J1265: A neoadjuvant immunologic study of androgen deprivation therapy combined with a GM-CSF–secreting allogeneic prostate cancer vaccine and low-dose cyclophosphamide in men with high-risk localized prostate cancer undergoing radical prostatectomy

NCT01696877

Protocol Date: November 30, 2012

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IRB #: NA_00073453 IND #: 9678

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Protocol Date: **November 30, 2012**

Protocol Type / Version # / Version Date: Protocol / Version 1.4 / November 30, 2012

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SYNOPSIS

Title	A neoadjuvant immunologic study of androgen suppression combined with a GM-CSF–secreting allogeneic prostate cancer vaccine and low-dose cyclophosphamide in men with high-risk localized prostate cancer undergoing radical prostatectomy
Institution	Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD
PI(s)	Emmanuel S. Antonarakis, MD (PI); and Edward M. Schaeffer, MD, PhD (Co-PI)
Sponsor	This is an investigator-sponsored trial. Charles Drake, MD, PhD is the IND-holder.
IRB #	NA_00073453
Investigational agents	1) GM-CSF–secreting cell-based (PC3/LNCaP) prostate cancer immunotherapy 2) Cyclophosphamide, intravenous 3) Degarelix acetate, subcutaneous
Phase	Immunologic study (phase I/II)
Indication	Men with high-risk localized prostate cancer, prior to radical prostatectomy
Target population	<u>Inclusion criteria:</u> • histologically confirmed prostate adenocarcinoma • clinical stage T1c–T3b, N0, M0 • Gleason sum 7-10 • at least 2 positive cores • prior decision to undergo radical prostatectomy • adult male > 21 years of age • ECOG performance status 0-1, or Karnofsky score ≥ 70% • adequate kidney, liver, and bone marrow function • willingness to sign informed consent and adhere to study requirements <u>Exclusion criteria:</u> • presence of known lymph node involvement or distant metastases • prior radiation, hormones, biologics, or chemotherapy for prostate cancer • prior immunotherapy/vaccine therapy for prostate cancer • previous or concurrent use of cyclophosphamide • concomitant treatment with hormonal therapy or 5α-reductase inhibitors • use of experimental agents for prostate cancer within the past 3 months • known allergy to cyclophosphamide or G-CSF/GM-CSF • known sensitivity to materials of bovine origin • history of autoimmune disease requiring systemic immunosuppression • other concurrent malignancy • uncontrolled major infectious, cardiovascular, pulmonary, hematologic, or psychiatric illnesses that would make the patient a poor study candidate
Start date/ Duration	Initial patients are expected to be entered in October 2012. With an estimated enrollment rate of 4 patients per month, accrual is expected to last 8 months.

Sample size

32 evaluable patients (*i.e.* those that undergo prostatectomy)

Rationale

Cancer immunotherapy refers broadly to approaches which attempt to treat cancer by activating immune responses directed against malignant tissue. Prostate GVAX is an allogeneic cell-based prostate cancer vaccine composed of two irradiated cell lines (PC3 and LNCaP) that have been genetically modified to secrete granulocyte-macrophage colony-stimulating factor (GM-CSF). The release of GM-CSF by these modified tumor cells serves to recruit dendritic cells which then present tumor antigens to T-cells, thus initiating antitumor immune responses.

However, abundant preclinical data show that, when used alone, cell-based immunotherapy is unable to break specific T-cell tolerance in tumor-bearing hosts. Studies in an autochthonous prostate cancer mouse model have shown that giving low-dose cyclophosphamide prior to a cell-based GM-CSF–secreting vaccine abrogates immune tolerance through augmentation of $CD8^+$ T cell infiltration in the prostate, transient depletion of regulatory T cells (T_{regs}), and increased expression of dendritic cell maturation markers. Enhancement of antitumor immunity has also been observed in other preclinical models where cyclophosphamide was given in sequence with GM-CSF–secreting immunotherapy for the treatment of breast and pancreatic cancers. These preclinical data are supported by early-phase clinical trials combining GVAX with low-dose cyclophosphamide in pancreatic and breast cancers.

Furthermore, emerging evidence suggests that androgen deprivation therapy (ADT) itself has profound effects on the host immune system, resulting in thymic regeneration and enhancement of antitumor immunity. In addition, preclinical and clinical studies demonstrate that ADT augments prostate cancer-specific immune responses induced by immunotherapy, suggesting that ADT may act synergistically with immunotherapy. Based on data from mouse models as well as human clinical trials, it has been suggested that prostate cancer immunotherapy may be most effective when administered in the setting of an androgen-suppressed environment.

Building on these findings, we have designed a study to assess the use of ADT given alone or administered following immunization with low-dose cyclophosphamide and prostate GVAX, in patients undergoing radical prostatectomy. We aim (1) to determine whether ADT is immunogenic in men with localized prostate cancer by evaluating T-cell infiltration in harvested prostate glands; (2) to determine whether administering ADT after low-dose cyclophosphamide and prostate GVAX augments immune infiltration into the prostate gland; and (3) to investigate whether this combinatorial immuno-hormonal approach is safe and feasible. We hypothesize that the combination of ADT and cyclophosphamide/GVAX will produce significantly greater antitumor immune responses than would ADT used alone.

Objectives

<u>Primary:</u>

- To quantify the extent of $CD8^+$ T cell infiltration into the prostate from harvested prostate glands in men with high-risk localized prostate cancer undergoing radical prostatectomy
- To evaluate the safety and tolerability of ADT following vaccination with cyclophosphamide and GVAX 4 weeks prior to radical prostatectomy

Secondary:

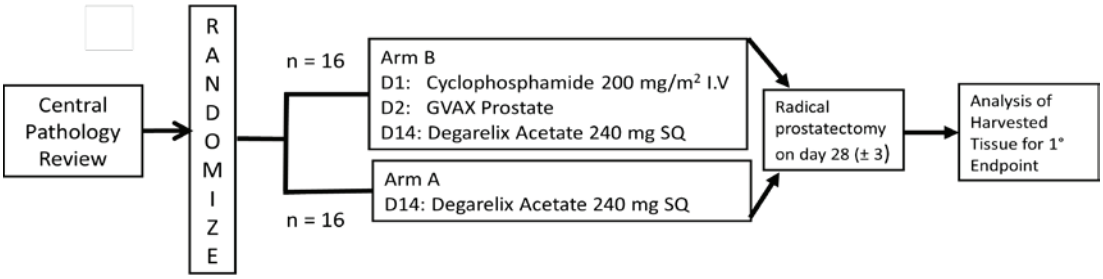
- To evaluate the feasibility of administering ADT after immunization with low-dose cyclophosphamide and prostate GVAX, beginning 4 weeks prior to radical prostatectomy
- To quantify the extent of $CD4^+$ T cell and T_{reg} infiltration into the prostate, and to quantify the $CD8^+/T_{reg}$ ratio as well as the $CD4^+/T_{reg}$ ratio in prostate specimens
- To quantify tissue androgen concentrations (testosterone, dihydrotestosterone), and to quantify androgen receptor (AR) protein expression in prostate specimens
- To quantify markers of apoptosis (activated caspase 3) and proliferation (Ki-67) in prostate tumor specimens
- To evaluate the proportion of pathological complete responses (pCR)
- To evaluate the generation of novel antibodies to prostate-associated antigens in the serum of patients, after the initiation of protocol therapy
- To determine the PSA response rate and time-to-PSA-recurrence after radical prostatectomy

Study design

This is a single-center, randomized, 2-arm, open-label, prospective clinical trial evaluating the immunogenicity and safety of a single administration of LHRH antagonist (degarelix) used alone, or degarelix administered following vaccination with low-dose cyclophosphamide and GVAX, given 4 weeks prior to radical prostatectomy in men with localized prostate cancer.

Patients will be recruited from the outpatient Urology clinic. Eligible patients will be randomized in a 1:1 ratio (stratified by Gleason score: ≤7 vs 8-10) to receive one of two treatment regimens (*see Schema below*), each beginning 28 (±3) days prior to radical prostatectomy. In Arm B, cyclophosphamide will first be given at a dose of 200 mg/m^2 as a single intravenous infusion. One day later, prostate GVAX will be administered as five 0.8-mL intradermal injections of PC3 (2.5×10^8 cells) and five 0.5-mL intradermal injections of LNCaP (1.6×10^8 cells), for a total dose of 4.1×10^8 cells. On day 14, degarelix acetate will be administered as three 80-mg subcutaneous injections, for a total dose of 240 mg. Prostate glands will be harvested 14 (±3) days later, at the time of radical prostatectomy, and prostate tissue will be examined for the primary endpoint. In Arm A, an identical dose of degarelix acetate will be administered, 14 (±3) days prior to surgery. A telephone follow-up evaluation (or an in-person visit) for adverse events will occur 28 (±21) days after surgery. Patients will then be followed by their urologists according to standard institutional practices, but will require PSA evaluations every 3 (±1) months during year 1 and every 6 (±2) months during years 2-3.

Treatment Schema:



Baseline evaluations:

- Informed consent
- Medical history and physical examination
- Review of medications
- Vital signs, including height and weight
- Performance status
- Central pathologic review of prostate core biopsies
- CT (If allergic to CT scan contrast, obtain MRI with contrast) and/or bone scan, if clinically indicated
- Hematology, coagulation, and chemistry laboratories
- Serum PSA measurement
- Sera for immunoassays (Pre-treatment)

On day 28 (±3 days):

- Medical history and focused physical examination (including vital signs)
- Review of medications
- Performance status
- Adverse events/toxicity evaluation
- Hematology and chemistry laboratories, as clinically indicated
- Serum PSA
- Sera for immunoassays
- Radical prostatectomy
- Pathologic review of surgical specimen according to standard procedures
- Evaluation of harvested prostate gland for $CD8^+$ T cell infiltration

Post-operative day 28 (±21 days):

- Performance status (by telephone interview or clinic visit)
- Adverse events/toxicity evaluation (by telephone interview or clinic visit)
- PSA every 3 months (post-op year 1), then every 6 months (post-op years 2-3)

Criteria for evaluation

Primary endpoints:

- Mean $CD8^+$ T cell density in tumor tissue from harvested prostate glands of patients in each of the 2 treatment groups
- Frequency, type, and severity of adverse events in each of the 2 treatment groups

Secondary endpoints:

- Mean T_{reg} density in tumor tissue from harvested prostate glands of patients in each of the 2 treatment groups
- Mean $CD8^+/T_{reg}$ and $CD4^+/T_{reg}$ ratios in tumor tissue from harvested prostate glands of patients in each of the 2 treatment groups
- Mean CD4+ T cell density in tumor tissue from harvested prostate glands of patients in each of the 2 treatment groups
- Mean levels of apoptotic markers (activated caspase 3) and proliferation markers (Ki-67) in prostate tumor specimens of patients in each of the 2 treatment groups
- Mean tissue androgen concentrations (testosterone, dihydrotestosterone) and mean androgen receptor (AR) protein expression in prostate specimens
- Proportion of pathological complete responses (pCR) in each treatment group

- Proportion of patients generating of novel antibodies to prostate-associated antigens after the initiation of protocol therapy in each treatment group
- Proportion of patients achieving a PSA response rate (PSA <0.1 ng/mL) at 3 months after prostatectomy in each treatment group
- Median time-to-PSA-recurrence (PSA ≥0.2 ng/mL) after radical prostatectomy in each of the 2 treatment groups

Statistical methods

Our primary hypothesis is that men receiving cyclophosphamide and GVAX followed by ADT (arm B) will have a 100% or greater $CD8^+$ T cell infiltration of the prostate than will men receiving ADT alone (arm A). In the absence of any intervention prior to prostatectomy, we have previously determined that the mean staining percentage for $CD8^+$ T cells in prostate tissue is 0.42% (standard deviation [SD] 0.36%). With 16 patients per arm, and assuming an 86% coefficient of variation for the average $CD8^+$ area/spot area ratios, a one-sided 0.05 α-level t-test of the logarithms of these ratios would have 82% power to detect a 2-fold (100%) increase of $CD8^+$ T cells in the cyclophosphamide/GVAX + ADT arm compared to the ADT alone arm. Treating Gleason score as a block factor reduces the variance, which would be adequate to offset the loss of power due to the 1 degree of freedom associated with the blocking factor.

The primary efficacy endpoint will be expressed as the mean $CD8^+$ T cell staining percentage in prostate tumor tissues, reported separately for each treatment arm. High density (saturation) tissue microarrays (TMAs) using the highest-grade/largest tumor per patient and sampling it with 100-fold redundancy will be used to determine $CD8^+$ T cell densities. Since the $CD8^+$ T cell quantity is a ratio variable and the distribution is skewed, the log transformation will be used for this analysis. Log-transformed mean $CD8^+$ T cell concentrations will be compared between treatment groups using a two-way analysis of variance (ANOVA) with the stratification variable (Gleason score) treated as a block factor. Boxplots and descriptive summaries by treatment arm will also be provided.

In other analyses, continuous variables will be summarized by descriptive statistics using the mean, standard deviation, median, and range, and 95% confidence intervals will be reported where appropriate. Two-way ANOVA will be used to investigate comparisons between the two treatment arms. Proportions will be compared using a Mantel-Haenszel test. Kaplan-Meier analysis will be used to determine median time-to-PSA-progression, and comparisons will be sought using the log-rank test, stratified by Gleason score. Statistical significance will be set at P <0.05.

Safety analysis

Frequency, types, and grades of adverse events in each treatment group will be measured using the NCI Common Toxicity Criteria version 4.0, and will be summarized using descriptive statistics. Formal safety assessments will be performed from the time of first administration of degarelix, cyclophosphamide or GVAX until the 28th postoperative day (±21 days). In addition, all patients that received GVAX will be enrolled on a separate long-term follow-up protocol and will be evaluated for late GVAX toxicities (*e.g.* autoimmune phenomena, second malignancies). These evaluations will occur annually for 3 years.

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1.1 Primary Objectives

- To quantify the extent of $CD8^+$ T cell infiltration into the prostate from harvested prostate glands in men with localized prostate cancer receiving neoadjuvant ADT alone (2 weeks prior to surgery), or cyclophosphamide and GVAX followed by ADT, (with CY/GVAX administered 4 weeks prior to prostatectomy, and ADT administered 2 weeks prior to prostatectomy).
- To evaluate the safety and tolerability of ADT used alone (2 weeks prior to surgery), or cyclophosphamide and GVAX followed by ADT (with CY/GVAX administered 4 weeks prior to prostatectomy, and ADT administered 2 weeks prior to prostatectomy).

1.2 Secondary Objectives

- To evaluate the feasibility of administering ADT used alone (2 weeks prior to surgery), or cyclophosphamide and GVAX followed by ADT (with CY/GVAX administered 4 weeks prior to prostatectomy, and ADT administered 2 weeks prior to prostatectomy).
- To quantify and compare the extent of $CD4^+$ T cell and T_{reg} infiltration into the prostate in prostate specimens of patients in each of the 2 treatment groups.
- To quantify and compare $CD8^+/T_{reg}$ and $CD4^+/T_{reg}$ ratios in prostate specimens of patients in each of the 2 treatment groups.
- To quantify and compare tissue androgen concentrations (testosterone, dihydrotestosterone), and to quantify androgen receptor (AR) protein expression in prostate specimens of patients in each of the 2 treatment groups.
- To quantify markers of apoptosis (activated caspase 3) and markers of cell proliferation (Ki-67) in prostate tumor specimens of patients in each of the 2 treatment groups.
- To evaluate and compare the proportion of pathological complete responses (pCR) in prostate tumor specimens of patients in each of the 2 treatment groups.
- To evaluate and compare the generation of novel antibody specificities to prostate-associated antigens in the serum of patients in each group, after the receipt of protocol therapy.
- To evaluate and compare PSA response rates and the time-to-PSA-recurrence after radical prostatectomy in each of the 2 treatment groups.

2. BACKGROUND AND RATIONALE

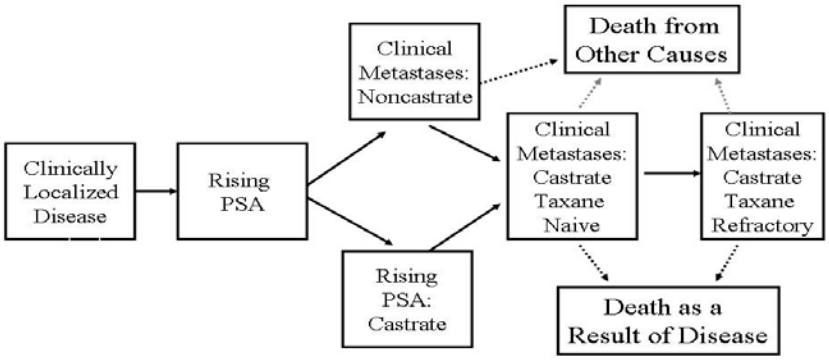
2.1 Disease Background

Prostate cancer is the second leading cause of cancer deaths in men. According to American Cancer Society estimates in 2008, as many as 186,320 American men will be diagnosed with prostate cancer, and nearly 28,660 will die of the disease (Jemal et al 2008). Yet, localized prostate cancer is often curable and even metastatic disease can respond to treatment.

The course of prostate cancer from diagnosis to death is best categorized as a series of clinical states (Figure 1). These clinical states involve the complex interplay of a network of signaling molecules that collectively promote net cell proliferation relative to cell death. Based on the extent of disease, hormonal status, and absence or presence of detectable metastases on imaging studies, the states are: localized disease, rising PSA after radiation therapy or surgery with no detectable metastases, and clinical metastases in the non-castrate or castrate settings.

Treatment of localized prostate cancer usually consists of surgery or radiation therapy or both. For patients with high-risk or locally advanced disease, a combination of radiation therapy and hormonal therapy is often used. However, even after definitive local therapy, approximately 30-50% of patients will have a local or distant recurrence (Pound et al 1999, D'Amico et al 2000). The risk of recurrence depends on multiple factors including tumor stage, tumor Gleason score, and serum PSA at the time of diagnosis. Recently, PSA doubling time at recurrence has been shown to be a powerful predictor of distant metastases and survival (Antonarakis et al 2012; Freedland et al 2007).

Figure 1 Clinical States of Prostate Cancer



Patients with metastatic prostate cancer have a median survival of 3-7 years, and most die of the disease. Treatment for metastatic disease involves surgical castration or hormonal manipulation using luteinizing hormone-releasing hormone (LHRH) agonists/antagonists, antiandrogens, or both. Although the majority of these patients initially respond to androgen deprivation therapy, all eventually progress to a state of castration-resistant prostate cancer (CRPC). Treatment options for these men are limited. Secondary hormonal manipulations, such as ketoconazole, are often used but these produce short-lived responses and do not improve survival (Small et al 2004). Moreover, these agents have unfavorable side effect profiles. Other acceptable approaches in these men include watchful waiting until the development of symptoms, or the initiation of cytotoxic chemotherapy. In this regard, docetaxel has been shown to improve overall survival in patients with CRPC, but only by a median of 2.9 months (Tannock et al 2004; Berthold et al 2008). However, because therapies for metastatic prostate cancer are palliative and not curative, there remains an urgent need to improve the outcome of primary therapies for prostate cancer.

2.2 Treatment Background

2.2.1 GM-CSF–secreting allogeneic cell-based vaccine as immunotherapy for prostate cancer

Cancer immunotherapy refers broadly to approaches which attempt to treat or cure cancer by activating an immune response directed against malignant tissue (Pardoll 2002). A number of different approaches fall broadly into this group, including dendritic cell vaccination, DNA vaccination, peptide vaccination, and others. Each of these methodologies has its own advantages and disadvantages. In particular, the use of granulocyte-macrophage colony-stimulating factor (GM-CSF) as an adjuvant has been widely evaluated in both murine models and in patients with cancer (Borrello and

Pardoll 2002). These studies suggest that GM-CSF based immunotherapy approaches function by recruiting and activating dendritic cells, now fairly well accepted as the most potent antigen presenting cells. Activated dendritic cells take up antigens from the tumor cell line or autologous tumor used in the biological formulation, and traffic to draining lymph nodes. In the lymphoid tissue, these activated dendritic cells present antigens derived from tumor peptides to T-cells, which then circulate to the sites of disease and (if successful) initiate a lytic immune response against the malignant tissue.

After initial evaluation in murine models (Dranoff et al 1993), the first clinical applications of GM-CSF transduced tumor cells employed autologous tumor in the setting of metastatic renal cell carcinoma (Simons et al 1997). These studies demonstrated the relative safety of this approach, but also underscored the technical difficulty of using autologous tumor as a source of antigens for immunotherapy. Recently, two significant findings have impacted the use of GM-CSF immunotherapies. First, it has been shown that GM-CSF need not come directly from the immunizing tumor cells, and that irradiated bystander erythroleukemia cells transduced to produce GM-CSF can provide identical response rates to directly transduced immunotherapy lines in murine models (Borrello et al 1999). Perhaps more significant, though, was the recognition that many common tumor antigens may indeed be shared between patients with the same tumor type, and that immortalized cell lines could thus be transduced with GM-CSF to serve as immunotherapies. The use of immortalized GM-CSF–secreting cell lines has been evaluated in patients with pancreatic cancer (Jaffee et al 2001), with preliminary results showing the safety of this immunotherapy, in addition to suggesting some degree of efficacy especially at higher immunotherapy doses.

