PROSTATE CANCER IN FOCUS

Current Developments in the Management of Prostate Cancer

Current Understanding of Resistance to Abiraterone and Enzalutamide in Advanced Prostate Cancer

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H&O How common is resistance to abiraterone and enzalutamide in castration-resistant prostate cancer (CRPC)?

ESA Approximately 15% to 25% of patients with CRPC do not respond to first-line treatment with either abiraterone (Zytiga, Janssen) or enzalutamide (Xtandi, Astellas/Medivation), meaning that their prostate-specific antigen (PSA) values do not decrease or their tumors do not regress. The other 75% to 85% of patients respond to abiraterone or enzalutamide initially, but a subsequent PSA increase or tumor progression occurs in nearly all of them with time. In the first-line CRPC setting, resistance typically develops after 9 to 15 months of treatment with either agent.

What is interesting is that patients who receive enzalutamide or abiraterone as first-line therapy and subsequently become resistant have only a 15% to 30% rate of response to the alternative agent as second-line CRPC treatment. That finding clearly shows that cross-resistance occurs between enzalutamide and abiraterone. Resistance to second-line therapy takes approximately 3 to 6 months to develop, so the duration of benefit of second-line CRPC therapy is decreased by at least 50% compared with that of first-line therapy.

H&O Do we understand what causes resistance?

ESA Resistance to enzalutamide and abiraterone is multifactorial, and we have only recently begun to understand the mechanisms behind it.

One way to consider abiraterone and enzalutamide resistance is to divide it into 3 biological categories (Figure). The first category is *reactivation or persistent activation of the androgen receptor (AR)*, which results in increased synthesis of androgen within the tumor and in the adrenal glands, the generation of *AR* mRNA splice variants, and the development of activating mutations of the *AR* gene.

AR bypass pathways, the second category, include glucocorticoid receptor activation and progesterone receptor activation. Both of these alternative steroid receptors may, in certain contexts, stimulate the transcription of androgen-responsive genes.

Androgen/AR-independent mechanisms, the third category, include multiple divergent mechanisms of resistance: mutation or inactivation of the tumor protein p53 gene *(TP53)* or retinoblastoma tumor suppressor gene *(RB)*; activation of the Wnt signaling pathway; loss of the phosphatase and tensin homolog tumor suppressor gene *(PTEN)*, which induces activation of the phosphoinositide 3-kinase (PI3K) and AKT pathway; and the transformation of classic prostate adenocarcinoma into a neuroendocrine or small-cell phenotype that is often associated with the amplification of N-Myc *(MYCN)* or Aurora kinase A *(AURKA)*. An additional mechanism that has been receiving a lot of attention recently is impairment of DNA damage repair pathways induced by mutations in the breast cancer type 2 susceptibility protein *(BRCA2)* and ataxia telangiectasia mutated *(ATM)* genes.

H&O How do the mechanisms of resistance to the 2 agents differ, and do some of them overlap?

ESA One mechanism of resistance that occurs with both drugs is upregulation of the cytochrome P-450 isoform

