

Androgen Deprivation Therapy for Prostate Cancer: Current Status and Future Prospects

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Androgens play a major role in promoting the development and progression of prostate cancer. As a result, androgen ablation or blockade of androgen action through the androgen receptor (AR) has been the cornerstone of treatment of advanced prostate cancer. Different strategies involving this hormonal therapy produce a significant clinical response in most of the patients, but most responders eventually lose dependency, resulting in mortality. Thus, whether hormonal therapy contributes to the improvement of overall survival rates, especially in patients with advanced prostate cancer, remains controversial. However, patients with advanced disease clearly have a benefit from androgen deprivation-based treatment for palliating their symptoms and for improving the quality of their lives. In order to improve overall survival, novel treatment strategies that prolong the androgen-dependent state and that are useful for androgen-independent disease based on specific molecular mechanisms need to be identified. © 2004 Wiley-Liss, Inc.

KEY WORDS: androgen receptor; androgen receptor coregulator; antiandrogen; androgen-independent prostate cancer; antiandrogen withdrawal syndrome

INTRODUCTION

The role and mechanism of androgen function have been studied in a variety of androgen target organs, including the prostate. As is the case with normal prostate development, the growth of prostatic neoplasms is generally dependent on androgens, especially on 5 α -dihydrotestosterone (DHT). Men castrated when young (eunuchs) or men with inherited deficiency of 5 α -reductase (type 2) do not develop prostate cancer. Since the first observation by Huggins and Hodges in 1941 [1], hormonal therapy remains the critical therapeutic option for advanced forms of prostate cancer. Multiple strategies have been used to reduce serum levels of androgens or interfere with their function via the androgen receptor (AR) (Fig. 1). However, the appropriate choice/timing and actual benefits of hormonal therapy in various situations still remain controversial. Indeed, prostate cancer is the second leading cause of cancer-related death among men in the United States [2]. This article reviews clinical and molecular evidence supporting current strategies of hormonal therapy and their controversies, and the

future prospects of prostate cancer treatment involving androgens and the AR.

ANDROGENS, THE AR, AND PROSTATE CANCER DEVELOPMENT

The AR is a member of the nuclear receptor superfamily, members of which function as ligand-inducible transcription factors that regulate the expression of target genes to mediate the transcriptional response to ligands in target cells [3,4]. These receptors include sex steroids (androgens, estrogens, and progestins), adrenal steroids (glucocorticoids and mineralcorticoids),

Grant sponsor: NIH; Grant sponsor: George Whipple Professor Endowment.

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Received 8 January 2004; Accepted 15 January 2004
DOI 10.1002/pros.20115

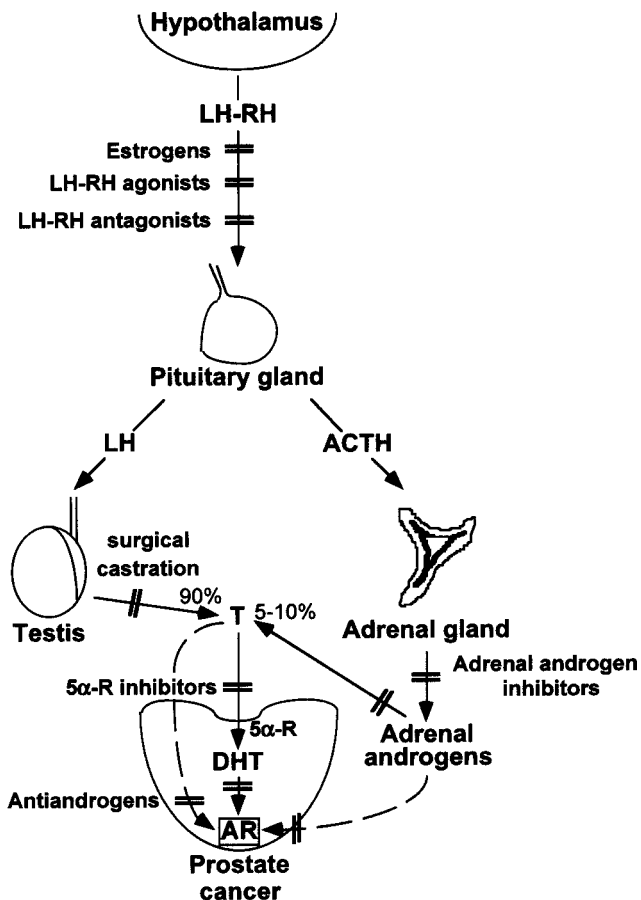


Fig. 1. Strategies for hormonal therapy. LH-RH, luteinizing hormone-releasing hormone; LH, luteinizing hormone; ACTH, adrenocorticotropic hormone; T, testosterone; 5 α -R, 5 α -reductase; DHT, 5 α -dihydrotestosterone; AR, androgen receptor.

retinoids, vitamin D, thyroid hormones, and fatty acids [3,5–7]. There is also a large group of orphan receptors, each of which is activated by an undetermined ligand or is active without ligand stimulation [8–10]. The AR, as well as other members of this family of nuclear receptors, contains four functional domains: the NH₂-terminal transactivation domain (A/B domain), the DNA-binding domain (DBD) (C domain), a hinge region (D domain), and the ligand-binding domain (LBD) (E domain). Deletion and mutational analyses of nuclear receptors in transfection experiments have identified two transcriptional functions: a ligand-independent NH₂-terminal activation function (AF-1), and a ligand-dependent AF-2 function located in the LBD [11–14]. Recent studies have also revealed that steroid hormone receptors modulate transcription by recruitment of coregulators that influence a number of functional properties of the receptors, including ligand selectivity and DNA binding capacity [15–18].

Testosterone (T) is secreted by Leydig cells in the testis and is the major sex hormone circulating within

the blood of males. In a variety of androgen-sensitive tissues like the prostate, T is irreversibly converted by 5 α -reductases to the more potent androgen, DHT, which has a higher affinity for the AR [4,19]. Besides T and DHT, several precursors of T, dehydroepiandrosterone (DHEA), its sulfate, Δ^4 -androstenedione, and Δ^5 -androstenediol, are classified as belonging to the adrenal androgen group. These can weakly stimulate the AR through their conversion to T/DHT in peripheral tissues, including the prostate, or by directly binding to the AR [20–23]. Upon binding of androgens, the androgen–AR complexes form homodimers, which constitute the active form of the transcription factor. The homodimers translocate into the nucleus and bind to androgen responsive elements (AREs) located on target genes, inducing transcription of genes for proteins such as prostate-specific antigen (PSA), which is clinically used for the detection and monitoring of prostate cancer recurrence and progression [24]. In this process, an interaction between the NH₂ and COOH terminals of the AR has been shown to be important in stabilizing bound ligand [14,25]. This NH₂/COOH interaction is facilitated by several AR coactivators, such as CBP, SRC-1, and ARA70 [18,26].

Although serum androgens alone do not significantly promote prostate carcinogenesis, androgen action and the functional status of the AR are believed to be important mediators of prostate cancer development. Indeed, men castrated when young do not develop prostate cancer. However, precise mechanisms by which androgens affect this process are largely unknown. Some epidemiological studies suggest that environmental factors, including exogenous hormones, are major risk determinants for prostate cancer, by driving cell proliferation leading to the accumulation of random genetic errors [27], but other studies have failed to establish a link between elevated serum androgens and prostate cancer risk [28]. Androgens have also been shown to enhance the carcinogenic activity of genotoxic carcinogens via AR-mediated mechanisms [26]. It is known that androgens regulate proliferation, differentiation, and survival of prostatic epithelial cells, although some of these functions are also controlled by a number of membrane receptors, such as the epidermal growth factor (EGF) receptor [29]. Superphysiological levels of serum androgens in rats and in humans who habitually use anabolic steroids increase cellular proliferation in the prostate [30,31]. Castration of adult male rats leads to a loss of 70% of the prostate's secretory epithelial cells due to apoptosis [32]. Although vascular endothelial cells of the prostate do not express the AR [33], castration also induces apoptosis and degeneration of prostatic capillaries and constriction of larger blood vessels that actually precedes the appearance of apoptosis in

prostatic epithelial cells [34]. Since androgens can regulate a subset of paracrine growth factors and thereby influence vascular survival [35,36], it is possible that castration initially changes growth factor production by prostatic stromal cells, resulting in a decreased vascular supply. Then, the resulting reduction of blood flow, combined with decreased expression of other androgen regulated proteins, might contribute to apoptosis of the secretory epithelia. Thus, prostatic cells have a wide range of responses to androgens, and the properties of the AR might play important roles in both the maintenance and homeostasis of normal prostatic epithelia, and in prostate cancer development.

