

Impact of androgen-deprivation therapy on the immune system: implications for combination therapy of prostate cancer

Jeanny B. Aragon-Ching¹, Kirsten M. Williams², James L. Gulley³

¹Medical Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD, ²Experimental Transplantation and Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD, ³Laboratory of Tumor Immunology and Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD

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1. ABSTRACT

Prostate cancer is the most common non-cutaneous malignancy in American men. Standard therapeutic strategies for systemic disease include androgen-deprivation therapy (ADT) and chemotherapy, both of which are palliative. However, there is a growing interest in the use of immunotherapy for prostate cancer. Evidence suggests that ADT may 1) enhance lymphopoiesis and thus potentially improve immune responses to vaccine, 2) renew thymopoiesis and thus reverse age-induced thymic involution, 3) augment B-cell development, and 4) mitigate tolerance to prostate cancer antigens. Although no vaccines are currently approved for prostate cancer, there are many promising agents under investigation. This review focuses on recent findings on immune regulation by androgens and immune-system regeneration with ADT, with emphasis on the rationale for the combination of ADT and vaccines in the clinical treatment of prostate cancer.

2. INTRODUCTION

2.1 Overview of prostate cancer

Prostate adenocarcinoma is the second leading cause of cancer-related mortality and the most common noncutaneous malignancy in American men (1). It was estimated that 218,890 men would be diagnosed with prostate cancer and 27,050 would die from the disease in 2007 (2). About 91% of prostate cancer patients are diagnosed when their disease is in the localized or regional stage, 5% are diagnosed after the cancer has metastasized (distant stage), and the remainder are unknown (1). Primary therapy typically includes radical prostatectomy, external beam radiation therapy (EBRT), brachytherapy, or watchful waiting, depending on multiple factors such as age, Gleason scores, comorbidities, and patient preference. Prostate cancer patients who are among the nearly 40% who fail primary local therapy (surgery or radiation), as evidenced by rising prostate-specific antigen (PSA) (3-5), are increasingly being treated with ADT, although there are

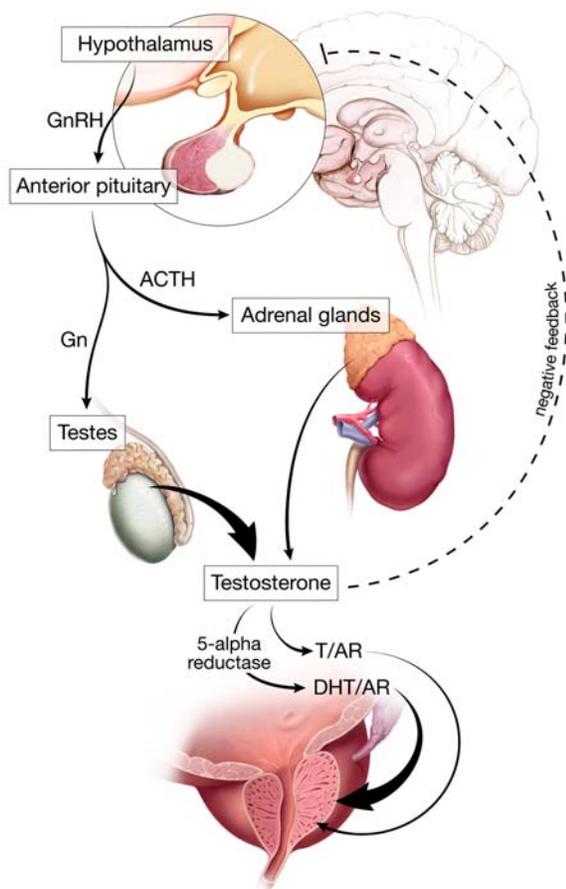


Figure 1. The Hypothalamic-Pituitary-Gonadal Axis. The hypothalamus/pituitary/testes endocrine loop is shown above. Gonadotropin-releasing hormone (GnRH) stimulates the secretion of gonadotropic hormone (Gn) from the anterior pituitary, and Gn in turn stimulates the production of testosterone. Circulating testosterone acts in a negative feedback loop to down-regulate the expression of GnRH. Adrenocorticotropic hormone (ACTH), also made by the pituitary, stimulates androgen synthesis in the adrenal gland. Testosterone (T) and dihydrotestosterone (DHT) bind to the androgen receptor (AR), causing increased expression of androgen-responsive genes and leading to cell growth.

no direct data demonstrating a survival advantage for ADT in this setting. In addition, ADT is frontline therapy for patients with metastatic disease and has been shown to improve survival as an adjunct to radiotherapy for high-risk patients (Gleason score ≥ 8 , stage $\geq T2c$), for patients with microscopic lymph node disease after radical prostatectomy, and as primary therapy for patients who are not candidates for local therapy (6). Unfortunately, nearly all prostate cancer patients develop disease progression, despite castrate levels of testosterone, within months or years after the initiation of ADT (7). While chemotherapy with docetaxel-based regimens has been shown to increase survival by 2 to 3 months in patients with androgen-independent prostate cancer (AIPC) (8, 9), to date no other therapy has been clearly demonstrated to improve survival

for patients with AIPC. As a result, novel therapies are needed to treat this prevalent and highly morbid disease. With the advent of new therapeutic vaccines for prostate cancer and emerging data on augmenting immune potential with ADT, the combination of ADT and immunotherapy may offer a tantalizing novel approach.

2.2. Overview of androgen signaling

To understand the rationale behind the use of ADT, one must first understand the effects of hormones on the prostate gland. Normal development of the prostate is dependent on endocrine stimulation, with testosterone being necessary for the prostate to develop into and remain a functional gland. Gonadotropin-releasing hormone (GnRH) is made in neurosecretory neurons in the hypothalamus (Figure 1). These neurons terminate in the hypothalamic-hypophysial portal system. This unique vascular bed shuttles the GnRH directly to the anterior lobe of the pituitary (which incidentally gets 90% of its blood from this venous plexus). Gonadotropin (also known as luteinizing hormone), produced in the anterior lobe of the pituitary, enters the circulation and subsequently binds to a specific high-affinity receptor on the plasma membrane of the testicular interstitial cells of Leydig. The end result of this interaction is an increase in production of testosterone by the testes.

Testosterone then enters the blood stream. Upon entering the cells of the prostate it may be converted by 5-alpha reductase into dihydrotestosterone (DHT). Either testosterone or DHT can bind directly to the cytoplasmic androgen receptor (AR) and initiate cell growth (DHT binds with higher affinity). The 10% to 15% of male androgens that come from the adrenal cortex play a lesser role in prostatic growth. While all these androgens are important for normal glandular growth, they also serve as strong growth factors for prostate cancer.

