Neoadjuvant Dupilumab in Men with Localized High-Risk Prostate Cancer

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SYNOPSIS

Title	Neoadjuvant Dupilumab in Men with Localized High-Risk Prostate Cancer
Institution	Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD
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Sponsor	Johns Hopkins University Regeneron, Inc.
IRB #	IRB00182718
Study agent	Dupilumab
Phase	Phase II Study
Indication	Men with high-risk localized prostate cancer, prior to radical prostatectomy
Target population	Inclusion criteria • Histologically confirmed prostate adenocarcinoma • Clinical stage T1c–T3b, N0, M0 • Gleason sum 7-10 • At least 2 positive cores at biopsy • Prior decision to undergo radical prostatectomy • Adult male ≥18 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 Exclusion criteria: • 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 • Concomitant treatment with hormonal therapy or 5□-reductase inhibitors • Use of experimental agents for prostate cancer within the past 3 months • History of autoimmune disease requiring systemic immunosuppression • History of other lethal malignancy within 3 years • Uncontrolled major infectious, cardiovascular, pulmonary, hematologic, or psychiatric illnesses that would make the patient a poor study candidate • significant eye disease
Start date/ Duration	Initial patients are expected to be entered in July 2018. With an estimated enrollment rate of 2-3 patients per month, accrual is expected to last 8-10 months.

Sample size: 20 evaluable patients (*i.e.* those that undergo prostatectomy)

Rationale: Once prostate cancer (PCa) metastasizes it is incurable and advanced PCa continues to kill more than 27,000 men per year in the United States (1). New supra-castration agents significantly extend life for men with metastatic castrate resistant prostate cancer (mCRPC) but progress has largely stalled in the last few years in the development of novel therapeutics. In particular, mCRPC has been particularly resistant to the newer immunotherapeutic anti-CTLA4 and anti-checkpoint strategies. For the field to move forward we need to know why PCa appears to be immunologically silent. We have previously identified alternatively activated Tumor associated macrophages (M2-TAMs) as key regulators of PCa tumorigenesis (2-10). Polarization of undifferentiated monocytes inot fully functional M2-TaMs requires activation of the IL4 receptor. Suppression of M2-TAMs by blocking the IL4 receptor in preclinical models inhibits tumor growth but effective clinical strategies remain to be developed. Simultaneously, primary and metastatic PCa lesions demonstrate poor infiltration by cytotoxic T cells. We hypothesize that M2-TAMs act in the tumor microenvironment to promote tumor growth (e.g., secrete MMPs,VEGF) while inhibiting the host immune response (e.g., express PD-L1 and secrete immunosuppressive cytokines such as IL-10).

We aim (Primary Outcome) to investigate whether inhibition of M2-TAMs via administration of dupliumab, an IL4 receptor antagonist approved for use in topic dermatitis, will inhibit the infiltration of M2-TAMs in primary prostate cancers in the neoadjuvant setting for men with localized high-risk prostate cancer (11, 12). This will be accomplished by a comparison of pre-dupilumab M2-TAM infiltration in the biopsy vs. post-dupilumab M2-TAM infiltration in the RP specimen. Secondary outcomes will include: 1) confirmation of the safety and feasibility of Dupilumab administered using a standard dose / schedule in the neo-adjuvant setting; 2) Quantify the extent of CD8+ T cell infiltration into the prostate from harvested prostate glands of treated patients; 3) Quantify the extent of CD4+ T cell and Treg infiltration into the prostate in prostate specimens of treated patients; 4) Quantify markers of apoptosis in prostate tumor specimens of treated patients using TUNEL staining and expressed as the mean staining percentage in tumor tissue; 5) Quantify markers of cell proliferation in prostate tumor specimens of treated patients using Ki-67 staining and expressed by the mean staining percentage in tumor; 6) Evaluate the proportion of pathological complete responses in prostate tumor specimens of treated patients; 7) Evaluate PSA response rates defined as the proportion of patients who achieve an undetectable PSA (<0.1 ng/mL) by 3 months after prostatectomy. We hypothesize that neoadjuvant Dupilumab will be feasible and safe (i.e. will not interfere with subsequent prostatectomy), and will produce a measurable decrease in M2-TAM infiltration into the primary tumor microenvironment.

