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Review Article

Androgen receptor targeted therapies in castration-resistant prostate cancer: Bench to clinic

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Abbreviations & Acronyms
3βHSD1 = 3β-hydroxysteroid dehydrogenase type 1
ADT = androgen deprivation therapy
AF-1 = activation function-1
AF-2 = activation function-2
AR = androgen receptor
AR-V = androgen receptor splice variant
CRPC = castration-resistant prostate cancer
CTC = circulating tumor cell
CYP17A1 = 17α-hydroxylase/17,20-lyase
D4A = Δ^4-abiraterone
DBD = DNA-binding domain
DHEA = dehydroepiandrosterone
DHT = dihydrotestosterone
FL-AR = full-length AR
GR = glucocorticoid receptor
HR = hinge region
IL-6 = interleukin-6
LBD = ligand-binding domain
LHRH = luteinizing hormone-releasing hormone
mCRPC = metastatic castration-resistant prostate cancer
mTOR = mechanistic target of rapamycin
N/C = N-terminal/C-terminal
NLS = nuclear localization signal
NTD = amino-terminal domain
PET = positron emission tomography
PI3K = phosphatidylinositol 3-kinase
PR = progesterone receptor
PSA = prostate-specific antigen
SRD5A = steroid-5α-reductase
TAU = transcription activation unit

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Abstract: The androgen receptor is a transcription factor and validated therapeutic target for prostate cancer. Androgen deprivation therapy remains the gold standard treatment, but it is not curative, and eventually the disease will return as lethal castration-resistant prostate cancer. There have been improvements in the therapeutic landscape with new agents approved, such as abiraterone acetate, enzalutamide, sipuleucel-T, cabazitaxel and Ra-223, in the past 5 years. New insight into the mechanisms of resistance to treatments in advanced disease is being and has been elucidated. All current androgen receptor-targeting therapies inhibit the growth of prostate cancer by blocking the ligand-binding domain, where androgen binds to activate the receptor. Persuasive evidence supports the concept that constitutively active androgen receptor splice variants lacking the ligand-binding domain are one of the resistant mechanisms underlying advanced disease. Transcriptional activity of the androgen receptor requires a functional AF-1 region in its N-terminal domain. Preclinical evidence proved that this domain is a druggable target to forecast a potential paradigm shift in the management of advanced prostate cancer. This review presents an overview of androgen receptor-related mechanisms of resistance as well as novel therapeutic agents to overcome resistance that is linked to the expression of androgen receptor splice variants in castration-resistant prostate cancer.

Key words: androgen receptor, castration-resistant prostate cancer, EPI-506, novel agents, prostate cancer, splice variants.

Introduction

Prostate cancer represents the second most frequently diagnosed cancer in men worldwide, accounting for 15% of all male cancers.[1] In 2015, there were 220 800 estimated new cases of prostate cancer and 27 540 deaths by prostate cancer, making this disease the second leading cause of cancer-related death for North American men.[2] Despite that most new patients are diagnosed in early stage, still approximately 4% of patients will have metastatic cancer, and after local therapy approximately 20–30% of patients will relapse and require systemic therapies.[3] ADT causes a temporary reduction in prostate cancer tumor burden, but the malignancy will begin to grow again despite the lack of testicular androgens to form CRPC. A rising level of serum PSA after ADT indicates biochemical failure, the emergence of CRPC, and re-initiation of the AR transcription program (Fig. 1). Most patients succumb to mCRPC within 2–3 years of biochemical failure. Hence, the AR pathway plays a critical role for survival and growth of most CRPC, and constitutes an attractive therapeutic target because most advanced tumors that are resistant to current therapies still express functional AR.[4–7] Although there have been improvements in the therapeutic landscape with new agents approved for CRPC, such as abiraterone,[8,9] enzalutamide,[10,11] sipuleucel-T,[12] cabazitaxel[13] and Radium-223,[14] new resistance mechanisms have been elucidated through these treatments in advanced disease. The present article reviews recent advances of AR-related resistance mechanisms as well as the novel AR-targeted therapeutic agents to overcome resistance linked to the expression of constitutively active truncated AR-Vs in CRPC.

AR as a therapeutic target for CRPC

AR is a transcription factor and validated drug target for all stages of prostate cancer. The FL-AR protein consists of approximately 919 amino acids with regions of polymorphisms in

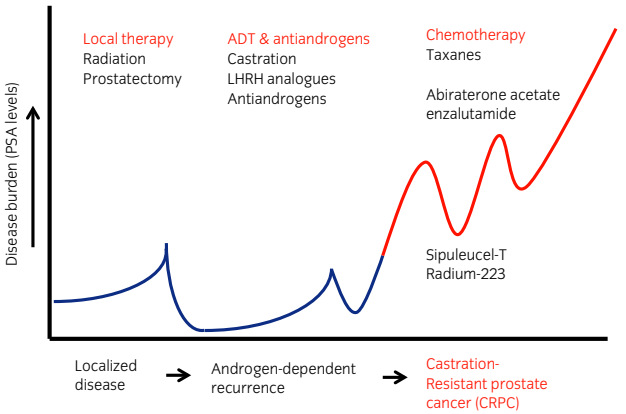

Fig. 1 Progression of prostate cancer to CRPC. Serum PSA levels correlate with disease burden. Localized disease can be cured by radiation therapy or radical prostatectomy. However, recurrent increasing PSA indicates regrowth of cancer. At the androgen-dependent disease state, ADTs reduce androgen produced by the testes. Ultimately, the disease will progress into a castration-resistant phenotype. Although there are several therapeutic agents available for CRPC, the cancer inevitably develops resistance to these therapeutic agents.

the proline, glutamine and glycine tracts. AR is a member of the steroid receptor family based on structural similarities that include three functional domains and a hinge region. Androgens and anti-androgens bind to the AR carboxy-terminal LBD, which is a folded domain; the nuclear translocation sequence is in the hinge region; the DBD is also a folded structure that binds to DNA sequences called AREs in the enhancers and promoters of target genes, such as PSA; and the NTD is predominantly intrinsically disordered and contains AF-1, which is necessary for the transcriptional activity of FL-AR and AR-Vs (Fig. 2).[15–17] The human *AR* gene is located on chromosome Xq11-12, and spans approximately 90 kb of DNA containing eight canonical exons. Exon 1 encodes the NTD, exons 2 and 3 encode the DBD, exon 4 encodes the hinge region and exons 5–8 encode the LBD.[18] The crystal structures of AR DBD and LBD have been resolved, but no crystal structures of the FL-AR or the NTD are available. There is little or no sequence homology between the AR NTD and the NTDs of other steroid or nuclear receptors.[19]

Androgens, such as testosterone and dihydrotestosterone, bind to LBD of FL-AR to initiate a cascade of events involving conformational changes and nuclear translocation, and binding of AR dimer to AREs on the DNA of target genes. AR LBD contains AF-2, which recruits co-activators and co-repressors to modulate its transcriptional activity on target genes. The NTD constitutes approximately 60% of the 110 kDa FL-AR protein and contains the transcriptional regulatory region, AF-1 (amino acid 142–485). Two TAUs exist

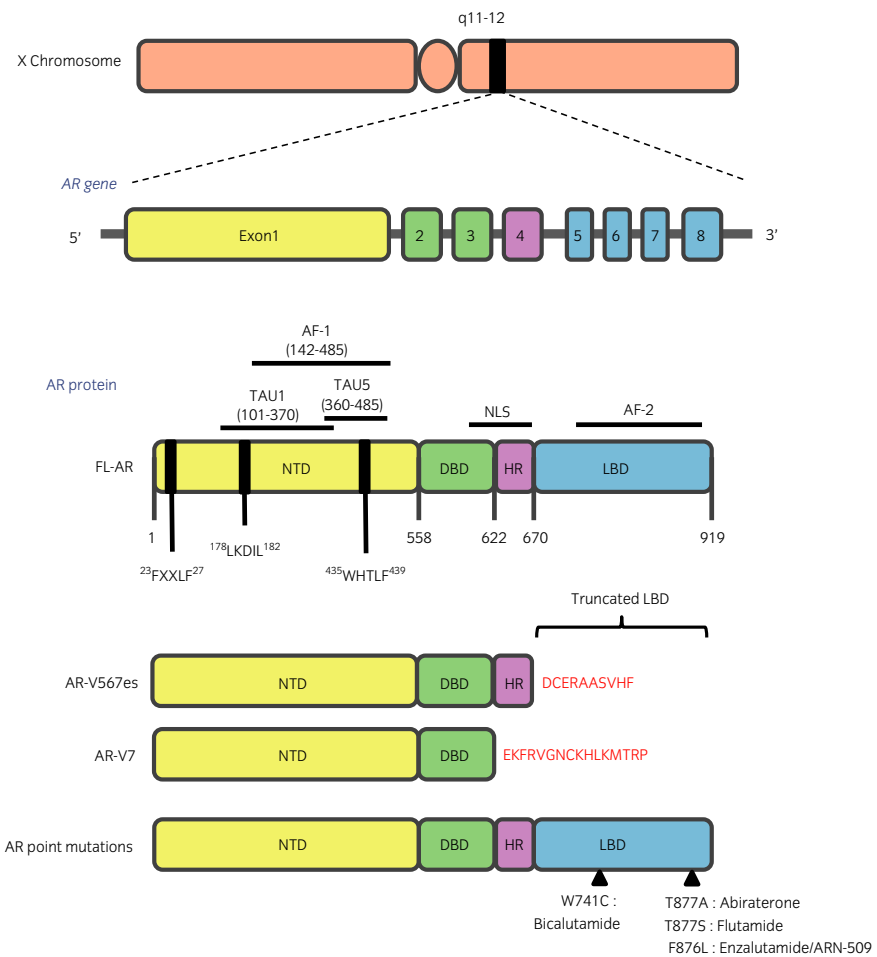

Fig. 2 Domain organization and structure of FL-AR and AR-Vs.[95] The human *AR* gene is located at q11-12 on the X chromosome. FL-AR has approximately 919 amino acid residues that comprise four distinctive functional domains: NTD, DBD, HR and LBD. Exon 1 encodes the full NTD, exons 2 and 3 encode the DBD, exon 4 encodes HR, and exons 5–8 encode the LBD. The NTD contains an AF-1 region, and two transcription activation units (TAU1 and TAU5). Tau1 core sequence motif is $_{178}$LKDIL$_{182}$, whereas Tau5 core motif is $_{435}$WHTLF$_{439}$. Two motifs involved in protein–protein interactions and AR N/C interactions are located in the NTD: FXXLF and WHTLF motifs. A NLS sequence is found in the HR, with part of it extending into the DBD. The LBD contains an AF-2 region, which androgen binds to induce interaction between AF-2 with the FXXLF motif of the NTD, resulting in AR N/C interaction. One of the AR-Vs, AR^{v567es}, is alternatively spliced to skip exons 5–7 with a missense stop codon in exon 8. AR-V7 contains exons 1–3, and leads to the addition of a novel cryptic exon 3. Translated protein products, AR^{v567es}, contain an exon 4 protein coding region (HR), whereas AR-V7 lacks HR, shown with the variant specific amino acids derived from the alternative splicing events. Some drug-specific resistance point mutations in AR-LBD are shown.[31,33,66,69,70]

