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Perspective

Steroid Hormone Receptors in Prostate Cancer: A Hard Habit to Break?

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The clinical data from abiraterone acetate and MDV-3100 confirm continued androgen receptor (AR) addiction in a significant proportion of castration-resistant prostate cancers (CRPC). However, patients nearly invariably progress with a rise in prostate-specific antigen, suggesting resumption of transcription of hormone-regulated genes. If CRPC remains addicted to steroid receptor signaling, including, but not exclusive, to AR, how does reactivation occur? Or if cancers lose this addiction, do they remain driven by the same oncogenic mechanisms? The future development of therapeutics for CRPC should be informed by an understanding of the mechanisms underlying disease progression following treatment with these novel agents.

Cancer of the prostate is the commonest cancer in men in western societies, accounting for 11% of male cancer deaths (Jemal et al., 2008). The proliferation and survival of prostate cancer cells is critically dependent on androgen stimulation (Huggins and Hodges, 1972), with treatment of incurable advanced prostate cancer being primarily based on androgen deprivation by castration, which is achieved chemically. Such treatment frequently results in dramatic remission, but the disease invariably relapses to what has historically been referred to as ''hormone-refractory'' prostate cancer. To explain this observation, Isaacs and Coffey (1981) proposed that both hormone-resistant and hormone-sensitive prostate cancer cells coexist and that androgen withdrawal results in the natural selection of the androgen-resistant component (Isaacs and Coffey, 1981). This general model is broadly consistent with recent evidence showing that the prostate cancer clonogenic stem cell population lacks androgen receptor (AR) (Collins et al., 2005). Evidence indicates, however, that despite the potential existence of ''androgen-independent'' clones, the growth of the majority of cancer cells in many patients with cancers recurring after castration remains dependent on the AR signaling axis:

- Studies of isogenic prostate cancer cell lines grown in androgen-deprived conditions report that progression invariably occurs following an increase in expression of AR mRNA (Chen et al., 2004).
- High expression of enzymes involved in steroid synthesis (Stanbrough et al., 2006) allows CRPC to maintain high intratumoral androgen levels despite ongoing castration (Mohler et al., 2004). These adaptive changes by CRPC suggest that the bulk of tumor remains dependent on ligand activation of the AR.
- Aberrations of the *AR* gene that could allow AR signaling occur with increasing frequency in multidrug-resistant CRPC (Buchanan et al., 2001).
- A rise in serum prostate-specific antigen (PSA), which is at least partly related to AR transcriptional activity, occurs in the majority of CRPC patients at progression.
- Nuclear AR expression persists in prostate cancer metastases from patients who progressed on multiple hormonal therapies (Shah et al., 2004).
- CRPC patients benefit from repeated further manipulations with agents targeting steroid receptor signaling. Critically, clinical data from recent phase I/II clinical trials of two oral agents, abiraterone acetate and MDV-3100, have reported a high level of antitumor activity in CRPC (Attard et al., 2009b; Tran et al., 2009).

These efforts by cancer to reactivate AR signaling at progression suggest continued addiction to AR signaling or other processes that modulate transcription of PSA and other hormone-regulated genes. The appealing corollary of this hypothesis would be that clinical castration resistance could develop via mechanisms that allow reactivation of the same downstream processes that drive therapy-naive cancer. We propose that therapeutic strategies aimed at modulating activation of AR targets are therefore likely to induce responses in multidrug-resistant prostate cancer.

Hormone-Driven Oncogenesis

The AR initiates the development and maintains regeneration of the prostate gland through regulation of genes involved in protein synthesis, secretion, apoptosis, and transcription. Despite this prodifferentiation function, androgen-regulated genes are overexpressed in primary prostate cancer and CRPC compared with benign prostate epithelia, and downregulation of these genes is associated with clinical responses to androgen ablation therapy (Massie et al., 2007). Tomlins et al. report that 40%–70% of prostate cancers have a chromosomal rearrangement that results in hormonal regulation of oncogenic ETS gene expression (Tomlins et al., 2005). This could explain one mechanism by which activation of the AR becomes oncogenic. Upregulation of genes, such as PSA, associated with prostate cancer could therefore be a ''bystander'' effect of the AR transcriptional activity required for prostate cancer progression. The most common ETS gene rearrangement results in fusion of the androgen-regulated

gene, *TMPRSS2*, with *ERG* (Tomlins et al., 2005). We have reported an AR-binding site in the genomic proximity of *TMPRSS2* and also of other less commonly occurring androgen-regulated ETS fusion partners, including *SLC45A3* and *ACSL3* (Attard et al., 2008a). In addition to AR, other steroid receptors could be involved in the regulation of ETS fusion partners (Setlur et al., 2008).