In prostate cancer, the initial immunotherapy approach also utilized irradiated autologous tumor cells transduced *in vitro* to secrete GM-CSF (Simons et al 1999; Dummer 2001). In a phase I trial, prostate tumor cells were harvested surgically and transduced with a replication-defective retrovirus. Doses of 10 million tumor cells were administered without significant adverse affects, and new delayed-type hypersensitivity (DTH) responses were documented in two out of eight treated patients. For phase II evaluation, an immunotherapy comprised of two common prostate cancer cell lines (LNCaP and PC3) has been developed (Higano et al 2008). LNCaP was originally isolated from a lymph node metastasis and expresses a number of prostate-restricted differentiation antigens including PSA and prostate-specific membrane antigen (PSMA) (Horoszewicz et al 1987; Israeli et al 1994). It also contains a mutant androgen receptor as well as the most common epigenetic abnormality in human prostate cancer: methylation of the glutathione-S-transferase gene (Lee et al 1994). The PC3 cell line was developed from a bone metastasis and expresses significant levels of proteases associated with metastatic prostate cancer. While LNCaP is androgen-sensitive, PC3 is considered androgen insensitive. Together, these 2 cell lines are likely to serve as a diverse source of prostate- and prostate tumor-specific antigens.

Two phase II studies have been performed using this product, officially registered as PC3/LNCaP immunotherapy for prostate cancer (or GVAX). In one of these studies, 41 hormone-naïve patients were primed with a single 500 million-cell immunotherapy dose, then boosted with 12 doses of 100 million cells at 2 week intervals. No autoimmunity was detected, and no life-threatening toxicities were observed. The most common adverse event was a reaction at the injection site. One patient had a partial PSA response (Simons et al 2006). In a second phase II trial, 55 hormone-refractory patients were treated with a 500 million-cell immunotherapy dose, then boosted with 12 doses of either 100 million or 300 million cells at 2 week intervals. One patient had a PSA

response, and there was a non-significant trend towards a longer median time to bone scan progression (140 days vs. 85 days, $P = 0.095$) in the high-dose arm (Small et al 2007). Taken together, these phase II data demonstrate the safety of this immunotherapy approach, but suggest a relatively low overall response rate. The most common adverse events in both studies were injection-site erythema, fever, fatigue, malaise, myalgias, and arthralgias.

Two large randomized phase III studies of GM-CSF–secreting cell-based immunotherapy for prostate cancer (GVAX) have been conducted: VITAL-1 and VITAL-2. The first study, VITAL-1, enrolled 626 men with asymptomatic chemotherapy-naïve castration-resistant prostate cancer, and randomized them to receive GVAX or docetaxel/prednisone, with the primary endpoint being overall survival (Higano et al 2009). The second study, VITAL-2, was planned initially to enroll 600 patients with symptomatic metastatic castration-resistant prostate cancer, randomizing them to docetaxel/prednisone or docetaxel/GVAX (Small et al 2009). Both trials were terminated early because of data observed at the time of interim analyses suggesting that the survival improvements initially hypothesized were unlikely to be observed if the trials were to be continued (Higano et al 2009; Small et al, 2009). In both studies, overall survival was not statistically superior in patients receiving GVAX. While a large number of potential reasons for this low activity may be postulated, perhaps the simplest is the well-known immunological observation that persistent antigen exposure tends to induce immune tolerance (Ramsdell and Fowlkes 1992; Rocha et al 1993; den Boer et al 2004). In addition, cancer immunotherapy might prove more efficacious in a minimal disease state, as has been observed in multiple experimental systems (Migita and Ochi 1993).

2.2.2 Cyclophosphamide as a potent enhancer of immune responses to GVAX

Efficient immunization against cancer requires a vaccine capable of eliciting a potent $CD4^+$ and $CD8^+$ T cell immune response. However, tumors have evolved several mechanisms to escape immune surveillance, including immune tolerance mediated by immunosuppressive T lymphocytes (Drake et al 2006). To this end, tumors have been shown to induce rapid expansion of $CD4^+CD25^+$ regulatory T cells (T_{regs}) in humans and mice, leading to delayed rejection of immunogenic cancers (Liyanage et al 2002; Woo et al 2001; Curiel et al 2004). Conversely, elimination of these T_{regs}, which constitute about 2-5% of the peripheral $CD4^+$ T cells in naïve mice, elicits potent antitumor immune responses leading to tumor eradication (Shimizu et al 1999; Sutmuller et al 2001). For many years, cyclophosphamide has been studied for its role in breaking immune tolerance. It is known that low-dose and high-dose cyclophosphamide have different biological activities (Motoyoshi et al 2006). High-dose cyclophosphamide has cytotoxic activity, whereas low-dose cyclophosphamide shows predominantly immune modulating effects. Cyclophosphamide, as an FDA-approved cytotoxic chemotherapy agent, is used in treating multiple types of cancer including breast cancer, lymphoma, leukemia, and sarcoma. In addition, cyclophosphamide is used as an immune suppressor in many autoimmune disorders.

Immune modulating doses of cyclophosphamide-containing chemotherapy have been studied in mouse tumor models in conjunction with vaccination. For example, Dr. Jaffee and colleagues have studied a *HER-2/neu* transgenic mouse model of mammary tumors, representing a clinically relevant model of human breast cancer. These mice develop spontaneous *HER-2/neu*–expressing mammary tumors due to activation of the transgene by the murine mammary tumor virus (MMTV) promoter in normal mammary tissue. It has been demonstrated that vaccination of these mice induces weak *HER-2/neu*–specific B cell and T cell responses that are not capable of controlling mammary

tumor growth regardless of the vaccine type. However, treatment of these mice with low-dose cyclophosphamide prior to vaccination resulted in tumor rejection in 10-30% of animals, while mice receiving vaccination alone all became tolerant to tumor growth (Ercolini et al 2005). In this study, it was shown that the immune enhancing effect of cyclophosphamide was mediated through selective inhibition of the cycling population of $CD4^+CD25^+$ T_{reg} cells and recruitment of high-avidity antitumor effector T cells. These findings were confirmed in a separate study using the same *HER-2/neu* transgenic mouse model of breast cancer. In that study, combined treatment with immune-modulating doses of cyclophosphamide and a GM-CSF–secreting whole-cell vaccine delayed tumor growth by overcoming immune tolerance and inducing antigen-specific antitumor immune responses (Machiels et al 2001). Importantly, because cyclophosphamide exerts its effect on the regulatory T cells of the immune system rather than on the cancer cells themselves, this approach can be applied to treat any type of cancer.

These animal studies have led to a phase I/II clinical trial testing the feasibility, safety, and immunogenicity of cyclophosphamide when given in sequence with a GM-CSF–secreting cell-based vaccine in breast cancer patients (Emens et al 2004). In this study, 21 women with metastatic breast cancer received one of three doses of cyclophosphamide (200, 250, or 350 mg/m^2) in conjunction with doxorubicin and an allogeneic, *HER-2/neu*$^+$, GM-CSF–secreting breast tumor vaccine. In addition, 6 women received immunotherapy alone. Patients on the combined-modality arms received three monthly immunizations and a booster 6 to 8 months later according to the following treatment schedule: cyclophosphamide was given on day –1, vaccination on day 0, and doxorubicin on day +7. Total *Her-2*–specific immune responses were observed in 33%, 50%, 21%, and 25% of patients receiving 0, 200, 250, and 350 mg/m^2 of cyclophosphamide respectively (Emens et al 2008). These clinical data suggest that addition of cyclophosphamide enhances *Her-2*–specific vaccine responses in patients with breast cancer, but that increasing doses of cyclophosphamide may abrogate these immune responses.

A second pilot study borrowing from these principles has recently been conducted in patients with metastatic pancreatic cancer (Laheru et al 2008). In this open-label study, 30 patients received up to six doses of a GM-CSF–secreting cell-based pancreatic cancer vaccine at 21-day intervals, while 20 patients received 250mg/m^2 of intravenous cyclophosphamide 1 day prior to the same vaccine. After 3 cycles of therapy, pancreatic cancer-specific (mesothelin-specific) $CD8^+$ T cell responses were augmented in only 50% of patients treated with vaccine alone compared to 90% of patients treated with cyclophosphamide plus vaccine. Mean immune response augmentation (as judged by mesothelin-specific $CD8^+$ T cell effects) after 3 cycles of treatment was 39% in patients receiving immunotherapy alone and 131% in patients receiving cyclophosphamide plus immunotherapy (Laheru et al 2008). In addition, median survival in patients on the vaccine arm was 2.3 months while survival on the cyclophosphamide/vaccine arm was 4.3 months. The administration of this cell-based immunotherapy in sequence with cyclophosphamide showed minimal treatment-related toxicity, although all patients developed erythema, induration and/or pain, soreness at the treatment sites following immunotherapy. Other common adverse events included fever, chills, fatigue, malaise, skin rash, arthralgias, and nausea.

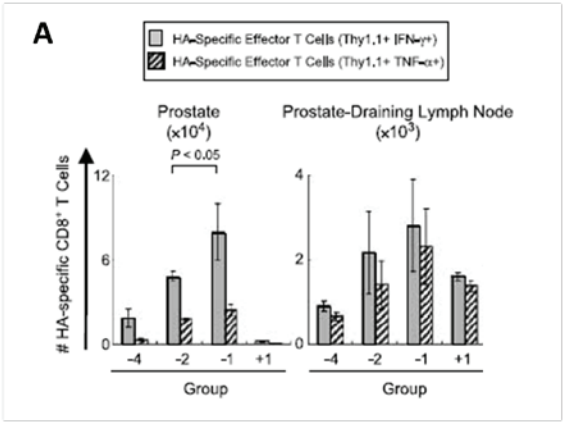
2.2.3 Cyclophosphamide combined with GVAX in prostate cancer

Recently, data evaluating the effect of cyclophosphamide on anti-tumor immune responses to a cell-based GM-CSF–secreting prostate cancer vaccine (GVAX) was

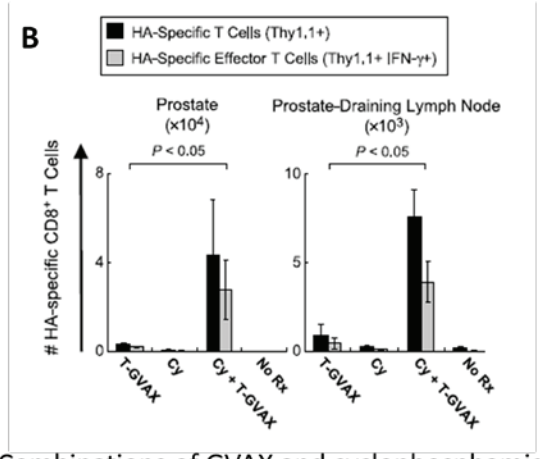
published by Dr. Drake and colleagues (Wada et al 2009). In this study, an autochthonous animal model was created using the transgenic adenocarcinoma of the mouse prostate (TRAMP) mouse crossed with analogous transgenic mice expressing the model antigen hemagglutinin (HA) in a prostate-restricted manner (ProHA). This clinically relevant murine model allowed investigation of immune responses to tissue-tumor–restricted HA as a function of immunotherapy and/or cyclophosphamide administration. In this model (ProHA × TRAMP), giving cyclophosphamide 1 day prior to GVAX immunotherapy induced maximal augmentation of HA-specific $CD8^+$ T cell responses both in the prostate and the prostate-draining lymph nodes (Figure 2a). Administering cyclophosphamide on day –4, day –2, or day +1 in relation to GVAX was not as effective. Using this treatment sequence, the optimal dose of cyclophosphamide with respect to antitumor immune responses was found to be 50 mg/kg given intraperitoneally. This dose produces serum levels equivalent to those achieved in humans using a cyclophosphamide dose of 200 mg/m^2 given intravenously. Importantly, higher doses of cyclophosphamide in this animal model resulted in a loss of immunotherapy efficacy and a suppression of specific $CD8^+$ T cell responses.

When comparing the antitumor immune responses of different permutations of GVAX and cyclophosphamide in ProHA × TRAMP mice (Wada et al 2009), it was shown that the combination of these two agents produced markedly superior HA-specific $CD8^+$ T cell responses in both the prostate and prostate-draining lymph nodes than either agent used alone (Figure 2b). This finding is particularly important, as it further demonstrates the weak antitumor immunogenicity of GVAX when used alone against prostate cancer. Further mechanistic experiments showed that immune augmentation by cyclophosphamide was associated with a transient depletion of regulatory ($CD4^+FOXP3^+$) T cells in the prostate and prostate-draining lymph nodes (Figure 2c) but not in the peripheral circulation. In addition, combined treatment resulted in increased ratios of $CD4^+$ and $CD8^+$ effector T cells to regulatory T cells (T_{eff}/T_{reg} ratio) in the prostate glands of these mice (Figure 2d). Alterations in T_{reg} function with cyclophosphamide use were not seen. Finally, combination of GVAX and cyclophosphamide resulted in increased expression of dendritic cell maturation markers, although a dramatic change in dendritic cell numbers was not observed. Taken together, these data provide a strong rationale for a strategy combining low-dose cyclophosphamide with allogeneic GVAX immunotherapy in patients with prostate cancer.

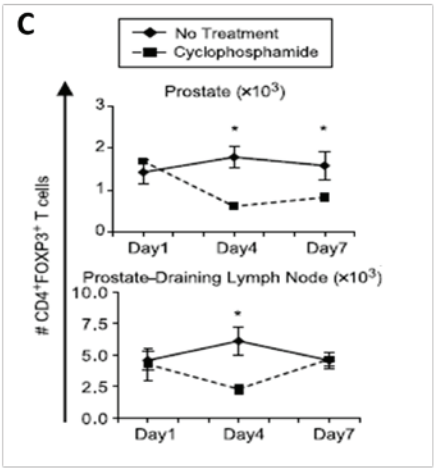
Figure 2 Cyclophosphamide Augments Antitumor Immunity Produced by GVAX



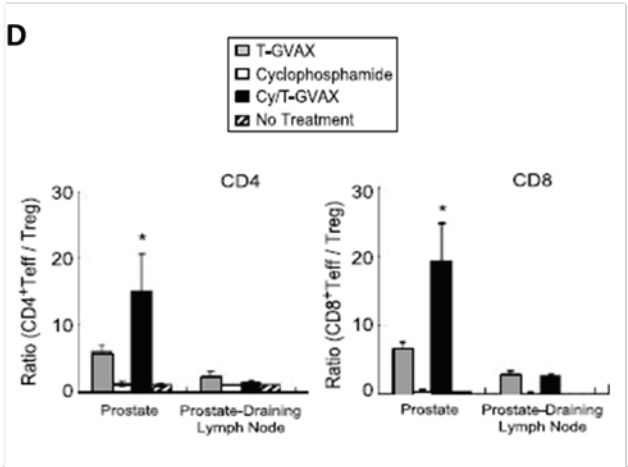
Cyclophosphamide in sequence with GVAX given to tumor-bearing ProHA × TRAMP mice



Combinations of GVAX and cyclophosphamide in tumor-bearing ProHA × TRAMP mice



Effect of cyclophosphamide/GVAX on T_{reg} numbers in ProHA × TRAMP mice



Effect of cyclophosphamide/GVAX combinations on T_{eff}/T_{reg} ratios in ProHA × TRAMP mice

2.2.4 *Immunological effects of androgen deprivation*

It is now appreciated that androgen deprivation therapy (ADT) has profound effects on the host immune system, resulting in enhancement of antitumor immune responses (Aragon-Ching et al 2007). Initial observations in this regard came from a study demonstrating that ADT administered before prostatectomy resulted in profuse $CD4^+$ T cell infiltration into human prostate glands, and that these T cells were oligoclonal suggesting an antigen-specific response (Mercader et al 2001). In a similar study of androgen-deprived prostate cancer patients, an increased $CD8^+$ T cell infiltrate was noted as well (Gannon et al 2009). Also supporting a pro-immunogenic role for ADT are recent data demonstrating induction of new antibody specificities in castrated patients (Morse et al 2010). In the laboratory, using an autocthonous prostate cancer mouse model, our group has previously shown that ADT decreases $CD4^+$ T cell tolerance to prostate cancer-associated antigens,

mitigating immune evasion (Drake et al 2005). Intriguingly, additional studies in both mice and humans have shown that ADT reverses age-related thymic involution, resulting in renewed thymopoiesis and increased output of naïve T cells (Sutherland et al 2005). Finally, expansion of the pre-B cell population is observed in the bone marrow after castration in mice, a process that may improve antigen presentation and T cell responses (Wilson et al 1995). Taken together, these data support the notion that ADT, through its augmentation of prostate-specific immune responses, may act synergistically with immunotherapy in the treatment of prostate cancer.

2.2.5 Combining androgen deprivation with immunotherapy

The addition of ADT to immunotherapy has been examined in several preclinical and clinical studies. Using our autocthonous prostate cancer mouse model, our group noted prostate-specific effector T cell expansion when a recombinant vaccinia virus vaccine was administered to castrated tumor-bearing mice, but this did not occur in non-castrated mice (Drake et al 2005). In a more recent study of tumor-free mice, vaccination with a prostate stem cell antigen (PSCA)-containing DNA-based vaccine produced greater numbers of PSCA-specific IFN-γ-secreting T cells in mice that were castrated compared to those that were not castrated (Koh et al 2009). Notably, this expansion of effector T cells was only seen when vaccination was performed prior to castration, and not when this treatment order was reversed. Another study using a PSA-expressing transgenic mouse model demonstrated proliferation of PSA-specific cytotoxic T cell in response to immunization with a PSA-based recombinant vaccinia virus but this effect was only noted in castrated mice (Arredouani et al 2010).

A small randomized clinical trial in men with non-metastatic castration-resistant prostate cancer examining the combination of antiandrogen therapy and a PSA-based recombinant poxviral vaccine showed a higher frequency of PSA-specific T cell responses in men who received vaccination before antiandrogen than in those receiving antiandrogen before vaccination (Arlen et al 2005). Intriguingly, longer follow up of this same study revealed that overall survival was increased in men who received vaccine followed by antiandrogen than in those who received this combination in the reverse order (median survival 6.2 vs 3.7 years, P = 0.045) (Madan et al 2008). Finally, a larger randomized study of sipuleucel-T versus placebo combined with ADT in men with non-castrate PSA-recurrent prostate cancer showed a slower post-treatment PSA doubling time (PSADT) in men receiving the combination therapy than in those receiving ADT alone (PSADT 5.1 vs 3.4 months, *P* = 0.038) (Beer et al 2011). In summary, these studies suggest that combined immuno-hormonal therapy may provide additive clinical benefit and is worthy of further evaluation.

2.3 Rationale

2.3.1 Rationale for conducting the study

In both animal models and humans with cancer (including prostate cancer), allogeneic cell-based GM-CSF–secreting immunotherapy is often unable to break specific T cell tolerance when this modality is used alone. Preclinical studies in an autochthonous prostate cancer mouse model have shown that giving low-dose cyclophosphamide prior to GVAX abrogates immune tolerance through enhancement of $CD8^+$ T cell infiltration into the prostate, transient depletion of regulatory T cells (T_{regs}), and increased expression of dendritic cell

maturation markers. Augmentation of antitumor immunity has also been observed in early human clinical trials where cyclophosphamide has been given in sequence with GM-CSF–secreting vaccines for the treatment of breast and pancreatic cancers.

Furthermore, emerging evidence suggests that androgen deprivation therapy (ADT) itself has profound effects on the host immune system, resulting in thymic regeneration and enhancement of antitumor immunity. In addition, preclinical and clinical studies demonstrate that ADT augments prostate cancer-specific immune responses induced by immunotherapy, suggesting that ADT may act synergistically with immunotherapy. Based on data from mouse models as well as human clinical trials, it has been suggested that prostate cancer immunotherapy may be most effective when administered in the setting of an androgen-suppressed environment.

The sequencing of androgen-ablation **after** immunotherapy is supported by preclinical data (Koh et al 2009)and more importantly by clinical data(Aragon-Ching et al 2007). In this sequence, the additive effect of these two treatment modalities likely represents a classical prime-boost regimen in which the well-established immune effects of androgen-ablation boost T cell responses initiated by vaccination. It should be noted that other sequences are possible; in fact a randomized phase II of sipuleucel-T testing two alternative sequences (NCT01431391) is currently accruing patients.

Building on these findings, we have designed a study to assess the use of ADT given alone or in sequence with low-dose cyclophosphamide and prostate GVAX, in patients scheduled to undergo radical prostatectomy. We aim (1) to determine whether ADT is immunogenic in men with localized prostate cancer by evaluating T-cell infiltration in harvested prostate glands; (2) to determine whether administering ADT in sequence with low-dose cyclophosphamide and prostate GVAX augments immune infiltration into the prostate gland; and (3) to investigate whether this combinatorial immuno-hormonal approach is safe and feasible. We hypothesize that the combination of ADT and cyclophosphamide/GVAX will produce significantly greater antitumor immune responses than ADT used alone.