within the AF-1 that are crucial for AR-dependent transcriptional activity: TAU1 (amino acid 101–370) and its overlapping region TAU5 (amino acid 360–485).[20] TAU1's core sequence motif, $_{178}$LKDIL$_{182}$, is suggested to be important for ligand-dependent transactivation of the FL-AR, whereas $_{435}$WHTLF$_{439}$ in TAU5 is suggested to play a role in ligand-independent transactivation of FL-AR and possibly truncated AR-Vs that lack LBD.[21] AR NTD also contains a FXXLF motif ($_{23}$FQNLF$_{27}$), which interacts with the ligand-bound LBD to form an N/C interaction that is required for transcriptional activity of FL-AR in response to ligand.[22,23] Unlike the other steroid hormone receptors, AR transcriptional activity requires AF-1 in the NTD with negligible activity being attributed to the AF-2 region in the LBD. The AF-1 region can form protein–protein interactions with AR co-activators, and recruits the general transcriptional machinery. Thus, AR NTD is the engine of AR transcriptional activity.[15,24]

Most CRPC is considered to still be driven by transcriptional active AR.[25] Several mechanisms have been proposed to explain the continued AR transcriptional activities despite castration levels of testosterone, as shown in Figure 3. These mechanisms include: (i) amplification of the *AR* gene and increased AR protein expression, which result in hypersensitivity to low levels of androgens as the case in CRPC;[26,27] (ii) AR gain-of-function mutations allowing activation by non-androgenic steroidal ligands or even anti-androgens;[28–33] (iii) overexpression of AR coactivators;[34–39] (iv) ligand-independent transactivation of the AR NTD by alternative pathways involving kinases or cytokines, such as IL-6;[40,41] (v) increased adrenal and intratumoral androgen biosynthesis;[42–44] and (vi) expression of constitutively active AR-Vs lacking the LBD.[45–48] Of these mechanisms, AR-Vs have emerged as a clinically relevant mechanism underlying continued AR transcriptional activities with expression of the variants correlated to poor prognosis.[49,50] Thus, blockade of AR and its signaling pathway by approaches with novel mechanisms of action remains of high interest in the field of prostate cancer.

Current AR targeted therapies for CRPC

In the past 10 years, the treatment of CRPC has advanced as a result of a much improved understanding of the molecular mechanism of the disease.[51] Although docetaxel plus prednisone chemotherapy was approved by the FDA to treat CRPC in 2004, the prolonged overall survival only lasted 2–3 months.[52,53] Prednisone is converted in the liver to its active metabolite prednisolone, which is an irreversible agonist for GR, and lesser so on the structurally-related mineralocorticoid receptor. There was no therapy available to treat post-docetaxel CRPC patients before 2010.[54] In 2010, cabazitaxel, a taxane derivative, was the first post-docetaxel therapy approved by the FDA to treat metastatic CRPC, as it increased survival for 2.4 months.[13] Carbazitaxel is also administered with prednisone. Taxane chemotherapy inhibits AR signaling, caused by drug-induced microtubule stabilization, to suppress nuclear translocation and transcriptional activity of AR.[55] The hinge region of AR is important for this activity. One of the AR-Vs, such as AR^{v567es}, which has the hinge region, is sensitive to microtubule stabilization induced by taxanes, whereas AR-V7, which lacks the hinge region, is unaffected.[56] Consistent with these data, AR-V7 expressing tumor xenografts were resistant to docetaxel

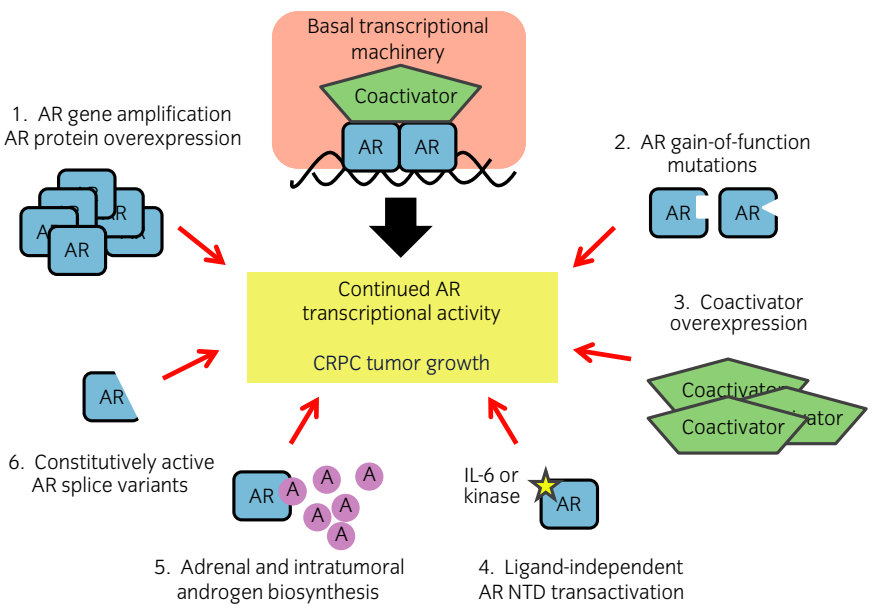

Fig. 3 Molecular biology of CRPC. Continued AR transcriptional activity is a major driver of most CRPC. There are several molecular mechanisms proposed to explain the aberrant AR activity despite castrated levels of testosterone. 1: Amplification of the *AR* gene and overexpression of AR protein, which provide hypersensitivity to low levels of androgen. 2: AR gain-of-function mutations that allow the AR to be activated by non-androgenic steroidal ligands, such as glucocorticoids, and convert anti-androgens into agonists. 3: Overexpression of AR co-activators that can enhance androgen-dependent and also promote ligand-independent AR transcriptional activities. 4: Androgen-independent AR transactivation through its NTD, such as cytokine IL-6, that can stimulate AR transcriptional activity in the absence of androgen. 5: Increased adrenal and/or intratumoral androgen biosynthesis, generating a low, but sufficient, level of androgen to support AR transcriptional activity. 6: AR splice variants with truncated LBD, which have the potential to be constitutively active regardless of the presence of androgen.

whereas AR^{v567es}-expressing xenografts were highly sensitive to docetaxel.[56] Thus, AR-Vs expression might influence sensitivity to taxanes. A retrospective study reported the possibility of cross-resistance between docetaxel and abiraterone with a lower PSA response rate observed in docetaxel-treated patients after abiraterone, as high intratumoral androgen and AR overexpression or mutation might contribute to docetaxel resistance.[57] Conflicting is the fact that detection of AR-V7 in CTCs from mCRPC patients is not associated with primary resistance to taxane chemotherapy, and such patients might retain sensitivity to taxanes.[58] A clinical study reported antitumor activity with cabazitaxel treatment after docetaxel and abiraterone or enzalutamide failures, which resulted from the expression of AR-Vs, which could be inhibited by taxanes.[59] Abiraterone-resistant cells also had impaired efficacy to docetaxel, cabazitaxel and enzalutamide, whereas impaired efficacy of docetaxel, cabazitaxel and abiraterone was observed in enzalutamide-resistant cells *in vitro*.[60]

Abiraterone (Fig. 4) is a small-molecule inhibitor of CYP17A1, which plays key roles in adrenal and intratumoral de novo biosynthesis of androgens. Recently, a study showed that abiraterone is converted by an enzyme to the more active D4A, which blocks multiple steroidogenic enzymes including CYP17A1, 3βHSD and SRD5A, which are required for DHT synthesis. The potency of abiraterone is increased by conversion to D4A with improved inhibition of tumor growth compared with abiraterone, and this D4A also has AR antagonistic activity comparable with enzalutamide.[61] Abiraterone was approved in 2011 to treat post-docetaxel mCRPC in combination with prednisone, as it increased survival to 3.9 months based on the COU-abiraterone-301 trial.[8,62] In 2012, the FDA also approved the use of abiraterone in combination with prednisone as first-line therapy for patients with metastatic CRPC before chemotherapy, as the COU-abiraterone-302 trial later provided evidence to support the benefit of such treatment.[9,63]

Enzalutamide is a second-generation anti-androgen with a similar structure to bicalutamide (Fig. 4), but has a higher affinity for AR LBD. Enzalutamide has an inhibitory effect on AR mutant W741C, which is resistant to bicalutamide.[64] Although enzalutamide is stated to inhibit nuclear translocation, confocal micrographs show that enzalutamide causes the majority of AR to become nuclear in the absence of androgens compared with in the absence of enzalutamide.[64] In 2012, the FDA approved enzalutamide as an agent to treat post-docetaxel second-line mCRPC, based on a AFFIRM trial that showed an improvement in overall survival by 4.8 months.[10] A recent PREVAIL trial showed that enzalutamide significantly reduced the risk of radiographic progression and death, and postponed the initiation of chemotherapy in patients with metastatic

