Novel Therapies in Prostate Cancer

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1. Introduction

Prostate cancer is one of the most common tumors in men and the second leading cause of cancer mortality in Western countries [1].

The management of the disease mainly depends on the stage of the tumor. Organ confined cancers and to some extent, locally advanced disease can be potentially cured by radical surgery or radiation therapy with or without hormonal manipulations [2]. Advanced or metastatic prostate cancer is unfortunately incurable at the moment. Disease progression and finally cancer related death occurs in the great majority of the patients [3].

Initial management of these patients consists of medical or surgical castration and/or antiandrogen therapy. However, resistance to hormonal therapies occurs usually within 24 months. Hormone refractory prostate cancer (HRPC) is a progressive disease and median survival at this stage is approximately 12 months [4].

Available cytotoxic chemotherapy regimens provide palliation in HRPC however, no survival benefit is yet demonstrated even with the newer combinations [5].

Thus, new forms of treatment are desperately needed for these patients.

Increased understanding of the main molecular steps in initiation and progression of prostate cancer hopefully will open up new avenues in the management of prostate cancer.

2. Immunotherapy

There has been a resurgence of interest in immunotherapy of prostate cancer during the last decade and the role of immunotherapy has been explored by a number of studies.

One of the main strategies of immunotherapy is to enhance the activity of the immune system by hematopoetic growth factors. GM-CSF is a promising molecule since it is a potent enhancer of cellular differentiation and proliferation of various progenitors, including monocytes, lymphocytes, dendritic cells, and NK cells.

In an experimental protocol, the efficacy of granulocyte macrophage colony stimulating factor (GM-CSF) was investigated. Patients with HRPC were given systemic GM-CSF 250 μg/m^2 with two different schedules of drug administration. Both groups had a PSA response. However, response rate was higher in the second cohort who received an initial daily treatment for 2 weeks and then 3 days per week. All but one patient experienced a decline in PSA (median decline, 32%), however, a PSA decline greater than 50% and sustained for more than 6 weeks was seen in only one patient (>99% decline in PSA) with an improvement in bone scan lasting for more than 14 months [6].

Another study [7] looked at the biologic effects of GM-CSF as measured by prostate-specific antigen (PSA) kinetics in patients with serologic progression of prostate cancer after definitive local therapy. Patients with prostate cancer who underwent previous definitive surgical or radiation therapy with non-metastatic, recurrent disease with a rising PSA, were enrolled in this phase II trial. A total of 30 patients received 250 μg/m^2/day of subcutaneous GM-CSF on days 1–14 of a 28-day cycle. Three patients achieved a 50% reduction in PSA and the median PSA doubling time increased from 8.4 months to 15 months ($p = 0.001$), accompanied by a decrease in the median slope of the PSA versus time curve ($p = 0.004$).

In another phase II trial, GM-CSF therapy was given to 7 hormonally naive and 9 hormone refractory prostate cancer patients via subcutaneous injections 3 times a week for up to 6 months. Six patients in this study demonstrated a 10–15% decline in their baseline PSA levels which was maintained during the entire treatment period. Five of these 6 patients demonstrated

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a rise in PSA levels following the cessation of therapy. Side effects were minimal [8].

Dose and duration of treatment with GM-CSF appears to be important in terms of efficacy. In a phase II clinical trial for prostate cancer 44 patients received GM-CSF (75 μg/m^2/day for 7 days) in addition to DC pulsed with HLA-A2-specific prostate-specific membrane antigen (PSMA) peptides. One complete and 8 partial responders were identified among 44 patients who received GM-CSF (RR 20.5%), as compared to 2 complete and 17 partial responders among 51 patients who did not receive GM-CSF (RR 37%). Thus the dose and duration of the GM-CSF therapy used in this trial was not found to be effective [9].