If this study shows significant enhancement of antitumor immunity when using ADT in sequence with low-cyclophosphamide and prostate GVAX, and this combination is feasible and safe, then future studies would aim to use this combinatorial approach in both the adjuvant and the salvage settings. For example, a trial could be designed to assess the efficacy of ADT combined with cyclophosphamide/GVAX given before and after radical prostatectomy, using PSA recurrence as the primary endpoint. Alternatively, a trial of ADT combined with cyclophosphamide/GVAX for patients with PSA recurrence could be designed, with metastatic progression as the primary endpoint.

2.3.2 Rationale for dosage selection

The dose of prostate GVAX in this study will be the same as the priming dose used in two large phase III studies evaluating this immunotherapy in patients with castration-resistant metastatic prostate cancer (Higano et al 2009; Small et al, 2009). Five 0.8-mL intradermal injections of PC3 (2.5×10^8 cells) and five 0.5-mL intradermal injections of LNCaP (1.6×10^8 cells) will be administered, for a total dose of 4.1×10^8 cells. In contrast to previous trials, the present study will use only a single dose of immunotherapy rather than a series of doses spread out over time. A single dose should be sufficient since we will be evaluating T cell infiltration in resected prostate glands (harvested three weeks later) as our primary endpoint.

Cyclophosphamide will be administered at 200 mg/m^2, as a single intravenous infusion. In the cyclophosphamide/GVAX treatment arm, cyclophosphamide will be given one day prior to GVAX, which itself will be administered two weeks prior to prostatectomy. We hypothesize that 200 mg/m^2 of cyclophosphamide will be the optimal immune priming dose in humans because this dose produces serum levels equivalent to those achieved using 50

mg/kg intraperitoneally in the ProHA × TRAMP mouse model, the dose which produced the largest antitumor immune responses (Wada et al 2009). In addition, the 200 mg/m^2 dose of cyclophosphamide was recently used in a human pancreatic cancer study, where it was shown to augment the immune effects of an allogeneic cell-based GM-CSF–secreting pancreatic cancer vaccine (Laheru et al 2008). In another study of breast cancer patients, 200 mg/m^2 of cyclophosphamide given in sequence with a cell-based GM-CSF–secreting breast cancer vaccine seemed to be the most immunogenic dose, while doses of 250 mg/m^2 and 350 mg/m^2 appeared to suppress *HER-2*–specific immune responses (Emens et al 2008).

Degarelix acetate will be administered as three 8 mg subcutaneous injections, for a total dose of 240 mg. This is the standard loading dose of degarelix, and is expected to induce castrate levels of serum testosterone for at least 4 weeks. Degarelix, an LHRH antagonist, was chosen rather than an LHRH agonist in order to produce rapid testosterone suppression without causing an initial testosterone surge. This rapid testosterone ablation more closely resembles the effect of surgical castration in preclinical animal models.

2.3.3 Rationale for immunobiologic endpoints

The primary goal of this study is to assess the degree of immune infiltration into the prostate after administering the protocol therapy, and to determine whether the combination of ADT and cyclophosphamide/GVAX may enhance intraprostatic immune infiltration compared to ADT administered alone. In studies using the ProHA × TRAMP mouse model, the primary endpoint was hemagglutinin (HA)-specific CD8$^+$ T cell responses in harvested prostate glands (Wada et al 2009). However, in humans with prostate cancer receiving GVAX, the exact immunogenic antigen is not known. Therefore, the primary endpoint for the present study will be assessment of total CD8$^+$ T cell infiltration into the prostate. Our previous studies have shown that the mean staining percentage for CD8$^+$ T cells in human prostate tumor tissue is 0.42% (standard deviation 0.36%, interquartile range 0.07%–0.71%). In the present study, we seek to augment the intratumoral CD8$^+$ T cell response by 100% in the experimental arm compared to the control arm. A 100% increase in tumor-infiltrating CD8$^+$ T cells was sought because in patients with advanced melanoma, adoptive T cell transfer studies have shown that a 100% increase in tumor-infiltrating CD8$^+$ T cells was required to cause regression of metastatic melanoma lesions (Rosenberg et al 2011); increases in tumor-infiltrating CD8$^+$ T cells that were less than 100% did not result in tumor regressions.

In the present study, CD8$^+$ T cell density will serve as a surrogate measure of prostate-/prostate cancer-specific T cell responses. By comparison, the human breast cancer trial used *Her-2*–specific immune responses as its primary endpoint (Emens et al 2008), while the pancreatic cancer trial used mesothelin-specific T cell responses as its primary endpoint (Laheru et al 2008). One of our goals in the future will be to identify a useful tumor-associated antigen in prostate cancer patients receiving GVAX, and to use this as a more rational marker of immunologic effects.

In addition to augmenting antitumor immune responses in ProHA × TRAMP mice, the combination of cyclophosphamide/GVAX also decreased the wet weight of the urogenital tract (a gross surrogate for tumor burden) compared to either agent used alone. Moreover, histologic tumor scores were lower and apoptotic bodies were more frequent in the combined treatment group compared with either single treatment alone (Wada et al 2009). To evaluate potential direct antitumor effects in the present study, we will measure tumor apoptosis and proliferation using validated markers such as activated caspase 3 (Marcelli et al 2000) and Ki-67 (Rubin et al 2002). These will be assessed by immunohistochemical staining of formalin-fixed tissue samples. Ki-67 is a nuclear antigen that is found in proliferating cells, whose expression correlates with the degree of apoptosis and cellular turnover in prostate cancer (Berges et al 1995). Apoptotic cells will also be identified histologically and quantified by the method of terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick end labeling (TUNEL assay) (Furuya et al

1995). We will also evaluate the proportion of patients achieving a pathological complete response (pCR) in each of the 2 treatment groups.

3. PATIENT SELECTION

3.1 Target Population

Subjects will include men with multifocal, Gleason 7 or greater, clinically localized prostate cancer for whom the decision has been made to perform radical prostatectomy at Johns Hopkins Hospital. Subjects will be identified and recruited through the Outpatient Clinic of the Department of Urology and from the Multidisciplinary Prostate Cancer Clinic.

3.2 Inclusion Criteria

To be eligible for this study, patients must meet *all* of the following criteria:

- Histologically confirmed adenocarcinoma of the prostate (clinical stage T1c–T3b, N0, M0) without involvement of lymph nodes, bone, or visceral organs
- Initial prostate biopsy is available for central pathologic review, and is confirmed to show at least 2 positive cores and a maximum Gleason sum of ≥7
- Radical prostatectomy has been scheduled at Johns Hopkins Hospital
- Age ≥21 years
- ECOG performance status 0-1, or Karnofsky score ≥ 70% (see Appendix A)
- Adequate bone marrow, hepatic, and renal function:
    - WBC >3,000 cells/mm^3
    - ANC >1,500 cells/mm^3
    - Hemoglobin >9.0 g/dL
    - Platelet count >100,000 cells/mm^3
    - Serum creatinine <2.0 mg/dL
    - Serum bilirubin <2 mg/dL
    - ALT <2 × upper limit of normal (ULN)
    - AST <2 × ULN
    - Alkaline phosphatase <2 × ULN
- Willingness to provide written informed consent and HIPAA authorization for the release of personal health information, and the ability to comply with the study requirements (***note***: HIPAA authorization will be included in the informed consent)
- Willingness to use barrier contraception from the time of cyclophosphamide and/or GVAX administration until the time of prostatectomy.

3.3 Exclusion Criteria

To be eligible for this study, patients should *not* meet *any* of the following criteria:

- Presence of known lymph node involvement or distant metastases
- Other histologic types of prostate cancers such as ductal, sarcomatous, lymphoma, small cell, and neuroendocrine tumors
- Prior radiation therapy, hormonal therapy, biologic therapy, or chemotherapy for prostate cancer

- Prior immunotherapy/vaccine therapy for prostate cancer
- Previous or concurrent use of cyclophosphamide
- Concomitant treatment with other hormonal therapy or 5α-reductase inhibitors
- Current use of systemic corticosteroids or use of corticosteroids within 4 weeks of enrollment (inhaled corticosteroids for asthma or COPD are permitted)
- Use of experimental agents for prostate cancer within the past 3 months
- Known allergy to cyclophosphamide or G-CSF/GM-CSF
- Known hypersensitivity to materials of bovine origin (*e.g.* fetal bovine serum), or other components of GVAX which include DMSO and hydroxyethyl starch as well as small amounts of porcine trypsin and DNase
- History or presence of autoimmune disease requiring systemic immunosuppression (including but not limited to: inflammatory bowel disease, systemic lupus erythematosus, vasculitis, rheumatoid arthritis, scleroderma, multiple sclerosis, hemolytic anemia, Sjögren syndrome, and sarcoidosis)
- Other concurrent malignancies, with the exception of non-melanoma skin cancers and superficial bladder cancer
- Uncontrolled major active infectious, cardiovascular, pulmonary, hematologic, or psychiatric illnesses that would make the patient a poor study candidate
- Known prior or current history of HIV and/or hepatitis B/C

3.4 Inclusion of Minorities

Men of all races and ethnic groups will be considered for study participation. Candidates must conform to all eligibility criteria to be accepted into the study. Minority patients who meet entry criteria will be actively recruited, although the trial is not designed to measure differences in outcomes between ethnic groups. The estimated breakdown of the target population by race and ethnicity is: 80% white/Caucasian, 15% black/African American, and 5% comprised of other ethnic minorities.

3.5 Prohibited Concomitant Medications

Concurrent use of other anticancer agents or therapies including other experimental treatments is not permitted. Patients may not currently be taking any other form of androgen deprivation therapy, antiandrogens, 5α-reductase inhibitors, chemotherapy, radiation therapy, biologic therapy, immunosuppressive medications, or systemic corticosteroids.

Within 30 days of administration of study drug(s), patients should ideally not receive other cancer immunotherapies or vaccines including but not limited to the live rotavirus vaccine, the live BCG (bacillus Calmette-Guerin) vaccine, the live influenza vaccine, the live measles vaccine, the live mumps vaccine, the live poliovirus vaccine, the live rubella vaccine, the smallpox vaccine, the typhoid vaccine, the varicella vaccine, and the yellow fever vaccine.

The following list of medications should generally be taken with caution by patients on chronic cyclophosphamide: allopurinol, cyclosporine, etanercept, hydrochlorothiazide, nevirapine, ondansetron, pentostatin, St John's wort, tamoxifen, trastuzumab, and warfarin. In the present study, because only a single low-dose administration of cyclophosphamide will be given (in treatment arm B), these medications are not prohibited. However, the principal investigator must be notified if a patient is taking any of these listed agents.

Because of the potential for unknown drug-drug interactions, concurrent use of all other agents, over-the-counter medications, herbal remedies, vitamins/minerals, and alternative therapies must be documented on the case report form (CRF).

4. REGISTRATION AND ENROLLMENT PLAN

4.1 Registration Procedure

Patients who are considered candidates for the study will first be evaluated for eligibility by one of the principal investigators, co-investigators, or the research nurse. After screening for eligibility, patients who are eligible to participate in the trial must be registered with the Sidney Kimmel Comprehensive Cancer Center (SKCCC) according to the instructions below. A record of patients who fail to meet entry criteria (*i.e.*, screen failures) will also be maintained. Registration must be completed before beginning any study-related activities.

To initiate registration at SKCCC, study personnel should forward copies of the signed informed consent form (with embedded research authorization/HIPAA form), the institutional registration form, plus any required pathology information/laboratory tests to the project manager by fax or email. Once eligibility is confirmed, each subject will be assigned a unique patient study identification number. Treatment must not commence until the patient has received his identification number.

Prior to protocol enrollment and initiation of treatment, subjects must sign and date an IRB-approved consent form. Authorized study personnel should fully explain the scope of the study to each patient before obtaining informed consent. Patients should be advised of any known risks inherent in the planned treatments/procedures, any alternative treatment options, their right to withdraw from the study at any time and for any reason, and their right to privacy. When obtaining informed consent, study personnel should: **first**, confirm that the patient has received and has had time to read the informed consent form (including the research authorization/HIPAA form); **next**, confirm eligibility as defined in Sections 3.2 and 3.3 (inclusion and exclusion criteria); and **finally**, obtain dated and signed informed consent. A copy of the signed informed consent should be supplied to the project manager.

To register a patient, the following documents must be completed and faxed (410-614-7287) or emailed (hcao7@jhmi.edu) to the study coordinator, Harry Cao:

- signed patient consent form
- institutional registration form
- copies of the prostate cancer pathology report and baseline laboratory studies including CBC with differential, liver and kidney function tests. Other materials may also be sent if considered pertinent for confirming patient eligibility.

The principal investigator and/or other authorized study personnel will then review these documents to confirm eligibility. To complete the registration process, the project manager will assign a patient study number (*i.e.*, protocol patient number). This number is unique to the patient and must be written on all data and correspondence for the patient. The project manager will also register the patient with SKCCC's Clinical Research Office (CRO).

4.2 Expected Enrollment

A total of 32 patients (16 in each arm) will be included in this study. The first patients are expected to be enrolled in October 2012, once the protocol has been approved by the IRB. With an estimated enrollment rate of 4 patients per month, accrual is expected to be completed in 8 months.

4.3 Study Centers

This is a single-institution study that will be conducted only at Johns Hopkins through a collaboration between the Brady Urological Institute and the Sidney Kimmel Comprehensive Cancer Center.

4.4 Recruitment

Subjects will be recruited from the outpatient facilities of the Brady Urological Institute, and from the outpatient Multidisciplinary Prostate Cancer Clinic of the SKCCC. Patients will not receive payment or reimbursement for participation. Every effort will be made to include patients of racial and ethnic minorities who fulfill the eligibility criteria.

4.5 Randomization

Patients will be randomized in a 1:1 ratio to one of 2 treatment arms (see *Study Schema*). Because our data showed that T cell function is associated with the tumor grade, the randomization will be stratified by Gleason score: ≤7 vs 8-10. Treatment allocation will be determined by a computer-generated permuted-block randomization scheme developed and maintained by the Department of Oncology Biostatistics at Johns Hopkins. Randomization will be performed by the study statistician, Marianna Zahurak, MS. This pre-generated randomization scheme has been approved by the Johns Hopkins Research Pharmacy, who will also maintain a copy of the scheme.

5. STUDY PLAN

5.1 Overview and Schema

This is a single-center, randomized, 2-arm, open-label, prospective clinical trial evaluating the immunogenicity and safety of a single administration of LHRH antagonist (degarelix) used alone, or degarelix administered in sequence with low-dose cyclophosphamide and GVAX, given 3 weeks prior to radical prostatectomy in men with localized prostate cancer.

Patients will be recruited from the outpatient Urology clinic. Eligible patients will be randomized in a 1:1 ratio (stratified by Gleason score: ≤7 vs 8-10) to receive one of two treatment regimens (*see Schema below*), each beginning 28 (±3) days prior to radical prostatectomy. In Arm B, Cyclophosphamide will be given at a dose of 200 mg/m^2 as a single intravenous infusion. 1 day later, prostate GVAX will be administered as five 0.8-mL intradermal injections of PC3 (2.5 × 10^8 cells) and five 0.5-mL intradermal injections of LNCaP (1.6 × 10^8 cells), for a total dose of 4.1 × 10^8 cells. On day 14, Degarelix acetate will be administered as three 80 mg subcutaneous injections, for a total dose of 240 mg. Prostate glands will be harvested 14 (±3) days later, at the time of radical prostatectomy, and prostate tissue will be examined for the primary endpoint. In Arm A, an identical dose of degarelix acetate will be administered 14 (±3) days prior to surgery. A telephone follow-up interview (or an in-person clinic visit) to evaluate for adverse events will occur 28 (±21) days after prostatectomy. Patients will then be followed by their urologists according to standard institutional practices, but will require PSA evaluations every 3 (±1) months during year 1 and every 6 (±2) months during years 2-3.

Treatment Schema:

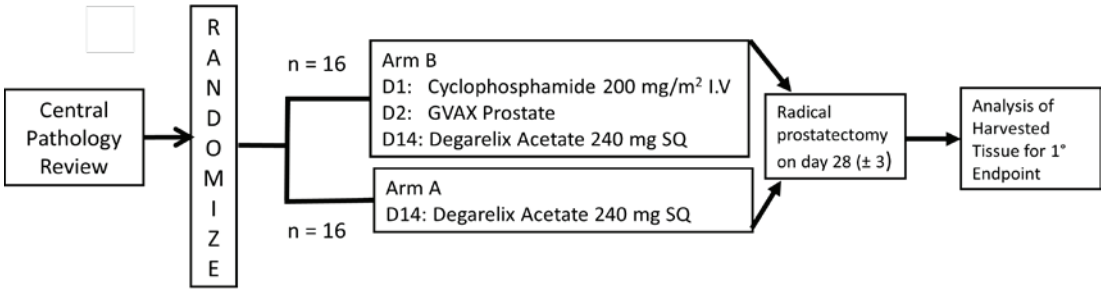


Table 1A STUDY CALENDAR for ARM A (Androgen-Ablation Alone)

Every effort should be made to keep visits, tests, and procedures on schedule. Acceptable deviations are listed below.

ARM A	Screening Evaluation [a]	Day 14	Radical Prostatectomy, Day 28 (±3)	Follow-up, 28 Days (±21) Post-op [l m]
Informed consent	X			
Medical history	X		X	
Review of medications	X		X	
Physical examination	X		X	
Vital signs	X		X	
Height and weight	X			
Performance status	X		X	X
CT and bone scan [b]	(X)			
Hematology labs [c]	X		X	X
Chemistry labs [d]	X		X	X
Serum PSA [e]	X		X	
HLA typing [f]		X		
Sera for immunoassays [g]		X	X	X
Blood (200 mL) for PBLs [h]		X		X
Degarelix acetate dose [i]		X		
Toxicity assessment	X----------			----------X
Vaccine site assessment	X----------			----------X
Surgical specimen [j]			X	
Pathologic review [k]	X		X	

a: The screening (pre-treatment) evaluation should be conducted within 3 weeks (±4 days) of starting protocol therapy.
b: Staging CT (if allergic to CT scan contrast, obtain MRI with contrast) and bone scans should only be performed if clinically indicated, and are not mandatory.
c: Hematology laboratories include hemoglobin, hematocrit, white blood cell count with differential (including absolute eosinophil count), and platelets. In addition, prothrombin time (PT/INR) and activated partial thromboplastin time (APTT) should be checked as clinically indicated.
d: Chemistry laboratories include sodium, potassium, chloride, bicarbonate, urea nitrogen, creatinine, glucose, calcium, albumin, total protein, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase.
e: Serum PSA should be obtained pre-treatment and on the day of surgery, and as clinically indicated thereafter according to institutional practices.
f: At baseline (day 14), collect 10 mL of blood into a 10-mL ACD (acid citrate dextrose) tube [test code 4470; CPT code 86813].
g: Blood samples will be collected at day 14 and again on the day of prostatectomy and at follow up visit. At each time point, 2 plain (red top) or SST (gold top) tubes should be collected, each containing ≥5mL of blood. After centrifugation, serum should be transferred into plastic cryovials and frozen at –20°C or colder until the time of analysis (see **Section 5.4**).
h: Peripheral blood lymphocytes (PBLs) will be obtained by collecting 200 mL of blood into 50-mL heparinized conical tubes. PBLs will be prepared by Ficoll-Hypaque density gradient centrifugation according to standard protocols, and will be cryopreserved in a liquid nitrogen freezer at –140°C for further batched analyses.
i: Degarelix acetate may be administered 2-14 days after the screening visit, and that day is designated as day + 14 for future reference. See also the treatment schema for further information on dosage and timing of degarelix acetate.
j: Harvested prostate gland to be evaluated for $CD8^+$ T cell infiltration, as well as other secondary endpoints.
k: Archival prostate core biopsies to be centrally reviewed at baseline prior to study entry; radical prostatectomy specimens to be processed at Johns Hopkins according to standard procedures.
l: The post-operative evaluation (28 days after prostatectomy) may take place over the telephone or in person, but patients are required to have hematology and chemistry labs collected at this time-point for safety purposes.
m: In addition, PSA should be measured every 3 (±1) months in the first year after prostatectomy, and every 6 (±2) months in the second and third years after prostatectomy.

Table 1B STUDY CALENDAR for ARM B (Experimental Arm)

Every effort should be made to keep visits, tests, and procedures on schedule. Acceptable deviations are listed below.

