccines. Another interesting and disruptive approach to targeting the AR and potentially reversing resistance to second-generation AR antagonists is the cyclic administration of high-dose testosterone, known as bipolar androgen therapy, which is currently being explored in multiple ongoing trials.

Keywords
Androgen receptor, prostate cancer

Introduction

Prostate cancer has an intrinsic dependence on androgens and androgen receptor (AR) regulation. As a result, the suppression of gonadal androgen synthesis either by orchiectomy or by pharmacologic

Table 1. FDA-Approved Androgen Receptor–Directed Therapies for Patients With Prostate Cancer

Setting	Anti-androgen	Main Outcomes	Approval
mCRPC	Abiraterone	Post-docetaxel median OS: 14.8 mo[3]	2011
		Pre-docetaxel median OS: 34.7 mo[108]	2012
	Enzalutamide	Post-docetaxel median OS: 18.4 mo[6]	2012
		Pre-docetaxel median OS: 35.3 mo[109]	2012
nmCRPC	Apalutamide	Median OS: 73.9 mo[110] Median MFS: 40.5 mo[111]	2018
	Enzalutamide	Median OS: 67.0 mo[7] Median MFS: 36.6 mo[112]	2018
	Darolutamide	Median OS: not reached[10] Median MFS: 40.4 mo[9]	2019
mHSPC	Abiraterone	Median OS: 53.3 mo[113]	2018
	Enzalutamide	Median OS: not reported/not reached[13] (3-year OS: 80%[14])	2019
	Apalutamide	Median OS: not reached[15]	2019

FDA, US Food and Drug Administration; mCRPC, metastatic castration-resistant prostate cancer; mHSPC, metastatic hormone-sensitive prostate cancer; MFS, metastasis-free survival; nmCRPC, non-metastatic castration-resistant prostate cancer; OS, overall survival.

modulation with luteinizing hormone-releasing hormone agonists—known as androgen deprivation therapy (ADT)—represents the treatment backbone of metastatic disease.[1] Unfortunately, resistance almost invariably develops and leads to a state called castration-resistant prostate cancer (CRPC), defined as disease that progresses despite serum testosterone levels in the castrate range.[2] Experience with drugs that target AR function, such as abiraterone,[3,4] enzalutamide (Xtandi, Astellas),[5-7] apalutamide (Erleada, Janssen),[8] and darolutamide (Nubeqa, Bayer),[9,10] have shown a benefit in patients with CRPC, supporting the hypothesis that the AR remains constitutively active and represents an important mechanism driving prostate cancer growth, even at low levels of testosterone.[2] Also, the treatment of patients with metastatic hormone-sensitive prostate cancer (HSPC) now incorporates the up-front use of medications that target the AR, along with ADT. Abiraterone,[11,12] enzalutamide,[13,14] and apalutamide[15] are now standard-of-care therapies for the treatment of metastatic HSPC (Table 1).

Since the recent approval of anti-androgens for nonmetastatic CRPC[7-9] and metastatic HSPC,[11,13,15] these therapies are being introduced earlier in the disease course, increasing the length and intensity of hormone deprivation therapy. This change in practice is notable because mechanisms of resistance to anti-androgens may develop earlier in the disease course, creating an urgent need for new strategies to overcome this problem. In addition, now that patients are starting anti-androgen therapies earlier—oftentimes in the absence of symptoms—and receiving AR-targeted therapies sequentially for years, long-term toxicities have become a concern. As a result, treatment approaches either to mitigate or to avoid the long-term toxicities of AR-targeted therapies are necessary. Novel approaches to target the AR and the AR pathway are being developed and tested in clinical trials. In this article, we review the most promising new approaches and therapies currently in development to target the AR and the AR pathway in prostate cancer.

The AR Structure and the AR Signaling Pathway

The AR is a nuclear receptor that belongs—along with the estrogen receptor, glucocorticoid receptor, and progesterone receptor—to the group of steroid hormone nuclear receptors.[16,17] The *AR* gene is located on the X chromosome at locus Xq11-Xq12, and the transcription factor has several functional domains: an N-terminal domain (NTD), encoded by exon 1[18]; a DNA-binding domain (DBD), encoded by exons 2 and 3; and a ligand-binding domain (LBD), encoded by exons 4 to 8.[19,20] Between the DBD and LBD is a flexible hinge region that contributes to nuclear localization and degradation, in addition to playing a complex role in DNA binding, recruitment of co-activators, and interaction between NTD and LBD.[21,22] This region is a target site for acetylation, ubiquitination, and methylation.[19,22] The activation function 1 (AF-1) region is the primary effector of the NTD; it contains the transcription activation units Tau-1 and Tau-5 (amino acids 110-370 and 360-485, respectively), implicated in the full activity of the AR. Interactions between the NTD and LBD occur by some mediators contained in Tau-1 and Tau-5, called FQNLF motif (amino acids 23-27)

