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Beyond simple castration: targeting the molecular basis of treatment resistance in advanced prostate cancer

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Abstract Over the past 20 years, research on hormonal treatments for prostate cancer focused on maximizing androgen ablation through combination therapy. Unfortunately, maximal androgen ablation increases treatment-related side effects and expense and has not significantly prolonged time to androgen-independent (AI) progression. Intermittent androgen suppression (IAS) is based on the hypothesis that if tumor cells surviving androgen withdrawal can be forced along a normal pathway of differentiation by androgen replacement, then apoptotic potential might be restored, androgen dependence may be prolonged and progression to androgen independence may be delayed. Observations from animal model studies suggest that progression to androgen independence is delayed by IAS and this strategy is now being evaluated in phase III trials. Another strategy for improving therapies in advanced prostate cancer involves targeting genes that are activated by either androgen withdrawal or chemotherapy to delay or prevent the emergence of the resistant AI phenotype. Targeted inhibition of stress-associated increases in gene expression precipitated by androgen withdrawal or chemotherapy may enhance treatment-induced apoptosis and delay progression to AI disease. Proteins fulfilling these criteria include antiapoptotic members of the Bcl-2 protein family, clusterin, Hsp27, and IGFBP-2 and IGFBP-5. The purpose of this paper is to review the rationale and progress in using targeted gene therapies to enhance tumor cell death after androgen withdrawal or taxane chemotherapy. Antisense oligonucleotides offer one approach to target genes involved in cancer progression, especially those not amenable to small molecule or antibody inhibition. The current status and future direction of several antisense oligonucleotides that have potential clinical use in cancer are also reviewed.

Keywords Androgen independence · Clusterin · Hsp27 · Intermittent therapy · bcl-2

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Introduction

More than 80% of men with advanced prostate cancer have symptomatic and objective responses following androgen suppression, and serum prostate-specific antigen (PSA) levels decrease in almost all patients. Surgical or medical castration results in a median progression-free survival of 12–33 months and a median overall survival of 23–37 months in patients with stage D2 disease [22, 53]. However, for reasons that are only partly defined, the apoptotic process induced by androgen ablation fails to eliminate the entire malignant cell population. Another limitation of conventional androgen ablation is that it accelerates the rate of progression of prostate cancer to an androgen-independent (AI) state [7], and after a variable period of time averaging 24 months, progression inevitably occurs with rising serum PSA levels and AI growth. Over the past two decades, many efforts have focused on maximizing the degree of androgen suppression therapy by combining agents that inhibit or block both testicular and adrenal androgens. Unfortunately, maximal androgen ablation (i.e., the addition of an antiandrogen to medical or surgical castration) increases treatment-related side effects and expense and has not significantly prolonged time to AI progression in most patients [18, 22, 58]. If we are to improve survival significantly, new therapeutic strategies designed to inhibit the emergence of this resistant phenotype, coupled with better prognostic factors, must be developed. This paper reviews various therapeutic strategies aimed at delaying progression of

late-stage prostate cancer to its lethal state of androgen independence, including the optimal timing of initiation of androgen ablation, the current status of intermittent androgen suppression (IAS) and the future biologic agents targeting the molecular basis of hormone resistance. The rationale and progress in targeted therapies to enhance tumor cell death after androgen withdrawal or taxane chemotherapy are also reviewed.

Optimal timing of hormone therapy

While early diagnosis and treatment is a long-held paradigm in oncology, the controversy surrounding optimal time to initiate hormone therapy in prostate cancer remains relevant, especially considering how wide the spectrum of advanced prostate cancer has become. Debates continue on whether to start treatment immediately after diagnosis of advanced or recurrent disease or wait until the disease burden is larger and causes symptoms. This issue is especially relevant in the PSA era, with its expanding proportion of patients with PSA-relapses after failed local therapy, many of whom may be candidates for androgen ablation therapy but have very long life expectancies.

Accumulating theoretical, preclinical and clinical evidence supports treatment at the time of diagnosis of locally advanced or metastatic disease (Table 1). The Goldie–Coldman [33] hypothesis of increasing somatic genomic alterations and tumor heterogeneity over time provides a theoretical basis to support initiation of therapy as early as possible. Also, data from several xenograft models support initiation of androgen ablation when tumor burden is small [37, 66]. For example, using the androgen-dependent Shionogi mouse model, So and colleagues [66] reported that large tumor volume and corresponding delay of castration reduced the time to AI recurrence and death. Earlier androgen ablation, at the time of subclinical (nonpalpable) disease, significantly delayed the rate and time to AI recurrence compared to delayed therapy when tumor burden was high.

