Targeting the androgen receptor: improving outcomes for castration-resistant prostate cancer

Howard I Scher, Grant Buchanan1, William Gerald2, Lisa M Butler1 and Wayne D Tilley1

Abstract

The categorization of prostate cancers that are progressing after castration as ‘hormone-refractory’ evolved from the clinical observation that surgical or medical castration (i.e. androgen ablation therapy; AAT) is not curative and, despite an initial response, virtually all tumors eventually regrow. Successful AAT is contingent on the dependence of prostate cancer cells for androgen signaling through an intracellular mediator, the androgen receptor (AR) for survival. Current preclinical and clinical data imply that the AR is expressed and continues to mediate androgen signaling after failure of AAT. As AAT does not completely eliminate circulating androgens, sufficient concentrations of dihydrotestosterone may accumulate in tumor cells to maintain AR signaling, especially in the context of upregulated receptor levels or increased sensitivity of the AR for activation. In addition, ligands of non-testicular origin or ligand-independent activation can contribute to continued AR signaling. In many cases, therefore, from the perspective of the AR, a ‘hormone-refractory’ classification after failure of AAT is inappropriate. Classifying prostate tumors that progress after AAT as ‘castration-resistant’ may be more relevant. Clinical responses to second- and third-line hormonal therapies suggest that the mechanisms of AR activation are in part a function of previously administered AAT. Accordingly, the increasing trend to utilize AAT earlier in the course of the clinical disease may have a greater influence on the genotype and phenotype of the resistant tumor. In this article, we detail strategies to inhibit the growth of prostate cancer cells that specifically target the AR in addition to those practiced traditionally that indirectly target the receptor by reducing the amount of circulating ligand. We propose that treatment regimes combining AAT with direct AR targeting strategies may provide a more complete blockade of androgen signaling, thereby preventing or significantly delaying the emergence of treatment-resistant disease.

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Introduction

The demonstration that exogenous estrogens or surgical orchidectomy could produce tumor shrinkage in the advanced disease setting ushered in the era of hormonal management of prostate cancer in 1941 (Huggins & Hodges 1941, Huggins et al. 1941). Benefits to the patient included palliation of pain and relief of urinary symptoms, with a concomitant decline in acid phosphatase concentrations, consistent with the clinical findings. The success of hormone treatment, or more specifically of androgen ablation therapy (AAT), is contingent on the dependence of prostate cancer cells on the more potent 5α-reduced metabolite of testosterone, 5α-dihydrotestosterone (DHT), for their growth and survival. The traditional view of hormones and the prostate is therefore
focused on the ligand, and in particular on reducing or blocking the action of DHT (Fig. 1). DHT binds to and activates the androgen receptor (AR), which regulates transcription of a diverse range of target genes involved in prostate cell homeostasis, angiogenesis, differentiation and apoptosis (Buchanan et al. 2001, Gelmann 2002, Tilley et al. 2003).

In the modern era, the clinical use of AAT has been expanded to include medical therapies such as luteinizing hormone-releasing hormone (LHRH) agonists/antagonists or estrogens that target the hypothalamic–pituitary–gonadal axis (Fig. 1). Combined androgen blockade strategies utilize LHRH agonists/antagonists and AR antagonists to inhibit respectively the production of testicular androgens and the binding of residual androgens to the AR (Labrie et al. 1983). Enzymatic inhibitors of adrenal synthetic enzymes are used to block the production of adrenal androgens. The common feature of these approaches is that they target the receptor indirectly, by (i) reducing circulating concentrations of the native ligand (i.e. testicular androgens) either medically or surgically, or (ii) blocking the ability of androgens to bind to the AR using receptor antagonists. Although these strategies were originally used to treat patients with metastatic disease, clinical use of AAT expanded to the neoadjuvant (before primary treatment) and, more commonly, to the adjuvant (during primary treatment) setting in combination with surgery or radiation, in addition to conditions of increasing prostate specific antigen (PSA) concentrations (no detectable disease on an imaging study). However, irrespective of the nature and timing of AAT, overall outcomes are similar: an initial response, then a period of stability, followed by biochemical, radiographic and, ultimately, clinical progression. What is not clear, however, are the mechanisms contributing to the failure of AAT. In particular, whether renewed growth of prostate tumors is the result of maintenance of AR signaling in a castrate setting or of activation of AR-independent survival pathways, is a topic of considerable debate. In this review, we develop
the case for the persistence of AR-dependent signaling mechanisms after failure of AAT, suggest novel strategies using existing hormonal treatments for prostate cancer, and discuss new therapies that directly target the AR, which may be more effective than conventional androgen ablation.

