Attacking the androgen receptor pathway in prostate cancer

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Since the 1940's the androgen receptor has been the main target for systemic therapy in prostate cancer. Classic hormonal therapy aims at lowering serum testosterone levels or block the androgen receptor ligand-binding domain. Despite disease progression, castration-resistant prostate cancer remains predominantly androgen-driven as novel secondary hormonal therapy with abiraterone acetate or enzalutamide has demonstrated increased overall survival. Promising androgen synthesis inhibitors (orteronel, galeterone), androgen receptor inhibitors (ARN-509, EPI-001, AZD3514) and heat-shock protein modulators are under investigation. Given the upcoming arsenal of systemic therapies and the molecular heterogeneity of castration-resistant prostate cancer, patient-tailored therapy strategies are being explored.

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Introduction

Prostate cancer (PCa) is the most common malignancy reported in men and is the third-most common cause of cancer-related death in Europe.[1,2] In 2010 8,863 new cases of PCa were diagnosed in Belgium.[3] The introduction of serum prostate-specific antigen (PSA) measurement has increased PCa detection with a stage migration towards more localised and more low-grade tumours.[4] PCa is either localised (T1-2 M0), locally advanced (T3-4 M0) or metastatic (M1).[5] Non-metastatic PCa is divided into low-risk (PSA <10 ng/ml, cT1-2a and Gleason ≤6), intermediate-risk (PSA 10-20 ng/mL, cT2b-c or Gleason 7), high-risk (PSA >20 ng/mL, cT3a or Gleason ≥8) and very high-risk disease (cT3b-4N0 or N1).[5,6] Patients with localised and locally advanced PCa can be offered treatment with curative intent (surgery or radiotherapy ± androgen deprivation therapy or a combination of both). Growing evidence of over-detection and overtreatment of low-risk PCa are popularising active surveillance in which patients are closely followed with PSA and digital rectal examination and only receive curative treatment in case of significant tumour progression.[7] Nevertheless, some patients will experience cancer recurrence or will present with multiple metastases at diagnosis. These patients require systemic therapy, starting with androgen deprivation therapy, which targets the androgen receptor (AR) pathway.[8] Recently novel AR-targeted therapies have become available in PCa treatment while more are on the horizon. The focus of this article is on the mechanisms and current indications of hormonal therapy, the mechanisms leading to castration-resistant PCa and novel and upcoming therapies targeting the AR.

The AR pathway and androgen deprivation therapy

The AR plays a central role in PCa development and progression (*Figure 1*). The AR gene (located at chromosome X locus q12) codes for the AR protein which consists of a COOH-terminal ligand-binding domain (LBD), a DNA-binding domain (DBD) and a NH2-terminal domain (NTD). This intracellular steroid receptor is stabilised by two chaperone heat shock proteins (HSP). Androgens, mainly testosterone and the more potent dihydrotestosterone, bind the AR LBD inducing a conformational change through which the AR loses its HSP, dimerises and translocates to the nucleus. The AR