Many clinical studies report prolonged time to androgen independence and improved survival with early therapy in men with advanced prostate cancer. While the first Veterans Administration Cooperative Urological Research Group study [45] reported that delayed endocrine therapy was equivalent to immediate treatment, reanalysis by Byar [10] indicated that earlier therapy, when corrected for the cardiovascular mortality associated with diethylstilbestrol (DES), was more effective. The Medical Research Council study from the United Kingdom randomized over 800 men with advanced M0 or M1 disease to immediate or deferred therapy, and showed that palliative surgery for bladder outlet or ureteral obstruction was reduced by almost 50% and disease-specific survival improved with immediate therapy [71]. Similarly, a European Organization for Research and Treatment of Cancer study demonstrated prolonged time to disease progression and 5-year survival (58% vs. 78%; $P < 0.05$) in patients with locally advanced disease treated with radiotherapy and immediate androgen ablation for 3 years compared to delayed therapy with symptomatic progression [5]. Messing and coworkers [46] reported dramatically improved overall and recurrence-free survival in patients with node-positive disease that underwent radical prostatectomy plus immediate androgen ablation compared to deferred therapy.

Data from the Early Prostate Cancer (EPC) trials show the potential benefits and risks of immediate versus deferred therapy. Overall, patients treated immediately with the antiandrogen bicalutamide had a lower risk of progression of disease [39]. Importantly, however, data from Trial 25 (Scandinavia) also reported that time to death is accelerated in men with T1/T2 prostate cancer treated with bicalutamide compared to watchful waiting alone [hazard ratio (HR) 1.47; $P = 0.0195$]. This decrease in overall survival in T1/T2 watchful waiting patients treated with bicalutamide is significant, appears to be drug-related and is biologically plausible. While the effects may be due to bicalutamide itself and not related to androgen receptor blockade, the effects are most plausibly due to androgen blockade. In contrast, in Trial 25 in T3/T4 prostate cancer patients, time to death was significantly longer (HR 0.67; $P = 0.0125$) in bicalutamide-treated compared to placebo-treated men. Accelerated rate of death in watchful waiting T1/T2 tumors with a reverse trend in watchful waiting T3/T4 patients is consistent with the hypothesis that bicalutamide treatment improves survival in men at high risk of progression, but worsens survival in men at low risk of progression.

Table 1 Clinical studies of intermittent androgen suppression

Investigator	Sample size	Clinical stage	Mean follow-up (months)	Cycle 1 start PSA (μg/l)	Cycle 2 start PSA (# of pts)	Cycle 3 start PSA	Mean cycle length (month) Cycle 1, 2, 3	Mean time off therapy (%)	Mean time to PSA nadir (months)
Goldenberg [31]	47	Local + met	30	128	15 ($n = 30$)	18 ($n = 15$)	18, 18, 16	47	5
Oliver [55]	20	Local + met	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Grossfeld [34]	47	Local	24	21	7.0 ($n = 17$)	20.5 ($n = 3$)	16, 11, N/A[a]	50	4
Higano [35]	22	Local + met	26	25	7.7 ($n = 12$)	N/A ($n = 0$)	15, 18	38	3.5
Crook [19]	54	Local	33	43	N/A	N/A	18	46	5
Hurtado [36]	70	Local + met	46	110	16 ($n = 56$)	12 ($n = 24$)	18, 18, 16	47	5

N/A not applicable
[a]Treatment was continued for 1–2 months after nadir PSA reached

Coincident with accumulating evidence supporting immediate therapy is a shift in the target population eligible to receive androgen ablation from metastatic disease to PSA failures after radical prostatectomy or radiotherapy. Use of PSA for early detection has produced a stage migration and a 50% decrease in the incidence of stage D2 disease over the past 10 years [67]. As the median survival in hormone-naïve M + disease is only 2–3 years, the long-term side effects of androgen ablation do not become apparent and are therefore less clinically relevant. However, use of PSA to detect biochemical recurrences following radical prostatectomy or radiotherapy identifies men at risk of recurrence and who may benefit from early adjuvant therapy but who have life expectancies exceeding 10 years. Hence, combinations of stage migration, earlier diagnosis of PSA recurrences, longer life expectancies and trend toward immediate therapy are forcing clinicians to balance the potential benefits of early adjuvant therapy with the risks of development of metabolic complications, as well as the increased expense, associated with long-term continuous androgen withdrawal therapy. These metabolic complications include: loss of bone mass (osteoporosis) and fractures; loss of muscle mass and anemia with easy fatigue and decreased energy levels; changes in lipid profile with increased risk of cardiovascular complications; glucose intolerance; and depressive and/or irritable personality changes [72]. Decreased overall survival in low-risk watchful waiting patients treated with bicalutamide in the EPC trials further emphasize the need carefully to select patients at high risk of disease progression who are most likely to benefit from early hormone therapy. In this regard, IAS may offer clinicians an opportunity to improve quality of life in patients with prostate cancer by balancing the benefits of immediate androgen ablation (delayed progression and prolonged survival), while reducing treatment-related side effects and expense.

Intermittent androgen suppression

Biologic rationale

The development of therapeutic resistance, after hormone- or chemotherapy for example, is the underlying basis for most cancer deaths. Therapeutic resistance and tumor progression result from multiple, stepwise changes in DNA structure and gene expression—a Darwinian interplay of genetic and epigenetic factors, many arising from selective pressures of treatment. This highly dynamic process cannot be attributed to singular genetic events, involving instead cumulative changes in gene expression that facilitate escape from normal regulatory control of cell growth and survival. Prostate cancer initially progresses as an androgen-dependent tumor, manifested by apoptosis, cell-cycle arrest and changes in androgen-regulated gene expression after androgen ablation [25, 69]. Unfortunately, progression to androgen independence nearly always occurs in prostate cancer after androgen ablation, illustrating that regimens used to kill or control cancer cells also trigger cascades of events that lead to a hormone-resistant phenotype.