**Evidence for maintenance of AR signaling after failure of AAT**

**Clinical studies**

Prostate cancers that progress despite castrate concentrations of testosterone in the blood have been categorized as ‘hormone-refractory’, implying that further hormonal treatments would be of limited clinical value. That PSA concentrations increase in virtually all cases of resistance to AAT argues against this categorization, because the increase in PSA is mediated through a specific androgen response element in the promoter of the PSA gene (Balk et al. 2003). Further evidence against a hormone-refractory categorization is the observation that more than 20–40% of prostate tumors that progress on AAT respond to second- and third-line hormonal treatments (Kojima et al. 2004). These therapies include anti-androgens, estrogens, progestational agents, inhibitors of adrenal steroid synthesis such as ketoconazole and glucocorticoids (Scher et al. 1995, Small 1997). The paradoxical responses to the discontinuation of anti-androgens, estrogens, glucocorticoids and progestational agents (Kelly & Scher 1993, Scher & Kelly 1993, Wirth & Froschmaier 1997), and disease flares that occur when exogenous androgens are administered (Fowler & Whitmore 1981, Manni et al. 1988), are additional illustrations of continued hormonal sensitivity despite failure of AAT.

Anti-androgen withdrawal responses have been documented in more than 30% of patients who received flutamide as part of a combined androgen blockade approach (Scher et al. 1995). Secondary clinical responses to bicalutamide observed in patients who have progressed on flutamide independent of a withdrawal response (Scher & Kolvenbag 1997), and the PSA response to nilutamide in patients with a previous anti-androgen withdrawal response, provide additional evidence of hormone sensitivity (Kassouf et al. 2003). A clinical example of secondary and tertiary responses to different androgen ablations is shown in Fig. 2. This patient was treated initially with a 6-month course of a gonadotropin-releasing hormone (GnRH) analog and bicalutamide,

![Figure 2](image)

**Figure 2** An example of a sequential hormonal response. A clinical example of sequential decreases in PSA concentration, associated with no progression in other sites such as bone, lymph nodes or viscera, and no new symptoms of disease, in a patient treated (i) with 6 months of a GnRH analog and bicalutamide (as indicated), and then (ii) a second course of combined blockade. At the time of progression, (iii) bicalutamide was discontinued with no response, following which (iv) nilutamide was added, producing a decline in PSA for more than 8 months. Later, an increase in PSA was noted, at which point (v) nilutamide was discontinued, with a tertiary response.
after which all treatment was discontinued. When the PSA increased, combined androgen ablation was reinstituted on a continuous basis until the PSA increased again and bicalutamide was discontinued. No response to bicalutamide discontinuation was observed, but the addition of nilutamide resulted in a decline in PSA concentrations for more than 8 months. Later, an increase in PSA was noted, at which time nilutamide was discontinued and the PSA declined again.

Immunohistochemical and other studies of clinical tumor samples have demonstrated that the AR is expressed in the majority of AAT-naive and -resistant tumors, and that the tissue concentrations of PSA and other androgen-responsive genes increase in the setting of castration-resistant tumor growth (Hobisch et al. 1995, Bentel & Tilley 1996, Culig et al. 1998). To investigate overall changes in gene expression during progression of clinical prostate cancer after androgen ablation, we undertook microarray gene profiling of both naïve and AAT-treated primary prostate cancers removed by radical prostatectomy, and castration-resistant metastatic tumors (Fig. 3) (Holzbeierlein et al. 2004). As expected, many of the genes with altered expression in primary tumors removed 3 months after initiation of androgen deprivation, and those in LNCaP cells after androgen manipulation, included known targets of the androgen receptor (e.g. KLK3, KLK2; Fig. 4a). Significantly, the gene expression profile of castration-resistant metastatic tumors is similar to that of hormone-naïve lesions, but quite distinct from that of primary tumors after...
short-term androgen ablation. Approximately 97% of the genes with altered expression after neoadjuvant AAT were not altered in castration-resistant tumors (e.g. KLK3, KLK2; Fig. 4a). The median level of AR expression was markedly increased (9–11-fold) in castration-resistant metastatic disease (Mann-Whitney U test; \( P = 0.028 \)) relative to untreated or neoadjuvant-treated primary tumors (Fig. 4a). Immunohistochemical analysis confirmed that AR RNA levels were concordant with the amount of receptor protein in individual tumor samples (Fig. 4b). These data strongly suggest that prostate tumors evolve mechanisms to reactivate AR expression and AR-responsive gene pathways after AAT, and that these changes have a key role in the development of resistance to hormonal treatment (Amler et al. 2000, LaTulippe et al. 2002, Holzbeierlein et al. 2004).