2.3. Androgen-deprivation therapy in prostate cancer

Ever since Huggins and Hodges established the link between androgens and prostate cancer 60 years ago (10, 11), ADT in the form of chemical or surgical castration has been a cornerstone of treatment for metastatic prostate cancer. The numerous clinical studies on the use of ADT in prostate cancer patients with different disease states and risk factors are reviewed elsewhere (6). Routine PSA testing after primary local therapy with surgery or radiation has helped to identify a greater number of patients with serologic or biochemical recurrence. A number of treatment options are available for these patients, including salvage radiotherapy or surgery (depending on the primary modality used), ADT, cryotherapy, observation, or enrollment in clinical trials with investigational agents. However, there is currently no standard of care defined for these patients. ADT, typically with GnRH agonists (GnRH-A), has been used in this treatment setting, but its true value is unknown because to date no large randomized trials for men with biochemical recurrence following local therapy have reported survival data. The presumed benefit can only be extrapolated from data on the use of ADT in patients at high risk for microscopic metastatic disease, in which the survival advantage of adjuvant ADT has been

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demonstrated. While immunotherapy combined with ADT is most promising for patients with early-stage disease, time to metastatic disease (or death) is a distant endpoint for these patients. Clinical trials in this patient population require large numbers of enrollees and long follow-up periods in order to document significant improvements.

2.4. Overview of immune system development: T and B cells

The immune system is composed of a variety of cell types whose function is to recognize and distinguish between foreign and self-antigens, and to eradicate destructive elements such as infections and tumors. Innate immunity, the body's first line of defense, is mediated by neutrophils, macrophages, and natural killer (NK) cells, and involves opsonization, phagocytosis, and release of mediators like cytokines, chemokines, and proteolytic enzymes. Importantly for antitumor responses, innate immunity signals the activation of acquired immunity. Acquired immunity, also known as active specific immunity, is mediated by B and T lymphocytes (12) and is subclassified as humoral and cellular immunity, respectively. In humoral immunity, B lymphocytes produce immunoglobulin, which activates the complement system and neutralizes the antigen, providing a major defense against bacterial infections. Cellular immunity, which is mediated by T lymphocytes, directly attacks invading antigens and is pivotal in defending against viruses, fungi, and some bacteria, such as the tubercle bacillus. Since B and T cells provide durable antigen-specific responses, these have been the target of research into antitumor immunity.

2.4.1. T-cell development

T cells are generated through 2 pathways: development in the thymus and peripheral expansion. Early T-cell progenitors migrate from the bone marrow to the thymus and undergo several sequential stages of maturation. T-cell receptors (TCRs) rearrange and generate a diverse array of TCRs that can recognize many different antigens. These can be defined by the presence of an episomal piece of DNA cleaved during the construction of the TCR, called T-cell receptor rearrangement excision circles (TRECs). The newly generated TCR undergoes positive and negative selection before the T cell migrates out of the thymus (Figure 2). Thus, T cells originating in the thymus have a diverse repertoire of TCRs. T cells produced by peripheral expansion are clones of their predecessor T cell and bear an identical TCR. In young children, the thymus is the primary pathway for T-cell generation. Thus, children have a diverse T-cell repertoire with the ability to recognize a wide array of antigens. However, older individuals acquire T cells predominantly through peripheral expansion. After cytoreductive therapies, adults reconstitute with a more restricted repertoire, nearly entirely the result of peripheral expansion.

The peripheral T-cell pool is diverse in young or older hosts whose immune system remains intact. Newly generated thymic T cells exit the thymus and enter the blood or lymphatic system where they are considered

“naïve” until presented with an antigen, which leads to activation, clonal expansion, and generation into effector T cells. Effector CD4⁺ T helper cells consist of subsets of TH1 and TH2 phenotypes, depending on their major cytokine profile. TH1 cells secrete IL-2 and gamma-interferon and are involved with cellular immunity; TH2 cells secrete IL-4 and IL-5 and interact mainly with B cells to affect humoral immunity. CD8⁺ T cells differentiate into functional cytotoxic T lymphocytes (CTL). Both effector T cells are important for host immune responses to tumor, particularly tumor-specific CTLs. Memory T cells, which persist along with memory B cells, readily convert to effector cells upon re-exposure to the initial antigen. Induction of specific immunity through the activation of T cells requires the presence of antigen-presenting cells (APCs), recognition of MHC by the TCR, and a second signal involving the ligation of costimulatory molecules (CD40, CD80, CD86) on the APCs to their respective ligands (CD40L for CD40, CD28 for CD80 and CD86), the absence of which can lead to T-cell anergy or apoptosis. Binding of CD80 or CD86 to CTLA-4 on activated T cells provides a negative feedback loop for regulating the immune response. These complex interactions between T cells and their receptors and costimulatory molecules are key factors in the development of vaccines that enhance immune responses.

Ongoing studies in autoimmunity and tumor immunology have uncovered various mechanisms involved in the recognition of self- and nonself-antigens, known as immune tolerance. Current evidence suggests that self- and nonself-antigens are copresented early in life, but T cells with potential reactivity for self-antigens are eliminated early in the process of T-cell differentiation due to tolerance (negative selection). Central tolerance for T cells occurs mainly in the thymus; tolerance for B cells occurs in the bone marrow (13). The ability of certain regulatory T cells (T_{regs}) to inhibit immune responses by blocking the activation or function of effector T cells (Figure 2) has been a topic of recent interest. It appears that the generation of T_{regs} is dependent on the transcription factor FOXP3 (14). Although the exact mechanism by which T_{regs} inhibit other effector T cells is yet unclear, this T cell-mediated suppression function appears to be one of the means by which tumor cells escape immune surveillance.

T cells play an essential role in immunizing the host against tumor antigens. Therefore, the vast majority of research to develop tumor vaccines has been geared toward enhancing cell-mediated immunity to tumors by up-regulating expression of costimulatory molecules, inducing cytokines, blocking inhibitory receptors, and stimulating T-cell proliferation.

