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REVIEW ARTICLE

Targeting the androgen receptor in the management of castration-resistant prostate cancer: rationale, progress, and future directions

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ABSTRACT

Since the year 2000, tremendous progress has been made in the understanding of castration-resistant prostate cancer (CRPC), a disease state now recognized to retain androgen receptor (AR)—dependency in most cases. That understanding led to the rational design of novel therapeutic agents targeting hormonal pathways in metastatic CRPC. Two new drugs—the CYP17 inhibitor abiraterone acetate and the potent AR antagonist enzalutamide—were recently shown to prolong overall survival after chemotherapy treatment in patients with metastatic disease, with the former agent also demonstrating impressive activity in the pre-chemotherapy setting. Other new drugs targeting the AR—as well as drugs targeting heat shock proteins that protect cytoplasmic AR from degradation—are currently undergoing clinical development.

This review briefly describes the molecular mechanisms underlying castration resistance and hormonal dependence in prostate tumours and summarizes the current ongoing and completed clinical trials that are targeting hormonal pathways in metastatic CRPC. Potential mechanisms of resistance to these novel hormonal agents are reviewed. Finally, future research directions, including questions about drug sequencing and combination, are discussed.

KEY WORDS

Prostate, androgen, castration, bicalutamide, abiraterone, enzalutamide, orteronel, galeterone, CYP17, ARN-509, ODM-201

1. INTRODUCTION

Since the 1940s, hormonal manipulation has been the mainstay of first-line treatment for metastatic prostate cancer. Unfortunately, that approach is not curative, and resistance inevitably arises. However, a conceptual shift in the understanding of prostate cancer has occurred recently, as it became apparent that castration resistance (that is, when systemic testosterone is measured below castrate levels) does not necessarily imply hormonal resistance altogether and that further hormonal manipulation can result in ongoing biochemical, clinical, and radiologic responses. Indeed, that understanding has changed the terminology used to define metastatic prostate cancer, now designated "castration-resistant" prostate cancer (CRPC) rather than "hormone-resistant." In this review, we briefly summarize the molecular evidence demonstrating that CRPC retains sensitivity to hormonal manipulation, and we describe the emergence of novel hormonal drugs for metastatic prostate cancer. The current ongoing clinical trials and literature on resistance to those drugs are reviewed, and future challenges are discussed.

2. THE MOLECULAR BASIS UNDERLYING RETAINED HORMONE SENSITIVITY IN CRPC

It was at the start of the 1990s that amplification of the androgen receptor (AR) locus was shown to occur in approximately 30% of castration-refractory prostate tumours, but not in tumours before therapy[1]. In parallel, novel promiscuous mutations in the AR were found in prostate cancer cells from patients with androgen-insensitive metastatic disease[2]. These pioneering observations suggested that cellular resistance to androgen-deprivation therapy is not necessarily a result of the acquisition of growth independence from testosterone, as was previously believed, but rather that it might be a result of cellular acquisition of mechanisms to overcome castrate-levels of testosterone. Other mechanisms found to be associated with retained hormonal sensitivity include enhanced intracellular conversion of adrenal androgens to testosterone and dihydrotestosterone in prostate cancer cells[3], intratumoral androgen synthesis[4], increased expression of AR messenger RNA (mRNA)[5], and ligand-independent AR activation[6]. Additionally, it was also realized that, in the absence

of androgen, AR is maintained in the cytoplasm by association with heat shock proteins (also called "chaperones") that maintain the AR in an inactive and yet highly responsive form and that protect it from proteolysis[7]. Those proteins thus also became attractive targets for indirect modulation of the AR signalling pathway.

3. CLINICAL DATA TO SUPPORT RETAINED HORMONAL SENSITIVITY IN CRPC

In parallel with the enormous progress in the molecular characterization of *in vitro* and *in vivo* castration resistance, clinical practice also supported the role of further hormonal treatment after the emergence of castration resistance. Ketoconazole, an imidazole antifungal agent, suppresses the multistep process of adrenal and intratumoral steroidogenesis by inhibiting the 17,20-lyase and 17α-hydroxylase enzymatic activities of CYP19, desmolase, and 11β-hydroxylase (reviewed in Reid *et al.*[8]). Formal clinical trials showed that ketoconazole was indeed able to elicit responses in some metastatic CRPC patients (reviewed in Keizman *et al.*[9]). Notwithstanding the fact that the responses to ketoconazole were generally of short duration, the relevant trials did provide clinical "proof-of-concept" to the retained hormonal sensitivity of prostate cancer cells in patients with castration-resistant disease.