STRATEGIES OF ANDROGEN DEPRIVATION

Multiple approaches at androgen deprivation have been used for the treatment of prostate cancer (see Fig. 1). The agents and strategies used for androgen deprivation therapies are listed in Tables I and II, respectively.

Surgical Castration

The classical form of androgen deprivation is surgical castration by bilateral orchiectomy. Surgical castration is the most immediate method to reduce

circulating T by >90% within 24 hr [37], and there is no risk of a paradoxical flare of the disease. Since 1960 the Veterans Administration Co-operative Urological Research Group (VACURG) trials, large-scale randomized studies regarding the treatment of prostate cancer, demonstrated the clinical effectiveness of surgical castration [38–40]. Compared to placebo, orchiectomy was more likely to decrease pain and improve performance status in patients with advanced disease. However, none of the studies showed a significant survival advantage for castration over placebo. Recent clinical studies are discussed later (i.e., surgical vs. chemical castration). Although surgical castration may be underused, some studies suggest that many patients prefer this approach for the reasons of convenience and cost [41]. On the other hand, other studies suggest that this treatment approach is unacceptable to many patients, causing considerable psychological problems, with libido and erectile function irreversibly impaired in most cases [42,43].

Medical Castration

Diethylstilbestrol (DES). In the 1940’s, the first reversible medical castration method was achieved by

TABLE I. Hormonal Agents for the Treatment of Prostate Cancer

Class	Mechanism/action	Major side effects
Estrogens		
Diethylstilbestrol	Suppresses LH-RH secretion, ↓LH, ↓T	Cardiovascular events, loss of libido, impotence
LH-RH agonists		
Leuprolide Goserelin	Suppress LH-RH secretion, ↓LH, ↓T	“Flare” phenomenon, hot flashes, loss of libido, impotence
LH-RH antagonists		
Abarelix	Antagonizes LH-RH receptor, ↓LH, ↓T	Histamine release, loss of libido, impotence, hot flashes
Steroidal antiandrogens		
Cyproterone acetate Megestrol acetate	Antagonize AR in target tissues, suppress LH-RH secretion, ↓LH, ↓T	Cardiovascular events, fluid retention, gynecomastia, impotence
Non-steroidal antiandrogens		
Flutamide Nilutamide Bicalutamide	Antagonize AR in target tissues, ↑T	Gynecomastia, hepatotoxicity (flutamide), visual and respiratory disturbances and alcohol intolerance (nilutamide), GI problems
5α-reductase inhibitors		
Finasteride Benzoquinoline	Inhibits type II 5α-reductase, ↑T, ↓DHT Inhibits types I/II 5α-reductase, ↑T, ↓DHT	Decreased libido, impotence
Adrenal androgen inhibitors		
Corticosteroids Ketoconazole	Suppresses ACTH secretion Inhibits P450 hydroxylase for adrenal and testicular steroidogenesis, ↓T	Mood changes Adrenal insufficiency, fatigue, GI problems

LH-RH, luteinizing hormone-releasing hormone; LH, luteinizing hormone; T, testosterone; AR, androgen receptor; GI, gastrointestinal; DHT, 5α-dihydrotestosterone; ACTH, adrenocorticotrophic hormone.

TABLE II. Treatment Options as Hormonal Therapy for Prostate Cancer

Modality	Methodology	Advantages	Disadvantages
Surgical castration	Bilateral orchiectomy	Rapid ablation of testicular T Relative simple procedure Lower cost	Definitive castration Associated psychological problems Irreversible loss of libido and sexual potency Reduced muscle mass and energy Hot flashes Anemia and osteoporosis Unaffected adrenal androgens
Medical castration	Estrogens LH-RH agonists LH-RH antagonists	Reversible castration Ablation of testicular T More acceptable than orchiectomy	Cardiovascular events (estrogens) "Flare" phenomenon (LH-RH agonists) Loss of libido and sexual potency Reduced muscle mass and energy Hot flashes Anemia and osteoporosis Unaffected adrenal androgens
CAB	Castration + antiandrogen	Ablation of testicular T + competitive inhibition of adrenal androgens	Increased side effects Antiandrogen withdrawal response
Antiandrogen monotherapy	Non-steroidal antiandrogen Steroidal antiandrogen	Competitive inhibition of both testicular and adrenal androgens Retaining sexual potency Less severe side effects Oral administration only CAB effect Oral administration only	Gynecomastia Less effective (?) Cardiovascular events Side effects due to lowering of serum T Gynecomastia
IAB	Intermittent hormonal therapy	Longer androgen-sensitive period (?) Reduced side effects and costs	Investigational may achieve continuous androgen ablation
TrAB	Intermittent CAB + 5 α R inhibitor	Superior to IAB (?)	Investigational
SAB	5 α R inhibitor + antiandrogen or LH-RH agonist	Superior to monotherapy (?)	Investigational

T, testosterone; LH-RH, luteinizing hormone-releasing hormone; CAB, complete androgen blockade; IAB, intermittent androgen blockade; TrAB, triple androgen blockade; 5 α R, 5 α -reductase; SAB, sequential androgen blockade.

administration of DES, a semi-synthetic estrogen compound [1]. Initial studies by the VACURG identified equivalent overall and superior disease specific survival rate in the DES group (5 mg/day) to the orchiectomy group, but non-cancer-related deaths, most of which were cardiovascular events, were noted [38,39]. Subsequent trials have shown the equivalence of DES at 3 mg/day and other treatment options in overall survival rates [44–48]. However, cardiovascular toxicity with events including myocardial infarction, deep vein thrombosis, edema, and transient ischemic attack occurred in 8–33% of patients.

Gynecomastia was also significantly observed in patients with 3 mg/day DES. In order to reduce the risk of cardiovascular side effects, low-dose of DES (1 mg/day) was evaluated [49–51]. Although it is still controversial, many question whether DES at 1 mg/day is as effective and safe as other treatment options. As a result, DES is only rarely used as first-line hormonal treatment in North America. There are also several studies evaluating the efficacy of DES as a salvage therapy after failure of first-line androgen deprivation. Recent reports have evaluated using 1–3 mg DES/day with or without antithrombotic agents

including warfarin and aspirin [52,53]. Response rates by PSA measurement were 43–79% with median durations of progression of 6–7.5 months, and with 0–28% cardiovascular events.

It was generally believed that the primary mechanism of action of DES was to decrease androgen levels through hypothalamic–pituitary suppression, but recent evidence indicates that the mechanism is probably more complex. Kitahara et al. [54] reported stronger suppression of T and follicle stimulating hormone (FSH) by DES than by surgical castration or other means of chemical castration, such as the administration of a luteinizing hormone-releasing hormone (LH-RH) agonist. The same group also suggested that DES might reduce serum DHEA sulfate, an androgen derived from the adrenal gland [55]. A direct cytotoxic effect of estrogens has also been suggested in prostate cancer *in vitro*, presumably through both estrogen receptor (ER)-dependent and ER-independent pathways [56–58]. This is consistent with the finding that phytoestrogens, which share estrogenic steroidal structures and are found in a variety of plant foods, have preventive effects for prostate cancer and inhibit prostate cancer cell proliferation [59]. Indeed, ER β has been detected in human prostate cancer cell lines, including LNCaP, PC-3, and DU145, and in normal and malignant prostate tissues, whereas ER α is expressed in PC-3 cells and in stromal (not epithelial) cells of the prostate [59–61]. Moreover, some studies suggest that loss of ER β in prostate cancer tissues is associated with tumor progression [61,62]. These findings might be able to explain the evidence that administration of DES could be more effective than other androgen ablation in suppressing prostate cancer growth if unfavorable side effects of DES are not considered [40]. On the other hand, we previously showed that a natural estrogen, 17 β -estradiol (E2), but not DES, promoted transcriptional activity of the AR in prostate cancer cells [63].

