Feature Article

Prostate Cancer Immunotherapy: Choices for Patients and Clinicians

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ABSTRACT

Once the standard treatments for adenocarcinoma of the prostate (surgery, hormones, radiation, and chemotherapy) fail to achieve a durable response, there are few, if any, effective options. Second-line therapies such as combination chemotherapy usually have little impact on disease progression. More and more medical professionals are increasingly choosing to follow novel methodologies, many of which are reviewed below, in response to advanced or relapsing disease. Despite the diversity of approaches, such methodologies share certain characteristics. Each seeks to take advantage of the body's natural antitumor immunity by stimulating antitumor responses beyond a threshold level needed for tumor regression or, at least, the slowing or stabilizing of progression. Immunotherapy is a broad topic; its targets are varied. Several therapies using components of cellular immunity are the focal point of much contemporary clinical research that already suggests the ability to improve disease-free or overall survival. In addition, immune cells, eg, dendritic cells and T lymphocytes, preserve an excellent quality of life for recipients. In vivo or ex vivo gene therapy—the modification of gene expression within an antigen-presented cell by the introduction of a vector, DNA, or RNA—has overcome many of the conceptual and technical hurdles impeding its development. Refinements involving gene delivery systems and target identification and characterization reflect the field's growth. Further, the monoclonal antibody approach is an established type of cancer immunotherapy now enjoying renewed interest. Advances in generating humanized or fully human antibodies, as well as novel moieties with which they can be coupled, bode well for enhancing their prospects for clinical benefit. Admittedly, much of the present work is limited to patients with advanced disease who are less likely to respond than healthier patients with earlier-stage disease. Nevertheless, the promise of effective immunotherapeutics for advanced prostate cancer is being met on several fronts. This review focuses on those approaches that have advanced (at least) to animal models.

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INTRODUCTION

Currently, both the medical and scientific communities are seeking better treatment options for men with prostate cancer—particularly men whose disease has progressed past the primary stage. This matter is of great consequence to the 20,000 Americans faced each year with newly diagnosed metastatic prostate cancer, many of whom will eventually die from their condition.[1] For most of these men, their cancer is an unstoppable progression, regardless of any initial response to primary hormone therapy treatment.[2] The numbers are ominous: for the year 2000, there were an estimated 180,400 new cases and nearly 32,000 deaths.[1] Prophylactic screening measures, such as digital rectal examination and serum prostate-specific antigen (PSA) determination, recently produced both a decrease in cancer occurrence and decreased mortality from early disease.[3] Improvements in early standards of care, predominantly radiotherapy, hormones, and surgery, decreased the number of men progressing to advanced disease. Still, fully one third of men with primary cancer will progress to metastatic disease for which no curative therapies exist. Furthermore, despite the improvement in mortality rates during the 1990s, deaths among African-Americans remain much higher as compared with whites, especially for those under 60 years of age.[4] Prostate cancer statistics for elderly African-Americans appear less alarming, but fewer live to an advanced age when the age-specific incidence of prostate cancer is much higher.

Prostate cancer is particularly troublesome when it comes to assessing treatment benefit. PSA, commonly used as a diagnostic indicator, also serves as a marker of

TALKING POINTS Physicians Pharmacy Formulary Cancer Nurses

Preliminary studies of immunotherapy for treating prostate cancer are promising.

The failure of second-line chemotherapy treatments to impact prostate cancer progression necessitates development of immunotherapies.

Immunotherapy research should include more basic studies of tumor immunology, as well as better coordination of dose regimens and treatment schedules.

Effective immunotherapies could improve both the quanity and quality of life for prostate cancer patients.

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response despite differences of opinion on what constitutes a meaningful decrease.[5,6] How the clinician defines progression profoundly impacts the course of treatment,[7] and there is intense debate involving classification. Prostate tumors are quite heterogenous and display widely disparate rates of progression.[8] Variable definitions of response[9] and a divergent patient population also contribute to the problem. Moreover, the lengthy nature of disease progression means that 10 years or more may be required to determine if one treatment has prolonged overall survival vs another regimen. One recent example is the 10-year study by Ragde et al,[10] who showed no statistically significant difference in treatment benefit in patients who received iodine-125 alone vs those who received iodine-125 with 45-Gy external beam irradiation. Still, an accompanying commentary declared that the duration of the study was inadequate.