4. NEW AND EMERGING AGENTS IN THE TREATMENT OF CRPC

The growing body of molecular and clinical knowledge in the castration-resistant setting led to rational design of novel agents targeting AR signalling. The resulting agents can generally be divided into two major classes based on their mechanism of action: agents that target steroidogenesis[8] and rationally designed second-generation pure AR antagonists (Table I, Figure 1).

4.1 Agents Targeting Steroidogenesis

4.1.1 Abiraterone Acetate

Abiraterone acetate [AA (Zytiga: Janssen Biotech, Horsham, PA, USA)] is an orally administered drug that inhibits 17α-hydroxylase and C17,20-lyase, two enzymatic steps that are required to synthesize androgens from cholesterol. The phase I trials suggested a daily dose of 1000 mg for further exploration on the basis of hormonal pharmacodynamics and provided further proof-of-concept that CRPC remains hormonally driven[10]. Two phase II trials ensued, demonstrating that the drug has activity in both the pre- and post-chemotherapy setting[11,12]. The first phase III trial was a randomized double-blind placebo-controlled trial of AA and prednisone compared with prednisone alone in almost 1200 CRPC patients who progressed after docetaxel treatment [COU-AA-301 (search for NCT00638690 at http://clinicaltrials.gov)]. The study was unblinded after the first interim analysis demonstrated an improvement in overall survival by approximately 4 months for the AA–prednisone group [14.8 months vs. 10.9 months; hazard ratio (HR): 0.65; 95% confidence interval (CI): 0.54 to 0.77; $p < 0.001$][13]. At the final update, with a median follow-up of 20.2 months, median overall survival was longer for the AA group than for the placebo group (15.8 months vs. 11.2 months; 95% CI: 14.8 to 17.0 months vs. 10.4 to 13.1 months; HR: 0·74; 95% CI: 0·64 to 0·86; $p < 0·0001$). Median time to prostate-specific antigen (PSA) progression (8.5 months in the AA group vs. 6.6 months in the placebo group; 95% CI: 8.3 to 11.1 months vs. 5.6 to 8.3 months; HR: 0.63; 95% CI: 0.52 to 0.78; $p < 0·0001$), median radiologic progression-free survival (5.6 months vs. 3.6 months; 95% CI: 2.9 to 5.5 months vs. 5.6 to 6.5 months; HR: 0.66; 95% CI: 0.58 to 0.76; $p < 0·0001$), and proportion of patients experiencing a PSA response [235 of 797 patients (29.5%) vs. 22 of 398 patients (5.5%), $p < 0·0001$] were all improved in the AA group compared with the placebo group.

A second clinical trial in the pre-chemotherapy setting [COU-AA-302 (search for NCT00887198 at http://clinicaltrials.gov)], recruited approximately 1000 patients with asymptomatic or minimally symptomatic CRPC to receive either AA and prednisone or prednisone alone. At the second planned interim analysis, it was found that AA plus prednisone produced a statistically significant improvement in radiologic progression-free survival (8.3 months vs. not reached; HR: 0.43; $p < 0.0001$) and a strong trend toward increased overall survival (27.2 months vs. not reached; HR: 0.75; $p = 0.0097$). On all secondary endpoints, AA plus prednisone resulted in clinically and statistically significant effects, including time to chemotherapy initiation, time to opiate use, time to deterioration in Eastern Cooperative Oncology Group performance status, and time to PSA progression[14]. No important new safety signals were seen. The independent data monitoring committee unanimously recommended unblinding the study and allowing crossover of patients to the treatment group, which approximately 25% of the patients did. Given that the trial was stopped after the second interim analysis (after 311 events), the p value for overall survival did not reach the pre-specified O'Brien–Fleming boundary for significance ($p = 0.008$). The impact of that result on regulatory ability to register the drug in the pre-chemotherapy setting remains to be seen.