Progression to androgen independence is a multifactorial process by which cells acquire the ability to both survive in the absence of androgens and proliferate using nonandrogenic stimuli for mitogenesis. It involves variable combinations of clonal selection, adaptive upregulation of antiapoptotic cytoprotective genes (e.g., Bcl-2, clusterin, Hsp27), androgen receptor transactivation in the absence of androgen from mutations or increased levels of coactivators and alternative growth factor pathways, including Her2/neu, EGFR, TGF-β and IGF-1 [7, 12, 17, 23, 27, 41, 49, 51, 59, 60, 64]. High-throughput bioprofiling using gene and tissue microarrays is used by many research groups to characterize genetic and signaling networks that enable tumors to progress and adapt to treatments. In general, there is a reproducible, programmatic drift in gene expression, in prostate tissues after androgen ablation. Of interest, clusterin, Bcl-2, Bcl-xL, Hsp27, IGFBP-2 and IGFBP-5 are all upregulated after castration and remain constitutively over-expressed in recurrent AI tumors. Increased expression of bcl-2 [27, 59], androgen receptor [12], clusterin [40, 49], IGFBP-5 [51], IGFBP-2 [41] and Hsp27 [60] after castration help confer androgen resistance and represent adaptive responses by malignant cells to activate survival and mitogenic pathways to compensate for androgen withdrawal (Fig. 1).

Based on the above observations, it is reasonable to postulate that the adaptive changes in gene expression precipitated by androgen ablation may be modulated by re-exposure to the differentiating effects of androgen (Fig. 1). Early observations that prostatic involution after castration is an active process involving rapid elimination of most epithelial cells led to the postulate that the replacement of androgens, even in small amounts, would have a conditioning effect on surviving cells and allow them to conserve or regain desirable traits of differentiation [6, 24, 54, 62]. The rationale behind IAS is based on the hypothesis that if tumor cells surviving androgen withdrawal are forced along a pathway of differentiation by androgen replacement, then apoptotic potential might be restored and progression to androgen independence delayed. It follows that if androgens are replaced soon after regression of tumor, it should be possible to bring about repeated cycles of androgen-stimulated growth, differentiation and androgen-withdrawal regression of tumor.

Preclinical studies of IAS

Several investigators [61, 73] reported no significant growth reduction in Dunning R3327 tumors treated with IAS, and concluded that IAS was inferior to early castration in inhibiting tumor growth. Although the intermittent regimen was successful in delaying tumor