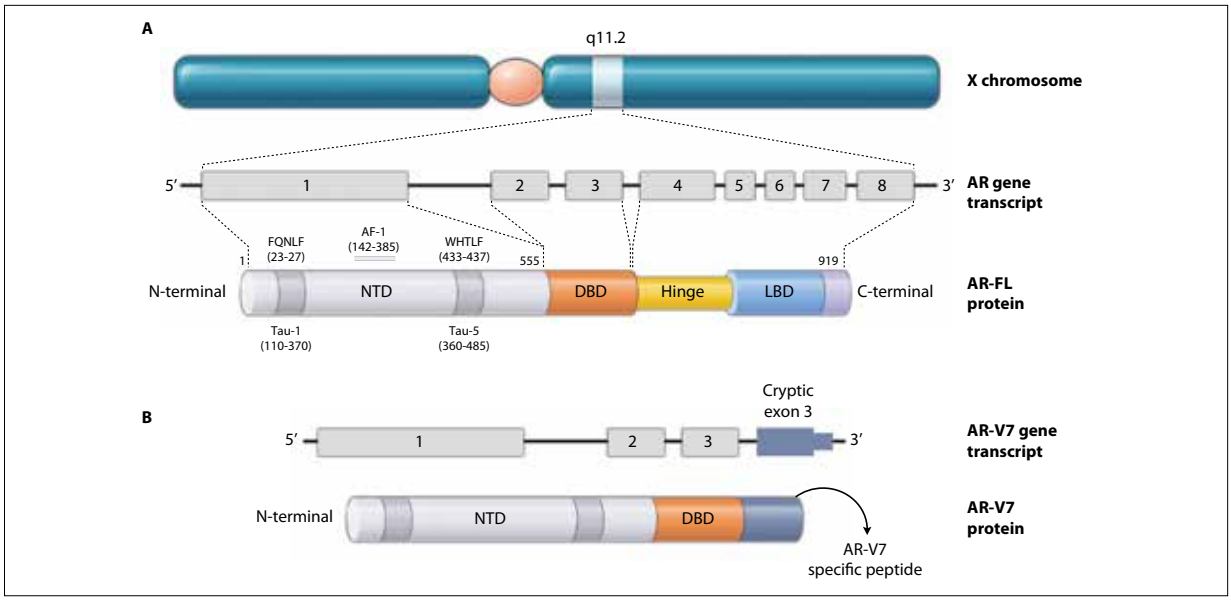

Figure 1. Structure of (A) AR-FL and (B) AR-V7.

AR, androgen receptor; AR-FL, full-length AR; AR-V7, AR splice variant 7; DBD, DNA-binding domain; LBD, ligand-binding domain; NTD, N-terminal domain.

and WHTLF motif (amino acids 433-437), respectively. These mediators are important in regulating many androgen-dependent genes, in addition to assisting in AR dimer complex stabilization and slowing the rate of ligand dissociation. The structures of the *AR* gene and AR protein are summarized in Figure 1.

Upon binding to a steroid hormone—specifically, testosterone or dihydrotestosterone—AR dimerizes and translocates to the nucleus, binding to DNA in the promoter regions of target genes, such as prostate-specific antigen (*PSA*) and transmembrane serine protease 2 (*TMPRSS2*). This in turn triggers cancer cell growth and survival (Figure 2A).[19] When the availability of testosterone from the bloodstream becomes limited after ADT, prostate cancer cells can maintain AR activity through other mechanisms, including *AR* overexpression, amplification and/or gain-of-function mutation of *AR,*[23-25] expression of AR splice variants (AR-Vs),[26,27] increased production of intracellular androgens, and changes in the activity or expression of AR co-activators and co-repressors.[23] All these mechanisms of resistance open windows of opportunity for the development of novel approaches and therapies to target the AR pathway. In addition, AR-independent mechanisms of resistance have been implicated in prostate cancer progression. The "darwinian" treatment-induced selective pressure triggered by ADT and anti-androgens in prostate cancer cells[28] may be responsible for the development of treatment-emergent small cell neuroendocrine prostate cancer,[29-31] an AR-independent lethal subtype of prostate cancer.

Morphologically distinctive populations of ARs—characterized by absence of the LBD—are called AR-Vs.[32] Most of these splice variants arise through the splicing of intronic sequences (ie, cryptic exons), the most notable examples being the AR-V7 and ARv567es variants.[32,33] These splice variants are constitutively active, allowing activation of the AR signaling pathway in the absence of a ligand (Figure 1B).[32] AR-V7 is the most abundant AR-V and the most widely studied thus far. In circulating tumor cells of patients with prostate cancer, AR-V7 expression increases remarkably upon androgen deprivation and disease progression during treatment with abiraterone or enzalutamide.[27] Expression of AR-V7 has emerged as one of the most important mechanisms of resistance to anti-androgens, being associated with progressive disease, resistance to abiraterone or enzalutamide,[26] and limited cancer-specific survival[26,27] despite partial responsiveness to taxane chemotherapy.[34]

BET/Bromodomain, CBP, and p300 Inhibitors

The normal gene expression of cells is maintained by multiple epigenetic mechanisms. Dysregulation of the epigenetic organization is frequently observed in cancer, leading to the overexpression of oncogenes and/or silencing of tumor suppressor genes.[35] Histone acetylation, a key component of epigenetic control and mediator of transcription, is regulated mainly by 3 classes of proteins:

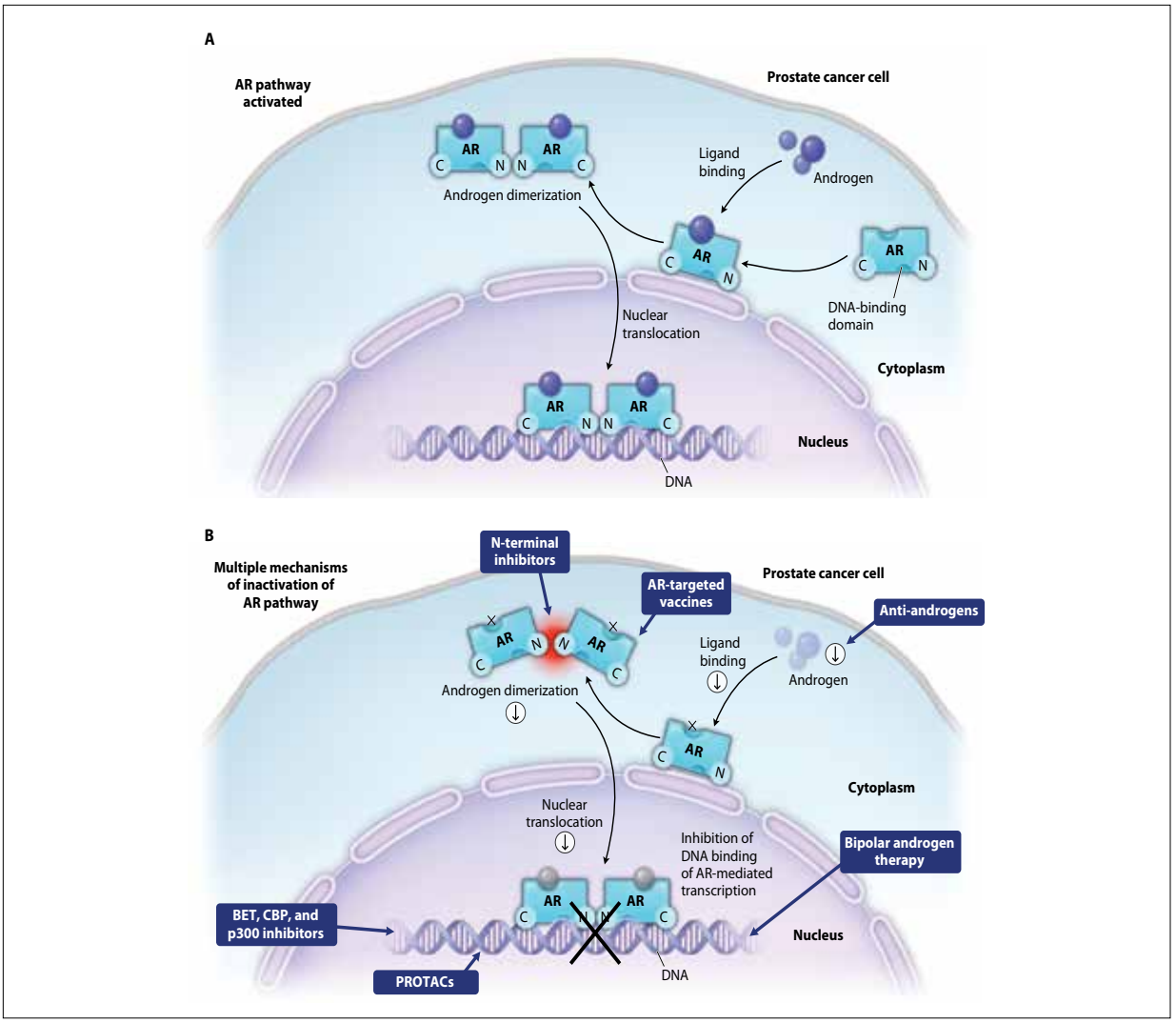

Figure 2. The androgen receptor pathway (A) and novel AR pathway inhibitors and repercussions in the AR pathway (B).

AR, androgen receptor; BET, bromodomain and extraterminal; CBP, CREB-binding protein; PSA, prostate specific antigen; PROTACs, proteolysis-targeting chimeras.

histone acetyltransferases, also called "writers," which covalently introduce acetyl groups; histone deacetylases, called "erasers," which remove these chemical modifications; and bromodomain and extraterminal (BET) proteins, called "readers," which bind to acetylated histones.[36] The histone acetyltransferases CREB-binding protein (CBP) and its homologue p300 are transcriptional co-activators of various sequence-specific transcription factors that are involved in multiple cellular processes, such as proliferation, cell cycle regulation, apoptosis, differentiation, and DNA damage response.[37,38]

In prostate cancer, the AR acts together with numerous co-activator proteins, including BET protein, CBP, and p300, in the regulation of cell growth in response to androgen.[39-42] Some evidence suggests that p300 and CBP may also play a role in CRPC transactivation of the AR (by interleukin 6 activation) in the absence of androgens.[43-46] Treatment with an anti-androgen induces the activation of CREB and promotes resistance in prostate cancer cells; CREB activation may be one of the unidentified mechanisms of resistance to anti-androgens in clinical practice.[47] As well, BET proteins can increase the expression of other oncogenic drivers, such as c-MYC[48] and the AKT/mammalian target of rapamycin (mTOR) complex.[49] BET proteins also may promote the repair of double-stranded DNA breaks (by the nonhomologous end-joining pathway)[50] and partially mediate the formation of *TMPRSS2-ERG* gene fusions.[50,51] In addition, the inhibition of CBP/p300 may increase the efficacy of programmed death ligand 1 (PD-L1) blockade, and BET

inhibition may reduce AR-V7 expression. These findings may open windows of opportunity to improve the efficacy of programmed death 1 (PD-1)/PD-L1 inhibitors[52] and anti-androgens[53] in prostate cancer. Resistance to BET inhibitors also has been studied and may open therapeutic vulnerabilities in CRPC.[54] Therefore, the development of bromodomain/BET inhibitors, CBP/p300 inhibitors, or dual inhibitors is a therapeutic opportunity to overcome conventional mechanisms of therapy resistance in prostate cancer.

Molibresib

Molibresib (GSK525762) is an orally bioavailable, small-molecule pure BET inhibitor.[55] A preclinical study demonstrated antitumor activity of molibresib in animal models of several types of cancer, including prostate cancer.[48] The first-in-human dose escalation study of molibresib in advanced solid tumors enrolled 196 patients, 23 of whom had advanced prostate cancer.[56,57] Treatment-related adverse events (AEs) occurred in 92% of patients, and discontinuation of therapy was required in 19%. Dose reductions because of AEs were necessary in 27% of patients. The most common AEs were thrombocytopenia (all grades, 64%; grade 3/4, 41%); nausea (all grades, 49%; grade 3/4, 6%); and decreased appetite (all grades, 46%; grade 3/4, 4%).