**Animal studies**

The CWR22 xenograft is an androgen-dependent tumor derived from a patient with metastatic prostate cancer grown subcutaneously in athymic nude mice (Pretlow et al. 1993, Wainstein et al. 1994, Nagabhushan et al. 1996, Tan et al. 1997). After androgen ablation, these tumors show regression, stability and later progression, similar to what is seen in the human condition. In most animals, castration-resistant CWR22 tumors emerge after 80–200 days after androgen withdrawal (Nagabhushan et al. 1996). A marked reduction in the expression of AR and markers of cellular proliferation is observed in CWR22 tumors two days post-castration (Agus et al. 1999).

However, subsequent proliferation during tumor regrowth is associated with re-expression of AR and androgen-regulated genes to levels comparable to those seen in tumors from intact mice (Gregory et al. 1998, Agus et al. 1999, Kim et al. 2002). Expression profiling of AAT-naïve and castration-resistant CWR22 tumors demonstrated that the expression of only a small proportion of genes (\(<5\%\)) was altered in the recurrent tumors (Amler et al. 2000). Collectively, these studies suggest that restoration of AR signaling pathways is associated with renewed growth of CWR22 tumors in a castrate environment. More recently, Chen and colleagues (2004), who compared the gene expression profiles of
isogenic androgen withdrawal-sensitive and -resistant xenograft tumors, demonstrated that, from seven human prostate cancer xenografts examined, the AR was the only gene consistently upregulated in castration-resistant tumors.

**Mechanisms of continued AR signaling during progression**

Studies of androgen-mediated signaling in animal models and human tumor specimens must be interpreted in the context of the point in the illness that the tumor sample represents. Different results will be obtained depending on the stage of disease, whether the sample was from the prostate or a particular metastatic site, whether the tumor has or has not been exposed to a specific form of androgen deprivation and whether it is proliferating (i.e. the disease is progressing) or non-proliferating (i.e. regressing or static). All these states are difficult to characterize, because human tumor samples often are not obtained in the course of routine medical management after diagnosis. Nevertheless, as discussed above, the findings of recent studies support the concept that AR signaling is maintained or upregulated in tumors that regrow after failure of AAT, and that the associated activation of androgen-regulated genes is sufficient to facilitate tumor survival. The specific alterations in prostate tumor cells that facilitate increased sensitivity of the AR signaling pathway can be considered at the level of the ligand and the receptor, the structure and function of the AR and its coregulators, or cross-talk with other signaling pathways (Fig. 5) (Tilley et al. 1996, Grossmann et al. 2001).

**Increased bioavailability of ligand**

Whereas testosterone and DHT concentrations in the blood are low in a patient whose tumor is progressing after castration, intratumor androgen concentrations may be sufficient to maintain tumor growth (Labrie et al. 1983, Geller et al. 1984a,b, Mohler et al. 2004). Tumor cells may acquire mechanisms to accumulate androgens, such as sequestration by steroid hormone binding globulin, which is synthesized and secreted by prostatic epithelial and stromal cells (Hryb et al. 2002), or by altered regulation of enzymes involved in the synthesis and metabolism of androgens. In support of this hypothesis, the comparative microarray analysis detailed above detected increased expression of enzymes in the steroid precursor synthesis pathway in castration-resistant tumors compared with that in castration-naive samples (Holzbeierlein et al. 2004). These enzymes included 3-hydroxy-3-methylglutaryl-coenzyme A synthase 1 and squalene epoxidase, which are considered to be rate-limiting enzymes in sterol biosynthesis (Chugh et al. 2003). Genes involved in fatty acid and steroid metabolism, which potentially could facilitate steroid production, were also upregulated. Recently, Mohler and coworkers (2004) measured the concentrations of AR and androgens in the tissues of locally recurrent prostate cancers after AAT. Similar concentrations of testosterone were detected in recurrent tumor samples and in control benign prostatic hyperplasia specimens. Moreover, whereas the concentrations of DHT, dehydroepiandrosterone and androstenedione were lower in recurrent prostate tumor tissues than in benign prostatic hyperplasia samples, there was a sufficient concentration of ligand to account for the expression of the AR-regulated PSA protein. These results agree that, despite androgen ablation, prostate tumors may never encounter a completely ‘androgen-independent’ environment (Mohler et al. 2004).