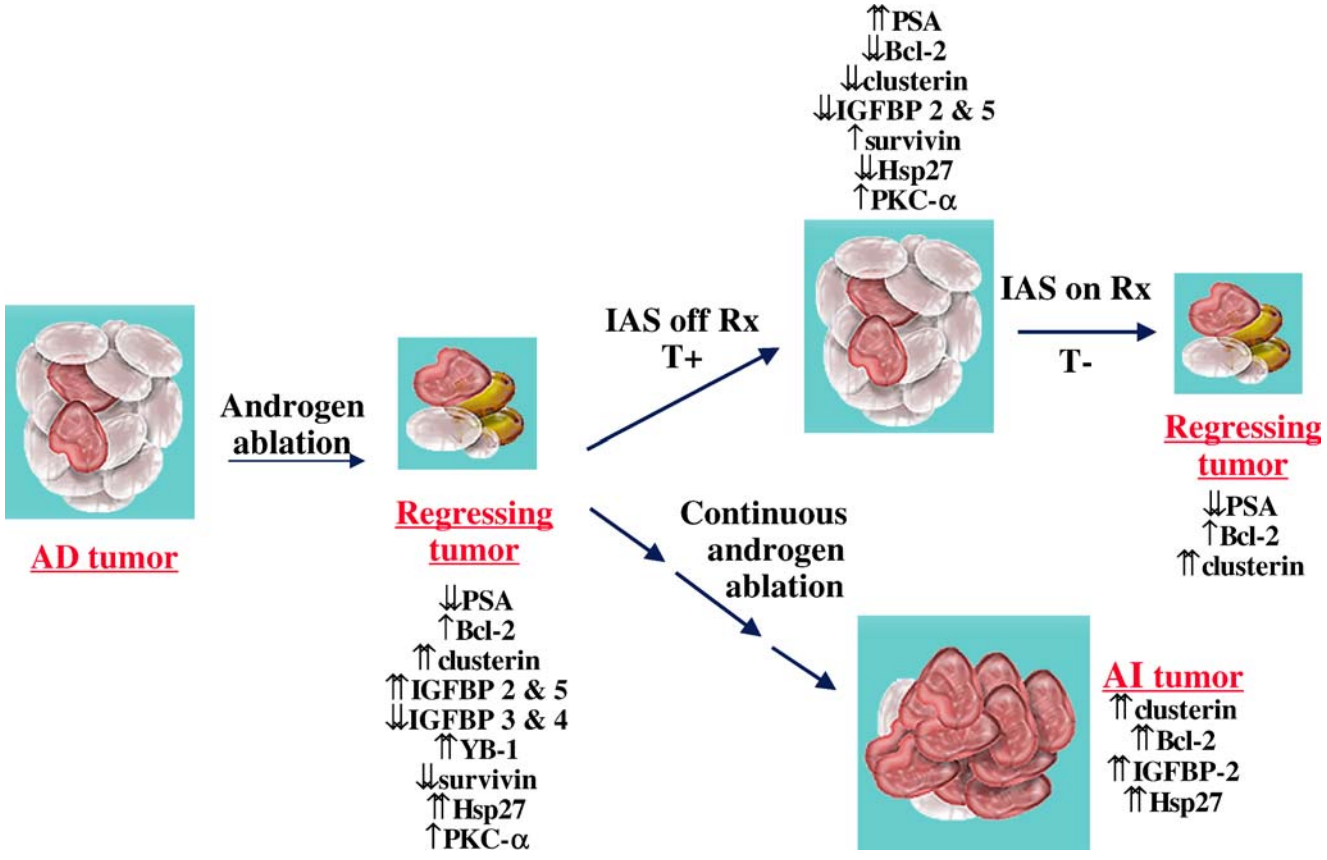

Fig. 1 Androgen ablation results in apoptosis and upregulation of previously androgen-repressed genes (e.g., Bcl-2, clusterin). However, not all cells are eliminated and in the absence of androgens, proliferating cells do not differentiate to become preapoptotic again, which results in development of the AI phenotype and constitutive overexpression of resistance-associated genes such as bcl-2, clusterin and Hsp27. With intermittent re-exposure to testosterone (T+), proliferating cells differentiate, expression of resistance-associated genes decrease, and cells become preapoptotic again, permitting another round of androgen ablation-induced apoptosis and tumor regression (T−)

growth, it did not yield a survival advantage and in this respect was inferior to castration. The foregoing experiments using the Dunning R3327 model draw attention to the importance of using cyclic androgen deprivation only for the treatment of androgen-dependent cancer. Dunning tumors are androgen-sensitive, not androgen-dependent, and apoptotic regression does not occur following castration. In this model, AI progression results from selective outgrowth of androgen-resistant clones after androgen ablation. Hence, the failure to improve outcome with IAS in Dunning tumors is not an unexpected result. Androgen ablation with the addition of cytotoxic agents appears to be the most effective therapy in this model [38].

In contrast to the Dunning model, IAS in the androgen-dependent Shionogi carcinoma induced multiple cycles of castration-induced apoptosis before growth became AI during the fifth cycle [1, 7]. The mean time to androgen independence increased three-fold compared to one time castration, consistent with a retarding effect of cyclic therapy on tumor progression. Some caution is required in the interpretation of these results since the procedure of transplanting a regressing tumor from a castrated to a noncastrated animal temporarily may reduce the rate of cell division and give rise to an apparent delay in progression. However, in addition to tumor growth, other markers of androgen dependence such as castration-induced apoptosis and clusterin gene expression were maintained three times longer with IAS compared to continuous therapy, paralleling and supporting the changes in tumor volume. Similarly, IAS in mice bearing LNCaP tumors prolonged AI regulation of the PSA threefold [63]. Taking an alternative approach, Thalmann and colleagues [70] reported evidence that suggests continuous androgen suppression might facilitate development of AI osseous metastasis. Additional, indirect evidence supporting the concept of IAS came from two independent reports that isolated androgen-repressed LNCaP cell lines that grew faster in castrated than in intact hosts, and exhibited growth rates that were actually suppressed by androgens [75, 78]. Collectively, these preclinical data emphasize the potentially diverse biologic effects that androgens have on a heterogeneous and adapting tumor population and supports the concept of IAS.

Clinical studies of IAS

The intermittent regulation of serum testosterone levels for therapeutic purposes in prostate cancer was first attempted with cyclic administration of estrogenic hormone [42]. Therapy-induced impotency was reversed in 9 of 10 men within 3 months of the break in treatment. The first nonsteroidal antiandrogens became available around the same time as this study. Subsequently, most clinical research focused on the combined use of luteinizing hormone-releasing hormone (LHRH) agonists and antiandrogens. Little attention has been given to the reversibility of action of LHRH agonists, the significance of which is far-reaching. The potential for a full recovery from therapy makes it possible to alternate a patient between periods of treatment and no treatment. Furthermore, serial serum PSA measurements permit accurate monitoring of disease activity and serve as trigger points for stopping and restarting therapy.