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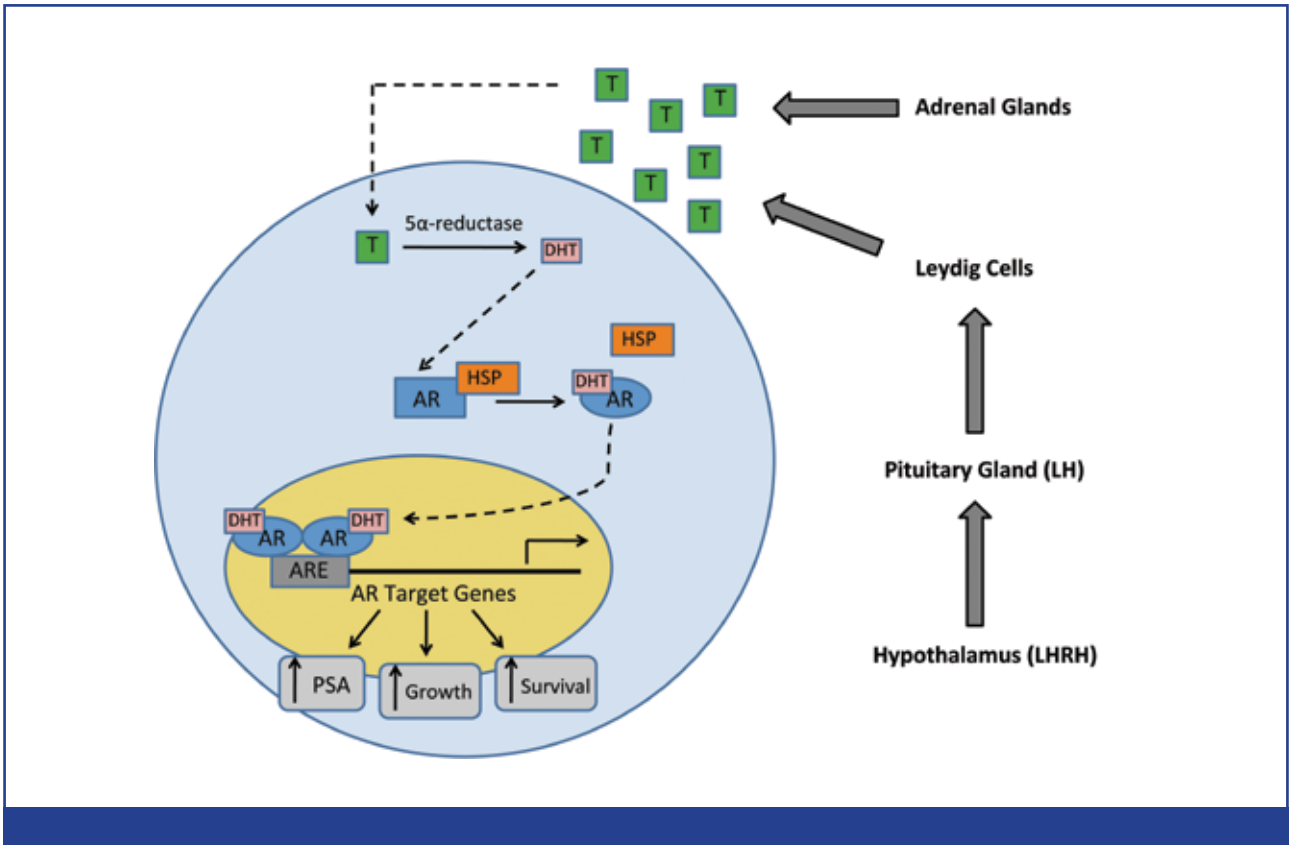

Figure 1. Androgen receptor pathway. The hypothalamic-pituitary axis drives the testicular Leydig cells to produce testosterone (T) which enters the prostate cell and is converted to the more potent dihydrotestosterone (DHT). Additionally, androgens are produced in the adrenal glands. Androgen receptor (AR)-DHT binding induces a conformational change in the AR, which loses its chaperone heat-shock proteins (HSP), dimerises and translocates to the nucleus. There it binds DNA androgen-responsive elements (ARE) and initiates cellular growth, proliferation and prostate-specific antigen (PSA) secretion. LH = luteinizing hormone. LHRH = LH-releasing hormone. *Reprinted from Saraon P, Jarvi K, and Diamandis E. Molecular Alterations during Progression of Prostate Cancer to Androgen Independence. Clinical Chemistry 2011; v. 57, p.1366-1375, with permission.*

dimer binds DNA AR-responsive elements to initiate transcription, which induces cell growth, proliferation and PSA secretion.[9] Testicular testosterone production is regulated by the hypothalamic-pituitary axis. The hypothalamus secretes luteinizing-hormone releasing hormone (LHRH) which stimulates the pituitary gland to produce luteinizing hormone (LH), which in turn stimulates the Leydig cells for testosterone production. In the prostate cell testosterone is converted into dihydrotestosterone by the enzyme 5α-reductase.

In PCa the AR pathway is classically attacked by either lowering ligand concentration or blocking the AR LBD.

Androgen synthesis inhibition

Androgen deprivation therapy (ADT) was first described by Charles Huggins in the 1940's, which earned him the Nobel prize for physiology or medicine in 1966.[10] By current standards, serum testosterone levels can be lowered <50 ng/dL by surgical (bilateral intratunical orchiectomy) or chemical castration. LHRH-agonists (gosereline, histreline, leuproreline, triptoreline) cause a burst in LH secretion in the pituitary gland and increased testosterone production in the testicular Leydig cells (flare phenomenon). Persistent LHRH stimulation causes a downregulation of LHRH-receptors and a fall in testosterone to castrate levels. Recently the LHRH-antagonist degarelix became available which does not cause testosterone flare and demonstrated longer time to PSA progression in metastatic patients with a pre-treatment PSA >20 ng/ml compared to LHRH-agonists.[11,12] In the past, oestrogen therapy (mostly diethylstilbestrol or DES) was applied to induce castration, but this has largely been abandoned due to an elevated cardiovascular risk.[13] ADT is indicated concomitantly with curative