ARM B	Screening Evaluation [a]	Day 1	Day 2	Day 14	Radical Prostatectomy, Day 28 (±3)	Follow-up, 28 Days (±21) Post-op [k l]
Informed consent	X					
Medical history	X				X	
Review of medications	X				X	
Physical examination	X				X	
Vital signs	X				X	
Height and weight	X					
Performance status	X				X	X
CT and bone scan [b]	(X)					
Hematology labs [c]	X				X	X
Chemistry labs [d]	X				X	X
Serum PSA [e]	X				X	
HLA typing [f]		X				
Sera for immunoassays [g]		X			X	X
Blood (200 mL) for PBLs [h]		X				X
Cyclophosphamide dose		X				
GVAX dose			X			
Degarelix acetate dose				X		
Toxicity assessment	X----------					----------X
Vaccine site assessment	X----------					----------X
Surgical specimen [i]					X	
Pathologic review [j]	X				X	

a: The screening (pre-treatment) evaluation should be conducted within 3 weeks (±4 days) of starting protocol therapy.
b: Staging CT (if allergic to CT scan contrast, obtain MRI with contrast) and bone scans should only be performed if clinically indicated, and are not mandatory.
c: Hematology laboratories include hemoglobin, hematocrit, white blood cell count with differential (including absolute eosinophil count), and platelets. In addition, PT/INR and APTT should be checked as clinically indicated.
d: Chemistry laboratories include sodium, potassium, chloride, bicarbonate, urea nitrogen, creatinine, glucose, calcium, albumin, total protein, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase.
e: Serum PSA should be obtained pre-treatment and on the day of surgery, and as clinically indicated thereafter according to institutional practices.
f: At baseline (day 1), collect 10 mL of blood into a 10-mL ACD (acid citrate dextrose) tube [test code 4470; CPT code 86813].
g: Blood samples will be collected at day 1 and again on the day of prostatectomy and at follow up visit. At each time point, 2 plain (red top) or SST (gold top) tubes should be collected, each containing ≥5mL of blood. After centrifugation, serum should be transferred into plastic cryovials and frozen at –20°C or colder until the time of analysis (see **Section 5.4**).
h: Peripheral blood lymphocytes (PBLs) will be obtained by collecting 200 mL of blood into 50-mL heparinized conical tubes. PBLs will be prepared by Ficoll-Hypaque density gradient centrifugation according to standard protocols, and will be cryopreserved in a liquid nitrogen freezer at –140°C for further batched analyses.
i: Harvested prostate gland to be evaluated for $CD8^+$ T cell infiltration, as well as other secondary endpoints.
j: Archival prostate core biopsies to be centrally reviewed at baseline prior to study entry; radical prostatectomy specimens to be processed at Johns Hopkins according to standard procedures.
k: The post-operative evaluation (28 days after prostatectomy) may take place over the telephone or in person, but patients are required to have hematology and chemistry labs collected at this time-point for safety purposes.
l: In addition, PSA should be measured every 3 (±1) months in the first year after prostatectomy, and every 6 (±2) months in the second and third years after prostatectomy.

5.2 Screening/Pretreatment Evaluation

Before initiating any screening activities, the scope of the study should be explained to each patient. Patients should be advised of any known risks inherent in the planned procedures, any alternative treatment options, their right to withdraw from the study at any time and for any reason, and their right to privacy. After this explanation, patients should be asked to sign and date an IRB-approved informed consent form that meets the requirements of the Code of Federal Regulations (Federal Register Vol. 46, No. 17, January 27, 1981, part 50).

The pretreatment/screening visit will determine patient eligibility according to the inclusion and exclusion criteria. All subjects must undergo a number of baseline evaluations as part of this screening visit, as detailed below. All of these evaluations should be conducted within 21 ± 4 days of starting the protocol. This information is also summarized in the Study Calendar (Tables 1A and 1B).

- informed consent (including research authorization)
- demographic information
- medical history, including review of systems
- performance status, using ECOG or Karnofsky scales (Appendix A)
- physical examination
- vital signs: temperature, pulse, sitting blood pressure, respiratory rate
- height and weight
- current medication list, including drug allergies/adverse events
- hematological laboratories (hemoglobin, hematocrit, white blood cell count with differential [including absolute eosinophil count], platelets); coagulation profile (INR, aPTT) if clinically indicated
- serum chemistry profile (sodium, potassium, chloride, bicarbonate, urea, creatinine, glucose, calcium, albumin, total protein, bilirubin, ALT, AST, alkaline phosphatase)
- serum PSA level
- sera for immunoassays (Pre-treatment) (see Section 5.4)
- CT (If allergic to CT scan contrast, obtain MRI with contrast) and/or bone scan, only if clinically indicated (not mandatory)
- central pathologic review of prostate core biopsies

After all relevant screening information is documented, registration should be finalized and appropriate documents (*i.e.*, signed informed consent, supporting source documentation for eligibility) should be faxed or emailed to the program manager.

Information on patients who do not meet eligibility criteria to participate in this study (*i.e.*, screening failures) should also be captured at the pretreatment visit.

5.3 Radical Prostatectomy

The following must be performed on the day of radical prostatectomy, which itself should occur 28 (±3) days after the administration of degarelix acetate.

- medical history
- performance status
- physical examination, including vital signs

- review of medication list
- review of toxicity/adverse events
- hematological laboratories and serum chemistry profile
- serum PSA
- sera for immunoassays (see Section 5.4)
- collection of prostatectomy tissue for analysis of study endpoints
- pathological processing of prostatectomy specimen according to standard procedures

5.4 Sera for Immunoassays

At each time point (Pre-treatment, at prostatectomy, and at follow-up visit), the following research blood samples should be collected and processed as outlined below:

- Draw approximately 10 mL of peripheral blood into 2 plain (red top) or SST (gold top) tubes, each containing ≥5 mL of blood per vacutainer.
- Allow blood to coagulate for 20 minutes, then centrifuge at 25°C, 1500 x g (2700-3000 rpm), for 15 minutes.
- Pipette the serum into 10 cryotubes (about 0.5 mL/tube).
- Store cryotubes frozen, below –20°C (–70°C preferred), until the time of analysis.

5.5 Follow-Up Evaluations

A follow-up visit scheduled for 28 (±21) days after radical prostatectomy may occur in the outpatient clinic or by telephone interview. Required evaluations during this follow-up visit are listed below. Patients should then continue to be followed by their treating urologist according to standard institutional practices.

- performance status
- review of toxicity/adverse events
- assessment for vaccine site reactions

Patients withdrawing from the study early because of adverse events should be followed until the adverse event has either resolved or stabilized. Reasons for premature withdrawal should be determined and documented.

In addition, patients will be required to have a structured assessment of post-operative PSA measurements for 3 years. In the first year after prostatectomy, PSA will be measured every 3 months (±1 mo). In the second and third years after prostatectomy, PSA will be measured every 6 months (±2 mo). These PSA measurements may be obtained outside of Johns Hopkins, but the results need to be made available to the study team.

Long-Term Follow-Up Protocol. All patients that have received GVAX will also be encouraged to enter a separate long-term follow-up protocol to assess for potential late toxicities of GVAX (*e.g.* autoimmune disease, second malignancies). This long-term follow-up protocol will involve annual visits for 3 years.

5.6 Duration of Therapy

Participation in this study will be terminated for any of the following reasons listed below:

- the patient decides to withdraw from the study (withdrawal of consent) due to unacceptable toxicities or for any other reason
- the patient completes all of the protocol procedures and follow-up requirements

- there are adverse events that, in the judgment of the investigator, may cause severe or permanent injury or are incompatible with continuation on study
- there are major violations to the study protocol or the patient is noncompliant with treatments, as judged by the investigator
- the patient experiences concurrent illness or a change in his condition that, in the judgment of the investigator, renders him unacceptable for further treatment on study
- the patient dies
- the patient is lost to follow-up
- the study is prematurely terminated for safety or feasibility concerns or other reasons

Patients should be removed from the study when any of the above criteria are met. Because an excessive rate of withdrawals can render the study uninterpretable, unnecessary withdrawal of patients should be avoided if possible. When a patient leaves the study early, the investigator should make every effort to contact the patient and to perform the final follow-up evaluation (even by telephone interview). The reason for removal of a patient from the study, and the date of removal, must be appropriately documented.

Patients will be replaced if they are removed from the study after signing the informed consent but before receiving degarelix, cyclophosphamide, or GVAX. Patients receiving at least one dose of the study drugs will be included in safety analyses, and those also undergoing prostatectomy will be used for the efficacy analyses.

6. THERAPEUTIC AGENTS

6.1 Prostate GVAX Immunotherapy

6.1.1 Dosage, Formulation, and Administration

The prostate GVAX immunotherapy will consist of 2.5×10^8 cells from the AAV genomic GM-CSF–transduced PC3 Clinical Lot and 1.6×10^8 cells from the AAV genomic GM-CSF–transduced LNCaP Clinical Lot. GVAX will only be given to patients in arm B.

Prostate GVAX will be administered as five 0.8-mL intradermal injections of PC3 (2.5×10^8 cells) and five 0.5-mL intradermal injections of LNCaP (1.6×10^8 cells), for a total dose of 4.1×10^8 cells. In the case of **PC3**, each vile is filled with 1mL of cells in suspension. The contents of <u>five</u> such vials should be distributed into 5 syringes. Because it is not possible to remove a full 1mL from each vial after thawing, a minimum of 0.8-mL should be drawn into each of the 5 syringes. In the case of **LNCaP**, each vile is also filled with 1mL of cells in suspension. The contents of <u>three</u> such vials should be distributed into 5 syringes. Because it is not possible to remove a full 1mL from each vial after thawing, approximately 0.5-mL should be drawn into each of the 5 syringes. Every attempt should be made to use the *entire* content of three vials of LNCaP cells, distributed among these 5 syringes (*i.e.* none of the LNCaP cells should be discarded).

The immunotherapy clinical lots were manufactured and released by the Cell Processing and Gene Therapy Facility, a cGMP-compliant facility, at the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins. The manufacturing and product release data for the PC3 and LNCaP cell lines were submitted to the FDA prior to initiating this study.

The transduced cells are irradiated prior to cryopreservation and stored frozen in the vapor phase of liquid nitrogen until the day of use. The cryoprotectant is in an injectable formulation: 6% hetastarch (in 0.9% sodium chloride) supplemented with 2% human serum albumin and 5% DMSO.

The cell lines used for the vaccine preparation have undergone extensive regulatory testing and have been determined to be sterile and free of viral contamination.

The details of production, irradiation, freezing, and preparation for administration are described in detail in the Investigational New Drug (IND) submission.

Released vials of the AAV genomic GM-CSF–transduced PC3 Clinical Lot and the AAV genomic GM-CSF–transduced LNCaP Clinical Lot will be transferred from the Cell Processing and Gene Therapy Facility to the CTL in a 'dry' liquid nitrogen shipper. The vials will be stored in a vapor-phase liquid nitrogen freezer until use, in accordance with existing standard operating procedures. The freezer is alarmed and monitored using an R & D Datatron System.

Administration of the cells will be given as intradermal injections using a 22-gauge needle and must be administered within 75 minutes from the time that the vials have thawed. Each cell line should be administered on a separate leg. Injections should use the upper portion of the thigh. Injections should be placed randomly, approximately 2 inches apart from each other. If the patient is unable to receive an injection in the legs, an acceptable alternative injection site is the abdomen. Do not place injections into skin that is ulcerated or infected. Patients must remain in the clinic for observation for ≥60 minutes following vaccination.

A lidocaine-based topical anesthetic cream (*e.g.* EMLA or ELA-MAX) will be recommended to be applied to each injection site approximately 1-2 hours (up to 2.5 hours) prior to vaccination to diminish the discomfort associated with the intra-dermal injections. The topical anesthetic is optional and participants are allowed to decline its use. Self-application of the topical anesthetic is allowed after the research participant receives instruction. If the research participant is non-compliant with topical anesthetic administration instructions, the topical anesthetic will be applied by the research nurse. If the subject is allergic to lidocaine, the topical anesthetic should not be used. All research participants will be seen in the Oncology Outpatient Center for immunotherapy administration and monitoring.

6.1.2 Potential Toxicities

Toxicities will be assessed using the most recent National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI CTCAE version 4.0 – see Appendix B).

6.1.2.1 Potential Toxicities of the Storage Solutions

Dimethyl Sulfoxide (DMSO)

The prostate GVAX vaccine is cryopreserved in a solution containing approximately 5% DMSO. DMSO is a cryoprotectant used to store a variety of infused cell products including hematopoietic stem cells and bone marrow. After cells are thawed, DMSO contained in the freezing solution is infused along with the cells. Toxicity associated with DMSO infusion is usually limited to minor changes in heart rate, blood pressure, fever, chills, sweats, nausea, vomiting and headaches. These are usually self-limited, lasting no more than a few hours. The most notable effect is a garlicky

odor due to exhalation of a DMSO metabolite that may last up to one day. More severe adverse effects include severe acute allergic reactions including anaphylactoid reactions, pulmonary embolism, respiratory failure, renal failure, cardiac arrhythmias, seizures, and death. These side effects are rarely observed and occur primarily in patients receiving much higher volumes of cryopreserved cell products than being infused in the present study. Nevertheless, patients will be carefully monitored for any adverse effects as outlined in Section 7. Any adverse events should be documented and recorded on the appropriate Case Report Form.

Human Serum Albumin (HSA)

The prostate GVAX vaccine is formulated in a cryoprotectant containing 2% human serum albumin. Albumin solutions are FDA-approved for volume resuscitation (treatment of hypovolemia, shock, and burns). Although hypersensitivity reactions are possible, reactions are not anticipated to the small amount of albumin administered in this study.

Hetastarch (6%)

The prostate GVAX vaccine is formulated in a cryoprotectant containing 6% hetastarch in 0.9% sodium chloride. Toxicities associated with the intravenous administration of 6% hetastarch include circulatory overload, abnormalities of coagulation (prolonged prothrombin, activated partial thromboplastin and clotting times), and hypersensitivity reactions characterized by wheezing, urticaria, hypotension, and anaphylactic/ anaphylactoid reactions.

6.1.2.2 Potential Toxicities of the Johns Hopkins Prostate GVAX Immunotherapy

The Johns Hopkins prostate GVAX immunotherapy will consist of two allogeneic prostate cell lines (PC3 and LNCaP). GM-CSF–secreting vaccines have been administered to over 300 patients at Johns Hopkins and toxicities have been limited to localized vaccine site reactions involving erythema and tenderness. Rarely, systemic reactions have been reported such as low-grade fevers and Grover's syndrome (transient acantholytic dermatosis).

The GM-CSF released by the PC3/LNCaP cells after injection may also cause side effects. Redness at old injection sites may return long after the administration of GVAX. The following side effects have also been reported by people taking GM-CSF: fever, chills, weakness, headache, bone pain, and muscle aches. Other side effects reported less often are difficulty breathing, rash, fluid overload/anasarca, tachycardia, arrhythmia, leukopenia, pain (*e.g.* abdomen, back, chest, and joint pain), venous thrombosis, abnormal liver function tests, and pleural/pericardial effusions.

GVAX contains porcine trypsin, fetal bovine serum, dextran sulfate and DNase and could cause an allergic reaction in sensitized individuals. There is a possible risk of a severe allergic reaction (anaphylaxis) to GVAX.

The LNCaP cell line contains very small amounts of fetal bovine serum. There is a remote possibility that bovine serum may contain known or unknown infectious agents that may cause human disease, such as Creutzfeldt-Jakob (mad cow) disease.

There is a slight possibility that GVAX could cause autoimmune disease. This has only been observed when GVAX was combined with the CTLA4-blocking monoclonal antibody, ipilimumab.

There is a theoretical concern that people who receive gene therapy may develop a new cancer.

A single dose of GVAX given 2 weeks prior to prostatectomy is not expected to cause side-effects that would potentially delay surgery or negatively affect surgical outcomes. Nonetheless, its influence on prostatectomy will be closely monitored.

GVAX is an experimental product that may have other side effects that are unknown and have not been predicted. These side effects may be serious.

6.1.2.3 Potential Toxicities Observed with the Cell Genesys Prostate GVAX®

Cell Genesys Prostate GVAX® has been given to at least 1200 men with hormone-refractory prostate cancer in prior clinical studies. Nearly all men developed swelling and redness where CG1940 (PC3) and CG8711 (LNCaP) were injected. Most of the men also had itching and pain at the injection site. In addition, other reported side effects were fatigue, malaise, headache, myalgias, arthralgias, fever, chills/rigors, rash, anemia, weight loss, nausea, decreased appetite, flu-like symptoms, weakness, constipation, general body pains, dizziness, back pain, and hives.

In a trial comparing Cell Genesys GVAX® to docetaxel, an increase in serious urinary tract obstructions was noticed in patients that received GVAX® as compared to those that received docetaxel. In that study, three patients had serious side effects that may have been related to GVAX®, and later died. An 82 year-old man died in his sleep four days after his first GVAX® treatment; the cause of his death was unknown. After a fall, another man had a subdural hematoma, thrombocytopenia, and later died. A third man had a stroke which resulted in death.

In another trial comparing docetaxel/GVAX® to docetaxel/prednisone, adverse events on the vaccine arm included infections, anemia, metabolic disturbances, altered mental status, abnormal coordination, seizure, cardiovascular complications, respiratory complications, abnormal taste, abdominal pain, chest pain, cough, hypertension, flushing, gastrointestinal disturbance, musculoskeletal effects, renal impairment, and fractures. It is unclear whether or not these side effects were related to GVAX®, docetaxel, or due to the omission of prednisone in that treatment arm. In addition, there were 8 deaths in the GVAX® arm in that trial that were not attributable to prostate cancer progression. These deaths were caused by cardiac arrest (2), respiratory arrest (1), myocardial infarction (1), intracranial bleed (1), ischemic encephalopathy (1), pneumonia (1), and failure to thrive (1).

Additional serious side effects have been reported as being related to GVAX® when given alone: anemia, deep venous thrombosis, sensorineural hearing loss, difficulty walking, weakness in the lower legs, severe joint pain, severe muscle pain, worsening of existing arrhythmia, dehydration resulting in kidney failure, hypercalcemia, nausea, vomiting, weight loss, pulmonary embolism, shortness of breath, fever, diarrhea, and malaise. Also, GVAX® may possibly be related to hypothyroidism and adrenal insufficiency.

6.1.3 Treatment Modifications and Dosing Delays

Since only a single dose of prostate GVAX will be administered to each patient (arm B only), dose modifications or treatment delays will not be permitted.

6.1.4 Supportive Care and Concomitant Medications

Injection site reactions: EMLA cream (or a similar product) should be applied to the site of vaccination at a dose of approximately 2.5 grams per site approximately one hour prior to vaccination to mitigate injection-site pain.

Nausea/vomiting: GVAX does not typically induce nausea or vomiting. However, prochlorperazine may be given for this indication at a dose of 10 mg by mouth or intravenously as frequently as every 6 hours. *Oral or intravenous corticosteroid use is not permitted.* However, prophylactic antiemetic therapy at the discretion of the treating physician is allowed.

During treatment (and for at least 60 minutes afterwards), patients should be observed for adverse reactions. Treatment areas should have ACLS-certified personnel on site, as well as access to appropriate supportive care modalities including but not limited to oxygen administration, corticosteroids, antihistamines and catecholamines. Any or all of these modalities may be used in the case of hypersensitivity reactions or anaphylactic reactions.

The interaction of PC3/LNCaP and other vaccines has not been studied, and there is a possibility that PC3/LNCaP may affect the immune response to other vaccines and *vice versa*. Whenever possible, patients should not receive another vaccine within 30 days of a dose of GVAX. However, medically indicated vaccines should not be delayed if doing so may be detrimental to the patient.

6.2 Low-Dose Cyclophosphamide

6.2.1 Dosage and Schedule

Immunomodulatory cyclophosphamide will be given in the outpatient setting as a single administration, about 4 weeks prior to prostatectomy. It will be given at a dose of 200 mg/m^2, one day before administration of prostate GVAX. It will only be given to patients in arm B.

6.2.2 Formulation and Preparation

Cyclophosphamide is a sterile, white, lyophilized powder containing 75 mg mannitol per 100 mg cyclophosphamide (anhydrous) supplied in 100 mg, 200 mg, 500 mg, 1000 mg, and 2000 mg vials for single-dose intravenous administration.

Lyophilized cyclophosphamide should be prepared for parenteral (intravenous) use by adding sterile water for injection (USP) to the vial, and shaking to dissolve. Vials containing 100 mg, 200 mg, 500 mg, 1000 mg, and 2000 mg should be reconstituted with 5 ml, 10 ml, 20 ml, 50 ml, and 100 ml respectively. Cyclophosphamide may be infused intravenously using the following solutions: 5% dextrose, 5% dextrose and 0.9% sodium chloride, 5% dextrose and Ringer's injection USP, lactated Ringer's injection USP, or sodium lactate injection USP.

Vials of cyclophosphamide are stable at room temperature and at temperatures less than 32°C. Reconstituted parenteral solutions are stable for 24 hours at room temperature (RT), or for 14 days if refrigerated at 2-8°C. After 24 hours at RT, or 14 days at 2-8°C, reconstituted cyclophosphamide must be discarded.

6.2.3 Drug Administration

Cyclophosphamide will be obtained from a commercial supplier and prepared as per the institutional standard procedures. Cyclophosphamide will be administered by intravenous infusion over 30 min in the outpatient setting. Cyclophosphamide should not be mixed or diluted with other drugs.

6.2.4 Potential Toxicities

Toxicity will be assessed using the most recent National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI CTCAE version 4.0 – see Appendix B).

The dose of cyclophosphamide proposed in this trial is below those commonly used when this agent is employed as a cytotoxic drug (*e.g.* typically 600 mg/m^2 of cyclophosphamide is used per treatment cycle in breast cancer chemotherapy). In addition, a 200 mg/m^2 dose of cyclophosphamide is at about 1/9th the dose used for pre-conditioning during bone marrow transplant or for primary management of some autoimmune disorders.