The first report of IAS using reversible medical castration and serum PSA as trigger points in 47 patients were reported by Goldenberg and coworkers [31] in 1995, and updated to include 80 patients in 1999 [32] and 2002 [36]. Patients with a minimum follow-up of 12 months and either recurrent or metastatic disease were followed for a mean of 46 months. Mean initial serum PSA was 110 μg/l. Treatment was initiated with combined androgen blockade and continued for an average of 9 months. Since prognosis is poor in patients who do not achieve normal PSA levels after androgen ablation, only patients with PSA nadir levels below 4 μg/l were allowed as candidates for this IAS protocol. Medication was withheld after 9 months of therapy until serum PSA increased to mean values between 10 and 20 μg/l. This cycle of treatment and no treatment was repeated until the regulation of PSA became AI. The first two cycles averaged 18 months in length with 45% of the time off therapy, while the third cycle averaged 15.5 months. Serum testosterone returned to the normal range within a mean of 8 weeks of stopping treatment. However, the return to baseline testosterone was delayed with advancing age and use of 3- and 4-month gonadotropin-releasing hormone depot formulations. The off-treatment period in all cycles was associated with an improvement in sense of well-being, and the recovery of libido and potency in the men who reported normal or near-normal sexual function before the start of therapy. No adverse effect of IAS on time to AI progression or survival was apparent.

Since these initial reports, others have confirmed the feasibility of IAS in patients with advanced or recurrent prostate cancer [3, 8, 19, 34, 35, 43, 55, 65] (Table 1). Each of these small series collectively illustrates that relatively short on-therapy periods may result in off-therapy intervals of several years in some men, and suggests that continuous androgen ablation may represent over-treatment in a proportion of men. Most studies use a 6–9-month treatment 'on' cycle based on time to PSA nadir data [26, 28]. Although the optimal time remains undefined and empirical, time off therapy should be long enough to permit normalization of improved quality of life and testosterone-induced tumor cell differentiation. In general, in patients with metastatic disease and high pretreatment PSA levels, therapy is restarted when PSA increases to 20 μg/l; in patients with locally recurrent disease and moderately elevated pretreatment PSA levels, therapy is restarted when PSA reaches 6–15 μg/l, and earlier for postradical prostatectomy recurrences.

Several large phase III trials are underway comparing continuous versus intermittent therapy. Both quality of life and cancer outcome events are being measured. For example, South-Western Oncology Group (SWOG) 9346 (National Cancer Institute of Canada PR.8) is enrolling newly diagnosed M+ prostate cancer. This group recently reported some early data on PSA normalization rates. PSA fell into the normal range during the induction (first) cycle in 84% of 527 patients. Target enrollment of 1,360 patients should be met by 2006. National Cancer Institute of Canada PR.7 is comparing IAS versus continuous androgen suppression in 1,350 men with PSA recurrence after radiotherapy. These two trials will help to determine whether intermittent therapy is equivalent to, better than, or worse than continuous therapy. Until that time, intermittent therapy should be considered investigational.

Combination multitargeted strategies to delay progression

The main obstacle to improved survival of advanced prostate cancer is our failure to prevent its progression to its lethal and untreatable stage of androgen independence. If we are to have a significant impact on survival, new therapeutic strategies designed to prevent AI progression must be developed. Characterization of changes in gene-expression profiles after androgen ablation and during progression to androgen independence suggest that the various therapies used to kill neoplastic cells precipitate changes in gene expression that lead to the resistant phenotype. Complexities of tumor heterogeneity and adaptability dictate that elimination of all cancer cells requires multitarget systemic therapies. We hypothesize that integration of combination therapy, including docetaxel-based chemohormonal regimens and biologic agents targeting cytoprotective or signal transduction pathways, will inhibit the emergence of the AI phenotype, and thereby delay AI progression and prolong survival (Fig. 2).

The role of systemic chemotherapy for prostate cancer is presently limited to the setting of symptomatic hormone-resistant prostate cancer (HRPC). Two recent randomized trials have shown a small but consistent survival advantage for docetaxel-based chemotherapy in this setting [57, 68]. These developments, coupled with the observation that hormonal therapies alone have an invariably finite efficacy, have led to further studies