If this study shows significant reduction in M2-TAM infiltration and administration of the study drug is feasible and safe, then future studies would aim to use Dupilumab in the mCRPC setting as an adjunct to cytotoxic and immunologic therapies.

Objectives

<u>Primary:</u>

- Evaluate the ability of dupilumab to inhibit M2-TAMs infiltration of prostate cancer by comparison of pre-dupilumab M2-TAM infiltration in the biopsy vs. post-

dupilumab M2-TAM infiltration in the RP specimen.

Secondary:

- To confirm the safety and feasibility of Dupilumab administered using a standard dose / schedule in the neo-adjuvant setting
- Quantify the extent of CD8+ T cell infiltration into the prostate from harvested prostate glands of treated patients
- Quantify the extent of CD4+ T cell and Treg infiltration into the prostate in prostate specimens of treated patients
- Quantify markers of apoptosis in prostate tumor specimens of treated patients using TUNEL staining and expressed as the mean staining percentage in tumor tissue
- Quantify markers of cell proliferation in prostate tumor specimens of treated patients using Ki-67 staining and expressed by the mean staining percentage in tumor
- Evaluate the proportion of pathological complete responses in prostate tumor specimens of treated patients
- Evaluate PSA response rates defined as the proportion of patients who achieve an undetectable PSA (<0.1 ng/mL) by 2 months after prostatectomy.

Study design

This is a single-center, single arm, open-label phase II study evaluating the safety, anti-tumor effect, and immunogenicity of neoadjuvant Dupilumab given prior to radical prostatectomy in men with high-risk localized prostate cancer (this trial will enroll men with at least high risk prostate cancer defined by NCCN Guidelines Version 2.2017 = clinical stage ≥T3a or PSA >20 ng/mL or Gleason score ≥8). Patients will be recruited from the outpatient Urology clinic. Men will be treated with dupilumab 600 mg s.q. on day 1, and then 300 mg s.q. on days 8,15, 22, 29, 36, 43. They will then undergo surgery on day 57. 14 days after the last dose of Dupilumab, prostate glands will be harvested at the time of radical prostatectomy, and prostate tissue will be examined for the secondary endpoints. Follow-up evaluation for adverse events will occur 30 days and 60 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.

Study 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 for histological dx / Gleason score
- 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

On treatment days (days 1, 8, 15, 22, 29, 36, 43):

- Performance status
- Adverse events/toxicity evaluation
- Hematology and chemistry laboratories, as clinically indicated
- Administration of Dupilumab days 1, 8, 15, 22, 29, 36, 43
- Interval history and focused physical examination (including vital signs before and 1 hour post subcutaneous injection)

On day 57:

- 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
- Radical prostatectomy
- Pathologic review of surgical specimen according to standard procedures
- Evaluation of harvested prostate gland for secondary endpoints

Post-operative day 30 and day 60:

- Performance status
- Adverse events/toxicity evaluation
- PSA every 3 months (post-op year 1), then every 6 months (post-op years 2-3)

Criteria for evaluation

Primary endpoint:

- A comparison of pre-dupilumab M2-TAM infiltration in the biopsy vs. post-dupilumab M2-TAM infiltration in the RP specimen

Secondary endpoints:

- Frequency, type, and severity of adverse events
- M2-TAM infiltration in the RP specimen
- Quantify the extent of CD8+ T cell infiltration into the prostate from harvested prostate glands of treated patients
- Quantify the extent of CD4+ T cell and Treg infiltration into the prostate in prostate specimens of treated patients
- Quantify markers of apoptosis in prostate tumor specimens of treated patients using TUNEL staining and expressed as the mean staining percentage in tumor tissue
- Quantify markers of cell proliferation in prostate tumor specimens of treated patients using Ki-67 staining and expressed by the mean staining percentage in tumor
- Evaluate the proportion of pathological complete responses in prostate tumor specimens of treated patients
  Evaluate PSA response rates defined as the proportion of patients who achieve an undetectable PSA (<0.1 ng/mL) by 2 months after prostatectomy.