STANDARDS OF CARE

Standard treatments already in widespread clinical use, such as hormone therapy, radiotherapy, chemotherapy, and surgery (prostatectomy and orchiectomy), have enhanced the quantity and quality of life for those with localized and/or hormone-sensitive prostate cancer. In contrast, men with metastatic and/or hormone-refractory prostate cancer (HRPC) do not enjoy the assurance of effective treatments. Surgery or radiation is contraindicated for patients with advanced disease. Maintaining testicular suppression yields minor improvement in survival.[11] Antiandrogen therapy, usually flutamide, nilutamide, and bicalutamide, often evokes only a transient decline in PSA, with a median duration of response of less than 5 months.[12,13] Estrogen therapy has achieved mixed results as a second-line hormonal agent.[14-16] Clinical responses using the opposite approach, antiestrogens, have response rates of less than 10%,[17,18] despite the abundant presence of estrogen receptors on most prostate cells.[19] Ultimately, the regrettable actuality is that many medical professionals are dissatisfied with the extent to which cytotoxic therapies have affected the natural course of advanced prostate cancer. In a recent example, the median survival following cytotoxic therapy of men with HRPC was reported to be less than 1 year, despite promising results with several agents, including mitoxantrone, estramustine, prednisone, and paclitaxel.[20-22]

RATIONALE FOR IMMUNE-BASED CANCER THERAPIES

Burnet's theory of immunologic surveillance in cancer provided the scientific genesis of the field of immunotherapy.[23] He hypothesized that lymphocytes acquired the ability to differentiate between self and non-self during their development. As a result, lymphocytes no longer reacted against self-molecules unless the latter were altered, such as occurs during neoplastic transformation.[24] Even today, immunotherapy remains predicated on a core set of fundamental beliefs: our immune system is able to recognize such differences; a biologic distinction exists between normal and cancer cells; and generating and promoting the degree of antitumor immunity might produce significant patient benefit.[25] Several of the crucial mechanisms involved in signaling pathways that are activated once T- and B-lymphocytes elicit antitumor immune reactivity have been resolved.[26-29] Many of the most hopeful approaches arising from advances in our fundamental understanding of antitumor immunity are quickly moving from the bench to the clinic.

DENDRITIC CELL THERAPY

Most dendritic cells (DCs) developing along specific pathways have unique immunoregulatory abilities, several of which make them arguably the most potent antigen-presenting cell (APC) in the immune arsenal. Immature DCs, or Langerhans' cells (LCs), residing in tissues are proficient in antigen capture,[30] which is logical considering their part in protective immunity. DCs not only uptake antigen, but migrate to and stimulate naïve T and B cells as well. During maturation, DCs lose the capacity for endocytosis, and the expression of adhesion and costimulatory molecules is upregulated.[31] Interestingly, DCs also appear to be able to help overcome some aspects of tumor escape from immune recognition, such as aberrant antigen presentation or immunosuppression,[32-34] by stimulating the innate, natural killer (NK) cell-mediated antitumor immunity. Non-major histocompatibility complex-restricted cytolysis occurs following direct DC-NK cell contact, indicating that DCs are intimately associated in the relationship between adaptive and innate immunity.[35]

Much of the initial work with DCs as prostate cancer vaccines used peptides and protein derived from prostate-specific membrane antigen (PSMA). PSMA is a type II transmembrane glycoprotein,[36,37] whose expression is abundant in, and highly restricted to, prostate cells.[38,39] Once the technical difficulties of growing sufficient DCs from prostate cancer patients were overcome,[40] a physician-directed Phase I clinical trial was initiated. Investigators demonstrated enhanced immunity in vitro following vaccination with autologous dendritic cells exogenously pulsed with two HLA-A2-restricted epitopes.[41,42] Using the modified criteria from the National Prostate Cancer Project,[9] therapeutic benefit was observed in a subset of patients with either HRPC or earlier localized disease.[43-46]