Despite those impressive results, a few points in clinical practice require discussion. First, the phase I clinical trial assessed AA in the fasting and the fed states, and although the recommended dose for further investigation was set at 1000 mg on an empty stomach, drug exposure was increased by a

TABLE I Novel pharmacologic agents targeting the androgen receptor (AR) signalling pathway

Mechanism of action	*Generic name*	*Stage in clinical development (at September 2012)*	*Remarks*
CYP17 modulator	Abiraterone acetate	Approved for post-chemotherapy setting; completed phase III for pre-chemotherapy	Prolongs OS in post-chemotherapy setting; trend towards OS prolongation in pre-chemotherapy setting
	TAK-700 (orteronel)	Phase III trials in pre- and post-chemotherapy settings	Relative selectivity for the 17,20-lyase enzymatic activity of CYP17
	TOK-001 (galeterone)	Completed phase I; awaiting phase II	Also a potent AR antagonist
Antiandrogen	MDV3100 (enzalutamide)	Approved for post-chemotherapy setting; phase III trial for pre-chemotherapy setting	Prolongs OS in post-chemotherapy setting
	ARN-509	Phase II	Competitive AR inhibitor, fully antagonistic
	ODM-201	Phase II	Blocks nuclear translocation of AR
	EZN-4176	Phase I	Antisense oligonucleotide to AR
Chaperone inhibitor	OGX-427	Phase II	Antisense oligonucleotide to Hsp-27
Androgen receptor modulator	EPR-001	Preclinical	Blocks transactivation of the N-terminal domain of AR

factor of more than 4 when the drug was administered with high-fat food. Several ongoing clinical trials are currently assessing the effect of food on the pharmacokinetics of, and the response to, AA at various dose levels (search for NCT01575587, NCT01543776, NCT01424930 at http://clinicaltrials.gov). Those studies may result in a novel paradigm to reduce the drug expenditure associated with AA. Although the potential for drug–drug interactions is less than that with ketoconazole, AA is an inhibitor of the CYP2D6 enzyme and a substrate of CYP3A4. Potential interactions of AA with commonly prescribed medications such as selective serotonin re-uptake inhibitors, clopidogrel, beta-blockers with ketoconazole and verapamil are commonly overlooked. Additionally, a phenomenon of "tumour flare"—namely, the appearance of radiologic progression on bone scans or of PSA increase, or both—during the first 12 weeks of AA treatment has been described in up to 50% of patients, most of whom were reported to have had a subsequent response or stabilization of disease[15]. Finally, recently presented retrospective data suggest that, in the context of PSA progression on AA, a clinically significant median lag time of 5.7 months until the appearance of radiologic or clinical progression (or both) was observed[16]. Indeed, in AA-receiving PSA progressors (n = 141), radiologic and clinical stable disease of 1 year or more, 2 years or more, and 3 years or more was observed in 43 (30.5%), 21 (14.9%), and 12 (8.5%) patients respectively. Interestingly, that observation suggests that biochemical escape does not necessarily signify significant disease progression and argues for a consideration of maintenance or continuation of therapy in future trials.

Future development plans for AA focus on assessing the drug in combination with a wide range of chemotherapeutic, biologic, immunologic, and hormonal agents to improve activity (Table II) or the drug's role in earlier disease states, and even in the high-risk curative setting.

4.1.2 Orteronel (TAK-700)

Orteronel (TAK-700) is a CYP17 inhibitor with relative selectivity for 17,20-lyase over 17α-hydroxylase[17], suggesting that it may not have the profound mineralocorticoid effects that AA does. In a phase I/II study in patients with metastatic CRPC, TAK-700 lowered the levels of testosterone and dehydroepiandrosterone