LH-RH agonists and antagonists. The development of LH-RH analogues, obtaining medical castration with significantly fewer cardiovascular events and lack of gynecomastia, has led to a dramatic change in the treatment of advanced prostate cancer [64]. In the United States, two LH-RH agonists are commercially available: leuprolide acetate and goserelin acetate.

LH-RH is generally secreted by the hypothalamus in pulses, leading to pulsatile secretion of LH and FSH by the pituitary. This in turn promotes T secretion by Leydig cells of the testes. However, constantly high levels of LH-RH that occur with agonist administration down-regulate the receptors in the pituitary, inhibit LH secretion, and thereby reduce T production. In addition, a direct inhibitory effect of LH-RH via LH-RH

receptors in prostate cancer cells has been suggested [65,66].

The equivalence of surgical castration and LH-RH agonist administration has been widely demonstrated [67,68]. Recently, depot LH-RH agonist preparations have been developed, which last 3–4 months and have the same efficacy as classical preparations [69]. The depot preparations have now become the most widely used form of androgen deprivation. Side effects of LH-RH agonists include hot flashes, loss of libido, and osteoporosis [70]. In addition, LH-RH agonists often cause an initial surge of LH release, with a corresponding increase in serum T lasting 1–2 weeks. This surge may stimulate prostate cancer growth with a worsening of related symptoms, which is known as the “flare” phenomenon [71]. In order to prevent this phenomenon, administration of an antiandrogen or estrogen for a week before and during the first few weeks of LH-RH agonist therapy is often employed [72].

LH-RH receptor antagonists recently have been developed for androgen deprivation. Since abarelix, a peptide antagonist, directly inhibits the binding of LH-RH to its receptor, but does not stimulate the LH-RH receptor, no initial flare phenomenon is observed after administration [73,74]. Recent clinical studies have demonstrated that abarelix monotherapy achieves medical castration and a reduction of serum PSA levels to the same extent achieved by LH-RH agonists [75–77]. However, long-term follow-up studies are necessary to determine whether LH-RH antagonists can be routinely used for advanced prostate cancer.

Combined Androgen Blockade (CAB)

Surgical or medical castration results in no or marginal decline of adrenal androgens that are likely to possess intrinsic androgenic activity [22,23,78]. Thus, men who undergo castration still have relatively high levels (up to 40%) of DHT and 5–10% of T, presumably derived from adrenal precursors [20,79]. This alternate source of DHT and T, in addition to adrenal androgens themselves, might explain the progression of most prostate cancers despite castration and positive clinical responses of adrenal ablation after failure from primary androgen deprivation. Indeed, Huggins and Scott [80] demonstrated that prostate cancer patients that progressed after initial successful treatment with androgen deprivation occasionally responded to bilateral adrenalectomy. The basis of CAB (also called maximal androgen blockade [MAB]) is to neutralize both testicular and adrenal sources of androgens. CAB consists of surgical castration or a LH-RH agonist combined with a non-steroidal antiandrogen. Antiandrogens include a number of compounds that competitively inhibit

binding of testicular and adrenal androgens by the AR in the target cell, which ultimately prevents the activation of AR pathways in those cells. CAB has been advocated as the most effective hormonal treatment for patients with advanced prostate cancer. However, this approach implies increased side effects (primarily of the antiandrogens, which can cause diarrhea and hepatic dysfunction) and cost, and the survival benefit achieved with CAB over castration is still debated [81,82].

Since 1989, several randomized trials have suggested that CAB significantly prolongs survival of the patients with advanced prostate cancer, compared to castration alone [83–86]. In 1998, however, Eisenberger et al. [87] reported a randomized trial of 1,387 patients with metastatic prostate cancer who were all treated with surgical castration with placebo or flutamide. There was no statistically significant survival advantage for CAB when using flutamide. Several hypotheses were proposed to explain the discrepancy between the results of this study and earlier reports. First, patients in this study might have had less aggressive disease. Second, castration with LH-RH agonists, especially daily injections in the first study [83], might not have been as complete as surgical castration. Third, the LH-RH agonist plus placebo group in the first study [83] may have experienced initial LH-RH agonist flare leading to worsening the disease. In 2000, the Prostate Cancer Trialists' Collaborative Group [82] performed a meta-analysis of 27 trials of CAB with steroidal or non-steroidal antiandrogen versus castration monotherapy involving 8,275 patients with advanced prostate cancer. The difference in the 5-year survival rate was not statistically significant (25.4% with CAB vs. 23.6% with castration alone). However, a statistically significant difference ($P < 0.02$) in favor of castration plus a non-steroidal antiandrogen was observed. More recently, another meta-analysis of 20 trials concluded that there was a 5% improvement in survival at 5 years (30% vs. 25%) with CAB [88]. However, only 7 of the 20 studies might be considered as high-quality trials and there was no significant improvement with CAB from these 7 studies. In summary, recent data show that CAB provides a minimal advantage (up to 5% improvement in 5-year survival) over castration monotherapy, which suggests that CAB may not be the first choice of hormonal therapy for advanced prostate cancer. However, to prevent possible symptoms of the flare, it is generally recommended to use an antiandrogen before and during the first several weeks of LH-RH agonist therapy.

Antiandrogen Monotherapy

Antiandrogens are generally used in combination with castration as CAB. However, castration based

approaches are usually associated with side effects, which have a negative impact on quality of life (QOL). There is increasing usage of antiandrogens as monotherapy, which can preserve gonadal function and, therefore, provide potential QOL benefits, particularly in terms of potency, libido, and bone demineralization. Indeed, most antiandrogens increase serum levels of androgens within normal physiologic range due to the suppression of the pituitary feed-back [89]. There are two types of antiandrogens, steroidal, such as cyproterone acetate (CPA) and megestrol acetate, and non-steroidal, such as flutamide, nilutamide, and bicalutamide.

CPA. CPA is a progestational antiandrogen and the first antiandrogen used for the treatment of advanced prostate cancer in Europe. It competes with androgens for the binding to the AR, as well as possesses antigonadotropic activity that results in a rapid and sustained 70–80% decrease in T levels [90]. Therefore, CPA, as a single agent, may yield CAB. In clinical studies, there were no significant differences in tumor response rates or disease specific survival between CPA and any other forms of androgen deprivation, such as surgical castration, estrogens, LH-RH agonists, and non-steroidal antiandrogens [91,92]. Unfortunately, CPA induces severe cardiovascular complications in about 10% of patients, which is dose-dependent, but lower than those of DES (up to 33%) [45]. Other complications include gynecomastia, loss of libido, erectile dysfunction, and central nervous system effects such as headache, fatigue, and weakness that are possibly attributable to the lowering of serum T levels. Therefore, the use of CPA as monotherapy might be limited to those who find surgical castration unacceptable. In addition, CPA can be used to block LH-RH induced flare reactions and to suppress surgical or medical castration-related hot flashes [90,91].

Flutamide. Flutamide was probably the first non-steroidal antiandrogen that was widely used as a component of CAB. However, the use of flutamide monotherapy for advanced prostate cancer has not been extensively studied in phase III trials [92]. Delaere and Van Thillo summarized initial open studies reporting the clinical efficacy of flutamide as monotherapy, along with their own results [92,93]. Among nearly 500 previously untreated patients with advanced prostate cancer, 68% showed a partial response. But most studies were relatively small, and there seemed to be differences in the criteria of response. Several trials have compared the efficacy of flutamide as monotherapy with that of DES, orchiectomy, or CAB. Boccardo [81] reviewed these studies and found no significant differences in response rates/duration among these

groups. In a double-blind randomized study to compare the efficacy of flutamide with DES at 3 mg/day [48], DES produced significantly longer overall survival than flutamide (43.2 months vs. 28.5 months). Hepatotoxicity, asymptomatic elevations in aminotransferases, diarrhea, and gynecomastia during treatment with flutamide were noted [94–96], and, thus, the rate of treatment withdrawal for any drug-related adverse events was highest with flutamide among three non-steroidal antiandrogens [92]. In order to reduce the incidence of flutamide-induced breast pain and enlargement, radiotherapy to the breast, an antiestrogen tamoxifen, and an aromatase inhibitor amastrozole have been being evaluated [97]. There have been no comparative studies of the efficacy of different non-steroidal antiandrogens as monotherapy.