**AR expression**

Immunohistochemical studies demonstrate that the AR is expressed in essentially all human prostate cancers, including those that regrow after failure of AAT, and that the level of AR expression is at least retained, and often increased, relative to untreated tumors (e.g. Fig. 4) (Sadi & Barrack 1993, Pertschuk et al. 1994, Tilley et al. 1994, Takeda et al. 1996, Culig et al. 1998, Prins et al. 1998, Mohler et al. 2004). One mechanism for increased receptor concentrations is amplification of the AR gene, which has been reported in 22% of castration-resistant metastatic tumors, and in 23–28% of recurrent primary tumors (Bubendorf et al. 1999). AR gene amplification is associated with increased concentrations of the AR and AR-regulated proteins (Koivisto et al. 1996, 1997, Koivisto & Helin 1999, Linja et al. 2001). Only eight castration-resistant tumors of 28 examined (29%) in our independent studies exhibited amplification of the AR gene, whereas 26 of the 28 (93%) overexpressed the AR protein (Holzbeierlein et al. 2004). Increased concentrations of AR in prostate tumors could result from increased AR protein stability, as observed in recurrent CWR22 and LNCaP xenograft tumors (Gregory et al. 2001a), or from increased activation of the AR promoter (Jarrard et al. 1998, Gregory et al. 2001b, Takahashi et al. 2002). Irrespective of the mechanism, after castration the concentration of AR protein in prostate tumors appears to be sufficient to allow continued AR signaling, particularly if tumor tissues retain significant concentrations of ligand as discussed above. In support of this hypothesis, increasing the AR concentration in prostate cancer cells using an AR-expressing lentivirus reduced the latency period for the development of LNCaP and LAPC4 xenograft tumors in castrate mice (Chen et al. 2004). An additional consequence in those studies was that
increased expression of AR reversed the antagonist function of bicalutamide such that it acted as a weak AR agonist (Chen et al. 2004). The precise consequences of increased expression of AR are not known, but recruitment and inactivation of pro-apoptotic factors by the AR can impair cell cycle arrest and apoptosis of prostate cancer cells (Li et al. 2003), suggesting that indirect mechanisms may, in part, facilitate survival of prostate cancer cells with higher concentrations of AR. The direct effects of increased AR concentrations probably derive from altered transcription of AR-responsive genes expressing products that are involved in both steroid biosynthesis and cell cycle control, apoptosis and differentiation (Nelson et al. 2002, Holzbeierlein et al. 2004).

Structure and activation of the AR

The AR protein has three major functional domains: a large amino-terminal domain (NTD) that contains at least two activation functions, AF-1 and AF-5; a DNA-binding domain (DBD); and a carboxy-terminal ligand binding domain (LBD) that contains a highly conserved
ligand-dependent transactivation function (AF-2). More than 85% of mutations detected in the AR LBD in clinical prostate cancer (Gottlieb et al. 1999), in addition to those identified in cell lines and animal models, collocate to a small number of discrete regions of the receptor (Fig. 6a) (Buchanan et al. 2001a,b). In all, 86% of mutations in the LBD in prostate cancer and 72% of inactivating mutations in the AR identified in the inherited form of androgen insensitivity collocate to regions that collectively encompass only 10% and 11% of the AR coding sequence respectively (Fig. 6a). The regions of collocation in prostate cancer, with the exception of that encompassing amino acids 739–755, are distinct from those in androgen insensitivity and have been implicated in modulating the specificity of the ligand binding, cofactor responses and transactivation capacity of the receptor (Buchanan et al. 2000, 2001b). It is hypothesized that, given the appropriate hormonal environment, mutations in these regions of collocation in prostate tumors facilitate increased AR function, resulting in a survival advantage. Although the
Table 1 Mutations detected in activation function 5 of the androgen receptor in clinical prostate cancer

