Androgen receptor antagonists for prostate cancer therapy

Christine Helsen¹, Thomas Van den Broeck¹², Arnout Voet³, Stefan Prekovic¹, Hendrik Van Poppel², Steven Joniau² and Frank Claessens¹

¹Laboratory of Molecular Endocrinology, Department of Cellular and Molecular Medicine, Katholieke Universiteit Leuven, Herestraat 49, B-3000 Leuven, Belgium
²Urology, Department of Development and Regeneration, University Hospitals Leuven, Herestraat 49, 3000 Leuven, Belgium
³Laboratory for Structural Bioinformatics, Center for Life Science Technologies, RIKEN, Yokohama, Japan

Correspondence should be addressed to F Claessens
Email frank.claessens@med.kuleuven.be

Abstract

Androgen deprivation is the mainstay therapy for metastatic prostate cancer (PCa). Another way of suppressing androgen receptor (AR) signaling is via AR antagonists or antiandrogens. Despite being frequently prescribed in clinical practice, there is conflicting evidence concerning the role of AR antagonists in the management of PCa. In the castration-resistant settings of PCa, docetaxel has been the only treatment option for decades. With recent evidence that castration-resistant PCa is far from AR-independent, there has been an increasing interest in developing new AR antagonists. This review gives a concise overview of the clinically available antiandrogens and the experimental AR antagonists that tackle androgen action with a different approach.

Key Words
- androgen receptor antagonist
- antiandrogen
- enzalutamide
- bicalutamide
- abiraterone
- prostate cancer

Introduction

Prostate cancer (PCa) remains a major healthcare problem and it is expected that the prevalence will only increase due to aging of the population. In 2013, it is estimated that PCa will account for 28% of all cancers in the USA with 29 720 expected PCa-related deaths (Siegel et al. 2013). Epidemiological studies from Europe show comparable data with an estimated incidence of 416 700 new PCa cases in 2012, representing 22.8% of cancer diagnoses in men. In total, 92 200 PCa-specific deaths are expected, making it one of the three cancers men are most likely to die from, with a mortality rate of 9.5% (Ferlay et al. 2013).

Due to its heterogeneity, the treatment of PCa has been shown to be an important challenge, for which careful selection of the most suitable treatment regimen is required (Fig. 1).

Currently, therapy selection is based on clinical staging (including TNM staging, Gleason scoring, and prostate specific antigen (PSA) levels) and patients’ overall health status. Low-risk, localized PCa is generally managed by active surveillance. For intermediate- and especially high-risk disease, more invasive therapy is required, for which surgery or radiotherapy are currently the standard treatment options whether or not within the context of a multimodal approach. In the metastatic setting, androgen deprivation therapy (ADT) is the mainstay treatment, targeting androgen receptor (AR) signaling. The first therapies, aimed at blocking AR activity, were already being used in the 1940s by Huggins (1942). He proposed castration and high doses of estrogens to create an androgen-deprived state of the tumor. This revolutionary method was refined during the years and has led to the use of luteinizing hormone-releasing hormone (LHRH) agonists that interfere with the hypothalamic–pituitary–testis axis, leading to androgen deprivation. This initially leads
Secondly, we will review the preclinical development of metastatic PCa, directly or indirectly targeting the AR. The recent understanding that the AR remains an important target, even in the castration-resistant setting, has led to the development of a plethora of treatment options for the treatment of metastatic prostate cancer (CRPC). Other promising non-AR-targeted therapies such as cabazitaxel (de Bono et al. 2010), sipuleucel-T (Higano et al. 2009), alpharadin (Harrison et al. 2013), and tasquinimod (Armstrong et al. 2013) are currently under development but are not within the scope of this review.

AR-targeted therapies

As androgens have a pivotal role in the development of the prostate gland and prostate carcinogenesis, the AR is the fundamental target of systemic therapy for PCa (Taplin & Balk 2004). The AR is a ligand-dependent transcription factor that is activated when androgens are present (Helsen et al. 2012a). In response to androgen binding, it can initiate expression of genes that contain response elements, which are recognized by the AR (Denayer et al. 2010). Indeed, in prostate cells, the AR controls the balance between cell differentiation and proliferation. In normal prostate tissue, the scale is tipped toward differentiation, while during progression of PCa from early to advanced cancer the scale gradually tips in favor of proliferation. This shift is accompanied by a downregulation of genes that support differentiation and upregulation of genes that promote proliferation (Hendriksen et al. 2006, Marques et al. 2011). Thus, the uncontrolled cell growth that is associated with PCa is reflected by a shift of the transcriptional program of the tumor from differentiation to proliferation. Therefore, it is crucial to inhibit androgen signaling either by depriving the tumor from androgens or by blocking the receptor activity. AR activity can be enhanced not only by steroids produced in the testis and adrenal glands but also by androgens that were synthesized by the tumor itself (Montgomery et al. 2008). This way, the tumor supports its own uncontrolled AR activity leading to CRPC.