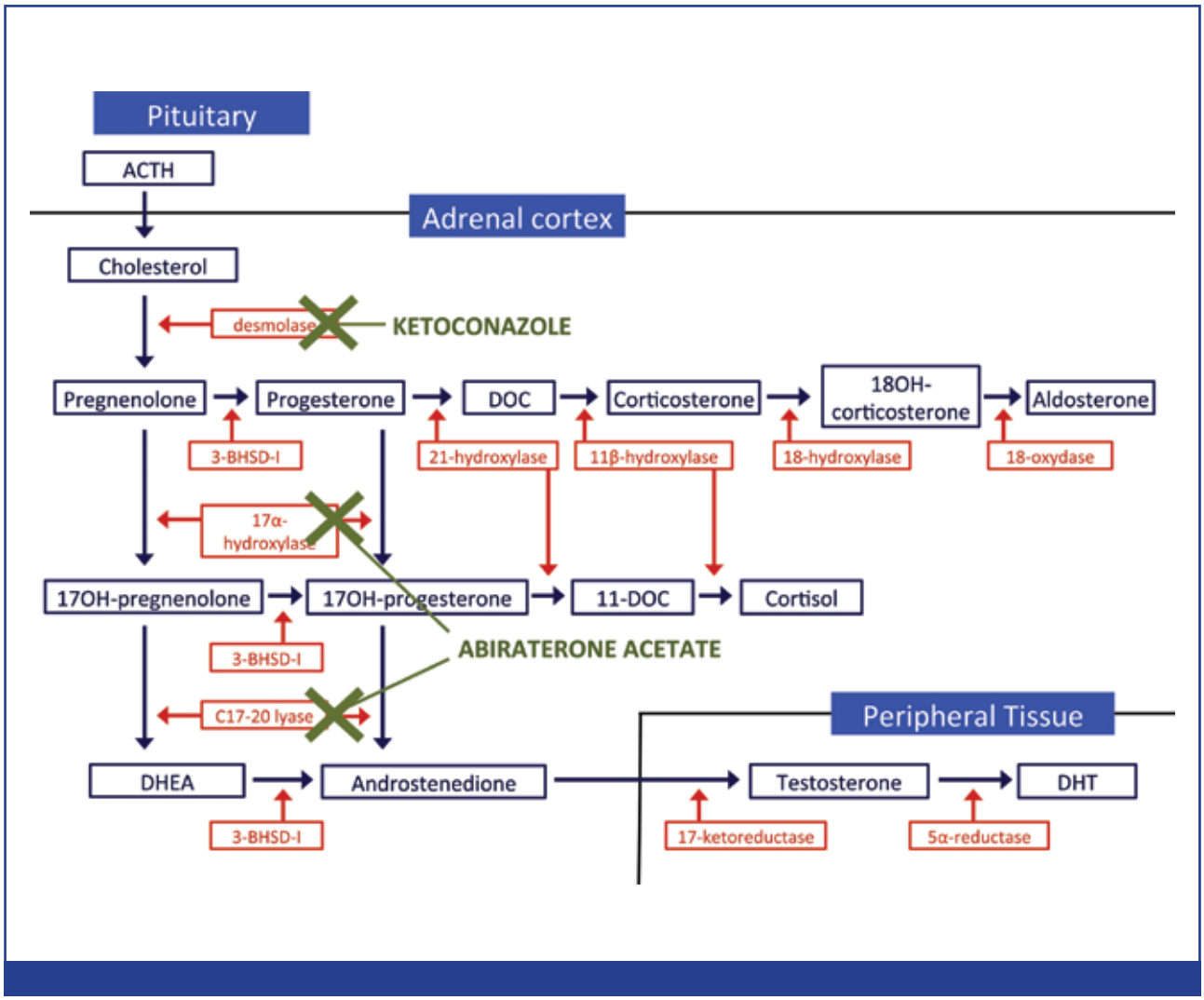

Figure 2. Abiraterone acetate and ketoconazole working mechanism. Both abiraterone acetate and ketoconazole inhibit CYP17 enzymes associated with steroid hormone synthesis, but abiraterone acetate does this more specifically (17α-hydroxylase and C17-20 lyase) than ketoconazole. Administration of abiraterone acetate leads to a decrease in molecules downward of this pathway (DHEA and androstenedione, but also cortisol), while aldosterone concentrations are increased. ACTH = adrenocorticotraphic hormone; DHEA = dihydroepiandrostenedione; DHT = dihydrotestosterone; BHSD = β-Hydroxysteroid dehydrogenase; DOC = deoxycorticosterone. *Modified from Update on Cancer Therapeutics, 2007, Vol 2, Aggarwal R, Ryan C. Development of abiraterone acetate, a 17-alpha hydroxylase C17,20-lyase inhibitor as a secondary hormonal therapy in prostate cancer. Pages 171-175. Copyright (2007), with permission from Elsevier.*

radiotherapy, ranging from six months (intermediate-risk PCa) to three years (high-risk PCa), and in locally advanced and metastatic PCa.

AR inhibition

Androgen-AR binding is inhibited by an anti-androgen (bicalutamide, cyproterone, flutamide). Anti-androgen therapy combined with ADT is prescribed to overcome the initial flare associated with LHRH-agonist therapy and following PSA progression under ADT. Anti-androgen monotherapy with bicalutamide 150 mg can also serve as an alternative to ADT in locally advanced non-metastatic PCa.[8]

ADT might not increase survival, but delays PCa progression, diminishes symptoms from metastases (e.g. pain) or the primary tumour (e.g. urinary obstruction) and prevents complications (vertebral fracture causing myelum compression).[14] Surgical or chemical castration itself can cause side effects including hot flushes (50-80%), loss of libido and erectile dysfunction (90%), metabolic syndrome (50%), cardiovascular events (24%), anaemia, osteoporosis, fatigue and depression. A varied

Mediterranean diet, regular physical activity, calcium and vitamin D supplementation and timely diagnosis and treatment of side-effects is therefore mandatory.[15-17] ADT is maintained ad vitam either continuously or intermittently, although recent studies indicate the assumed equivalent efficacy and reduced side-effects of intermittent ADT as compared to continuous ADT might be overestimated.[18,19]