The trials using peptides are being followed up with FDA-approved studies using whole, recombinant PSMA protein osmotically loaded into DCs. The DCs are cultured from monocytic precursors for 6 days in granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4, then exposed overnight to bacillus Calmette-Guerin (BCG). BCG serves three important functions: it matures the DCs; acts as an adjuvant capable of nonspecific immunostimulation; and serves as a marker for immune monitoring. Men with HRPC and progressive disease are candidates for the study. They receive four monthly intradermal injections of between 5 and 20×10^6 DCs. This Phase I/II multicenter trial is well advanced, with over

25 patients already enrolled. Over 100 total injections have been given safely, with no serious adverse events reported. Although still preliminary, initial results strongly demonstrate the vaccine's ability to elicit both vaccine-specific cellular and humoral immunity in a majority of patients. Further, many subjects have seen a promising drop in PSA levels, as well as a stabilization of bone or soft tissue involvement.[47] A Phase III trial is expected.

Prostatic acid phosphatase (PAP) is a prostate-specific isoenzyme amid a heterologous group of acid phosphatases produced by prostatic cells. After the immunogenicity of PAP was established,[48] clinical trials were quickly undertaken. Burch and associates[49] used PA2024 antigen, a recombinant protein combination consisting of human PAP fused through its COOH terminus to the NH_2 terminus of GM-CSF. Patients were given two intravenous infusions of loaded antigen-presenting cells (only 18.6 ± 9.4% of the administered cells were $CD54^{bright}$, and this marker is not exclusive to DCs), followed by three subcutaneous injections of soluble antigen alone. Interestingly, more patients developed anti-GM-CSF than anti-PAP antibodies. Some patients demonstrated in vitro proliferation of T cells stimulated with the vaccine components. Yet, only 3 of 13 patients had decreases in PSA levels, and no clinical outcomes were reported. The booster vaccinations with soluble antigen alone were not beneficial. In a follow-up report, 3 of 31 patients achieved a 50% decline in PSA. Although time-to-disease progression was associated with DC dose and in vitro immunity to PAP, no other clinical data were reported.[50]

MONOCLONAL ANTIBODIES

Monoclonal antibodies are capable of producing cell death by activating the complement fixation pathway or antibody-dependent cell-mediated cytotoxicity (ADCC). The development of chimeric human/mouse and fully humanized mouse monoclonals over the past few years should overcome the human antimouse activity (HAMA) that proved the death knell for the so-called magic bullet cancer treatment of the 1970s and 1980s. Combining monoclonals with more specific molecules should improve the ability to compete with nonspecific antibodies to stimulate ADCC. Conjugation to more potent toxins and radioisotopes should enhance tumor penetrance and lethality.[51]

Since PSMA is largely membrane-bound, it is a promising target of antibody-based vaccines. A group of second-generation, humanized, and fully human monoclonal antibodies specific for protein conformational epitopes on the extracellular domain were recently produced. Flow cytometric analysis of several fully human monoclonals showed strong specific binding to live prostate cells and, consequently, recognition of native epitopes.[52] Another reagent, CYT-356, contains 7E11.C5, monoclonal reactive with an epitope on PSMA.[53] Generated from a hybridoma from mice immunized with a human prostate adenocarcinoma cell line,[36] CYT-356 is currently being investigated in several human studies as a potential agent for diagnostic imaging.[54] Quite recently, a construct consisting of an α-particle-emitting anti-PSMA antibody ([^{213}Bi]J591) significantly impacted tumor-free survival in an athymic nude mouse model.[55] Overall, PSMA is an ideal molecule for targeting prostatic cancer cells with antibodies and DCs alone or in combination with other modalities.[56] Lastly, the new discovery that PSMA is highly expressed in the neovasculature of a wide variety of malignant neoplasms[57] makes it a promising target of antibody-based therapeutics for many tumor types.