Androgen deprivation therapy

Inhibition of testicular androgen synthesis  ADT leads to a systemic decrease in the level of circulating androgens resulting in an androgen-depleted environment that prevents activation of the AR (Fig. 2). ADT, achieved by chemical or surgical castration, still is the mainstay of treatment for patients with metastatic PCa. Due to convincing evidence showing comparable efficacy, surgical castration is generally replaced by chemical castration in the Western world (Soloway et al. 1991, Vogelzang et al. 1995, Seidenfeld et al. 2000).

Chemical castration is achieved by blocking the hypothalamic–pituitary–testis feedback system with
LHRH analogues (Fig. 2). Currently, the available LHRH agonists are leuprolide, goserelin, triptorelin, and histrelin, which need to be administered, monthly to yearly, by injection or implantation. There is an initial release of luteinizing hormone, causing a downregulation of LHRH receptors at the hypothalamus, disrupting the hypothalamic–pituitary–testicular axis and therefore reducing testosterone production (Schally et al. 1992). Due to the initial release of luteinizing hormone (increase by up to tenfold), there is an initial rise in testosterone levels (approximately twofold), occurring during the initial 72 h of treatment, which leads to a transient increase in PSA levels (Thompson 2001). Testosterone levels are being suppressed at 10–20 days from administration, however, at a lower efficacy compared with orchidectomy, as a significant number of patients treated with the LHRH agonists fail to reach castrate levels of testosterone (Wex et al. 2013).

It has been suggested that this ‘biochemical flare’, occurring in 4–63% of patients, could cause clinical ‘flares of disease’, with reports of increased pain from bone metastases, spinal cord compression, pathological fractures, bladder outlet obstruction, and even death (Bubley 2001, Heidenreich et al. 2011). Besides the initial flare, mini-flares are observed within 12 h after the second or subsequent administration of the LHRH agonist (Sarosdy et al. 1999, Sharifi & Browneller 2002). To prevent these flares, antiandrogens (see below) are being combined with LHRH agonistic treatment, before and during the first weeks, to reach a total androgen blockade. While the antiandrogens will block the action of testosterone and adrenal androgens on the AR, they will not inhibit the hormonal surge and some of the potentially harmful effects that it produces (Brawer 2001).

To circumvent the flares, LHRH antagonists have been developed, resulting in an equally effective, but more rapid chemical castration as LHRH agonists (Van Poppel et al. 2008, Crawford et al. 2011, Garnick & Mottet 2012, Ozono et al. 2012). Moreover, the LHRH antagonists do not have increased risk for cardiovascular diseases as is the case for LHRH agonists (Levine et al. 2010).

Despite the lack of evidence on the contribution of antiandrogens, the current EAU Guidelines recommend ADT therapy in all (symptomatic and asymptomatic) metastatic patients combined with short-term administration of antiandrogen therapy to reduce the risk of the ‘flare’ phenomenon in patients receiving LHRH agonists. They emphasize that, especially in patients at high risk of ‘clinical flare’, LHRH agonists should not be administered as monotherapy, even though there is no evidence of long-term effects (Heidenreich et al. 2011).

**Inhibition of adrenal and intracellular androgen synthesis** Despite the initial good response to ADT, PCa progresses toward a castration-resistant stage after a median time of 2–3 years (Knudsen & Kelly 2012). This means that the tumor has adapted to survive the castrate levels of
androgens or the presence of antiandrogens. Frequently, AR signaling has been restored not only by an upregulation of the AR, by mutation of the AR, by an imbalance of AR co-regulatory proteins but also by the synthesis of androgens in the tumor cells itself (Taplin & Balk 2004).