ETS gene fusions appear to precede the development of metastases, as there is significant concordance in ETS gene status of all CRPC metastases and therapy-naive prostate cancer (Attard et al., 2009c; Mehra et al., 2008). However, ERG remains overexpressed in end-stage *ERG*-rearranged CRPC (Attard et al., 2009c; Cai et al., 2009), and in fact, Tomlins et al. first confirmed the existence of ETS gene fusions in CRPC metastases obtained at autopsy that overexpressed ERG or ETV1 (Tomlins et al., 2005). This provides further evidence that CRPC undergoes adaptations in order to maintain overexpression of hormone-regulated ETS genes.

ETS gene rearrangements and other genomic aberrations are not present in stromal cells, but there is strong preclinical evidence that stromal-epithelial interactions play an important role in prostate carcinogenesis (Cunha et al., 2002). Growth factors produced by prostate stromal cells can activate steroid receptor signaling pathways, and the efficacy of current hormonal therapies in targeting such paracrine loops is unclear. Steroid receptor-mediated transcriptional activity is altered in malignant stroma (Cano et al., 2007), and it therefore cannot be assumed that ablation of stromal steroid receptor signaling is necessarily therapeutic. Current xenograft models fail to accurately mimic human tumor stromal conditions, and a better understanding of the stromal-epithelial interplay is dependent on more clinically representative preclinical models.

Abiraterone Acetate and MDV3100

Abiraterone acetate is a highly specific inhibitor of CYP17 and results in significant suppression of serum androgenic steroids and estrogens (Attard et al., 2008b). We and others have reported declines in PSA by $\geq$50% and $\geq$90%, with abiraterone acetate in 50%–60% and 20%–30% of CRPC patients, respectively (Attard et al., 2009a). Importantly, declines in PSA were associated with radiological tumor regression, declines in circulating tumor cell (CTC) count, and symptomatic benefit (Attard et al., 2009a). MDV-3100 is a potent AR antagonist that also induces tumor responses in CRPC patients that have failed other hormonal therapies (Tran et al., 2009). This antitumor activity in heavily pretreated (standard antiandrogens, low-dose steroids, estrogens, and docetaxel) CRPC patients suggests these agents are superior at inhibiting the AR signaling axis and, importantly, confirms that further hormonal manipulations in CRPC are an effective therapeutic strategy. Although "androgen-independent clones" may ultimately give rise to an "androgen-independent tumor," these clinical data indicate that therapeutic attempts to completely ablate processes that associate with steroid receptor signaling will give patients real clinical benefit. These observations have led to a very substantial investment in the development of novel drugs that target the AR signaling axis, and both of these agents are now undergoing evaluation in large, randomized, double-blind, phase III studies designed to identify a survival benefit and obtain regulatory approval to treat CRPC.

Abiraterone acetate is well tolerated, and no dose-limiting toxicities were observed at the highest dose evaluated (2000 mg daily continuously). The main toxicities reported were related to a syndrome of secondary mineralocorticoid excess that was reversed by either a mineralocorticoid antagonist or suppression of raised ACTH with low-dose daily exogenous glucocorticoids (Attard et al., 2008b). MDV-3100 is also well tolerated, although full publication of the phase I results is currently awaited (Tran et al., 2009). The long-term toxicities associated with inhibition of the AR in men treated for long periods of time with these agents remain unstudied.

Reports to date suggest resistance to these agents commonly develops within 12–36 months and is nearly invariably characterized by a rising PSA. This suggests that although CRPC resistant to these agents may contain androgen-independent clones, a significant portion of the tumor remains "addicted" to activation of the AR or its downstream targets. We hypothesize that strategies that improve on the efficacy of the current phase III combination of these drugs with castration will improve outcome. These could include (1) the sequential or combinatorial use of these agents; (2) the continuation of treatment beyond objective progression and in combination with subsequent treatments in order to achieve long-term supercastration or androgen blockade; (3) the development of novel drugs that ablate ligand-binding domain (LBD)-independent AR signaling with the aim of reversing resistance to abiraterone or MDV-3100; or (4) the direct targeting of rearranged ETS genes or their downstream effectors which could abrogate the need for therapeutic castration. The observation or absence of responses to one drug after progression on the other will inform on the best strategy.