Another immunotherapy approach utilizes dendritic cells (DC) to increase the presentation of antigens related to the tumor cells to activate the immune response. A phase II trial was conducted to assess the efficacy of infusions of dendritic cells and two HLA-A2-specific prostate-specific membrane antigen peptides (PSM-P1 and -P2) in a cohort of 37 patients with recurrent prostate cancer [10]. Six infusions of DC pulsed with PSM-P1 and -P2 at 6-week intervals were given which resulted in 1 complete (2.7%) and 10 partial responses (27%). This study suggested that DC-based cancer vaccines may provide an alternative therapy for prostate cancer patients whose primary treatment failed.

A recently introduced immunotherapy product (Provenge) consists of autologous dendritic cells loaded ex vivo with a recombinant fusion protein (prostatic acid phosphatase (PAP) linked to GM-CSF). Sequential phase I and phase II trials revealed that fever is the most common adverse event and 38% of the patients developed immune responses to PAP. More than 50% decline in serum PSA level was observed in 3 patients (9.6%), and another 3 patients had 25% to 49% decreases in PSA. The time to disease progression correlated with development of an immune response to PAP and with the dose of dendritic cells received [11].

In several small phase II trials, dendritic cells pulsed with HLAA2.1-restricted PSMA peptides were believed to have enhanced tumor killing, since they achieved a decrease in serum PSA levels [12,13]. Preliminary results of a phase II trial by Tjoa et al. showed that in patients with metastatic prostate cancer, immunotherapy using PSMA as an antigenic stimulant resulted in an 8% complete response rate (CRR) and 24% partial response rate (PRR). For hormone refractory prostate cancer, the CRR was 3%, and PRR was 27%, with median survival time of 20 months compared to historical survival of less than 6 months [13].

A phase I trial has also been reported with DCVax^{TM}-Prostate, a dendritic cell-based immunotherapy developed by Northwest Biotherapeutics. It is produced by using a patient's leukopheresed autologous peripheral mononuclear cells matured with IL-4, GM-CSF, and inactivated BCG, and subsequently pulsed with recombinant PSMA and then re-infused into the patient. Patients with androgen-independent low volume prostate cancer were eligible. The vaccination had minimal toxicity. Patients had a minimum follow-up of 6 months following initiation of vaccination. Twenty four patients received at least one of the planned four monthly intradermal injections. Analysis of study data revealed that 21% of the patients have completed 6 months of follow-up without disease progression, while another 6/19 (31%) had PSA increases (≥50% increase compared to baseline) with stable radiographic findings. The majority (80%) of the patients had significant humoral and/or cell-mediated responses to PSMA. The median time to progression was 20 weeks [14].

3. Gene therapy

Gene therapy involves the transfer of genetic material to tumor cells with subsequent expression resulting in a therapeutic response. It may be in the form of corrective gene therapy to restore the normal function of a deleted or mutated tumor suppressor gene or interfere with the increased activity of an oncogene. Genes that are transferred may kill the tumor cells with direct or indirect activity resulting in cytoreductive gene therapy. Lastly it can be in the form of immunomodulatory gene therapy. Unfortunately, all gene therapy approaches are of limited value at present due to lack of efficient delivery systems.

In a clinical trial using a replication defective adenoviral vector that encodes wt p53 gene the vector was injected into the prostate using a transperineal approach with transrectal ultrasound guidance. In 3 of 17 treated patients, a 25% tumor size reduction was seen by magnetic resonance imaging, and no serious adverse events were recorded [15].

Suicide gene therapy, an example of cytoreductive strategy, involves the transfection of a gene responsible for the production of an enzyme that converts an otherwise inactive substance (prodrug) into a toxic agent that kills the cancer cells. Most of the enzymes used in suicide gene therapy are not normally encoded by mammalian cells but originate from viruses, bacteria, or fungi, and thus, are foreign to the transfected malignant mammalian cell. The HSV-*tk* gene is such an

example and most often the vehicle to transfer this gene is a replication defective adenovirus. This is then followed by administration of agents, such as ganciclovir, acyclovir, or valacyclovir. These prodrugs are poor substrates for mammalian thymidine kinases, but are phosphorylated into effective cytotoxic drugs by HSV-*tk* [16,17]. The resulting molecules are nucleotide analogues, which are incorporated into DNA during cell division, leading to termination of DNA replication and cell death [18].