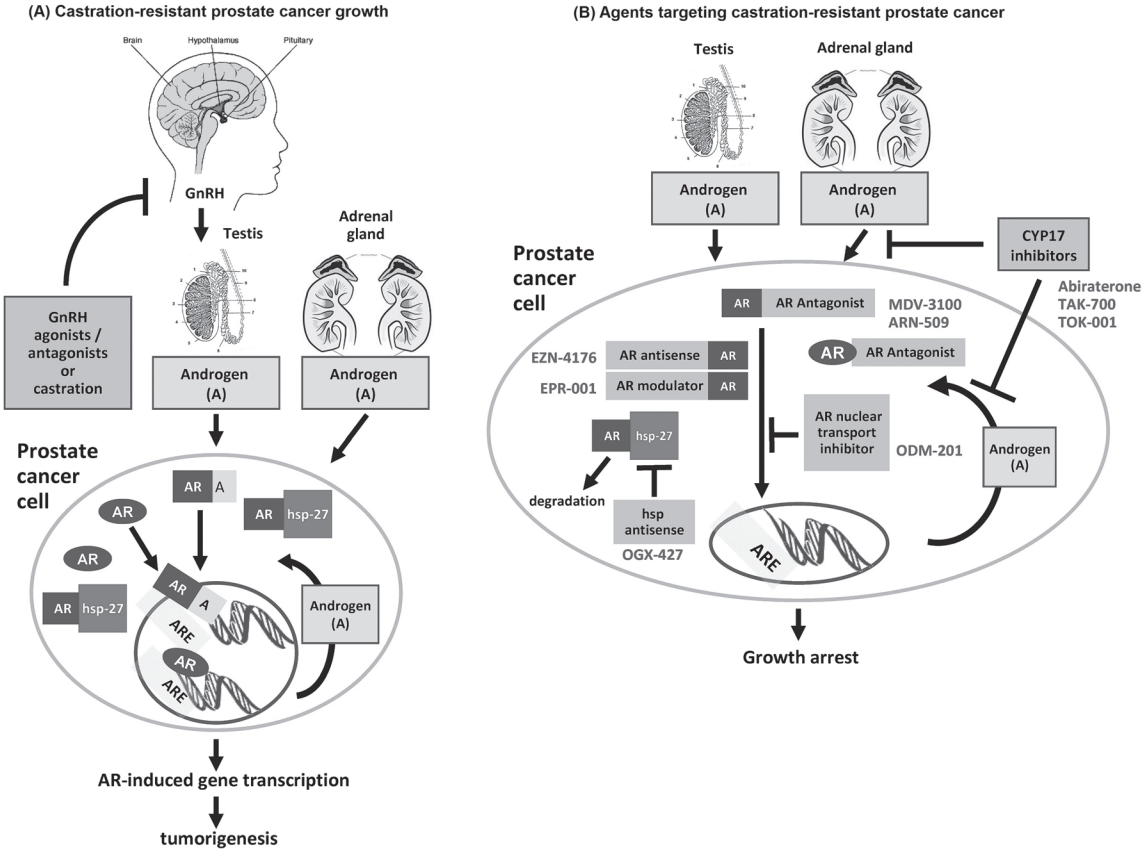

FIGURE 1 (A) Prostate cancer cell growth in the presence of castration can be maintained through intratumoral or adrenal production of androgen (light blue box), by overexpression of wild type androgen receptor (AR, rectangular blue box) or mutated or alternatively-spliced AR (oval blue box). Heat shock protein 27 (Hsp-27, red box) prevents degradation of cytoplasmic unbound AR. Androgen-bound AR or mutated or spliced AR transports to the nucleus, binds to androgen-responsive elements on the DNA, leading to AR-induced gene transcription and cellular growth. (B) Agents targeting castration-resistant prostate cancer include CYP17 inhibitors, AR antagonists, AR modulators, AR antisense molecules, AR nuclear transport inhibitors, and Hsp antisense molecules (grey boxes). The generic names of the compounds currently being evaluated in clinical trials are depicted in red. GnRH = gonadotropin-releasing hormone; ARE = androgen response element.

sulfate consistent with 17,20-lyase inhibition and lowered PSA levels in all patients treated with doses of 300 mg or more twice daily[18]. The phase II portion of the study, which accrued 96 chemotherapy-naïve subjects with castrate levels of testosterone, included 4 additional dose cohorts and showed that at a dose of 400 mg twice daily with prednisone 5 mg twice daily, 52% of patients experienced a PSA reduction of 50% or more at 12 weeks. The most common grade 3 or greater adverse events were fatigue and diarrhea[19]. The response and side-effect profile in patients receiving 300 mg twice daily without prednisone was recently presented, highlighting the appearance of dyspnea, hypertension, hypokalemia, and pneumonitis as potential new serious side effects and showing that a decline of more than 50% in PSA and a significant decline in testosterone was obtained in most of the cohort[20].