The median age of patients in the prostate cancer cohort was 63.8 years, 70% had an Eastern Cooperative Oncology Group (ECOG) performance status of 1, and all patients had CRPC and had received at least 3 previous therapies.[57] A partial response occurred in 1 patient, and 5 patients (22%) has stable disease as their best response. The median progression-free survival (PFS) in the CRPC cohort was 8 months (95% CI, 5.5-11.7), and median overall survival (OS) was 9.1 months (6.7-11.7).[57] Molibresib is now being investigated in combination with either abiraterone or enzalutamide in patients with metastatic CRPC (NCT03150056).

Birabresib

Birabresib (MK-8628/OTX015) is a bromodomain inhibitor that acts by competition with the binding of BRD2, BRD3, and BRD4.[58] Preclinical data have shown that birabresib has activity in several types of cancer cell lines. The dose-finding phase 1b study of birabresib enrolled 46 patients with multiple solid tumors, including non–small cell lung cancer (10 patients), nuclear protein of the testis (NUT) midline carcinoma (10 patients), and metastatic CRPC (26 patients).[59] Patients were treated according to 2 different dose schedules in 2 parallel cohorts: cohort A, 80 mg once daily continuously (21 of 21 days); cohort B, 100 mg once daily for 7 consecutive days every 3 weeks (7 of 21 days).

Dose-limiting toxicities occurred in 6 patients, all within cohort A, and consisted of anorexia, nausea, thrombocytopenia (4 patients), alanine aminotransferase (ALT) elevation, and hyperbilirubinemia abnormalities. Treatment-related AEs were seen in 83% of patients, the most common being diarrhea (37%), nausea (39%), decreased appetite (30%), vomiting (28%), and thrombocytopenia (22%). The most common treatment-related serious AE was thrombocytopenia. Grade 3/4 thrombocytopenia occurred in 28% of patients, all in cohort A. Dose reductions, interruptions, or treatment withdrawals secondary to AEs occurred in 39% of patients. On the basis of these results, the recommended phase 2 dose of birabresib was 80 mg once daily with continuous dosing.[59]

Birabresib demonstrated clinical antitumor activity in patients with the 3 solid tumor types enrolled; however, the best responses (partial responses) were seen in the patients with NUT midline carcinomas. Of the 24 patients with metastatic CRPC who were evaluable for efficacy, 15 (63%) had stable disease as the best response. At the time of the published analysis, 2 patients with CRPC were free of progression—one at 3.5 and the other at 7.8 months.[59] It is unclear if this agent will be developed further in prostate cancer.

ZEN-3694

ZEN-3694 is an orally bioavailable second-generation pan-BET/bromodomain inhibitor. It has demonstrated strong inhibitory activity—either as monotherapy or in combination with enzalutamide—in the AR signaling pathway, AR splice variants, MYC, glucocorticoid receptor, and other CRPC oncogenes.[60] On the basis of these preclinical findings, researchers designed a phase 1b/2 study of ZEN-3694 in combination with enzalutamide.[61] ZEN-3694 was evaluated in a multicenter, open-label, 3+3 dose escalation study that enrolled 75 patients with metastatic CRPC that had progressed on abiraterone and/or enzalutamide. The initial dose of ZEN-3694 was 36 mg orally once per day. All patients received the standard dose of enzalutamide (160 mg orally once per day). Co-primary endpoints were safety and the recommended phase 2 dose of ZEN-3694 in combination with enzalutamide. Pharmacokinetic assessment of ZEN-3694 and enzalutamide, PSA_{50} response (ie, percentage of patients with a ≥50% decline in PSA), duration of response, and radiographic PFS were the secondary endpoints. Tumor genomics, transcriptional profile, and protein expression were also evaluated and correlated with clinical outcomes.

Grade 3 or higher AEs were reported in 14 patients (18.7%), with nausea (4%), thrombocytopenia (4%), anemia (2.7%), fatigue (2.7%), and hypophosphatemia (2.7%) the most common. AEs leading to dose reduction

and/or treatment discontinuation occurred in 24 patients (32%), with most events occurring at dose levels of 120 to 144 mg/d. The maximum tolerated dose was not reached. Of the 35 patients enrolled in the dose escalation part of the study, only one patient experienced a dose-limiting toxicity at the dose level of 96 mg/d (grade 3 nausea); therefore, the maximum tolerated dose was not reached.

PSA_{50} and PSA_{90} responses were seen in 8% and 5.3% of patients, respectively. The median PSA PFS was 3.2 months (95% CI, 3.2-5.1); however, the subgroup of patients who exhibited PSA declines had sustained responses, with a median duration of response of 21.1 months (95% CI, 19.0-23.2). The median radiographic PFS in the overall cohort was 9.0 months (95% CI, 4.6-12.9). Among the patients with disease progression on abiraterone, the median radiographic PFS was 7.8 months (95% CI, 4.9-10.6), in comparison with 10.1 months for the patients with disease progression on enzalutamide (95% CI, 4.4-12.9). At 12 and 24 months, 17% and 5% of patients, respectively, remained without progression. Post hoc analyses showed that radiographic PFS in the patients with a lower rate of canonical AR transcriptional activity in baseline tumor biopsy specimens was longer than radiographic PFS in the patients with a high rate of AR transcriptional activity (median radiographic PFS, 10.4 vs 4.3 months).

