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## “Understanding the mechanisms of androgen deprivation resistance in prostate cancer at the molecular level”

Theodoros Karantanos, MD<sup>1</sup>, Christopher Evans, MD<sup>2</sup>, Bertrand Tombal, MD, Ph.D<sup>3</sup>, Timothy C. Thompson, Ph.D<sup>1</sup>, Rodolfo Montironi, MD<sup>4</sup>, and William B. Isaacs, Ph.D<sup>5</sup>

<sup>1</sup> Department of Genitourinary Medical Oncology – Research, The University of Texas MD Anderson Cancer Center, Houston, TX

<sup>2</sup> Department of Urology, UC Davis Comprehensive Cancer Center, Sacramento, CA

<sup>3</sup> Cliniques universitaires Saint Luc, Université catholique de Louvain, Brussels, Belgium

<sup>4</sup> Section of Pathological Anatomy, Polytechnic University of the Marche Region, School of Medicine, Ancona, Italy

<sup>5</sup> Brady Urological Institute, Johns Hopkins Medical Institutions, Baltimore, MD

### Abstract

**Context**—To evaluate molecular mechanisms that play a role in the development of resistance to androgen deprivation therapy in castration-resistant prostate cancer.

**Objective**—The understanding of mechanisms and biological pathways associated with the progression of prostate cancer under systemic androgen depletion or administration of novel anti-androgens abiraterone, enzalutamide and ARN-509. This review also examines the introduction of novel combinational approaches for patients with castrate resistant prostate cancer.

**Evidence Acquisition**—Pubmed was the data source and “castrate resistant prostate cancer”, “abiraterone, enzalutamide resistance mechanisms”, “resistance to androgen deprivation”, “AR mutations”, “amplifications”, “splice variants” and “AR alterations” were the keywords for the search. Papers published before 1990 were excluded from the review and only English papers were included.

**Evidence Synthesis**—This review summarizes the current literature regarding the mechanisms implicated in the development of castrate resistant prostate cancer and the acquisition of resistance to novel anti-androgen axis agents. It focuses on androgen biosynthesis in the tumor microenvironment, AR alterations and post-transcriptional modifications, the role of glucocorticoid receptor, pathways of cellular stress and alternative oncogenic signaling which are de-repressed upon maximum AR inhibition promoting cancer survival and progression.

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**Correspondence to:** Theodoros Karantanos, University of Texas, MD Anderson Cancer Center, Department of Genitourinary Medical Oncology – Research, Unit 18-3, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA. Tel.: +1 713-517-7400; theodoroskarantanosmd@gmail.com, William B. Isaacs, Brady Urological Institute, Johns Hopkins Medical Institutions, 115 Marburg Bldg., 600 N. Wolfe St., Baltimore, MD 21205, USA. Tel.: 410-955-2518; Fax: 410-955-0833; wisaacs@jhmi.edu.

**Conclusions**—The mechanisms implicated in the development of resistance to AR inhibition in prostate cancer are multiple and complex, involving virtually all classes of genomic alteration and leading to a host of selective/adaptive responses. Combinational therapeutic approaches targeting both AR signaling and alternative oncogenic pathways may be reasonable for patients with castrate resistant prostate cancer.

**Patient Summary**—In this review we looked for mechanisms related to the progression of prostate cancer in patients undergoing hormonal therapy and treatment with novel drugs targeting the androgen receptor. Based on recent data, the combination of maximal androgen receptor inhibition with novel agents targeting other tumor compensatory, non-AR related pathways may improve the survival and quality of life of patients with castrate-resistant prostate cancer (CRPC).

### Keywords

Castrate resistant prostate cancer; androgen receptor; novel anti-androgens; alternative signaling

## INTRODUCTION

Prostate cancer (PCa) remains the second leading cause of death by cancer in western societies [1]. Usually PCa is diagnosed as localized disease and the management includes surveillance or radical prostatectomy, radiation therapy or even combination approaches such hormonal therapy prior to prostatectomy. Few patients undergoing prostate cancer screening present with metastatic disease, while metastatic disease is present in up to 10% or more of unscreened populations at first presentation, usually in bone, the predominant site of advanced and lethal PCa [2], highlighting critical differences between screened and unscreened populations. Despite the obvious increase in the overall survival of patients with PCa over the past decade, recent data indicate that improvement of survival among patients with metastatic PCa has not significantly contributed to this decline in mortality [3]. Patients with metastatic disease usually receive hormonal therapy which decreases the production of testosterone by the testes. However, after an initial response which varies significantly among patients, the disease eventually progresses despite the low levels of testosterone in the systemic circulation ( $<20\text{ng/dl}$ ) [4]. This state of disease is known as metastatic castrate resistant PCa (CRPC) and the average overall survival is 1.5 years with significant variability between patients with lymph node metastasis, bone metastasis and metastasis in both lymph nodes and bone [3]. Docetaxel and Cabazitaxel are the only chemotherapy regimens approved for this state of disease [5, 6]. Ra 233 (Xofigo injection) was recently approved for the treatment of patients with CRPC, symptomatic bone metastases, and no known visceral metastatic disease [7].