Therefore, we anticipate that the risk of toxicity related to the use of a single dose of cyclophosphamide at 200 mg/m^2 is very small. Based on the toxicity evaluations of higher dose of cyclophosphamide (600 mg/m^2), the most common adverse events with this agent include fatigue, alopecia, nausea, vomiting, diarrhea, fluid retention, skin rash, cytopenias, and infections. In addition, hemorrhagic cystitis, interstitial pneumonitis, and cardiomyopathy have been observed. However, these side effects have not been reported with the low doses of cyclophosphamide that will be used in this study. With standard-dose cyclophosphamide, secondary leukemia and myelodysplastic syndrome have also been reported as rare late occurrences.

Allergic and anaphylactic reactions to cyclophosphamide have been reported. In addition, rare cases of Stevens-Johnson syndrome/toxic epidermal necrolysis have occurred.

A single dose of cyclophosphamide given 3 weeks prior to prostatectomy is not anticipated to cause side-effects that would potentially delay surgery or negatively affect the outcomes of surgery.

6.2.5 Treatment Modifications and Dosing Delays

Since only a single dose of cyclophosphamide will be administered to each patient (arm B only), dose modifications or treatment delays will not be permitted.

6.2.6 Supportive Care and Concomitant Medications

Nausea/vomiting: Low-dose cyclophosphamide does not typically induce nausea or vomiting. However, prochlorperazine may be given for this indication at a dose of 10 mg by mouth or intravenously as frequently as every 6 hours. *Oral or intravenous corticosteroid are not permitted*. However, prophylactic antiemetic therapy at the discretion of the treating physician is allowed.

During treatment (and for at least 60 minutes afterwards), patients should be observed for adverse reactions. Treatment facilities should have ACLS-certified personnel on site, as well as access to appropriate supportive care modalities including but not limited to oxygen administration, corticosteroids, antihistamines and catecholamines. Any or all of these modalities may be used in the case of hypersensitivity reactions or anaphylactic reactions.

The following medications should generally be taken with caution by patients on chronic cyclophosphamide: allopurinol, cyclosporine, etanercept, ondansetron, hydrochlorothiazide, nevirapine, pentostatin, St John's wort, tamoxifen, warfarin, and trastuzumab. In the present study, because only a single administration of low-dose cyclophosphamide will be given, these medications are not prohibited. However, the Principal Investigator should be notified if a patient is taking any of these listed agents.

6.3 Degarelix Acetate

6.3.1 Dosage and Schedule

Degarelix will be given in the outpatient setting as three 80 mg subcutaneous injections, for a total dose of 240 mg, approximately 2 weeks prior to radical prostatectomy. It will be given to patients in both treatment arms, A and B.

6.3.2 Formulation and Preparation

Degarelix is supplied as a powder for subcutaneous injection. One vial of degarelix contains 80 mg of degarelix. Each vial is to be reconstituted with 2 mL of sterile water for injection, USP. 2 mL is withdrawn to deliver 80 mg degarelix at a concentration of 40 mg/mL. One dose of degarelix comprises 240 mg given as three 2 mL injections of 80 mg each.

6.3.3 Drug Administration

Degarelix is administered as a subcutaneous injection in the abdominal region. As with other drugs administered by subcutaneous injection, the injection site should vary periodically. Injections should be given in areas of the abdomen that will not be exposed to pressure, *e.g.* not close to waistband or belt.

Degarelix is supplied as a powder to be reconstituted with sterile water for injection, USP. The reconstitution procedure needs to be carefully followed. Administration of other concentrations is not recommended. See "Instructions for Proper Use" below.

Instructions for Proper Use

• Gloves should be worn during preparation and administration.

• Reconstituted drug must be administered within one hour after addition of sterile water for injection, USP.

• Keep the vial vertical at all times

• Do not shake the vials

• Follow aseptic technique

Preparation

• Draw up 2 mL of sterile water for injection, USP (WFI) using a reconstitution needle (21G/2 in)

• Inject the WFI slowly into the degarelix 80 mg vial. To keep the product and syringe sterile, do not remove the syringe and the needle.

• Keeping the vial in an upright position, swirl it very gently until the liquid looks clear and without undissolved powder or particles. If the powder adheres to the vial over the liquid surface, the vial can be tilted slightly to dissolve powder. Avoid shaking to prevent foam formation. A ring of small air bubbles on the surface of the liquid is acceptable. The reconstitution procedure may take up to 15 minutes.

• Tilt the vial slightly and keep the needle in the lowest part of the vial. Withdraw 3 mL of degarelix 80 mg without turning the vial upside down.

• Exchange the reconstitution needle with the administration needle for deep subcutaneous injection (27G/1.25 in). Remove any air bubbles.

• Inject 2 mL of degarelix 80 mg subcutaneously immediately after reconstitution. Grasp the skin of the abdomen, elevate the subcutaneous tissue. Insert the needle deeply at an angle of not less than 45 degrees. Gently pull back the plunger to check if blood is aspirated. If blood appears in the syringe, the reconstituted product can no longer be used. Discontinue the procedure and discard the syringe and the needle (reconstitute a new dose for the patient).

• Repeat reconstitution procedure for the second dose. Choose a different injection site and inject 3 mL.

6.3.4 Potential Toxicities

The most commonly observed adverse reactions with degarelix therapy include injection site reactions (*e.g.* pain, erythema, swelling or induration), hot flashes, increased weight,

fatigue, and increases in serum levels of transaminases and glutamyltransferase (GGT). The majority of the adverse reactions are grade 1 or 2, with grade 3/4 adverse reactions occurring at incidences of ≤1%.

The most frequently reported adverse reactions at the injection sites are pain (28%), erythema (17%), swelling (6%), induration (4%) and nodule (3%). These adverse reactions are mostly transient, of mild to moderate intensity, occur primarily with the starting dose and lead to few discontinuations (<1%). Grade 3 injection site reactions occur in ≤2% of patients receiving degarelix. Hepatic laboratory abnormalities are primarily grade 1 or 2 and are generally reversible. Grade 3 hepatic laboratory abnormalities occur in <1% of patients.

Other potential toxicities of degarelix include fatigue, asthenia, chills, night sweats, hypertension, arthralgias, back pain, diarrhea, constipation, erectile dysfunction, gynecomastia, hyperhidrosis, headache, dizziness, and insomnia.

6.3.5 Treatment Modifications and Dosing Delays

Since only a single dose of degarelix acetate will be administered to each patient, dose modifications or treatment delays will not be permitted.

6.3.6 Concomitant Medications

Degarelix is not a substrate for the human CYP450 enzyme system. Degarelix is not an inducer or inhibitor of the CYP450 system *in vitro*. Therefore, clinically significant CYP450 pharmacokinetic drug-drug interactions are unlikely.

6.4 Concomitant Medications and Supportive Care

Because of the potential for drug-drug interactions, the concurrent use of all other drugs, over-the-counter medications, and alternative therapies must be documented on the case report form (CRF).

Concurrent use of other therapies (including other experimental modalities) intended for the treatment of prostate cancer is not permitted. Patients may not currently be taking any other form of androgen deprivation therapies, antiandrogens, 5α-reductase inhibitors, chemotherapy, radiation therapy, biologic therapy, or immunosuppressive drugs.

Because glucocorticoids (*e.g.* dexamethasone) may contribute to immunosuppression, patients should generally not initiate steroid therapy while on study. Accordingly, intermittent use of dexamethasone as an antiemetic agent is discouraged. The Principal Investigator must be notified if steroid use is being considered. Subjects may receive other forms of non-cancer-directed endocrine replacement therapy (e.g. thyroxine replacement and insulin replacement).

Injection site reactions: For patients receiving GVAX, EMLA cream (or a similar product) should be applied to the site of vaccination at a dose of approximately 2.5 grams per site approximately one hour prior to vaccination to mitigate injection-site pain.

Nausea/vomiting: GVAX and low-dose cyclophosphamide do not typically induce nausea or vomiting. However, prochlorperazine may be given for this indication at a dose of 10 mg by mouth or intravenously as frequently as every 6 hours. Prophylactic antiemetic therapy at the discretion of the treating physician is allowed.

During treatment (and for at least 60 minutes afterwards), patients should be observed for adverse reactions. Treatment areas should have ACLS-certified personnel on site, as well as access to appropriate supportive care modalities including but not limited to oxygen administration, corticosteroids, antihistamines and catecholamines. Any or all of these modalities may be used in the case of hypersensitivity reactions or anaphylactic reactions.

Diet will not be regulated on this study, except as required by standard pre-operative protocols. The use of vitamins, nutritional supplements, homeopathic medications, and herbal remedies is

discouraged and must be approved by the principal investigator. Multivitamin tablets and calcium supplementation are generally permitted.

Patients should receive full supportive care as appropriate, including transfusions of blood and blood products, erythropoietin, antibiotics, antiemetics, analgesics, electrolyte replacement, and other supportive measures. The indications, doses, and dates of such treatments should be recorded on the CRFs. Use of growth factors such as filgrastim/peg-filgrastim (G-CSF) or sargramostim (GM-CSF) is discouraged.

6.5 Drug Interactions/Cautions

Within 30 days of administration of study drug(s), patients should not receive other vaccines including but not limited to the live rotavirus vaccine, the live BCG (bacillus Calmette-Guerin) vaccine, the live influenza vaccine, the live measles vaccine, the live mumps vaccine, the live poliovirus vaccine, the live rubella vaccine, the smallpox vaccine, the typhoid vaccine, the varicella vaccine, and the yellow fever vaccine.

The following list of medications should generally be taken with caution by patients on chronic cyclophosphamide: allopurinol, cyclosporine, etanercept, hydrochlorothiazide, nevirapine, ondansetron, pentostatin, St John's wort, tamoxifen, trastuzumab, and warfarin. In the present study, however, because only a single low-dose administration of cyclophosphamide will be given, these medications are not prohibited. Nevertheless, the principal investigator must be notified if a patient is taking any of these listed agents.

6.6 Drug Dispensing Log

Study site personnel should record all study drugs administered during this trial on the drug-dispensing log. This log may also be reviewed by the research pharmacist. The drug dispensing log will contain the following information:

- patient study identification number
- date(s) of study drug administered
- doses/quantities of study drug(s) administered
- signature of the investigator or authorized research nurse

6.7 Pregnancy

Men with partners of child-bearing potential must agree to use adequate contraception (hormonal and/or barrier methods; or abstinence) for the duration of the study and for 3 months after the last dose of study drug(s).

7. ADVERSE EVENTS AND REPORTING REQUIREMENTS

An adverse event (AE) is defined as any untoward medical occurrence (symptom, sign, illness or experience) that develops or worsens in a research patient during a clinical study or within 30 days post-treatment, regardless of causality. This includes adverse clinical or laboratory findings, any adverse drug reaction (ADR), an illness with onset during the study, or an exacerbation of a preexisting illness or condition. Cancer progression should not be considered an AE, unless the investigator believes that the study treatment exacerbated the patient's condition. Exceptions are if disease progression results in death or hospitalization while a patient is on study, in which case the disease progression is considered a serious AE. Abnormal findings on physical examination or diagnostic procedures are also considered AEs if: they are associated with clinical signs or symptoms; they require therapeutic intervention or additional diagnostic testing; they lead to dose modifications/termination of the study drug; or they are considered clinically significant by the

investigator. All observed or reported AEs, regardless of their suspected causal relationship to the study drug, should be recorded on the Case Report Form (CRF).

The NCI CTCAE version 4.0 will be used for adverse event descriptions and grading (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf). These criteria are summarized in Appendix B. The type and severity of an AE, as well as its potential link to the study drug(s), will determine whether the event requires expedited reporting in addition to routine reporting. For all AEs, the investigator must pursue and obtain information to adequately determine the causality and outcome of the event, and to assess whether it meets criteria for a serious AE. In addition, follow-up of AEs should continue until the event and any sequelae resolve or stabilize at a level acceptable to the investigator and/or the medical monitor.

7.1 Recording and Grading

7.1.1 Recording

All observed or volunteered adverse events, regardless of treatment group, severity, suspected causality, expectedness, or seriousness will be documented on the CRF.

A clinically significant change in a physical examination finding or an abnormal test result (*i.e.*, laboratory value) should be recorded as an AE, if it:

- is associated with accompanying symptoms
- requires additional diagnostic testing or medical or surgical intervention
- leads to a change in study dosing or discontinuation from the study
- requires additional concomitant drug treatment or other therapy, or
- is considered clinically significant by the investigator

An abnormal test result that is subsequently determined to be an error does not require recording as an AE even if it originally met one or more of the above criteria.

7.1.2 Grading severity

All adverse events will be graded for intensity on a scale of 0 to 5, according to the NCI CTCAE version 4.0 (see Table 2 and Appendix B). These criteria can be found at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf

Table 2 Intensity of Adverse Event

Grade	Description
0 (none)	No adverse event or within normal limits.
1 (mild)	Transient or mild discomfort; generally non-progressive; no limitation in daily activities; no medical intervention required.
2 (moderate)	Mild/moderate limitation in daily activities; some assistance may be required; no/minimal medical intervention required.
3 (severe)	Marked limitation in daily activities; some assistance usually required; medical intervention is required.
4 (life-threatening)	Extreme limitation in daily activities; major assistance required; significant medical intervention required.
5 (death)	Fatal adverse event.

7.1.3 Attributing causality

After grading for severity, the investigator must evaluate all clinical AEs and abnormal laboratory values for possible causal relationship to the study drug(s). Causality attribution will be decided using the criteria outlined in Table 3.

Table 3 Relationship of Adverse Event to Study Drug

Relationship	Description
Unrelated	AE is clearly not related to the study drug (An event that does not meet any of the criteria below).
Unlikely	AE is doubtfully related to the study drug (An event that follows a reasonable temporal sequence after drug administration; that follows a known or expected response pattern; but that could more reasonably be explained by other characteristics of the patient's clinical state).
Possible	AE may be related to the study drug (An event that follows a reasonable temporal sequence after drug administration; that follows a known or expected response pattern; but that could just as readily be attributed to a number of other factors).
Probable	AE is likely related to the study drug (An event that follows a reasonable temporal sequence after drug administration; that follows a known or expected response pattern; that is confirmed by stopping/reducing the drug dose; and that could not be reasonably explained by the characteristics of the patient's clinical state).
Definite	AE is clearly related to the study drug (An event that follows a reasonable temporal sequence after drug administration; that follows a known or expected response pattern; and that is confirmed by improvement on stopping/reducing the drug dose and reappearance on repeated exposure).

Abnormal laboratory values of clinical significance that were present at baseline and did not change in severity or frequency during experimental therapy and those that can obviously be attributed to underlying disease will be recorded as unrelated and will not be considered when evaluating study drug toxicity.

7.2 Unexpected Adverse Events

An unexpected adverse event is any event not associated by nature or intensity with the investigational agent(s) under study. A comprehensive list of adverse events and potential risks related to prostate GVAX and cyclophosphamide are provided in this Protocol and in the Consent form. Both of these agents have been reported to cause allergic reactions in very rare instances. A severe allergic reaction could be life-threatening. Examples of allergic reactions include: rash; shortness of breath; wheezing; sudden drop in blood pressure; swelling around the mouth, throat, or eyes; fast pulse; and sweating.

7.3 Serious Adverse Events and Serious Adverse Drug Reactions

The investigator must assess each event to determine if it meets the criteria for classification as a serious adverse event (SAE) or serious adverse drug reaction (ADR). An SAE/ADR is defined in the Code of Federal Regulations (21CFR312.32) as any event that:

- results in death
- is life-threatening
- results in inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability or incapacity
- results in congenital anomaly or birth defect
- is medically significant in the opinion of the investigator

All SAEs that occur any time while a patient is on study (*i.e.*, as soon as the informed consent has been signed) or within 30 days of the last dose of study drug administration must be documented, regardless of the suspected relationship to the investigational agent(s). Any SAE occurring more than 30 days after the last dose of the study drug(s) must be recorded if a causal relationship to the investigational agent(s) is suspected.

7.3.1 Progression of malignancy

Progression of a patient's malignancy should *not* be considered an AE unless, in the investigator's opinion, study treatment resulted in an exacerbation of the patient's condition. If disease progression results in death or hospitalization while on study or within 30 days of the last dose of study drug administration, progressive disease will be considered an SAE.

7.3.2 Life-threatening events

A life-threatening event is any AE that places the patient at immediate risk of death from the reaction as it occurs. It is not a reaction that, had it occurred in a more severe form, might have caused death.

7.3.3 Hospitalization or prolongation of hospitalization

Hospitalization encompasses any inpatient admission (even if < 24 hours) resulting from a precipitating treatment-emergent adverse event. For chronic or long-term patients, inpatient admission also includes transfer within the hospital to an acute or intensive care inpatient unit. Hospitalizations for administrative reasons or a non-worsening preexisting condition should not be considered AEs (*e.g.*, admission for workup of a persistent pretreatment laboratory abnormality, yearly physical exam, protocol-specified admission, or prostatectomy surgery). Hospitalization because of an unplanned event will be deemed an SAE. Preplanned treatments or surgical procedures should be noted in the baseline documentation. In the case of this study, all patients will have a preplanned prostatectomy surgery.

Prolongation of hospitalization is any extension of an inpatient hospitalization beyond the stay anticipated or required for the original reason for admission.

7.3.4 Significant disability

This is defines as a substantial disruption of the patient's ability to conduct normal life functions and activities of daily living.

7.3.5 Congenital anomaly

If the female partner of a male patient becomes pregnant during the course of the study, the treating physician must be notified immediately. All confirmed pregnancies must be immediately reported to the principal investigator and the medical monitor, and recorded in the CRF. All pregnancies will be followed until resolution (*i.e.*, voluntary or spontaneous termination or birth) and assessed for congenital anomalies and birth defects.

7.3.6 Medical significance

An event that is not fatal or life-threatening and that does not necessitate hospitalization may be considered serious if, in the opinion of the investigator, it jeopardizes the patient's status and might lead to medical or surgical intervention to prevent any of the above outcomes. Such medically significant events could include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias that do not result in inpatient hospitalization, or the development of drug dependency or abuse.

7.4 Perioperative Adverse Events

Complications of surgery or those occurring in the early post-operative period will be recorded. These include wound complications, estimated blood loss, post-operative infections, delayed wound healing, abnormal laboratory values, etc. Duration of hospital stay will be recorded. Additional medical examinations will be allowed at the request of patients during drug administration or at the discretion of the principal investigator for the evaluation of new adverse events that warrant physical examination. During and following completion of the study, patients should notify the study staff of any problems that occur between visits or following study termination by telephone and, if necessary, will be evaluated by the investigator or study personnel at an unscheduled interim visit.

Operative/perioperative events will be recorded as described in Section 7.1, and their severities will be categorized using the NCI CTCAE version 4.0 criteria. The relationship of these events to the investigational drug(s) will be determined by the principal investigator together with urologist co-investigators and, if necessary, with the primary urologic surgeon. Reporting of these events will follow the same guidelines described in Section 7.5.

7.5 Reporting Adverse Events

7.5.1 Reporting serious adverse events (SAEs)

All SAEs and unknown/unexpected reactions should be reported to the PI (Emmanuel Antonarakis, MD) within 24 hours. In addition, all SAEs must be reported by the PI to the IND Sponsor (Charles Drake, MD, PhD) within 24 hours.

Harry Cao (phone 443-287-6882, fax 410-614-7287, hcao7@jhmi.edu) should be contacted when reporting an SAE or death. If this person cannot be reached within 24 hours, the PI (Emmanuel Antonarakis MD) should be contacted at 443-287-0553 or at eantona1@jhmi.edu. Alternatively, the co-PI (Edward Schaeffer MD, PhD) can be contacted at (410) 502-2733 or at eschaef4@jhmi.edu.

The initial report for each SAE or death should include the following information (see also Appendix C for SAE Reporting Form):

- protocol # and title
- patient initials, study identification number, sex, age
- date the event occurred
- description of the SAE
- investigational agent received (and dose level) at the time the SAE occurred
- description of the patient's condition
- indication of whether the patient remains on study

Follow-up information including severity, causality, action taken, concomitant medications, and outcome should be communicated to the principal investigator and IND Sponsor as soon as possible with an indication whether an amendment will need to be made to the protocol, the consent form, or both, as a result of this event.

7.5.2 Reporting requirements for the Sidney Kimmel Comprehensive Cancer Center (SKCCC)

The principal investigator will notify the appropriate regulatory agencies of any SAEs occurring during the study period, regardless of causality. These agencies include the Sidney Kimmel Comprehensive Cancer Center (SKCCC) Data and Safety Monitoring Committee (DSMC), and the Institutional Review Board (IRB) and the Institutional Biosafety Committee (IBC) of the Johns Hopkins Medical Institutions (JHMI). Expedited reporting to the IND Sponsor by the PI within 24 hours is required for all SAEs (see Section 7.4.1). For SAEs that are fatal, life-threatening, or treatment-related but non-life threatening: IRB/IBC reporting by the PI is required within 3 days. For unrelated SAEs, IRB/IBC reporting by the PI is required within 15 days. All other AEs should be documented on CRFs and submitted according to the standard data management guidelines.