The pharmacodynamic data of this study indicated the existence of a plateau of effect in the downregulation of BET target gene expression at doses above 96 mg/d. Also, because of the high percentage of patients requiring dose interruptions/reductions at the same dose, 96 mg/d was chosen as the recommended phase 2 dose of ZEN-3694. Because of the promising activity of ZEN-3694 in combination with enzalutamide, a phase 2 study evaluating these 2 therapies in combination with pembrolizumab (Keytruda, Merck) was designed. This study began enrolling patients with metastatic CRPC that had progressed on a prior anti-androgen in December 2020 (NCT04471974).

CCS1477

CCS1477 is a potent, selective, and orally bioavailable bromodomain inhibitor developed to inhibit p300 and CBP. This drug also showed consistent capability to inhibit the expression and function of full-length AR (AR-FL), AR splice variants, and c-MYC, demonstrating a sustainable effect in bicalutamide-resistant xenograft models either as monotherapy or in combination with enzalutamide.[62] Preclinical studies have shown that monotherapy with CCS1477 caused PSA reductions and tumor regression in xenograft models of CRPC, with continued blockage of tumor growth following drug withdrawal.[62,63]

The first trial (NCT03568656) to evaluate the safety and efficacy of CCS1477, either as monotherapy or in combination with abiraterone or enzalutamide, in patients with metastatic CRPC was designed to enroll approximately 150 patients. This multicohort phase 1/2a study is enrolling patients with metastatic CRPC in the monotherapy dose escalation part.[64] Patients who have received previous treatment with abiraterone, enzalutamide, and taxanes (unless ineligible or refused) and whose disease has progressed on these therapies are eligible. After further study expansion to include patients in whom other solid tumors have been diagnosed, patients presenting with somatic CBP or p300 mutations will be enrolled. In phase 2, following a determination of the recommended dose and schedule for monotherapy, it is planned to open 3 expansion arms in parallel: CCS1477 monotherapy, CCS1477 plus abiraterone, and CCS1477 plus enzalutamide.[64]

NEO2734

NEO2734 is a novel small-molecule dual inhibitor of both the BET family and CBP/p300. It has shown antiproliferative activity against a variety of solid tumor cell lines, including triple-negative breast cancer, colorectal cancer, hematologic cancers, and CRPC.[65,66] NEO2734 has shown superior antiproliferative activity in comparison with molibresib, a pure BET inhibitor, in multiple cancer cell lines, such as leukemia, lymphoma, and prostate cancer. In CRPC xenograft models, NEO2734 has shown antitumor activity and PSA reduction. It has also demonstrated activity in speckle-type pox virus and zinc finger (POZ) protein (*SPOP*-mutant) prostate cancer.[67] *SPOP* is the most commonly mutated gene in primary prostate cancer, with an estimated incidence of 10% to 15%, depending on the patient cohort studied.[68,69]

Among all the genotypically distinct subtypes of prostate cancer, the *SPOP*-mutated form has the highest rate of AR transcriptional activity,[68] being extremely sensitive to treatment with ADT (superior PFS and OS).[70] Some specific *SPOP* hotspot mutations (F133V and W131R) confer resistance to BET inhibitors owing to the upregulation of BET proteins and aberrant occupancy of BRD4 in the genome.[49,71] A preclinical study with NEO2734 in *SPOP*-mutant prostate cancer cell lines, organoids, and xenografts showed that simultaneous inhibition of the BET family and CBP/p300 proteins with the dual pathway inhibitor resulted in an antitumor effect that was superior or at least equivalent to that achieved by co-targeting both pathways with individual inhibitors.[67] These findings provide a rationale for the design of clinical trials testing NEO2734 in patients with advanced *SPOP*-mutated CRPC, especially those with the F133V and W131R hotspot mutations.

PLX2853

PLX2853 is a BET inhibitor developed to exhibit a unique binding mode in addition to a short terminal half-life, to improve tolerability.[72,74] Preclinical and animal studies with PLX2853 demonstrated antitumor activity in aggressive MYC-driven lymphomas, despite limited activity in BCL-2–driven diffuse large B-cell lymphoma.[72] A clinical trial evaluating PLX2853 in combination with abiraterone, prednisone, and olaparib (Lynparza, AstraZeneca) is enrolling patients (NCT04556617), and preliminary results are expected in February 2023.

N-Terminal Domain Inhibitors

Even though the NTD is the critical region of the AR that drives its transcriptional activity, all modern AR-targeted therapies depend on the presence of the LBD to act. The link between these therapies and the LBD results in the blockage of AR dimerization and nuclear translocation, and consequently the inhibition of DNA and protein synthesis.[19] The essential step for AR transactivation lies in the AF-1 region (Figure 1), which is contained within the NTD; therefore, deletions of this region cause AR transcriptional silencing,[74-76] opening a window of opportunity to overcome the limitations of current LBD-targeting therapies.[77]

Because the NTD is required for all AR transcriptional activities and is present in all forms of the AR, NTD inhibitors may affect a broader AR population, including AR splice variants as well as AR species harboring gain-of-function LBD mutations, in contrast with current therapies, which affect only AR populations that possess an intact LBD.[77] Preclinical studies have demonstrated promising results of NTD inhibitors (small molecules and bispecific antibodies) in prostate cancer cells, including enzalutamide-resistant cells.[78,79] Some AR NTD inhibitors that are in clinical development are discussed below.

EPI-506

EPI-506 is a first-in-class prodrug; it is a highly specific small molecule that binds the NTD, inhibiting AR transcriptional activity and blocking the interaction between the AR and transcriptional proteins.[77,80] EPI-506 is rapidly (within 5 minutes) and completely metabolized to PI-002, which is the active compound.

