Second-Generation Antiandrogen Therapy for Prostate Cancer

Prostate cancer therapy for locally advanced and metastatic diseases includes androgen deprivation therapy (ADT). Second-generation antiandrogens have a role in castration-resistant prostate cancer. Nevertheless, some patients do not respond to this therapy, and eventually all the patients became resistant. This is due to modifications to intracellular signaling pathways, genomic alteration, cytokines production, metabolic switches, constitutional receptor activation, overexpression of some proteins, and regulation of gene expression.

1. Abiraterone

Abiraterone is an orally administered small molecule that irreversibly inhibits the products of the cytochrome P450 family 17 (CYP17) gene (including both 17,20 lyase and 17-alpha-hydroxylase). It blocks the synthesis of androgens in the tumor as well as in the testes and adrenal glands. Altered steroidogenesis was discussed as one mechanism by which CRPC develops. Adrenal androgen levels are not affected by ADT. Dehydroepiandrosterone (DHEA) and its sulfated form (DHEA-S) are converted to dihydrotestosterone (DHT) via a backdoor pathway [1].

The molecules are converted to androstenedione (AD) either in the prostate or adrenal gland by 3β-hydroxysteroid dehydrogenase (3βHSD), encoded by HSD3B. There are two isoforms, 3βHSD1 in the prostate and peripheral tissues, and 3βHSD2 in the adrenal gland. The conversion from AD to DHT, in the absence of ADT, goes through testosterone as an intermediary and requires 17βHSD3, AKR1C3 and steroid-5α-reductase. However, in the presence of ADT, this sequence can be reversed, leading to 5α-AD (5α-androstenedione) that acts as an intermediary agent, bypassing testosterone completely. This alternative pathway, known as the "5α-dione" pathway, has been demonstrated to predominate in CRPC. So, abiraterone-naive CRPC cells use the 5α-dione pathway to produce intra-tumoral DHT but are still dependent on adrenal androgens. By irreversibly inhibiting this critical upstream enzyme in the steroidogenesis pathway, abiraterone effectively causes a significant decrease in intratumoral androgen levels by preventing production of adrenal androgens. However, despite its effectiveness in inhibiting the steroidogenesis pathway, abiraterone's effect is incomplete. Overexpression or mutations of CYP17A1 may also contribute to abiraterone resistance. HSD3B1 (1245C) mutation contributing to progression to CRPC has also been found in abiraterone-resistant xenograft models [2]. Mostaghel et al. demonstrated that in abiraterone-treated prostate cancer cells there is overexpression of the isoform CYP17A1, as well as other enzymes involved in steroidogenesis, such as AKR1C3 and HSD17B3 [3]. AKR1C3 facilitates the conversion of androstenedione and 5 α-androstenedione (5 α-dione) that are weak androgens to the more effective testosterone and DHT. It is not only enzyme mutations that can lead to abiraterone resistance. Another important role in resistance to abiraterone in prostate cancer cells is played by androgen accumulation, such as pregnenolone and progesterone. The progesterone receptor (PR) has been found overexpressed in mCRPC [4]. In addition, PR high density is an independent poor prognostic factor [5]. The PR and AR have 88% sequence homology in the ligand-binding domain and share common response elements. Therefore, the accumulation of progesterone could lead to transcription of androgen-dependent genes. A novel therapeutic target could be the inhibition of PR caused by onapristone, a progesterone receptor antagonist that is currently under evaluation in CPRC that has progressed after abiraterone, enzalutamide, or two lines of chemotherapy: preliminary data has shown this is safe and feasible [6].

2. Enzalutamide

Enzalutamide is an androgen receptor signaling inhibitor without agonistic activity. It suppresses the nuclear translocation of active androgen receptors to prevent recruitment of androgen response elements. This leads to cellular apoptosis and inhibition of CRPC cell proliferation. Despite his efficacy, eventually all of the enzalutamide-treated patients became

resistant. This could be related to different mechanisms. One of these is the androgen receptor reactivation. Yuan et al. observed that AR is highly expressed and transcriptionally active in CRPC despite enzalutamide treatment [7]. AR-persistent activation could be secondary to somatic mutations, such as the amplification of an acquired androgen receptor enhancer located at 650-kb centromere to the AR [8]. Another study [9] found that galectin-3, a member of the animal lectin family, significantly inhibited the therapeutic effect of enzalutamide by increasing the expression of several androgen receptor target genes, such as kallikrein-related peptidase 3 (KLK3) and transmembrane protease serine 2 (TMPRSS2). Such enhancement of androgen receptor transcriptional activity and expression of androgen receptor-related genes can contribute to enzalutamide resistance. Androgen hormone potentiation could also participate. Liu et al. demonstrated that AKR1C3, a critical enzyme in the biological pathways that lead to the production of testosterone and DHT from weak androgens (androstenedione and 5 α-androstenedione), is upregulated in enzalutamide-resistance prostate cancer cells and that indomethacin, an inhibitor of AKR1C3 activity, could overcome this resistance, reducing cell proliferation [10]. Another mechanism is the inhibition of AR-protein degradation by preventing E3 ligase-mediated ubiquitination. This is due to the binding of the AR by a polycomb group protein, B lymphoma Moloney murine leukemia virus insertion region 1 (BMI1). In an enzalutamide-resistant xenograft model, it was found that the BMI1 inhibitor significantly decreased the development of enzalutamide-resistant CRPC [11]. Another cellular pathway involved in AR activation is c-Myc. One study showed induction of c-Myc's expression by erythropoietin-producing human hepatocellular (Eph) receptors, which is a key factor for enzalutamide resistance [12].