2.4.2. B-cell development

B cells arise from progenitor hematopoietic stem cells and undergo a series of maturational steps within the bone marrow. Throughout the early stages of maturation, the developing B cell undergoes proliferative expansion in response to IL-7, produced by bone marrow stromal cells. The B cell's immunoglobulin receptor is generated during bone marrow development and formed through somatic

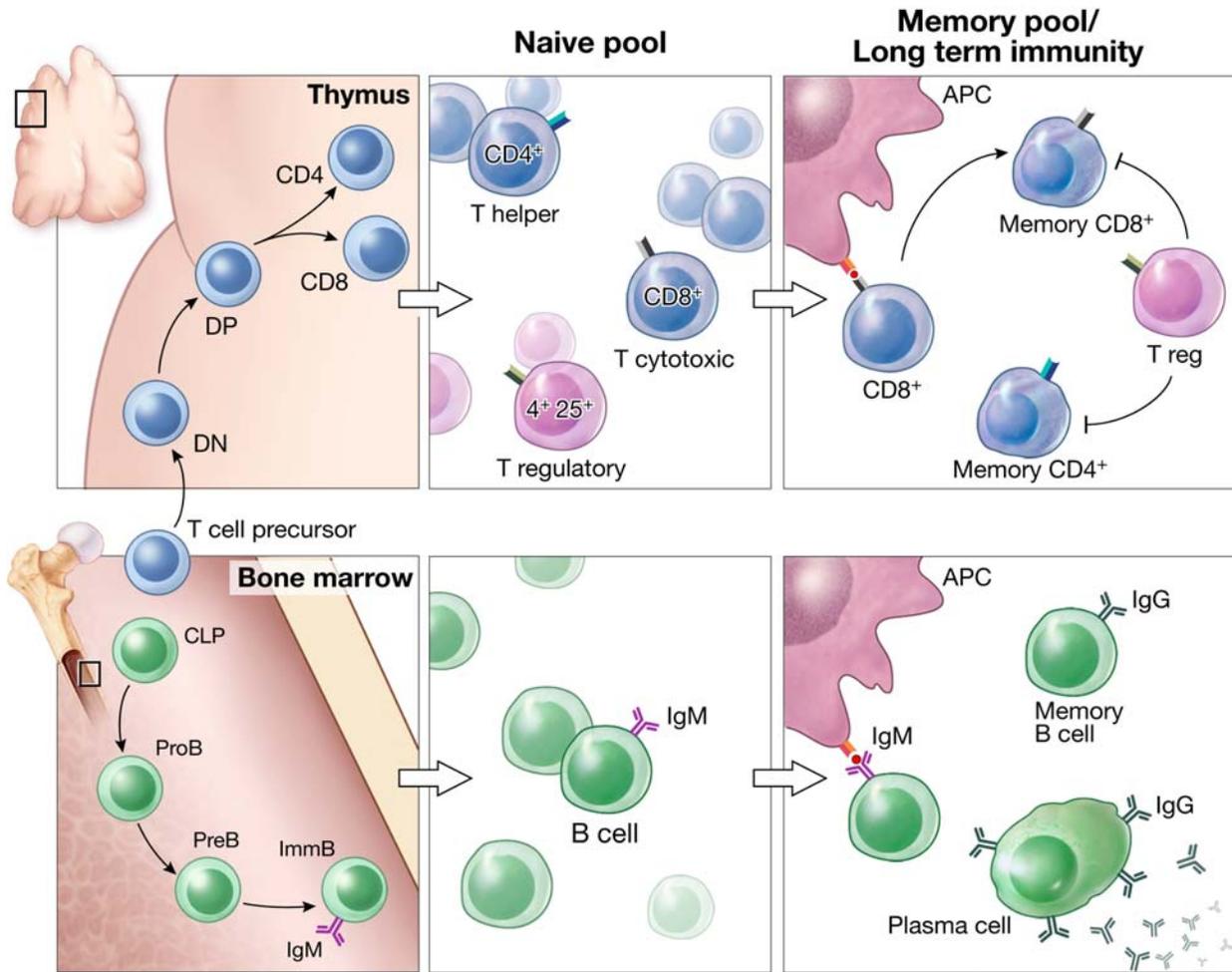


Figure 2. Overview of Immune System. Bone marrow-derived precursors enter the thymus (T-cell development) and undergo intrathymic development that includes sequential stages of phenotypic maturation, beginning with the DN (double negative) stage. This is followed by expression of CD4 and CD8 DP (double positive) thymocytes and subsequent positive and negative selection events, leading to the generation of MHC class I-restricted cytotoxic CD8⁺ T cells and MHC class II-restricted helper CD4⁺ T cells that exit to the periphery as effector T cells in the naïve pool. Memory T cells, as depicted in the memory pool, confer long-term immunity. T_{regs} characterized by surface expression of CD4+CD25⁺, some of which are generated from the thymus, are thought to suppress CD4⁺ and CD8⁺ T cells by unknown mechanisms. B-cell development in the bone marrow follows differentiation from common lymphocyte precursors (CLP) to the most immature B lineage-committed cells (pro-B) and immunoglobulin heavy chain gene rearrangements in pre-B cell stage. Naïve mature B cells emerge from the marrow bearing the IgM receptor and migrate to secondary lymphoid tissues, where they encounter antigens. Activated B cells secrete IgM and may isotype switch to IgG. Some will further differentiate into plasma cells, secreting antigen-specific IgG antibodies essential for long-term immune response.

recombination, whereby genes are rearranged to create diverse receptor sequences. Like T cells, B cells undergo positive selection through interaction with self-antigens presented within the marrow. If these naïve mature B cells bind the self-antigen too strongly, they are deleted or undergo a subsequent rearrangement to alter the receptor by receptor editing (15).

Naïve mature B cells emerge from the bone marrow bearing the IgM receptor and migrate to secondary lymphoid tissues. Once a B cell encounters an antigen, it has 2 important functions. First, the B cell becomes activated and begins to secrete IgM. Second, the B cell

processes the antigen for presentation to helper T cells. After T-cell stimulation, the B cell may isotype switch to express an IgG immunoglobulin both on its surface as a receptor and as a secreted antibody. This activated B cell may proliferate and form the basis of a germinal center within the secondary lymphoid tissues. The B cell receptor/immunoglobulin molecule can then undergo somatic hypermutation, increasing the avidity of the antibody/epitope interaction. Some of these cells will be retained as long-lived plasma cells, producing and releasing the specific antibody for a given antigen that can be detected in the serum, and demonstrating long-term immunity (16). B cells may therefore play 2 significant

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roles in tumor immunotherapy: presenting antigen for T cells and providing long-lasting protein recognition in the form of vaccine-induced immunity.

3. INFLUENCE OF ANDROGENS ON LYMPHOCYTE DEVELOPMENT AND ACTIVATION

3.1. Immune system subsets bearing sex steroid receptors

Expression of AR in thymocytes and thymic stromal and epithelial cells has been demonstrated in a number of assays, but not clearly established in peripheral T cells (17-22). Similar to T cells, AR has been demonstrated in both immature B cells and bone marrow stromal cells, but has not been consistently demonstrated in peripheral B cells (23-25). The paucity of evidence for existing AR in mature T and B lymphocytes suggests that the mechanism of ADT is likely on the developing lymphocyte—in the bone marrow for B cells and the thymus for T cells.