Statistical methods

For the primary outcome: pre- and post-treatment M2-TAM infiltration will be compared using a paired t-test, or nonparametric signed rank test if data don't conform to a Gaussian distribution. Power is based on the paired t-test. With 20 patients, we have the power (≥ 81%) to detect a decrease of ≥0.67 standard deviation unit (effect size = 0.67) (14). This is likely to be conservative as we observed a much larger difference in M2-TAM levels between primary and metastatic tumors in our data. Power calculated using PASS v. 11 (NCSS Software, Kaysville, UT).

For the secondary analysis of safety, we will use a continuous toxicity monitoring approach with sequential dose-limiting toxicity boundaries (13). Patients will be evaluated for AEs after neoadjuvant dupilumab. We consider a 15% rate of Grade ≥3 AEs to be unacceptable and indication to halt the trial. Accrual will continue until the number of Grade ≥3 AEs equals or exceeds the boundary for a given number of patients, or the completion of 20 patients without crossing any toxicity boundaries. The table below shows the schedule of accrual and associated toxicity boundaries.

Number of patients:	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Toxicity Boundary:	3	3	4	4	4	4	5	5	5	6	6	6	6	7	7	7	7	7

The AE rate and 95% confidence interval will be calculated, either at trial halt or completion of accrual.

Secondary analyses of other immune infiltrate cells will be compared using a paired t-test, or nonparametric signed rank test if data don't conform to a Gaussian distribution. Other secondary analyses will include calculating the rate and 95% confidence interval (CI) for complete response rates, and undetectable post-treatment PSA, and paired t-tests for the secondary outcome biomarkers listed above. In exploratory analyses we will evaluate whether # of biopsy cores evaluated, patient, or clinical factors correlate with extent of decrease in infiltration, although the small sample size limits these analyses purely to hypothesis generating.

Power. For the primary outcome of pre- vs. post-treatment decrease in M2-TAM infiltration, power is based on the paired t-test. With 20 patients, we have the power (≥ 81%) to detect a decrease of ≥0.67 standard deviation unit (effect size = 0.67) (14). This is likely to be conservative as we observed a much larger difference in M2-TAM levels between primary and metastatic tumors in our data. Power calculated using PASS v. 11 (NCSS Software, Kaysville, UT). The continuous toxicity monitoring design with n=20 above has probability = 0.048 of early stopping if the Grade ≥3 AE rate is <15%. Toxicity boundaries calculated using software developed by Ivanova (13) (http://cancer.unc.edu/biostatistics/program/ivanova/ContinuosMonitoringForToxicity.aspx).

Safety

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 Dupilumab until the 60th postoperative day.

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1. OBJECTIVES

1.1 Primary Objective

- Evaluate the ability of dupilumab to inhibit M2-TAMs infiltration or prostate cancer by comparison of pre-dupilumab M2-TAM infiltration in the biopsy vs. post-dupilumab M2-TAM infiltration in the RP specimen.

1.2 Secondary Objectives

- To evaluate the safety and tolerability of DUPILUMAB administered 600 mg s.q. on day 1, and then 300 mg s.q. on days 8,15, 22, 29, 36, 43. They will then undergo surgery on day 56.
- To evaluate the feasibility of administering DUPILUMAB administered 600 mg s.q. on day 1, and then 300 mg s.q. on days 8,15, 22, 29, 36, 43.
- Quantify the extent of CD8+ T cell infiltration into the prostate from harvested prostate glands of treated patients
- Quantify the extent of CD4+ T cell and Treg infiltration into the prostate in prostate specimens of treated patients
- Quantify markers of apoptosis in prostate tumor specimens of treated patients using TUNEL staining and expressed as the mean staining percentage in tumor tissue
- Quantify markers of cell proliferation in prostate tumor specimens of treated patients using Ki-67 staining and expressed by the mean staining percentage in tumor
- Evaluate the proportion of pathological complete responses in prostate tumor specimens of treated patients
- Evaluate PSA response rates defined as the proportion of patients who achieve an undetectable PSA (<0.1 ng/mL) by 2 months after prostatectomy.

2. BACKGROUND AND RATIONALE

2.1 Disease Background

Once prostate cancer (PCa) metastasizes it is incurable and advanced PCa continues to kill more than 27,000 men per year in the United States (1). Yet, localized prostate cancer is often curable and even metastatic disease can respond to treatment.