The two ongoing phase III clinical trials—one in the pre-chemotherapy setting (search for NCT01193244 at http://clinicaltrials.gov), the other in the post-chemotherapy setting (search for NCT01193257 at http://clinicaltrials.gov)—have added prednisone 5 mg twice daily to the treatment regimen. The trial in the pre-chemotherapy setting has now been closed to accrual, but crossover of patients to AA may hamper achievement of an overall survival endpoint.

4.1.3 Galeterone (TOK-001)

Galeterone (also known as TOK-001 and initially designated VN/124–1) was rationally designed to inhibit the human CYP17 enzyme, but was also found to be a potent pure AR antagonist and to effectively prevent the binding of synthetic androgens to both mutant and wild-type AR[21,22]. The results of the phase I trial of TOK-001 (search for NCT00959959 at http://clinicaltrials.gov) were published during the American Society of Clinical Oncology 2012 annual meeting, demonstrating that galeterone was generally

TABLE II Clinical trials of combination therapies with abiraterone acetate (AA)

Combination type	*Drug (mechanism of action)*	*Trial*	*Phase*	*Rationale*
Chemotherapy	Cabazitaxel Docetaxel	NCT01511536 NCT01400555	I/II I	Addition of AA to chemotherapy may prolong PFS and ultimately OS by additive mechanisms of action
Biologic therapy	AT-13387 (Hsp-90 inhibitor)	NCT01685268	I/II	Dual mechanism of androgen receptor blockage
	Dasatinib (Src inhibitor)	NCT01685125	II	Src expression may contribute to androgen resistance; may be synergistic in bone
	ABT-888, veliparib (PARP inhibitor)	NCT01576172	II	Response to treatment will be stratified according to the existence of *ETS* gene fusions
	GDC-0068 (Akt inhibitor) or GDC-0980 (PI3K or Torc1/2 inhibitor)	NCT01485861	IB/II	Suggestion from preclinical models that Akt pathway blockade would be synergistic
	OGX-427 (Hsp-27 antisense)	NCT01681433	II	Blocking Hsp-27 may enable cancer cells to regain sensitivity to AA after PSA progression
	AMG-386 (anti-angiogenic)	NCT01553188	II	Angiogenesis as a mechanism of escape from hormonal treatment
	Metformin	NCT01677897	II	Metformin perturbates the PI3K/Akt pathway and through activation of AMPK
	XL-184, cabozantinib (Met or VEGFR inhibitor)	NCT01574937	I	Synergistic or additive effect on bone metastases
	Sunitinib *or* dasatinib	NCT01254864	II	Combined targeting of tyrosine kinase and hormonal pathways
	BEZ235, BKM120 (Akt or MTOR inhibition)	NCT01634061	IB	Suggestion from pre-clinical models that Akt pathway blockade would be synergistic
Hormonal therapy	Dutasteride	NCT01393730	II	Increasing potency of testosterone blockade
	Enzalutamide	NCT01650194	II	Complementary mechanisms of action and *in vitro* evidence of cross-resistance
Immunologic therapy	Sipuleucel-T	NCT01487863	II	Safety only
	Ipilimumab (CTLA-4 antagonist)	NCT01688492	I/II	Tumour death induced by AA may provoke more potent immune response
Combination therapy	Zoledronic acid–docetaxel–AA	NCT00268476	II	Explore mechanisms to increase utility of androgen deprivation

PFS = progression-free survival; OS = overall survival; PSA = prostate-specific antigen; VEGFR = vascular endothelial growth factor receptor; MTOR = mammalian target of rapamycin.

well tolerated in CRPC patients and demonstrated some clinical activity[23]. Galeterone is being reformulated after additional pharmacokinetic study, and phase II trials are planned (search for NCT01709734 at http://clinicaltrials.gov).

4.2 Agents That Block the AR

4.2.1 Enzalutamide (MDV3100)

Enzalutamide (MDV3100) was chosen for further investigation after a screen for nonsteroidal