Several compounds, such as ketoconazole and abiraterone, are able to inhibit the intracellular biosynthesis of androgens (CYP17A1) and thereby prevent activation of the AR (Attard et al. 2009; Fig. 2). Compared with ketoconazole, abiraterone acetate is a potent, selective, and safe drug. It inhibits the androgen synthesis not only in the testis but also in the adrenal glands and the prostate (Attard et al. 2009). Ketoconazole and abiraterone have to be administered together with prednisone to avoid symptoms of mineralocorticoid excess, caused by an excess of ACTH secretion (Danila et al. 2010).

**Abiraterone acetate** Abiraterone acetate was developed to manage metastatic PCa, progressing after chemotherapy. One of the observed adaptive mechanisms in PCa cells resulting in castration resistance is an increase of the intracellular androgen levels (Montgomery et al. 2008). CYP17A1, which is an enzyme involved in the androgen biosynthesis pathway, is specifically inhibited by abiraterone. By suppressing testosterone synthesis in the adrenals and intratumorally, administration of abiraterone results in decreased intraprostatic testosterone levels.

After the efficacy had been proved in clinical trials on both chemotreated and chemonaive mCRPC patients (de Bono et al. 2011, Ryan et al. 2013), the drug was approved by the FDA and EMA for use in patients with mCRPC. Recently, Richards et al. (2012) demonstrated that part of the mechanism of action of abiraterone (steroidal) can be attributed to AR binding and inhibition (Fig. 2).

**Novel CYP17A1 inhibitors** Other CYP17A1 inhibitors are under development (Vasaitis et al. 2008, Yamaoka et al. 2012; Fig. 3). Indeed, abiraterone is an inhibitor of both the C17,20-lyase activity and the 17α-hydroxylase activity of CYP17A1 and inhibition of the latter causes symptoms

![Figure 3](http://erc.endocrinology-journals.org) Steroidal antiandrogens and CYP17 inhibitors. The CYP17 inhibitors with a steroidal structure also display antiandrogenic effects by binding directly to the AR. DHT, dihydrotestosterone; CPA, cyproterone acetate; MPA, medroxyprogesterone acetate.
of mineralocorticoid excess. Therefore, compounds with higher specificity against the C17,20-lyase activity of CYP17A1 have been developed to prevent the need for prednisone. TAK700, a non-steroidal CYP17A1 inhibitor, is an example of more selective CYP17A1 inhibitor; however, the phase 3 clinical trial with TAK700 has ended preliminary because of the observation that TAK700 plus prednisone would not likely meet the primary endpoint of improved overall survival compared with placebo plus prednisone. For TOK-001, it is known that it is not only a CYP17 inhibitor, but also a competitive AR antagonist that results in the downregulation of the AR (Vasaitis et al. 2008, Bruno et al. 2011).

**Antiandrogens**

AR antagonists or antiandrogens prevent androgens from carrying out their biological activity by directly binding and blocking the AR LBD, and by inducing repressive activity (Fig. 4).

The current roles of antiandrogen therapy are limited to preventing the flare-up of LHRH-agonistic therapies (see above) and usage in combination with LHRH-agonistic or -antagonistic therapy to achieve complete androgen blockade (Fig. 1).

Despite the fact that chemical or surgical castration reduces 95% of testosterone levels, an intraprostatic androgen stimulus is still present as a result of circulating androgens and androgen precursors of adrenal origin. Adding an antiandrogen to castration blocks the action of these adrenal androgens, resulting in complete androgen blockade. However, as the clinical advantage of this combined treatment is still questionable, the current EAU guidelines do not recommend this as standard treatment (Heidenreich et al. 2011).

In the next section, we will discuss the current evidence on the role of (steroidal and non-steroidal) antiandrogens in treating advanced PCa.

**Steroidal antiandrogens** In analogy with the structure of androgens, the first antiandrogens contained a steroidal skeleton to ensure binding to the receptor (Fig. 3). Several steroidal antiandrogens (cyproterone acetate (CPA), megestrol acetate, and medroxyprogesterone acetate) were initially used for obtaining maximal androgen blockade in patients; however, severe drawbacks such as hepatotoxicity, interference with libido and potency, cardiovascular side effects, and low efficacy have limited their clinical use (Jacobi et al. 1980, Pavone-Macaluso et al. 1986, 1989, Moffat 1990, Patel et al. 1990, Dawson et al. 2000, Schroder et al. 2004).