Although GnRH receptors have been demonstrated in the thymus and spleen in mice and on human peripheral T and B lymphocytes (26), GnRH action on these cells is not thought to be immune stimulatory. Therefore, ADT, which involves the administration of a large dose of GnRH, likely influences T- and B-cell development through the interaction of testosterone with the thymus (for T cells) and bone marrow (for B cells), rather than through direct action on T and B cells.

3.2. Effects of androgen deprivation on T cells

When androgen levels increase, such as during puberty or exogenous administration, the thymus rapidly involutes, suggesting a link between androgens and thymopoiesis. Androgens affect thymic epithelial or stromal cells directly, and thus indirectly influence thymocytes (17, 18, 27, 28). One hypothesis is that androgens cause thymic regression by stimulating secretion of factor(s) by thymic epithelial cells (29), as shown in a chimeric experiment using mice engrafted with testicular feminization (Tfm) bone marrow cells (30). Analyses of these data suggest that AR expressed by thymic epithelium play an important role in thymocyte development, and could explain why androgens induce apoptosis of thymocytes *in vivo* but not *in vitro* (31). In subsequent studies, androgen withdrawal led to increased thymopoiesis and reversal of thymic atrophy in post-pubertal male mice (32) and even in aged mice (33, 34). Furthermore, thymopoiesis decreased with the administration of testosterone (35, 36). Castration also results in increased T-cell export in aged mice and increased naive splenic T cells compared to aged controls (34).

Although persistent thymic function is evident in older individuals, it is decreased, as demonstrated by lower TREC levels (37). However, studies show that ADT can induce thymic renewal in older individuals (38). In one study, elderly prostate cancer patients given GnRH-A experienced a notable increase in TRECs in 6 out of 10

cases, indicating renewed thymopoiesis (34). These studies suggest that the effects of androgen ablation are not limited to the young, as evidenced by restoration of thymic function and export of naïve T cells after surgical (orchiectomy) or medical (GnRH-A) castration.

The enhanced thymopoiesis associated with ADT has important clinical implications for the treatment of immunocompromised patients and for immunotherapy for prostate cancer (see Figure 3 for a summary of ADT's effects on the T-cell compartment). Thymic renewal in these patients may increase the diversity of the T-cell repertoire, increasing the pool of antigens recognized by the immune system. In the setting of vaccine therapy, an increased naïve T-cell compartment may enhance the response to immunotherapy.

3.3. Effects of androgen deprivation on B cells

Androgen levels also affect the production of B cells. Studies in nonmammalian species showed deceleration of normal age-related bursal involution upon castration and reverse acceleration with testosterone implantation (39). In mice, expansion of the pre-B-cell population in the bone marrow is observed after castration (40, 41) and DHT supplementation may suppress B-cell precursors (42). In normal male mice, castration leads to a dramatic increase in IgM+ naïve splenic B cells (23, 41). Furthermore, this was reversed with testosterone supplementation, implicating androgens in naïve B-cell production and export. In a study of prostate cancer patients treated with GnRH-A, 5 of 12 patients (58%) showed decreased lymphocytes on day 7 during the time when testosterone levels transiently increase, but by day 28, 13 of 17 patients had increased lymphocytes compared to day 0 (77%) (43).

In sum, androgens can be manipulated to increase de novo T- and B-cell production (Figure 3), which may have implications for immunotherapy in prostate cancer patients on ADT. By increasing the number of naïve T cells from the thymus, ADT may be able to broaden the potential repertoire of the immune response. ADT may enhance production of newly generated, IgM+ naïve B cells from bone marrow. This enhanced B-cell generation post-ADT may improve antigen presentation and T-cell responses.

4. EVIDENCE FOR ANDROGEN DEPRIVATION IMMUNOTHERAPY IN PROSTATE CANCER

4.1. Evidence for T cell-directed immunotherapy in prostate cancer

Although the immune system is generally indifferent to the prostate, there is some evidence that prostate cancer can induce productive immune system responses with the addition of ADT. T cells likely show tolerance for prostate cancer because prostate-specific proteins are expressed in both nonmalignant specialized prostate epithelial cells and malignant prostate cells. Drake *et al.* created transgenic mice that expressed a model antigen in a prostate-restricted pattern (Pro-HA) and crossed them with TRAMP mice that developed spontaneous prostate cancer. They demonstrated that naïve

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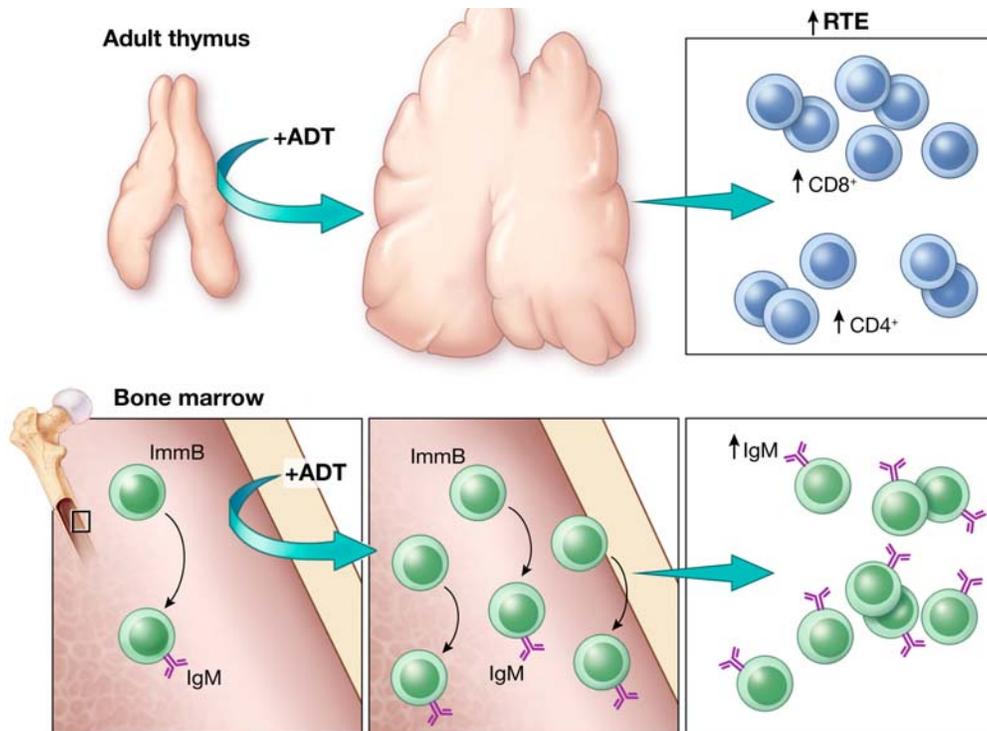


Figure 3. Effects of Androgen-Deprivation Therapy (ADT) on T and B Cells. ADT's effect on the T-cell compartment consists of thymic enlargement, with increased export of recent thymic emigrants (RTE). ADT's effect on the B-cell compartment consists of increased B lymphopoiesis.

prostate-specific CD4 T cells generally ignore a noncancerous prostate gland. However, in the Pro-HA mice crossed with TRAMP mice, naïve T cells were able to recognize the prostate gland, but the recognition was tolerogenic, leading to abortive proliferation, absence of effector function, and inability to mount an antitumor response following vaccination (44). ADT, on the other hand, mitigated immune tolerance of prostate tissue in mice with prostate cancer, allowing T cells to develop effector function in response to vaccination. Further evidence was provided by Roden *et al.*, who demonstrated an increase in T-cell proliferation in response to CD28-mediated costimulation after androgen deprivation (45).