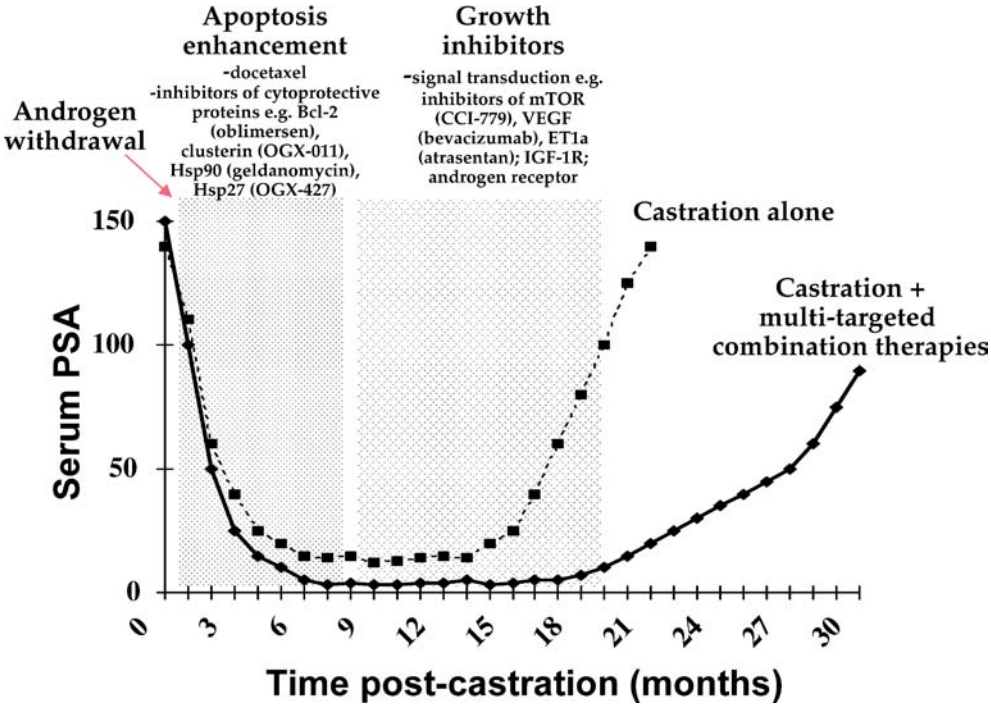

Fig. 2 Illustrative example of how integration of targeted biologic agents with active cytotoxic agents such as docetaxel into cycles of intermittent androgen suppression (IAS) may prolong the length of each IAS cycle by enhancing castration-induced apoptosis and or inhibiting activation of alternative growth factor signaling pathways

aimed at demonstrating the effectiveness of chemotherapy given earlier in the course of the disease and in combination with hormonal therapies, for example in the neoadjuvant setting [30] with or without androgen ablation and in combination with androgen ablation postoperatively (Radiation Therapy Oncology Group 99-02 and SWOG S9921).

While the evaluation of therapeutic regimens aimed at better disease control is essential, the necessary preclinical data supporting the combination of androgen withdrawal and cytotoxic chemotherapy for prostate cancer are limited. Furthermore, clinical trials in the setting of breast cancer treatment have failed to demonstrate any significant improvement in disease-free or overall survival for the use of combination chemo/hormonal therapy, and have shown a superiority of sequential treatment when compared to combination therapy [2, 4, 11]. Recent preclinical studies in our laboratory tested the effect of taxane-based chemotherapy given either pre-, concurrent with, or postcastration in an animal model of prostate cancer, and to characterize chemotherapy-induced changes in gene expression to understand better the biologic basis for different responses observed. In Shionogi and LNCaP models of prostate cancer, simultaneous androgen deprivation plus paclitaxel proved to be more effective than sequential treatment (Fig. 3). These findings provide preclinical proof-of-principle for ongoing clinical trials addressing the role and timing of systemic therapies in prostate cancer.

A markedly diminished tumor regression observed upon castration of paclitaxel-treated mice was also observed. One potential explanation for this finding is that paclitaxel treatment induces a stress response and may result in the upregulation of genes involved in antiapoptosis and/or androgen independence. It has already been demonstrated, for example, that paclitaxel treatment can induce Bcl-2 protein phosphorylation, and that clusterin and hsp27, both stress-associated cytoprotective proteins are upregulated by chemotherapy and known to play a role in the progression to AI growth. RT-PCR analysis of paclitaxel-treated LNCaP cells demonstrated a significant upregulation of bcl-2, bcl-xl, clusterin and hsp 27. These results all lend credence to the hypothesis that chemotherapy of hormone-naïve cancers can induce or select for the AI phenotype, and illustrate once again that our treatments can induce the upregulation of survival genes that can, in turn, become therapeutic targets.

Improving chemo- and hormonal therapies by targeting cell survival genes using antisense oligonucleotides

Research during the past decade has identified many gene products that may promote progression and resistance by inhibiting apoptosis. Of special relevance to the development of AI progression and HRPC are those survival proteins upregulated after apoptotic triggers such as androgen ablation that function to inhibit cell death. Proteins fulfilling these criteria include antiapoptotic members of the Bcl-2 protein family, clusterin, Hsp27 and IGFBP-2 and IGFBP-5 (reviewed in 29). Targeting genes upregulated after chemotherapy or androgen withdrawal that function to