PCa cells exist in a complex habitat within their microenvironment, co-existing with other normal host cells, including hematopoietic precursors, stromal cells, osteoblasts, osteoclasts, nerve cells, adipocytes, endothelial cells, and multiple cell types of the immune system including macrophages (2). Within the ecosystem of the tumor, these host cells provide the cancer cells with a multitude of growth factors and cytokines that result in tumor promotion and growth. In turn, the cancer cells provide growth factors to the host cells that result in their proliferation and survival. Disruption of this cooperation is a paradigm that could be explored and exploited as a way to suppress tumorigenesis and overall, eradicate tumors both at primary and metastatic sites.

Malignant tumors are associated with a leukocytic infiltrate as part of the reactive stroma that is enriched for macrophages (3-7). Macrophages play an important role in the regulation of angiogenesis in both normal and diseased tissues, including malignant tumors (7-9). It is not clear whether TAMs are derived from peripheral blood monocytes

recruited into the tumor from the circulation or from resident macrophages already in the healthy tissue before tumor develops and metastasizes(10). However, elevated expression of a number of monocyte chemoattractants, including chemokines CCL2, CCL3, CCL4, CCL8 and CCL5 (RANTES) by both tumor and stromal cells within the tumor microenvironment has been shown to positively correlate with increased M2-TAM recruitment within several human tumor types (15-20). When associated with tumors, macrophages demonstrate functional "polarization" towards one of two phenotypically different subsets of macrophages: T_H1 (also known as M1 macrophages) or T_H2 (also known as M2 macrophages) (14). M1 macrophages are known to produce pro-inflammatory cytokines and play an active role in cell destruction. Conversely, M2 macrophages primarily scavenge debris and promote angiogenesis and wound repair (2-10, 15-20). The M2 macrophage population is phenotypically similar to the TAM population that promotes tumor growth and development. Others and we have demonstrated the importance of TAMs in promoting PCa tumorigenesis. TAMs have been demonstrated to make up to 50% of the population of cells in PCa bone metastases, contributing to cancer cell growth by promoting a permissive growth environment through the secretion of matrix degrading enzymes, angiogenic factors such as VEGF, and multiple growth factors while simultaneously secreting multiple factors that suppress the host immune response (15-20).

We have demonstrated that Inhibition of IL-4 by blocking IL4rα blocks polarization to the M2 phenotype and significantly inhibits prostate tumor growth. Human macrophages were polarized using phased polarization protocol, each condition in triplicate (21). On day 5, 10 ng /mL of the anti-IL4rα antibody Dupilumab was added to the cultures. On day 9, % of cells exhibiting CD206 and CD163 as markers of M2 polarization was determined by FACS sorting per published protocol (**Table 2**). We next tested the ability of IL4rα inhibition to inhibit tumor growth *in vivo* in preclinical mouse models. Parallel studies were conducted in human SCID mice using the human cell line PC3 as well as the Hi-Myc B6CaP model. For the PC-3 model, 500,000 cells were implanted into mice (n=12 per group). When these tumors reached $2mm^3$ (week 2), animals began treatment. Docetaxel was given as a single dose of 5mg/kg at week 2 per published protocol (22-27). Mice were treated weekly (weeks 2,3,4,5,6) with a murine specific anti-IL4rα antibody (rat anti-mouse IL-4R alpha clone 129801 (R&D Systems MAB530) since Dupilumab is human specific. Tumor growth was measured weekly with caliper measurements. As single agents, both docetaxel and anti-IL4rα antibody inhibited tumor growth by 33% ($p<0.05$). The combination of docetaxel + anti-IL4rα antibody inhibited tumor growth by 75% ($p<0.01$) (**Figure 1**). At week 7, tumors were excised and FACS analysis was performed to determine the number of CD68+ macrophages as a percent of cellular mass as per published protocol (27). IL4rα inhibition significantly decreased macrophage infiltration into the tumor mass as compared to control and docetaxel treated alone tumors, $p<0.001$ (**Table 3**) Similar significant results were demonstrated utilizing the B6CaP mouse model (results not shown).

2.2 Treatment Background

2.2.1 Study Agent (DUPILUMAB) Background

Dupilumab is a human monoclonal IgG4 antibody that inhibits interleukin-4 (IL-4) and interleukin-13 (IL-13) signaling by specifically binding to the IL-4Rα subunit shared by the IL-4 and IL-13 receptor complexes. Dupilumab inhibits IL-4

signaling via the Type I receptor and both IL-4 and IL-13 signaling through the Type II receptor (11, 12).