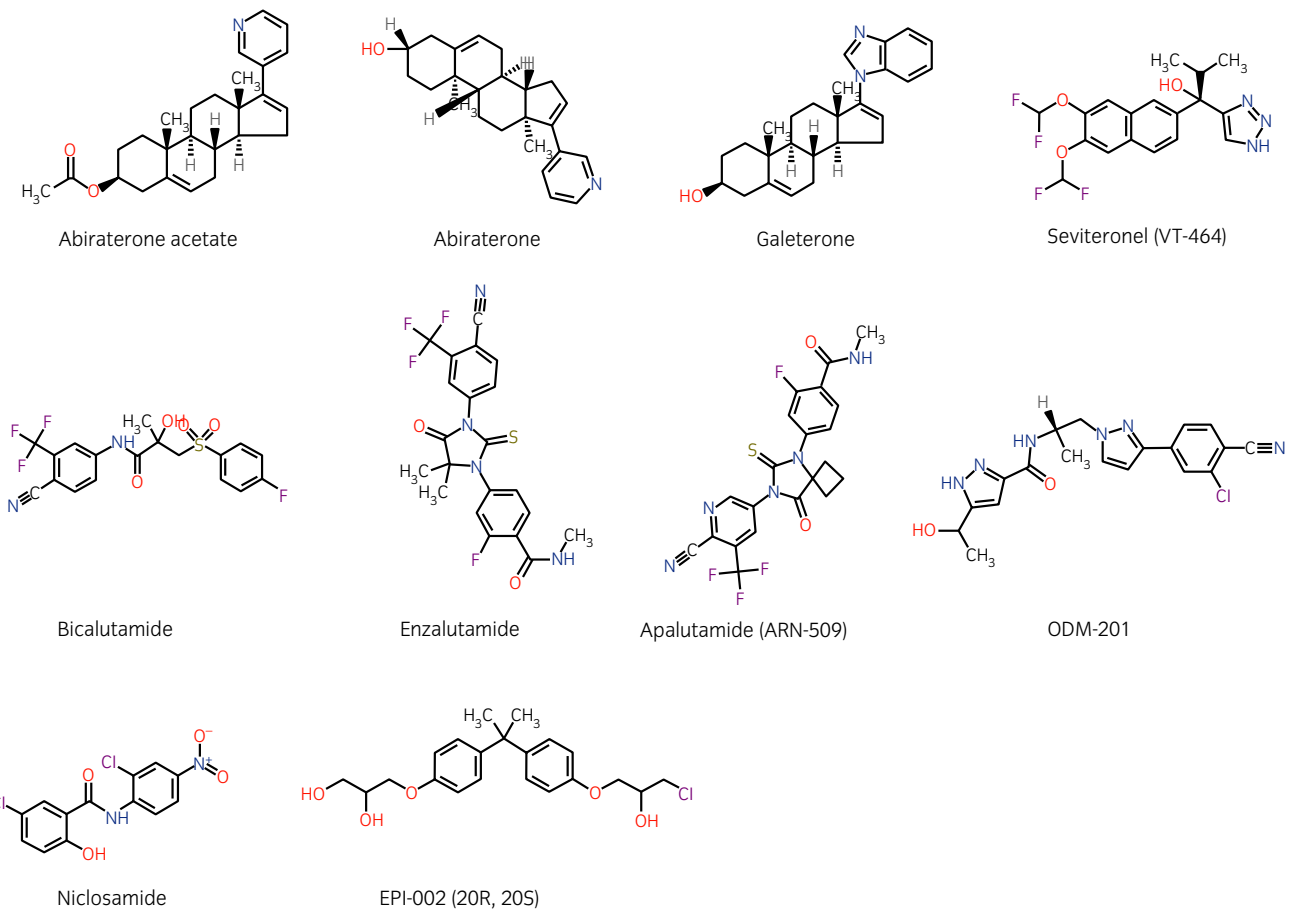

Fig. 4 Chemical structures of drugs used for hormone therapy. Small molecule inhibitors that block the androgen axis through inhibition of CYP17, competitive inhibition of the AR LBD or the AR NTD.

prostate cancer before chemotherapy.[11] As a result, the FDA approved the application of enzalutamide as a first-line therapy to treat CRPC patients before chemotherapy in 2014. The past 10 years represent a new era in molecular-targeted drug research and development for CRPC.

Mechanisms of resistance to abiraterone and enzalutamide

Unfortunately, most CRPC patients treated with the next-generation AR-targeting therapy, abiraterone or enzalutamide, will eventually develop resistance and succumb to the disease. Here, recent conceivable mechanisms are described below and are shown in Figure 5.

Increased androgen synthesis

Resistance to abiraterone in CRPC patients is linked to reactivation of androgen synthesis in prostate cancer cells. Mostaghel *et al.* detected an increase in enzymes modulating steroid metabolism including CYP17A1 in abiraterone-treated LuCaP human prostate cancer xenografts.[65] Upregulation of CYP17 expression itself in the steroidogenesis pathway is a likely contributor to both CRPC progression and abiraterone resistance. The clinical relevance is supported by analyses of tumor biopsies from CRPC patients after CYP17 inhibitor therapy, which showed markedly elevated intratumoral CYP17 expression.[66]

Chang *et al.* reported that DHT can be synthesized from androstanedione instead of testosterone.[67] Abiraterone induces a gain-of-function mutation (N367T) in 3βHSD1, which catalyzes the initial rate-limiting step in conversion of the adrenal-derived steroid DHEA to DHT, renders the enzyme resistant to ubiquitination and degradation, leading to profound accumulation for DHT synthesis.[67]

AR point mutation

Point mutations to the AR in the LBD are implicated in enzalutamide resistance, and it is estimated that 10–30% of CRPC

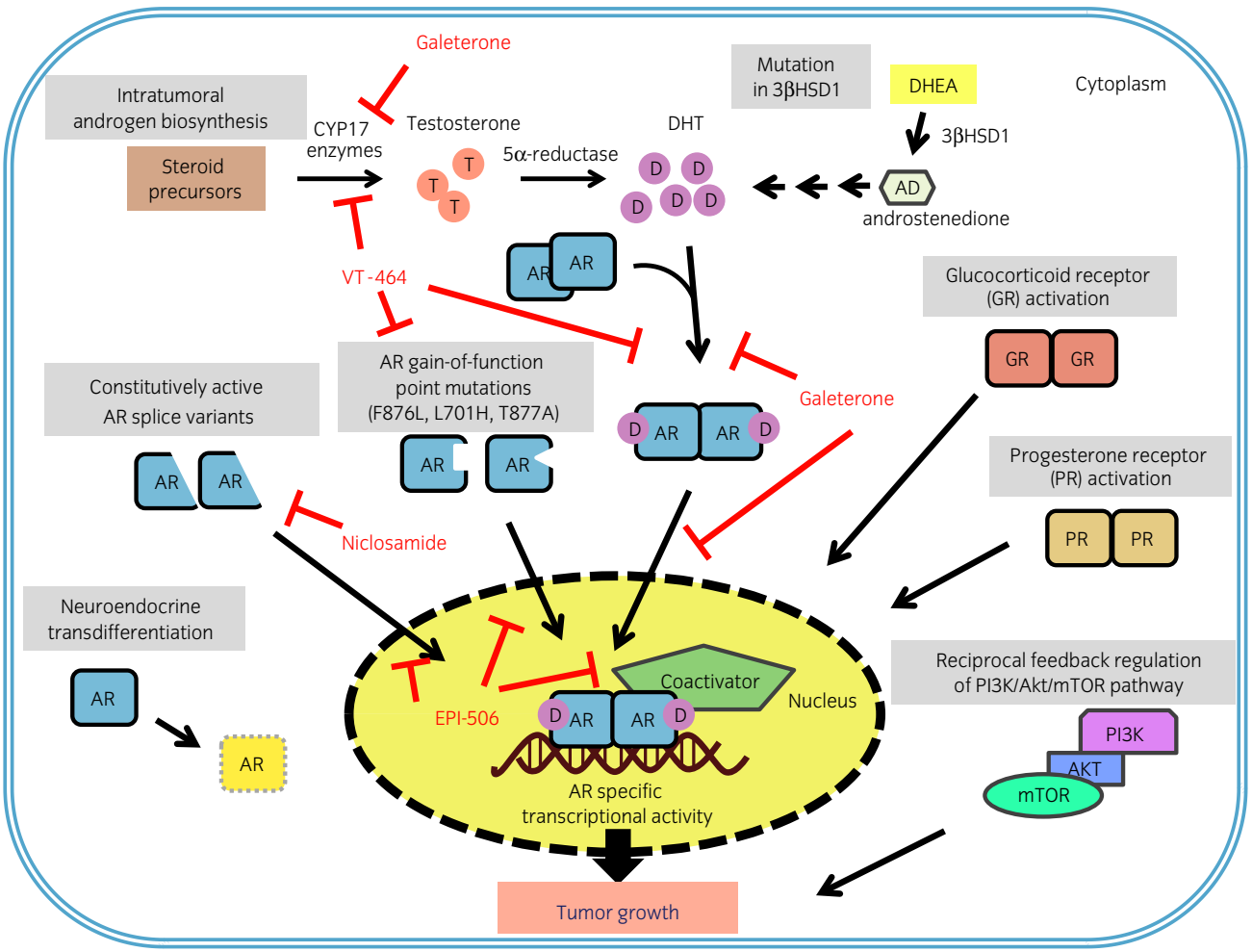

Fig. 5 Conceivable mechanisms of resistance to abiraterone and enzalutamide. There are several molecular mechanisms proposed to explain resistance to abiraterone and enzalutamide: increased intratumoral androgen biosynthesis as a result of upregulation of CYP17 enzymes or a gain-of-function mutation (N367T) in 3βHSD1; AR gain-of-function mutations that allow the AR to be activated by anti-androgens; alternative steroid receptors, such as GR or PR activation, to bypass AR; reciprocal feedback regulation of PI3K/Akt/mTOR pathway; neuroendocrine transdifferentiation; and constitutively active AR-Vs with truncated LBD. Some novel AR-targeted agents that are in clinical development with potential to overcome resistance are shown.