antiandrogens that retain antagonistic activity in the setting of increased AR expression[24]. Enzalutamide was shown to bind to the AR with 10 times greater affinity relative to bicalutamide, to reduce the efficiency of AR nuclear translocation, and to impair both DNA binding to androgen response elements and recruitment of co-activators. Importantly, in contrast to bicalutamide, which exhibited context-dependent agonistic activity, enzalutamide was shown to be a pure antagonist of the AR[24]. Antitumour responses were observed at all dose levels tested in the phase I/II trial, and the most common grade 3 or 4 adverse event was fatigue[25]. A phase III trial of enzalutamide in CRPC patients post-chemotherapy was soon initiated, randomizing participants 2:1 in favour of the drug compared with placebo, with a primary endpoint of overall survival (AFFIRM study). That trial was positive, with a median overall survival of 18.4 months in the enzalutamide group compared with 13.6 months in the placebo group (HR for death in the enzalutamide group: 0.63; 95% CI: 0.53 to 0.75; $p < 0.001$). The superiority of enzalutamide over placebo was shown with respect to all secondary endpoints examined, including biochemical, radiologic, and palliative endpoints. Fatigue, diarrhea, and hot flashes occurred more frequently in the treatment arm, and seizure occurred in 0.6% of the patients in that arm[26]. The study was closed, and crossover of the remaining patients was allowed. The PREVAIL study, a phase III study of enzalutamide in the pre-chemotherapy setting is now closed to accrual (search for NCT01212991 at http://clinicaltrials.gov), and other ongoing studies are exploring the use of enzalutamide in the neoadjuvant setting (search for NCT01547299 at http://clinicaltrials.gov) and in comparison with bicalutamide (search for NCT01288911 and NCT01664923 at http://clinicaltrials.gov).

4.2.2 ARN-509

ARN-509, a competitive AR inhibitor, is fully antagonistic to AR overexpression. ARN-509 was shown to bind to the AR and to inhibit growth and androgen-mediated gene transcription *in vitro,* to impair nuclear localization and DNA binding of AR, and to inhibit tumour growth in a xenograft model[27]. The results of the phase I study were recently presented. In the 30 patients enrolled, the most common grades 1 and 2 treatment-related adverse events were fatigue, nausea, and pain. Pharmacokinetics were shown to be linear and dose-dependent. At 12 weeks, 42% of the patients had experienced a 50% or greater decline in PSA, and at 4 weeks, imaging by ^{18}F-fluoro-5α-dihydrotestosterone positron-emission tomography demonstrated AR blockade across multiple dose levels. That trial selected the optimal biologic dose of 240 mg daily for phase II investigation[28]. The phase II portion of a multicentre phase I/II study (search for NCT01171898 at http://clinicaltrials.gov) is evaluating the activity of ARN-509 in 3 distinct patient populations of men with CRPC (high-risk non-metastatic CRPC, metastatic treatment-naïve CRPC, and progressive disease after AA), and further phase III trials are planned.

4.2.3 ODM-201

ODM-201 was designed as an AR antagonist that works by blocking AR nuclear translocation, with no agonistic activity in the context of AR overexpression. The results of a first-in-humans, multicentric phase I/II dose-escalation trial in progressive metastatic CRPC [the ARADES trial (search for NCT013117641 at http://clinicaltrials.gov)] were recently presented. ODM-201 was administered orally to patients either before or after docetaxel treatment. In 21 treated patients at several dose levels, ODM-201 has been well tolerated, its most common reported side effects being asthenia, nausea, and diarrhea. A PSA decline of 50% or more was obtained in 13 of 15 patients (87%) at 12 weeks, including all 6 patients previously treated with docetaxel[29]. The dose-escalation part of the study is still ongoing at a dose level of 700 mg twice daily, and an expansion of the phase II trial started in June 2012.

4.2.4 EZN-4176

EZN-4176 is a nucleic acid–based antisense oligonucleotide targeted against the mRNA of AR. When administered as a single agent, it was shown to specifically downregulate AR mRNA and protein, in concert with inhibition of the growth of both androgen-sensitive and CRPC tumours *in vitro* and *in vivo*—an effect that was not seen with a nonspecific antisense oligonucleotide[30]. This agent is currently being evaluated in a phase I clinical trial (search for NCT01337518 at http://clinicaltrials.gov).