Blocking IL-4Rα with dupilumab inhibits IL-4 and IL-13 cytokine-induced responses, including the release of proinflammatory cytokines, chemokines and IgE

Dupilumab is produced by recombinant DNA technology in Chinese Hamster Ovary cell suspension culture.

DUPILUMAB (dupilumab) Injection is supplied as a sterile, preservative-free, clear to slightly opalescent, colorless to pale yellow solution for subcutaneous injection. DUPILUMAB is provided as a single-dose pre-filled syringe with or without needle shield in a 2.25 mL siliconized Type-1 clear glass syringe. The needle cap is not made with natural rubber latex. Each pre-filled syringe delivers 300 mg dupilumab in 2 mL which also contains L-arginine hydrochloride (10.5 mg), L- histidine (6.2 mg), polysorbate 80 (4 mg), sodium acetate (2 mg), sucrose (100 mg), and water for injection, pH 5.9.

Mechanism of Action

Dupilumab is a human monoclonal IgG4 antibody that inhibits interleukin-4 (IL-4) and interleukin-13 (IL-13) signaling by specifically binding to the IL-4Rα subunit shared by the IL-4 and IL-13 receptor complexes. Dupilumab inhibits IL-4 signaling via the Type I receptor and both IL-4 and IL-13 signaling through the Type II receptor.

Blocking IL-4Rα with dupilumab inhibits IL-4 and IL-13 cytokine-induced responses, including the release of proinflammatory cytokines, chemokines and IgE.

Pharmacodynamics

Consistent with receptor blockade, serum levels of IL-4 and IL-13 were increased following dupilumab treatment. The relationship between the pharmacodynamic activity and the mechanism(s) by which dupilumab exerts its clinical effects is unknown.

Pharmacokinetics

Absorption

Following an initial subcutaneous (SC) dose of 600 mg, dupilumab reached peak mean ±SD concentrations (Cmax) of 70.1±24.1 mcg/mL by approximately 1 week post dose.

Steady-state concentrations were achieved by Week 16 following the administration of 600 mg starting dose and 300 mg dose either weekly (twice the recommended dosing frequency) or every other week. Across clinical trials, the mean ±SD steady-state trough concentrations ranged from 73.3±40.0 mcg/mL to 79.9±41.4 mcg/mL for 300 mg administered every 2 weeks and from 173±75.9 mcg/mL to 193±77.0 mcg/mL for 300 mg administered weekly.

The bioavailability of dupilumab following a SC dose is estimated to be 64%

Distribution
The estimated total volume of distribution was approximately 4.8±1.3 L.
Elimination

The metabolic pathway of dupilumab has not been characterized. As a human monoclonal IgG4 antibody, dupilumab is expected to be degraded into small peptides and amino acids via catabolic pathways in the same manner as endogenous IgG. After the last steady-state dose of 300 mg Q2W or 300 mg QW dupilumab, the median times to non-detectable concentration (<78 ng/mL) are 10 and 13 weeks, respectively.

Dose Linearity

Dupilumab exhibited nonlinear target-mediated pharmacokinetics with exposures increasing in a greater than dose-proportional manner. The systemic exposure increased by 30-fold when the dose increased 8-fold following a single dose of dupilumab from 75 mg to 600 mg (i.e., 0.25 times to 2-times the recommended dose).

Weight
Dupilumab trough concentrations were lower in subjects with higher body weight.

Immunogenicity

Development of antibodies to dupilumab was associated with lower serum dupilumab concentrations. A few subjects who had high antibody titers also had no detectable serum dupilumab concentrations.

Specific Populations

Geriatric Patients

In subjects who are 65 years and older, the mean ±SD steady-state trough concentrations of dupilumab were 69.4±31.4 mcg/mL and 166±62.3 mcg/mL, respectively, for 300 mg administered every 2 weeks and weekly. No dose adjustment in this population is recommended.

Renal or Hepatic Impairment

No formal trial of the effect of hepatic or renal impairment on the pharmacokinetics of dupilumab was conducted.

Drug Interaction Studies

Drug interaction studies have not been conducted with DUPILUMAB.