patients have AR mutations.[68] Many of these mutations result in gain-of-function that confer anti-androgens to agonists. One example is the F876L mutation in AR LBD that converts enzalutamide into an AR agonist to sustain AR signaling in the presence of enzalutamide.[32] The clinical relevance of the AR F876L mutation is shown by the detection of the mutant from progressive CRPC patients failing second-generation anti-androgen therapy. The highly structurally-related investigational drug, apalutamide (Fig. 4; previously known as ARN-509), also shows agonist properties to the F876L mutation.[69] Importantly, AR F876L mutation is sensitive to bicalutamide and hydroxyflutamide.[70] Thus, sequencing of anti-androgens might be beneficial if the mechanism of resistance involves gain-of-function AR mutations. CRPC cells commonly express a progesterone-responsive T877A mutant AR, which is related to abiraterone resistance, and AR activity remains steroid-dependent and mediated by upstream CYP11A1-dependent intratumoral pregnenolone/progesterone synthesis.[66]

Alternative steroid receptors bypassing AR

Activation of GR has been proposed to confer enzalutamide resistance, because AR and GR cistromes and transcription programs show significant overlap.[71] This suggests that glucocorticoids and GR could retain the AR pathway under androgen-deprived conditions in CRPC patients. Inhibition of AR activity by enzalutamide causes increases of GR in a subset of prostate cancer cells as a result of relief of AR-mediated feedback repression of GR expression.[72] Preclinical studies suggest GR inhibition might have therapeutic benefit for enzalutamide-resistant CRPC.[73] However, as GR overexpression was observed in samples from a small subset of patients who responded poorly to enzalutamide, the clinical significance of GR-mediated resistance to enzalutamide remains to be validated.[72] Contrary to these data, several clinical trials have shown glucocorticoids to have beneficial activity for patients with mCRPC as a monotherapy;[74] and the wide application of prednisone, an irreversible GR agonist, administered with taxanes or abiraterone does not appear to exacerbate the disease.

PR is a steroid hormone receptor that is the most structurally-related protein to AR in the human proteome. As with GR, it is possible that PR could transcriptionally regulate a subset of AR target genes in prostate cancer. PR expression has been shown in prostate tumor cells in some,[75] but not all, studies.[76] Recently, high PR staining in primary prostate cancer was reported and associated with clinical failure in a large retrospective analysis.[75] Most anti-androgens are excellent inhibitors of PR because of the high homology of their LBDs.

Reciprocal feedback regulation of PI3K and AR

The PI3K–Akt–mTOR pathway is a key oncogenic pathway, and is linked to resistance to ADTs in prostate cancer.[77] AR inhibition could lead to upregulation of the PI3K pathway and vice versa, suggesting cross-regulation. A reciprocal feedback regulation of PI3K and FL-AR signaling in PTEN-deficient prostate cancer has been reported.[78] Although the effects of inhibiting PI3K signaling on AR are controversial, co-targeting the PI3K pathway together with inhibitors of AR is considered a promising approach for the treatment of CRPC.[79,80]

Neuroendocrine transformation

mCRPC shows molecular heterogeneity, and some patients might relapse with clinically aggressive disease regardless of AR expression. Markers of neuroendocrine differentiation include chromogranin A or synaptophysin and/or detection of histological features of small-cell carcinoma, which is an AR-negative prostate cancer. Currently, it is unclear whether AR-negative prostate cancers arise from typical AR-positive adenocarcinomas by a process of transdifferentiation or from AR-negative neuroendocrine cells present in the normal prostate. Support for transdifferentiation comes from evidence of the presence of AR-regulated *TMPRSS2–ERG* genomic translocation in AR-negative small-cell carcinoma that is similar to that seen in AR-positive adenocarcinoma.[81,82] The amount of neuroendocrine differentiation of prostate adenocarcinoma increases with disease progression and in response to ADTs.[83,84] Recent studies suggest that the placental gene, *PEG10*, is de-repressed during the adaptive response to AR inhibition, and subsequently highly upregulated in clinical tissue of neuroendocrine prostate cancer. *PEG10* is regulated by AR, and promotes growth and invasion of neuroendocrine prostate cancer cells in the context of RB1 and TP53 loss.[85] Long-term use of next-generation AR inhibitors might increase the loss of AR and neuroendocrine differentiation.[86]

Cross-resistance

Clinical studies on the efficacy of abiraterone on CRPC patients progressed after enzalutamide treatment reported modest responses and a brief duration of effect.[87,88] The clinical activity of enzalutamide on CRPC patients progressed after abiraterone treatment was also shown to be limited.[89–91] These observations suggest cross-resistance between abiraterone and enzalutamide, which might involve common mechanisms of resistance.

Recent findings that a metabolite of abiraterone, D4A, is also a potent AR antagonist comparable with enzalutamide might provide an explanation for cross-resistance between enzalutamide and abiraterone.[61] Neuroendocrine differentiation of prostate cancer might also partly explain cross-resistance.

Constitutively active AR-Vs

AR is suspected to play an important role in CRPC[92,93] with resistance to therapies that target the AR LBD possibly as a result of expression of constitutively active AR-Vs that lack LBD.[48,94] These AR-Vs are detected in prostate cancer cell lines (e.g. LNCaP95, VCaP and 22Rv1) and in CRPC tissues.[45–47] More than 20 AR-Vs have been reported,[49,95] but just two of these are considered to be clinically relevant,

AR^{v567es} and AR-V7, because their levels of expression are correlated to poor survival and CRPC.[49] AR^{v567es} is solely expressed in 20% of metastases.[48] Enzalutamide resistance is associated with expression of AR-Vs,[50,96] with enzalutamide shown to increase levels of AR-V7 in prostate cancer cells and xenografts.[94] The molecular adaptations to counter CYP17 inhibition by abiraterone might also involve increased levels of expression of FL-AR and AR-Vs.[65] Clinical evidence supporting AR-Vs as a resistance mechanism can be drawn from the detection of AR-V7 in CTCs of patients treated with abiraterone or enzalutamide that correlated to lower PSA response, shorter progression-free and overall survival compared with patients without CTCs that were positive for AR-V7.[97] Importantly, detection of AR-V7 in CTCs from mCRPC patients is not associated with primary resistance to taxane chemotherapy, and such patients might retain sensitivity to taxanes.[98] Taken together, these findings show that constitutively active AR-Vs might be a common mechanism of resistance to enzalutamide and abiraterone.

Treatments in development that might overcome current mechanisms of resistance

Although abiraterone and enzalutamide have been approved for CRPC patients, these treatments eventually fail by secondary resistance mechanisms. Clinical trials of sequential therapies or combination therapies with distinct mechanisms are currently underway in the hope to provide better efficacy, outcome and prognosis for optimal treatment.[3,51,99,100] Here, some AR targeted agents with novel mechanisms or improved qualities are described below and summarized in Table 1.

Apalutamide (ARN-509)

Apalutamide is an anti-androgen with high structural similarity to enzalutamide (Fig. 4), but better affinity for the AR LBD. It is fully antagonistic to AR overexpression and does not induce AR nuclear translocation or DNA binding. Furthermore, ARN-509 has less blood–brain barrier penetration, at least in preclinical studies, which might reduce seizures that are associated with anti-androgens binding to the GABA-A receptor in the brain.[101,102] The phase 3 SPARTAN trial is ongoing in patients with non-mCRPC (NCT01946204). However, the clinical niche for apalutamide is not clearly apparent because of the overlap with enzalutamide in structure, mechanism and pharmacology.

ODM-201 (BAY-1841788)

ODM-201 is an anti-androgen with superior potency to enzalutamide and apalutamide. To date, all anti-androgens have a similar chemical scaffold, and each yields gain-of-function mutations in AR LBD. Although ODM-201 antagonizes AR mutants, such as F876L, W741L and T877A, known to mediate resistance to other anti-androgens because of its chemical similarity (Fig. 4) and mechanism of action with those anti-androgens, ODM-201 is also predicted to promote gain-of-function mutations in AR LBD.[103] In preclinical models, ODM-201 does not cross the blood–brain barrier, which should reduce the potential for seizure. ODM-201 inhibits overexpressed AR, and impairs its nuclear translocation.[104] A phase 3 clinical trial is currently underway in non-mCRPC (NCT02200614). Currently, it is unclear if this agent will be able to impact disease that is resistant to abiraterone and/or enzalutamide.

Seviteronel (VT-464)

Seviteronel (Fig. 4) is a CYP17 inhibitor with 17,20-lyase selectivity. The inhibition of 17,20-lyase activity is enough to reduce androgen levels, and its preserving of 17α-hydroxylase activity largely avoids interference with the production of other steroidal hormones.[105] Seviteronel has shown AR-antagonist activity independent of CYP17 enzyme inhibition,

Table 1 Novel AR targeted drugs in clinical trials

Agents	Mechanism	Advantage	Target	Clinical study ID	Phase
Apalutamide (ARN-509)	AR antagonist (AR-LBD)	High affinity, less blood–brain barrier penetration	Non-mCRPC patients	NCT01946204	3
ODM-201 (BAY-1841788)	AR antagonist (AR-LBD)	High affinity, does not cross blood–brain barrier	High-risk non-mCRPC patients	NCT02200614	3
Seviteronel (VT-464)	CYP17 inhibitor (17,20-lyase selective inhibition)	Does not need corticosteroid	CRPC patients progressing on enzalutamide or abiraterone	NCT02445976	2
			CRPC patients	NCT02361086	1/2
			CRPC patients	NCT02012920	1/2
			CRPC patients previously treated with enzalutamide	NCT02130700	2
Galeterone	CYP17A1 inhibitor (17,20-lyase selective inhibition), AR antagonist, AR degrader	Does not need corticosteroid	mCRPC patients positive for AR-V7	NCT02438007	3
			CRPC patients	NCT01709734	2
Niclosamide	Proteasome-dependent AR degrader (inhibit AR-V7)	Enhances response to enzalutamide	mCRPC patients who are AR-Vs positive	NCT02532114	1
EPI-506	AR antagonist (AR-NTD, AF-1)	Targets FL-AR and AR-Vs	mCRPC patients who failed enzalutamide or abiraterone	NCT02606123	1/2

which might extend to mutated forms of AR, such as F876L.[105,106] A phase 2 clinical trial is underway in patients with CRPC who have been previously treated with enzalutamide or abiraterone (NCT02445976).