A phase 1/2 adaptive 3+3 dose escalation study evaluated EPI-506 in patients with metastatic CRPC and an ECOG performance status of 0 or 1 whose disease had progressed after prior treatment with abiraterone and/or enzalutamide. Of the 18 patients enrolled in the first part of the study, 90% had received EPI-506 as the fifth or later line of therapy, and 40% had received chemotherapy. The dose escalation part of the study enrolled 18 patients, who received EPI-506 by mouth once a day at a starting dose of 80 mg/d. The primary endpoints of this part of the study were safety and tolerability; the maximum tolerated dose, recommended phase 2 dose, and pharmacokinetic profile were also evaluated. In exploratory analyses, circulating tumor cells (AR-V7 status) and pain response (by brief pain inventory) were evaluated.

The median duration of treatment at the time of data cutoff was 87 days (range, 21-444). Treatment was prolonged in 3 patients (median, 325 days). Pharmacokinetic data demonstrated dose-proportional profiles for peak serum concentration (C_{max}) and area under the curve (AUC). A negative effect of food was reported in patients receiving doses of up to 640 mg/d (60% decrease in AUC), and a positive effect of food was reported at doses of 1280 mg (40% increase in AUC) and 2400 mg (20% increase in AUC).

The most common AEs reported were diarrhea (38%), nausea (33%), pain in the extremities (29%), decreased appetite (19%), and fatigue (19%). Grade 3 or higher AEs that were considered related to the drug were rare and occurred in 2 patients. Elevated aspartate aminotransferase (AST) developed in 1 patient receiving a dose of 1280 mg/d (which was a dose-limiting toxicity), and elevated amylase developed in 1 patient receiving a dose of 640 mg/d.

Declines in PSA ranging from 4% to 29% occurred in 4 patients (22.2%), all of whom received daily doses greater than 1280 mg. This study showed that EPI-506 is well tolerated, with a favorable safety profile. The second part of this study was terminated owing to excessively high pill burden.[81] This agent will not be developed further in prostate cancer, however, and has been superseded by EPI-7386, which is discussed below.

EPI-7386

EPI-7386 is an NTD inhibitor designed to inhibit transcriptional activity of the AR by interacting with the NTD, so that it is active against both AR-FL and AR splice variants.[82] A preclinical study evaluating the 2 N-terminal inhibitors, EPI-506 and EPI-7386, and various anti-androgens, including enzalutamide, apalutamide, bicalutamide, and darolutamide, demonstrated profound inhibition of androgen-induced transcriptional activity by EPI-7386. In this study, EPI-7386 induced a dose-dependent decrease in AR transcriptional activity in LNCaP cells, which bear the *AR* T877A mutation, having decreased affinity for apalutamide and darolutamide.

Proliferation assays in vitro demonstrated activity in multiple prostate cancer cell lines, including activity in AR-V7–driven cellular models. In enzalutamide-resistant CRPC xenografts, EPI-7386 induced superior tumor

Table 2. Published Clinical Trials Evaluating BAT in Prostate Cancer

Study First Author	Inclusion Criteria	Therapies	Design	PSA Response Rate	Radiographic Response Rate
Schweizer[91] (N=16)	mCRPC after first- or second-generation anti-androgens	BAT + oral etoposide	Phase 1/2, single arm	PSA_{50}: 28.6%	50%
Schweizer[92] (N=33)	mHSPC or biochemically recurrent disease	BAT	Phase 2, single arm	PSA <4 ng/mL: 59%	80%
Teply[93] (N=30)	mCRPC after enzalutamide	BAT	Phase 2, single arm	PSA_{50}: 30%	50%
Markowski[94] (N=59)	mCRPC after abiraterone or after enzalutamide	BAT	Phase 2, multicohort	PSA_{50} after abiraterone: 17% PSA_{50} after enzalutamide: 30%	After abiraterone: 29% After enzalutamide: 50%
Denmeade[95] (N=179)	mCRPC after abiraterone	BAT vs enzalutamide	Phase 2, randomized	PSA_{50} with BAT: 27.1% PSA_{50} with enzalutamide: 25.3%	BAT: 24.2% Enzalutamide: 4.2%

BAT, bipolar androgen therapy; mCRPC, metastatic castration-resistant prostate cancer; mHSPC, metastatic hormone-sensitive prostate cancer; PSA, prostate-specific antigen; PSA50, percentage of patients with a decline in PSA of 50% or greater.

regression in comparison with enzalutamide, and the 2 agents in combination have achieved a synergistic antitumor response.[82] On the basis of these results, EPI-7386 has emerged as a potential new investigational drug for patients with CRPC, and a clinical trial was recently started (NCT04421222). This phase 1, open-label study will enroll 40 patients with metastatic CRPC, who will receive 1 of 5 doses of EPI-7386. The study started enrolling patients in June 2020 and is estimated to have results in December 2022.

Proteolysis-Targeting Chimeras

Proteolysis-targeting chimeras (PROTACs) are nanomolecules with the capacity to recruit proteins for cancer cell death through ubiquitin-proteasome–mediated protein degradation. The ubiquitin-proteasome system is an important pathway that controls the cell protein levels and regulates other important cell functions, such as protein localization, cell cycle, apoptosis, autophagy, and DNA repair.[83] The PROTAC mechanism of action relies on the creation of a chimeric molecule that recruits any cancer-related protein to an E3 ligase for subsequent ubiquitination and degradation. Because of the presence of several E3 ubiquitin ligases in the human genome, many possible PROTAC combinations can be developed to target cancer-specific proteins.[83]

PROTAC technology was used to develop the orally bioavailable small-molecule AR degrader ARV-110. This agent has demonstrated robust AR degradation in multiple cell lines and also in enzalutamide-resistant prostate cancer xenograft models.[84] Recently, a first-in-human phase 1 study of ARV-110 was reported in 22 patients with metastatic CRPC who had received at least 2 prior lines of therapy, including abiraterone or enzalutamide.[85] In this preliminary report, a PSA decline of at least 50% occurred in 2 of 20 evaluable patients, and the drug demonstrated an overall acceptable safety profile. The study is ongoing, and neither the maximum tolerated dose nor the recommended phase 2 dose has yet been determined.

