REVIEW

Molecular underpinnings of enzalutamide resistance

S Prekovic¹, T Van den Broeck^{2,3}, S Linder¹, M E van Royen^{4,5}, A B Houtsmuller^{4,5}, F Handle², S Joniau³, W Zwart^{1,6,*} and F Claessens^{2,*}

¹Division of Oncogenomics, Oncode Institute, The Netherlands Cancer Institute, Amsterdam, The Netherlands

²Laboratory of Molecular Endocrinology, KU Leuven, Leuven, Belgium

³Department of Urology, University Hospitals Leuven, Leuven, Belgium

⁴Department of Pathology, Erasmus MC, Rotterdam, The Netherlands

⁵Erasmus Optical Imaging Centre, Erasmus MC, Rotterdam, The Netherlands

⁶Department of Biomedical Engineering, Laboratory of Chemical Biology and Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, The Netherlands

Correspondence should be addressed to S Prekovic or F Claessens: s.prekovic@nki.nl or frank.claessens@med.kuleuven.be

*(W Zwart and F Claessens share senior authorship)

Abstract

Prostate cancer (PCa) is among the most common adult malignancies, and the second leading cause of cancer-related death in men. As PCa is hormone dependent, blockade of the androgen receptor (AR) signaling is an effective therapeutic strategy for men with advanced metastatic disease. The discovery of enzalutamide, a compound that effectively blocks the AR axis and its clinical application has led to a significant improvement in survival time. However, the effect of enzalutamide is not permanent, and resistance to treatment ultimately leads to development of lethal disease, for which there currently is no cure. This review will focus on the molecular underpinnings of enzalutamide resistance, bridging the gap between the preclinical and clinical research on novel therapeutic strategies for combating this lethal stage of prostate cancer.

Key Words

- ▶ enzalutamide
- ▶ abiraterone
- ▶ resistance
- ▶ androgen receptor
- ▶ glycolysis
- ▶ hexosamine biosynthesis
- ▶ autophagy
- ▶ cancer
- ▶ prostate cancer
- ▶ AKR3C1
- ▶ Wnt
- ▶ IL-6
- ▶ mCRPC
- ▶ mutations

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Introduction

Metastatic prostate cancer (PCa) is an incurable disease with relative 5-year survival rate of 29% (Sartor & de Bono 2018). While current therapies are effective at slowing down progression of the disease, eradication of metastatic cancer is currently not achievable.

Several approaches to treat this lethal disease exist, including chemotherapy and targeted therapy (Cornford *et al.* 2017). Blocking the androgen receptor (AR), a main driver of disease progression is a preferred therapeutic strategy. Currently, enzalutamide is one of the most

frequently used antiandrogens in treatment of the advanced disease. However, antiandrogen treatment is inevitably counteracted by a selection process, resulting in the emergence of resistant tumor clones. Prostate cancer cells have various ways to escape the shackles of AR-directed therapies, even the strongest enzalutamide treatment. This review will focus on specific resistance mechanisms discovered *in vitro*, but anticipated to occur in the clinics and vice versa.

AR mutations as mediators of enzalutamide resistance

Gain-of-function mutations in the AR gene are almost exclusively found in mCRPC and not in primary disease (Grossmann et al. 2001). While nucleotide substitutions most frequently occur within the exon coding for the ligand-binding domain, mutations in other regions of the AR gene have been reported (Watson et al. 2015, Nadal et al. 2017, Prekovic et al. 2017). For example, N-terminal domain and hinge region mutations have been shown to change the activation potential of the AR. A small deletion between positions 388 and 390 resulted in the loss of a sumoylation site, which is also known to potentiate AR activity *in vitro* (Callewaert et al. 2004). Furthermore, several mutations in the hinge region also seem to result in gain of function (Haelens et al. 2007).

As mentioned earlier, most frequently, changes are located within the ligand-binding pocket (LBP). These can cause promiscuity of the receptor for alternative ligands or alter the mode of action of antiandrogens resulting in their conversion to potent activators of the receptor (Grossmann et al. 2001). The first clinically relevant AR mutation in PCa was detected in a metastatic specimen

of a patient who underwent flutamide treatment (Taplin et al. 1999). Following that discovery, various other mutations in the AR LBP have been detected in treatment-resistant mCRPC (Coutinho et al. 2016).