Numerous studies during the last decade have highlighted the role of androgen receptor (AR) in the development of mCRPC showing that despite the systemic androgen depletion, AR signaling remains active and supports the survival and growth of PCa cells. Based on these results two novel agents have been recently evaluated in clinical trials, namely abiraterone acetate (AA), an inhibitor of androgen synthesis and enzalutamide, a potent anti-androgen. In particular, AA is a CYP17A1 inhibitor blocking the production of androgens in the testes, adrenal glands and tumor microenvironment by inhibiting both  $17\alpha$  hydroxylase

and 17,20 lyase activities of the CYP17A1 enzyme [8]. AA has been recently approved for chemotherapy naïve patients with mCRPC improving the overall survival by 4 months [11].

Enzalutamide is a novel antagonist of AR, inhibiting nuclear translocation, chromatin binding and interactions with AR co-regulators [10]. Enzalutamide prolongs the survival of patients who failed chemotherapy [11] while more recent data suggest that in chemotherapy-naïve mCRPC patients, enzalutamide increases the overall and progression free survival and delays the need for chemotherapy [12]. ARN-509, a next generation anti-androgen was found to be more effective than enzalutamide in CRPC preclinical models in terms of tumor growth [13]. According to a recent phase I clinical trial, ARN-509 is safe, well-tolerated and displays dose-proportional pharmacokinetics demonstrating pharmacodynamic and anti-tumor activity across all dose levels examined [14].

Despite the significant advances in the targeting of AR which have been translated to survival improvement for patients with mCRPC, this state of disease remains incurable and is associated with significant morbidity and mortality [15]. While the introduction of novel anti-androgens has provided survival benefits through tumor growth inhibition, two critical clinical concerns arise: 1. Which patients really benefit from these agents and which biomarkers can be used to identify these patients and 2. What alternative approaches can be used if the disease progresses during treatment with these agents.

The aim of this review is to summarize the recent advances in the evaluation of the multiple levels of resistance development to androgen deprivation and AR inhibition that occur before and after the introduction of novel anti-androgen axis agents. The heterogeneity of prostate cancer diversity of pathways involved demands a critical consideration of numerous possible explanations of these biological events.

### **Paracrine/Autocrine androgen synthesis as a mechanism of resistance to systemic hormonal therapy and novel inhibitors of androgen biosynthesis**

It is well documented that in normal prostate tissue and low grade PCa the prostate stroma secretes active androgens (e.g. DHT) and other growth factors supporting the survival and proliferation of overlying epithelium by a paracrine loop [16]. During PCa progression, this paracrine dependence is lost and converted to an autocrine phase where cancer cells produce numerous factors including androgens supporting their own growth and survival [16]. Although circulating androgens are initially the driver of disease progression, explaining the initial response of metastatic disease to systemic androgen depletion, this treatment selects for cancer cells capable of surviving and growing by virtue of a variety of paracrine and autocrine mechanisms resulting in AR activation despite the reduced systemic testosterone below “castration” levels (20ng/dl).

Montgomery et al found that intratumoral levels of testosterone and dihydrotestosterone (DHT) are higher in mCRPC compared to primary localized disease derived from untreated patients [17]. Moreover, the intratumoral testosterone levels in patients with mCRPC were found to be in a range known to stimulate AR and promote PCa growth and proliferation [17]. Chang et al showed that in mCRPC androstenedione is converted to 5 $\alpha$  androstenedione which is finally converted to DHT leading to prostate cancer progression

despite systemic androgen depletion [18]. The authors showed that expression of key enzymes, such as HSD3B1 and CYP17A1, required for metabolism of progestins to adrenal androgens and their subsequent conversion to testosterone, were significantly higher in mCRPC compared to primary tumors [18]. Recently, Chang et al found that CRPCs harbor a gain-of-stability N367T variant in the gene encoding for the 3 $\beta$ -hydroxysteroid dehydrogenase type 1 (3 $\beta$ HSD1) enzyme which catalyzes the conversion of DHEA to DHT, which accumulates and activates AR despite systemic androgen deprivation [19].

Importantly, CYP17A1, which mediates the synthesis of 17-OH progesterone and 17-OH pregnenolone from progesterone and pregnenolone, respectively, and promotes the synthesis of androgen mediators, was found to be upregulated in mCRPC. This finding led to the introduction of AA, which suppresses testosterone concentrations in blood and tumor microenvironment to levels lower than picograms per milliliter, while increased nuclear localization of AR and induced CYP17A1 are predictors of good response to this agent [20].

Increased copy numbers of genes encoding enzymes implicated in testosterone synthesis such as HSD17B3 and decreased copy numbers of genes encoding enzymes promoting the conversion of testosterone to the less active androstenedione have been reported in mCRPC [21]. Ishizaki et al in a recent report demonstrated that androgen deprivation leads to upregulation of HSD17B6 which was associated with increased incidence of biochemical recurrence [22]. HSD17B6 catalyzes the conversion of androgen metabolites such as 5 $\alpha$ -androstane-3 $\alpha$ / $\beta$ ,17 $\beta$ -diol (3 $\alpha$ / $\beta$ -diol) to dihydrotestosterone and human PCa xenografts produce this metabolite from acetate and cholesterol [23]. Recently, Lee et al found that increased cholesterol synthesis through downregulation of ABCA1 is associated with increased prostate cancer aggressiveness [24]. Thus, combination of androgen deprivation and cholesterol synthesis inhibition may be a reasonable approach for some mCRPC.

Collectively, upon systemic androgen depletion, metastatic PCa shifts from an endocrine-driven to a paracrine/autocrine-driven disease, where the local tumor microenvironment and cancer cells produce androgens leading to persistent AR activation, or the cancer cells become less dependent upon androgen ligand altogether (see below). The paracrine and autocrine androgen biosynthesis pathways associated with the development of CRPC are summarized in the figure 1.