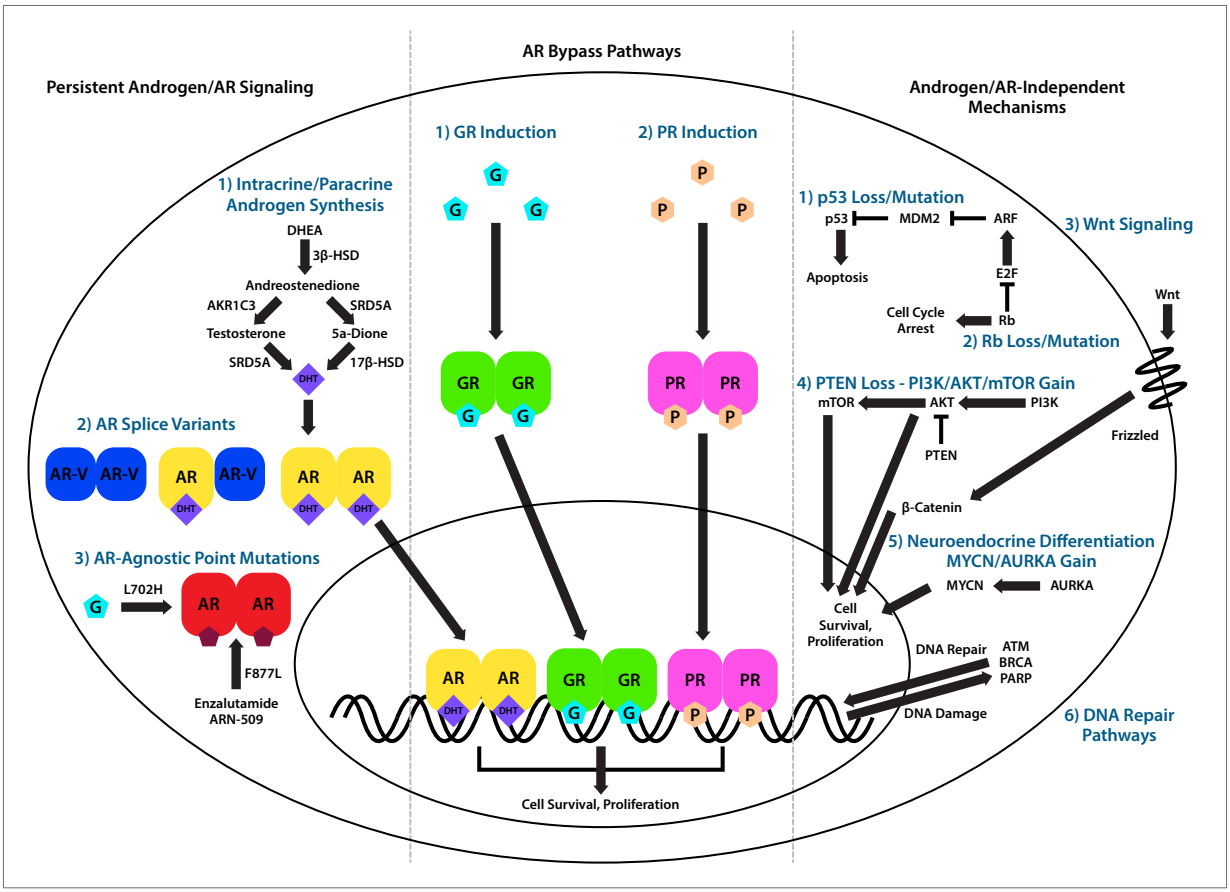

Figure. Signaling pathways implicated in resistance to novel androgen/AR-directed therapies. These resistance mechanisms are conceptualized in 3 broad biological categories: (1) reactivation of androgen/AR signaling, leading to persistent AR signaling; (2) AR bypass pathways, leading to activation of androgen-regulated genes by alternative steroid receptors; and (3) a large number of androgen/AR-independent pathways.

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17 (CYP17) enzyme, which plays a key role in the synthesis of androgen by the adrenal glands and by the prostate cancer tumor cells themselves.

A second mechanism that is common to both agents is upregulation of the AR. This may occur because either the *AR* gene is amplified or the AR protein is overexpressed.

A third mechanism that is common to the 2 drugs is the emergence of *AR* splice variants, in which abnormal splicing of the *AR* messenger RNA (mRNA) leads to the formation of a prematurely truncated AR protein that is constitutively active in a ligand-independent fashion.

Regarding mechanisms of resistance that are unique to abiraterone, the first of these is the L702H mutation in the ligand-binding domain of *AR*, which results in activation of the AR by glucocorticoids such as prednisone. This activation causes resistance to abiraterone because abiraterone is usually prescribed in combination with prednisone.

The second of these is the T878A mutation in *AR*, which makes the AR responsive to progesterone. Abiraterone increases blood levels of progesterone, which can stimulate the AR if this mutation is present.

Regarding mechanisms of resistance that are unique to enzalutamide, the first of these is the F877L mutation, which is also in the ligand-binding domain of *AR*. This mutation converts enzalutamide from an antagonist into an agonist, so that enzalutamide stimulates rather than inhibits the AR in patients with this mutation.

The second mechanism of resistance to enzalutamide is induction of the glucocorticoid receptor. It has been shown that after enzalutamide inhibits the AR, the glucocorticoid receptor can sometimes take over its role—some people say that it "hijacks" the AR's androgen response elements in DNA. The result is that the glucocorticoid receptor activates the transcription of genes that allow the tumor to proliferate.