Similar evidence of prostate-directed immune response has been seen in humans. Mercader *et al.* demonstrated that ADT induced T-cell priming to prostatic antigens. They noted T-cell infiltration of benign human prostates and prostate tumors after androgen ablation (46). The use of ADT in inducing trafficking of activated T cells to the area of prostate tumor bears important implications in tumor immunity since this represents a potential for increased antigen presentation, as evidenced by concomitant increase in the tissue levels of several APCs such as macrophages and dendritic cells. This T-cell infiltration is apparent after 1 to 4 weeks of treatment and is composed predominantly of CD4+ T cells and comparatively fewer CD8+ T cells. Increased T-cell response following ADT may be related to enhanced APC-mediated prostate antigen presentation (47-50). The role of

CD4+ T cells in promoting successful antitumor response has been demonstrated (51, 52) and underscores the importance of not only CD8+ CTLs in lysing target tumor cells, but the role of CD4+ T helper cells in engaging with APCs and thus activating the CTLs.

Given that augmentation of CD8+ T lymphocytes is important for homing of antigen-specific CTLs (53), strategies that enhance CD8+ T lymphocytes have become a principal focus of prostate cancer research. This is exemplified by adoptive immunotherapy, whereby activated CD8 T cells are generated *ex vivo* and reinfused to generate antitumor response (54).

The role of CD8+ CTLs in antitumor immunity has been demonstrated by the fact that depletion or loss of CD8+ T cells renders vaccines ineffective in eliciting antitumor response (55, 56). In prostate cancer, several studies have shown that vaccines can generate specific CD8+ T-cell responses (57) and that specific CTLs are able to kill autologous prostate cancer cells (58). The efficacy of these CTLs may depend more on the avidity of the CTLs induced than their quantity (59). Therefore, strategies that enhance antitumor immune responses by inducing higher-avidity T cells through costimulatory signals or prime-and-boost vaccine regimens may bring about more efficient tumor cytolytic activity.

However, CTL response and proliferation may not be the only means of augmenting host immunity to

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tumors; decreasing T_{regs} may be another strategy. Accumulating evidence suggests that immunologic self-tolerance may also play a role in tumor recognition or surveillance. In normal mice and humans, maintenance of peripheral tolerance is mediated by the T-cell subpopulation of CD25+CD4+ T (T_{reg}) cells, which constitute about 5% to 10% of peripheral CD4+ T cells (60). It has been demonstrated that T_{regs} suppress the activation of other CD4+ or CD8+ T cells upon APC processing (61). Removing T_{regs} can therefore enhance immune response to nonself-antigens, such as tissue grafts (62), or elicit autoimmunity (63). Removing T_{regs} has also been shown to improve immune recognition and clearance of autologous tumors *in vitro* and *in vivo*, leading to the spontaneous development of tumor-specific and -nonspecific effector cells in otherwise nonresponding individuals (60). T_{regs} are found in a variety of solid tumors (64, 65) and are associated with poor prognosis (66, 67). In a randomized trial done in 12 healthy men treated with GnRH-A or GnRH-A plus testosterone, results showed that androgen deprivation was associated with a significantly reduced percentage of CD4+CD25+ T cells (68). Although this may in part reflect a dilution of T_{regs} , it is possible that even a relative decrease in T_{regs} compared to conventional T cells would enhance immunotherapy.

These studies demonstrate the diverse effects of androgen ablation in the T-cell compartment. There is mounting evidence that androgen ablation is a potentially viable approach for activating the immune response against certain tumor antigens by increasing naïve T cells, as demonstrated by TREC analysis, with concomitant enhanced infiltration into prostate tumors and a potential relative decrease in T_{regs} .

4.2. Evidence for B cell-directed immunotherapy in prostate cancer

Although most of the available literature in this field addresses the effects of T cell-directed immunotherapy, a number of studies suggest a promising role for B cell-directed immunotherapy. In a study of 25 metastatic prostate cancer patients with lymph node involvement compared to 26 control patients, histopathologic analysis of the lymph nodes showed decreases in CD20+ B lymphocytes, CD38+ activated lymphocytes, and CD68+ macrophages, with reduced follicular and sinus hyperplasia and fibrosis (69). Several studies have shown that vaccines can elicit specific antibodies against tumor antigens, demonstrating a B-cell response (57, 70). One study showed that standard hormonal and radiation therapy can elicit tumor-specific autoantibody response (71). Seventy-three men with nonmetastatic prostate cancer and 50 controls were evaluated with western blot and antigen array to determine whether autoantibody responses occurred during the course of treatment with neoadjuvant hormonal therapy, EBRT, and brachytherapy, compared with patients undergoing radical prostatectomy and controls. Treatment-associated autoantibody responses to tumor antigens developed in patients undergoing neoadjuvant hormone therapy (7 of 24 patients, 29.2%), brachytherapy (5 of 20 patients, 25%), and EBRT (4 of 29 patients, 13.8%). Responses arose early

and were durable in most cases. Although the exact mechanism for the emergence of immunogenicity following hormonal or radiation therapy is unclear, these findings suggest a potential role for manipulating humoral immunity to target prostate cancer-specific tumor antigens.

5. VACCINE THERAPY FOR PROSTATE CANCER

Immunotherapy in prostate cancer is an active field of investigation, with an increasing number of trials utilizing a variety of approaches. The concept of using immunotherapy to treat cancer initially derived from studies on experimental grafts of chemically induced tumors in syngeneic mice (72, 73). Potent antigens identified by the immune system stimulate CTLs. These antigens are peptide fragments of intracellular proteins transported to the endoplasmic reticulum, bound to major histocompatibility complex (MHC) molecules type 1 or 2, depending on the cell type, then carried to the cell surface (74). Any intracellular protein expressed by a tumor can be a potential target antigen that can be used for T cell-based immunotherapy. In prostate cancer, PSA, a 34-kD cell-surface protein located in the acini and ducts of the prostate, represents one such target (75). Though PSA is secreted in increased amounts by a prostatic tumor, it is also present in the normal prostatic epithelium. The goal, therefore, of developing PSA-based vaccines is to induce antiprostata cancer immunity by overcoming immune tolerance against normal prostate cells, which could obviate the host immune response (76). Evidence shows that circulating CTLs specific for PSA-derived peptides can be increased by immune stimulation (77). Vaccine strategies that would enhance response against PSA and other targets, such as prostate-specific membrane antigen and prostatic acid phosphatase (PAP), are thus an attractive approach.