Given the critical role of CYP17A1 in the paracrine androgen biosynthesis novel inhibitors targeting this enzyme have been introduced in the clinical setting. AA inhibits the 17 $\alpha$  hydroxylase and 17, 20 lyase activities of this enzyme while Orteronel (TAK700) inhibits mainly the 17, 20 lyase activity of the enzyme (Figure 1). Interestingly, treatment of castration-resistant VCaP xenografts with AA generated relapsed tumors with upregulated CYP17A1, [25] while, LNCaP cells express a mutant form of AR (T877A) activated by progesterone which is the product of CYP11A1 in the initial step of steroidogenesis, rendering AR sensitive to steroid metabolites produced upstream of CYP17A1 [26]. This concept could explain the failure of Orteronel (TAK700) which affects mainly 17,20 lyase, to provide significant survival benefits in patients with mCRPC [27]. On the contrary AA affects both 17, 20 lyase activity and the upstream 17 $\alpha$  hydroxylase activity decreasing the

accumulation of steroid metabolites which can induce PCa growth through binding to AR (Figure 1).

The transcriptional activity of AR has been found to be significantly altered in CRPC and many of the androgen-regulated genes become up-regulated during the progression of the disease to CRPC [28] supporting that AR signaling remains active in this state of disease. In the next sections molecular events implicated in the induction of AR signaling and their impact on development resistance to androgen depletion are critical and will be analyzed.

### **AR mutations and resistance to androgen ablation and AR inhibition**

It is known that AR mutations, which are exceedingly uncommon in primary, hormonally naïve disease have been reported to occur in comparatively high incidence (>10%) in patients with CRPC, especially in tumors progressing under systemic hormonal therapy [29 – 32]. Recently, Grasso et al, found that AR is among the nine genes that are significantly mutated in mCRPC [33]. Of note, treatment with AR antagonists increases the incidence of mutations in the ligand binding domain (LBD) of AR in metastatic PCa compared to hormonal therapy alone and T877A mutant is one of the most frequently observed variants [34]. This mutant broadens the ligand binding specificity of AR, sensitizing it to other steroid hormones such as progesterone and estrogens [35, 36] and, strikingly, to some anti-androgens which are converted to strong agonists [37].

H874Y mutation, initially identified in CRPC tumors treated with flutamide [32] enhances the binding of AR co-regulators increasing AR transcriptional activity through conformational change of AR protein [38]. Moreover AR in metastatic disease treated with AR antagonists carries mutations in the N-terminal domain, such as W435L affecting its interaction with AR co-regulators promoting androgen independent AR activation [39]. Korpál et al and Joseph et al found that the F876L mutation of AR was present in all the enzalutamide and ARN-509 strongly resistant clones [40, 41]. This mutation switches the effect of these novel AR inhibitors from antagonistic to agonistic. Finally, circulating tumor DNA from patients with progressive disease under ARN-509 presented higher incidence of F876L-encoding mutations [41]. These results suggest that much like mutations in other therapeutically targeted oncogenes, AR mutations provide a survival advantage to PCa cells and promote resistance to novel anti-androgens [42].

### **AR variants as a mechanism of resistance to ADT and anti-androgens**

The finding that CWR-22Rv1 cells express two different AR protein species at 112kDa and 75-80kDa while the smaller form lacked the AR LBD and was constitutively localized in the nucleus and remained active [43] suggested that variant forms of AR could be implicated in the development of resistance to hormonal therapy. AR species with a similar mobility to this AR isoform were frequently expressed in human PCa tissues [44] while increased levels of particular AR variants in prostatectomy samples are associated with increased incidence of relapse [45].

Androgen dependent gene expression and cell growth are mediated by the full-length AR while androgen independent transcriptional activity and cell growth are attributed to COOH-terminal truncated LBD lacking AR variants [46, 46-50]. Sun et al identified the presence of

an AR variant lacking the exons 5, 6 and 7, which encode the LBD of the AR (AR<sup>v567es</sup>) in xenografts derived from mCRPC, after prolonged exposure to ADT [50]. This AR variant was found to be constitutively active in PCa cell lines promoting the expression and the activity of the full length AR (AR<sup>fl</sup>) [50]. The authors demonstrated that castration selected for AR<sup>v567es</sup> variant expression in PCa xenografts models while this variant was found to be upregulated in mCRPC [50]. Finally, the AR<sup>v567es</sup> variant presented distinct transcriptional activity compared to the AR<sup>fl</sup> [50]. Consistently, Hörnberg et al showed that AR<sup>v567es</sup> mRNA levels are higher in mCRPC compared to hormone naïve metastatic disease and were associated with higher nuclear AR and shorter survival [51].

It was shown that inhibition of AR by small interfering RNA or enzalutamide promotes the expression of the ARV7 variant [52]. Interestingly this particular AR variant is expressed at a higher level in mCRPC compared to hormone naïve metastatic disease and was found to be associated with disturbed cell cycle regulation and shorter survival [51]. It was also discovered that AR<sup>fl</sup> mainly induces genes related to biosynthesis and metabolism while ARV7 promotes the expression of genes related to cell cycle progression including the UBE2C gene [52]. These results were confirmed in vivo since treatment of LuCaP35CR with abiraterone upregulated both AR and ARV7 but only the expression of the latter was found to be associated with UBE2C induction [52].