Galeterone

Galeterone is a unique antihormonal agent. It is a novel CYP17A1 inhibitor, an anti-androgen and also an AR-degrading agent.[107] Galeterone selectively and irreversibly inhibits CYP17A1 to prevent intratumoral androgen synthesis similar to structurally-related abiraterone (Fig. 4). However, galeterone only inhibits 17,20-lyase, whereas abiraterone inhibits both 17,20-lyase and 17α-hydroxylase. Inhibition of 17α-hydroxylase can lead to the overproduction of progesterone and pregnenalone, causing symptoms such as hypokalemia, hypertension and fluid retention, thereby patients require corticosteroid therapy.[62] Galeterone can therefore block androgen synthesis without causing symptoms of secondary mineralocorticoid excess, which means there is no need for concomitant corticosteroid therapy. Galeterone is also a competitive anti-androgen. *In vitro* studies show galeterone degrades the mutated T878A (also known as T877A) AR, but not wild-type AR, and impairs AR binding to chromatin.[108] There are some discrepancies in the literature that challenge the ability of galeterone to degrade AR-V7.[107,108] Endogenous AR-V7 in 22Rv1 cells is not affected by galeterone.[108] However, the phase 2 ARMOR2 trial (NCT01709734) showed that galeterone might still be effective in the treatment of CRPC that is positive for AR-Vs.[109] A randomized phase 3 trial (ARMOR3-SV) is now underway to compare galeterone and enzalutamide in abiraterone or enzalutamide treatment-naive mCRPC patients with AR-V7-positive CTCs (NCT02438007).

Niclosamide

Niclosamide is an anthelmintic, also used as a piscicide, that was recently found to inhibit AR-V7 through a proteasome-dependent mechanism of degrading it.[110] Niclosamide inhibits prostate cancer cell growth *in vitro* and tumor growth *in vivo*. Importantly, the combination of niclosamide and enzalutamide causes significant inhibition of enzalutamide-resistant tumor growth, suggesting that niclosamide enhances enzalutamide therapy.[110] Niclosamide is currently in phase 1 clinical trials as a therapeutic together with enzalutamide for AR-V-positive mCRPC patients (NCT02532114).

EPI-506

Most investigational drugs in clinical trials still target the AR LBD either directly or indirectly, and do not directly inhibit (bind) AR-Vs that are associated with resistance. An inhibitor to AR NTD would have efficacy against both FL-AR and AR-Vs, because the AF-1 region within this domain is essential for transcriptional activity.[24,111,112] EPI-001 and its analogs (EPI compounds) directly interact with AF-1 to inhibit the transcriptional activity of all AR species.[111,112] This inhibition is achieved by EPI blocking necessary protein–protein interactions required for transcription.[111–113] EPI compounds are the first small molecules known to bind to the NTD of any steroid receptor. As predicted for an AF-1 inhibitor, EPI significantly reduces the growth of LNCaP xenografts, CRPC xenografts that express AR-Vs, such as VCaP and LNCaP95 xenografts, and causes atrophy of androgen-dependent benign tissue in mature male mice.[80,111,112] An analog of EPI compound, EPI-506, is currently in phase 1/2 clinical trials in the USA and Canada for CRPC patients that have failed abiraterone and/or enzalutamide (NCT02606123).[114]

Advantages of AR NTD inhibitors

To date, all hormonal therapies in the clinic target AR LBD directly or indirectly. Unfortunately, all of these therapies eventually fail by mechanisms that might involve constitutively active AR-Vs, breakthrough of androgen/steroid blockade, increased expression of AR and coactivators, and gain-of-function mutations in the LBD. EPI-506 has just entered clinical testing, so there are no long-term studies. However, it is predicted that EPI-506 will not likely cause a resistance mechanism involving gain-of function point mutations in the AR NTD where it binds, because this domain is intrinsically disordered.[115] Thus, point mutations will unlikely have a large impact on the structure of this domain. Importantly, resistance of current therapies is considered to involve transcriptionally active AR species. The strong rationale for blocking the AR NTD or more precisely, AF-1, within this domain is because functional AF-1 is essential for transcriptional activity mediated through protein–protein interactions. Thus, AR NTD inhibitors should block the activities of all AR species regardless of androgen, other steroids and agonistic gain-of-function mutations in the LBD, which anti-androgens cannot achieve. Consequently, the advantages of EPI, an NTD inhibitor include: (i) it does not cause nuclear translocation of the AR in the absence of ligands unlike anti-androgens, including enzalutamide; (ii) it does not cause the AR to bind AREs; (iii) it inhibits protein–protein interactions that are necessary for transcription, such as CREB-binding protein, RAP-74 and N/C interactions; and (iv) it is the only known direct inhibitor of all AR species, including constitutively active AR-Vs.[24,115]

Future directions (indicators of treatment response)

Tumor heterogeneity is defined in both space and time with anatomically distinct regions of the same primary tumor and their respective metastases showing clear differences in genomic architecture.[116–118] A single patient with mCRPC can have many lesions throughout the body and skeleton, and each tumor can have differing levels of expression of AR.[119] Biopsy of all metastatic tumors in a patient to determine AR species is not feasible, and is complicated because of the bone being the dominant site of metastases. The biology of the metastases might also differ from the primary tumor. Thus, it is essential to develop non-invasive approaches to detect the expression of all AR species for the molecular classification of tumors based on the level and extent of expression of AR-Vs to identify patients with potentially

aggressive disease and poor prognosis, or to identify patients that will not respond to ADTs that target the AR LBD.[49] Currently, the optimal choice of therapeutic options such as sequencing and/or combinations remains unclear, and might be based on patient risk factors and preferences, as well as individualized treatment goals.[120,121] Although the relevance of heterogeneity in personalized medicine is yet to be defined, more comprehensive methods of overall tumor content are required for optimal therapy selection.[117]

Liquid biopsy

In clinical samples, AR-Vs are detected at the mRNA level based on reports that AR-Vs are predominantly generated by a splicing event, with no clinical evidence to date that supports genomic rearrangement to contribute substantially to the generation of these variants. This means that approaches using circulating tumor-derived cell-free DNA will miss the bulk of these AR-Vs, and instead approaches that ensure integrity of the RNA are required. CTCs provide easy access for cancer characterization, and are considered a sample of the entire pool of metastases. The detection of CTCs can be carried out by several approaches, such as CellSearch, AdnaGen, EpicSciences and a geometrically-enhanced differential immunocapture-based method GEDI,[122] or commonly achieved by immunostaining, microscopy or by polymerase chain reaction-based methods for epithelial-specific proteins or mRNA species.[123,124] Kits for the isolation of CTCs and analysis of RNA are commercially available from AdnaGen. CTCs are theorized to contribute to metastatic progression,[125] and the number of CTCs are an indicator of poor prognosis.[126] Detection of AR-V7 mRNA in CTCs is a strong indicator of resistance to both abiraterone and enzalutamide, and is a treatment-selection marker in mCRPC.[97,98] CTCs might enhance cancer diagnosis and prognosis, and thus show the resistance and sensitivity profile of prostate cancer. However, to date, CTC studies that have applied multiple DNA, RNA, and protein-based assays have shown limited reproducibility, sensitivity and specificity for detecting or isolating pure CTCs. Importantly, the predictive value of the assay is limited to patients with detectable CTCs. It is also important to note that the majority of methods for isolation of CTCs and detection rely on the presence of a surface antigen, such as HER2, EPCAM or CD45, and might not capture the entire CTC population.

Molecular imaging

New imaging modalities, such as PET-based or single-photon emission computed tomography-based technologies, providing molecular events from the spatiotemporal dimension could be useful to elucidate the intracellular signaling pathways both in the tumor as well as in the surrounding tissues.[127] Molecular imaging provides a method to detect metastases, but also could provide biological information about the lesion.[127] Several targets have been identified, such as FL-AR,[128–132] prostate-specific membrane antigen,[133–136] PSA[137] or prostate stem cell antigen,[138,139] for direct-molecular imaging. For example, ^{18}F-FDHT can be used to study the FL-AR status of tumors.[128] In metastatic CRPC patients, the numbers of bone lesions on CT, FDG PET and ^{18}F-FDHT PET, as well as the intensity of ^{18}F-FDHT uptake, are significantly associated with overall survival.[130] PET imaging with ^{18}F-DCFBC, a small-molecule prostate-specific membrane antigen-targeted radiotracer, detected more lesions than conventional imaging modalities in patients with metastatic prostate cancer.[136] Molecular classification of tumors based on the level and extent of expression of AR-Vs might identify patients that will not respond to abiraterone, anti-androgens or other approaches that target the AR LBD. Molecular imaging agents that can detect AR-Vs could be used to follow AR-related molecular events directly during drug therapy, and to determine therapeutic effectiveness. Proof-of-concept was provided by radioactive iodine EPI compound (^{123}I-EPI) that specifically binds to the AR NTD, and was able to detect both FL-AR and AR-Vs. The clinical application for such an imaging compound includes that it could provide direct visualization of AR-driven cancer in individual lesions in a heterogeneous disease to enhance the clinical assessment of advanced prostate cancer.[140] By using sequential imaging, a discordant distribution or discordant level of uptake between ^{18}F-FDHT and a radiolabeled AR NTD imaging agent would show the presence of AR-Vs. An AR NTD-targeted molecular imaging probe, such as ^{123}I-EPI, might be useful for selecting patients for subsequent anti-androgen or taxane therapies, monitor treatment response and provide insight into the role of all AR species in resistance mechanisms.

Conclusions

There have been improvements in the therapeutic landscape of CRPC with new agents approved and elucidation of the mechanisms of resistance. Persuasive evidence supports that constitutively active AR-Vs are an aspect of AR-related resistant mechanisms underlying CRPC. In the near future, with indicators of treatment response, ongoing research and trials will optimize the sequencing or combination use of currently available AR-targeted therapies, and further facilitate the emergence of new treatments with the potential to overcome resistance mechanisms in this incurable disease.