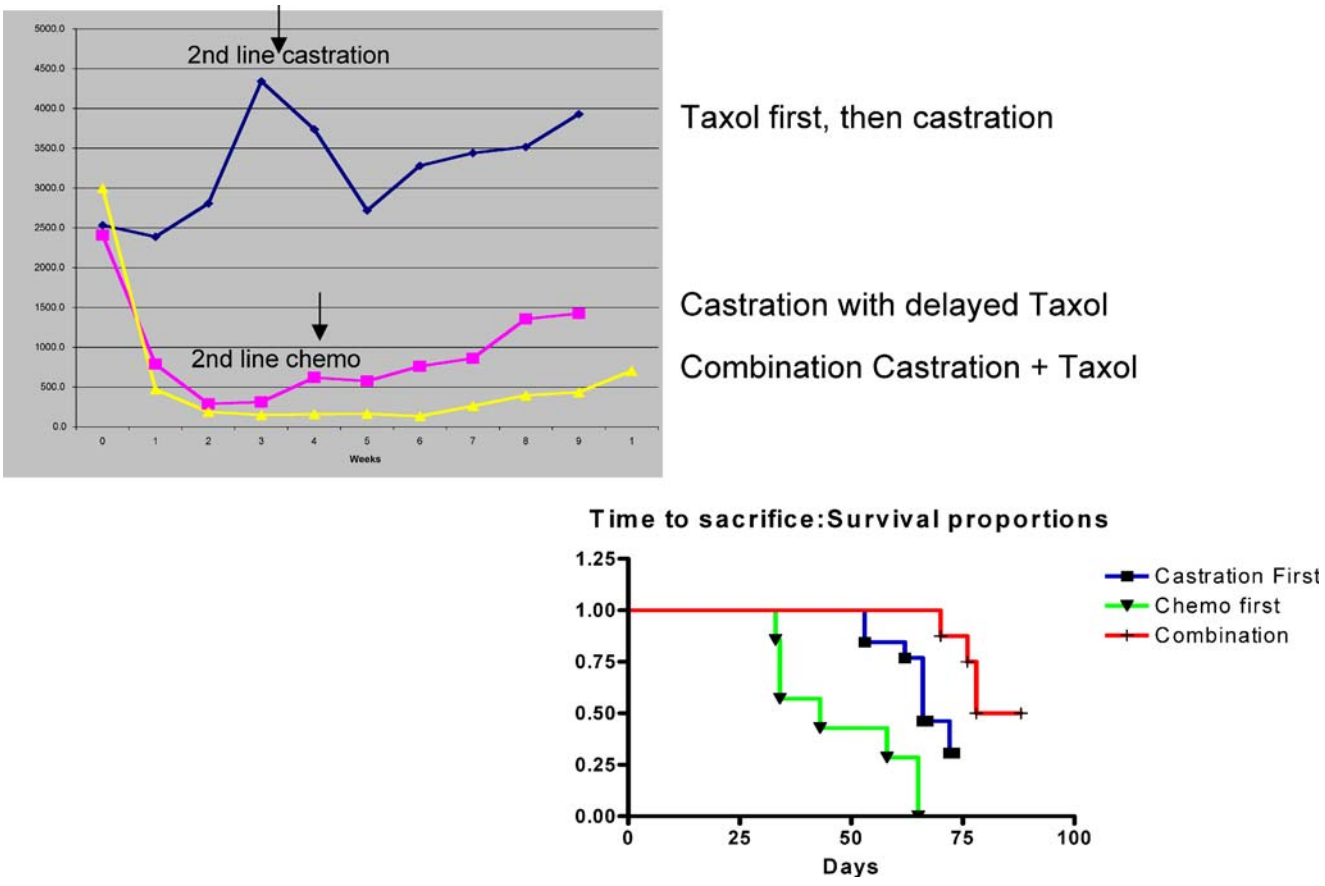

Fig. 3 To assess the effects of simultaneous chemo-hormonal therapy compared to sequential chemo-hormonal therapy on tumor growth in vivo, intact male mice bearing Shionogi xenografts were divided into three different treatment groups. Mice treated with simultaneous chemo-hormonal therapy had a more pronounced tumor regression and longer time to recurrence than either of the sequentially treated groups

either prevent castration-induced apoptosis or activate alternative growth-factor pathways may prolong time to overt recurrence.

Bcl-2

Bcl-2 belongs to a family of related genes whose proteins regulate a final common pathway regulating programmed cell death in both normal and abnormal cell populations. Bcl-2 levels increase after androgen withdrawal and in HRPC, which supports the hypothesis that Bcl-2 expression confers resistance to androgen withdrawal by blocking the usual apoptotic signal from androgen manipulation [27, 59]. One method to inhibit Bcl-2 function is to block translation using antisense oligonucleotides (ASOs) [21, 29]. Several small molecule inhibitors of Bcl-2 are now in preclinical development. Bcl-2 ASOs have also shown good hormone or chemo-sensitization activity in various preclinical models systems [27, 47, 48]. Oblimersen sodium (G3139, Genasense, Genta Inc.), is an 18-mer phosphorothioate ASO complimentary to the first six codons of the initiating sequence of the human bcl-2 mRNA. In preclinical prostate cancer models, oblimersen inhibited expression of bcl-2, delayed AI tumor growth, and enhanced the effects of chemotherapy by increased apoptosis. Oblimersen has moved forward into advanced clinical trials based on promising activity in preclinical models systems. Several trials in HRPC demonstrated that standard doses of docetaxel or mitoxantrone could be delivered with oblimersen without apparent increased toxicity, and when used in combination with docetaxel produced a promising 55% PSA response rate [13, 14, 20]. Based on these phase II data, a randomized phase III trial was scheduled to begin in 2005, but with the negative results from trials in melanoma and myeloma, and the subsequent breakup of the Aventis/Genta partnership, this trial has been put on hold. Issues persist about the dosing and regimen of this first-generation ASO, and whether 6 days of 7 mg/kg/day treatment is enough to suppress target sufficiently.