A series of clinical studies have been carried out with CC49, a murine IgG1 antibody recognizing TAG-72. TAG-72, a tumor-associated mucin, is expressed in a variety of adenocarcinomas, including prostate, breast, colon, and pancreas.[58] Following preliminary work in colorectal cancer, a Phase II study with ^{131}I-CC49 was initiated in 15 men with hormone-independent prostate cancer. They received 75 mCi/m^2 infusions of the radioimmunoconjugate, and although side effects were mild and transitory, no objective responses were realized.[58] Interferon-γ (IFN-γ) or tumor necrosis factor-α (TNF-α) was then added to stimulate the surface expression of tumor antigens. Slovin and associates[59] pretreated patients with an IFN-γ dose of 0.017 mg/m^2 for 7 days prior to ^{131}I-CC49 administration. A few subjects achieved the radiographic criteria for stable disease, although none reached a >50% PSA decrease. Adjuvant cytokine treatment with IFN-α resulted in somewhat improved outcomes. In a Phase II study using ^{131}I-CC49 in concert with IFN-α, participants were given four doses of the cytokine ($3x10^6$ IU) over the course of 8 days preceding the antibody administration. Although thrombocytopenia again proved the dose-limiting toxicity, there was none of the marrow suppression so prevalent when the radioconjugate was given as a stand-alone agent. In addition, minor treatment impact was noted: five of six subjects experienced pain relief and two patients also displayed some radiographic improvement.[60]

GENE THERAPY AND IMMUNITY

Gene therapy seeks to alter or elicit gene expression within a tumor or immune cell by the introduction of DNA or RNA. Although some consider this approach distinct from immunotherapy per se, in many instances the immune system is affected in either an afferent or efferent manner. The list of promising gene therapy targets is growing rapidly. Among these are mdm-2, a negative potentiator of p53, and cell adhesion molecules such as C-CAM161 and E-cadherin.[62] A novel approach is to use chemically inducible effector caspases to generate programmed cell death (apoptosis) in prostate cancer cells. Replication-deficient Adv vectors expressing caspase-1 or caspase-3—critical mediators of apoptosis—produced significant abrogation of tumor growth in the TRAMP-C2 murine model.[63] Li and associates[64] showed that adenovirally mediated overexpression of another proapoptotic molecule, Bax, produced strong antitumorgenicity in vivo. Coming from the standpoint of prolonging cell life, Pirtskhalaishvili et al[65] showed that DCs engineered to overexpress the

antiapoptotic protein, Bcl-x_L, enhanced their efficiency and produced a significant decrease in the growth of RM-1 murine prostate tumors. Studies utilizing such targets have not yet progressed into the clinic. Two recent reviews provide an excellent, in-depth update of gene therapy targets for prostate cancer.[66,67]

CYTOKINES AND EX VIVO GENE TRANSFER

Initially, cytokines were quite promising as individual antitumor agents until frequent and severe toxicities were observed in most early human trials. It was subsequently thought that the expression of such cytokines within gene-modified cells could still elicit the desired outcome without serious adverse effects. One of the most commonly studied cytokines for clinical development is GM-CSF,[68] first examined due to the abundant expression of receptors on the surface of prostate cancer cells.[69] The immunotherapeutic potential of GM-CSF was conclusively shown using the Dunning rat model in a series of animal studies.[70] In humans, GM-CSF as a stand-alone agent was recently used in Phase I and II clinical trials for men with progressive adenocarcinoma of the prostate in the face of androgen withdrawal. Transient malaise and fever were the only vaccine-associated toxicities observed. Only one patient achieved a robust, stable (14+ months) decline in PSA in concert with a decrease in tumor burden.[71] GM-CSF has also been used as part of an ex vivo gene transfer immunotherapy. Eight patients with adenocarcinoma of the prostate that was actually metastatic at the time of radical prostatectomy were enrolled.[72] Participants were given three to six vaccinations of $1x10^7$ or $5x10^7$ autologous prostate cells retrovirally transduced to produce GM-CSF (143–1,403 ng/10^6 cells). Following treatment, five of eight subjects seroconverted to a positive DTH test result when challenged with autologous tumor. Humoral immunity, as indicated by prostate tumor cell-specific antibodies, was also noted. Although the small number of subjects prohibited statistical analysis, a transient decrease in median PSA levels was observed after the first pre- vs posttreatment vaccination. Ultimately, all subjects had disease progression based on elevated PSA levels.