Molecular screens designed for detection of AR mutations that cause the antagonist-to-agonist switch for enzalutamide led to description of F877L, a mutation that was reported to convert enzalutamide to a partial agonist of the AR (Balbas et al. 2013, Joseph et al. 2013, Korpai et al. 2013). However, this mutation is yet to be found in patients who progressed on enzalutamide treatment. Strikingly, for now a single mutation able to convert enzalutamide to a full agonist has not been identified, despite sequencing a rather large number ($n=72$) of enzalutamide-treated patients (Robinson et al. 2015).

However, several double mutant ARs which convert enzalutamide to a strong partial agonist were reported by the Vancouver Cancer Agency (Azad et al. 2015, Lallous et al. 2016, Wyatt et al. 2016). In their cohort, enzalutamide treatment led to the emergence of F877L/T878A and M896V/S889G double mutants, which were associated with resistance (Fig. 1). A detailed molecular analysis of the F877L and the double mutant F877L/T878A revealed that the second mutation is essential for enhancing the N/C interactions, co-regulator interactions and transactivation (Prekovic et al. 2016). The second mutation expands the LBP and potentially induces repositioning of helix 11 and helix 12 (H12) toward the LBP, in turn facilitating the transition into the agonistic conformation of the receptor (Prekovic et al. 2016). This mutant AR is still inhibited by Abiraterone Acetate, Epi-001 and Galaterone (Attard et al. 2009, Andersen et al. 2010, Njar & Brodie 2015). For the M896V/S889G double mutant, it is proposed that the M896V also leads

Figure 1

Androgen receptor mutations may cause antagonist-to-agonist switch for enzalutamide. (A) Double-mutant F877L/T878A AR has enhanced affinity for enzalutamide, an increase in N/C interactions, co-regulator interactions and transactivation upon enzalutamide binding. (B) Changes in LBP volume and mobility of H12 that occur in S889G/M896V AR double mutant convert enzalutamide to a partial agonist of the receptor.

to the expansion of the LBP, while the introduction of a flexible glycine side chain might increase the mobility of H12 and facilitate the formation of activation-function 2 (AF2) (Lallous *et al.* 2016). The single mutant remains inhibited by enzalutamide, while for the double mutant AR enzalutamide is a partial agonist.

In conclusion, mutational events in the AR gene that lead to conversion of enzalutamide to an agonist are rare and do not contribute to resistance in the majority of the patients.

AR splice variants

While it is certain that hormone therapy response and resistance in PCa are marked by the emergence of truncated AR splice variants (Antonarakis *et al.* 2014, Seitz *et al.* 2017), the functional mode of these AR variants is still under debate (Watson *et al.* 2010, Li *et al.* 2013, Luo *et al.* 2017). The AR variants are structurally diverse; however, most of them lack parts or the whole ligand-binding domain resulting in the inability of ligands to control their function. However, there are conflicting reports on whether truncated ARs initiate the same transcriptional program as the full-length AR and whether they can activate the expression of AR target genes without the presence of full-length protein (Fig. 2A).

Truncated ARs could very well serve as markers of response and resistance to therapy in advanced PCa (Antonarakis *et al.* 2014, Cao *et al.* 2014). Both in experimental and clinical setting, enzalutamide treatment indeed induces the expression of truncated ARs. Moreover, the splice variants are expressed at high levels in cell lines resistant to the enzalutamide. The truncated ARs are suggested to drive resistance to enzalutamide

by recapitulating the AR signaling even when the full-length AR is blocked (Li *et al.* 2013). Interestingly, recent data suggest that suppressing AR splice variants can restore sensitivity to enzalutamide in xenograft models, implying that this could be used as a strategy to overcome antiandrogen resistance (Luo *et al.* 2017, Tummala *et al.* 2017).