Immunomodulatory gene therapy was used in a study by Belldegrun et al. In their phase I study, utilizing a DNA-lipid complex encoding IL-2 in locally advanced prostate cancer, they demonstrated that over 50% of patients responded to therapy, with decreasing PSA levels and increasing levels of peripheral blood lymphocytes as well as lymphocyte infiltration of tumor at injection sites [19].

3.1. Antisense oligonucleotide (ASO) therapy

Modulation of genetic activity by ASO is another form of gene therapy. The design of ASOs depends on which target is aimed. Progression to androgen independent state in prostate cancer is a complex process and involves clonal selection [20], adaptive up-regulation of survival genes [21], transactivation of androgen receptor in the absence of androgens [22] and activation of growth factor pathways [23].

Targeting the molecules that promote the growth of tumor cells may enhance the efficacy of cytotoxic therapies. Available experimental data highly supports the notion that inactivation of up-regulated survival genes by ASOs would result in delay of disease progression and enhance the effectiveness of hormone and chemotherapy [24–26].

Pre-clinical studies investigating an antisense strategy to down-regulate molecules such as type I insulin-like growth factor receptor [27,28], MMP-9 [29], MDM2 [30], androgen receptor [31], Bcl-2 [32,33] and TRPM-2 [34] demonstrated prominent anti-proliferative activity in prostate cancer models and interfere with the progression of the tumors.

In a phase I clinical study, a phosphorothioate antisense oligonucleotide complementary to the bcl-2 mRNA open reading frame and mitoxantrone combination was tested in 26 HRPC cases. The dose of ASO ranged from 0.6 to 5.0 mg/kg/day and mitoxantrone from 4 mg/m^2 to 12 mg/m^2. No dose-limiting toxicities were observed. and 2 (7.7%) patients had >50% reductions in PSA levels [32].

A novel antisense phosphorodiamidate morpholino oligomer directed against c-*myc*, AVI-4126, inhibited c-*myc* translation in a sequence-specific manner in PC-3 androgen-independent human prostate cancer xenograft murine model and caused a 75–80% reduction in tumor burden. Phase I safety trials in humans via i.v. route of administration showed no toxicity or serious adverse events demonstrating that inhibition of c-*myc* expression by antisense phosphorodiamidate morpholino oligomer can be a promising new and safe therapeutic strategy for prostate cancer [35].

4. Growth factor related pathway inhibition

EGFR is known to be over-expressed and mutated in prostate cancer. Stimulation of this pathway enhances tumor cell mobility, adhesion and invasion [36].

EGFR expression is also associated with resistance to chemotherapy and hormonal treatment, thus a poor clinical outcome [2].

EGFR and c-erbB-2 expression were evaluated by immunohistochemistry in a consecutive series of 74 prostate cancer patients [37]. EGFR expression was found in 12 of 29 (41.4%) patients who underwent radical prostatectomy, and in 16 of 16 (100%) metastatic prostate cancer patients with resistance to hormonal therapy ($p < 0.005$). A significant association was found between EGFR expression and a high Gleason score ($p < 0.01$) and between EGFR expression and higher serum prostate-specific antigen values ($p < 0.02$) in all groups of patients. In a Cox multivariate analysis, the only parameter with an independent prognostic effect on disease-free survival was EGFR expression (relative hazard, 11.23; $p = 0.0014$).

Thus EGFR and related pathways appear as attractive anticancer targets.

Basically two types of therapeutic approaches have been developed to deal with the aberrant growth factor pathways. The first type involves the use of monoclonal antibodies or toxin-attached ligand conjugates targeting the cell surface receptors. The second type of therapy utilizes small specific molecules that target the tyrosine kinase component of the growth factors involved in intracellular signaling, including the EGFR as well as Ras, Raf-1, MAPK and MAPK/extracellular signal-regulated kinase (MEK).