IFN-α is the other general biologic response modifier common to prostate cancer immunotherapy. From the mid-1980s to mid-1990s, three studies described the effect of low- (2.5–5 million U/m^2) and high-dose (10 million U/m^2) therapy of patients with stage D1 or D2 disease.[73-75] Similar to systemic IL-2 administration, toxicity limited efficacy in the two studies in which adverse events were reported. Chang et al[73] could only evaluate nine patients, and the trial was ended due to severe (grade 3 or 4) weight loss, fatigue, neurotoxicity, leukopenia, or gastrointestinal distress in 29 other participants. Moreover, only one partial response was obtained. Several years later, a Phase II study of 40 patients achieved a disappointing 5% response rate, and toxicity was again severe and frequent.[74] The outcome of subsequent studies combining IFN-α with agents such as 5-fluorouracil or retinoic acid, as well as those utilizing the closely-related IFN-β, were also discouraging.[75,76]

Not surprisingly, the huge increase in attention given IL-12, known to augment a wide range of immune functions,[77] has expanded into immune approaches to fighting prostate cancer. A single vaccination with an adenovirus expressing IL-12 yielded improved survival and reduced the number of murine lung metastases, using the poorly immunogenic orthotopic model, RM-9.[78] In a later report, an adenoviral vector coexpressing IL-12 and B7-1 (AdmIL-12/B-7) improved IL-12 secretion and B7-1 cell surface expression by RM-9 cells. This combination vector provided a greater survival advantage vs the AdmIL-12 vector.[79] Preclinical research involving IL-15, a pleiotropic cytokine critical in both adaptive and innate immunity, is planned since it has the beneficial function of enhancing NK cell-mediated antitumor immunity.[80]

Continued progress with newer viral delivery systems should provide additional proof-of-principle work in animal models, with clinical trials as the ultimate goal. Certain avipoxviruses, such as fowlpox and canarypox, have proven nonreplicative in mammalian cells (enhancing biosafety) and are somewhat less immunogenic.[81] A human study with a PSA-containing fowlpox vector has been discussed by the Eastern Cooperative Oncology Group.[82] ALVAC, a nonreplicating canarypox vector, was well tolerated in Phase I studies of several types of cancer,[83] and prostate cancer trials are being designed.

Cytokine gene therapy directed toward evoking antitumor immunity—as with cancer in general—still must surmount key problems. A threshold degree of gene expression needs to be maintained in a sufficient percentage of tumor cells for treatment benefit to occur. Critical abnormalities present in a majority of tumors still need to be identified and characterized. Such hurdles are not exclusive to prostate cancer, and it is hoped that advances in other cancers or infectious diseases will benefit this tumor type as well.

THE NEXT GENERATION OF PROSTATE CANCER TARGETS

Mucins are glycoproteins secreted by epithelial carcinomas, such as prostate, colon, ovary, and breast.[84-86] Even though their expression is not limited to the prostate, mucins such as MUC-1 and -2 are the target of intense clinical study because they are abnormally glycosylated in tumor vs corresponding normal tissue.[84] Moreover, immune recognition of tumor mucins is not restricted by the major histocompatibility complex (MHC).[87] A number of clinical studies were conducted with glycoprotein (MUC-1 and MUC-2) or carbohydrate targets (Globo H and GM2). Subjects with metastases and/or rising PSA titers were given five monthly vaccinations of a 32-amino acid oligomer from MUC-1. Anti-MUC-1 IgG and IgM titers rose considerably during the regimen, then decreased soon after. Despite PSA stabilization for most participants, only 2 of 20 subjects achieved biochemical and radiographic stabilization of their progressive disease. A follow-up study involving MUC-2 and Globo H also achieved a notable, albeit transient, decrease in PSA level and radiographic

progression. Larger Phase II/III trials are currently in progress.[86]

The advancement of immunotherapeutics directed against *HER-2/neu*, the gene product of the *erbB2/neu* proto-oncogene, followed several reports linking it with the androgen pathway that is so fundamental to prostate cancer progression.[88] Further, the expression of *HER-2/neu* was recently associated with the long-term outcome of those with both metastatic and localized prostate cancer.[89] It was shown that anti-*HER-2* antibodies suppressed tumor growth in nude mice,[89] and vaccination with helper peptides produced T-cell immunity in cancer patients.[90] As a result, clinical trials utilizing both humoral and cellular approaches are being advanced.