Androgens in CRPC

Up to 20% of serum androgens in the noncastrate male are nongonadal in origin: serum androstenedione and dehydroepiadrostenedione (DHEA) levels in castrate men are similar to levels in noncastrate men and could activate the AR and other steroid receptors. Similarly, the recent use of super-sensitive LC-MS/MS assays has confirmed detectable serum testosterone levels in the majority of castrate CRPC patients (Attard et al., 2008b). Intraprostatic testosterone levels fall immediately after castration in cancer patients but rise to precastration levels after development of castration resistance (Mohler et al., 2004). This could either be secondary to upregulation of enzymes involved in the conversion of adrenal androgens to testosterone that has been reported in CRPC (Stanbrough et al., 2006) or to intratumoral synthesis of androgens from pregnenolone and other precursors. De novo synthesis of androgens has been demonstrated in androgen-dependent xenograft tumor models when exposed to androgen-deprived conditions (Locke et al., 2008), and several enzymes, including CYP17, involved in steroid biosynthesis are highly expressed in CRPC (Montgomery et al., 2008). These data introduce the possibility that inhibition of intratumoral CYP17 expression and intracrine tumor steroid synthesis could account for the significant antitumor activity of abiraterone. This remains to be confirmed clinically in prospective clinical trials and, in

fact, suppression of serum steroids, which mostly originate from the adrenal glands, could be sufficient to explain the tumor responses observed with abiraterone acetate (Attard et al., 2008b). Also, pretreatment serum androgen and estradiol levels are associated with tumor responses to CYP17 inhibition with either abiraterone acetate or the nonspecific CYP17 inhibitor ketoconazole (Attard et al., 2009a; Ryan et al., 2007), further supporting a role for serum hormones in driving CRPC. In contrast to treatment with ketoconazole, no rise in serum hormones at disease progression on abiraterone has been reported to date (Attard et al., 2008b; Small et al., 2004). It is however possible that more sensitive tests could detect very low levels of serum androgens that could support the growth of CRPC cells in patients treated with abiraterone. Also, although no evidence to support this has been reported, CRPC could potentially become resistant to abiraterone following synthesis of androgens via CYP17-independent pathways or genetic changes in CYP17 that prevent its inhibition.

Androgen-Independent Activation of the AR

AR gene amplification is reported in one-third of CRPC tumors. Since this is significantly less common in untreated tumors, it has been proposed that gene amplification is the result of selective pressure exerted by androgen deprivation (Bubendorf et al., 1999; Visakorpi et al., 1995). Similarly, the prevalence of *AR* mutations in tumor tissue increases with exposure to castration, antiandrogens, and other lines of hormone treatment (Buchanan et al., 2001; Taplin et al., 2003).

The vast majority of these *AR* gene mutations collocate to the signature sequence and Activation-Function-2 (AF-2), two discrete regions of the ligand-binding domain LBD, and result in loss of ligand specificity of the AR (Buchanan et al., 2001). For example, the human prostate cancer cell line LNCaP has an *AR* point mutation (Thr-Ala877) that has been detected in up to 15% of clinical samples and results in inappropriate activation by several ligands, including progestins, estrogens, adrenal androgens, steroidal compounds such as the diuretic spironolactone, and antiandrogens (Grigoryev et al., 2000; Luthy et al., 1988; Taplin et al., 2003; Veldscholte et al., 1992). Also, mutations involving cofactor binding sites can lead to enhanced coactivator binding and stabilization of the AR, or a loss of interaction with corepressors (Brooke and Bevan, 2009). An increase in AR mRNA expression is sufficient in vitro to convert the action of bicalutamide from an overall AR antagonist to an agonist (Chen et al., 2004). These data provide a molecular explanation for the phenotypic phenomenon whereby currently available antiandrogens become agonistic in at least one in five patients (Small et al., 2004). Similarly, ligands other than testosterone or dihyrdotestosterone could result in potent activation of AR signaling (Culig et al., 1993; Fenton et al., 1997; Zhao et al., 2000). MDV-3100 binds to the AR LBD but has shown no AR agonistic activity in preclinical models (Tran et al., 2009).