A variety of antibody and related complexes are developed for clinical use which include IMC-C225 (Cetuximab). Small molecular inhibitors include Gefitinib (ZD1839; Iressa), OSI774 (Tarceva), CI-1033, PD182905 and PKI-166.

4.1. Cetuximab

This is a chimeric monoclonal antibody that inhibits EGFR signaling cascade by binding to the external

domain of the receptor. Fc regions of the antibody have been substituted with the equivalent human sequences to limit the generation of antibodies in the patient. In a phase I/II study where combination of cetuximab with adriamycin have been used, stable disease was observed in 38% of the patients after 4 courses of therapy in patients with HRPC [38].

4.2. Gefitinib

ZD1839 (Iressa^{TM}) is an orally active quinazoline-based inhibitor of the epidermal growth factor receptor (EGFR) tyrosine kinase, and blocks signal transduction pathways implicated in proliferation and survival of cancers. It has promising anti-tumor activity in pre-clinical models and patients with HRPC displayed responses at a certain level in early clinical trials. In a phase II trial either 250 or 500 mg of gefitinib was combined with docetaxel and estracyte [39]. PSA and pain response was similar at both dose levels of gefitinib (41.7%) and addition of this agent did not cause any increase in toxicity.

Another similar trial combined gefitinib with mitoxantrone and prednisone. A PSA response of >50% decline for at least 4 weeks was observed in 25% of the 250 mg arm and 23% of the 500 mg arm [40].

Dual inhibitor of both EGFR and HER2, GW572016, is another promising agent which entered clinical trials [41].

5. Endothelin axis

Functionally, endothelin-1 (ET-1) is a weak mitogen for prostate cancer cell lines but it enhances the effects of other growth factors [42,43].

ET-1 also inhibits apoptosis in prostate cancer cells when induced by a variety of stimuli, including paclitaxel and antibodies to the FAS ligand [44].

There are two types of receptors for ET-1. ET-A receptor antagonists blocks the effects of ET-1, while inactivation of ET-B receptor does not, suggesting that the effects may be mediated entirely through the ET-A receptor. Shortly after the identification of ET-1, a large number of endothelin receptor antagonists were developed. ABT-627 (atrasentan) was the first of the available agents to be used in the clinical setting for the treatment of patients with cancer.

Phase I trials were performed using Atrasentan in men with HRPC. A total of 22 men with prostate cancer were included and the most common adverse events were rhinitis, headache, asthenia and peripheral edema [45].

Interestingly, 68% of the men (15 of 22) experienced declines in PSA after 28 days of treatment, ranging from a 4% to 89% decrease from baseline. Also a significant improvement in pain score was achieved, as measured by visual analog scales.

These studies were followed by two phase II clinical trials in men with androgen independent prostate cancer. These were double-blind and placebo controlled, with men receiving either 2.5 or 10.0 mg atrasentan daily [46,47].

There was significant improvement in the pain score and quality of life of patients in receiving atrasentan. Stabilization in the rate of increase in PSA and improvement in some markers of bone metabolism were observed in the active drug arms.

The median time to clinical progression was significantly increased by atrasentan therapy (placebo 129 days, 2.5 mg was 184 ($p = 0.035$) and 10 mg 196 ($p = 0.021$) days, respectively). The median time to PSA progression was 71, 134 ($p = 0.13$) and 155 ($p = 0.002$) days, respectively. An estimation of 6 months disease free survival by the use of the Kaplan–Meier method was 35% for placebo, 53% for 2.5 mg ($p = 0.022$ versus placebo) and 54% for 10 mg ($p = 0.018$ versus placebo). Atrasentan did not increase the incidence of severe side effects and quality of life measurement demonstrated that atrasentan had a longer duration of treatment benefit compared to placebo.

6. Thalidomide

This drug is originally developed in late 1950s, resulted in severe congenital malformations with its use in pregnant women. It was later on demonstrated that thalidomide inhibits basic fibroblast growth factor (bFGF)- and VEGF-induced angiogenesis [48,49].

The role of angiogenesis in prostate cancer was documented in prostate cancer by the demonstration of microvessel density as a predictor of the extent of the tumor and prognosis [50].