Enzalutamide resistance can also be achieved by AR splice variants, as discussed for abiraterone. Antonarakis et al. demonstrated a significant overexpression of AR–V7 in enzalutamide-treated patients. TK activated cdc42-associated kinase 1 (ACK1) (TNK2) could work in an epigenetic circuit, contributing to the overexpression of AR-V7 and leading to enzalutamide resistance [13]. Another possible target in AR-V7 amplification could be the vasopressin1A (V1A) receptor (AVPR1A) [14]. AR-V7 overexpression could also be related to ubiquitin E3 ligase proteasome degradation: the interaction between heat shock protein family member HSP70 and functional E3 ubiquitin ligase STIP1 homology and U-box containing protein 1 (STUB1) was required for androgen receptor variant 7 proteostasis. Suppression of HSP70 inhibited tumor growth and enzalutamide resistance by decreasing AR-V7 levels [15]. Inhibition of ubiquitination is also achieved by activation of anti-apoptotic B cell lymphoma-2 (BCL2) protein [16].

3. Darolutamide and Apalutamide

Darolutamide is a novel AR antagonist that has the capacity of binding both wild-type and mutated AR, trying to overcome enzalutamide resistance. Moreover, compared to apalutamide and enzalutamide, it has a low penetration of the brain blood barrier and a low binding affinity for the gamma-aminobutyric acid (GABA) type A receptor. Borgman et al. evaluated the efficacy of darolutamide in inhibiting cell growth and tumor progression in enzalutamide-resistant CRPC and in mutated AR patients previously treated with abiraterone, enzalutamide, or bicalutamide. Darolutamide significantly inhibited cell growth and AR transcription activity in vitro while decreased tumor volume and serum prostate-specific antigen levels in vivo. In addition, it had a significative inhibition of transcriptional activity of AR-mutated variants, such as F877L, F877L/T878A and T878G, that, as said before, transform enzalutamide into an agonist (Borgmann et al.).

Apalutamide is a new nonsteroidal AR antagonist that binds directly to the ligand-binding domain of the AR and prevents its translocation and its related transcriptional pathway. Apalutamide has demonstrated an improvement in overall survival (OS) compared to placebo in nonmetastatic CRPC and a longer metastasis-free survival [17].

Currently, there are not many studies that evaluate darolutamide's and apalutamide's unique resistance mechanisms. It has been demonstrated that there is a cross-resistance mechanism between darolutamide, apalutamide, enzalutamide, and abiraterone.

4. Cross-Resistance

It is interesting to highlight that those patients who receive abiraterone or enzalutamide as the first line of therapy have a 15–30% response rate to the alternative agents as second-line CRPC treatment. This underlines that there clearly exists a cross-resistance between enzalutamide and abiraterone. Resistance to second-line therapy takes about 3–6 months to develop, significantly shortening the duration of benefits from this treatment by at least 50% compared with that of the first-line [18].

As suggested before, some biological mechanisms of resistance to these drugs overlap. Upregulation of CYP17 occurs both in resistance to enzalutamide and abiraterone. A second mechanism that leads to cross-resistance is the upregulation of the AR, due to the amplification of the gene or the overexpression of the protein. Another mechanism

common to the two drugs is the emergence of AR splice variants (for example ARV-7), in which abnormal splicing of the AR messenger RNA (mRNA) leads to the formation of a truncated AR protein that is constitutively active without the necessity of a ligand [19].

What is interesting is that cross-resistance can also involve darolutamide and apalutamide, in addition to enzalutamide and abiraterone. Zhao et al. demonstrated that enzalutamide- and abiraterone-resistant prostate cancer cells are also resistant to apalutamide and darolutamide. In particular, the presence of ARV-7 conferred resistance to enzalutamide and apalutamide [20].

Moreover, the emergence of resistance to antiandrogens such as enzalutamide determine the potential for accelerating metastatic disease, as Simon et al. discovered. This implies that the treatment must not be interrupted, even when drug resistance has been achieved [21].

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