Further support for the hypothesis that a promiscuous AR becomes a "driving" oncogene is provided by reports of PSA responses in 25% of patients progressing on abiraterone acetate alone when low-dose corticosteroids were added, despite prior documented tumor progression on the same corticosteroid dose and regimen (Attard et al., 2008b, 2009a). Although alternative are possible, these data suggest that AR signaling was initially abrogated by suppression of androgen LBD and estrogens downstream of CYP17 and subsequently reactivated by a rise in the level of upstream steroids driven by high levels of adrenocorticotrophic hormone that are suppressed by exogenous corticosteroids. Overall, these observations support the current clinical development of abiraterone acetate in combination with prednisone.

Genetic aberrations of the *AR*, including high copy number amplification of the *AR*, and changes in the AR coactivator/corepressor balance could also result in constitutive activation. Recent reports suggest that naturally occurring AR splice variants lacking the LBD but retaining the Activation-Function-1 (AF-1) region in the N-terminal domain, which in isolation is a potent activator of AR transcriptional activity and could induce canonical androgen-responsive gene expression in the absence of androgens, could be selected for by androgen deprivation (Dehm et al., 2008). This could potentially lead to resistance to drugs that inhibit steroid synthesis and antiandrogens that bind the AR LBD, including MDV-3100.

Alternative strategies for targeting the AR signaling axis that are not dependent on the AR LBD are therefore required. One strategy that is undergoing clinical evaluation is the targeting of AR post-translational maturation by blocking chaperone proteins. Preclinical reports indicate that aberrant (mutated) oncoproteins may be more dependent on chaperone proteins for maintaining stability and avoiding ubiquitination (Banerji et al., 2008), suggesting increased sensitivity of aberrant AR in multidrug resistant disease. AR is maintained in the cytoplasm in a complex that includes the heat shock protein HSP90; following ligand binding, HSP27 displaces HSP90 from this complex and chaperones AR into the nucleus (Zoubeidi et al., 2007). It is unclear whether AR splice variants are also dependent on heat shock proteins and other components of the AR transcription complex. Nonetheless, both HSP90 and HSP27 are potential therapeutic targets in prostate cancer. HSP27 is an ATP-independent chaperone that is currently being targeted therapeutically using the antisense nucleotide OGX-427 (Zoubeidi et al., 2007). Several specific HSP90 inhibitors are undergoing clinical evaluation, although better blockade of HSP90 could be achieved with alternative strategies: for example, concurrent inhibition of HSP70 and HSP72 (Powers et al., 2008). We have reported suppression of HSP90 in clinical studies of the histone deacetylase inhibitor (HDACi) LAQ824 (de Bono et al., 2008) but very limited antitumor activity in CRPC with the HDACi depsipeptide (Molife et al., 2009). Recent preclinical data support the further clinical development for CRPC of HDACi that have improved tolerability and superior pharmacological properties (Welsbie et al., 2009). These strategies could achieve complete ablation of the AR and improve significantly on current therapeutic strategies, especially if unliganded AR and LBD-lacking AR variants serve as cytoplasmic or cell membrane signaling proteins as do other steroid receptors, including the ER (Pedram et al., 2006), or regulate tumorigenesis via distinct mechanisms, including through upregulation of M-phase cell-cycle genes (Wang et al., 2009). Also, therapeutic targeting of the AF-2 region in the N-terminal domain

would be pharmacologically challenging but could potentially achieve complete abrogation of AR transcriptional activity.

There is strong preclinical evidence to support crosstalk between receptor tyrosine kinases: for example, members of the family of epidermal growth factor (EGF) receptors and their downstream targets and steroid receptor networks resulting in activation of steroid-regulated transcription in the absence of ligand (Craft et al., 1999; Culig et al., 1994). For example, HER2 kinase activity results in prostate tumor growth in ''androgen-independent'' preclinical models. These data led to the clinical evaluation of therapeutics targeting EGFR or HER2 in CRPC that, however, failed to demonstrate any clinical benefit, despite proven antitumor activity with the same dose and regimen in other tumor types (de Bono et al., 2007; Pezaro et al., 2009; Ziada et al., 2004). Similarly, other networks such as the Wnt/β-catenin pathway that have been reported to cause androgen-independence through inappropriate activation of the AR (Verras et al., 2004) are undergoing early clinical evaluation. As it becomes increasingly evident that the AR and other steroid receptors remain activated in CRPC, the role of crosstalk should be considered inadequately explored in the clinic by single-agent testing of these agents—future trials must evaluate therapeutics targeting crosstalk pathways in patients in whom AR and other steroid receptor signaling has been truly abrogated.