The side effects that occur for these drugs are related to their effects on other steroid receptors such as the progesterone receptor and the glucocorticoid receptor, for...
which they are (weak/partial) agonists (Pridjian et al. 1987, Schroder 1993). Interference with libido and potency is the result of their antigonadotropic effects leading to reduced secretion of LH and follicle-stimulating hormone (FSH) and as a consequence decreased plasma levels of T and E. Unlike the steroidal antiandrogens, flutamide (flut) increases T and E levels which could lead to gynecomastia (Schroder 1993). In concert with LHRH treatment, the steroidal antiandrogens seem to reduce gynecomastia and hot flushes. The effectiveness of the LHRH therapy, however, cannot be increased as was shown in a prospective, randomized study comparing goserelin acetate vs CPA vs a combination of the two, and this study concluded that CPA as monotherapy was inferior and the combined treatment was not superior to goserelin acetate alone (Thorpe et al. 1996). With regard to reducing hot flushes, Irani et al. (2010) performed a randomized double-blind trial, comparing efficacies of these drugs, concluding that medroxyprogesterone acetate could be considered as the standard treatment for hot flushes.

Non-steroidal antiandrogens

Non-steroidal antiandrogens (NSAAs) were first introduced in 1989 in clinical practice as treatment for advanced and metastatic PCa. Currently, they are mainly used in combination with LHRH agonists in preventing clinical ‘flare-up’ and in combination with LHRH agonists/antagonists to achieve ‘complete androgen blockade’ (see above). Available drugs are the first-generation antiandrogens flut, bicalutamide (bic), and nilutamide (nil), and the second-generation compound enzalutamide (enz) (previously known as MDV3100) (Fig. 5). They antagonize the actions of androgens at the receptor level and thereby inhibit tumor growth. The first-generation antiandrogens bic and nil were derived from flut and thus have a similar non-steroidal, chemical structure (Fig. 5). The non-steroidal structure avoids the typical constraints associated with the previous steroidal antiandrogens. Of them, bic is the best tolerated and most stable antiandrogen currently used in clinical practice. It has a half-life of seven days compared with 6–8 h for flut and 2 days for nil which allows once-a-day dosing (McLeod 1997). The efficacy of bic in clinical trials was reported to be at least equivalent to the efficacy of flut (Schellhammer et al. 1996) and it is better tolerated in terms of diarrhea, a common adverse effect in flut-treated patients (Sarosdy 1999). No beneficial effects were observed for nil over flut and nil has the least favorable safety profile (Sarosdy 1999). While peripheral selectivity was observed in intact rats due to a low passage across the blood–brain barrier (Furr 1996), this was not the case in men with advanced PCa. Their serum LH and serum T levels were significantly increased due to bic therapy (Mahler & Denis 1990).

Enzalutamide was first introduced in 2009 as a second-generation antiandrogen with several advantages over bic (Tran et al. 2009). In contrast to the ‘classical antiandrogens’, enz does not only block androgen binding, but it also inhibits translocation of the AR to the nucleus and impairs AR binding to DNA (Tran et al. 2009). It has been approved by the FDA and EMA for treating patients with chemo-resistant CRPC (Ning et al. 2013). Further structural optimization for inhibition of proliferation, pharmacokinetics, and in vivo efficacy resulted in the development of a compound called ARN-509 (Fig. 5). It has a higher efficacy than enz, as 30 mg/kg per day has the same therapeutic effect as 100 mg/kg per day enz (Clegg et al. 2012). Moreover, due to the lower required dosage of ARN-509, it has a lower tendency to induce seizures, a typical side effect of antiandrogens from the bic class (Foster et al. 2011).

Clinical use

Several studies have been conducted to determine the efficacy and to assess whether NSAAs in monotherapy have an advantage over LHRH analogues regarding side effects such as erectile dysfunction, loss of libido, fatigue, etc. Both the first- and second-generation NSAAs demonstrate an identical off-target on GABAA receptors in the brain, leading to seizures (Foster et al. 2011). In light of this, ARN-509 has been developed, which has a reduced passage through the blood–brain barrier. We will briefly discuss the clinical use of the first- and second-generation antiandrogens.

Flutamide

Flut was the first NSAA available for clinical use, approved by the FDA in 1989. It is indicated to use in combination with LHRH agonists for the management of locally confined/advanced and metastatic PCa.