Clusterin

The clusterin gene on chromosome 8 encodes a chaperone protein involved in numerous physiological processes. Also known as testosterone-repressed prostate message-2 (TRPM-2), or sulfated glycoprotein-2,

clusterin is associated with numerous tumors including prostate, neuroblastoma, breast, lymphoma, urothelial and renal cell carcinoma, and with various pathological conditions including Alzheimer's Disease and nephrotoxic injury (reviewed by Wilson and Easterbrook-Smith [76]). Clusterin levels increase dramatically during castration-induced apoptosis in rat prostate epithelial cells, and in many hormone-sensitive prostate and breast cancer xenografts. In human prostate cancer, clusterin levels are low or absent in most untreated hormone-naïve tissues, but increase significantly within weeks following neoadjuvant hormone therapy [41]. Clusterin binds to a wide variety of biological ligands and is regulated by transcription factor heat shock factor 1 (HSF1). This has led to the view that clusterin functions similarly to heat shock protein (HSP), i.e., to chaperone and stabilize conformations of proteins at time of cell stress. Indeed, clusterin is more potent than other HSPs at inhibiting stress-induced protein precipitation.

Experimental and clinical studies associate clusterin with the development of hormone and drug resistance, and it has been shown to have a protective role against apoptotic cell death from androgen withdrawal, chemotherapy and radiation [49, 50, 52, 77]. OGX-011 (OncoGeneX Technologies Inc.) is an ASO complementary to the clusterin mRNA. OGX-011 incorporates a phosphorothioate backbone with second-generation chemistry in the form of 2′-O-methoxyethyl (MOE) modifications to the four bases on either end of the 21-mer molecule. Such "MOE gap-mer" modifications maintain the improved tissue pharmacokinetic profile of the second-generation chemistry, but preserve high affinity for target mRNA and recruitment of RNase H necessary for activity [77]. In preclinical models, OGX-011 improves the efficacy of chemotherapy, radiation and androgen withdrawal by inhibiting expression of clusterin and enhancing the apoptotic response. Furthermore, because of the second-generation chemistry and enhanced tissue half-life of OGX-011, more relaxed dosing schedules are possible while maintaining biological efficacy of target inhibition. OGX-011 recently completed two phase I trials administered weekly as a single agent or in combination with docetaxel. The single-agent study has a unique design in that patients with localized prostate cancer are treated with the OGX-011 prior to radical prostatectomy, allowing determination of an optimal biologically effective dose and tissue drug levels in addition to the usual parameters of toxicity [15]. Twenty-five patients were enrolled to six cohorts with doses of up to 640 mg OGX-011 delivered. Toxicity was limited to grades 1 or 2, including fevers, rigors, fatigue and transient elevations in alanine and aspartate aminotransferases. Prostate tissue concentrations of OGX-011 increased with dose, and tissue concentrations associated with preclinical effects could be achieved. Dose-dependent decreases in prostate cancer cell clusterin expression were observed. At 640 mg dosing, clusterin mRNA was decreased to a mean of 8% (SD = 4%) compared with lower dose levels and historical controls as assessed by RT-PCR of microdissected cancer cells. The fraction of cancer cells staining with zero intensity for clusterin protein at 640 mg dosing increased to 54% from < 10% for lower dose levels and historical controls. Phase II studies of OGX-011 in combination with hormone and chemotherapy are underway in patients with prostate, breast and lung cancers.

Hsp27

Many components of survival and apoptotic pathways are regulated by molecular chaperones. HSPs have attracted much attention as new therapeutic targets for cancer, especially since the discovery and characterization of geldanamycin as an inhibitor of Hsp90 and the targeting of the clusterin gene, whose product has small HSP-like function. Hsp27 is an ATP-independent molecular chaperone highly induced during stress responses to interact with client proteins and prevent their precipitation. The cytoprotective effects of Hsp27 result from its chaperone function, direct interference of caspase activation, modulation of oxidative stress and regulation of the cytoskeleton [16, 56]. Higher levels of Hsp27 are commonly detected in many cancers including breast, ovarian and prostate, and is induced by hormone- or chemotherapy to inhibit treatment-induced apoptosis via multiple mechanisms [9, 44].

As an important regulator of cell survival and treatment stress at many different points along the apoptotic pathway, Hsp27 is now recognized as an important therapeutic target. Recently, Hsp27 ASO and siRNA targeting the human translation initiation site were reported to potently inhibit Hsp27 expression in human prostate PC3 cells with increased caspase-3 cleavage and apoptosis [60]. Hsp27 ASO and siRNA also enhanced paclitaxel chemosensitivity in vitro. Meanwhile, in vivo, systemic administration of Hsp27 ASO in athymic mice decreased PC-3 tumor progression and enhanced paclitaxel chemosensitivity. Overexpression of Hsp27 in human prostate LNCaP cells caused these normally androgen-dependent cells to become AI and more resistant to cytotoxic chemotherapy. These findings suggest that increased levels of Hsp27 after androgen withdrawal provide a cytoprotective role during development of androgen independence and that ASO-induced silencing can enhance apoptosis and delay tumor progression. A second-generation MOE gap-mer ASO targeting Hsp27 (OGX-427) is planned to enter clinical trials in solid cancers and multiple myeloma in 2006 (OncoGenex Technologies, Inc.).

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