Glucocorticoid receptor takeover

There is a high similarity of molecular mechanisms behind DNA binding by the AR and the other oxosteroid hormone receptors (glucocorticoid, estrogen, progesterone and mineralocorticoid receptor) (Fig. 2B) (Schoenmakers *et al.* 1999, 2000, Shaffer *et al.* 2004, Denayer *et al.* 2010). For that reason, it is conceivable that they are interchangeable in some cases of enzalutamide resistance. In PCa cell lines, the glucocorticoid receptor (GR) can indeed recapitulate part of the AR transcriptional program under castrate conditions and promote growth (Sahu *et al.* 2013). Furthermore, Arora *et al.* (2013) demonstrated that GR is able to support the cell cycle of PCa cells when the AR is antagonized by enzalutamide. Moreover, they showed an increase in GR expression in tumor samples from patients receiving enzalutamide therapy. Treatment with enzalutamide is also accompanied by loss of an enzyme (11 β -HSD2) that inactivates cortisol, further boosting GR activity and enzalutamide resistance (Li *et al.* 2017). The possible role of the GR as an oncogene in PCa is suggested by the *post hoc* analysis of the AFFIRM study, which has shown that patients co-treated with glucocorticoids have inferior clinical characteristics and OS rate in both placebo- (9.3 vs 15.8 months) and enzalutamide-treated (12.3 vs OS not reached) groups (Montgomery *et al.* 2014).

Figure 2

Androgen receptor splice variants and glucocorticoid takeover may mediate enzalutamide resistance. (A) AR splice variants without the ligand-binding domain are not under the control of the ligand, and may act either in pair with the full-length receptor or as homodimers to drive the growth despite presence of antiandrogens targeting the ligand-binding domain. (B) Glucocorticoid receptor may hijack the androgen response elements and drive the growth of prostate cancer when the AR is inhibited by enzalutamide. E, element.

Surprisingly, a tumor suppressor role of GR has also been described elaborately. Glucocorticoids suppress tumor angiogenesis (Yano *et al.* 2006) and inhibit PCa cell growth *in vitro* (Nishimura *et al.* 2001, Yemelyanov *et al.* 2007). Furthermore, several clinical studies have shown that glucocorticoid co-treatment is beneficial for mCRPC patients in different settings (Storlie *et al.* 1995, Tannock *et al.* 1996, Fosså *et al.* 2001, De Bono *et al.* 2011, Ryan *et al.* 2013). Possibly, whether the GR acts as an oncogene or a tumor suppressor gene might be influenced by interacting partners and/or chromatin remodeling proteins. For instance, one could hypothesize that pioneering factors such as FOXA1 might be involved, as it is known to regulate AR and GR transcriptional activity in a cell type-specific manner (Sahu *et al.* 2013).

Intratumour production of androgens mediated by AKR1C3 enzyme

Intratumour production of androgens is able to drive PCa progression and resistance toward androgen deprivation (Locke *et al.* 2008). Increase in expression of genes involved in androgen biosynthesis has been observed after ADT and antiandrogen treatment (Locke *et al.* 2008, Cai *et al.* 2011, Liu *et al.* 2015). One of these enzymes, AKR1C3, is an emerging therapeutic target in PCa and is upregulated in advanced disease (Fig. 3) (Fung *et al.* 2006, Hamid *et al.* 2012). The AKR1C3 is an NADPH-dependent

Figure 3

Enzymes involved in steroidal biosynthesis mediate intratumoral production of testosterone. Increase in expression of AKR1C3 gene leads to increase in conversion of androstenedione to testosterone within the cancer cells; this allows reactivation of the AR signaling despite the presence of antagonists.

reductive enzyme that converts weak androgens (DHEA and androstenedione) to more potent androgens in the prostate (testosterone and DHT) (Lin *et al.* 1997). Besides that, AKR1C3 is also responsible for synthesis of several prostaglandins, which could also drive tumor progression (Matsuura *et al.* 1998, Sales *et al.* 2004). Evidence for a role of AKR1C3 in advanced PCa has been found in both cell line models and clinical samples of advanced disease. AKR1C3 is upregulated in PCa cell lines when grown in androgens-depleted conditions and enzalutamide (Pfeiffer *et al.* 2011). Furthermore, in xenograft models, it was shown that addition of AKR1C3 substrate led to an increase in transcription of KLK3 gene, which was abrogated by a selective blocker of the AKR1C3 enzyme (indomethacin) (Cai *et al.* 2011). Importantly, upregulation of AKR1C3 was associated with clinical progression and aggressiveness of CRPC tumors (Stanbrough *et al.* 2006, Wako *et al.* 2008). These data converge to the conclusion that AKR1C3 may be a driver of androgen biosynthesis and growth in advanced PCa. Inhibition of AKR1C3 by indomethacin was already seen to be able to increase sensitivity of cancer cells to several different compounds (Byrns & Penning 2009, Verma *et al.* 2016). Recently, it was shown that this compound could re-sensitize enzalutamide-resistant PCa cells to enzalutamide both *in vitro* and *in vivo* (Liu *et al.* 2015). These data suggest that combining enzalutamide with AKR1C3 inhibitors may be a potential therapeutic strategy. Two clinical trials are investigating the clinical benefit of indomethacin in locally advanced PCa (NCT02849990) and mCRPC (NCT02935205). Interestingly, it was recently described that intratumoral androgen profile differs between TMPRSS2-ERG-positive and -negative PCa (Knuuttila *et al.* 2018). The positive status was associated with increased DHT/testosterone ratios, suggesting that TMPRSS2-ERG status could be used to identify patients that may benefit from inhibitors targeting the DHT biosynthesis (Knuuttila *et al.* 2018).