It was also found that the expression of AR variants in CWR-22Rv1 cells is sufficient to promote growth under enzalutamide while knockdown of the AR variant increases the sensitivity to AR inhibitors [53]. Nadiminty et al showed that NF-κB2/p52 promotes resistance to enzalutamide in LNCaP C4-2B and CWR-22Rv1 through upregulation of AR variants [58]. The authors found that knockdown of full length AR and AR V7 increased the sensitivity of these two cell lines to enzalutamide [54]. Liu et al using luciferase activity assay to determine the activity of AR V7 after treatment with various compounds demonstrated that niclosamide, an FDA approved anti-helminthic drug significantly downregulated AR V7 protein levels through protein degradation and decreased AR V7 transcriptional activity [55]. Moreover, the addition of nicosamide significantly enhances the activity of enzalutamide in vitro and in vivo in the androgen insensitive C4-2B and CWR22Rv1 cells [55]. Finally, Antonarakis et al recently showed that the detection of AR V7 mRNA in circulating tumor cells (CTC) in patients with mCRPC predicts primary resistance to enzalutamide and abiraterone [56]. In particular, it was found that detectable AR V7 mRNA from CTC is associated with decreased progression free survival and absence of PSA response [56].

It should be noted that all current therapies for CRPC targeting AR such as enzalutamide and ARN-509 are dependent on the presence of LBD which as analyzed above is missing in numerous AR variants upregulated under novel anti-androgens. Moreover, the majority of mutations related to the development of resistance to these agents such as T877A are located in the LBD. These conclusions led to the introduction of small molecules such as EPI-001 targeting the N-terminal AR domain [57] as promising novel therapeutic candidates.



## Combinational approaches targeting AR

As analyzed above alterations of androgen levels in the tumor microenvironment and alterations of AR gene and protein levels are implicated in the development of resistance to hormonal therapy and novel anti-androgens. AA decreases the testosterone levels both in systemic circulation and bone microenvironment [20] but early after the initiation of treatment AR copy number is increased while enzalutamide is associated with increases of testosterone levels in both systemic circulation and bone microenvironment [58]. 57 patients with mCRPC received AA and enzalutamide and were monitored every 4 weeks and significant declines in PSA levels in the majority of patients were observed [58]. These results suggest that combinational therapy including an androgen synthesis inhibitors and an AR inhibitor may lead to more effective inhibition of AR signaling.

## Post-translational alterations of AR and transcriptional activity: alternative oncogenic signaling implications

Tyrosine phosphorylation is significantly higher in hormonal resistant xenografts compared to their hormone sensitive counterparts [59] and was associated with increased AR transcriptional activity and increased PCa cells growth under androgen depletion and clinical progression of PCa [59]. Ueda et al found that AR activation is induced by Src-1 and interleukin 6 (IL-6) in the absence of androgens while inhibition of mitogen-activated protein kinase (MAPK) abrogated this effect [60]. Moreover, downregulation of Src-1 reduces PCa growth and AR target genes' transcription, while higher Src-1 expression in localized PCa is associated with higher Gleason score, extracapsular extension and pelvic lymph node metastases [61]. Moreover, IL-6 promotes resistance to bicalutamide, an AR inhibitor, through upregulation of the AR co-activator TIF-2 [62], which has been related to increased incidence of biochemical recurrence after radical prostatectomy [63]. These results highlight the critical role of Src-1 and IL-6 in the regulation of AR in multiple levels and demonstrate that these effects are mediated by MAPK signaling.

Epidermal Growth Factor Receptor (EGFR) and its dimerization partner HER2 have been implicated in activation of AR and promotion of PCa growth [64]. Mellinghoff et al found that knockdown of HER2 and not EGFR inhibits the AR transcriptional activity in LNCaP and LAPC4 cells, while HER2 and HER3 stabilize AR and increase its binding to androgen responsive elements in the promoters of AR regulated genes such as PSA and KLK2 [65]. Chen et al found that androgen depletion increases HER2 and ERBB3 promoting AR stabilization and PSA production while dual inhibition of HER2 and EGFR with trastuzumab and erlotinib abrogated these events, sensitized the LNCaP cells to androgen depletion and showed synergistic effects with castration decreasing the growth of the androgen independent CWR22 PCa cells [66]. Interestingly, the inhibition of PI3K signaling by BEZ235 leads to increased AR protein levels and transcriptional activity through induced HER2/HER3 pathway [67]. These data suggest that the inhibition of HER2/HER3 may sensitize PCa cells to androgen depletion, providing a rationale for combination therapy.

The loss of tumor suppressor gene PTEN and activation of PI3K signaling take place in almost 70% of metastatic prostate cancers [68]. Akt mediated AR phosphorylation at the Ser 81 residue increases the interaction of AR with the transcriptional factor p300 inhibiting AR

ubiquitination and degradation [69]. Also, deletion of p300 in a prostate specific PTEN deletion transgenic animal model decreases the incidence of high grade intraepithelial neoplasia and invasive cancer increasing the survival of these mice [69]. Finally high expression of p300 is critical for the androgen dependent and independent transactivation of AR [70] and was correlated with higher AR protein levels in human PCa specimens [69]. The implications of alternative oncogenic pathways in the post-transcriptional activation of AR under low androgen levels are summarized in the figure 2.