<table>
<thead>
<tr>
<th>Nucleotide change</th>
<th>Amino acid substitution</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCG → CTG</td>
<td>Pro389Leu</td>
<td>Taplin et al. 2001</td>
</tr>
<tr>
<td>CCT → CTT</td>
<td>Pro502Leu</td>
<td>Tilley et al. unpublished</td>
</tr>
<tr>
<td>CCC → TCC</td>
<td>Pro512Ser</td>
<td>Hytinen et al. 2002</td>
</tr>
<tr>
<td>AGT → GGT</td>
<td>Ser513Gly</td>
<td>Tilley et al. unpublished</td>
</tr>
<tr>
<td>ATG → GTG</td>
<td>Met521Val</td>
<td>Tilley et al. unpublished</td>
</tr>
<tr>
<td>GGC → AGC</td>
<td>Gly522Ser</td>
<td>Hytinen et al. 2002</td>
</tr>
<tr>
<td>GGC → GAC</td>
<td>Gly522Asp</td>
<td>Hytinen et al. 2002</td>
</tr>
<tr>
<td>TGG → TAG</td>
<td>Trp524STOP</td>
<td>Hytinen et al. 2002</td>
</tr>
<tr>
<td>GAT → GTG</td>
<td>Asp526Gly</td>
<td>Tilley et al. 1996</td>
</tr>
<tr>
<td>CCT → TCT</td>
<td>Pro531Ser</td>
<td>Hytinen et al. 2002</td>
</tr>
<tr>
<td>ATG → AGG</td>
<td>Met535Arg</td>
<td>Tilley et al. unpublished</td>
</tr>
<tr>
<td>ATG → GTG</td>
<td>Met535Val</td>
<td>Tilley et al. unpublished</td>
</tr>
</tbody>
</table>

*Numbering according to Tilley et al. 1989.

AR gene mutation collocation data are currently less compelling for the AR NTD than for the LBD, only a few studies have examined the coding sequence of the AR NTD for mutations (Fig. 6b). This is particularly relevant to resolving conflicting reports of the frequency of AR gene mutations in prostate cancer, as the findings of recent animal model and clinical studies suggest that surgical and medical castration result in the preferential accumulation of mutations in the AR NTD (Han et al. 2001, Hytinen et al. 2002). Studies by Hytinen and colleagues (2002) and our own unpublished work found that more than 50% of the AR gene mutations detected in cohorts of patients with prostate cancer receiving combined androgen blockade were located within a C-terminal 34 amino acid region (amino acids 502–535) of the AF-5 activation function in the NTD (Fig. 6b, Table 1). AR gene mutations that confer enhanced responsiveness to putative AR coregulators have also been identified in the AF-1 activation function (Han et al. 2001). One of these mutations (Glu231Gly), located in the highly conserved AR NTD signature sequence, is of particular interest, as enforced expression of the receptor variant in the mouse prostate confers rapid development of prostatic intraepithelial neoplasia that progresses to invasive and metastatic disease in 100% of mice (N. Greenberg, personal communication). In contrast, enforced expression of the wild-type AR has no observable effect on the prostate. The findings of that study highlight the potential functional significance of mutations in the AR NTD, and demonstrate that specific mutations can turn the AR into a potent oncogene sufficient to promote metastatic prostate cancer.

Level and function of AR coregulators

High-affinity ligand binding causes conformational changes to the AR that result in the recruitment of coregulator proteins that act to enhance (coactivators) or repress (corepressors) receptor function. The p160 coregulators interact with activation functions in both the LBD and NTD of the AR to enhance the transactivation capacity of the receptor in the presence of the native ligand, DHT. Amplification, overexpression, or both, of p160 cofactors (such as TIF2 and SRC1) has been reported in human prostate cancers after the failure of AAT, and in castrate-resistant CWR22 xenografts (Anzick et al. 1997, Gregory et al. 2001b). In clinical studies, the concentrations of key AR coregulators, including p300/CBP, have been shown to increase after AAT (Debes et al. 2003). Overexpression of these coactivators increases AR transactivation capacity in the presence of physiological concentrations of non-classical ligands such as estradiol, progesterone and adrenal androgens (Gregory et al. 2001b). In addition, Yeh and colleagues (1998, 1999a) have shown that specific AR coregulators, namely ARA54, ARA55 and ARA70, selectively enhance the ability of 17β-estradiol, hydroxyflutamide and androst-5-ene-3ß,17ß,diol, a precursor to testosterone, to activate the AR. Therefore, increased concentrations of AR coactivators in prostate tumors could contribute to continued AR signaling after androgen ablation, by sensitizing the receptor to lower concentrations of native androgens, or by altering the specificity of AR activation. A similar effect could be achieved by decreasing the concentrations of AR corepressors such as SMRT and NCoR, which inhibit AR function in a ligand-dependent manner (Cheng et al. 2002, Dotzlaw et al. 2002, Liao et al. 2002) and probably compete with p160 and other coactivators for the same interaction surfaces (G Buchanan & W Tilley, unpublished observations). In support of this hypothesis, NCoR was recently shown to mediate the antagonist action of bicalutamide, flutamide and mifepristone for the AR in prostate cancer cells (Berrevoets et al. 2004). These observations suggest that the ratio of critical AR coregulators is likely to be a key determinant of AR function in prostate cancer cells.