Its role as a monotherapeutic agent is still unclear, with only one randomized controlled trial (RCT) published, comparing the therapeutic efficacy of flut with surgical castration in patients with metastatic PCa. However, the results remained inconclusive on its efficacy (Boccon-Gibod et al. 1997). Furthermore, the EORTC group could not show a difference in outcome between flut and CPA either (Schroder et al. 2004). The same EORTC trial 30892 showed that only 20% of men treated with flut remained sexually active for 7 years (Schroder et al. 2004). Due to the poor evidence for its efficacy and its side effects on sexual activity in men, flut is currently not indicated as a monotherapeutic drug in patients with metastatic PCa.
Bicalutamide  Bic is currently the most investigated and most widespread NSAA in clinical practice, with recommended doses of 50 mg/day in complete androgen blockage and 150 mg/day for monotherapy. The latter has been investigated most extensively, with several prospective RCTs being performed. When comparing bic in monotherapy with castration, bic was associated with worse survival rates, although the difference in median survival was only 6 weeks (Tyrrell et al. 1998). Two small RCTs, comparing bic with complete androgen blockade, remained inconclusive, although Boccardo et al. showed an increased risk of death in poorly differentiated tumors (Fourcade et al. 1998, Boccardo et al. 2002).

Despite these inconclusive results, the American Society of Clinical Oncology (ASCO) has suggested bic monotherapy as an alternative for the gold standard (ADT) in well-informed patients with favorable clinical parameters (Loblaw et al. 2007).

Nilutamide  There are no comparative trials of nil monotherapy with castration or with other antiandrogens, because of which nil is currently not licensed for monotherapy.

Enzalutamide  In patients who progressed during docetaxel therapy, enz has been shown to increase overall survival in comparison with placebo in mCRPC patients in the AFFIRM trial, leading to its FDA approval (Scher et al. 2012, Ning et al. 2013). A recent report from the AFFIRM trial showed comparable efficacy, safety, and tolerability in patients aged >75 years as well, suggesting its possible future widespread use (Sternberg et al. 2014). Two small

**Figure 5**
Non-steroidal antiandrogens. Both the first- and second-generation antiandrogens that are currently being used in clinical practice share a structural motif consisting of an anilide substituted with a trifluoromethyl group in meta-position and a nitro- or cyano-group in para-position. In nilutamide, enzalutamide, and ARN-509, the amide of the anilide is a part of (thio)hydantoin.

**Non-steroidal antiandrogens**

First-generation

- **Flutamide**

Second-generation

- **Enzalutamide**

- **ARN-509**

**Bicalutamide**

Bic is currently the most investigated and most widespread NSAA in clinical practice, with recommended doses of 50 mg/day in complete androgen blockage and 150 mg/day for monotherapy. The latter has been investigated most extensively, with several prospective RCTs being performed. When comparing bic in monotherapy with castration, bic was associated with worse survival rates, although the difference in median survival was only 6 weeks (Tyrrell et al. 1998). Two small RCTs, comparing bic with complete androgen blockade, remained inconclusive, although Boccardo et al. showed an increased risk of death in poorly differentiated tumors (Fourcade et al. 1998, Boccardo et al. 2002).

Despite these inconclusive results, the American Society of Clinical Oncology (ASCO) has suggested bic monotherapy as an alternative for the gold standard (ADT) in well-informed patients with favorable clinical parameters (Loblaw et al. 2007).

**Nilutamide**

There are no comparative trials of nil monotherapy with castration or with other antiandrogens, because of which nil is currently not licensed for monotherapy.

**Enzalutamide**

In patients who progressed during docetaxel therapy, enz has been shown to increase overall survival in comparison with placebo in mCRPC patients in the AFFIRM trial, leading to its FDA approval (Scher et al. 2012, Ning et al. 2013). A recent report from the AFFIRM trial showed comparable efficacy, safety, and tolerability in patients aged >75 years as well, suggesting its possible future widespread use (Sternberg et al. 2014). Two small
Endocrine-Related Cancer