Shift to aerobic glycolysis

Over 80 years ago, Warburg hypothesized that cancer is a metabolic disorder (Warburg & Dickens 1930). While most types of normal cells produce molecules with high-energy bonds predominantly by using the oxidative decarboxylation of pyruvate, cancer cells shift toward glucose as the main source for ATP production and decrease mitochondrial respiration (Gatenby & Gillies 2004).

The metabolism of the prostate epithelial cells is unique as it favors citrate production and secretion

(Costello & Franklin 2000). The Krebs cycle of these cells is altered and interrupted, resulting in low levels of citrate oxidation, diminished respiration and ATP production (Costello & Franklin 2000, Dakubo *et al.* 2006). While in most cancer types there is an initial switch toward aerobic glycolysis (less energy-efficient process per glucose molecule), in primary prostate cancer (PCa), there is a restoration of the Krebs cycle, which results in more energy being produced per glucose molecule (Costello & Franklin 1998, 2000). Fascinatingly, it seems that the PCa cells induce the Warburg effect in their neighboring stromal fibroblasts, which hence secrete lactate and pyruvate that can be used by cancer cells (Di Vizio *et al.* 2009). Furthermore, during the course of disease progression of PCa, there is yet another metabolic shift leading to an increase in glycolytic flux and hypoxic signaling within the cancer cells, which are correlated with poor prognosis (Pertega-Gomes *et al.* 2015).

Shift toward or increase in flux of glycolysis has been observed in various types of drug-resistant cancer, as well as PCa resistant to enzalutamide (Cui *et al.* 2014, Bhattacharya *et al.* 2016). Overexpression of the NFκB family member p52 leads in PCa cells to an increase in glycolytic capacity and to enzalutamide-resistant phenotype in preclinical models (Fig. 4A) (Cui *et al.* 2014). High levels of p52 intensify the flux of both glycolysis and pentose phosphate pathway, leading to an increase in ATP production, which supports evasion of apoptosis and fast

growth (Lunt & Vander Heiden 2011, Cui *et al.* 2014). The shift to aerobic glycolysis can be achieved through other pathways besides the NFκB, such as IGF and PI3K signaling. Interestingly, these are recurrently mutated in CRPC (Roberts 2004, Robinson *et al.* 2015). Whether they contribute to resistance to antiandrogens via increase in aerobic glycolysis remains to be determined.

As there is multiple evidence that high glycolytic flux supports aggressiveness and drug resistance in PCa, targeting glucose metabolism could be a therapeutic alley. Although not effective as monotherapy, 2-dexoglucose (a glucose analog that has the 2-hydroxyl group replaced by hydrogen) could be an alternative therapy in combination with radiation therapy or other drugs such as autophagy inhibitors (Dwarakanath *et al.* 2009, Gupta *et al.* 2009, Stein *et al.* 2010).

Changes in the hexosamine biosynthetic pathway

Recent reports have suggested that the hexosamine biosynthetic pathway (HBP) may play a role in antiandrogen resistance (Fig. 4A). This pathway accounts for about 2–5% of total glucose metabolism and is important for post-translational protein modifications, synthesis of glycolipids, proteoglycans and glycosylphosphatidylinositol anchors (Chatham *et al.* 2008, Munkley *et al.* 2016).