H&O What clinical studies have looked at these mechanisms of resistance?

ESA Most of the studies in patients with CRPC have focused primarily on *AR* mutations, *AR* amplification, *AR* splice variants, or the glucocorticoid receptor.

At least 2 prospective studies have looked at *AR* mutations, which occur in approximately 5% to 15% of patients receiving enzalutamide or abiraterone. The first study, which was published by Romanel and colleagues in *Science Translational Medicine* in 2015, looked at circulating tumor DNA in patients receiving abiraterone. The researchers found that outcomes with abiraterone were much better in patients who had the wild-type *AR* gene than in those who had either *AR*-activating mutations or *AR* amplification.

The second study, which was published by Azad and colleagues in *Clinical Cancer Research* in 2015, evaluated circulating tumor DNA in patients receiving enzalutamide or abiraterone. Much as in the previous study, the researchers found that patients with the wild-type *AR* gene had a better prognosis than did those who had either *AR*-activating mutations or *AR* amplification.

Several prospective studies have looked at the importance of *AR* splice variants, which are abnormal splice isoforms of *AR* mRNA. The majority of these splice variants lead to a truncated AR protein that retains the transcriptionally active N-terminal domain but is missing the C-terminal domain, which contains the ligand-binding pocket to which all the androgens and anti-androgens bind. Despite absence of the ligand-binding domain, these splice variants function as ligand-independent transcription factors and can stimulate cancer growth. The most important of the splice variants in humans is AR-V7.

The first clinical study on *AR* splice variants in CRPC was conducted here at Johns Hopkins and was published in the *New England Journal of Medicine* in 2014. In that study, we prospectively evaluated 62 patients who were starting either enzalutamide or abiraterone for the first time and analyzed AR-V7 with a circulating tumor cell (CTC) assay that we had developed. We found that outcomes of treatment with enzalutamide and abiraterone were significantly worse in patients who harbored AR-V7 in their CTCs than in those who did not have detectable AR-V7 in their CTCs. The results of that study were supported by another trial, conducted in Germany. That study, which was performed by Steinestel and colleagues and published in *Oncotarget* in 2015, also found that the presence of AR-V7 in CTCs was a negative prognostic factor for response to enzalutamide and abiraterone.

Interestingly, the presence of AR-V7 does not appear to be associated with primary resistance to chemotherapeutic agents commonly used in prostate cancer, such as docetaxel and cabazitaxel (Jevtana, Sanofi-Aventis). In fact, emerging evidence suggests that patients with AR-V7 in their CTCs may be better served by treatment with a chemotherapeutic agent such as docetaxel or cabazitaxel than by treatment with enzalutamide or abiraterone. Therefore, AR-V7 may be one of the first markers for treatment selection that we have in CRPC, which is an exciting prospect. These data clearly require further prospective validation before AR-V7 can be used in routine clinical practice.

Regarding investigations of glucocorticoid receptor expression, an important study was published by Arora and colleagues in *Cell* in 2013. In that study, patients underwent a bone marrow biopsy immediately before and 8 weeks after starting treatment with enzalutamide. It was found that the glucocorticoid receptor protein was more likely to be detected in bone marrow by immunohistochemistry in the patients who had either primary or acquired resistance to enzalutamide, and higher levels of the protein were associated with worse outcomes. This was the first study in humans to suggest that glucocorticoid receptor expression may correlate with enzalutamide resistance in patients with CRPC.

H&O What strategies are used to overcome resistance?

ESA Primary resistance is difficult to prevent, but a number of different approaches attempting to delay acquired resistance are being studied. For example, a large phase 3 study being led by the Alliance for Clinical Trials in Oncology is comparing enzalutamide plus abiraterone vs enzalutamide alone as first-line treatment of CRPC, to see whether the combination of these 2 agents will be more effective at improving overall survival (NCT01949337). One of the rationales for this approach is that the combination of the 2 drugs might prevent or slow the emergence of acquired *AR* mutations and *AR* splice variants or induction of the glucocorticoid receptor.

Another potential strategy would be to use the 2 agents in the optimal sequence for reducing acquired resistance. In a large, randomized phase 2 trial that is being conducted by Dr Kim Chi and colleagues at the Vancouver Cancer Centre of the BC Cancer Agency in Canada, patients are randomly assigned to start with abiraterone or enzalutamide and then switch to the other agent after disease progression. This trial will inform us whether either sequence is superior to the other, and a number of biomarkers embedded in this study will help clarify resistance mechanisms (NCT02125357).