467
Ligand-independent activation of the AR

Studies using in vitro systems indicate that the AR can be activated in the absence of native ligand by growth factors (keratinocyte growth factor, insulin-like growth factor-I and epidermal growth factor), cytokines (interleukin-6) or protein kinase-A, or by overexpression of the tyrosine kinase receptor, HER2/neu (Culig et al. 1994, Nazareth & Weigel 1996, Craft et al. 1999, Yeh et al. 1999b, Grossmann et al. 2001, Ueda et al. 2002a,b, Gregory et al. 2004). Activation of the AR by these factors in the absence of ligand enhances transcription of PSA and other androgen-regulated genes (Yeh et al. 1999b), can enhance the proliferation of prostate cancer cells in vitro and in vivo (Lee et al. 2003) and increases tumor cell survival during androgen deprivation (Wen et al. 2000). These observations have led to the proposal that ligand-independent activation of the AR may facilitate continued prostate cancer growth after AAT, although more evidence that these signaling pathways are active in in vivo systems is required. In particular, the critical molecular events leading to activation of the AR in vivo by ligand-independent pathways in prostate cancer cells need to be defined precisely. For example, evolving data suggest that ligand-independent activation of the AR may result, in part, from modification of cofactors by phosphorylation (e.g. SRC1 by MAPK), which serves to create a more efficient platform for recruitment of the basal transcription complex (Rowan et al. 2000, Gregory et al. 2004). The recent findings discussed above suggest that the growth of prostate tumors after the failure of conventional AAT is not necessarily a result of the evolution of a growth state that circumvents the androgen-signaling axis, but rather is the result of increased sensitivity to activation or increased activity of the AR in tumor cells. This represents a paradigm shift in the accepted understanding of what is commonly called hormone-refractory prostate cancer, and extends the understanding of the molecular mechanisms involved in disease progression. As each of the emerging pathways to AAT-resistant prostate cancer detailed above is dependent on the presence of functional AR, targeting the receptor itself potentially offers a more effective approach to treatment of this disease.

Strategies for targeting the AR

Reducing AR concentrations

Various approaches, including double-stranded RNA interference (Caplen et al. 2002), antisense oligonucleotides (Eder et al. 2000, 2002), hammerhead ribozymes (Zegarra-Moro et al. 2002) and, more recently, the ansamycin antibiotic geldanamycin or its analog 17-allylamino-17-demethoxygeldanamycin (17-AAG) (Grenert et al. 1997, Prodromou et al. 1997, Stebbins et al. 1997, Solit et al. 2002) have been tested for their ability to reduce the concentrations of AR and suppress the proliferation of human prostate cancer cells both in vitro and in vivo (Solit et al. 2002, 2003). Phase II clinical trials of 17-AAG for the treatment of solid tumors are in progress. Although these approaches show promise as novel therapeutic agents for use in prostate cancer, all have the potential to disrupt androgen signaling in several tissues which, although not life-threatening, may cause debilitating side effects similar to those evoked by current hormonal treatments. In the case of 17-AAG, there is the added complication of specificity of action, as this agent will also suppress the functional maturation of other Hsp90 substrates, including Raf and HER2/neu, on which a range of cell types are dependent.