In an open label randomized phase II clinical trial HRPC patients were treated with either low (200 mg/day) or high (up to 1200 mg/day) dose thalidomide [51].

A total of 63 patients with a median PSA of 326 ng/ml and median Gleason score of 8 were enrolled in the trial. A reduction of $\geq$40% in serum PSA levels was achieved in 28% of the cases. In the low dose arm 18% of the patients achieved a reduction of $\geq$50% in PSA. Two patients who received long term thalidomide therapy had improvement in bone scan lesions and their pain score.

In an attempt to further enhance the effectiveness of chemotherapy a randomized phase II trial of docetaxel with or without thalidomide was employed in patients with HRPC [52]. None of the patients received prior chemotherapy nor thalidomide were given docetaxel (30 mg/m^2, weekly for 3 weeks) either as single agent or in combination with thalidomide (200 mg/day, PO). Serum PSA response was identified in 35% of the docetaxel only group vs. 53% of the combination arm.

The results of early clinical trials are promising due to possible synergistic effects of thalidomide with antineoplastic agents. It has a low side effect profile and relatively low cost.

7. HER-2 (c-erbB-2), heat shock protein 90 and 17-allylamino-17-demethoxygeldanamycin

HER-2 over-expression is one of the abnormal genetic activities implicated in hormone-independent progression of prostate cancer. HER-2, by stimulating two parallel kinase cascades, the MAP kinase [53] and Akt pathways [54], promotes phosphorylation of the androgen receptor at multiple sites. This, in turn, results in a high transcriptional activity even in the presence of low androgen concentrations. Importantly, inhibition of either pathway alone fails to completely abrogate androgen receptor-driven transcription which suggests that at least a proportion of hormone refractory prostate cancers have established a self-perpetuating transcriptional loop, which promotes autocrine activation of the androgen receptor in the presence of castrate levels of androgen [55].

A novel small molecule, 17-allylamino-17-demethoxygeldanamycin (17-AAG), was reported to induce the degradation of the androgen receptor, HER-2 and Akt in prostate cancer xenografts, thereby inhibiting their growth [56]. This molecule has a novel target and a novel mechanism of action. It does not interact directly with HER-2, Akt, or the androgen receptor. Instead, the drug binds to and alters the activity of the molecular chaperone heat shock protein 90 (HSP90). Unlike other molecular chaperones, HSP90 does not participate in general protein folding but instead interacts with a certain set of signaling proteins, including HER-2 [57], Akt [58], Raf-1 [59], and the androgen receptor [60]. HSP90 interaction regulates the half-lives of the proteins that it interacts by forming conformation-dependent higher order chaperone complexes. 17-AAG stabilizes an HSP90 conformation that recruits co-chaperones and directs HSP90 bound proteins to the proteasome where they are degraded in a ubiquitin-dependent manner [61]. Thus, 17-AAG, by modulating HSP90 activity, promotes the destruction of key components of the dual kinase cascades that have been found to promote activation of the androgen receptor in advanced, hormone-refractory prostate cancer [62].

In addition, the androgen receptor itself is an HSP90 client protein, and it was demonstrated that 17-AAG is able to promote the degradation of both mutant and wild-type forms of the androgen receptor both *in vitro* and *in vivo* [56]. In light of its novel target and mechanism of action, the future clinical prospects of 17-AAG and other HSP90 inhibitors look promising. Initial studies strongly suggested the use of 17-AAG in hormone refractory prostate cancer, especially in the presence of HER-2 over-expression. Multiple sites of action are anticipated and 17-AAG should inhibit multiple kinase-dependent upstream signaling pathways that promote androgen receptor activation; the stability of both wild-type and mutated androgen receptor proteins; as well as the transcriptional activity of androgen receptors. Ongoing clinical trials will certainly provide more information about the effects of this agent.

Trastuzumab (herceptin) which is a humanized monoclonal antibody blocking the activity of HER2 receptor may also prove effective under the light of these findings.

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