A third potential strategy would be to combine enzalutamide or abiraterone with a second agent to target one of the other resistance pathways. For example, several trials are now combining enzalutamide or abiraterone with a PI3K inhibitor or an AKT inhibitor to see whether this combination might delay the development of secondary resistance (NCT02215096, NCT02525068, and NCT01884285).

A fourth potential strategy, which may overcome resistance induced by the glucocorticoid receptor, would be to add the glucocorticoid receptor antagonist mifepristone to enzalutamide treatment. An ongoing phase 2 trial is randomly assigning patients to either enzalutamide alone or enzalutamide plus mifepristone to determine whether the combination will prolong responses and delay resistance (NCT02012296).

Another approach that we have begun to test here at Johns Hopkins is the use of high-dose testosterone in patients with CRPC that has become resistant to abiraterone or enzalutamide (NCT02090114). Preclinical work has supported the idea that exposing CRPC cells to very high doses of testosterone can induce cell death by causing double-strand breaks in DNA as well as by preventing DNA relicensing during the cell cycle. The first of these studies, which was published by Schweizer and colleagues in *Science Translational Medicine* in 2015, found that monthly intramuscular injections of high-dose testosterone produced significant clinical responses in approximately half of patients with CRPC. One of our emerging hypotheses is that high-dose testosterone therapy may also work by eliminating *AR* splice variants.

H&O Can biomarkers be used to determine whether enzalutamide and abiraterone will work?

ESA This is an area of great interest right now. I would say that the 2 most promising clinical biomarkers are AR-V7 in circulating tumor cells and *AR* mutations in circulating tumor DNA. I predict that these will enter the clinic within the next 3 years, and it will be possible to use a simple blood test to determine whether a patient is a good candidate for enzalutamide or abiraterone treatment. For example, patients with normal (wild-type) *AR* would be very likely to benefit from either abiraterone or enzalutamide, those with an activating mutation in the *AR* gene might be resistant to one or the other agent, and those with AR-V7 splice variants might not respond to either agent. Of course, prospective studies will need to be carried out to validate the clinical utility of these biomarkers before they can be used to make clinical decisions. Such validation studies are ongoing (NCT02269982).

H&O What other studies are looking at ways to overcome resistance?

ESA Right now, a phase 3 trial is examining at whether enzalutamide or galeterone, an experimental AR antagonist, is more effective in patients with CRPC positive for AR-V7 (NCT02438007). Galeterone may be able to overcome resistance caused by *AR* splice variants by degrading the AR-V7 protein. In addition, a phase 1/2 trial is looking at the use of an experimental agent called EPI-506. EPI-506 is the first drug to target the N-terminal of the AR, which theoretically should inhibit both mutant *AR* and *AR* splice variants in patients with treatment-resistant CRPC (NCT02606123).

Ongoing trials are also investigating agents that might target activating mutations in the *AR* gene, such as the AR inhibitors ODM-201 (NCT02200614) and VT-464 (NCT02130700). These agents may have clinical activity in men with certain activating *AR* mutations. As we discussed earlier, researchers are also investigating whether mifepristone, a glucocorticoid receptor inhibitor, can improve results in patients receiving enzalutamide. Moreover, a trial that is being led by Dr Gerhardt Attard at the Royal Marsden Institute in London is studying the use of onapristone, an agent that blocks the activated progesterone receptor, in patients with metastatic CRPC (NCT02049190).

Finally, in terms of AR-independent mechanisms of escape from abiraterone and enzalutamide, the experimental AURKA inhibitor alisertib is being studied as a possible way to overcome resistance in patients with neuroendocrine prostate carcinoma (NCT01799278).

Disclosures

Emmanuel S. Antonarakis has served as a paid consultant/advisor for Janssen, Astellas, Sanofi, Dendreon, ESSA, and Medivation and has received research funding from Janssen, Johnson & Johnson, Sanofi, Dendreon, Exelixis, Genentech, Novartis, and Tokai. He is also the coinventor of a biomarker technology that has been licensed to Tokai.

Suggested Readings

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