Figure 4

Metabolic alterations can lead to sustained growth and enzalutamide resistance. (A) Increase in glycolytic flux or hexosamine biosynthesis may drive cellular growth and annul the inhibitory effect of enzalutamide. (B) Androgen receptor inhibition leads to activation of AMPK, which regulates autophagy through inhibition of mTOR and activation of Ulk1, this in turn leads to cell survival.

The HBP gives yield to UDP-GlcNAc, an amino-sugar conjugate that is used to modify proteins. Upregulation of crucial enzymes for UDP-glycosylation (OGT) and elevated glycosylation has been associated with poor prognosis, possibly by promoting metabolic reprogramming of PCa cells (Lynch *et al.* 2012, Kamigaito *et al.* 2014, Itkonen *et al.* 2016). The HBP was identified as one of the biochemical drivers of CRPC progression by mediating the metabolic re-wiring which supports cell growth (Itkonen *et al.* 2016, Kaushik *et al.* 2016). This pathway may also increase aerobic glycolysis which was seen to be one of the main characteristics of advanced PCa (Ma & Vosseller 2014).

Recently, it was found that proliferation of CRPC-like cells can be inhibited by treatment with UDP-N-acetylglucosamine and the combination with enzalutamide led to further enhancement in efficacy of the therapy (Kaushik *et al.* 2016). Furthermore, inhibition of OGT, which is the enzyme that processes the UDP-GlcNAc, can also induce cancer cell death (Itkonen *et al.* 2016). Direct inhibitors that target the OGT enzyme have been developed (Trapannone *et al.* 2016), but their specificity should be enhanced before they can be tested in preclinical and clinical development.

Autophagy

Besides relaying on glucose metabolism, cancer cells can resort to autophagy for their survival. Similar to glycolysis, autophagy has been linked to drug resistance in several cancer types as well as in the survival of cells under unfavorable conditions including androgen deprivation (Li *et al.* 2008, Chhipa *et al.* 2011).

As discussed earlier, the PI3K/Akt signaling is altered in CRPC (Robinson *et al.* 2015). This signaling cascade results in activation of mTOR, which is an enticing therapeutic target as shown in preclinical settings (Sparks & Guertin 2010). Multiple clinical trials with mTOR inhibitors have been conducted in the CRPC setting. In a systematic review, it was concluded that unfortunately these drugs lack efficacy in the clinical setting. However, the analysis also encouraged combined therapy with AR or PI3K inhibitors (Statz *et al.* 2016).

The AR is a negative regulator of autophagy and the inhibition of the AR stimulates autophagic activity in PCa cells (Boutin *et al.* 2013). It was observed that cells insensitive to enzalutamide have high rates of autophagy (Fig. 4B). Inhibition of the AR leads to activation of autophagy through the activation of AMP-dependent protein kinase (AMPK) and suppression of mTOR signaling (Nguyen *et al.* 2014b). Combined therapy with enzalutamide and

metformin was able to reduce tumor growth significantly more than either of them alone. These results suggest that using autophagy modulators such as metformin may be beneficial for patients on enzalutamide therapy or after the therapy (Nguyen *et al.* 2014b). Even though current clinical studies show that use of metformin has only a slight beneficial effect as monotherapy for advanced PCa (Rothermundt *et al.* 2014), the study to evaluate the effect of metformin in combination with enzalutamide was started (NCT02339168). To date, it was observed that combination of the two was well tolerated and the efficacy results supported continued study in patients with CRPC (Parikh *et al.* 2018). Other than that, a phase II clinical trial evaluating whether the combination of enzalutamide and metformin in CRPC patients progressing on ADT is better than enzalutamide alone (NCT02640534).

Activation of canonical and non-canonical Wnt signaling

Under normal physiological conditions, the Wnt pathway regulates growth of the embryo and maintenance of the stem cell populations (Logan & Nusse 2004). There are two types of Wnt signaling: canonical and non-canonical. The canonical Wnt signaling is mediated by binding of Wnt ligand to Frizzled protein, which in turn stabilizes beta-catenin, the main executor of the canonical pathway (Logan & Nusse 2004). The non-canonical Wnt signaling is an alternative mode in which beta-catenin is not involved (Gómez-Orte *et al.* 2013). Changes in both canonical and non-canonical Wnt signaling have been observed in CRPC. Mutations in *CTNNB1* are frequent in metastatic samples (Chesire *et al.* 2000). Expression levels of Wnt-1 and beta-catenin were increased in ~80% of metastatic hormone refractory tumors (Chen *et al.* 2004). Recently, by looking at genome-wide changes in advanced metastatic tumors, Robinson *et al.* were able to identify Wnt pathway genomic alterations in 18% of the samples (Robinson *et al.* 2015). Interestingly, the Wnt pathway is also enriched in enzalutamide-treated LNCaP cells suggesting that it might compensate for AR loss and be involved in clinical progression under antiandrogen treatment (Lee *et al.* 2015).