Adverse event information will be collected continuously throughout the duration of the study. Participants will be instructed to notify their treating provider of any new signs or symptoms, and providers will actively assess patients for adverse events at each visit (including by evaluation of laboratory studies). The investigator will assess each AE for its severity and for its relationship to the study drug, and all events Grade 1 or higher will be documented on CRFs and then reported as described above within the required time frame. Any AE occurring while a patient is on study (*i.e.* after informed consent has been signed) or within 30 days of study termination requires reporting. AEs occurring later than this must still be reported if a causal relationship with the study drug is suspected.

For all AEs, the investigator must pursue and obtain information to adequately determine the causality and outcome of the event, and to assess whether it meets criteria for a SAE. In addition, follow-up of an AE is required until the event either resolves or stabilizes. Initial reporting of an AE should include at a minimum the patient number, age, the dose at which the event occurred, and the type and severity of the event. Follow-up information including causality, duration, outcome, action taken, and concomitant medications should be reported soon thereafter.

The principal investigator must keep copies of all CRFs and other AE information, including correspondence with the IRB and/or FDA, for as long as required to comply with national and international regulations (generally at least 3 years after study completion).

7.5.3 FDA reporting requirements

Annual IND Report

All SAEs are reported to the FDA via the IND annual report per 21 CFR 312.33. SAEs deemed unexpected and related to the investigational product qualify for expedited reporting and must be submitted by the IND Sponsor-Investigator to the FDA per 21 CFR 312.32 as shown immediately below.

7 Calendar-day telephone or fax report
The IND Sponsor (Charles Drake, MD, PhD) is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the use of prostate GVAX. An unexpected adverse event is one that is not already described in this Protocol or the Consent form. Such reports are to be telephoned or faxed (301-827-9796) to the FDA within 7 calendar days of first learning of the event. Each telephone call or fax transmission should be directed to the FDA new drug review division in the product review division for the Center for Biologics Evaluation and Research (CBER), whichever is responsible for the review of the IND.

15 Calendar-day written report
The IND Sponsor (Charles Drake, MD, PhD) is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE

that is considered reasonably or possibly related to the use of prostate GVAX. An unexpected adverse event is one that is not already described in this Protocol or the Consent form.

Written IND Safety Reports should include an "Analysis of Similar Events" in accordance with regulation 21 CFR 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous similar reports.

Written IND safety reports with "Analysis of Similar Events" are to be submitted to the FDA and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on the FDA 3500A Form but alternative formats are also acceptable (*e.g.* summary letter).

Long-Term Follow-Up Protocol
All patients that have received GVAX will also be encouraged to enter a separate long-term follow-up protocol to assess for potential late toxicities of GVAX (*e.g.* autoimmune disease, second malignancies). This long-term follow-up protocol will involve annual visits for 3 years after the last dose of GVAX has been administered to a given patient.

7.5.4 RAC reporting requirements

All AEs and SAEs will be reported to the Johns Hopkins Institutional Biosafety Committee (IBC) per current institutional standards, and to the Recombinant DNA Advisory Committee (RAC) as described in Section 7.5.3. These reporting procedures will be in accordance with Appendix M of the NIH Guidelines for Research Involving Recombinant DNA Molecules (http://oba.od.nih.gov/oba/rac/guidelines_02/APPENDIX_M.htm#_Toc7255846).

7.6 Pregnancy

Pregnancy is not an AE unless it results in congenital anomaly or birth defect, in which case it is a SAE. If the partner of a male patient should become pregnant while he is participating in the study, the patient should inform his treating physician immediately. All pregnancies must be reported at once to the principal investigator and medical monitor. All confirmed pregnancies will be followed until birth or until voluntary or spontaneous termination.

8. OUTCOME ASSESSMENT

8.1 Radical Prostatectomy Specimen

Most of the study outcomes in this immunologic trial will depend on collection of prostate tissue from prostatectomy specimens. All pathologic specimens will be handled in routine fashion by the operating room (OR) staff, except that as soon as the specimen is removed the OR nurse will directly page a tissue harvesting technician that is part of the Brady Urological Research Institute Prostate Specimen Repository Team. Accessioning of pathology specimens will be coordinated in the OR areas by the Departments of Urology and Pathology. A study technician will be available to the pathologist receiving the specimen to assure that the tissue is handled appropriately for the intended bioassays. At the time of harvesting, the pathologist will apply ink and cut the prostate specimen in transverse sections. The specimen will be fixed in 10% phosphate buffered formalin, and tissue blocks will be paraffin-embedded and sectioned at 4 μm thickness for routine histologic evaluation and for immunohistochemical determinations. Fixation should occur as soon as possible after operative removal of the prostate, and ideally within 30-60 minutes. Assessment of index tumors for Gleason grade, nodal involvement, and pathologic staging will be conducted in usual fashion and will be provided to the patient for prognostic information. After the pathology report is available, a database will be established and all information from the pathology reports of all samples will be included. Tumor blocks and/or additional unstained slides will be collected for study-specific quantitative immunohistochemical evaluations.

8.2 Primary Endpoints

8.2.1 CD8⁺ T cell infiltration

The primary measure of treatment effect in this study will be achieved by quantifying the extent of $CD8^+$ T cell infiltration into the prostate from harvested prostate glands in men with high-risk localized prostate cancer receiving neoadjuvant ADT alone or in sequence with low-dose cyclophosphamide and GVAX (administered three weeks prior to radical prostatectomy).

This will be done using immunohistochemical staining methods. We will also attempt to quantify prostatic $CD4^+$ T cell infiltration and T_{reg} infiltration, as well as to determine the $CD8/T_{reg}$ ratio and the $CD4/T_{reg}$ ratio. The primary endpoint will be expressed as the mean $CD8^+$ T cell staining percentage in harvested tumor tissue, and will be analyzed separately for each treatment arm.

Analysis of the primary endpoint will be achieved by preparing tissue microarrays (TMAs) using the highest-grade/largest tumor per patient and sampling it with 100-fold redundancy. All tissues will first be fixed in 10% neutral buffered formalin and processed into paraffin blocks. For each immunohistochemical stain (*e.g.* $CD8^+$ T cell stain, $CD4^+$ T cell stain, T_{reg} stain), TMA slides will be scanned using the Aperio ScanScope® CS instrument (Aperio Technologies, Vista, CA) and TMA cores will first be assigned a diagnosis by the study pathologist and will then be subjected to semi-automated image analysis using the Aperio system. For each biomarker, we will divide the area of brown DAB staining by the area of epithelial cells on the TMA core, obtaining a staining percentage. The area of epithelium will be obtained on each TMA core by staining with cytokeratin-8 and using automated image analysis. Cores with both tumor and normal tissue will be excluded if they contained >10% of the other component.

For CD8 staining, slides will be steamed for 20 minutes in citrate antigen retrieval solution (Vector Laboratories, Burlingame, CA) followed by incubation with a mouse monoclonal anti-CD8 antibody for 45 minutes at room temperature (Dako, Carpinteria, CA). For CD4 staining, slides will be steamed for 40 minutes in high pH antigen retrieval solution (Dako, Carpinteria, CA) and then incubated with a mouse monoclonal anti-CD4 antibody for 45 minutes at room temperature (Serotec, Kidlington, UK). For T_{reg} analysis, cells will be stained for the FoxP3 protein by steaming slides for 40 minutes in high pH antigen retrieval solution (Dako, Carpinteria, CA) and incubating them with a mouse monoclonal anti-FoxP3 antibody for 45 minutes at room temperature (eBioscience, San Diego, CA, 1:1000 dilution). In all cases, poly-HRP-conjugated anti-mouse IgG Ab (Dako, Carpinteria, CA) will be used as the secondary antibody. Staining will be visualized with diaminobenzidine (Sigma, Saint Louis, MO) and slides will be counterstained with hematoxylin.

Images of each TMA core will be captured by automated scanning of TMA slides using the Aperio ScanScope® CS instrument (Aperio Technologies, Vista, CA). Captured images will be imported into the TMAJ Images Application program (http://tmaj.pathology.jhmi.edu). Histological diagnoses (normal, atrophy, prostatic intraepithelial neoplasia, cancer) will be applied to all images used for the analyses by a pathologist. In addition, for TMA spots containing more than one type of lesion, the percentage of each diagnosis will be noted. All images and data will be available for viewing/downloading at http://demarzolab.pathology.jhmi.edu/Pubs.html. For image analysis, we will use a custom open source software package, FRIDA (Framework for Image Dataset Analysis; http://sourceforge.net/projects/fridajhu), for the evaluation of red-green-blue (RGB) color image datasets, including those generated from scanning of tissue microarray slides. To analyze CD4, CD8 and FoxP3 (T_{reg}) staining, hue-saturation-brightness (HSB) segmentation ranges for brown DAB staining will be defined from the tissue microarray image set, and the total number of pixels in every image that fall within the defined parameters for brown DAB

staining will be calculated, reflecting the total area of brown DAB staining for each spot. For every spot, a "staining ratio" for each of the three proteins will be calculated by dividing the total area (in pixels) of brown DAB staining by the average TMA spot area.

Previous TMA studies conducted by Angelo DeMarzo, MD PhD and Charles G. Drake, MD PhD have defined the extent of T cell infiltration into human prostate glands using prostatectomy specimens. In normal prostate tissue, the mean staining percentage for $CD8^+$ T cells is 0.29% (interquartile range, IQR, 0.13% - 0.39%) while in tumor tissue, the mean percentage of $CD8^+$ T cells is 0.42% (IQR 0.07% - 0.71%). The corresponding values for $CD4^+$ T cell infiltration in normal and tumor tissue are 0.16% (IQR 0.04% - 0.18%) and 0.25% (IQR 0.01% - 0.32%), respectively. The corresponding values for T_{reg} infiltration in normal and tumor tissue are 0.03% (IQR 0.02% - 0.04%) and 0.06% (IQR 0.02% - 0.08%), respectively (Gurel and DeMarzo, unpublished data). These values are helpful for the determination of sample size/power calculations in the present study (see Section 10.1).

The above analyses will be performed in the laboratory of Angelo DeMarzo, MD PhD. A detailed description of these methodologies has previously been published (Zha et al 2001; Faith et al 2004; Gurel et al 2008). The contact information for the DeMarzo laboratory is listed below:

Angelo DeMarzo, MD, PhD
CRB1 - Room 151
1650 Orleans Street
Baltimore, MD 21231
Phone: (410) 614-5686
Fax: (410) 502-9817
Email: ademarz@jhmi.edu

8.2.2 Rate of adverse events

All subjects receiving at least one dose of the study drug(s) will be evaluated for safety by monitoring symptoms, physical examinations, and laboratory tests. Adverse events will be classified and graded according to the NCI Common Toxicity Criteria version 4.0 (see Appendix B). The absolute number and frequency of each adverse event will be reported, and subdivided according to toxicity grade. A description of adverse events by treatment arm will also be reported. A particular adverse event occurring more than once in the same subject will be counted only once and at its worse grade.

8.3 Secondary Endpoints

8.3.2 Regulatory T cell (T_{reg}) infiltration

The method for quantifying T_{reg} density from harvested prostate tissue is as described in Section 8.2.1. This endpoint will be expressed as the mean staining percentage in tumor tissue, and will be reported separately for each treatment arm.

8.3.1 $CD4^+$ T cell infiltration

The method for quantifying $CD4^+$ T cell density from harvested prostate tissue is as described in Section 8.2.1. This endpoint will be expressed as the mean staining percentage in tumor tissue, and will be reported separately for each treatment arm.

8.3.3 Tissue androgen concentrations

Tissue concentrations of testosterone and 5α-dihydrotestosterone (DHT) will be measured using a highly sensitive liquid chromatography–electrospray ionization tandem mass spectrometry method using a high proton affinitive derivatization of the 17β-hydroxyl group of testosterone and DHT with picolinic acid, and a mobile phase consisting of MeCN–MeOH–

H_2O–formic acid and a conventional octadecylsilica (ODS) column (Yamashita et al 2009). Purification of the derivatives will be carried out using solid-phase extraction with the ODS cartridge. By this method, testosterone and DHT will be determined simultaneously with limits of quantification of 0.5 pg and 1 pg/3 mg of prostate tissue, respectively. Tissue androgen concentrations will be reported separately for each treatment arm.

8.3.4 Androgen receptor (AR) quantification

The method for quantifying androgen receptor (AR) density from harvested prostate tissue is similar to that described in Section 8.2.1, and will rely on immunohistochemical staining for the AR protein. This endpoint will be expressed as the mean staining percentage in tumor tissue, and will be reported separately for each treatment arm.

8.3.5 Markers of apoptosis

TMAs from formalin-fixed tumor samples will be analyzed for the degree of tumor apoptosis using immunohistochemical staining for activated caspase 3 (Marcelli et al 2000), or by using the method of terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick end labeling (*i.e.* TUNEL assay) (Furuya et al 1995). The *in situ* Cell Death Detection Kit (Roche, Indianapolis, IN) may be used to perform TUNEL reactions. Quantification of staining percentage will be achieved using the Aperio ScanScope® CS instrument (Aperio Technologies, Vista, CA) as described in Section 8.2.1. This endpoint will be expressed as the mean staining percentage in tumor tissue, and reported separately for each treatment arm. Analyses will be performed in the laboratory of Dr Angelo DeMarzo.

8.3.6 Markers of cell proliferation

TMAs from fixed tumor samples will be analyzed for the degree of tumor cell proliferation using the validated marker, Ki-67. This will be achieved by immunohistochemical staining (Berges et al 1995; Rubin et al 2002), using the Ki-67 monoclonal antibody (Dako North America, Carpineteria, CA). Quantification of staining percentage will be performed using the Aperio ScanScope® CS instrument (Aperio Technologies, Vista, CA) as described in Section 8.2.1. This endpoint will be expressed as the mean staining percentage in tumor tissue, and reported separately for each treatment arm. Assays will be performed in the laboratory of Dr DeMarzo.

8.3.7 Pathological complete responses (pCR)

This will be defined as the absence of tumor identification by the study pathologist on standard histological analysis of the resected prostate specimens. The proportion of men achieving a pCR will be reported separately for each treatment arm.

8.3.9 Novel anti-prostate antibodies

Patient sera will be cryopreserved and archived at the Johns Hopkins Specimen Acquisition Core (SAC) facility. Novel induced antibody specificities will be investigated by interrogating patient sera using the high-throughput immunoblot (HTI) assay developed by our collaborator, Dr. Douglas McNeel, as previously described (Smith et al 2011; Zabransky et al 2012). Briefly, λ-phage encoding 126 unique prostate-associated antigens will spotted in triplicate in a 16×24 array onto *E. Coli* bacterial lawns using a Biomek FX liquid handling robot. These individual antigens include 29 cancer-testis antigens, 40 antigens identified in patients with chronic prostatitis, and 57 antigens identified in individual patients, some of whom were treated with androgen ablation or other immunomodulatory therapies. Encoded proteins will be transferred to nitrocellulose membranes, washed, blocked, and probed with sera from patients pre- or post-treatment, diluted 1:100 in isotonic buffer. Human IgG will then be detected with an IgG-specific secondary antibody conjugated to alkaline phosphatase and immunoreactivity will be detected by development with 0.3 mg/mL nitro blue tertazolium chloride (NBT) (Fisher Biotech, Hampton, NH) and 0.15

mg/mL 5-bromo 4-chloro 3-indoylphosphate (BCIP) (Fisher Biotech). Membranes will be digitally scanned and then assessed visually, with individual plaques scored "positive" or "not positive" by 3 independent observers, blinded to the treatment arm, timing of sample acquisition, and membrane layout. Triplicate samples will be evaluated for each antigen, and immunoreactivity to individual antigens will be scored positive if there is concordance among 2 of 3 observers, and if immunoreactivity is scored positive in at least two of the three replicates. Novel antibody specificities with potential biomarker and/or therapeutic potential will be independently confirmed by developing individual ELISA assays for those particular antigens.

8.3.9 PSA response rates

This will be defined as the proportion of patients who achieve an undetectable PSA (<0.1 ng/mL) by 3 months after prostatectomy. The proportion of men achieving a PSA response will be reported separately for each treatment arm.

8.3.10 Time to PSA recurrence

This will be defined as the interval from the time of prostatectomy to the time when the serum PSA is ≥0.2 ng/mL. PSA will be measured every 3 (±1) months during the first post-operative year and every 6 (±2) months during the second and third post-operative years. For subjects who have not yet demonstrated PSA relapse at the time of censoring, patients will be censored at the date of the last assessment that shows a lack of PSA recurrence. This outcome will be expressed as a median for each treatment arm, and will be determined using the Kaplan-Meier method.

9. REGULATORY AND REPORTING REQUIREMENTS

Contact details for personnel connected with this study are provided on the title page at the front of this protocol. Patient registration procedures are described in Section 4.

9.1 Regulatory Responsibilities

9.1.1 Protocol chair

The protocol chair (=PI), Emmanuel Antonarakis is responsible for the following tasks:

- Coordinating, developing, writing, submitting, and obtaining IRB-approval for the protocol as well as its subsequent amendments.
- Assuring that all study personnel are using the correct version of the protocol.
- Taking responsibility for the overall conduct of the study and for monitoring the progress of the study.
- Reviewing and ensuring reporting of serious adverse events (SAEs).
- Reviewing data from all patients.

9.1.2 Study Coordinator

The study coordinator, Harry Cao, is responsible for the following tasks:

- Ensuring that IRB approval has been obtained prior to patient registration, and maintaining copies of IRB approvals (including approval of amendments).
- Managing patient registration.
- Collecting and compiling data from each patient.
- Establishing procedures for documentation, reporting, and submission of AEs/ SAEs to the protocol chair (Charles G. Drake, MD PhD) and other applicable parties.

- Facilitating audits by securing selected source documents and research records from participating patients for audit.

9.1.3 Study personnel

Study personnel (co-investigators, research nurses) are responsible for these tasks:

- Following the protocol as written, and Good Clinical Practice (GCP) guidelines.
- Submitting data to the project manager.
- Registering all patients by submitting the patient registration form and signed informed consent form promptly.
- Providing sufficient experienced clinical and administrative staff and adequate facilities and equipment to conduct the trial according to the protocol.
- Maintaining regulatory binders and providing copies of all required documents to the project manager.
- Collecting/submitting data according to the schedule specified by the protocol.

9.2 Data Management

Data collected during this study will be entered into a secure database. The study coordinator will be responsible for the initial study configuration and setup of the database and for any future changes.

9.2.1 Case report forms

Case report forms (CRFs) will be generated by the study coordinator for the collection of all study-related data. Investigators and study personnel will be responsible for ensuring that the CRFs are kept up-to-date.

9.2.2 Source documents

Investigators and study personnel will record clinical data in each patient's source documents (*i.e.*, the patient's medical record). Source documentation will be made available to support the patient research record. Study monitors will review entries on the CRFs at regular intervals, comparing the content with the source documents.

9.2.3 Record retention

The investigator will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. After study closure, the investigator should maintain all source documents, study-related documents, and the CRFs. Because the length of time required for retaining records depends upon a number of regulatory and legal factors, documents should be stored until the investigator is notified that the documents may be destroyed. In this study, records are to be retained and securely stored for a minimum of 3 years after the completion of all study-related activities.

9.3 Study Monitoring and Quality Assurance

This is a DSMP Level III study under the Johns Hopkins Sidney Kimmel Comprehensive Cancer Center (SKCCC) Data Safety Monitoring Plan (herein after referred to as either JHU SKCCC DSMP or DSMP; please see Appendix E for complete plan). The protocol will be internally monitored by the principal investigator. External data monitoring will be performed by the SKCCC Clinical Research Office Quality Assurance Program (CRO QA) at least quarterly, but may occur more or less frequently depending on the rate of accrual (per the DSMP). Additional data and safety monitoring oversight will also be performed by the SKCCC Safety Monitoring Committee (SMC - as defined in the DSMP) and a Medical Expert Committee (MEC) as detailed below.

Authorized representatives of SKCCC are permitted to perform audits or inspections, including source data verification. The purpose of these audits or inspections is to systematically and independently examine all trial-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (GCP) guidelines, and any applicable regulatory requirements.

Regularly scheduled registration reports will be generated to monitor patient accrual and the completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and the extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period, and potential problems will be brought to the attention of the principal investigator for discussion and action.

The Medical Expert Committee (MEC) for this clinical study contains two medical oncologists and one urologist (see below) from other disciplines who are not affiliated with this clinical trial protocol. The MEC will review safety data on at least a semi-annual basis. The MEC will provide a written summary of each assessment to the IND Sponsor-Investigator after each meeting. In turn, the study team will forward these summaries to the JHU IRB and JHU SKCCC SMC. The operating plan of the MEC will be as follows:

- Meetings will be held at least semi-annually, and potentially more frequently if needed.
- Meetings will be conducted in-person or via video/teleconference, with a participant sign-in sheet collected at each meeting.
- Approximately one week prior to each MEC meeting, the study team will submit the following items to MEC personnel for review and discussion at the meeting (the PI may join the MEC meeting in order to answer any questions the MEC might have):
    - A summary of the clinical trial's progress to date;
    - The latest IRB-approved consent document;
    - A summary of all AEs, SAEs, deaths, and withdrawals to date

Note that the MEC reserves the right to halt trial accrual or all study activity if, after review, serious safety concerns warrant this action. If the MEC halts study accrual or all study activity, then the study team must notify the JHU SKCCC SMC, JHU IRB, FDA, and NIH RAC immediately.