Nilutamide. No randomized studies of monotherapy with nilutamide or comparative studies with any other hormonal therapy have been reported. One small study (26 patients) evaluated the efficacy of nilutamide as monotherapy, finding that 21 (91%) of the 23 evaluable previously untreated patients with metastatic prostate cancer had a response, with a median duration of overall survival of 23 months [98]. Although nilutamide as monotherapy demonstrated antitumor activity, the survival rate in this study might be less than that achieved by CAB with nilutamide [98]. In addition, a high incidence (31%) of visual problems (adverse light-dark adaptation) was observed in the 26 patients [99]. Other unique adverse effects of nilutamide, when used as a component of CAB, include alcohol intolerance and respiratory disturbance (rarely interstitial pneumonitis) [96,99]. Nilutamide has been reported to cause a higher incidence of nausea and vomiting than the other non-steroidal antiandrogens, whereas the incidence of diarrhea and gynecomastia is lower with nilutamide than flutamide [92,99]. These results may discourage conducting larger trials with nilutamide monotherapy.

Bicalutamide. Of available non-steroidal antiandrogens, bicalutamide as monotherapy has been most extensively studied. Early clinical studies showed the effectiveness of bicalutamide monotherapy at daily doses of 30 mg, with increasing efficacy up to 200 mg [100]. Early comparative trials using bicalutamide at 50 mg/day showed castration was superior to bicalutamide monotherapy, in terms of survival rate in patients with metastatic disease [101]. However, subsequent trials with bicalutamide at 100 or 150 mg/day have revealed equivalent efficacy between bicalutamide monotherapy and surgical or medical castration [102,103]. Other comparative studies also showed no statistically significant differences in survival between

bicalutamide at 150 mg/day monotherapy and CAB (castration with flutamide or nilutamide) with better tolerability in the bicalutamide monotherapy group [104,105]. Bicalutamide at 150 mg/day has been shown to have a more favorable side effect profile than flutamide and nilutamide [92], although there was still a high risk of gynecomastia and breast pain. As with flutamide, bicalutamide gynecomastia can be prevented by short-course, prophylactic, external beam radiation [100]. Since bicalutamide has a longer elimination half-life of approximately 6 days than flutamide (6 hr), it can be given once daily (vs. flutamide dosed three-times daily) [106,107]. The most recent and largest trials of 8,113 patients confirmed these observations (clinical efficacy, QOL benefit, and tolerability) [108]. Thus, bicalutamide at 150 mg/day is thought to be an appropriate dosage, and this treatment, either alone, referred to as peripheral androgen blockade (PAB), or as adjuvant therapy, could be a standard option in patients with localized or advanced prostate cancer.

Intermittent Androgen Deprivation

Intermittent androgen blockade (IAB) aims at delaying the onset of androgen-independent growth of prostate cancer, as well as reducing side effects and costs. Laboratory studies support the hypothesis that IAB prolongs the initial androgen-sensitive period. Langelier et al. [109] showed that intermittent androgen suppression could delay the emergence of androgen-independent clones induced in androgen-sensitive prostate cancer cell line LNCaP after long-term culture with androgen deprivation. Akakura et al. [110] and Sato et al. [111] confirmed this finding using castrated animals bearing androgen-dependent tumors with intermittent exposure to androgens, suggesting that IAB can induce multiple apoptotic regressions of androgen-dependent prostate cancer and that both tumor progression and effects of the treatment can be monitored by PSA measurement.

In 1986, Klotz et al. [112] reported a first attempt to treat 20 patients with advanced prostate cancer with intermittent hormonal therapy (DES in 19 cases and flutamide in 1 case). These patients were treated until a clinical response was demonstrated, with a mean initial treatment duration of 10 months (range 2–70 months). The treatment was then stopped, and re-started when tumors relapsed, with mean interval times of 8 months (range 1–24 months). All relapsed patients responded to re-administration of the drug. Patients had better QOL during the break in the treatment and DES-induced erectile dysfunction recovered in 9 of 10 patients within 3 months of treatment interruption.

Since medical castration by LH-RH agonists and serial serum PSA measurements became available after

mid-1980s, it became easier to introduce IAB and to determine the relapse of the tumors earlier. Several clinical studies of IAB have been reported [113–116]. These intermittent hormonal therapies consist of an initial androgen deprivation period by an LH-RH agonist with or without a non-steroidal antiandrogen of usually between 6 and 9 months, followed by an off-therapy interval (6–15 months). When PSA values meet threshold criteria (>5 – 10 ng/ml), treatment is re-started. Most of the initial responders (57–100%) respond to re-treatment. This cyclic treatment continues until the patient develops androgen-independent tumors. While off-treatment, many patients had improvement in their libidos, erections, hot flashes, and energies. Retrospective comparison of survival in these patients was similar to those who were treated with continuous androgen blockade. Interestingly, in certain patients, especially in those who received androgen deprivation for longer periods, their gonadal function and serum T levels did not recover. Indeed, 6 months after cessation of long-term (25–82 months) LH-RH agonist treatment, serum T levels were invariably below the normal range [117]. These findings suggest that intermittent administration of LH-RH agonists may achieve continuous androgen deprivation, resulting in reduction of cost. A recent article also showed that the median duration of castrated levels of serum T was 5.5 months (range 3.5–10 months) after a single injection of long-acting (3-month) depot LH-RH agonist and that the method of re-dosing LH-RH agonists based on serum T levels appeared efficacious, safe, and cost-effective [118].

The debate continues as to whether IAB improves survival. Large, multicenter, randomized, phase III clinical trials, comparing intermittent versus continuous androgen deprivation are currently ongoing to assess endpoints including survival, time to androgen-independent progression, and QOL. Furthermore, another form of IAB using a 5α -reductase inhibitor, finasteride, during off-treatment periods is also being evaluated [119].

Neoadjuvant/Adjuvant Hormonal Therapy With Radical Prostatectomy

Neoadjuvant hormonal therapy. Neoadjuvant therapy is defined as a systemic therapy, which is administered after the diagnosis, but before starting definitive local therapy. Radical prostatectomy is a treatment modality which can offer the possibility of cure of prostate cancer if surgical margins are negative. However, surgical attempts for a cure in patients with apparently localized prostate cancer often fail because the cancer is incompletely resected possibly due to clinical understaging before the surgery or microme-

tastases existing at the time of surgery. The purpose of neoadjuvant androgen deprivation is to lower the pathological stage, reduce positive margins, eliminate micrometastasis, and consequently increase patient survival [120].

Laboratory experiments using the Shionogi tumor model support this rationale [121]. Mice were divided into two groups: group 1 received wide tumor excision (with castration on tumor recurrence) and group 2 was treated with neoadjuvant castration 10 days prior to wide excision of the progressed tumor. Pathologically positive surgical margins were detected in 66% of group 1 and in 33% of group 2. Subsequent androgen-independent tumor recurrences were seen in 92% of group 1 and in 44% of group 2. There was a statistically significant difference in overall cancer-free survival (group 1: 20% vs. group 2: 56%, $P < 0.05$). Thus, the authors concluded that neoadjuvant hormonal therapy can reduce positive surgical margins and local recurrences by approximately 50% in this animal model.