Inhibiting AR function

Based on AR variants deficient in autologous transactivation, dominant negative AR inhibitors provide a potentially effective approach to inhibition of endogenous AR function in prostate cancer cells (Palvimo et al. 1993). In a recent study, an AR inhibitor created by fusion of an AR deleted for the core region of AF-1 to the Kruppel-associated box transcriptional repressor domain was able to inhibit AR function significantly in human prostate cancer cells (Bramlett et al. 2001). However, as titration of cellular repressor complexes by the Kruppel-associated box transcriptional repressor domain may interfere with signaling by other pathways, and delivery of large expression constructs to cancer cells in vivo remains a significant challenge, the clinical usefulness of this particular AR inhibitor strategy is uncertain. In an alternative approach, we have generated small autologous human AR inhibitors, based on an AR inhibitor reported for the rat AR (Palvimo et al. 1993), by deletion of the majority of the AR NTD. These constructs have little or no intrinsic activity in the presence of androgen, but can inhibit more than 95% of the activity of wild-type AR and gain-of-function AR variants that are activated by either native androgens or non-classical ligands (L M Butler & W D Tilley, unpublished observations). These AR inhibitors therefore have the potential to block AR-dependent growth of prostate tumors irrespective of the level or structure of the receptor. Nonetheless, the success of this and other gene-based strategies depends in a large part on the development of suitable viral delivery approaches that specifically introduce AR inhibitor constructs into prostate cancer cells, or alternatively on the development of small-molecule inhibitors of AR function that are more amenable to therapeutic delivery and targeting.
Microinjection of a commercially available antibody to the AR has been shown to inhibit AR function (Zegarra-Moro et al. 2002). Notably, the antibody suppressed the proliferation of androgen-sensitive and castrate-resistant prostate cancer cells that express the AR, reduced PSA concentrations and caused morphological changes indicative of differentiation (Zegarra-Moro et al. 2002). Transfection of a ‘decoy’ double-stranded DNA fragment containing an androgen response element into LNCaP cells was able to compete with endogenous androgen response elements for AR binding, and induce apoptosis of the cells in the presence of DHT (Kuratsukuri et al. 1999). However, delivery of these types of agents to prostate cancer cells in vivo currently is not feasible. Recently, the histone deacetylase inhibitors suberoylanilide hydroxamic acid and phenylbutyrate, which inhibit the activity of chromatin remodeling enzymes recruited by AR coregulators such as NCoR and SMRT (Marks et al. 2001), have been shown to suppress the growth of prostate cancer cells in vitro and in vivo (Butler et al. 2000, Gore & Carducci 2000). These agents are currently being evaluated in a clinical setting for the treatment of various solid tumors, including prostate cancer (Carducci et al. 2001, Kelly et al. 2003).

Inhibition of the ligand-independent pathways leading to AR activation is a potentially viable alternative therapeutic strategy that may be best implemented in combination with strategies that target the AR directly. There are several inhibitors of the MAPK pathway currently undergoing clinical trial for treatment of cancer or inflammatory diseases (English & Cobb 2002), and specific inhibitors of JAK or Akt kinases are in advanced stages of preclinical development (Mills et al. 2003, Luo & Laaja 2004). In addition, agents such as the ansamycin antibiotics (discussed above) have the potential to target simultaneously both ligand-dependent and ligand-independent activities of AR by promoting degradation of both AR and HER2/neu (Solit et al. 2002).

### Selection pressures associated with AAT influence AR status

There is emerging evidence that different mechanisms facilitating continued signaling through the AR in tumor cells may depend on the specific therapy to which a tumor has been exposed during treatment of the disease. Given the often protracted natural history of prostate cancer, and that treatments, both local and systemic, alone or in combination, are being administered earlier in the clinical course, we are now potentially creating a new series of diseases as a result of this selection. We have termed this ‘therapy-mediated selection pressure’. This concept is exemplified by studies of the autochthonous transgenic adenocarcinoma of the mouse prostate (TRAMP) model of prostate cancer (Greenberg et al. 1995, Gingrich & Greenberg 1996, Gingrich et al. 1996, 1997), in which androgen ablation results in the selection of AR gene mutations in distinctly different regions of the receptor than in intact mice. In all, six of the eight AR gene mutations identified in tumors of intact TRAMP mice at 24–28 weeks of age were located in the AR LBD, whereas in mice castrated at 12 weeks, seven of seven AR gene mutations identified in their recurrent tumors at 24–28 weeks of age were located in the AR NTD (Han et al. 2001).