2.2.2 Safety of DUPILUMAB

Carcinogenesis, Mutagenesis, Impairment of Fertility

Animal studies have not been conducted to evaluate the carcinogenic or mutagenic potential of dupilumab.

No effects on fertility parameters such as reproductive organs, menstrual cycle length, or sperm analysis were observed in sexually mature mice that were subcutaneously administered a homologous antibody against IL-4Rα at doses up to 200 mg/kg/week.

2.2.3. Clinical Trial Experience

CLINICAL STUDIES
Three randomized, double-blind, placebo-controlled trials (Trials 1, 2, and 3) enrolled a total of 2119 subjects 18 years of age and older with moderate-to-severe atopic dermatitis (AD) not adequately controlled by topical medication(s). Disease severity was defined by an Investigator's Global Assessment (IGA) score ≥3 in the overall assessment of AD lesions on a severity scale of 0 to 4, an Eczema Area and Severity Index (EASI) score ≥16 on a scale of 0 to 72, and a minimum body surface area involvement of ≥10%. At baseline, 59% of subjects were male, 67% were white, 52% of subjects had a baseline IGA score of 3 (moderate AD), and 48% of subjects had a baseline IGA of 4 (severe AD). The baseline mean EASI score was 33 and the baseline weekly averaged peak pruritus Numeric Rating Scale (NRS) was 7 on a scale of 0-10.

In all three trials, subjects in the DUPILUMAB group received subcutaneous injections of DUPILUMAB 600 mg at Week 0, followed by 300 mg every other week (Q2W). In the monotherapy trials (Trials 1 and 2), subjects received DUPILUMAB or placebo for 16 weeks.

In the concomitant therapy trial (Trial 3), subjects received DUPILUMAB or placebo with concomitant topical corticosteroids (TCS) and as needed topical calcineurin inhibitors for problem areas only, such as the face, neck, intertriginous and genital areas for 52 weeks.

All three trials assessed the primary endpoint, the change from baseline to Week 16 in the proportion of subjects with an IGA 0 (clear) or 1 (almost clear) and at least a 2-point improvement. Other endpoints included the proportion of subjects with EASI-75 (improvement of at least 75% in EASI score from baseline), and reduction in itch as defined by at least a 4- point improvement in the peak pruritus NRS from baseline to Week 16.

In Trial 3, of the 421 subjects, 353 had been on study for 52 weeks at the time of data analysis. Of these 353 subjects, responders at Week 52 represent a mixture of subjects who maintained their efficacy from Week 16 (e.g., 53% of DUPILUMAB IGA 0 or 1 responders at Week 16 remained responders at Week 52) and subjects who were non-responders at Week 16 who later responded to treatment (e.g., 24% of DUPILUMAB IGA 0 or 1 non-responders at Week 16 became responders at Week 52). Treatment effects in subgroups (weight, age, gender, race, and prior treatment, including immunosuppressants) in Trials 1, 2, and 3 were generally consistent with the results in the overall study population.

In Trials 1, 2, and 3, a third randomized treatment arm of DUPILUMAB 300 mg QW did not demonstrate additional treatment benefit over DUPILUMAB 300 mg Q2W

2.3 Rationale

2.3.1 Rationale for conducting the study

Once prostate cancer (PCa) metastasizes it is incurable and advanced PCa continues to kill more than 27,000 men per year in the United States (1). Yet, localized prostate cancer is often curable and even metastatic disease can respond to treatment.

PCa cells exist in a complex habitat within their microenvironment, co-existing with other normal host cells, including hematopoietic precursors, stromal cells, osteoblasts, osteoclasts, nerve cells, adipocytes, endothelial cells, and multiple cell types of the immune system including macrophages (2). Within the ecosystem of the tumor, these host cells provide the cancer cells with a multitude of growth factors and cytokines that result in tumor promotion and growth. In turn, the cancer cells provide growth factors to the host cells that result in their proliferation and survival. Disruption of this cooperation is a paradigm that could be explored and exploited as a way to suppress tumorigenesis and overall, eradicate tumors both at primary and metastatic sites.