### **The role of Glucocorticoid receptor (GR) in the development of resistance to novel AR inhibitors**

The glucocorticoid receptor (GR) has been implicated in cancer progression while dexamethasone has been used in treatment of CRPC associated with PSA response [71]. Glucocorticoids were found to inhibit lymphangiogenesis through VEGF downregulation [71] while induction of GR led to inhibition of PCa cells proliferation associated with upregulation of p21 and p27 and downregulation of oncogenic molecules such as MAP kinases, Nuclear factor Kappa B and STAT1 [72]. Sahu et al evaluated the AR and GR target genes in different PCa cells and concluded that these two receptors present overlapping sets of gene targets suggesting that GR may be implicated in the development of resistance to ADT [73].

Arora et al in a recent study treated LNCaP xenografts expressing wild type AR (LNCaP/AR) with novel AR inhibitors including enzalutamide, ARN-509 and RD162 until the tumors regressed [74]. The authors discovered that numerous common targets of AR and GR were upregulated in the resistant tumors, while GR mRNA and protein levels were found significantly higher in enzalutamide and ARN-509 resistant tumors [74]. Moreover, knockdown of GR in cells derived from resistant tumors retained the sensitivity to enzalutamide and dexamethasone induced resistance to enzalutamide when administrated in VCaP cells [74] supporting the idea that GR upregulation promotes resistance to novel anti-androgens. The authors examined the expression of GR in metastatic PCa obtained from patients treated with enzalutamide and showed that poor responders presented higher levels of GR 8 weeks after the initiation of treatment compared to good responders at the same time-point and compared to baseline levels [74]. It was also shown that in a subset of PCa cells AR represses GR expression while AR inhibition leads to GR induction due to de-repression of the gene [74]. This result was in consistency with a previous report by Davies et al showing that the GR expression in the ventral rat prostate is increased after castration [75]. These conclusions highlight the role of GR in the development of resistance to novel anti-androgens despite the earlier evidence that GR exhibit a tumor suppressor role in prostate cancer.

### **AR inhibition de-represses numerous genes implicated in the activation of oncogenic signaling promoting resistance to ADT**

In the previous section the ADT mediated de-repression of GR signaling was discussed. It could be hypothesized that androgen depletion or AR inhibition in general acts as a selective pressure favoring the survival of PCa cells maintaining this negative feedback between AR and genes implicated in oncogenic signaling promoting cancer cell survival.



Cai et al showed that numerous genes implicated in DNA synthesis and repair, DNA metabolism and cell cycle which are upregulated in CRPC xenografts are repressed by androgens [76]. Given that AR signaling is also upregulated in CRPC, the androgen levels at this state of disease are adequate to induce AR regulated genes but are not high enough to stimulate AR activity on suppressor elements and inhibit the expression of genes implicated in cell cycle and DNA metabolism [76]. Thus, the maximal androgen depletion and AR inhibition may further de-repress these cell cycle and DNA synthesis genes in a subset of PCa potentially leading to resistant phenotypes associated with activation of oncogenic and growth promoting pathways very early during treatment.

AR has also been found to inhibit the phosphorylation of Akt in PTEN conditional knockout transgenic animal models and PCa cell lines such as LNCaP and LAPC4 [67, 77]. This phenomenon was mainly attributed to the upregulation of FKBP5 and PHLPP leading to decreased Akt phosphorylation by AR [67, 77]. PTEN deletion and subsequent Akt activation was also found to decrease AR protein levels and transcriptional activity through HER3 signaling alteration [67]. Akt was also found to increase AR ubiquitylation and degradation providing another reasonable mechanism explaining this phenomenon [78]. Papamycin, a mTOR inhibitor led to increased tumor regression in PTEN knockout models when combined with castration compared to single agents [77]. However, according to a phase II clinical trial in patients with CRPC RAD001, another mTOR inhibitor in combination with bicalutamide failed to improve response compared to bicalutamide alone [79]. This result may be attributed to the complex adaptive resistance pathways to PI3K/ mTOR inhibition [80].

The Enhancer of zeste homolog 2 (EZH2) which functions as an epigenetic gene silencer is induced in numerous human malignancies including PCa [81]. This molecule is downregulated by androgens while the administration of androgens in LNCaP cells decreased their migration but knockdown of E-cadherin a gene target of EZH2 abrogated these effects [82]. According to a recent study by Xu et al, EZH2 is more critical for the survival and growth of the castration-resistant LNCaP cells compared to the castration-sensitive LNCaP cells [83] providing rationale for introducing EZH2 inhibitors as novel therapeutic approach in CRPC especially in combination with hormonal therapy.

STAT3 transcriptional factor is associated with metastatic potential in PCa cells [84] while Schroeder et al found that AR inhibition and androgen depletion induced STAT3 activation and promoted the development of prostate cancer stem-like cells [85]. Liu et al showed that overexpression of IL-6 promotes the development of resistance to enzalutamide through STAT3 activation, and autocrine IL-6 promotes AR transactivation through STAT3 induction, while the addition of AG490, a STAT3 inhibitor increases the sensitivity of LNCaP cells to enzalutamide [86]. These results support that STAT3 signaling may represent another example of AR depressed oncogenic pathway while targeting STAT3 in combination with AR inhibition may be a reasonable approach for patients with advanced PCa.