The MEC consists of the following members:

- Dr Dung Le (medical oncology); phone: 443-287-0002; email: dle@jhmi.edu
- Dr Evan Lipson (medical oncology); phone: 410-502-5977; email: elipson2@jhmi.edu
- Dr Ronald Rodriguez (urology); phone: 410-614-6662; email: rrodrig@jhmi.edu

All clinical work conducted under this protocol is subject to Good Clinical Practice (GCP) guidelines. This includes inspection of study-related records by the principal investigator, project manager, sponsor or its designee, or health authority representatives at any time.

10. STATISTICAL CONSIDERATIONS

10.1 Study Design and Sample Size

This is a single-center, randomized, 2-arm, open-label, prospective clinical trial evaluating the immunogenicity and safety of ADT used alone (arm A), or ADT given in sequence with low-dose cyclophosphamide and prostate GVAX (arm B), each administered 3 weeks prior to radical prostatectomy in men with high-risk localized prostate cancer. The primary goal of this study is to quantify the extent of $CD8^+$ T cell infiltration into the prostate gland from patients in the two treatment arms, and to compare the two arms. Our primary hypothesis is that men on the combination arm receiving cyclophosphamide/GVAX in addition to ADT (arm B) will have a 100% or

greater $CD8^+$ T cell infiltration of the prostate than will men receiving ADT alone (arm A). A difference smaller than this in the primary endpoint would not be considered biologically meaningful.

In the absence of any intervention prior to prostatectomy, we have previously determined that the mean staining percentage for $CD8^+$ T cells in prostate tumor tissue is 0.42% (standard deviation 0.36%) (Gurel, De Marzo and Drake, unpublished data). We hypothesize that men on the control arm (arm A) would have similar $CD8^+$ T cell staining percentages as those quoted above. In the combination arm (arm B), a biologically meaningful treatment effect would be achieved if prostatic $CD8^+$ T cell responses were augmented by 100% or more compared to arm A. Therefore, with 16 patients per arm, and assuming an 86% coefficient of variation for the average CD8 area/spot area ratios, a one-sided 0.05 α-level t-test of the logarithms of these ratios would have 82% power to detect a 2-fold (100%) increase of $CD8^+$ T cells in the degarelix plus cyclophosphamide/GVAX arm (arm B) compared to the degarelix alone arm (arm A).

The safety of ADT monotherapy and the combination of ADT with cyclophosphamide/GVAX delivered before prostatic surgery is an important co-primary endpoint. We hypothesize that combining ADT with low-dose cyclophosphamide and prostate GVAX in the neoadjuvant setting will be feasible and safe.

Patients will be replaced if they are removed from the study after signing the informed consent but before receiving the study drug(s). Patients receiving at least one dose of the study drug(s) will be included in both safety and efficacy analyses.

10.2 Study Endpoints

10.2.1 Analysis of the primary endpoints

10.2.1.1 $CD8^+$ T cell infiltration

The primary efficacy objective of this study is to quantify the extent of $CD8^+$ T cell infiltration into the prostate gland in the two treatment arms, and to compare them. This outcome will be expressed as the mean $CD8^+$ T cell staining percentage in harvested prostate tissues, and will be reported separately for each treatment arm. The standard deviation, 95% confidence interval, median, and range of values will also be reported where appropriate. Since the $CD8^+$ T cell quantity is a ratio variable and the distribution is skewed, the log transformation will be used for the analysis.

Tissue microarrays (TMAs) using the highest-grade/largest tumor per patient and sampling it with 100-fold redundancy will be used for $CD8^+$ T cell quantification. We expect that 3-50% of the spots per patient will be assigned a carcinoma diagnosis by the study pathologist. Cores with both tumor and normal tissue will be excluded if they contain >10% of the other component. The percent positive staining score for $CD8^+$ T cells in the spots classified as tumor will be used to quantify the primary outcome. The mean will be used to pool multiple spot measurements for each patient. Following a log transformation, means will be compared between treatment arms using a two-way analysis of variance (ANOVA) with the stratification variable (Gleason score) treated as a block factor. Boxplots and descriptive summaries by treatment arm will also be provided as appropriate. To account for co-morbidities and other immunosuppressive factors as potential confounding factors for treatment comparisons, multivariable regression analysis will be performed. The model will consider Gleason score and disease stage as covariates.

10.2.1.2 Safety and feasibility

An additional co-primary endpoint is safety. To this end, the frequency of adverse events will be described separately for each treatment arm using summary statistics. The proportion of patients in each arm with an adverse event will be reported with an exact binomial 95%

confidence interval. All subjects receiving at least one dose of the study drug(s) will be evaluable for toxicity.

Early stopping rules for safety: Safety will be monitored closely in the degarelix plus cyclophosphamide/GVAX arm of the study (arm B). The stopping rule for safety is based on the posterior probability that the adverse event (AE) rate is too high. Any systemic symptoms (fevers, rash, myelosuppression) compromising the planned surgery or any grade-3/4 local reactions (erythema, swelling, pain) attributable to the cyclophosphamide or GVAX immunotherapy will be considered AEs for purposes of this stopping rule. We expect that the adverse event rate will be low. Trial enrollment will be suspended for a safety evaluation if the AE rate convincingly exceeds 25%. This stopping rule will halt enrollment if the posterior probability of the AE rate exceeding 0.25 is 70% or higher. The prior probability for this toxicity monitoring rule will be a Beta-distribution with parameters of 2 and 8, assuming that 2 of 8 patients may experience an AE. This distribution corresponds to assuming a 1-in-4 chance that the risk of AEs is 25% or higher and 90% certainty that the risk is between 4% and 43%. The stopping rule applies this prior distribution to the observed number of patients experiencing an AE and computes the resulting probability that the rate is too high. If the posterior certainty that the rate is too high based on Bayes' rule and these assumptions is 70% or higher, the study should stop. The following table shows the resulting stopping rules.

Study termination if:	2 AEs	3 AEs	4 AEs	5 AEs	6 AEs
And number of patients between:	2	3 - 5	6 - 9	10 - 12	13 - 16

For example, the rule will call for stopping the study if 4 patients out of the first 6 experience AEs. The next table shows the percentage of the time that the stopping rule will terminate the study under different hypothetical risks of AEs, along with the average sample size (based on 5000 simulations).

Risk of AE	0.10	0.20	0.25	0.30	0.35	0.40
% of Time study stops	2.2%	16%	29%	45%	60%	75%
Expected sample size	15.8	14.6	13.5	12.3	11.0	9.5

Early stopping rule for feasibility: Androgen deprivation therapy (ADT) does not independently alter the surgical approach to prostatectomy and we do not anticipate an increase in the surgical difficulty with the use of neoadjuvant degarelix. Men who have had acute (post-biopsy) and prolonged issues (chronic prostatitis) with prostate infection/inflammation are fairly routine in urologic surgical practice, and we anticipate that the immune infiltrate potentially induced by GVAX would not pose a substantial increase in surgical difficulties. However, in addition to safety, feasibility will be monitored separately in both arms of the study. The feasibility rule for this study will be based on a change in surgical outcomes beyond what may be expected for patients without presurgical interventions and that may be attributable to the study drugs (degarelix, or the combination of degarelix plus cyclophosphamide/GVAX treatment). These events would include: (1) average blood loss in excess of 2500 mL, (2) average operative time in excess of 3.5 hours, and (3) average hospital stay in excess of 4 days. These values are approximately 2 standard deviations above the average surgical outcomes for men undergoing radical prostatectomy at Johns Hopkins Hospital.

We require that the probability that the surgery will not be complicated by these events to be high (≥90%). We will monitor this endpoint after every patient. If it becomes apparent that the surgeries are being negatively impacted by the pre-surgical investigational treatments, then the study will be suspended for review. This stopping rule will halt

enrollment if the posterior probability of no complications <0.90 is greater than 80%. The prior probability for this rule has a Beta-distribution with parameters of 9 and 1, based on the expectation that these treatments will not impact the feasibility of surgery. Instances where the study would be temporarily suspended are listed below.

Stopping rules:

- Not feasible if both of the first 2 patients in an arm have surgical complications.
- Not feasible if two of the first 3 patients in an arm have complications.
- Not feasible if 2 out of the first 4 patients in an arm have complications.
- Not feasible if 3 out of the first 6 patients in an arm have complications.
- Not feasible if 4 out of the first 8 patients in an arm have complications.
- Not feasible if 5 out of the first 10 patients in an arm have complications.
- Not feasible if 6 out of the first 12 patients in an arm have complications.
- Not feasible if 7 out of the first 14 patients in an arm have complications.
- Not feasible if 8 out of 16 patients in an arm have complications.

10.2.2 Analysis of the secondary endpoints

The secondary endpoints of this study have previously been defined (see Section 8.3). The statistical analysis of these endpoints is described below. Data transformation will be performed when they are not normally distributed.

- **Regulatory T cell (T_{reg}) density**. The methods for analyzing T_{reg} infiltration will be similar to those described for the primary endpoint. Descriptive statistics and graphical summaries by treatment arm for these outcomes will be provided. In addition, the $CD8^+/T_{reg}$ ratio and the $CD4^+/T_{reg}$ ratio will be computed, and reported using descriptive statistics. Comparisons between treatment arms will be made using two-way ANOVA.
- **$CD4^+$ T cell density.** The methods for quantifying and analyzing $CD4^+$ T cell infiltration will be similar to those described for the primary endpoint (*i.e.* $CD8^+$ T cell infiltration). Descriptive statistics and graphical summaries by treatment arm for these outcomes will be provided. Comparisons between treatment arms will be made using two-way ANOVA.
- **Tissue androgen concentrations**. Intraprostatic androgen concentrations (*i.e.* testosterone and dihydrotestosterone) will be summarized descriptively by study arm, and comparisons across arms will be made using two-way ANOVA.
- **Androgen receptor (AR) quantification**. Androgen receptor (AR) staining concentrations will be summarized descriptively by study arm, and comparisons across arms will be made using two-way ANOVA.
- **Apoptotic markers.** Caspase 3 staining (and/or TUNEL assay results) will be expressed as the mean staining percentage in tumor samples, and reported separately for each treatment group. Standard deviations, 95% confidence intervals, and ranges will also be reported where appropriate. Means will be compared across treatment arms using two-way ANOVA.
- **Proliferation markers.** Ki-67 staining will be expressed as the mean staining percentage in tumor samples, reported separately for each treatment arm. Standard deviations, 95% confidence intervals, and ranges will also be reported where appropriate. Means will be compared across treatment arms using two-way ANOVA.
- **Pathological complete responses (pCR)**. This will be defined as an absence of tumor identification on standard histological analysis of the resected prostate specimens. The proportion of patients achieving a pCR will be compared between treatment arms using the Mantel-Haenszel test.

- **Novel anti-prostate antibodies**. Induction of novel anti-prostate antibodies will be assessed with a high-throughput immunoblot (HTI) assay, before and after treatment initiation. The change in immunoreactivity will be evaluated for each antigen and scored as a gain, loss, or no change. These data will be visualized per patient for both treatment groups using heatmaps. A comparison of the proportions of patients with these ordered outcomes between the treatment arms will be made using an exact version of the Cochran-Armitage test for trend (Armitage 1955). Antibody responses will be dichotomized as "gain" versus "loss or no change" and correlated with PSA response and pathologic complete response (pCR) using χ^2-tests or Fisher's exact tests. Antibody responses will be correlated with time-to-PSA-recurrence using Kaplan-Meier curves and log-rank tests. The correlation of antibody responses with CD8 concentrations, CD4 concentrations, and T_{reg} concentrations will be explored using Spearman's correlation coefficient (ρ).
- **PSA response rates**. This will be defined as an undetectable PSA (<0.1 ng/mL) at 3 months after prostatectomy. The proportion of patients achieving a PSA response will be compared between treatment arms using the Mantel-Haenszel test.
- **Time to PSA recurrence.** This will be defined as the interval from prostatectomy to the time when the serum PSA is ≥0.2 ng/mL. For subjects who have not yet demonstrated PSA recurrence at the time of censoring, patients will be censored at the date of the last assessment that shows a lack of PSA recurrence. For each treatment arm, the median time to PSA recurrence after prostatectomy (*i.e.*, the median PSA-recurrence-free survival) will be estimated with 95% confidence intervals using Kaplan-Meier survival analysis. Comparisons will be sought using the log-rank test, stratified by Gleason score.

10.3 Analysis Populations

10.3.1 Intention-to-treat population

All patients who meet eligibility criteria and receive at least one dose of the study drug(s) will be included in the analysis of the primary and secondary endpoints, even if there are subsequent protocol deviations. However, in cases where prostatectomy is not performed or if adequate surgical tissue is not collected, then determination of the primary endpoint (tissue $CD8^+$ T cell analysis) and some secondary endpoints (apoptosis/proliferation marker analysis) will not be possible. Patients will be replaced if they are removed from the study after signing the informed consent but before receiving the study drug(s).

10.3.2 Safety population

All patients enrolled in the study will be included in the safety analysis population and considered evaluable for toxicity from the time of their first dose of the study drug(s). Patients never receiving any of the study drugs will not be included in this analysis. Demographic and baseline characteristics for the safety population will be summarized by number and percent for categorical data and by descriptive statistics for continuous data.

10.4 Safety Analysis

10.4.1 Evaluation of adverse events

Treatment-emergent adverse events will be translated from investigator terms to MedDRA version 6.0 terminology and summarized (number and percentage of patients) for all patients who receive at least one dose of the study drug(s). Adverse event summaries will be organized by body system, frequency of occurrence, intensity (*i.e.*, severity grade), and causality or attribution. Patients who experience an adverse event more than once will be counted only once. The occurrence with the maximum severity will be used to calculate intensity.

10.4.2 Evaluation of serious adverse events and premature withdrawals

Adverse events deemed serious and those resulting in early treatment withdrawal or death will be summarized separately. Narrative paragraphs will be generated to describe the circumstances surrounding each SAE and each death.

10.4.3 Evaluation of laboratory parameters and assays

Abnormal laboratory parameters (*e.g.* electrolyte levels, liver function tests, renal function tests, complete blood counts) will be summarized, and clinically significant changes from baseline will be discussed.

10.5 Statistical Procedures

10.5.1 General

Most study outcomes will be reported using descriptive statistics: number of observations, means, standard deviations, medians, minimum, and maximum values. 95% confidence intervals will be provided where appropriate. A *P*-value of ≤0.05 will be used to denote statistical significance.

10.5.2 Sample size calculation

Our primary hypothesis is that men on the combination arm receiving cyclophosphamide/ GVAX in addition to ADT (arm B) will have a 100% or greater $CD8^+$ T cell infiltration of the prostate than will men receiving ADT alone (arm A). A difference smaller than this in the primary endpoint would not be considered biologically meaningful. In the absence of any intervention, we have previously determined that the mean staining percentage for $CD8^+$ T cells in tumor tissue is 0.42% (standard deviation 0.36%) (Gurel and DeMarzo, unpublished data). We hypothesize that men on the control arm (arm A) would have similar $CD8^+$ T cell staining percentages as those quoted above. In the combination arm (arm B), a biologically meaningful treatment effect would be achieved if prostatic $CD8^+$ T cell responses were augmented by 100% or more compared to arm A. Therefore, with 16 patients per arm, and assuming an 86% coefficient of variation for the average CD8 area/spot area ratios, a one-sided 0.05 α-level t-test of the logarithms of these ratios would have 82% power to detect a 2-fold (100%) increase of $CD8^+$ T cells in the degarelix plus cyclophosphamide/GVAX arm (arm B) compared to the degarelix alone arm (arm A). Treating Gleason score as a block factor reduces the variance, which would be adequate to offset the loss of power due to the 1 degree of freedom associated with the blocking factor.

In summary, 16 patients per arm will yield a total sample size of 32 men. With an estimated enrolment rate of 4 men per month, accrual is expected to last 8 months.

10.5.3 Stratification factors

The randomization will be stratified by Gleason score: ≤7 vs 8-10.

10.5.4 Statistical analysis of primary and secondary endpoints

For a description of statistical methods used to evaluate the primary and secondary study outcomes, please see Section 10.2.

11. PROTECTION OF HUMAN SUBJECTS

11.1 Ethical Considerations

This study will be conducted in compliance with the protocol, Good Clinical Practice (GCP) guidelines established by the International Conference on Harmonization (ICH), and the ethical standards set

forth in the Declaration of Helsinki of 2004 (these documents may be found at www.wma.net/e/policy/b3.htm and www.laakariliitto.fi/e/ethics/helsinki.html). Review of this protocol by the Institutional Review Board (IRB)/Ethics Committee (EC), and the performance of all aspects of the study including acquisition of informed consent, must also be in accordance with the principles elaborated in the Declaration, as well as the ICH guidelines (Code of Federal Regulations (CFR), Title 21: Part 50 and Part 312). The principal investigator will be responsible for submitting documents to the IRB/EC, and obtaining written approval for the protocol prior to study initiation. The approval of both the protocol and the informed consent must specify the date of approval, protocol number and version, and amendment number. The principal investigator is also responsible for notifying the IRB/EC of any serious deviations from the protocol, or other circumstances that may result in added risk to participating patients.

11.2 Protocol Amendments

Before starting the study, the protocol must be approved by the IRB/EC, the JHU Institutional Biosafety Committee (IBC), the FDA, and the Recombinant DNA Advisory Committee (RAC). Amendments to the protocol are subject to IRB approval before instituting. Any amendments made after IRB/EC approval is granted must be resubmitted to the IRB/EC for new approval.

11.3 Written Informed Consent

Before obtaining consent, members of the study team must review the rationale for the treatment program with the patient. The discussion will review the alternatives available, the potential benefits of this program, the risks and the probability of their occurrence, and the procedures to minimize these risks. Should an adverse event occur, the provisions available to ensure medical intervention must also be reviewed. Why the risks are reasonable in relation to the anticipated benefits, incentives, or costs that will/may be incurred as a result of participating in the study, as well as the efforts to maintain confidentiality, should also be discussed with the patient.

Patients will be required to sign and date (in triplicate) a statement of informed consent that meets the requirements of the Code of Federal Regulations (Federal Register Vol. 46, No. 17, January 27, 1981, part 50) and the IRB. The consent form should be submitted with the protocol for review and approval by the IRB/EC. The medical record should include a statement that written informed consent was obtained (and should document the date that it was obtained) before the patient is enrolled in the study. The original signed consent document will become part of the patient's medical record, a copy will be forwarded to the project manager pursuant to registration, and a copy will be sent home with each patient.

The consent form must include the following information:

- the nature and objectives, potential toxicities, and benefits of the intended study
- the length of therapy and follow-up required
- alternatives to the proposed therapy (including standard and investigational therapies)
- the name of the investigator(s) responsible for the protocol
- the right of the patient to accept or refuse treatment and to withdraw from participation in the study at any time

11.4 Protection of Privacy

Patients will be informed of the extent to which their confidential health information generated from this study may be used for research purposes. After this discussion, they will be asked to sign a Notice of Privacy Practice research authorization/HIPAA form. This may be embedded within the informed consent document. The original signed documents will become part of the patient's medical records, and each patient will receive a copy of the signed documents. The use and disclosure of protected health information will be limited to the individuals described in the research

authorization form. The research authorization form must be prepared by the principal investigator and approved by the IRB.

In compliance with US federal regulations, the investigator is required to permit representatives of the US Food and Drug Administration (FDA) or other regulatory authorities to review and/or copy any medical records relevant to the study in accordance with local laws. Patients will be informed of the extent to which their confidential health information generated from this study may be disseminated to other parties. Should direct access to medical records require a waiver or authorization separate from the subject's statement of informed consent, it is the responsibility of the investigator to obtain such permission in writing from the patient.

11.5 Study Termination or Modification

Adverse events and laboratory data from this trial will be assessed by the principal investigator and/or medical monitor on an ongoing basis. At least quarterly, data from the clinical database will be reviewed. The results of this review will be shared with all investigators either in writing or as part of a teleconference. SAEs will be reviewed as they are reported to the principal investigator or project manager, and the medical monitor will make an assessment regarding the safety of continuing or modifying the study. This assessment will be shared with the investigators either in writing or as part of a teleconference. Should the assessment of either the principal investigator or the medical monitor be that the trial should be terminated, the study will then be closed to further accrual. Follow-up safety assessments will be performed for all patients who are taken out of the study prematurely.

12. REFERENCES

1. Antonarakis ES, Trock BJ, Feng Z, et al. The natural history of metastatic progression in men with PSA-recurrent prostate cancer after radical prostatectomy: long-term follow-up. BJU Int 2012; 109: 32-39.

2. Aragon-Ching JB, Williams KM, Gulley JL. Impact of androgen-deprivation therapy on the immune system: implications for combination therapy of prostate cancer. Front Biosci 2007; 12: 4957-4971.