The non-canonical Wnt signaling was found to be upregulated in human PCa supporting growth via potentiating AR signaling (Takahashi *et al.* 2011). In bone metastases of patients treated with antiandrogens, it was found that non-canonical Wnt might mediate castration resistance (Lee *et al.* 2014). Furthermore, non-canonical Wnt signaling is increased in circulating tumor cells from

patients progressing under treatment with AR inhibitors. This activation seems to combat the negative effects of AR blockade (e.g. enzalutamide) and allows cells to continue proliferating (Miyamoto *et al.* 2015).

As the Wnt signaling is a crucial pathway in somatic stem cell homeostasis and regenerative processes after injury, therapies blocking this pathway could have serious adverse effects (Kahn 2014). However, due to progress in the development of Wnt inhibitors, there are several clinical trials that have just been completed or are currently running, e.g. trials for Vantictumab (NCT01957007, NCT01973309 and NCT02005315), OMP-54F28, (NCT02069145, NCT02092363 and NCT02050178) and PRI-724 (NCT01606579 and NCT02413853).

Changes in the interleukin 6 signaling pathway

Inflammation has an important role in PCa pathogenesis by modulating the tumor microenvironment. Among many molecules that are involved in the process of inflammation, interleukin 6 (IL-6) has been studied extensively in the context of PCa biology. IL-6 controls the acute phase response, regulates immune cell differentiation and activation and supports cell proliferation and survival. By binding to a membrane receptor, IL-6 triggers phosphorylation of Stat3 by JAK, and concomitant translocation of dimeric Stat3 to the nucleus and regulation of its target genes (Yu *et al.* 2014).

It is clear that IL-6 plays a role in PCa progression and aggressiveness. For example, the serum levels of this cytokine are high in patients with bone metastases (Shariat *et al.* 2001, Ara & DeClerck 2010) and are also associated with shorter survival time in CRPC setting (George *et al.* 2005). The role of IL-6 in prostate carcinogenesis and progression has been well reviewed by Nguyen *et al.* (2014a), Culig *et al.* (2005) and Smith *et al.* (2001). Involvement of IL-6 in resistance to enzalutamide has been studied in cell line models. The constitutive expression of IL-6 led to persistent activation of Stat3 and loss of sensitivity to enzalutamide (Liu *et al.* 2014b). The inhibition of Stat3 leads to restoration of sensitivity to enzalutamide. Niclosamide (inhibitor of AR-V7) also inhibits Stat3 phosphorylation and signaling. This drug might therefore be a promising candidate for overcoming enzalutamide resistance and advanced PCa (Liu *et al.* 2014a).

The IL-6 axis inhibitors have been subjected to clinical investigation in numerous trials. For now, only

one IL-6 axis inhibitor has been used for treatment of PCa. CNTO328, a monoclonal antibody to IL-6, showed minimal activity as monotherapy for treatment of men with CRPC who progressed on docetaxel (Dorff *et al.* 2010). As the IL-6 axis might be one of the drivers of PCa progression and therapy resistance, development of novel agents and initiation of clinical trials for PCa is needed.

Lineage plasticity and antiandrogen resistance are mediated by SOX2

It is becoming clear that cells could switch lineage from a cell type that is sensitive to a certain compound to another cell type that is resistant. Recent publications by Mu *et al.* (2017) and Ku *et al.* (2017) describe the mechanism behind cell lineage plasticity in prostate cancer and how this influences therapy.

Alterations in TP53 and RB1 can be found in 39% of mCRPC with adenocarcinoma histology and 74% of mCRPC with neuroendocrine histology, whereas these alterations are only found in 5% of primary PCa specimens. Upon loss of expression of these two molecules, there is a concomitant increase in SOX2 expression. SOX2 is a transcription factor essential for maintenance of pluripotency and has a role in embryonic and neuronal stem cell maintenance. Activation of SOX2 transcriptional program induces lineage plasticity and allows switch in cell type, which in turn enables cells to adapt under selective pressure of the treatment. Cells expressing SOX2 would be able to escape AR inhibition by adopting a more neuroendocrine phenotype.