It is known that hepatocyte growth factor derived from prostate stroma enhances PCa cell proliferation, motility and invasion through activation of the c-Met protein [87 – 90]. Verras

et al showed that AR represses c-Met expression directly through binding in its promoter while castration was found to induce c-Met expression in LNCaP xenografts [91]. Interestingly, long term androgen deprivation leads to a signaling pathway switch from AR to c-Met which could be predictive of a more aggressive disease [92], while cabozatinib, a c-Met inhibitor leads to increased progression-free survival, improvement of bone scans and reduction of soft tissue lesions patients with mCRPC [93]. However, two phase III clinical trials evaluating the efficacy Cabozatinib in combination with prednisone and mitoxantrone for patients with mCRPC failed to reach their primary endpoint of improved survival compared to prednisone and mitoxantrone alone (COMET-1, NCT01605227 and COMET-2, NCT01522443).

ADT and AR inhibition were recently found to increase the expression of c-Myb [94], a transcriptional factor which is upregulated during the progression of breast, prostate and head and neck cancers [95, 96]. AR suppresses the expression of MYB, which mediates growth and induces the metastatic potential of PCa cells [94]. AR and c-Myb co-regulate a signature of DNA damage response related genes, strongly associated with tumor recurrence, castration resistance and metastasis [94]. These data suggest that c-Myb regulates a resistance pathway which can be targeted to develop novel combinational therapeutic approaches for patients with PCa.

Based on these observations, a critical function of AR in a subset of PCa is to repress numerous genes implicated in oncogenic signaling promoting the development of a more aggressive form of PCa. Some of the oncogenic pathways that are de-repressed by AR inhibition are presented in the figure 3. AR inhibition by novel anti-androgens may select for these cells leading to rapid development of resistance. Further studies are needed for the discovery of reliable biomarkers to identify this subset of patients and new targets for the introduction of novel therapeutic approaches.

## CONCLUSION

In conclusion, mCRPC remains an incurable disease despite the introduction of novel agents targeting androgen action. Increased androgen biosynthesis in the tumor microenvironment and alterations of AR signaling including AR mutations mainly in the LBD, AR variants and AR gene amplifications all play roles in the development of resistance to systemic hormonal therapy. Especially after the introduction of AA and enzalutamide which maximally decrease AR activity, these and other mechanisms related to resistance emerge (Fig. 3). The combination of these two agents is predicted to maximally inhibit AR signaling. Cancers presenting with upfront resistance to this approach should be more dependent on post-translational modifications of AR and alternative oncogenic signaling. Moreover, the finding that AR represses critical oncogenic mediators explains the clinical observation that a subset of patients becomes rapidly resistant to hormonal therapy and does not respond to novel anti-androgens (Fig. 3). Finally, a new classification of patients with mCRPC based on molecular biomarkers predicting this subset of patients and novel therapeutic approaches targeting these pathways are required to change the natural history of currently incurable mCRPC.

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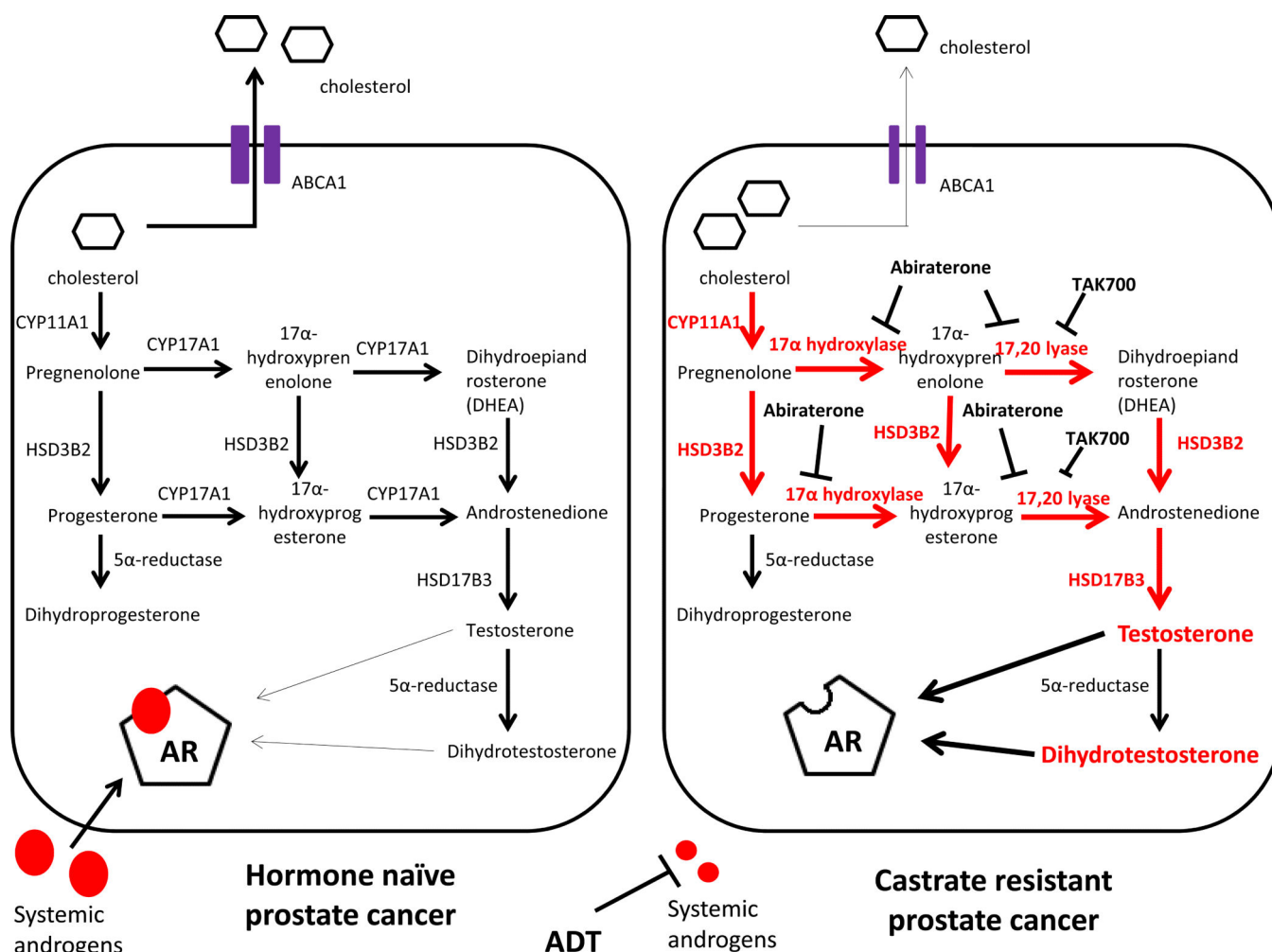