In the past few years, a number of prostate-associated antigens have been discovered and the list is steadily increasing. These include, but certainly are not restricted to, PSCA,[91-93] STEAP,[94] PART-1,[95] the PTEN tumor suppressor gene,[96] prostein,[97] PCGEM1,[98] and PSGR.[99] Sufficient experimentation has been completed with PSCA (prostate stem-cell antigen) to demonstrate its ability to evoke cytotoxic T cells in vitro[91] and antitumor activity in vivo.[92] To date, work suggesting the clinical value of most other prostate-associated genes has been limited to expression studies involving immunohistochemistry or mRNA analysis. Clearly, this is insufficient. When selecting a target for clinical development, it is essential to demonstrate that the particular gene chosen is a putative tumor-rejection antigen or, at least, immunogenic. Regardless of the extent of tumor reactivity shown via mRNA or protein analysis, this criterion remains valid because it is not realistic or desirable to bring every potential candidate to the clinic.

CONCLUSIONS

During the past two decades, biologic therapy has developed as an effective approach for improving the status and survival of prostate cancer patients, even for those with advanced disease. The two-pronged strategy of directly attacking the tumor and stimulating host antitumor immunity has achieved the goal of disease regression or stabilization, and significant advances in our fundamental understanding of tumor immunology and the immune system have made this possible. Also crucial was the evolution of biotechnology to the point where it became possible to manufacture large amounts of purified reagents, such as cytokines. Consequently, immunotherapy is cautiously and deliberately making its way to the patient's bedside along with the standard modalities of radiotherapy, chemotherapy, and surgery. Prostate cancer immunotherapy has progressed to large, multicenter Phase II and III clinical trials. Many more Phase I studies are under way or have been approved by the necessary regulatory agencies. Although most therapeutic agents have been evaluated by a limited number of clinical trials, the results are encouraging for their future clinical use. Continued clinical trials and basic research should provide insight as to the best methods of generating and prolonging antitumor immunity.

Immunotherapy has evolved from promising to beneficial in the treatment of many forms of cancer, and, in the realm of prostate cancer, preliminary studies are encouraging. The realization of stimulating a patient's natural immunity has produced clinical responses, and without the serious adverse events so common with chemotherapy and radiotherapy. It is much too early to draw conclusions on which forms of immunotherapy should be emphasized. There must be more coordination in dose regimens and treatment schedules so investigators can get vital information on how to optimally stimulate in vivo antitumor immunity. Currently, it remains difficult to compare even those trials using similar reagents. There should also be more basic research into tumor immunology, both in antigen discovery and in how to overcome the detrimental effects of a patient's age, prior therapy, and tumor burden on the immune system.[100] As with most cancers, defective antigen presentation and, consequently, recognition remains a conceptual Achilles' heel of immunotherapy.[101,102] Some argue that the therapies described in this review merely promote tumor escape from immune surveillance, thereby limiting their utility to the adjuvant setting with minimal residual disease.[103] It is fitting and proper that immunotherapy is challenged with such skepticism. Still, those biased toward refinements of standard treatments must recognize that a significant fraction of patients harbor dissatisfaction and regret with what is currently available.[104] Pessimism about the value of immunotherapy is prediction rather than prophesy—and what patient would turn down a safe, effective treatment to lengthen and improve life, even if it could not promise a cure? Who among us would reject the hope of a few extra years with less pain, which is what immunotherapy is offering with stronger and stronger evidence? Only future clinical studies will determine to what extent immunotherapy fulfills its promise or validates its critics. OS

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