Bipolar Androgen Therapy

Preclinical experiments indicate that re-exposure of castrate-resistant tumors to exogenous testosterone can cause cell death by different mechanisms of action.[86-90] In recent clinical experience, the predictable and controlled administration of supraphysiologic doses of exogenous testosterone has been shown to benefit a subset of patients with CRPC, an approach termed bipolar androgen therapy (BAT).[91-95]

Mechanisms of action of BAT include the following: (1) stabilization of the link between DNA and the AR, which prevents AR degradation and DNA re-licensing, resulting in cell death during the cell cycle; and (2) induction of breaks in double-stranded DNA (dsDNA), which leads to chromosomal rearrangements and cell death. Other speculated potential mechanisms are the following: inhibition of the expression of AR-V7,[86-90] modulation of the expression of some oncogenes, such as c-*MYC*[96,97] and *SKP2*,[98,99] and inhibition of the progression to a neuroendocrine prostate cancer phenotype.[95] BAT has shown impressive results in clinical trials, in

Table 3. Clinical Trials Evaluating Anti-androgen Re-challenge After BAT

Study First Author	Inclusion Criteria	Decline in AR-V7+ Status With BAT	Post-BAT Therapy	Responses to Post-BAT Therapy
Schweizer[91] (N=16)	mCRPC after first- or second-generation anti-androgens	Not reported	First- or second-generation anti-androgens, abiraterone, or enzalutamide	Abiraterone PSA_{50}: 100% Enzalutamide PSA_{50}: 75%
Teply[93] (N=30)	mCRPC after enzalutamide	–100% (3/3)	Enzalutamide	PSA_{50}: 52%
Markowski[94] (N=59)	mCRPC after abiraterone or after enzalutamide	–90% (9/10)	Abiraterone or enzalutamide	Enzalutamide rechallenge PSA_{50}: 68% Abiraterone rechallenge PSA_{50}: 16%
Denmeade[95] (N=195)	mCRPC after abiraterone	Not reported	Enzalutamide	PSA_{50}: 77.8%

AR-V, AR splice variant; BAT, bipolar androgen therapy; mCRPC, metastatic castration-resistant prostate cancer; PSA50, percentage of patients with a decline in PSA of 50% or greater.

both hormone-sensitive and castration-resistant disease,[91-95] which are summarized in Table 2.

Other studies evaluating BAT in different settings (NCT02090114, NCT03522064), in combinations (NCT03516812, NCT03554317, NCT04558866), and with biomarkers of response (NCT04424654) are ongoing. BAT has shown promising activity not only with respect to PSA and radiographic responses but also for its capability to (re-)sensitize tumors to anti-androgens.[93-95] In at least 3 studies, re-challenge with anti-androgens (after BAT) has demonstrated significant and durable PSA responses, in addition to encouraging clinical benefit (with some patients achieving long-term responses). Also, some studies have demonstrated that BAT may decrease AR-V7 expression, perhaps one reason why tumors are (re-)sensitized to anti-androgens after BAT (Table 3).

One of these studies is the phase 2 TRANSFORMER trial, which randomly assigned 195 patients with metastatic CRPC following abiraterone to receive either BAT or enzalutamide.[95] Crossover was permitted at progression. The patients who received BAT and then crossed over to enzalutamide (BAT→enzalutamide) had superior outcomes in comparison with those who received enzalutamide and then crossed over to BAT (enzalutamide→BAT). The rates of PSA_{50} response to the crossover therapy were 77.8% vs 23.4% (P<.001), the objective response rates were 28.6% vs 7.3% (P=0.03), and the times to PSA progression were 10.9 vs 1.1 months (P<.001), all endpoints favoring the BAT→enzalutamide sequence.[95] Median delayed PFS (PFS2, defined as the time from the initiation of therapy to progression on crossover) and OS in the patients who received BAT→enzalutamide were superior to median delayed PFS2 and OS in the patients who received enzalutamide→BAT: PFS2, 28.2 vs 19.6 months (P=.02); OS, 37.2 vs 29 months (P=.01).[95]

The reasons why BAT restores the activity of anti-androgens may be related to the following mechanisms: (1) decrease or eradication of *AR* overexpression[100] and (2) inhibition, delay, or reversal of the development of AR splice variants (including AR-V7),[93-95] all of which are mechanisms of resistance to anti-androgens and associated with a poor prognosis.[26,27,101,102] In addition, BAT may mitigate the "darwinian" treatment-induced selective pressure triggered by ADT and anti-androgens[28] in prostate cancer cells. This phenomenon is responsible for the development of treatment-emergent small cell neuroendocrine prostate cancer, which is an AR-independent lethal subtype of prostate cancer that is identified in up to 17% of patients.[29-31] Studies evaluating BAT in combination with other therapies are ongoing (see eTable at www.hematologyandoncology.net).