4.3 Targeting Heat Shock Proteins

Heat shock protein 27 (Hsp27) is a cytoprotective chaperone of AR that is expressed in response to many stress signals and that regulates key effectors of the apoptotic machinery. Its levels increase after androgen ablation and facilitate cancer growth[31,32], suggesting it as a therapeutic target. Indeed, antisense knockdown of Hsp27 delayed prostate cancer xenograft growth in a mouse model[31]. OGX-427, a second-generation antisense oligonucleotide that inhibits Hsp27 expression, was well tolerated and showed single-agent activity in phase I studies. Results of the first stage in the phase II randomized study of OGX-427 plus prednisone compared with prednisone alone were recently presented. A PSA decline of 50% or more occurred in 11 of 22 patients (50%) on OGX-427 plus prednisone and in 4 of 20 patients (20%) treated with prednisone alone. A measurable disease response occurred in 4 of 9 patients (44%) on OGX-427 plus prednisone (1 complete, 3 partial) and in 0 of 12 patients on prednisone alone. The main

adverse events were infusion-related[33]. The second stage in this phase II trial (search for NCT01454089 at http://clinicaltrials.gov) is continuing to accrue now, and a trial assessing the addition of OGX-427 to AA upon PSA progression is about to start accrual (search for NCT01681433 at http://clinicaltrials.gov).

5. MECHANISMS OF RESISTANCE AND CROSS-RESISTANCE, AND THERAPEUTIC IMPLICATIONS

Unfortunately, resistance to these newly emerging hormonal drugs inevitably occurs, and some patients never respond to hormonal manipulations beyond castration.

Theoretically, resistance can occur as a result of nonhormonal signalling pathways "taking over" cellular growth in an AR-independent manner or of acquisition of resistance within an intact and functional AR hormonal axis. Clearly, the exact mechanism of resistance has significant implications for further disease management, and thus it is currently the object of intense research efforts.

5.1 Abiraterone Acetate

In prostate cancer xenograft models, AA treatment imposed selective pressure for increased intratumoral expression of CYP17A1[34] and led to induction of AR and AR splice variants that confer ligand-independent AR transactivation[35]. Indeed, intratumoral expression of CYP17A1 was markedly increased in tumour biopsies from CRPC patients after CYP17A1 inhibitor therapy[34]. Recently, it was hypothesized that AA can also inhibit 3β-hydroxysteroid dehydrogenase or isomerase, which is absolutely required for the intratumoral synthesis of dihydrotestosterone in CRPC; and indeed, *in vitro* work in CRPC cell lines demonstrated such inhibitory activity. That observation led the authors to hypothesize that resistance to AA treatment could potentially be overcome by dose escalation[36]. Parallel *in vitro* work also suggested that resistance may occur secondary to glucocorticoid-induced activation of mutated AR. That resistance was able to be reversed *in vitro* with the addition of AR antagonists such as enzalutamide or with a higher dose of AA[37].

5.2 Enzalutamide

Mechanisms of resistance to enzalutamide are currently obscure. Given that alternative AR splice variants were shown to arise as part of the acquisition of the castration-resistant phenotype (reviewed in Guo and Qiu[38]), it is tempting to speculate that this phenomenon underlies, at least in part, the "escape" of AR from its very potent antagonist. Some data support the concept that full-length AR is required for signalling even in presence of AR-splice variants, but more recent results demonstrate that estrogen receptor splice variants are constitutively active regardless of ligand or full-length AR[39]. Dysregulation of the PTEN/PI3K pathway was also associated with resistance to conventional antiandrogen therapies[40], and PTEN loss or PI3K pathway activation was suggested to function in a cell-autonomous manner to promote androgen- or AR-independent prostate cancer progression to castration resistance[41]. Indeed, recent work suggests that resistance to enzalutamide is associated with the upregulation of epithelial growth factor receptor and human epidermal growth factor receptor 2 expression *in vitro,* and that lapatinib treatment reduces the growth of enzalutamide-resistant prostate cancer cell lines[42].

Interestingly, very recent data suggest the presence of cross-resistance between AA and enzalutamide. A combined retrospective analysis of two abstracts from the recent European Society for Medical Oncology meeting, which reported on approximately 60 patients receiving AA after progression on enzalutamide, showed a minor PSA response rate of 5%[16,43]. Should that observation be confirmed in larger patient cohorts, it will have important treatment implications.

6. FUTURE RESEARCH DIRECTIONS IN HORMONAL TREATMENT OF CRPC

The treatment paradigm of metastatic CRPC has changed tremendously in only a very few years, with hormonal treatment now being re-established as a valid and survival-prolonging option in the post- and (probably) pre-chemotherapy setting. However, a number of issues about how to integrate these agents into clinical practice persist, and many registration challenges also remain to be addressed.