DNA response elements, recruitment of coactivators, etc. such as inhibition of entry to the cell nucleus, binding to results in the inhibition of many required actions of the AR, The conformation of the receptor induced by antagonists such as the amino terminal domain (NTD), which was repositioned from its active position or helix 12 is forced into a flexible position (Kauppi et al. 2003, Hodgson et al. 2008, Lusher et al. 2011). This disables the binding of coactivators and/or enables the formation of another platform to which corepressors can bind. Moreover, antiandrogens can recruit corepressors via other domains such as the carboxy-terminal end of helix 12 is anchored by the -sheet leading to a less flexible helix 12. In absence of antagonist-bound AR crystals, molecular modeling techniques have predicted that, for the AR, a displacement of helix 12 could occur in the presence of antiandrogens, although involvement of residues in helix 5 and helix 11 cannot be excluded (Georget et al. 2002, Bohl et al. 2005a, Osguthorpe & Hagler 2011). The conformation of the receptor induced by antagonists results in the inhibition of many required actions of the AR, such as inhibition of entry to the cell nucleus, binding to DNA response elements, recruitment of coactivators, etc. Each step is important for the execution of the normal function of AR, and each step is therefore a potential target for antiandrogen therapy. The precise mechanism of action of bic and enz is described below (Fig. 4).

Mechanism of action of antiandrogens Antiandrogens bind to the ligand-binding pocket of the AR and thereby prevent its activation. When androgens such as dihydrotestosterone (DHT) bind to the AR, a very specific conformational change occurs in the ligand-binding domain, leading to an active receptor (Pereira de Jesus-Tran et al. 2006; Fig. 4). Crystal structures of steroid receptor ligand binding domains (LBDs) with an agonist have shown that after binding, helix 12 closes off the pocket and forms a platform for the binding of coactivators, called activation function 1 (AF1; Shiau et al. 1998, Williams & Sigler 1998). This specific position of helix 12 is required and can only be induced by agonists. When antagonists bind, they induce a distinct inactive LBD conformation in which helix 12 is repositioned from its active position or helix 12 is forced into a flexible position (Kauppi et al. 2003, Hodgson et al. 2008, Lusher et al. 2011). This disables the binding of coactivators and/or enables the formation of another platform to which corepressors can bind. Moreover, antiandrogens can recruit corepressors via other domains such as the amino terminal domain (NTD), which was demonstrated for CPA (Dotzlaw et al. 2002). For the AR, the carboxy-terminal end of helix 12 is anchored by the formation of a -sheet leading to a less flexible helix 12. In absence of antagonist-bound AR crystals, molecular modeling techniques have predicted that, for the AR, a displacement of helix 12 could occur in the presence of antiandrogens, although involvement of residues in helix 5 and helix 11 cannot be excluded (Georget et al. 2002, Bohl et al. 2005a, Osguthorpe & Hagler 2011). The conformation of the receptor induced by antagonists results in the inhibition of many required actions of the AR, such as inhibition of entry to the cell nucleus, binding to DNA response elements, recruitment of coactivators, etc. Each step is important for the execution of the normal function of AR, and each step is therefore a potential target for antiandrogen therapy. The precise mechanism of action of bic and enz is described below (Fig. 4).

Bicalutamide When bic binds to the ligand-binding pocket of the AR, a displacement of helix 12 and consequently a distortion of the coactivator platform were suggested by molecular dynamics-based simulations (Osguthorpe & Hagler 2011). Furthermore, it leads to the assembly of a transcriptionally inactive complex due to the inability of the bic-bound AR to recruit coactivators and/or the preferential recruitment of corepressors, NCoR and SMRT (Masiello et al. 2002). By fluorescence recovery after photobleaching (FRAP) analysis with a GFP-tagged AR, the immobile fraction of nuclear AR disappeared when bic was present, meaning that the receptor was unable to bind DNA (Farla et al. 2005). Moreover, the AR was shown to be destabilized in the presence of bic (Waller et al. 2000). All these observations explain why bic can decrease androgen-induced gene expression and reduce the weight of rat ventral prostate and seminal vesicles after oral administration (Furr & Tucker 1996).