Malignant tumors are associated with a leukocytic infiltrate as part of the reactive stroma that is enriched for macrophages (2-10). Macrophages play an important role in the regulation of angiogenesis in both normal and diseased tissues, including malignant tumors (2-10). It is not clear whether TAMs are derived from peripheral blood monocytes recruited into the tumor from the circulation or from resident macrophages already in the healthy tissue before tumor develops and metastasizes. However, elevated expression of a number of monocyte chemoattractants, including chemokines CCL2, CCL3, CCL4, CCL8 and CCL5 (RANTES) by both tumor and stromal cells within the tumor microenvironment has been shown to positively correlate with increased M2-TAM recruitment within several human tumor types (2-10, 15-20). When associated with tumors, macrophages demonstrate functional “polarization” towards one of two phenotypically different subsets of macrophages: T_H1 (also known as M1 macrophages) or T_H2 (also known as M2 macrophages). M1 macrophages are known to produce pro-inflammatory cytokines and play an active role in cell destruction. Conversely, M2 macrophages primarily scavenge debris and promote angiogenesis and wound repair (2-10, 15-20). The M2 macrophage population is phenotypically similar to the TAM population that promotes tumor growth and development. Others and we have demonstrated the importance of TAMs in promoting PCa tumorigenesis. TAMs have been demonstrated to make up to 50% of the population of cells in PCa bone metastases, contributing to cancer cell growth by promoting a permissive growth environment through the secretion of matrix degrading enzymes, angiogenic factors such as VEGF, and multiple growth factors while simultaneously secreting multiple factors that suppress the host immune response (15-20).

We have demonstrated that Inhibition of IL-4 by blocking IL4rα blocks polarization to the M2 phenotype and significantly inhibits prostate tumor growth. Human macrophages were polarized using phased polarization protocol, each conditi in triplicate (21). On day 5, 10 ng /mL of the anti-IL4rα antibody Dupilumab was added to the cultures. On day 9, % of cells exhibiting CD206 and CD163 as markers of M2 polarization was determined by FACS sorting per published protocol (**Table 2**). We next tested the ability of IL4rα inhibition to inhibit tumor growth *in vivo* in preclinical mouse models. Parallel studies

were conducted in human SCID mice using the human cell line PC3 as well as the Hi-Myc B6CaP model. For the PC-3 model, 500,000 cells were implanted into mice (n=12 per group). When these tumors reached $2mm^3$ (week 2), animals began treatment. Docetaxel was given as a single dose of 5mg/kg at week 2 per published protocol (22-27). Mice were treated weekly (weeks 2,3,4,5,6) with a murine specific anti-IL4rα antibody (rat anti-mouse IL-4R alpha clone 129801 (R&D Systems MAB530) since Dupilumab is human specific. Tumor growth was measured weekly with caliper measurements. As single agents, both docetaxel and anti-IL4rα antibody inhibited tumor growth by 33% ($p<0.05$). The combination of docetaxel + anti-IL4rα antibody inhibited tumor growth by 75% ($p<0.01$). At week 7, tumors were excised and FACS analysis was performed to determine the number of CD68+ macrophages as a percent of cellular mass as per published protocol (27). IL4rα inhibition significantly decreased macrophage infiltration into the tumor mass as compared to control and docetaxel treated alone tumors, $p<0.001$. Similar significant results were demonstrated utilizing the Hi-MYC mouse model (results not shown).

2.3.2 Rationale for dosage selection

In the Phase III studies utilizing DUPILUMAB the prescribed dosage proposed in this trial was considered to be the safe and effective dose. Specifically, a third randomized treatment arm of DUPILUMAB 300 mg QW did not demonstrate additional treatment benefit over DUPILUMAB 300 mg Q2W (11,12)

2.3.3 Rationale for immunobiologic endpoints

The major efficacy goal of this study is to assess a decrease in M2-TAM infiltration in tumors based on our preliminary data. To do this, we have developed a CD206 antibody for immunohistochemical analysis (IHC) and demonstration of M2-TAM infiltration into primary and metastatic prostate cancers. The analysis of M2-TAM infiltration into human tumors has previously not been possible because of lack of a specific marker for M2 macrophages. Current markers, e.g., CD68, are specific for all human macrophages but do not reflect polarization states. Working with our collaborator Angelo De Marzo, Director of the Tissue Core for the Sidney Kimmel Cancer Center (SKCCC) at Johns Hopkins School of Medicine, utilizing stringent protocols to assess and quantify antibody staining, we have developed an anti-CD206 reagent to allow staining of human tissue microarrays to assess M2-TAM infiltration (28, 29, and submitted). Utilizing this antibody, we have demonstrated a significant increase in M2-TAMs in prostate tumors compared to normal tissue.