6.1 Determining the Sequence of Treatment

With the very recent U.S. Food and Drug Administration approval of two new hormonal drugs in the post-chemotherapy setting, the obvious question of how to sequence those two drugs is currently unanswered. Both drugs are indicated after docetaxel, and optimal timing or switching strategies remain unclear. As mentioned earlier, the activity of AA after enzalutamide appears to be small, and our initial anecdotal clinical experience suggests that the reverse sequence is equally unlikely to be a useful strategy. Those scenarios are further complicated by the availability of cabazitaxel and exposures to all of those drugs in the pre-chemotherapy setting.

6.2 Assessing Drug Combinations

Given that castration resistance is likely a consequence of several concurrent biologic processes, another potential avenue for investigation is combinations of

two or more hormonal drugs. Recent data provide a potential theoretical rationale for combined treatment with AA and enzalutamide, because it was suggested that progressive disease may occur on AA secondary to activation of mutated AR by either exogenous glucocorticoids, endogenous plasma prednisolone, or pharmacologic mineralocorticoid receptor antagonists often given to treat side effects, and that such activation might be mitigated by the addition of enzalutamide[44]. That approach is currently being explored (search for NCT01650194 at http://clinicaltrials.gov).

The idea of combining hormonal therapy and chemotherapy has also emerged, drawing its theoretical basis from the observations that taxanes exert their effect by preventing AR translocation to the nucleus[45,46]. In breast cancer, hormonal–chemotherapeutic combinations have proven to be ineffective and toxic[47], but the same case may not hold in prostate cancer. A multicentre open-label phase I/II study of orteronel in combination with docetaxel is currently evaluating the safety and pharmacokinetics of that combination in men with metastatic CRPC (search for NCT01084655 at http://clinicaltrials.gov). Although that approach may not emerge as a standard of care, it may be that the field evolves *de facto* rather than *de jure* into a model whereby these novel targeted drugs are continued through chemotherapy (as occurs with luteinizing-hormone releasing-hormone agonists and antagonists), because most men will now be receiving the new hormonal drugs in the pre-chemotherapy setting.

Lastly, a rationale for combining hormonal and biologic drugs in CRPC is emerging. Inhibition of AR led to activation of Akt signalling in a murine model, and combined pharmacologic inhibition of PI3K and AR signalling caused significant regressions of prostate cancer in a PTEN-deficient murine prostate cancer model and also in human prostate cancer xenografts, indicating that both pathways coordinately support survival[48]. Several trials of AA combined with biologic agents are ongoing (Table II) and will help to clarify that hypothesis (for example, search for NCT01485861 at http://clinicaltrials.gov).

6.3 Novel Methods to Target the AR

As a direct result of the improved understanding of the structure, cellular localization, and function of the AR in recent years, several novel methods of targeting it have been proposed and are now being tested preclinically. The N-terminal domain of the AR is crucial for its transcriptional activity independent of the existence of ligand[49]. This realization brought about the development of several drug candidates (for example, sintokamides, decoy peptides) that disrupt AR function by targeting that specific domain (reviewed in Sadar[50]).

For example, EPI-001 is a small molecule that was shown to block the transactivation of the N-terminal domain of AR. It reduced AR interaction with androgen response elements on target genes and blocked androgen-induced proliferation, leading to decreased tumour growth in xenograft models of CRPC[51]. Importantly, it inhibited constitutively active AR devoid of a ligand binding domain, suggesting that it may retain action in the setting of AR splice variants.

AZD-3514 is an AR downregulator that binds to the AR ligand binding domain, but in a manner distinct from that used by enzalutamide. This agent is currently being pursued in two phase I clinical trials (search for NCT01162395 and NCT01351688 at http://clinicaltrials.gov).

7. CONCLUDING REMARKS

The rapid and significant advancement in prostate cancer therapy in the last few years is clearly one of the more exciting examples of "bench to bedside" research, combining molecular delineation of cellular pathways with rational drug design and well-performed clinical trials. Agents with activity in the metastatic CRPC arena are now being thoroughly explored in earlier disease states (such as the neoadjuvant setting) or in localized high-risk patients in the hope of improving cure rates. Nonetheless, it is becoming clear that the heterogeneity of resistance will mandate the design of "personalized" sophisticated clinical trials to find appropriate therapeutic solutions.

8. CONFLICT OF INTEREST DISCLOSURES

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