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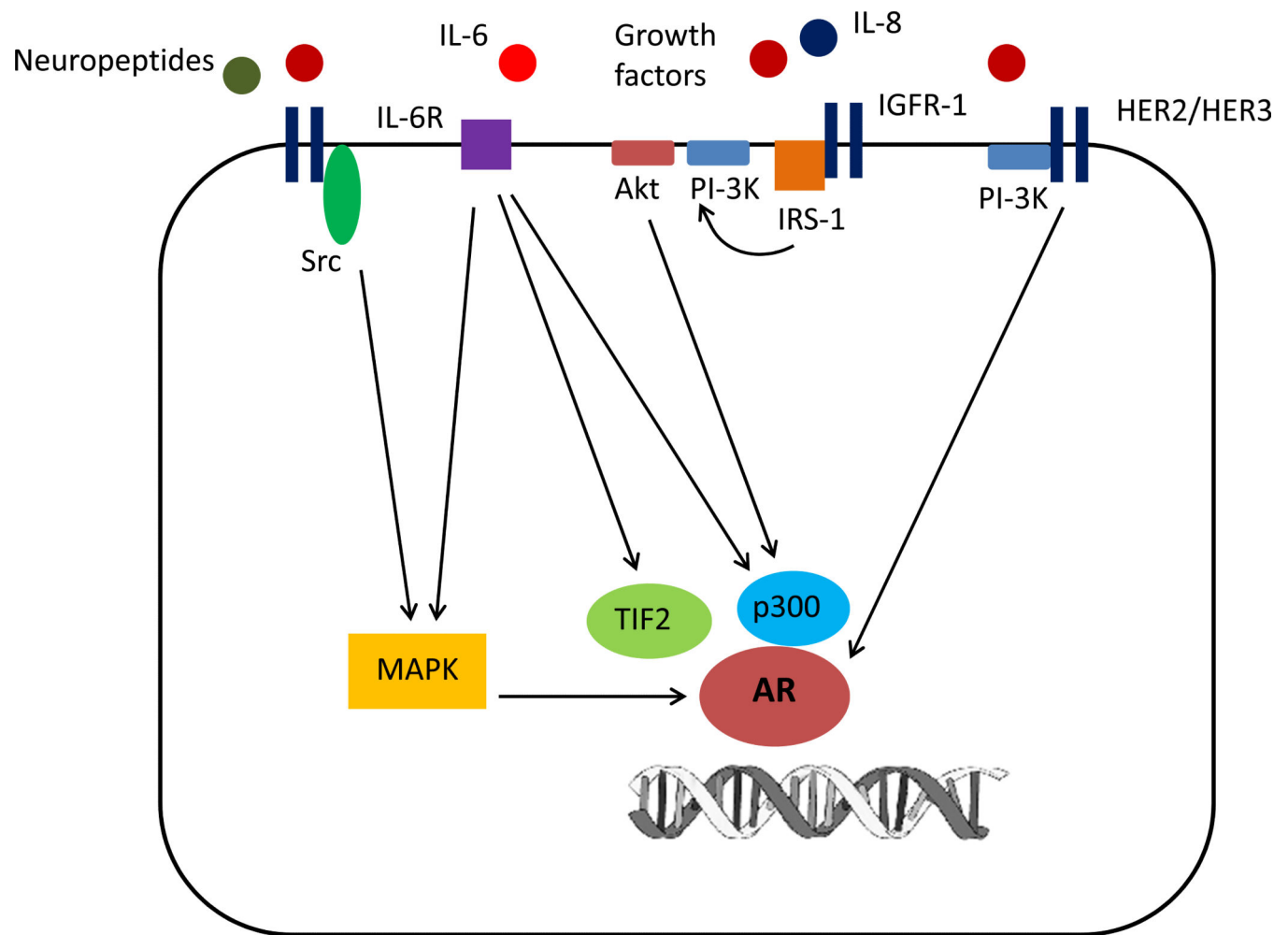
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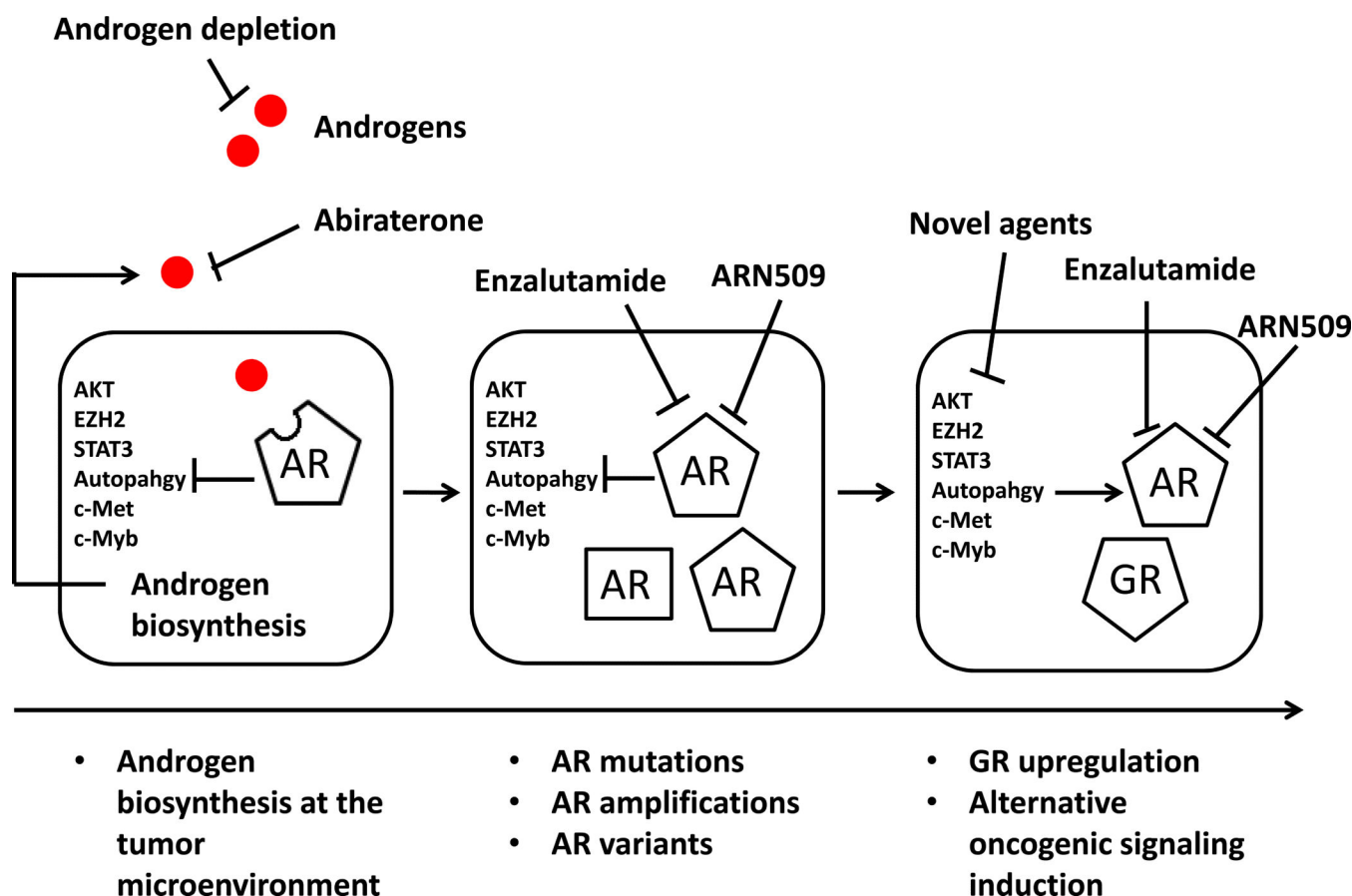
**Figure 1.**