The different approaches to and clinical outcomes of therapeutic vaccines in prostate cancer are reviewed elsewhere (78, 79). We focus here on the various prime-and-boost strategies for prostate cancer vaccines currently in use and how these strategies can be enhanced by the addition of other therapies, including ADT. Different approaches for delivering vaccine have been explored, each with advantages and disadvantages, including dendritic cell-based vaccines, DNA and recombinant protein vaccines, recombinant viral vector vaccines, antibody-based vaccines, and whole tumor-cell vaccines (79). Adjuvants such as Bacillus Calmette-Guerin (BCG), granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-2, and costimulatory molecules have also been examined for their ability to enhance T-cell responses (80, 81).

Poxviral vectors infect a wide range of cells and can contain multiple transgenes, making them an attractive vector for therapeutic vaccines. Two of these viruses, vaccinia and fowlpox, have been widely used in cancer-related clinical trials. Repeated administration of vaccinia-based vaccines resulted in the generation of neutralizing antivaccinia antibodies capable of preventing effective antigen presentation and subsequent T-cell proliferation. The inability of additional vaccinations to further enhance

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the immune response limited the efficacy of repeated administrations. Gullely *et al.* noted that the rate of inoculation-site reactions decreased dramatically from 74% of patients following the initial injection to 37% and 19% in subsequent injections (82). This finding led to the development of a prime-and-boost strategy that involves priming with vaccinia vectors and boosting multiple times with avipox vectors such as folwpox. Indeed, preclinical and clinical studies showed optimal immune response (with corresponding survival advantage in murine models and clinical benefit in clinical trials) with these prime-and-boost regimens (83, 84).

The prime-and-boost strategy was also investigated by the Eastern Cooperative Oncology Group (ECOG), which reported a randomized phase II study in which 64 patients with rising PSA following definitive local therapy with no evidence of disease on scans were randomized to receive 4 doses of vaccines with rV-PSA (designated V) and/or rF-PSA (designated A for avipox). The arms were thus AAAA, AAAV, and VAAA. Of the eligible patients in the ECOG study, 45.3% in the VAAA arm were progression-free at 19.1 months and 78.1% demonstrated clinical progression-free survival, suggesting that strategies that improve the ability to mount an optimal immune response are associated with improved time to PSA progression in this patient population (85). Updated results with a median follow-up of 50 months showed median time to PSA progression of 9.2, 9.1, and 18.2 months, respectively, for the 3 treatment arms. Median time to clinical progression has not been reached for any of the treatment groups, but 80% of men in the AAAA and AAAV arms and 90% of men in the VAAA arm are disease-free (86).

Several vaccines have completed initial clinical trials with promising results. A poxviral vector-based vaccine containing the genes for PSA and multiple T-cell costimulatory molecules (PSA/TRICOM) has been tested in 2 phase II studies. The first demonstrated that this vaccine could induce immune responses and that immune responses correlated with evidence of clinical benefit, including objective response and declines in PSA (87). A multicenter randomized phase II study of PSA/TRICOM vs. placebo was conducted in 125 patients with metastatic androgen-independent asymptomatic prostate cancer. While this study did not meet its primary endpoint of progression-free survival, patients' overall survival data, which are currently being accumulated, show provocative results. Median overall survival thus far is 16.3 months for the control cohort (fowlpox wild-type vector; n = 41) vs. 24.4 months for patients receiving PSA/TRICOM vaccines (n = 84) (88).

Another vaccine that has undergone an initial clinical trial is GVAX (Cell Genesys, South San Francisco, CA), a GM-CSF-transduced tumor-cell vaccine for patients with AIPC that was developed from 2 prostate cancer cell lines (PC-3 and LNCaP). GM-CSF is used in GVAX as an adjuvant to recruit dendritic cells to the vaccine site. Two phase II trials (G-9803 and G-0010) have been completed and 2 phase III trials (VITAL-1 and VITAL-2 for

asymptomatic and symptomatic AIPC patients, respectively) are currently open for accrual. The G-9803 phase II trial of an earlier generation of GVAX included 34 patients with radiographic evidence of metastatic disease and 21 patients with biochemical progression only. Enrolled patients had no bone pain and were chemotherapy- and immunotherapy-naïve. In the cohort with metastatic disease, patients received 1 of 2 dose levels of GVAX. In the high-dose group, 24 patients received a 500 million-cell prime followed by up to 12 boosts of 100 million cells each at 2-week intervals. In the low-dose group, 10 patients received the same priming dose and up to 12 boosts of 300 million cells each at 2-week intervals. Doses were equally split between the 2 constituent cell lines. The primary endpoints were safety, PSA response, and radiographic response. Other endpoints were time to progression measured by PSA and clinical disease, immunogenicity, and PSA velocity measured by the slope of PSA rise before and after treatment (89). The combined median survival for both dose groups was 26.2 months. PSA control (including PSA response and stable disease) was observed in 2 of 34 patients with metastatic AIPC in the G-9803 study. One patient in the high-dose group achieved a complete response, with PSA normalization and lesion regression on bone scan; another patient had stable PSA levels for more than 90 days.

The second phase II trial of GVAX immunotherapy for prostate cancer (G-0010) was carried out in 80 patients with metastatic AIPC who had no bone pain and were chemotherapy- and immunotherapy-naïve. The study explored various dose schedules and 5 dose levels of GVAX (90). Endpoints included maximum tolerated dose, GM-CSF pharmacokinetics, safety, PSA response, and time to PSA progression. One patient in the low-dose group had a partial PSA response ($\geq 50\%$ decrease by PSA Working Group criteria) and 13 patients had stable PSA levels ($< 25\%$ increase) for more than 90 days (91). Delayed declines of PSA (one or more PSA measurements documenting a $\geq 25\%$ reduction from peak value) after initial progression were noted in 12 of 80 patients overall and in 6 of 19 patients in the high-dose group, which is consistent with the potential for delayed effects associated with immunotherapy. For the 22 patients treated with a dose comparable to that being employed in the subsequent phase III trials, median survival is 35 months (92, 93). Predicted survival was estimated for each patient on these phase II GVAX studies using a nomogram; the median measured survival was greater than 6 months longer than the predicted survival for both of these studies.