Enzalutamide As mentioned earlier, enz is considered as a more effective inhibitor of AR compared with bic due to a higher binding affinity, the inhibition of AR nuclear translocation and DNA binding by AR (Tran et al. 2009, Guerrero et al. 2013). FoxA1 is a PCa-involved pioneering factor that makes DNA more accessible for binding by the AR. Enz prevented the AR from binding to DNA response elements in presence of FoxA1, while bic could not (Belikov et al. 2012). Further optimization of enz is ongoing and has led to the development of ARN-509, a compound with optimized pharmacokinetics, phase 1 and 2 clinical studies of which are currently running (Clegg et al. 2012, Rathkopf et al. 2013). Due to its structural similarities (Fig. 5), a similar binding mode to AR and thus a similar working mechanism are attributed to ARN-509 (Clegg et al. 2012, Balbas et al. 2013). Unfortunately, so far no antagonist-containing WT AR LBD crystal structures are available (Balbas et al. 2013, Joseph et al. 2013, Korpal et al. 2013). The discovery of the AR W741C/L mutation in bic-resistant cells, AR T877A mutation in flut-resistant cells, and AR F876L mutation in enz-resistant cells has given some insights. A model for the binding of enz to the ligand-binding pocket of WT AR has been proposed (Fig. 6; Balbas et al. 2013). A focused chemical screen, based on this in silico model, has led to a compound that circumvents this escape mutation and remains antagonistic (Balbas et al. 2013).
This was confirmed not only by transfection experiments with AR F876L (where ARN-509 also performed as an agonist), but also by the detection of the AR F876L mutation in the plasma DNA of ARN-509-treated CRPC patients (Balbas et al. 2013, Joseph et al. 2013).

Structural similarities for the current antiandrogens Strikingly, the available first- and second-generation antiandrogens share a common structural element, an anilide core motif (Fig. 6). For some compounds, the amide of the anilide can be a part of (thio)hydantoin. The benzene ring of the anilide has a trifluoromethyl cyano benzyl group in meta-position and a nitro- or cyano-group in para-position. The chemical scaffold of Bic has been studied well by means of structure–activity relationships (Kirkovsky et al. 2000, Bohl et al. 2005b) and has also served as a template for the development of several selective AR modulators (SARMs), such as Ostarine and LGD-4033 (Dalton et al. 2013). The structures of RD162, MDV3100 (enz), and ARN-509 are based on that of RU59063, a non-steroidal arythiohydantoin AR agonist that binds AR with the highest known affinity (in the nanomolar range) (Jung et al. 2010). Holding on to this structural element, however, diminishes the structural diversity of AR ligands and could therefore be the cause of cross-resistance in case of sequential therapy. Molecular modeling of bic and enz in the ligand-binding pocket of AR shows a highly conserved interaction required for binding to AR (Voet et al. 2013; Fig. 6). Structurally more diverse AR LBD antagonists could thus provide an innovative way of inhibiting AR, by introducing a different conformational change in AR and hence also a different helix 12 position.

From the latest studies on enz resistance and the much earlier reports of bic and flut resistance, it is almost certain that eventually every LBD inhibitor, which has been and will be developed, will lead to the introduction of a somatic mutation that causes a switch to agonist. Therefore, it seems that the future of AR antagonists lies in the development of compounds with different targets or different strategies. For instance, compounds that do not act via the ligand-binding pocket but via other sites on the LBD, the NTD or compounds that could affect AR protein levels will be of interest. Furthermore, the combination of compounds with a complementary action mechanism could lead to a more efficient inhibitory action of AR and thus a better control of the disease.

Experimental AR-targeted therapies

Inhibitors binding to the ligand-binding pocket We and others have screened for novel AR antagonists belonging to classes that are structurally different from those that are currently used in the clinic (Helsen et al. 2012b, Voet et al. 2013). Although such molecules have the advantage that they might still work in case of resistance to bic, flut, or enz, they still need to be developed further before they could be tested in a clinical trial setting.

ODM-201 is a novel AR antagonist that binds to AR with a higher affinity than enz. Antiproliferative effects of ODM-201 have been demonstrated in subcutaneous VCaP xenografts and, due to its unique pharmacological properties, it has also been shown to have superior preclinical efficacy in phase 1 studies with no side effects on the CNS. The most common adverse effects were asthenia, diarrhea, and nausea (Fizazi et al. 2012).

Inhibitors of AR that do not bind to the ligand-binding pocket EPI-001 is a bisphenol A diglycidyl ether-like compound that has been reported to affect the transactivation mediated by the AR NTD. It inhibits the AR regardless of ligand and inhibits
androgen-induced gene expression and proliferation both in vitro and in LNCaP xenograft mice models (Andersen et al. 2010, Myung et al. 2013). EPI-001 is an alternative drug candidate for PCa as it does not act via the LBD. EPI-001 might circumvent escape mechanisms such as LBD mutation or truncation and should therefore still be active in many forms of CRPC.