AR-Targeting Vaccines

A novel strategy that is being explored in prostate cancer is the use of AR-targeting vaccines.[103] Although immunotherapy has not demonstrated clinically meaningful efficacy results in unselected patients with prostate cancer so far, with the exception of sipuleucel-T (Provenge, Dendreon), a continuous effort is being made to discover novel ways to elicit an immune response that could lead to clinical benefit in patients with advanced disease. The AR LBD has been identified and explored as an immunotherapeutic target because its sequence is identical among humans and other species.[104,105]

In a preclinical study of a DNA vaccine encoding the LBD of the AR (pTVG-AR, MVI-118), Olson and colleagues demonstrated that administering the pTVG-AR

vaccine to male mice elicited AR-specific CD8+ T cells and led to the prolonged survival of prostate-bearing mice with no damage to normal tissues.[105,106] The recently published first-in-human phase 1 study of the pTVG-AR vaccine for advanced prostate cancer included 40 patients with metastatic HSPC, 10 in each of the 4 study arms; different treatment schedules with or without granulocyte-macrophage colony–stimulating factor (GM-CSF) were evaluated.[107] In terms of safety, no grade 3 or 4 AEs were observed, and the treatment was well tolerated. A Th1-type response toward the AR LBD developed in 47% of the 30 patients with available samples for immune activation analysis,, and the patients with T-cell immunity demonstrated a significantly prolonged time to PSA failure in comparison with those without the antigen-specific immune response. This strategy of using the AR as an immune target is currently being explored in ongoing clinical trials in which AR-targeting vaccines are being combined with programmed death 1 (PD-1) checkpoint inhibitors to treat patients with advanced prostate cancer (NCT04090528, NCT03600350).

Future Perspectives

Even after the development and introduction of newer and more potent anti-androgens, the AR pathway continues to be active and is responsible for prostate cancer progression as resistance to these therapies develops over time. Therefore, use of the novel AR pathway inhibitors that have been addressed in this review will certainly gain ground in the therapy of patients with prostate cancer during the next several years. Given the pivotal role of inhibition of the AR pathway in the treatment of prostate cancer, the AEs caused by the introduction of anti-androgens earlier in the disease history need to be mitigated. Because novel anti-androgens are now part of the standard-of-care therapies for earlier stages of the disease, including nonmetastatic CRPC and hormone-sensitive disease, the development of chronic AEs will become more common in clinical practice. Therefore, novel approaches and therapies that target the AR pathway without the use of "classic" anti-androgens may improve patients' survival and quality of life.

Disclosures

Dr Antonarakis is a paid consultant/advisor to Janssen, Astellas, Sanofi, Dendreon, Pfizer, Amgen, Lilly, Bayer, AstraZeneca, Bristol Myers Squibb, Clovis, and Merck; has received research funding to his institution from Janssen, Johnson & Johnson, Sanofi, Dendreon, Genentech, Novartis, Tokai, Bristol Myers Squibb, AstraZeneca, Clovis, and Merck; and is the co-inventor of an AR-V7 biomarker technology that has been licensed to Qiagen. This work by Dr Antonarakis was partially supported by National Institutes of Health Cancer Center Support Grant P30 CA006973 and by Department of Defense grants W81XWH-16-PCRP-CCRSA and W81XWH2010079. Drs Velho and Bastos report no conflicts of interest.

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eTable. Ongoing Clinical Trials Evaluating New Approaches Targeting AR-Related Pathways

Approach	Inclusion Criteria	Design	Number of Patients	Principal Outcomes	Identifier
Bipolar androgen therapy					
BAT + radium 223	mCRPC	Phase 2, single arm	47	Median rPFS	NCT04704505
BAT + darolutamide	mCRPC after abiraterone	Phase 2, single arm	47	Rate of rPFS at 12 mo	NCT04558866
BAT + nivolumab	mCRPC after abiraterone or after enzalutamide	Phase 2, single arm	44	PSA response	NCT03554317
BAT	mCRPC after abiraterone	Pilot, biomarker	20	PSMA gallium Ga 68 uptake and response to BAT	NCT04424654
BAT	Cohort A: mCRPC after enzalutamide; Cohort B: mCRPC after abiraterone; Cohort C: mCRPC after first-line castration-only therapy; Cohort D: mCRPC with inactivating mutations in ≥2 of the genes *TP53*, *PTEN*, and *RB1*	Multicohort	110	PSA response to BAT; PSA response to abiraterone or enzalutamide after BAT; PSA response to castrate levels of testosterone after BAT	NCT02090114
BAT	mCRPC with deleterious mutation in one of the homologous recombination genes	Phase 2, single arm	30	PSA response to BAT	NCT03522064
BAT + olaparib	mCRPC	Phase 2, single arm	30	PSA response to BAT; safety	NCT03516812
BET/bromodomain, CBP, and p300 inhibitors					
CCS1477 monotherapy CCS1477 + abiraterone or enzalutamide	mCRPC after abiraterone/enzalutamide and docetaxel	Phase 1/2a, single arm	120	Safety; biomarker analysis; PSA response	NCT03568656
PLX2853	Advanced malignancies	Phase 1/2a	166	Safety; overall response rate	NCT03297424
PLX2853 + abiraterone + olaparib	mCRPC	Phase 1/2a	110	Disease response; safety	NCT04556617
ZEN-3694 + enzalutamide + pembrolizumab	mCRPC and small cell carcinoma	Phase 2	54	Response rate	NCT04471974
N-terminal domain inhibitor					
EPI-7386	mCRPC after abiraterone/enzalutamide and docetaxel	Phase 1	40	Safety	NCT03888612
Proteolysis-targeting chimera (PROTAC)					
ARV-110	mCRPC after abiraterone/enzalutamide and docetaxel	Phase 1/2	150	Safety	NCT03888612
AR-targeted vaccine					
pTVG-HP +/- pTGV-AR and pembrolizumab	mCRPC after abiraterone or enzalutamide	Phase 2, randomized	60	PFS	NCT04090528

AR, androgen receptor; BAT, bipolar androgen therapy; BET, bromodomain and extraterminal; CBP, CREB-binding protein; mCRPC, metastatic castration-resistant prostate cancer; mo, months; No., number; PFS, progression-free survival; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; rPFS, radiographic progression-free survival.