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Conflict of interest

Yusuke Imamura and Marianne D Sadar are inventors of ^{123}I-EPI, and have licensed the technology to ESSA Pharma. Marianne D Sadar has shares in ESSA Pharma, is a Director and Officer of ESSA, and receives consulting fees.

References

1 Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J. Clin.* 2015; **65**: 87–108.
2 Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J. Clin.* 2015; **65**: 5–29.

3 Buttigliero C, Tucci M, Bertaglia V *et al.* Understanding and overcoming the mechanisms of primary and acquired resistance to abiraterone and enzalutamide in castration resistant prostate cancer. *Cancer Treat. Rev.* 2015; **41**: 884–92.
4 Debes JD, Tindall DJ. The role of androgens and the androgen receptor in prostate cancer. *Cancer Lett.* 2002; **187**: 1–7.
5 Saraon P, Jarvi K, Diamandis EP. Molecular alterations during progression of prostate cancer to androgen independence. *Clin. Chem.* 2011; **57**: 1366–75.
6 Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. *Nat. Rev. Cancer* 2001; **1**: 34–45.
7 Katsogiannou M, Ziouziou H, Karaki S, Andrieu C, Henry de Villeneuve M, Rocchi P. The hallmarks of castration-resistant prostate cancers. *Cancer Treat. Rev.* 2015; **41**: 588–97.
8 Fizazi K, Scher HI, Molina A *et al.* Abiraterone acetate for treatment of metastatic castration-resistant prostate cancer: final overall survival analysis of the COU-AA-301 randomised, double-blind, placebo-controlled phase 3 study. *Lancet Oncol.* 2012; **13**: 983–92.
9 Ryan CJ, Smith MR, Fizazi K *et al.* Abiraterone acetate plus prednisone versus placebo plus prednisone in chemotherapy-naive men with metastatic castration-resistant prostate cancer (COU-AA-302): final overall survival analysis of a randomised, double-blind, placebo-controlled phase 3 study. *Lancet Oncol.* 2015; **16**: 152–60.
10 Scher HI, Fizazi K, Saad F *et al.* Increased survival with enzalutamide in prostate cancer after chemotherapy. *N. Engl. J. Med.* 2012; **367**: 1187–97.
11 Beer TM, Armstrong AJ, Rathkopf DE *et al.* Enzalutamide in metastatic prostate cancer before chemotherapy. *N. Engl. J. Med.* 2014; **371**: 424–33.
12 Kantoff PW, Higano CS, Shore ND *et al.* Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N. Engl. J. Med.* 2010; **363**: 411–22.
13 de Bono JS, Oudard S, Ozguroglu M *et al.* Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. *Lancet* 2010; **376**: 1147–54.
14 Parker C, Nilsson S, Heinrich D *et al.* Alpha emitter radium-223 and survival in metastatic prostate cancer. *N. Engl. J. Med.* 2013; **369**: 213–23.
15 Jenster G, van der Korput HA, van Vroonhoven C, van der Kwast TH, Trapman J, Brinkmann AO. Domains of the human androgen receptor involved in steroid binding, transcriptional activation, and subcellular localization. *Mol. Endocrinol.* 1991; **5**: 1396–404.
16 Rundlett SE, Wu XP, Miesfeld RL. Functional characterizations of the androgen receptor confirm that the molecular basis of androgen action is transcriptional regulation. *Mol. Endocrinol.* 1990; **4**: 708–14.
17 Simental JA, Sar M, Lane MV, French FS, Wilson EM. Transcriptional activation and nuclear targeting signals of the human androgen receptor. *J. Biol. Chem.* 1991; **266**: 510–8.
18 Gao W, Bohl CE, Dalton JT. Chemistry and structural biology of androgen receptor. *Chem. Rev.* 2005; **105**: 3352–70.
19 McEwan IJ. Intrinsic disorder in the androgen receptor: identification, characterisation and drugability. *Mol. BioSyst.* 2012; **8**: 82–90.
20 Jenster G, van der Korput HA, Trapman J, Brinkmann AO. Identification of two transcription activation units in the N-terminal domain of the human androgen receptor. *J. Biol. Chem.* 1995; **270**: 7341–6.
21 Dehm SM, Regan KM, Schmidt LJ, Tindall DJ. Selective role of an NH2-terminal WxxLF motif for aberrant androgen receptor activation in androgen depletion independent prostate cancer cells. *Cancer Res.* 2007; **67**: 10067–77.
22 Schaufele F, Carbonell X, Guerbadot M *et al.* The structural basis of androgen receptor activation: intramolecular and intermolecular amino-carboxy interactions. *Proc Natl Acad Sci U S A* 2005; **102**: 9802–7.
23 He B, Kemppainen JA, Voegel JJ, Gronemeyer H, Wilson EM. Activation function 2 in the human androgen receptor ligand binding domain mediates interdomain communication with the NH(2)-terminal domain. *J. Biol. Chem.* 1999; **274**: 37219–25.
24 Sadar MD. Small molecule inhibitors targeting the "achilles' heel" of androgen receptor activity. *Cancer Res.* 2011; **71**: 1208–13.
25 Chen CD, Welsbie DS, Tran C *et al.* Molecular determinants of resistance to antiandrogen therapy. *Nat. Med.* 2004; **10**: 33–9.
26 Visakorpi T, Hyytinen E, Koivisto P *et al.* In vivo amplification of the androgen receptor gene and progression of human prostate cancer. *Nat. Genet.* 1995; **9**: 401–6.
27 Koivisto P, Kononen J, Palmberg C *et al.* Androgen receptor gene amplification: a possible molecular mechanism for androgen deprivation therapy failure in prostate cancer. *Cancer Res.* 1997; **57**: 314–9.
28 Zhao XY, Malloy PJ, Krishnan AV *et al.* Glucocorticoids can promote androgen-independent growth of prostate cancer cells through a mutated androgen receptor. *Nat. Med.* 2000; **6**: 703–6.
29 Sun C, Shi Y, Xu LL *et al.* Androgen receptor mutation (T877A) promotes prostate cancer cell growth and cell survival. *Oncogene* 2006; **25**: 3905–13.
30 Fenton MA, Shuster TD, Fertig AM *et al.* Functional characterization of mutant androgen receptors from androgen-independent prostate cancer. *Clin. Cancer Res.* 1997; **3**: 1383–8.
31 Yoshida T, Kinoshita H, Segawa T *et al.* Antiandrogen bicalutamide promotes tumor growth in a novel androgen-dependent prostate cancer xenograft model derived from a bicalutamide-treated patient. *Cancer Res.* 2005; **65**: 9611–6.
32 Korpal M, Korn JM, Gao X *et al.* An F876L mutation in androgen receptor confers genetic and phenotypic resistance to MDV3100 (enzalutamide). *Cancer Discov.* 2013; **3**: 1030–43.
33 Taplin ME, Bubley GJ, Ko YJ *et al.* Selection for androgen receptor mutations in prostate cancers treated with androgen antagonist. *Cancer Res.* 1999; **59**: 2511–5.
34 Ueda T, Mawji NR, Bruchovsky N, Sadar MD. Ligand-independent activation of the androgen receptor by interleukin-6 and the role of steroid receptor coactivator-1 in prostate cancer cells. *J. Biol. Chem.* 2002; **277**: 38087–94.
35 Agoulnik IU, Vaid A, Bingman WE 3rd *et al.* Role of SRC-1 in the promotion of prostate cancer cell growth and tumor progression. *Cancer Res.* 2005; **65**: 7959–67.
36 Debes JD, Schmidt LJ, Huang H, Tindall DJ. p300 mediates androgen-independent transactivation of the androgen receptor by interleukin 6. *Cancer Res.* 2002; **62**: 5632–6.
37 Debes JD, Sebo TJ, Lohse CM, Murphy LM, Haugen DA, Tindall DJ. p300 in prostate cancer proliferation and progression. *Cancer Res.* 2003; **63**: 7638–40.
38 Agoulnik IU, Vaid A, Nakka M *et al.* Androgens modulate expression of transcription intermediary factor 2, an androgen receptor coactivator whose expression level correlates with early biochemical recurrence in prostate cancer. *Cancer Res.* 2006; **66**: 10594–602.
39 Tien JC, Liu Z, Liao L *et al.* The steroid receptor coactivator-3 is required for the development of castration-resistant prostate cancer. *Cancer Res.* 2013; **73**: 3997–4008.
40 Sadar MD. Androgen-independent induction of prostate-specific antigen gene expression via cross-talk between the androgen receptor and protein kinase A signal transduction pathways. *J. Biol. Chem.* 1999; **274**: 7777–83.
41 Ueda T, Bruchovsky N, Sadar MD. Activation of the androgen receptor N-terminal domain by interleukin-6 via MAPK and STAT3 signal transduction pathways. *J. Biol. Chem.* 2002; **277**: 7076–85.
42 Stanbrough M, Bubley GJ, Ross K *et al.* Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. *Cancer Res.* 2006; **66**: 2815–25.
43 Locke JA, Guns ES, Lubik AA *et al.* Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer. *Cancer Res.* 2008; **68**: 6407–15.
44 Montgomery RB, Mostaghel EA, Vessella R *et al.* Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth. *Cancer Res.* 2008; **68**: 4447–54.
45 Hu R, Dunn TA, Wei S *et al.* Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. *Cancer Res.* 2009; **69**: 16–22.
46 Guo Z, Yang X, Sun F *et al.* A novel androgen receptor splice variant is up-regulated during prostate cancer progression and promotes androgen depletion-resistant growth. *Cancer Res.* 2009; **69**: 2305–13.
47 Dehm SM, Schmidt LJ, Heemers HV, Vessella RL, Tindall DJ. Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate cancer therapy resistance. *Cancer Res.* 2008; **68**: 5469–77.
48 Sun S, Sprenger CC, Vessella RL *et al.* Castration resistance in human prostate cancer is conferred by a frequently occurring androgen receptor splice variant. *J. Clin. Invest.* 2010; **120**: 2715–30.

49 Hornberg E, Ylitalo EB, Crnalic S *et al.* Expression of androgen receptor splice variants in prostate cancer bone metastases is associated with castration-resistance and short survival. *PLoS ONE* 2011; **6**: e19059.