Several prospective randomized trials have been performed to investigate the significance of neoadjuvant androgen deprivation for 3 months before radical prostatectomy [120,122–125]. Most studies showed a significant reduction in prostate volume and margin-positive rates in the patient groups with neoadjuvant androgen deprivation. Unfortunately, all of the studies failed to show a significant improvement in seminal vesicle or lymph node invasion, or PSA recurrence. The studies are too immature to yet evaluate survival. Possible reasons for this discrepancy include an insufficient duration of neoadjuvant hormonal therapy. Gleave et al. [126] reported a randomized comparative study of 3- versus 8-month neoadjuvant CAB, prior to radical prostatectomy. They showed that positive margin rates were significantly lower in the 8-month than 3-month group (12% vs. 23%, respectively), and concluded that the optimal duration of neoadjuvant therapy is longer than 3 months. However, rates of local or biochemical recurrence and long-term survival were not reported in this study. A prospective randomized trial conducted by the Canadian Urological Oncology Group evaluating this concept has closed to accrual and results will soon be forthcoming. An 8 months delay of surgery might carry a high risk for patients with androgen-independent tumor. Currently, neoadjuvant hormonal therapy, therefore, should remain investigational.

Adjuvant hormonal therapy. Several studies have suggested that adjuvant hormonal therapy following radical prostatectomy contributes to improvement of local control and survival in patients at high risk for recurrence. However, followings remain unanswered: (1) how to select appropriate candidates; (2) optimal

type, timing, duration, and specific regimen of adjuvant therapy; and (3) whether adjuvant hormonal therapy really improves patient outcome.

Options for adjuvant hormonal therapy include surgical or medical castration, antiandrogens, or CAB. There are a few reports showing a significantly positive effect of adjuvant hormonal therapy on cause-specific survival. Zincke et al. retrospectively reviewed 707 patients with stage pT3b disease, and found 157 of them received adjuvant hormonal therapy [127]. Adjuvant hormonal therapy significantly improved the mean survival rates at 10 years, including cause-specific (95% vs. 87%), systemic progression-free (90% vs. 78%), and biochemical progression-free survival (67% vs. 23%). Messing et al. [128], in a prospective randomized clinical trial, investigated the effect of adjuvant hormonal therapy in 98 patients with clinically localized prostate cancer and lymph node metastases. Androgen deprivation (goserelin or surgical castration) was initiated within 12 weeks of radical prostatectomy and pelvic lymphadenectomy in the adjuvant group, whereas, in the observation group, androgen deprivation was delayed until clinical progression. After 7.1 years median follow-up, overall and cause-specific survival were significantly better in the adjuvant group (85% vs. 65% and 93% vs. 68%, respectively). An updated analysis of this study with the mean follow-up of 10 years still showed statistically significant difference in overall (72% vs. 49%) and cause-specific (87% vs. 57%) survival [129]. They concluded that immediate adjuvant androgen deprivation provides a highly significant survival benefit in patients with node-positive prostate cancer following radical prostatectomy and lymphadenectomy. Adjuvant therapy with antiandrogens, such as flutamide [130] and bicalutamide [108], have also been shown to reduce biochemical recurrence and to prolong time to progression in a broad spectrum of post-prostatectomy patients, although in these studies more than 20% of patients were withdrawn from the study secondary to adverse events. These studies have too brief a follow-up period to evaluate effects on survival yet.

The beneficial effect of adjuvant hormonal therapy for patients after clinically curative surgery remains controversial. However, in those at great risk of recurrence such as those with positive nodes (in both the study by Messing et al. [128] and other clinical series [131] fewer than 20% of observed patients after surgery are free of PSA recurrence and thus likely cured, at 7-year follow-up, by surgery alone), adjuvant androgen deprivation following surgery seems strongly supported [127,128]. For less advanced disease, current data are insufficient to establish a definite role for adjuvant hormonal therapy after radical prostatectomy.

Hormonal Therapy With Radiation Therapy/Brachytherapy/Chemotherapy

Radiation therapy. Several randomized clinical trials have demonstrated the effect of hormonal therapy (castration or CAB) plus external-beam radiotherapy on localized or locally advanced/high-risk prostate cancer. Most of the studies have compared the clinical benefit between radiation therapy plus adjuvant or neoadjuvant androgen deprivation (for 4 months to life-time) and radiation therapy alone [132–135]. In the majority of these studies, adding androgen deprivation has been shown to significantly improve long-term local/distant and biochemical controls. Most of them were also able to show a statistically significant improvement in cause-specific survival, but not in overall survival [136]. Interestingly, in the studies that failed to show a statistically significant difference in clinical outcome, patients with high-grade (Gleason scores 8–10) [132] or low- to intermediate-grade (Gleason scores 2–6) [133] cancers had a statistically significant benefit from androgen deprivation therapy. Based on the published trials' data, Horwitz et al. [137] suggested the current consensus that patients with clinically localized or locally advanced high-grade tumors benefit from definitive radiation therapy and long-term adjuvant androgen deprivation.

Brachytherapy. Brachytherapy is increasingly used in patients with localized, low- to intermediate-grade prostate cancer. Neoadjuvant androgen deprivation therapy is commonly given to patients who have a large prostate, to downsize the prostate, making the brachytherapy procedure easier and more feasible. Indeed, it has been reported that prostate volume is reduced by up to 40% after 3 months of androgen deprivation therapy [126,138]. Other possible reasons for combining hormonal therapy with prostate brachytherapy include reducing brachytherapy-related morbidity and improving patient outcome. In the clinical studies with neoadjuvant hormonal therapy (LH-RH agonist or CAB) before prostate brachytherapy, significant reduction of prostate size was observed. However, no additional benefits of adjuvant hormonal therapy over the prostate brachytherapy on survival were apparent [138]. Because no prospective, randomized studies have been published, the impact of hormonal therapy in conjunction with brachytherapy remains unclear.

Chemotherapy. Previous studies have established the role of chemotherapy in the palliation of symptoms in patients with prostate cancer after failure of hormonal therapy [139,140], although its clear survival benefit is

not reported. Among a variety of drugs, mitoxantrone- and estramustine-based regimens have been extensively studied and shown to have a significant palliative benefit [140]. Estramustine has an estradiol moiety and has been used in prostate cancer patients for several decades. Estramustine, as a single agent, decreases serum T to castrating levels, with significant cardiovascular toxicity. During the last 10 years, *in vitro* studies of estramustine combined with other anti-cancer agents have demonstrated synergistic effects [140,141]. Combination regimens of chemotherapy with other hormonal therapies have also been investigated for locally advanced, presumably androgen-sensitive, prostate cancer. In a study by Pettaway et al. [142], 33 high-risk patients (either clinical stage T3 or Gleason score >7) were treated with ketoconazole, doxorubicin, vinblastine, and estramustine plus an LH-RH agonist and an antiandrogen for 12 weeks before radical prostatectomy. No patients were downstaged to P0 status, but 33% of them were found to have prostate-confined disease at the time of surgery. In another multicenter study, 50 locally advanced patients were treated with paclitaxel, estramustine, and carboplatin plus an LH-RH agonist for 4–6 months. Of the 23 patients who underwent radical prostatectomy, 45% of them attained organ-confined disease although no patients were downstaged to P0 status [140]. There were no comparisons of combination regimen to hormonal therapy alone.

Several *in vitro* studies investigated combinations of chemotherapy and hormonal therapy. Kreis et al. [141] showed synergistic effects on growth inhibition of either androgen-sensitive prostate cancer cell line LNCaP, androgen-insensitive lines DU145 and PC-3, or all cell lines, using combinations of estramustine plus flutamide and PSC833 (Sandoz) plus bicalutamide. Other studies demonstrated that androgen deprivation can trigger apoptosis of androgen-sensitive cancer cells via a transient increase in cytosolic calcium, resulting in activation of Ca²⁺- and Mg²⁺-dependent endonucleases [143,144]. Therefore, chemotherapy may become more effective when combined with androgen deprivation. In contrast, androgen deprivation was shown to promote androgen-dependent cells to enter the G0 phase of the cell cycle instead of undergoing apoptosis [145]. It is speculated that these cells might be more difficult to eradicate with subsequent chemotherapy.

An ongoing randomized study in high-risk patients is comparing goserelin plus bicalutamide to goserelin plus bicalutamide plus mitoxantrone and predonisone after radical prostatectomy. At present, the role of chemotherapy in conjunction with hormonal therapy in androgen-sensitive prostate cancer remains unclear.