The first of 2 phase III trials of GVAX immunotherapy for prostate cancer (VITAL-1) opened to accrual in July 2004. VITAL-1 compares the survival benefit of GVAX vs. docetaxel plus prednisone in asymptomatic metastatic AIPC patients, with a target accrual of 600. The second phase III trial (VITAL-2) opened to accrual in June 2005 and compares the survival benefit of GVAX plus docetaxel vs. docetaxel plus prednisone in symptomatic metastatic AIPC patients, with a target accrual of 600.

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Table 1. Candidate vaccines in combination with ADT in hormone-sensitive prostate cancer

Hormone agonist/vaccine	No. of patients/disease stage	Study phase	Dose schedule	Immunologic response	Clinical response	Reference
GnRH-A x 1 mo/PROSTVAC (rV-PSA) on D7 after GnRH-A	6/T3N0 to T3N1	1	2.65 x 10 ⁸ PFU and 2.65 x 10 ⁷ PFU D7 after GnRH-A	Primary anti-PSA IgG antibody activity in 1 pt	1 pt with undetectable serum PSA (< 0.2 ng/mL) for > 8 mos after N testosterone	99
GnRH-A x 6 mos/GVAX (GM-CSF-secreting whole cell)	120/D0	3	ADT x 6 mos alone vs. ADT x 6 mos with concurrent vaccine	Anti-PSA response	PSA progression to be measured	104
GnRH-A x 3 mos/ONY-P1 whole cell	42/D0	2.5	ONY-P1 with BCG D1 and D15, then vaccine alone D29 and q 4 wks thereafter x 52 wks vs. placebo	ELISPOT assay for CD8+ PSA-specific responses	PSA progression to be measured	Gulley <i>et al.</i> (personal communication)
GnRH-A x 3 mos/sipuleucel-T (DC-based cultured with PA2024)	176/D0	3	ADT x 3 mos followed by sipuleucel-T alone vs. ADT x 3 mos with placebo; 1 booster at biochemical progression	ELISPOT assay and T-cell stimulation index	PSADT increased 35% vs. placebo (p = 0.046); time to distant failure and overall survival to be measured	105

GnRH-A = gonadotropin-releasing hormone agonist; rV = recombinant vaccinia; GM-CSF = granulocyte macrophage-colony stimulating factor; D = day; PFU = plaque-forming units; ADT = androgen-deprivation therapy; BCG = Bacille Calmette-Guerin; PSA = prostate-specific antigen; pt = patient; DC = dendritic cell; PA2024 = fusion protein composed of prostatic acid phosphatase (PAP) and GM-CSF

Another prostate cancer vaccine currently in phase III trials is sipuleucel-T (APC8015 [Provenge®; Dendreon, Seattle, WA]). Sipuleucel-T is an autologous, APC-enriched vaccine composed of processed cells cultured with a recombinant fusion protein consisting of PAP and GM-CSF, termed PA2024. In a prior phase II study, 22 patients received bevacizumab and sipuleucel-T until disease progression with doubling of baseline or nadir PSA, or when toxicity occurred (94). There was an approximately 90% increase in PSA doubling time (6.7 months pretreatment vs. 12.7 months on treatment; p = 0.004) and no objective disease progression. In the first phase III trial of sipuleucel-T, 127 patients with asymptomatic metastatic AIPC were randomly assigned to 3 infusions of sipuleucel-T or placebo every 2 weeks (95). Results showed that the primary endpoint was not reached, with median time to progression of 11.7 weeks for sipuleucel-T and 10 weeks for placebo (hazard ratio [HR] = 1.45; 95% confidence interval [CI]: 0.99 to 2.11; p = 0.052). However, overall survival was 25.9 months for sipuleucel-T vs. 21.4 months for placebo (HR = 1.70; 95% CI: 1.13 to 2.56; p = 0.010 log rank). The 36-month survival was 15% for placebo and 33% for vaccine, a difference that could not be explained by an obvious imbalance in patient baseline characteristics or subsequent chemotherapy treatment. A confirmatory phase III clinical trial employing sipuleucel-T is currently ongoing, with survival as a primary endpoint.

6. IMPLICATIONS FOR COMBINING ADT AND IMMUNE THERAPY

Mounting evidence on the role of testosterone in immunity and the beneficial effects of androgen ablation on immune response provides a rationale for combining ADT and vaccine therapy in prostate cancer. It is likely that tumor-specific T cells are either deleted, nonfunctional, or present in insufficient numbers in patients who develop prostate cancer. Therefore, any therapy, such as ADT, that can increase the pool of naïve T cells and B cells can enhance the likelihood of generating a clinically

meaningful antitumor immune response. It is possible that these “holes” in the tumor-specific immunotherapy armamentarium could be filled by these naïve T cells, which could subsequently be expanded by an appropriate vaccine. In fact, there are some data supporting this idea. Using a murine model, Hsueh *et al.* showed that androgen ablation with an oral AR antagonist augmented immune response to a whole tumor-cell vaccine (B16) (96). Although the model used was melanoma, these data are proof of principle that ADT can enhance anticancer vaccine responses. Since immunotherapy would likely be most effective when administered during the early stages of disease or prophylactically after primary local treatment of prostate cancer, patients in the early stages of prostate cancer are an attractive population for combination ADT and vaccine strategies. However, the very biology that makes immunotherapy appealing in biochemically recurrent (D0) prostate cancer creates difficulties for clinical endpoint trials, given that time to metastatic disease can be about 8 years and median overall survival about 13 years (97, 98).

Table 1 outlines the clinical trials utilizing this relatively new approach to prostate cancer treatment. In 1999, Sanda *et al.* reported a study in which 6 patients with D0 prostate cancer were treated with a one-month depot of GnRH-A, followed 7 days later by a single vaccination with recombinant vaccinia (rV) expressing human PSA (99). In this small phase I study, no dose-limiting toxicity was observed and one patient maintained undetectable PSA for 8 months after testosterone recovery.