Paracrine and autocrine androgen biosynthesis as a mechanism of resistance to systemic androgen depletion. Upon systemic androgen deprivation the tumor microenvironment and prostate cancer cells produce androgens through androgen biosynthesis. In particular numerous steroidogenic enzymes (these enzymes are presented in red) implicated in the androgen biosynthesis are induced during the development of castrate resistant prostate cancer (CRPC). The novel agent Abiraterone Acetate (AA) targets the enzyme CYP17A1 and has been found to prolong the survival of patients with chemotherapy naïve and resistant prostate cancer.



**Figure 2.**

Alternative oncogenic signaling implicated in the postranscriptional activation of AR. Upon androgen deprivation and AR inhibition numerous alternative oncogenic pathways are activated and promote the transcriptional activities of AR. Src and IL-6 signaling promote AR activation through MAPK signaling during the development of castration resistance. Moreover, IL-6 promotes the upregulation of TIF2, an AR co-regulator under androgen depletion. IL-6 and Akt signaling increase the interaction between p300 and AR increasing the stabilization of AR under low androgen levels. Finally, HER2 and HER3 downstream signaling has been associated with increased AR activity during prostate cancer progression.



**Figure 3.**

Prostate cancer progression under androgen deprivation and AR inhibition. Systemic androgen depletion by hormonal therapy leads to progressive activation of numerous survival mechanisms including androgen biosynthesis in the tumor microenvironment while more effective AR inhibition through abiraterone, enzalutamide and ARN509 promotes the emergence of AR mutations, amplifications and variants maintaining disease progression. Finally, sustained AR inhibitions leads to alternative oncogenic signaling de-repression such as AKT, EZH2, STAT3 and c-Met and induction of Glucocorticoid receptor (GR) providing survival advantage to cancer cells under maximum AR inhibition.