5 α -Reductase Inhibitors

Two 5 α -reductase enzymes have been isolated: type 1, the predominant enzyme in extraprostatic tissues, such as skin and liver; and type 2, predominantly expressed in the prostate [19,146]. The type 2 5 α -reductase has been implicated in at least partially regulating early prostate growth as well as later hyperplastic growth. Therefore, finasteride, the first 5 α -reductase inhibitor specific for the type 2 enzyme, significantly decreases levels of both serum and intraprostatic DHT by 70–80% and thereby reduces the size of the total prostate gland [146]. Finasteride treatment has been a useful form of androgen deprivation for benign prostatic hyperplasia (BPH), with fewer adverse effects than antiandrogen treatment. However, the therapeutic activity of finasteride itself on prostate cancer has not been identified. The effect of finasteride in conjunction with other forms of hormonal therapy has been investigated. In addition to intermittent triple androgen blockade (TrAB) [119] consisting of CAB plus finasteride, followed by finasteride maintenance therapy, combination therapy with finasteride plus an antiandrogen or an LH-RH agonist, termed sequential androgen blockade (SAB) has been evaluated [147,148]. This approach (finasteride plus antiandrogen) was shown to substantially decrease the PSA levels in men with metastatic prostate cancer while maintaining sexual potency in most patients. Although phase III studies, comparing SAB with traditional hormonal therapy, such as CAB, have not been conducted and the survival benefit thus remains unknown, the above findings suggest that SAB may be a potent therapeutic option in the future for advanced prostate cancer patients.

The benzoquinoline, LY320236, is a newer and dual (type 1/2) 5 α -reductase inhibitor currently in phase I trials of prostate cancer [149]. The antitumor activity of benzoquinoline has been shown in human prostate cancer xenograft models.

FAILURE OF HORMONAL THERAPY

Androgen-Independent Prostate Cancer

As noted, development and progression of prostate cancer are strongly associated with AR activation. Prostate cancer cells are thus mostly androgen-sensitive and, therefore, respond to androgen deprivation therapy. However, this treatment very rarely results in the “cure” of advanced disease, and the majority of the patients eventually develop symptomatic recurrences. In this state, termed androgen-independent or hormone-refractory prostate cancer, hormonal therapy is no longer effective and cancer cells continue to

proliferate. There are also some patients, mostly with high-grade metastatic tumors, in whom initial hormonal therapy does not produce any or minimal clinical responses. A number of molecular mechanisms may underlie androgen-independent growth of prostate cancer, but exact mechanisms are still far from being fully understood. The proposed mechanisms follow (Fig. 2).

Hypersensitive AR pathway. The first possibility is that the AR is “superactive” in androgen-independent tumors. Earlier theories postulated that loss of AR expression in cancer cells was a major reason for lack of androgenic growth control. Nevertheless, the majority of the tumors in the androgen-independent state were found to express the AR, even at increased levels as compared to localized androgen-sensitive tumors [150,151]. Moreover, in those androgen-independent tumors, the AR is thought to be transcriptionally active and necessary for cell proliferation [152,153]. Therefore, it is likely that the AR remains important in growth of most prostate cancer cells from patients clinically defined as having androgen-independent disease. This is particularly likely since an increase in serum PSA level virtually always heralds emergence of clinically

significant androgen independence and currently no mechanisms other than those that work through an activated AR has been shown to induce PSA expression in vivo. AR gene amplification is a mechanism for increased AR expression. The AR gene has been shown to be amplified in approximately one-third of androgen-independent tumors, and in none of the paired samples collected from the same patients prior to hormonal therapy [151,154]. A mutation in the AR gene is also a mechanism for increasing receptor activity. A number of different mutations of the AR gene, mainly occurring in the LBD, have been reported with much higher frequency (up to 50%) in advanced prostate cancer specimens [155,156]. Significantly, most mutations were found in patients treated with flutamide in conjunction with castration, but not in those treated with castration alone [153,156], while other studies showed that AR mutations were relatively frequent in metastatic tumors even prior to hormonal therapy [157,158]. When compared to the wild-type receptor, these mutations within the AR LBD can alter ligand binding specificity and increase the sensitivity to hormonal agents other than T and DHT, such as adrenal androgens, estrogens, progestins, and even antiandrogens [21–23,159,160]. This activation by antiandrogens might help explain the antiandrogen withdrawal syndrome (discussed below). Since transcriptional activity of the AR is mediated by a number of interacting proteins that function as coregulators [18,161], involvement of these AR coregulators are highly possible. Recent studies have revealed that expression of some AR coregulators is increased in tissue samples from androgen-independent prostate cancer [162,163]. All of the above observations suggest that “superactive” or “hypersensitive” AR plays an important role in the transition to the androgen-independent state. Additionally, androgen-independent prostate cancer cells may possess enzymes capable of converting adrenal androgens to DHT, providing higher intracellular levels of DHT than would be expected from low levels of serum T occurring in the androgen deprived state [164].

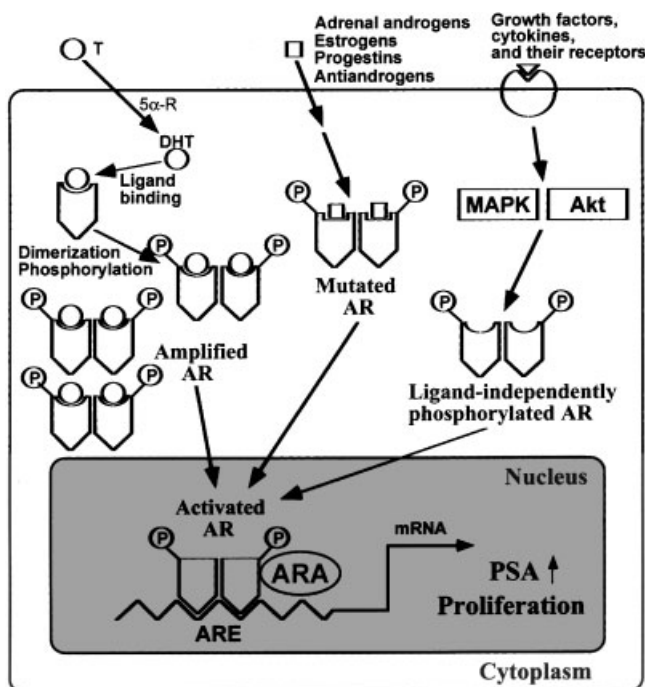


Fig. 2. Potential AR-related pathways to androgen-independence in prostate cancer cells. T, testosterone; 5α-R, 5α-reductase; DHT, 5α-dihydrotestosterone; AR, androgen receptor; P, phosphorylation; MAPK, mitogen-activated protein kinase; ARA, AR-associated protein; ARE, androgen response element; PSA, prostate-specific antigen.

Ligand-independent activation of the AR pathway. A second possible mechanism is that the AR is inappropriately activated by ligand-independent mechanisms. The AR exists as a phosphoprotein, and the functional status of the AR is associated with its phosphorylation status both in the presence and absence of androgens [165–167]. In vitro studies have demonstrated that several peptide growth factors, such as insulin-like growth factor-1 (IGF-1), keratinocyte growth factor, and EGF, increase AR transcriptional activity in the absence of androgens [36,168]. These growth factors serve as ligands for receptor

tyrosine kinases, such as the EGF receptor and HER-2/neu (also known as erbB-2). Therefore, this means of activating the AR may be mediated through signal transduction pathways, such as the mitogen-activated protein kinase (MAPK) and Akt (also known as protein kinase B), which can specifically bind to and phosphorylate the AR [169,170]. Other molecules, including a cytokine, interleukin-6, protein kinase A, and protein kinase C, have also been shown to activate the AR in an androgen-independent manner [171–173]. Some of these androgen-independent activations can be completely inhibited by bicalutamide [168]. However, these findings do not match with clinical observations of androgen-independent prostate cancer, which respond only briefly (if at all) to secondary antiandrogen treatment. Clinical data also show that some of the growth factors or IL-6 are increased in the prostate (or metastatic sites) and serum of the patients with androgen-independent disease [174–176]. In addition, AR acetylation may involve AR transcriptional activity in both androgen-dependent and androgen-independent manners [177,178]. Thus, AR transactivation, induced by the factors that can regulate AR phosphorylation/acetylation in the absence of androgens and are active in the tumors, may provide a mechanism for androgen-independent progression.