Taplin and colleagues (1999) reported that the AR from tumors of patients treated with hydroxyflutamide, as part of a combined androgen blockade strategy, harbored mutations that exhibited a marked increase in activity in response to hydroxyflutamide, but not to DHT or other androgenic ligands (Fenton et al. 1997). In contrast, AR gene mutations in tumors from patients treated with orchidectomy or bicalutamide, or both (Haapala et al. 2001, Taplin et al. 2003), are located in different regions of the receptor. Recent clinical studies by us and others have determined that the majority of mutations identified in patients after complete androgen blockade are located in a discrete region of the AR NTD (see above, Structure and activation of the AR). These findings are consistent with specific alterations in AR signaling being selected for by different hormonal treatments.

In an attempt to minimize the potential detrimental effects of continuous AAT, we have begun pharmacological repletion of testosterone in a rapid hormonal cycling strategy. We hypothesize that this strategy will limit tumor regrowth between cycles while maintaining the sensitivity of prostate cancer cells to subsequent androgen withdrawal, resulting in a net decrease in tumor mass with each successive cycle. A 3:1 ratio of depletion and repletion is used. Conceptually, the approach mimics the female menstrual cycle, but does not allow for the equivalent of
a prolonged luteal phase to restore the endometrial lining to a fully functioning level. A representative case is illustrated in Fig. 7, showing successive declining peaks and troughs in serum PSA concentrations after administration of testosterone to a patient undergoing intermittent AAT. The effect of testosterone repletion on chemosensitivity is currently under study in a trial in which chemotherapy is given after a 7-day course of testosterone, following which the patches are withdrawn for a 3-week period and cycles are repeated.

**Implications**

To date, the general approach to the hormonal management of prostate cancer has been to administer AATs that predominantly target AR signaling by reducing circulating concentrations of ligand on a continuous basis, to observe the effect, and to administer an additional therapy when the first is no longer effective (i.e. when the tumor is regrowing). This scenario is illustrated by the CWR22 xenograft model of prostate cancer in Fig. 8. Second-line approaches, be they additional hormone treatments or cytotoxic drugs, are used after tumor progression on first-line therapy. The results of AAT studies with CWR22 prostate cancer xenografts led us to propose a functional time-course classification of the disease with potential points of therapeutic attack (Fig. 8). Alternative therapeutic approaches should be directed toward targeting the AR in addition to targeting the ligand, with the aim of completely inhibiting AR signaling, both in non-proliferating or dormant tumor cells (Fig. 8, points i, ii) and in proliferating cells (Fig. 8, point iii), thereby preventing or delaying the emergence of truly hormone-refractory disease. Until AR function can be completely abrogated, it will not be possible to say conclusively whether regrowth of prostate tumors after failure of AAT is strictly dependent on continued AR signaling, or is initiated by non-AR-dependent growth regulatory pathways.

On the basis of current evidence implicating the AR as a key factor in maintaining the growth of prostate cancer cells in an androgen-depleted state, receptor-targeted treatments should effectively eliminate AR-dependent prostate cancer cells. Moreover, different androgen-ablation and receptor-targeting therapies could be applied simultaneously to enhance the proportion of cells undergoing apoptotic cell death, or in a cyclical manner to limit selection of clones with a specific adaptation in the
androgen signaling pathway, thereby countering enhanced sensitivity of AR signaling and preventing tumor survival. The challenge, however, is to develop targeted strategies that completely knock out the AR in clinical prostate cancer without affecting other androgen target tissues. These approaches could improve quality of life, especially for those patients being treated earlier in the course of disease with AAT and who face the prospect of significant side effects, including bone and muscle loss, loss of libido and a range of psychosocial effects resulting from prolonged androgen ablation. Complete abrogation of AR signaling by application of both AAT and AR-targeted therapies at a time of low-volume disease before selection of tumor cells that can survive and grow in a castrate environment may afford the best opportunity to advance the management of prostate cancer since the concept of androgen ablation was introduced by Huggins, Stevens and Hodges more than 60 years ago (Huggins et al. 1941).

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Figure 8 Timing and points of intervention. A functional time-course classification for combining different approaches with AAT (CASTRATION), based on changes in gene expression and regulatory proteins in CWR22 xenografts. Potential points of therapeutic attack: (i) non-proliferating tumor cells; (ii) dormant tumor cells; (iii) proliferating cells. Modified from Scher (2003).


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