50 Zhang X, Morrissey C, Sun S *et al.* Androgen receptor variants occur frequently in castration resistant prostate cancer metastases. *PLoS ONE* 2011; **6**: e27970.

51 Suzman DL, Antonarakis ES. Castration-resistant prostate cancer: latest evidence and therapeutic implications. *Ther. Adv. Med. Oncol.* 2014; **6**: 167–79.

52 Tannock IF, de Wit R, Berry WR *et al.* Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N. Engl. J. Med.* 2004; **351**: 1502–12.

53 Petrylak DP, Tangen CM, Hussain MH *et al.* Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. *N. Engl. J. Med.* 2004; **351**: 1513–20.

54 Yap TA, Zivi A, Omlin A, de Bono JS. The changing therapeutic landscape of castration-resistant prostate cancer. *Nat. Rev. Clin. Oncol.* 2011; **8**: 597–610.

55 Zhu ML, Horbinski CM, Garzotto M, Qian DZ, Beer TM, Kyprianou N. Tubulin-targeting chemotherapy impairs androgen receptor activity in prostate cancer. *Cancer Res.* 2010; **70**: 7992–8002.

56 Thadani-Mulero M, Portella L, Sun S *et al.* Androgen receptor splice variants determine taxane sensitivity in prostate cancer. *Cancer Res.* 2014; **74**: 2270–82.

57 Mezynski J, Pezaro C, Bianchini D *et al.* Antitumour activity of docetaxel following treatment with the CYP17A1 inhibitor abiraterone: clinical evidence for cross-resistance? *Ann. Oncol.* 2012; **23**: 2943–7.

58 Antonarakis ES, Lu C, Luber B *et al.* Androgen Receptor Splice Variant 7 and Efficacy of Taxane Chemotherapy in Patients With Metastatic Castration-Resistant Prostate Cancer. *JAMA Oncol.* 2015; **1**: 582–91.

59 Pezaro CJ, Omlin AG, Altavilla A *et al.* Activity of cabazitaxel in castration-resistant prostate cancer progressing after docetaxel and next-generation endocrine agents. *Eur. Urol.* 2014; **66**: 459–65.

60 van Soest RJ, van Royen ME, de Morree ES *et al.* Cross-resistance between taxanes and new hormonal agents abiraterone and enzalutamide may affect drug sequence choices in metastatic castration-resistant prostate cancer. *Eur. J. Cancer* 2013; **49**: 3821–30.

61 Li Z, Bishop AC, Alyamani M *et al.* Conversion of abiraterone to D4A drives anti-tumour activity in prostate cancer. *Nature* 2015; **523**: 347–51.

62 de Bono JS, Logothetis CJ, Molina A *et al.* Abiraterone and increased survival in metastatic prostate cancer. *N. Engl. J. Med.* 2011; **364**: 1995–2005.

63 Ryan CJ, Smith MR, de Bono JS *et al.* Abiraterone in metastatic prostate cancer without previous chemotherapy. *N. Engl. J. Med.* 2013; **368**: 138–48.

64 Tran C, Ouk S, Clegg NJ *et al.* Development of a second-generation antiandrogen for treatment of advanced prostate cancer. *Science* 2009; **324**: 787–90.

65 Mostaghel EA, Marck BT, Plymate SR *et al.* Resistance to CYP17A1 inhibition with abiraterone in castration-resistant prostate cancer: induction of steroidogenesis and androgen receptor splice variants. *Clin. Cancer Res.* 2011; **17**: 5913–25.

66 Cai C, Chen S, Ng P *et al.* Intratumoral de novo steroid synthesis activates androgen receptor in castration-resistant prostate cancer and is upregulated by treatment with CYP17A1 inhibitors. *Cancer Res.* 2011; **71**: 6503–13.

67 Chang KH, Li R, Kuri B *et al.* A gain-of-function mutation in DHT synthesis in castration-resistant prostate cancer. *Cell* 2013; **154**: 1074–84.

68 Waltering KK, Urbanucci A, Visakorpi T. Androgen receptor (AR) aberrations in castration-resistant prostate cancer. *Mol. Cell. Endocrinol.* 2012; **360**: 38–43.

69 Joseph JD, Lu N, Qian J *et al.* A clinically relevant androgen receptor mutation confers resistance to second-generation antiandrogens enzalutamide and ARN-509. *Cancer Discov.* 2013; **3**: 1020–9.

70 Balbas MD, Evans MJ, Hosfield DJ *et al.* Overcoming mutation-based resistance to antiandrogens with rational drug design. *Elife* 2013; **2**: e00499.

71 Sahu B, Laakso M, Pihlajamaa P *et al.* FoxA1 specifies unique androgen and glucocorticoid receptor binding events in prostate cancer cells. *Cancer Res.* 2013; **73**: 1570–80.

72 Arora VK, Schenkein E, Murali R *et al.* Glucocorticoid receptor confers resistance to antiandrogens by bypassing androgen receptor blockade. *Cell* 2013; **155**: 1309–22.

73 Isikbay M, Otto K, Kregel S *et al.* Glucocorticoid receptor activity contributes to resistance to androgen-targeted therapy in prostate cancer. *Horm. Cancer* 2014; **5**: 72–89.

74 Venkitaraman R, Lorente D, Murthy V *et al.* A randomised phase 2 trial of dexamethasone versus prednisolone in castration-resistant prostate cancer. *Eur. Urol.* 2015; **67**: 673–9.

75 Grindstad T, Andersen S, Al-Saad S *et al.* High progesterone receptor expression in prostate cancer is associated with clinical failure. *PLoS ONE* 2015; **10**: e0116691.

76 Yu Y, Liu L, Xie N *et al.* Expression and function of the progesterone receptor in human prostate stroma provide novel insights to cell proliferation control. *J. Clin. Endocrinol. Metab.* 2013; **98**: 2887–96.

77 Kaarbo M, Mikkelsen OL, Malerod L *et al.* PI3K-AKT-mTOR pathway is dominant over androgen receptor signaling in prostate cancer cells. *Cell Oncol.* 2010; **32**: 11–27.

78 Carver BS, Chapinski C, Wongvipat J *et al.* Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer. *Cancer Cell* 2011; **19**: 575–86.

79 Sarker D, Reid AH, Yap TA, de Bono JS. Targeting the PI3K/AKT pathway for the treatment of prostate cancer. *Clin. Cancer Res.* 2009; **15**: 4799–805.

80 Kato M, Banuelos CA, Imamura Y *et al.* Cotargeting androgen receptor splice variants and mTOR signaling pathway for the treatment of castration-resistant prostate cancer. *Clin. Cancer Res.* 2016; **22**: 2744–54.

81 Williamson SR, Zhang S, Yao JL *et al.* ERG-TMPRSS2 rearrangement is shared by concurrent prostatic adenocarcinoma and prostatic small cell carcinoma and absent in small cell carcinoma of the urinary bladder: evidence supporting monoclonal origin. *Mod. Pathol.* 2011; **24**: 1120–7.

82 Tomlins SA, Rhodes DR, Perner S *et al.* Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science* 2005; **310**: 644–8.

83 Hirano D, Okada Y, Minei S, Takimoto Y, Nemoto N. Neuroendocrine differentiation in hormone refractory prostate cancer following androgen deprivation therapy. *Eur. Urol.* 2004; **45**: 586–92; discussion 592.

84 Epstein JI, Amin MB, Beltran H *et al.* Proposed morphologic classification of prostate cancer with neuroendocrine differentiation. *Am. J. Surg. Pathol.* 2014; **38**: 756–67.

85 Akamatsu S, Wyatt AW, Lin D *et al.* The placental gene PEG10 promotes progression of neuroendocrine prostate cancer. *Cell Rep.* 2015; **12**: 922–36.

86 Yuan TC, Veeramani S, Lin MF. Neuroendocrine-like prostate cancer cells: neuroendocrine transdifferentiation of prostate adenocarcinoma cells. *Endocr. Relat. Cancer* 2007; **14**: 531–47.

87 Noonan KL, North S, Bitting RL, Armstrong AJ, Ellard SL, Chi KN. Clinical activity of abiraterone acetate in patients with metastatic castration-resistant prostate cancer progressing after enzalutamide. *Ann. Oncol.* 2013; **24**: 1802–7.

88 Loriot Y, Bianchini D, Ileana E *et al.* Antitumour activity of abiraterone acetate against metastatic castration-resistant prostate cancer progressing after docetaxel and enzalutamide (MDV3100). *Ann. Oncol.* 2013; **24**: 1807–12.

89 Badrising S, van der Noort V, van Oort IM *et al.* Clinical activity and tolerability of enzalutamide (MDV3100) in patients with metastatic, castration-resistant prostate cancer who progress after docetaxel and abiraterone treatment. *Cancer* 2014; **120**: 968–75.

90 Bianchini D, Lorente D, Rodriguez-Vida A *et al.* Antitumour activity of enzalutamide (MDV3100) in patients with metastatic castration-resistant prostate cancer (CRPC) pre-treated with docetaxel and abiraterone. *Eur. J. Cancer* 2014; **50**: 78–84.

91 Schrader AJ, Boegemann M, Ohlmann CH *et al.* Enzalutamide in castration-resistant prostate cancer patients progressing after docetaxel and abiraterone. *Eur. Urol.* 2014; **65**: 30–6.

92 Nelson PS, Clegg N, Arnold H *et al.* The program of androgen-responsive genes in neoplastic prostate epithelium. *Proc Natl Acad Sci U S A* 2002; **99**: 11890–5.

93 Scher HI, Sawyers CL. Biology of progressive, castration-resistant prostate cancer: directed therapies targeting the androgen-receptor signaling axis. *J. Clin. Oncol.* 2005; **23**: 8253–61.

94 Hu R, Lu C, Mostaghel EA *et al.* Distinct transcriptional programs mediated by the ligand-dependent full-length androgen receptor and its splice variants in castration-resistant prostate cancer. *Cancer Res.* 2012; **72**: 3457–62.