Bypassing the AR pathway. The third possible mechanism is that androgen-independent prostate cancer cells bypass the AR pathway. These cells may use other pathways for proliferation even when the AR remains active. An apoptosis inhibitor, Bcl-2, most likely involves this bypass [152], although AR-mediated anti-apoptotic effects have also been implicated [179]. Indeed, overexpression of Bcl-2 has been found in advanced prostate cancer [180]. Other genetic alterations, especially in cell cycle modulators, some of which are also involved in activation of the AR, contribute to proliferation of prostate cancer cells in the absence of activated AR. Such genes include tumor suppressor genes *p53*, retinoblastoma (*Rb*), and *PTEN*, and several oncogenes, such as *c-myc* [167,181]. The prevalence of neuroendocrine cells, which lack AR expression, in prostate tumors is also postulated to contribute to the development of androgen-independent tumors [167,182].

Clonal selection theory. Additionally, Isaacs [183] proposed the clonal expansion theory. Androgen-independent tumor cells, possibly epithelial stem cells [184], which co-exist with androgen-dependent cells at the time of initial treatment, might become predominant (selected) during hormonal therapy, and then continue to proliferate.

Thus, many possible mechanisms for the transition to androgen-independent progression have been proposed. It is likely that no single mechanism is utilized in every case of androgen-independent prostate cancer.

Antiandrogen Withdrawal Syndrome

Some patients with disease progression during CAB therapy experience a decline of PSA value, often correlated with clinical improvement, after discontinuation of antiandrogen treatment. This withdrawal response was first reported with flutamide [185], but similar phenomena have been observed after cessation of other non-steroidal or steroidal antiandrogens, as well as DES and progestational agents [186]. Therefore, what initially was called ‘flutamide withdrawal syndrome’ was renamed ‘antiandrogen withdrawal syndrome’ or even more recently ‘steroid hormone withdrawal syndrome.’ It is noted that patients on antiandrogen monotherapy apparently do not manifest a withdrawal response. Cumulatively, the withdrawal responses, as determined by PSA decline of more than 50%, can be observed in a significant number (15–80%) of treated patients. Clinical regression of the primary lesion and metastases are occasionally seen. The duration of response in most patients is 4–8 months, and tumors then progress into an androgen-independent state. Small and Srinivas [187] found no prolongation of survival time after initiation of antiandrogen withdrawal syndrome (13 months for responders to the antiandrogen withdrawal versus 12 months for non-responders), although total survival time from starting hormonal therapy was slightly increased (44.5 months for responders vs. 35 months for non-responders). There are no established prognostic parameters to separate patients who will respond to antiandrogen withdrawal from those with androgen-independent cancer. It has been demonstrated that patients who respond to initial hormonal therapy quickly (e.g., normalization of PSA values within 3 months) and over a prolonged period tend to have a higher rate of developing antiandrogen withdrawal syndrome [186–188].

Molecular mechanisms responsible for antiandrogen withdrawal syndrome have not yet been determined. Three possible mechanisms follow.

AR gene mutations. One potential mechanism is AR mutations in the tumors that enable antiandrogens to function as AR agonists. Flutamide has been shown to act as an agonist in LNCaP cells with a point mutation of codon 877 in the AR gene [159,189]. This type of mutation was often found in patients with the antiandrogen withdrawal syndrome, whereas all other reported mutations were only

occasionally detected [190]. As discussed above, antiandrogen treatment may more easily induce AR mutations (or select for cells which have such mutations), particularly at the codon 877, in tumor cells [153,156]. Some mutated ARs can be activated by antiandrogens [191]. Therefore, withdrawal responses may occur in cases where antiandrogens with agonist activity are the major stimulators for cell proliferation.

AR coregulator involvement. AR coactivators, such as ARA70 and ARA55, have similarly been reported to enhance the ability of antiandrogens to induce AR activity [161,192–195]. If expression of such AR coregulators is regulated by CAB treatment, antiandrogens might act as agonists to activate the AR pathway. The increasing expression of SRC-1 and TIF-2 after androgen deprivation therapy has been observed [163], but these coactivators only weakly promote the androgenic effect of flutamide. We have recently applied the yeast two-hybrid system, using codon 877 mutant AR as bait in the presence of hydroxyflutamide, to screen AR interacting proteins that are involved in the flutamide withdrawal syndrome. One of the positive clones, gelsolin, a multifunctional actin-binding protein, significantly enhanced AR transcriptional activity in the presence of androgen and/or hydroxyflutamide [196]. Interestingly, after androgen depletion, gelsolin expression was up-regulated in LNCaP cells, LNCaP xenografts, and prostate cancer specimens. These results suggest that weak agonist effect of flutamide may be amplified by increasing the amount of gelsolin after CAB treatment. Up-regulation of expression after CAB treatment was also observed with ARA70 (Yeh et al., unpublished data). Furthermore, we have recently shown that several mutants of AR coactivators, including ARA54, ARA55, and ARA70, which function as dominant-negative inhibitors of AR transcription, reduce hydroxyflutamide- or E2-enhanced PSA expression and cell growth in prostate cancer [197–199]. These findings confirm the involvement of AR coregulators in promoting the agonist effect of antiandrogens. Thus, patients with certain AR mutations or coactivator imbalances may be more likely to respond to antiandrogen withdrawal.

Activation of MAPK pathway. Recently, Lee et al. [200] have found the activation of the MAPK pathway by hydroxyflutamide in prostate cancer cells. In both the AR-negative cell line DU145 and AR-positive cell lines, hydroxyflutamide was found to activate MAPK via the Ras/Raf pathway. Hydroxyflutamide also stimulated DU145 cell proliferation, and this effect could be inhibited by an EGF receptor inhibitor and

an EGF receptor-neutralizing antibody. These results suggest that the activation of the Ras/MAPK signaling pathway by hydroxyflutamide can be (e.g., in DU145 cells) a non-AR-mediated action. In addition, it was shown that the levels of active MAPK in the prostate cancer specimens from patients whose tumors progressed on CAB therapy with flutamide were elevated, compared to those in the specimens from the same patients prior to CAB treatment. This mechanism may also contribute to the withdrawal responses of antiandrogens.

CONCLUSION AND FUTURE DIRECTIONS

Many options involving the AR, androgens, and their antagonists are available for the treatment of prostate cancer. Numerous clinical studies have shown equivalent effects on therapeutic benefits by different hormonal treatment strategies. Each treatment strategy/hormonal agent has favorable and unfavorable effects. Patients with advanced prostate cancer will clearly benefit from androgen deprivation-based treatment for palliating their symptoms and for improving their QOL. However, whether these therapies prolong survival when administered before there are symptoms caused by disease progression remains controversial. Importantly, advantages versus disadvantages of the treatment as well as patient preference and cost should be considered when treatment options are determined. A fundamental decision is the timing of hormonal therapy. Data from multiple recent studies indicate that an earlier treatment in patients' disease course likely leads to better outcome [128,131,136], but it is not easy to predict the best timing of hormonal therapy for patients with asymptomatic advanced disease. Observation may be still a reasonable choice for these patients.

To improve overall survival of patients with advanced prostate cancer, we need to determine: (1) the way to prolong the androgen-dependent state, (2) precise mechanisms responsible for the emergence of androgen-independent prostate cancer, and (3) novel treatment strategies for androgen-independent prostate cancer based on these molecular mechanisms.

For the purpose of delaying the onset of androgen-independent growth of prostate cancer, different regimens of IAB have been applied to patients [112–117]. Larger phase III trials are ongoing to evaluate the beneficial effect of IAB as well as to identify the most effective form of IAB. Since the majority of available antiandrogens have been reported to induce antiandrogen withdrawal syndrome [186], possibly resulting from their partial agonist effects, the identification of new antiandrogenic compounds with lower androgenic activity could be another potential approach. We screened DHEA metabolites/derivatives and

found that 3 β -acetoxyandrost-1,5-diene-17-ethylene ketal (ADEK) acted as an antiandrogen *in vitro* and could inhibit DHT-induced PSA expression and proliferation of LNCaP cells [201]. More interestingly, ADEK had only marginal agonist activity on both the wild-type AR and mutant (codon 877) AR, which could not be induced further by AR coactivators. Moreover, we demonstrated that ADEK [201] and several other DHEA metabolites [202] could also inhibit transactivation of the AR induced by the adrenal androgen, Δ^5 -androstenediol. Pharmacological concentrations of hydroxyflutamide and bicalutamide were unable to do this in prostate cancer cells [22]. Further *in vivo* studies might facilitate the development of better antiandrogens that are able to provide a longer period of sensitivity to hormonal therapies.