A phase II clinical trial in nonmetastatic AIPC cancer patients that employed an AR antagonist and a PSA-targeted poxviral vaccine also yielded interesting data (100). Patients who were not surgically castrate remained on GnRH-A and were randomized to vaccine (n = 21) versus AR antagonist therapy with nilutamide (n = 21). After 6 months, patients with rising PSA who remained nonmetastatic could receive a combination of both treatments. The primary endpoint of the study was to

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Table 2. Antiandrogen, vaccine, and combination therapy in patients with nonmetastatic hormone refractory prostate cancer

Treatment	N	Median overall survival (yrs)	3-yr overall survival	5-yr overall survival	Log rank p
Initial vaccine	21	Not reached	81%	59%	0.13
Initial nilutamide	21	3.4	62%	38%	

compare time to treatment failure for both arms. The median pretreatment PSA velocity of 6.6 ng/mL/month in the vaccine arm decreased following 6 months of vaccine therapy to 4.5 ng/mL/month ($p = 0.025$). Median time to treatment failure was 9.9 months in the vaccine arm versus 7.6 months in the nilutamide arm ($p = 0.28$). Twelve patients on the vaccine arm had nilutamide added at the time of PSA progression. Median time to treatment failure with the combined therapy was 13.9 months from the addition of nilutamide, for a total of 25.9 months from initiation of therapy. In contrast, 8 patients from the nilutamide arm had vaccine added at the time of PSA progression. Median time on study with the combined therapy was 5.2 months, with a total duration from onset of study of 15.9 months. Thus, while both vaccine and nilutamide showed clinical activity, patients appeared to respond better to nilutamide after receiving vaccine. Recently updated survival data from these patients further support this notion (101). In this very small study, patients randomized to receive vaccine up front appeared to have a much longer, albeit not statistically significant, overall survival (Table 2), with a 5-year overall survival of 38% for those patients who received nilutamide first (nilutamide alone or nilutamide then vaccine) versus a median overall survival of 59% for those patients who received vaccine first (vaccine alone or nilutamide plus vaccine), supporting the preclinical finding that AR antagonists may enhance the effectiveness of immune-based therapies (102).

7. ONGOING AND PLANNED CLINICAL TRIALS

Recent clinical trials have yielded provocative findings on the potential synergy between ADT and vaccine therapy, and emerging research is providing more potent vaccines that elicit more vigorous immune responses. These developments signal a need for further investigation of a strategy that combines ADT to enhance and regenerate immune function with vaccines that target prostate tumor antigens. Based on the promising data from the phase II clinical trial mentioned above (100), a randomized clinical trial of AR antagonist with or without a PSA/TRICOM vaccine (07-C-0107) is currently underway in patients with nonmetastatic AIPC. In addition, a double blind randomized phase 2.5 trial of ONY-P1 vaccine vs. placebo in men with D0 prostate cancer following limited androgen ablation is slated to open in early 2007 at the NCI. Eligible patients must have biochemical progression (e.g., rising PSA) following local definitive therapy, with no radiographic evidence of disease. Forty-two patients will be enrolled and randomized to 2 arms. Arm A will receive limited ADT with 12 weeks of GnRH-A followed by ONY-P1 vaccine. Arm B will receive limited ADT with 12 weeks of GnRH-A followed by placebo vaccine. Vaccines will be administered on days 1, 15, and 29, then every 4 weeks for up to 52 weeks. The initial 2 active vaccines will be given with BCG. Immunologic response will be measured by ELISPOT assay, which is sensitive and quantitative and

can be used without prolonged *ex vivo* manipulation of peripheral blood mononuclear cells (103). The primary endpoint of this study is to determine whether ONY-P1 vaccine can increase the time to PSA-defined progression in patients with stage D0 AIPC. Secondary endpoints include evaluation of immunologic response, comparison of immunologic response between the 2 arms, TREC analysis, thymic index, PSA kinetics, toxicity, and time to testosterone recovery following limited androgen ablation.

ECOG E3806 is another proposed clinical trial to assess the benefit of ADT combined with vaccination. In this study, 120 patients with D0 prostate cancer will be randomized 1:2 (based on PSA, PSA doubling time, and primary local therapy) to either ADT alone for 6 months or ADT for 6 months with concurrent vaccination. Patients will be evaluated monthly until PSA rises to > 0.5 ng/mL, at which time they will be given ADT for an additional 6 months (104).

Another trial with ongoing clinical endpoint analysis is PROTECT (PROvenge® Treatment and Early Cancer Treatment), or P-11, a randomized, double-blind, placebo-controlled trial designed to evaluate the safety and biologic activity of sipuleucel-T in men with nonmetastatic hormone-sensitive prostate cancer who have had biochemical recurrence following radical prostatectomy (105). A total of 176 patients at 19 sites in the United States were randomized 2:1 to receive sipuleucel-T or placebo following a 3-month course of hormonal therapy. Patients were then followed with serial PSA measurements to evaluate the impact of sipuleucel-T on PSA and PSA doubling time (PSADT). At biochemical progression, defined as ≥ 3 ng/mL PSA, patients were eligible for one booster infusion of either sipuleucel-T or placebo, depending on the treatment arm to which they were randomized. Preliminary analysis at biological progression showed a 35% increase in PSADT for patients in the sipuleucel-T arm compared to patients in the placebo arm ($p = 0.046$). To adjust for potential variations in the rate of testosterone recovery following hormone therapy, PSADT was calculated after testosterone levels returned to baseline. Patients in the sipuleucel-T arm had a 49% increase in PSADT compared to patients in the placebo arm ($p = 0.038$). Immune response to the recombinant antigen PA2024 was measured at baseline and at 4 and 13 weeks after dosing. Significant responses were seen in the sipuleucel-T arm but not in the placebo arm, as determined by measuring T-cell proliferation (by stimulation index) and the number of T cells that secrete IFN-gamma (by ELISPOT). Further data will be available for presentation at the American Society of Clinical Oncology Meeting in June 2007.

8. CONCLUSIONS

ADT is the cornerstone of treatment for metastatic prostate cancer, even though it is not curative at

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this stage of disease. However, data on ADT in the adjuvant setting suggest that a subset of prostate cancer patients are “cured” of prostate cancer. It is tempting to speculate that this improvement in overall survival is mediated in part by the immunologic changes caused by ADT, and that further directing the immune system with specific immunotherapy will provide even greater clinical benefit.

There is currently significant interest in vaccine therapy for prostate cancer, with 3 ongoing randomized phase III clinical trials with a primary endpoint of survival. Decades of research showing that ADT enhances immune response, along with increased understanding of the immune response and its negative regulators, have also created rapidly growing interest in further characterizing ADT’s ability to potentiate an immune response to vaccine. Ongoing and planned clinical endpoint studies combining ADT and vaccine show significant promise, and may begin to answer the question of whether this combination can achieve improved clinical outcomes compared to either modality alone. These trials can also serve as proof of concept studies for testing ADT and therapeutic vaccines in other cancers.

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Send correspondence to: James L. Gulley MD, PhD, FACP, Director, Clinical Immunotherapy Group, Laboratory of Tumor Immunology and Biology, Center for Cancer Research, National Cancer Institute, 10 Center Drive, Room 8B09, MSC 1750, Bethesda, MD 20892, Tel: 301-431-1355, Fax: 301-480-5094, E-mail: gulleyj@mail.nih.gov

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