Development of neuroendocrine prostate cancer

The neuroendocrine phenotype of prostate cancer (NEPC; Fig. 5) has a high metastatic and proliferative potential marked by overexpression of key cell cycle genes (Beltran *et al.* 2011, Tzelepi *et al.* 2012). Even though the phenotype of NEPC is complex and can vary greatly (e.g. small-cell carcinoma, large cell carcinoma, paneth cell-like neuroendocrine differentiation, etc.), clinically these tumors are characterized by positive staining for chromogranin, synaptophysin, neuron-specific enolase and CD56 (Abrahamsson 1999, Isshiki *et al.* 2002, Vashchenko & Abrahamsson 2005, Evans *et al.* 2006). On a molecular level NEPC is characterized by low transcriptional activity of the AR, loss of tumor suppressors (RB1, PTEN and TP53), genomic instability, downregulation of REST, changes in IL-6 signaling and

Prostatic adenocarcinoma

Neuroendocrine prostate cancer

Clinical markers

- AR
- PSA
- Gleason score

Clinical markers

- Chromogranin
- Neuron specific enolase
- Synapthophysin
- CD56
- Cellular features

Molecular characteristics

- High AR activity
- TMPRSS2-ERG fusion
- MYC amplifications
- REST high
- PTEN loss
- SPOP mutations
- Loss of tumor suppressors

Molecular characteristics

- Low activity of the AR
- Genomic instability
- Changes in IL-6 signaling
- REST low
- MYCN amplifications
- AURKA amplifications
- Loss of tumor suppressors

Figure 5

Histology and characteristics of prostate adenocarcinoma and neuroendocrine prostate cancer. Prostate adenocarcinoma (image taken from [Gordetsky & Epstein 2016](#)) and neuroendocrine disease (image taken from [Grigore *et al.* 2015](#)) have different histology and molecular characteristics.

MYCN amplifications ([Smith *et al.* 2008](#), [Lapuk *et al.* 2012](#), [Beltran & Rubin 2013](#), [Kani *et al.* 2013](#), [Logothetis *et al.* 2013](#)). As these tumors are clinically hormone refractory and resistant to antiandrogens such as enzalutamide, the question is which therapy could be used to treat this aggressive subtype.

Small-cell lung cancer and extrapulmonary small-cell carcinomas are treated with platinum-based chemotherapy; however, due to the lack of prospective data, it is not clear whether this approach is effective in NEPC ([Nadal *et al.* 2014](#)). As NEPC is marked by upregulation of cell cycle genes, one possible way of combating NEPC is targeting the cell cycle machinery. A multi-institutional single-arm, open-label phase II trial evaluating alisertib, inhibitor of AURKA ([Kelly *et al.* 2012](#)), in patients with histologically confirmed or clinically suspected metastatic NEPC (NCT01799278) failed to meet its primary endpoint ([Sheahan & Ellis 2018](#)). On the other hand, data from this trial suggests that in a subset of patients alisertib may be highly effective. A potential alternative therapeutic approach would be to use disulfiram in combination with copper chloride which acts through the increase in reactive oxygen species production and inhibition of DNA

methyltransferase and ubiquitin-proteasome pathway ([Safi *et al.* 2014](#)). The use of this drug has shown success in preclinical models of PCa. At this moment, a clinical trial is being set up for evaluating the efficacy of this therapy in patients with CRPC and NEPC (NCT02963051).

General conclusions

There are many possible mechanisms that have the potential to lead to enzalutamide resistance. The pathways involving the AR signaling are well characterized; however, new concepts are still emerging (e.g. somatically acquired enhancer as a non-coding driver of enzalutamide resistance ([Takeda *et al.* 2018](#))). The adaptations of cancer cells to the presence of enzalutamide by changes in metabolic pathways (glycolysis, hexosamine), in alternative pathways like autophagy, Wnt, interleukin signaling and even by the development of NEPC clearly shows that we need to do a better job in classifying the advanced disease. Only through such more detailed classifications, patient-tailored rationales for personal or precision medicine can be developed.

Declaration of interest

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