95 Haile S, Sadar MD. Androgen receptor and its splice variants in prostate cancer. *Cell. Mol. Life Sci.* 2011; **68**: 3971–81.
96 Li Y, Chan SC, Brand LJ, Hwang TH, Silverstein KA, Dehm SM. Androgen receptor splice variants mediate enzalutamide resistance in castration-resistant prostate cancer cell lines. *Cancer Res.* 2013; **73**: 483–9.
97 Antonarakis ES, Lu C, Wang H *et al.* AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *N. Engl. J. Med.* 2014; **371**: 1028–38.
98 Antonarakis ES, Lu CX, Chen Y *et al.* AR splice variant 7 (AR-V7) and response to taxanes in men with metastatic castration-resistant prostate cancer (mCRPC). *J. Clin. Oncol.* 2015; **33**(Suppl): 138.
99 Fujimoto N. Novel agents for castration-resistant prostate cancer: early experience and beyond. *Int. J. Urol.* 2016; **23**: 114–21.
100 Wyatt AW, Gleave ME. Targeting the adaptive molecular landscape of castration-resistant prostate cancer. *EMBO Mol. Med.* 2015; **7**: 878–94.
101 Clegg NJ, Wongvipat J, Joseph JD *et al.* ARN-509: a novel antiandrogen for prostate cancer treatment. *Cancer Res.* 2012; **72**: 1494–503.
102 Foster WR, Car BD, Shi H *et al.* Drug safety is a barrier to the discovery and development of new androgen receptor antagonists. *Prostate* 2011; **71**: 480–8.
103 Fizazi K, Albiges L, Loriot Y, Massard C. ODM-201: a new-generation androgen receptor inhibitor in castration-resistant prostate cancer. *Expert Rev. Anticancer Ther.* 2015; **15**: 1007–17.
104 Moilanen AM, Riikonen R, Oksala R *et al.* Discovery of ODM-201, a new-generation androgen receptor inhibitor targeting resistance mechanisms to androgen signaling-directed prostate cancer therapies. *Sci. Rep.* 2015; **5**: 12007. doi:10.1038.
105 Toren PJ, Kim S, Pham S *et al.* Anticancer activity of a novel selective CYP17A1 inhibitor in preclinical models of castrate-resistant prostate cancer. *Mol. Cancer Ther.* 2015; **14**: 59–69.
106 Moore WR, Norris JD, Wardell S *et al.* Direct effects of the selective CYP17 lyase (L) inhibitor, VT-464, on the androgen receptor (AR) and its oral activity in an F876L tumor mouse xenograft model. *J. Clin. Oncol.* 2015; **33**: 263.
107 Njar VC, Brodie AM. Discovery and development of Galeterone (TOK-001 or VN/124-1) for the treatment of all stages of prostate cancer. *J. Med. Chem.* 2015; **58**: 2077–87.
108 Yu Z, Cai C, Gao S, Simon NI, Shen HC, Balk SP. Galeterone prevents androgen receptor binding to chromatin and enhances degradation of mutant androgen receptor. *Clin. Cancer Res.* 2014; **20**: 4075–85.
109 Yaqub F. Galeterone activity in castration-resistant prostate cancer. *Lancet Oncol.* 2015; **16**: e10.
110 Liu C, Lou W, Zhu Y *et al.* Niclosamide inhibits androgen receptor variants expression and overcomes enzalutamide resistance in castration-resistant prostate cancer. *Clin. Cancer Res.* 2014; **20**: 3198–210.
111 Andersen RJ, Mawji NR, Wang J *et al.* Regression of castrate-recurrent prostate cancer by a small-molecule inhibitor of the amino-terminus domain of the androgen receptor. *Cancer Cell* 2010; **17**: 535–46.
112 Myung JK, Banuelos CA, Fernandez JG *et al.* An androgen receptor N-terminal domain antagonist for treating prostate cancer. *J. Clin. Invest.* 2013; **123**: 2948–60.
113 De Mol E. Structure, dynamics and interactions of the N-terminal domain of the androgen receptor. 2014. [Cited 2 Jun 2016.] Available from URL: http://hdl.handle.net/10803/147275
114 Montgomery RB, Antonarakis ES, Hussain M *et al.* A phase 1/2 open-label study of safety and antitumor activity of EPI-506, a novel AR N-terminal domain inhibitor, in men with metastatic castration-resistant prostate cancer (mCRPC) with progression after enzalutamide or abiraterone. *J. Clin. Oncol.* 2015; **33**(Suppl): abstr TPS5072.
115 Sadar MD. Advances in small molecule inhibitors of androgen receptor for the treatment of advanced prostate cancer. *World J. Urol.* 2012; **30**: 311–8.
116 Swanton C. Intratumor heterogeneity: evolution through space and time. *Cancer Res.* 2012; **72**: 4875–82.
117 Krebs MG, Metcalf RL, Carter L, Brady G, Blackhall FH, Dive C. Molecular analysis of circulating tumour cells-biology and biomarkers. *Nat. Rev. Clin. Oncol.* 2014; **11**: 129–44.
118 Greaves M, Maley CC. Clonal evolution in cancer. *Nature* 2012; **481**: 306–13.
119 Roudier MP, True LD, Higano CS *et al.* Phenotypic heterogeneity of end-stage prostate carcinoma metastatic to bone. *Hum. Pathol.* 2003; **34**: 646–53.
120 Omlin A, Pezaro C, Gillessen Sommer S. Sequential use of novel therapeutics in advanced prostate cancer following docetaxel chemotherapy. Ther. *Adv. Urol.* 2014; **6**: 3–14.
121 Saad F, Fizazi KS. Androgen deprivation therapy and secondary hormone therapy in the management of hormone-sensitive and castration resistant prostate cancer. *Urology* 2015; **86**: 852–61.
122 Gleghorn JP, Pratt ED, Denning D *et al.* Capture of circulating tumor cells from whole blood of prostate cancer patients using geometrically enhanced differential immunocapture (GEDI) and a prostate-specific antibody. *Lab Chip* 2010; **10**: 27–9.
123 Harouaka R, Kang Z, Zheng SY, Cao L. Circulating tumor cells: advances in isolation and analysis, and challenges for clinical applications. *Pharmacol. Ther.* 2014; **141**: 209–21.
124 Alix-Panabieres C, Schwarzenbach H, Pantel K. Circulating tumor cells and circulating tumor DNA. *Annu. Rev. Med.* 2012; **63**: 199–215.
125 Zieglschmid V, Hollmann C, Bocher O. Detection of disseminated tumor cells in peripheral blood. *Crit. Rev. Clin. Lab. Sci.* 2005; **42**: 155–96.
126 de Bono JS, Scher HI, Montgomery RB *et al.* Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. *Clin. Cancer Res.* 2008; **14**: 6302–9.
127 Evans MJ. Measuring oncogenic signaling pathways in cancer with PET: an emerging paradigm from studies in castration-resistant prostate cancer. *Cancer Discov.* 2012; **2**: 985–94.
128 Dehdashti F, Picus J, Michalski JM *et al.* Positron tomographic assessment of androgen receptors in prostatic carcinoma. *Eur. J. Nucl. Med. Mol. Imaging* 2005; **32**: 344–50.
129 Larson SM, Morris M, Gunther I *et al.* Tumor localization of 16beta-18F-fluoro-5alpha-dihydrotestosterone versus 18F-FDG in patients with progressive, metastatic prostate cancer. *J. Nucl. Med.* 2004; **45**: 366–73.
130 Vargas HA, Wassberg C, Fox JJ *et al.* Bone metastases in castration-resistant prostate cancer: associations between morphologic CT patterns, glycolytic activity, and androgen receptor expression on PET and overall survival. *Radiology* 2014; **271**: 220–9.
131 Zanzonico PB, Finn R, Pentlow KS *et al.* PET-based radiation dosimetry in man of 18F-fluorodihydrotestosterone, a new radiotracer for imaging prostate cancer. *J. Nucl. Med.* 2004; **45**: 1966–71.
132 Beattie BJ, Smith-Jones PM, Jhanwar YS *et al.* Pharmacokinetic assessment of the uptake of 16beta-18F-fluoro-5alpha-dihydrotestosterone (FDHT) in prostate tumors as measured by PET. *J. Nucl. Med.* 2010; **51**: 183–92.
133 Evans MJ, Smith-Jones PM, Wongvipat J *et al.* Noninvasive measurement of androgen receptor signaling with a positron-emitting radiopharmaceutical that targets prostate-specific membrane antigen. *Proc Natl Acad Sci U S A* 2011; **108**: 9578–82.
134 Chen Y, Foss CA, Byun Y *et al.* Radiohalogenated prostate-specific membrane antigen (PSMA)-based ureas as imaging agents for prostate cancer. *J. Med. Chem.* 2008; **51**: 7933–43.
135 Hillier SM, Maresca KP, Femia FJ *et al.* Preclinical evaluation of novel glutamate-urea-lysine analogues that target prostate-specific membrane antigen as molecular imaging pharmaceuticals for prostate cancer. *Cancer Res.* 2009; **69**: 6932–40.
136 Rowe SP, Macura KJ, Ciarallo A *et al.* Comparison of PSMA-based 18F-DCFBC PET/CT to Conventional Imaging Modalities for Detection of Hormone-Sensitive and Castration-Resistant Metastatic Prostate Cancer. *J. Nucl. Med.* 2015; **57**: 46–53.
137 Ulmert D, Evans MJ, Holland JP *et al.* Imaging androgen receptor signaling with a radiotracer targeting free prostate-specific antigen. *Cancer Discov.* 2012; **2**: 320–7.
138 Lepin EJ, Leyton JV, Zhou Y *et al.* An affinity matured minibody for PET imaging of prostate stem cell antigen (PSCA)-expressing tumors. *Eur. J. Nucl. Med. Mol. Imaging* 2010; **37**: 1529–38.
139 Leyton JV, Olafsen T, Lepin EJ *et al.* Humanized radioiodinated minibody for imaging of prostate stem cell antigen-expressing tumors. *Clin. Cancer Res.* 2008; **14**: 7488–96.
140 Imamura Y, Tien AH, Jian KZ *et al.* Development of an imaging approach to detect splice variants of androgen receptor in prostate cancer. *J. Clin. Oncol.* 2015; **33**(Suppl): abstr 5058.