Resistance mechanisms leading to castration-resistant disease

Patients with metastatic PCa under ADT will invariably progress after 12-24 months to castration-resistant PCa (CRPC), which is the lethal stage of PCa.[20] Given the effect of secondary hormonal treatments such as abiraterone (see below), the former term hormone-refractory PCa is no longer applicable. CRPC is defined by three consecutive PSA rises, including two 50% over the nadir under castrate serum testosterone levels (<50 ng/dL) following anti-androgen withdrawal phenomenon.[5] PCa progresses to CRPC through a combination of several AR-dependent and AR-independent mechanisms.[9,20]

AR-dependent mechanisms

PCa cells can become over-sensitised to low androgen levels by AR overexpression. This happens mostly due to AR gene amplification which occurs in 20-30% of CRPC patients.[21] Extra-testicular production of androgens from steroid precursors such as cholesterol in the adrenals and the tumour itself has also been postulated.[22] Next to androgens, other molecules can function as AR ligand due to decreased specificity of the AR LBD, including growth factors, cytokines, steroids, and even anti-androgens such as flutamide or bicalutamide.[23] This could partially explain the occasional anti-androgen withdrawal phenomenon, where tumour activity decreases 4-6 weeks after anti-androgen discontinuation.[24] Ligand-independent mechanisms of CRPC progression include production of AR splice variants lacking the LBD which are constitutively active (thus without binding of a ligand).[25] Furthermore, outlaw pathways have been described that directly activate the AR through growth factor (e.g. epithelial or insulin-like growth factor), cytokine (IL-6 or IL-8) or kinase action.[26,27]

AR-independent mechanisms

Bypass pathways are independent of AR function. They facilitate tumour progression by up-regulation of oncogenes (Bcl2, HER-2/neu) or down-regulating of tumour suppressor genes (p53, PTEN).[28,29] Epithelial to mesenchymal transition (EMT) is another example through which PCa cells can gain more aggressive and metastatic potential.[30]

All these different mechanisms underline the genetic, molecular and phenotypical heterogeneity of CRPC tumours and the need for targeted patient-tailored therapy.