In patients whose PSA relapse during androgen deprivation is not caused by the antiandrogen withdrawal syndrome, second-line hormonal agents have been investigated before considering other forms of treatment such as chemotherapy. These hormonal agents include non-steroidal antiandrogens bicalutamide (high-dose: 150–200 mg/day) and nilutamide, estrogens DES and PC-SPEs (an herbal combination with strong estrogenic activity that is no longer available in the United States), and adrenal steroidogenesis inhibitors corticosteroids and ketoconazole [203,204]. Oh [204] reviewed recent phase III clinical studies showing that 20–80% of patients with androgen-independent prostate cancer who received these secondary hormonal therapies had a PSA decline of >50% and symptomatic improvement, with a response duration of 2–6 months. Nevertheless, no studies have demonstrated a survival benefit with the use of these treatments. Further studies are necessary to define the optimal use of secondary hormonal therapy, including the appropriate timing and sequencing of treatments, whether combination with chemotherapy or other agents would improve efficacy, and to evaluate survival benefits.

Other strategies for androgen-independent prostate cancer based on currently understood concepts might provide therapeutic benefits. Since the AR pathway likely remains important for androgen-independent prostate cancer cell proliferation, down-regulation of AR activity might remain a major target for the development of novel therapies.

One possible therapeutic modality is to block interactions of the AR with AR coregulators, possibly using gene therapy [197–199,205]. These proteins include AR coactivators or corepressors. We have found that mutant AR coactivators (e.g., ARA54, ARA55, ARA70) inhibit AR transcription and AR-mediated prostate cancer cell growth in a dominant-negative fashion, presumably through the disruption of AR coactivator

dimerization [197–199]. We confirmed that disruption of AR coactivators in cells using double-stranded short interfering RNA produced similar inhibitory effects [198,199]. These results suggest that these dominant-negative mutant coactivators can be effective for both androgen-dependent and androgen-independent prostate cancers. However, prostate cancer cells appear to be capable of overexpressing more than one coactivator simultaneously [162,163]. Therefore, this approach would presumably be feasible only if interacting proteins/peptides can block the AR interaction with multiple coactivators. For example, several peptides (40–50 amino acids) from the AR LBD or DBD could block gelsolin-enhanced AR activity [196]. However, since different AR coactivators interact with different AR domains [18], it might be difficult to design enough proteins/peptides to effectively block multiple coactivator binding sites of the AR. Further study is necessary to test whether the AR peptides also interfere with functions of other AR coregulators.

In addition to general transcription coregulators, several AR modifiers, such as cyclins, cyclin-dependent kinases, and cyclin-dependent kinase inhibitors, have been shown to be able to regulate androgen-independent prostate cancer cell proliferation [153]. Modulation of signal transduction pathways might be a useful way to induce apoptosis in prostate cancer cells. It has been suggested that some growth factors can modulate apoptosis through phosphorylation of multiple target proteins. At times, one of the targets so phosphorylated is the AR without its ligand, which results in AR activation. Thus, interruption of interactions between the AR and these modulating proteins could be a possible target for novel AR antagonists. Indeed, inhibition of growth factors has been tested through decreasing availability of growth factors, modulation of intracellular kinase activity, immunological targeting of growth factor receptors, or inhibition of receptor tyrosine kinase activity [206,207]. Initial clinical studies, using IGF-1 modulators, including somatostatin analogues and vitamin D analogues, or humanized monoclonal antibody to the Her-2 receptor trastuzumab, showed some benefits in androgen-independent prostate cancer patients [208–210]. Since the activity of signal transduction pathways, via MAPK and Akt, occur both through the AR and independent of AR signals, specific inhibitors to these pathways could also be potential therapeutic agents [170,200,211,212]. These agents might be able to be combined with classical androgen deprivation strategies.

Inhibition of AR function through the mechanisms interfering with AR expression, protein stability, nuclear translocation, and interactions of the NH₂ and COOH terminals of the AR, all of which are expected to

decrease AR transcriptional activity, might provide another therapeutic benefit. Relatively few compounds are known to inhibit prostate cancer cell growth through the above mechanisms. Resveratrol [213], found in grapeskins and used in some Asian medicines with weak estrogenic activity, and Vitamin E succinate [214] are reported to suppress AR expression and androgen-induced LNCaP cell growth. Curcumin, a perennial herb used as a yellow coloring and flavoring agent in foods, can inhibit proliferation of both AR-positive and AR-negative prostate cancer cells through inducing apoptosis [215,216]. We also found that an analogue of curcumin inhibited DHT-induced PSA expression and tumor growth only in AR-positive cells, by (1) competing with ligands for AR binding, (2) reducing AR protein expression in the presence and absence of androgens, (3) promoting AR degradation, (4) retarding androgen-induced AR translocation into the nucleus, and/or (5) interrupting androgen-mediated ARNH₂/COOH interaction (Miyamoto et al., unpublished data). Thus, these compounds might be expected to inhibit proliferation of both androgen-dependent and androgen-independent prostate cancer cells. Further investigations are necessary to determine their ultimate therapeutic use. Furthermore, since many of the mechanisms are mediated through an activated AR, means of interfering with the AR-ARE interaction would appear to be important approaches in delaying the emergence of true androgen independence.

Finally, hormonal therapy is used for prostate cancer chemoprevention. Target populations for primary and secondary prevention may include healthy men aged >50 years with or without a strong family history, men with borderline and/or sustained elevations of PSA levels, men with histologic changes, such as atypia, prostatic intraepithelial neoplasia, and low-grade/low-volume localized (clinical stage T1a or T1c) carcinoma, as well as men with a high-risk for recurrence after radical treatments [217]. Most of the strategies for androgen-dependent disease described above can be used for this purpose, but the strategies/agents must have significant preventive effects and minimal adverse effects if they are to be used in a preventive setting [218]. They must also be inexpensive due to the many years needed for therapy. Therefore, natural compounds, such as soy isoflavones and mammalian lignans, which inhibit prostate cell proliferation through androgen-dependent and androgen-independent mechanisms [59], might be potential agents. A large study, the Prostate Cancer Prevention Trial, involving >18,000 men aged ≥55 years with PSA <3 ng/ml has recently identified that finasteride leads to approximately 25% decrease in biopsy-detected incidence of prostate cancer at 7 years, as compared

to the placebo group [219]. Conversely, 4 years of finasteride treatment in BPH patients failed to reduce the occurrence of prostate cancer, as compared to the placebo group (4.7% vs. 5.1%) [220]. In addition, epidemiological, in vitro, in vivo animal and/or clinical studies have suggested potential natural and pharmaceutical agents for prostate cancer chemoprevention. These agents include non-steroidal antiandrogens, antiestrogens, antioxidant nutrients (e.g., vitamin E, selenium, lycopene, green tea polyphenols), non-steroidal anti-inflammatory drugs, antiangiogenesis inhibitors, antiproliferation agents, peroxisome proliferator-activated receptor modulators/ligands, farnesyl transferase inhibitors, growth factor modulators, protein tyrosine kinase inhibitors, and gene-based vaccines (e.g., PSA, prostate-specific membrane antigen, p53, Rb) [217]. Furthermore, several rational combinations have been tested for their additive or synergistic effects or for preventing proliferation of "resistant" clones to single agents.

In conclusion, currently available options for hormonal therapy almost never lead to cures in patients with advanced prostate cancer. Therefore, novel treatment strategies that prolong the androgen-dependent state and that are effective for the androgen-independent disease need to be identified. In addition, it may be necessary to explore more individualized approaches, such as selectively blocking the activated AR pathway in cancer cells.

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