An alternative way of action compared with enz and bic was discovered for ASC-J9, an AR-binding compound that destabilizes full-length AR as well as the AR splice variants involved in castration resistance of PCa (Yamashita et al. 2012). They all inhibit PCa proliferation and reduce PSA levels, but only ASC-J9 was able to suppress cell invasion and thus the formation of metastases in in vitro and in vivo models (Lin et al. 2013a). A different modulation of the STAT3–CCL2 signaling pathway was suggested to lie at the base of this observed difference (Lin et al. 2013b).

**Affecting AR localization** SNARE-1 binds WT and mutant ARs via the LBD. It not only inhibits nuclear translocation of the AR, but also facilitates nuclear export leading to reduced nuclear AR levels. Furthermore, it promotes degradation of the AR via the ubiquitination pathway. Its mechanism of action is completely different compared with the clinically available antiandrogens and its activity has been demonstrated on PCa xenografts in mice (Narayanan et al. 2010).

**Affecting AR mRNA half-life** EZN-4176 is a 16-mer antisense oligonucleotide that decreases full-length AR protein expression by binding to exon 4 of AR mRNA (the hinge). Both in vitro and in animal models, EZN-4176 can inhibit the proliferation of androgen-sensitive and CRPC cells (Zhang et al. 2011). A small study with 22 CRPC patients who were progressive (prior abiraterone or enzalutamide treatment was allowed) demonstrated only little biochemical and soft tissue response and only three out of eight patients showed a reduction in circulating tumor cells at the doses used (Bianchini et al. 2013b); side effects such as fatigue and liver toxicity prevented further dose escalation (Bianchini et al. 2013b).

**Inhibitors binding to BF3 of AR** Next to the ligand-binding pocket, a newly discovered surface pocket of AR, called binding function 3 (BF3), could be targeted to influence coactivator binding (Estebanez-Perpina et al. 2007). Several structural scaffolds have been identified through virtual screening as binders to this BF3 region and were confirmed to have antagonistic effects not only on AR transcriptional activity (Lack et al. 2011), but also on proliferation of LNCaP and enz-resistant cells (Munuganti et al. 2013).

**Future perspectives**

A new era has started in treating mCRPC patients thanks to the development of multiple new potent AR-targeted therapies. These agents first have to prove their efficacy in the post-chemotherapeutic setting, as have abiraterone and enzalutamide, leading to their FDA and EMA approval. For ARN-509, a safety, pharmacokinetic, and proof-of-concept study is currently ongoing (NCT01171898). The efficacy of these AR-targeted compounds in these advanced stages has confirmed that CRPC is not an AR-independent disease and these novel treatments are even being considered to be used at an earlier stage during the course of the disease. When resistance to these novel agents will arise, the elucidation of the underlying mechanisms will tell us what the targets of the next generation of compounds should be. The emergence of AR splice variants and the antagonist-agonist switching mutation AR F876L indicate that the AR can also circumvent the new LBD inhibitors, which are being developed and might necessitate the development of structurally or strategically different AR inhibitors. Will the AR keep its critical role in PCa treatment or will it be replaced by completely different targets?

---

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

**Funding**

This work was supported by the fund from the KU Leuven (OT08-11) and the FWO (G.0684-12/N and G.0830-13N), and the authors gratefully acknowledge the financial support from the company Ravago Distribution Center NV (LUOR funds).

**Acknowledgements**

The authors would like to acknowledge Servier for producing Figs 2 and 4 using Servier Medical Art (www.servier.com).

**References**


http://erc.endocrinology-journals.org DOI: 10.1530/ERC-13-0545 © 2014 Society for Endocrinology Printed in Great Britain

Published by Bioscientifica Ltd.


Fourcade RO, Chatelain C & Poterne M. 1998 An open multicentre randomised study to compare the effect and safety of ‘Casodex’ (bicalutamide) 150 mg monotherapy with castration plus nilutamide in metastatic prostate cancer. In 13th EAU Congress, p88 (abstract #349).


Hodgson MC, Shen HC, Hollenberg AN & Balk SP 2008 Structural basis for nuclear receptor coexpressor recruitment by antagonist-agnetized androgen receptor. Molecular Cancer Therapeutics 7 3187–3194. (doi:10.1158/1535-7163.MCT-08-0461)


and the antagonism of this interaction by tamoxifen. Cell **95** 927–937. (doi:10.1016/S0092-8674(00)81717-1)


Received in final form 21 February 2014
Accepted 13 March 2014
Made available online as an Accepted Preprint 17 March 2014