Novel secondary hormonal therapies

Despite the failure of classical hormonal treatment, CRPC appears to remain an AR-dependent disease. Systemic treatment for CRPC includes secondary hormonal manipulations, chemotherapy (docetaxel or cabazitaxel next to older and less active drugs like mitoxantrone), immunotherapy (sipuleucel-T) or radionuclides (alpharadin). Alongside anti-tumoural treatment, bone-targeted therapy in case of bone metastases (zoledronic acid or denosumab) and symptomatic treatment should be the standard of care.[8] This chapter is restricted to AR-targeted products that have demonstrated efficacy in phase III trials. Chemotherapy, radionuclides or other therapies such as estramustine and corticosteroids (e.g. dexamethason) will not be covered.[31]

Androgen synthesis inhibitors

Ketoconazole

This anti-fungal drug impairs extra-testicular androgen synthesis through inhibition of several CYP enzymes. In a phase III trial patients were randomised to undergo anti-androgen withdrawal alone or with 400mg ketoconazole and 40mg hydrocortisone. Ketoconazole was associated with increased PSA decline >50% (27% versus 11%, p=0.002) and objective response rate (ORR: 20% versus 2%, p=0.02).[32] Notably, patients with high baseline androgen level were more likely to benefit from therapy.[33]

Abiraterone acetate (AA, Zytiga®)

This is a potent and specific inhibitor of CYP17A1, which is responsible for pregnenolone (Preg) and progesterone (Prog) hydroxylation to 17OH-Preg and 17OH-Prog and hydroxylation of 17OH-Preg to dehydroepiandrosterone (DHEA), a precursor molecule to both androgens and oestrogens (*Figure 2*).[22,34,35] It might also have a direct anti-AR effect.[36] AA is registered in metastatic CRPC, both before and after docetaxel and is reimbursed for use in mCRPC after docetaxel failure in Belgium today. In a phase III study 1g of AA + prednisone 10mg daily led to a significant increase in overall survival compared to placebo + prednisone (15.8 months versus 11.2 months, p<0.001) in CRPC patients previously

treated with docetaxel.[37] A PSA decline ≥50% and ORR were seen in 30% and 15% of patients on AA respectively. Again patients with higher baseline androgen levels were more likely to respond.[38] More recently a phase III trial in chemotherapy-naive patients was unblinded at the first interim analysis after a relative reduction of 25% (p=0.01) for death was seen in the AA arm.[39] A PSA decline ≥50% and ORR were seen in 62% and 36% of patients on AA respectively. Blockade of CYP17 also inhibits cortisol but not mineralocorticoid production, which is upregulated following increased adrenocorticotropic hormone (ACTH) levels (*Figure 2*). Concomitant steroid treatment is necessary to reduce mineralocorticoid excess and side-effects such as fluid retention, hypertension and hypokalaemia. Furthermore, rise in liver enzymes has been described which requires frequent monitoring.[37]

AR inhibitor

Enzalutamide (Xtandi®)

Enzalutamide (formerly known as MDV-3100) is a more potent anti-androgen, that blocks androgen-AR binding over five times more potently than bicalutamide and inhibits nuclear AR translocation, DNA binding and recruitment of AR coactivators.[40] A phase III trial (AFFIRM) recently demonstrated a significant increase in overall survival post-docetaxel (18.4 months versus 13.6 months, p<0.001).[41] A PSA decline ≥50% and ORR was observed in 54% and 29% of patients respectively. Common side-effects included fatigue (34%), diarrhoea (21%) and hot flushes (20%). Enzalutamide should be used with caution in men at risk of seizures: an elevated seizure rate was seen in phase I-II trials and men at risk of seizures were excluded from the phase III trial, where the seizure rate was about 1%. In June 2013 treatment with enzalutamide post-docetaxel was approved by the European Medicines Agency (EMA).

A look at the future

The following chapter is restricted to novel AR-targeted treatments and indications currently under investigation and registered on *Clinicaltrials.gov*.

New indications

Abiraterone Acetate

Current phase I-II trials investigate the potential role of AA in combination therapy or in earlier PCa stages. A combination of AA in pre- and post-chemotherapy patients is being investigated with chemotherapy, AR antagonists, 5α-reductase inhibitors and several novel non-AR targeted therapies (see 'other targeted pathways' below). Furthermore, AA is being investigated in PSA-recurrent non-metastatic PCa following ADT (monotherapy and combination), in primary locally advanced or metastatic PCa (monotherapy and combination) and in the neo-adjuvant setting for high-risk localised PCa patients undergoing radical prostatectomy or radiotherapy. Finally, a possible resistance mechanism to AA is upregulation of adrenal CYP17A1 synthesis. Trials to assess whether AA dose escalation tackles this mechanism in CRPC are ongoing.