Estrogen and Other Steroid Receptors

Evidence is emerging that steroid receptors other than the AR regulate prostate cancer cell proliferation and survival. In the prostate cancer cell line NCI-H660, transcription of *TMPRSS2:ERG* is modulated by functional ERα and ERβ in a yin-yang fashion (Setlur et al., 2008). Computational analysis of expression array data suggests a central role for ER-related pathways in prostate cancer. Furthermore, increased expression of ERα and decreased expression of ERβ has been associated with prostate cancer progression (Ellem and Risbridger, 2007). Estrogens are suppressed by abiraterone, and this could account for some of its antitumor activity. Similarly, response elements upstream of *TMPRSS2* can be activated by other steroid receptors, including vitamin D (Attard et al., 2008a). The role of these other steroid receptors and their variants in CRPC appear increasingly relevant. Although phase II clinical studies of the ER antagonist, tamoxifen, and aromatase inhibitors have reported no activity in CRPC, a role for these drugs may be found in combination with agents that target the AR. One important clinical trial could be an evaluation of whether aromatase inhibitors such as letrozole can reverse resistance to novel antiandrogens such as MDV3100.

Future Directions

We recently reported highly heterogeneous loss of *PTEN* and gain of *AR* in CTC, suggesting the existence of multiple different malignant clones that could have developed different mechanisms of resistance to castration in the same CRPC patient (Attard et al., 2009c). The intrapatient heterogeneity of *PTEN* loss and *AR* gain in CRPC was, however, associated with highly homogeneous *ERG* gene rearrangement status (Attard et al., 2009c). This supports our hypothesis that although multiple different mechanisms for inducing overexpression of ERG may exist in the same patient, an *ERG*-rearranged tumor does not change its underlying *ERG* gene status. Similarly, other hormone-regulated oncogenic mechanisms could persist throughout all stages of the disease. This introduces the possibility of overcoming resistance to hormone treatments by therapeutically targeting ETS gene fusions or their downstream targets. Importantly, this could achieve therapeutic benefit but avoid the inevitable toxicities of androgen deprivation. We have reported an association between the presence of an *ERG* gene rearrangement and magnitude of PSA decline and falls in CTC counts with abiraterone acetate (Attard et al., 2009c); we postulate that further molecular characterization of this disease using analytically validated, predictive biomarkers in a combination of tumor tissue and CTC could allow the molecular stratification of patients to ensure they are enrolled to trials of appropriately matched targeted drugs, preferably in drug combinations to simultaneously target molecularly heterogeneous clones. Ongoing phase III studies of abiraterone acetate and MDV-3100 are also evaluating whether a change in CTC count with treatment is a robust surrogate of survival, with the aim of validating a decline in CTC count as an intermediate endpoint for accelerating drug approval in future regulatory phase III trials.

In conclusion, clinical studies with improved inhibitors of AR signaling indicate that a significant proportion of CRPC finds AR signaling a ''hard habit to break.'' Further studies must now confirm that this disease commonly remains truly addicted to nuclear steroid receptor signaling. Key research questions for the identification of future therapeutic targets include:

- Is the oncogenic mechanism underlying steroid receptor activation entirely attributable to overexpression of hormone-regulated gene fusions?
- How does steroid receptor activation occur in patients whose cancers are resistant to drugs such as abiraterone and MDV-3100?
- Can prostate cancer lose its addiction to steroid receptor activation and yet remain driven by the same downstream targets?

Answers to these questions will inform the future development of therapeutics for CRPC. Efforts to develop strategies to overcome resistance to abiraterone and MDV-3100 must commence now, in advance of the conclusion of regulatory studies evaluating their use in the treatment of CRPC. Moreover, future investigations should also focus on developing predictive biomarkers that subdivide CRPC into distinct molecular entities to allow the conduct of trials and clinical use of targeted therapies in an enriched sensitive population. This is vital to accelerating anticancer drug approval and fixing the broken drug development pipeline for this disease.

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