3. Arlen PM, Gulley JL, Todd N, et al. Antiandrogen, vaccine and combination therapy in patients with nonmetastatic hormone refractory prostate cancer. J Urol 2005; 174: 539-546.

4. Armitage P. Test for linear trend in proportions and frequencies. Biometrics 1955; 11: 375-386.

5. Arredouani MS, Tseng-Rogenski SS, Hollenbeck BK, et al. Androgen ablation augments human HLA2.1-restricted T cell responses to PSA self-antigen in transgenic mice. Prostate 2010; 70: 1002-1011.

6. Beer TM, Bernstein GT, Corman JM, et al. Randomized trial of autologous cellular immunotherapy with sipuleucel-T in androgen-dependent prostate cancer. Clin Cancer Res 2011; 17: 4558-4567.

7. Berges RR, Vukanovic J, Epstein JI, et al. Implication of the cell kinetic changes during the progression of human prostatic cancer. Clin Cancer Res 1995; 1: 473-480.

8. Berthold DR, Pond GR, Soban F, et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer: updated survival in the TAX 327 study. J Clin Oncol 2008; 26: 242-245.

9. Borrello I, Sotomayor EM, Cooke S, et al. A universal granulocyte-macrophage colony-stimulating factor–producing bystander cell line for use in the formulation of autologous tumor cell-based vaccines. Hum Gene Ther 1999; 10: 1983-1991.

10. Borrello I, Pardoll D. GM-CSF–based cellular vaccines: a review of the clinical experience. Cytokine Growth Factor Rev 2002; 13: 185-193.

11. Cohen J. Statistical power analysis for the behavioral sciences (2nd ed). Hillsdale, NJ: Lawrence Earlbaum Associates, 1988.

12. Curiel TJ, Coukos G, Zou L, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med 2004; 10: 942-949.

13. D'Amico AV, Schultz D, Loffredo M, et al. Biochemical outcome following external beam radiation therapy with or without androgen suppression therapy for clinically localized prostate cancer. JAMA 2000; 284: 1280-1283.

14. den Boer AT, van Mierlo GJ, Fransen MF, et al. The tumoricidal activity of memory $CD8^+$ T cells is hampered by persistent systemic antigen, but full functional capacity is regained in an antigen-free environment. J Immunol 2004; 172: 6074-6079.

15. Diggle PJ, Liang K, Zeger SL. Analysis of longitudinal data. New York, NY: Oxford University Press, 1994.

16. Drake CG, Doody AD, Mihalyo MA, et al. Androgen ablation mitigates tolerance to a prostate/prostate cancer-restricted antigen. Cancer Cell 2005; 7: 239-249.

17. Drake CG, Jaffee E, Pardoll DM. Mechanisms of immune evasion by tumors. Adv Immunol 2006; 90: 51-81.

18. Dranoff G, Jaffee E, Lazenby A, et al. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. Proc Natl Acad Sci USA 1993; 90: 3539-3543.

19. Dummer R. GVAX (Cell Genesys). Curr Opin Investig Drugs 2001; 2: 844-848.

20. Emens LA, Armstrong D, Biedrzycki B, et al. A phase I vaccine safety and chemotherapy dose-finding trial of an allogeneic GM-CSF–secreting breast cancer vaccine given in a specifically timed sequence with immunomodulatory doses of cyclophosphamide and doxorubicin. Hum Gene Ther 2004; 15: 313-337.

21. Emens LA, Asquith JM, Leatherman JM, et al. Increasing doses of cyclophosphamide suppress antigen-specific T helper-dependent immunity induced by a GM-CSF–secreting breast tumor vaccine. J Clin Oncol 2008; 26: abstract 3009.

22. Ercolini AM, Ladle BH, Manning EA, et al. Recruitment of latent pools of high-avidity $CD8^+$ T cells to the antitumor immune response. J Exp Med 2005; 201: 1591-1602.

23. Faith DA, Isaacs WB, Morgan JD, et al. Trefoil factor 3 overexpression in prostatic carcinoma: prognostic importance using tissue microarrays. Prostate 2004; 61: 215-227.

24. Freedland SJ, Humphreys EB, Mangold LA, et al. Death in patients with recurrent prostate cancer after radical prostatectomy: prostate-specific antigen doubling time subgroups and their associated contributions to all-cause mortality. J Clin Oncol 2007; 25: 1765-1771.

25. Furuya Y, Krajewski S, Epstein JI, et al. Expression of Bcl-2 and the progression of human and rodent prostatic cancers. Clin Cancer Res 1995; 1: 473-480.

26. Gannon PO, Poisson AO, Delvoye N, et al. Characterization of the intra-prostatic immune cell infiltration in androgen-deprived prostate cancer patients. J Immunol Methods 2009; 348: 9-17.

27. Gurel B, Iwata T, Koh CM, et al. Nuclear MYC protein overexpression is an early alteration in human prostate carcinogenesis. Mod Pathol 2008; 21: 1156-1167.

28. Higano CS, Corman JM, Smith DC, et al. Phase 1/2 dose-escalation study of a GM-CSF–secreting, allogeneic, cellular immunotherapy for metastatic hormone-refractory prostate cancer. Cancer 2008; 113: 975-984.

29. Higano C, Saad F, Somer B, et al. A phase III trial of GVAX immunotherapy for prostate cancer versus docetaxel plus prednisone in asymptomatic, castration-resistant prostate cancer. Genitourinary Cancer Symposium 2009; abstract LBA150.

30. Horoszewicz JS, Kawinski E, Murphy GP. Monoclonal antibodies to a new antigenic marker in epithelial prostatic cells and serum of prostatic cancer patients. Anticancer Res 1987; 7: 927-935.

31. Israeli RS, Powell CT, Corr JG, et al. Expression of the prostate-specific membrane antigen. Cancer Res 1994; 54: 1807-1811.

32. Jaffee EM, Hruban RH, Biedrzycki B, et al. Novel allogeneic granulocyte-macrophage colony-stimulating factor–secreting tumor vaccine for pancreatic cancer: A phase I trial of safety and immune activation. J Clin Oncol 2001; 19: 145-156.

33. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2008. CA Cancer J Clin 2008; 58: 71-96.

34. Koh YT, Gray A, Higgins SA, Hubby B, Kast WM. Androgen ablation augments prostate cancer vaccine immunogenicity only when applied after immunization. Prostate 2009; 69: 571-584.

35. Laheru D, Lutz E, Burke J, et al. Allogeneic granulocyte-macrophage colony-stimulating factor–secreting tumor immunotherapy alone or in sequence with cyclophosphamide for metastatic pancreatic cancer: a pilot study of safety, feasibility, and immune activation. Clin Cancer Res 2008; 14: 1455-1463.

36. Lee WH, Morton RA, Epstein JI, et al. Cytidine methylation of regulatory sequences near the π-class glutathione S-transferase gene accompanies human prostatic carcinogenesis. Proc Natl Acad Sci USA 1994; 91: 11733-11737.

37. Liyanage UK, Moore TT, Joo HG, et al. Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironments of patients with pancreas or breast adenocarcinoma. J Immunol 2002; 169: 2756-2761.

38. Machiels JP, Reilly RT, Emens LA, et al. Cyclophosphamide, doxorubicin, and paclitaxel enhance the antitumor immune response of granulocyte/macrophage-colony stimulating factor–secreting whole-cell vaccines in *HER-2/neu* tolerized mice. Cancer Res 2001; 61: 3689-3697.

39. Madan RA, Gulley JL, Schlom J, et al. Analysis of overall survival in patients with nonmetastatic castration-resistant prostate cancer treated with vaccine, nilutamide, and combination therapy. Clin Cancer Res 2008; 14: 4526-4531.

40. Marcelli M, Marani M, Li X, et al. Heterogeneous apoptotic responses of prostate cancer cell lines identify an association between sensitivity to staurosporine-induced apoptosis, expression of Bcl-2 family members, and caspase activation. Prostate 2000; 42: 260-273.

41. Mercader M, Bodner BK, Moser MT, et al. T cell infiltration of the prostate induced by androgen withdrawal in patients with prostate cancer. Proc Natl Acad Sci USA 2001; 98: 14565-14570.

42. Migita K, Ochi A. The fate of anergic T cells *in vivo*. J Immunol 1993; 150: 763-770.

43. Morse MD, McNeel DG. Prostate cancer patients on androgen deprivation therapy develop persistent changes in adaptive immune responses. Hum Immunol 2010; 71: 496-504.

44. Motoyoshi Y, Kaminoda K, Saitoh O, et al. Different mechanisms for anti-tumor effects of low- and high-dose cyclophosphamide. Oncol Rep 2006; 16: 141-146.

45. Pardoll DM. Spinning molecular immunology into successful immunotherapy. Nat Rev Immunol 2002; 2: 227-238.

46. Pound CR, Partin AW, Eisenberger MA, et al. Natural history of progression after PSA elevation following radical prostatectomy. JAMA 1999; 281: 1591-1597.

47. Ramsdell F, Fowlkes BJ. Maintenance of *in vivo* tolerance by persistence of antigen. Science 1992; 257: 1130-1134.

48. Rocha B, Tanchot C, von Boehmer H. Clonal anergy blocks *in vivo* growth of mature T cells and can be reversed in the absence of antigen. J Exp Med 1993; 177: 1517-1521.

49. Rosenberg SA, Yang JC, Sherry RM, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res 2011; 17: 4550-4557.

50. Rubin MA, Dunn R, Strawderman M, et al. Tissue microarray sampling strategy for prostate cancer biomarker analysis. Am J Surg Pathol 2002; 26: 312-319.

51. Shimizu J, Yamazaki S, Sakaguchi S. Induction of tumor immunity by removing $CD25^{+}CD4^{+}$ T cells: a common basis between tumor immunity and autoimmunity. J Immunol 1999; 163: 5211-5218.

52. Simons JW, Jaffee EM, Weber CE, et al. Bioactivity of autologous irradiated renal cell carcinoma vaccines generated by *ex vivo* granulocyte macrophage colony-stimulating factor gene transfer. Cancer Res 1997; 57: 1537-1546.

53. Simons JW, Mikhak B, Chang JF, et al. Induction of immunity to prostate cancer antigens: results of a clinical trial of vaccination with irradiated autologous prostate tumor cells engineered to secrete granulocyte-macrophage colony-stimulating factor using *ex vivo* gene transfer. Cancer Res 1999; 59: 5160-5168.

54. Simons JW, Carducci MA, Mikhak B, et al. Phase I/II trial of an allogeneic cellular immunotherapy in hormone-naive prostate cancer. Clin.Cancer Res 2006; 12: 3394-3401.

55. Small EJ, Halabi S, Dawson NA, et al. Antiandrogen withdrawal alone or in combination with ketoconazole in androgen-independent prostate cancer patients: a phase III trial. J Clin Oncol 2004; 22: 1025-1033.

56. Small EJ, Sacks N, Nemunaitis J, et al. Granulocyte macrophage colony-stimulating factor–secreting allogeneic cellular immunotherapy for hormone-refractory prostate cancer. Clin Cancer Res 2007; 13: 3883-3891.

57. Small E, Demkow T, Gerritsen WR, et al. A phase III trial of GVAX immunotherapy for prostate cancer in combination with docetaxel versus docetaxel plus prednisone in symptomatic, castration-resistant prostate cancer. Genitourinary Cancer Symposium 2009; abstract 7.

58. Smith HA, Maricque BB, Eberhardt J, et al. IgG responses to tissue-associated antigens as biomarkers of immunological treatment efficacy. J Biomed Biotechnol 2011; 2011: 454861.

59. Sutherland JS, Goldberg GL, Hammett MV, et al. Activation of thymic regeneration in mice and humans following androgen blockade. J Immunol 2005; 175: 2741-2753.

60. Sutmuller RP, van Duivenvoorde LM, van Elsa A, et al. Synergism of cytotoxic T lymphocyte-associated antigen 4 blockade and depletion of $CD25^+$ regulatory T cells in antitumor therapy reveals alternative pathways for suppression of autoreactive cytotoxic T lymphocyte responses. J Exp Med 2001; 194: 823-832.

61. Tannock IF, de Wit R, Berry WR, et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. N Engl J Med 2004; 351: 1502-1512.

62. Tu XM, Zhang J, Kowalski J, et al. Power analyses for longitudinal study designs with missing data. Stat Med 2007; 26: 2958-2981.

63. Wada S, Yoshimura K, Hipkiss EL, et al. Cyclophosphamide augments antitumor immunity: studies in an autochthonous prostate cancer model. Cancer Res 2009; 69: 4309-4318.

64. Wilson CA, Mrose SA, Thomas DW. Enhanced production of B lymphocytes after castration. Blood 1995; 85: 1535-1539.

65. Woo EY, Chu CS, Goletz TJ, et al. Regulatory $CD4^+CD25^+$ T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. Cancer Res 2001; 61: 4766-4772.

66. Yamashita K, Miyashiro Y, Maekubo H, et al. Development of highly sensitive quantification method for testosterone and dihydrotestosterone in human serum and prostate tissue by liquid chromatography–electrospray ionization tandem mass spectrometry. Steroids 2009; 74: 920-926.

67. Zabransky DJ, Smith HA, Thoburn CJ, et al. Lenalidomide modulates IL-8 and anti-prostate antibody levels in men with biochemically-recurrent prostate cancer. Prostate 2012; 72: 487-498.

68. Zha S, Gage WR, Sauvageot J, et al. Cyclooxygenase-2 is up-regulated in proliferative inflammatory atrophy of the prostate, but not in prostate carcinoma. Cancer Res 2001; 61: 8617-8623.

APPENDIX A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		*Karnofsky Performance Scale*	
Grade	**Description**	**%**	**Description**
0	Normal activity. Fully active, able to continue all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease
		90	Able to carry on normal activity, minor signs or symptoms of disease
1	Symptoms, but ambulatory. Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort, some signs or symptoms of disease
		70	Cares for self, unable to carry on normal activity or to do active work
2	In bed < 50% of the time. Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance but is able to care for most needs
		50	Requires considerable assistance and frequent medical care
3	In bed > 50% of the time. Capable of only limited self-care, confined to bed or chair > 50% of waking hours.	40	Disabled, requires special care and assistance
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled, cannot carry on any self-care, totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly
5	Dead	0	Dead

APPENDIX B: COMMON TOXICITY CRITERIA, VERSION 4.0

Adverse events will be described and graded using the NCI Common Toxicity Criteria (Version 4.0). A copy of this document can be downloaded from the CTEP website (http://ctep.cancer.gov/forms). All treatment areas must have a copy of this document, or must be able to access a copy.

In general, the grading system can be summarized as follows:

Grade:	***Severity:***	***Description:***
Grade 1	Mild	Transient or mild discomfort; generally non-progressive; no limitation in daily activities; no medical intervention required.
Grade 2	Moderate	Mild/moderate limitation in daily activities; some assistance may be required; no/minimal medical intervention required.
Grade 3	Severe	Marked limitation in daily activities; some assistance usually required; medical intervention is required.
Grade 4	Life-threatening	Extreme limitation in daily activities; major assistance required; significant medical intervention required.
Grade 5	Death	Death related to an adverse event.

APPENDIX C: SERIOUS ADVERSE EVENT (SAE) REPORTING FORM

Please notify Dr. Charles Drake within 24 hours

Protocol Title:	A neoadjuvant immunologic study of androgen deprivation therapy combined with a GM-CSF–secreting allogeneic prostate cancer vaccine and low-dose cyclophosphamide in men with high-risk localized prostate cancer undergoing radical prostatectomy		
Protocol Number: J12XX	**Principal Investigator:** Dr. Emmanuel Antonarakis	**Signature of PI:**	**Date:**
Report Date:	**Hospital Admission Date:**	**Date of Discovery of Event:**	**Report Type:** ☐ **Initial** ☐ **Follow-up** ☐ **Final Follow-up** ☐ **Death** ☐ **Addendum to:**

Section A: Subject Information		
Subject ID:	**Subject Initial:**	**Subject Gender:** ☐ **Male** ☐ **Female**

Section B: Event Information		
Event diagnosis or symptoms:	**Date of CY Dose:** **Date of GVAX Dose:**	**Action taken regarding the study drugs:** ☐ **None** ☐ **Interrupted** ☐ **Discontinued**
Event Onset Date:		**Event End Date:**

Relationship to:	**Cyclophosphamide**	**Prostate GVAX**	**Underlying Disease**
Unrelated	☐	☐	☐
Probably Unrelated	☐	☐	☐
Possible Related	☐	☐	☐
Probably Related	☐	☐	☐
Definitely Related	☐	☐	☐

Section C: Brief Description of the Event:
Section D: Relevant Medical History

Section E: Concomitant Drug (Not related to SAE)					
Name of the Drug	**Start Date**	**Stop Date**	**Route**	**Dose**	**Frequency**

Section F: Comments
Additional Documents: ☐ Please specify

APPENDIX D: ABBREVIATIONS AND ACRONYMS

AAV	adeno-associated virus
ACLS	Advanced Cardiac Life Support
ADR	adverse drug reaction
ADT	androgen deprivation therapy
AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
ANOVA	analysis of variance
APTT	activated partial thromboplastin time
AR	androgen receptor
AST	aspartate aminotransferase
BCG	bacillus Calmette-Guerin vaccine
bid	bis in die (twice a day)
BP	blood pressure
BSA	body surface area
BUN	blood urea nitrogen
^{o}C	degrees Celsius
Ca^{++}	calcium
CBC	complete blood count
$CD4^{+}$ T cells	cluster determinant 4–positive T lymphocytes
$CD8^{+}$ T cells	cluster determinant 8–positive T lymphocytes
CFR	Code of Federal Regulations
CG1940	PC3-derived prostate cancer cell line expressing human GM-CSF (Cell Genesys, Inc.)
CG8711	LNCaP-derived prostate cancer cell line expressing human GM-CSF (Cell Genesys, Inc.)
CI	confidence interval
Cl^{-}	chloride
cm	centimeter
COPD	chronic obstructive pulmonary disease
CR	complete response
CRO	Clinical Research Office
CRF	case report form
CRPC	castration resistant prostate cancer
CRRMC	Clinical Research Review and Monitoring Committee
CT	computerized tomography
CTL	Cell Therapy Laboratory
CTLA-4	cytotoxic T lymphocyte-associated antigen 4
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program

DAB	3,3′-diaminobenzine tetrahydrochloride
dL	deciliter
DLT	dose-limiting toxicity
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
DPBS	Dubelcco's phosphate buffered saline
DSMC	data and safety monitoring committee
DTH	delayed-type hypersensitivity
EC	ethics committee
ECOG	Eastern Cooperative Oncology Group
EMLA	eutectic mixture of local anesthetics
FDA	Food and Drug Administration
GCP	good clinical practice
G-CSF	granulocyte-colony stimulating factor
GM-CSF	granulocyte-macrophage colony-stimulating factor
GnRH	gonadotropin-releasing hormone
GVAX	allogeneic cell-based GM-CSF–secreting immunotherapy for prostate cancer
HA	hemagglutinin
HIPAA	Health Insurance Portability and Accountability Act
HR	heart rate
HRPC	hormone-refractory prostate cancer
HSA	human serum albumin
HSB	hue-saturation-brightness
IBC	Institutional Biosafety Committee
ICH	International Conference on Harmonisation
IHC	Immunohistochemistry
ID	intradermal
IND	investigational new drug
INR	international normalized ratio
IQR	interquartile range
IRB	Institutional Review Board
IV	intravenous
JHMI	Johns Hopkins Medical Institutions
K^+	potassium
LDH	lactate dehydrogenase
LHRH	luteinizing hormone releasing hormone
LNCaP	AR-positive human prostate cancer cell line derived from a lymph node metastasis
LOI	letter of intent
MedDRA	Medical Dictionary for Regulatory Activities

MMTV	murine mammary tumor virus
MTD	maximum tolerated dose
NA	not applicable
N/A	not available
NCI	National Cancer Institute
OR	operating room
PC3	androgen-independent human prostate cancer cell line derived from a bone metastasis
PI	principal investigator
PO	per os (by mouth)
PSA	prostate-specific antigen
PSMA	prostate-specific membrane antigen
PT	prothrombin time
PTT	partial thromboplastin time
qd	quaque die (every day)
RAC	Recombinant DNA Advisory Committee
RP	radical prostatectomy
RT	room temperature
SAE	serious adverse event
SC	Subcutaneous
SD	standard deviation
SKCCC	Sidney Kimmel Comprehensive Cancer Center
TdT	terminal deoxynucleotidyl transferase
tid	ter in die (3 times a day)
TMA	tissue microarray
TRAMP	transgenic adenocarcinoma of the mouse prostate(mouse model)
T_{regs}	regulatory T lymphocytes
TUNEL	TdT-mediated deoxy uridine triphosphate (UTP) nick end-labeling assay
ULN	upper limit of normal
USP	United States Pharmacopeia (sterile, hypotonic, nonpyrogenic water for injection)
WBC	white blood cell

APPENDIX E: DATA AND SAFETY MONITORING PLAN (DSMP), Version 4.0 (9/22/11)