Enzulatamide

Current phase I-II trials investigate enzalutamide in combination with AA or docetaxel in CRPC patients. A Phase III trial (PREVAIL) on enzalutamide in the pre-docetaxel setting has completed accrual. Furthermore, the effect of enzalutamide is being assessed as compared to bicalutamide following ADT failure (STRIVE and TERRAIN), in first-line advanced PCa (monotherapy) and in the neo-adjuvant setting prior to radical prostatectomy for localised PCa (combination).

New molecules

Androgen synthesis inhibitors

Orteronel (TAK-700) is a potent, non-steroidal imidazole inhibitor of CYP17A1, more specifically the 17.20 lyase component of the enzyme, that demonstrated a PSA decline >50% in 54% of patients in phase II.[42] Two clinical phase III trials are underway examining orteronel in chemotherapy-naïve and docetaxel-treated CRPC patients.

Galeterone (TOK-001) inhibits both CYP17A1 and AR directly.[43] One phase II study is assessing efficacy of galeterone without prednisone in chemotherapy-naive CRPC patients.

AR inhibitors

ARN-509 is a non-steroidal anti-androgen, binding AR with seven- to tenfold greater affinity than bicalutamide.[44] One phase I-II trial is currently underway in chemotherapy-naïve CRPC patients.

EPI-001, unlike previous anti-androgens, exclusively blocks the AR NH2-terminal domain, both in wild-type AR and constitutively active AR splice variants lacking the LBD. Its effect is irrespective of ligand concentration.[45] No clinical trials are currently registered.

AZD3514 binds to the AR, reducing PCa cell growth in both wild-type and mutated AR, reducing the expression of AR.[46] One phase I trial has completed recruitment.

Key messages for clinical practice

1. The androgen receptor plays a key role in prostate cancer progression. Even in the castration-resistant state, prostate cancer remains hormone-driven.
2. Abiraterone acetate is available in Belgium for docetaxel-treated prostate cancer patients, but has demonstrated its efficacy pre-docetaxel as well.
3. Enzalutamide has demonstrated efficacy in docetaxel-treated prostate cancer patients.
4. Several new androgen receptor-targeted molecules are currently under investigation in phase I-II clinical trials.
5. Given the heterogeneity of castration-resistant prostate cancer, these novel therapies could provide an opportunity for patient-tailored therapy in the future.

Heat shock protein inhibitors

Heat shock proteins are chaperone molecules involved in the process of folding, activation, trafficking, and transcriptional activity of most steroid receptors, including AR.

HSP90 is an ATP-dependent chaperone that accounts for the maturation and functional stability of several client proteins, including AR and the HER-2 oncogene. Inhibition of HSP90 is an attractive therapeutic strategy as it simultaneously modulates several client proteins associated with PCa progression. 17-AAG and IPI-504 were the first HSP90 inhibitors that showed promising phase I results, but failed in phase II studies.[47,48] Multiple derivatives are currently under investigation in phase I-II trials (*AT11387, 17-DMAG* and *STA-9090*).

HSP27 is a stress-inducible, ATP-independent nuclear chaperone that enhances AR stability, transport, and transcriptional activity.[49] *OGX-427* is a second-generation antisense drug targeting HSP27 that has shown promising results in phase I studies and is now studied in a phase II study in combination with low dose prednisone in chemotherapy-naive CRPC patients and in a phase II trial in combination with AA.[50]

Other targeted pathways

Multiple other targeted agents are under investigation, including TKIs (dovitinib, dasatinib, cabozantinib, sunitinib, saracatinib), immunomodulatory treatments (ipilimumab), chemotherapy (carboplatin, ixabepilone, eribulin, patupilone), PARP-inhibitors (veliparib), pan-class I PI3K-inhibitors (BKM120), hepatocyte growth factor (HGF)-inhibitors (AMG-102), IGF-1 receptor inhibitors (figitumumab, cixutumumab), Notch signaling inhibitors (RO4929097) and anti-angiogenic agents (aflibercept, tasquinimod, trebananib).

Conclusion

The AR has been the target for most systemic therapies in PCa for over 70 years and counting. ADT remains the gold standard first-line therapy in metastatic PCa. The rising number of available and upcoming AR-targeted therapies underline the pivotal role of AR even in the CRPC setting and will leave clinicians with the challenge to determine which treatment schedule is most beneficial to these patients. Furthermore, increased understanding of PCa progression and heterogeneity could allow for patient-tailored therapy in the future.

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