We will also quantify the extent of CD8+ T cell and CD4+ T cell infiltration into the prostate from harvested prostate glands of treated patients. We will quantify markers of apoptosis in prostate tumor specimens of treated patients using TUNEL staining and expressed as the mean staining percentage in tumor tissue and quantify markers of cell proliferation in prostate tumor specimens of treated patients using Ki-67 staining and expressed by the mean staining percentage in tumor. We will evaluate the proportion of pathological complete responses in prostate tumor specimens of treated patients and evaluate PSA response rates defined as the proportion of patients who achieve an undetectable PSA (<0.1 ng/mL) by 2 months after prostatectomy.

3. Patient Selection

3.1 Target Population

Subjects will include men with multifocal, Gleason 7 or greater, clinically localized prostate cancer (NCCN High Risk / Very High Risk) 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 Gleason sum of ≥7
- Radical prostatectomy has been scheduled at Johns Hopkins Hospital
- Age ≥18 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 <3 × upper limit of normal (ULN)
  - Serum bilirubin <3 × ULN
  - ALT <5 × ULN
  - AST <5 × ULN
  - Alkaline phosphatase <5 × 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 first dose of DUPILUMAB 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
- 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 from time of screening
- 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)
- History of malignancy within the last 3 years, 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
- Significant eye disease

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, 5□-reductase inhibitors, chemotherapy, radiation therapy, biologic therapy, immunosuppressive medications, or systemic corticosteroids.

Within 30 days of administration of study drug, patients should not receive 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.

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.

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 study coordinator.

4.2 Expected Enrollment

A total of 20 patients will be included in this study. The first patients are expected to be enrolled in August 2018, once the protocol has been approved by the IRB. With an estimated enrollment rate of 2-3 patients per month, accrual is expected to be completed in 10 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.

5. STUDY PLAN

5.1 Overview and Schema

This is a single-center, single arm, open-label phase II study evaluating the safety, anti-tumor effect, and immunogenicity of neoadjuvant DUPILUMAB given prior to radical prostatectomy in men with high-risk localized prostate cancer. Patients will be recruited from the outpatient Urology clinic. Men will be treated with dupilumab 600 mg s.q. on day 1, and then 300 mg s.q. on days 8, 15, 22, 29, 36, 43. They will then undergo surgery on day 57. 14 days after the last dose of Dupilumab, prostate glands will be harvested at the time of radical prostatectomy, and prostate tissue will be examined for the secondary endpoints. Follow-up evaluation for adverse events will occur 30 days and 60 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.

Table 1 STUDY CALENDAR

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

	Screening Evaluation[a]	Treatment Days (1, 8,15, 22, 29, 36, 43) (+/- 1 day)	Radical Prostatectomy Day 57 (+/- 3 days)	Follow-up, 30, and 60 Days Post-op (+/- 1 wk)[h,i]	LTFU (+/- 1 Month)[i]
Informed consent	X				
Medical history	X				
Review of medications	X	X	X	X	
Physical examination	X	X			
Vital signs	X	X	X		
Height and weight	X	X	X		
Performance status	X	X	X	X	
CT and bone scan [b]	(X)				
Hematology labs, coagulations [c]	X	X	X	X	
Chemistry labs [d]	X	X	X	X	
Serum PSA [e]	X		X	X	X
DUPILUMAB dose		X			
Toxicity assessment	X----------	----------	----------	----------X	
Surgical specimen [f]			X		
Pathologic review [g]	X		X		

a: The screening (pre-treatment) evaluation should be conducted within 42 days (±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 at screening, then 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: Harvested prostate gland to be evaluated for tumor apoptosis, CD8+ T cell infiltration, as well as other secondary endpoints.
g: 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.
h: The post-operative evaluations (30 and 60 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.
i: PSA should be measured every 3 months (±1 month) in the first year after prostatectomy, and every 6 months (±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 (Table 1).

- informed consent
- 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
- 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 Treatment Visits

The following must be performed on treatment days (days 1, 8,15, 22, 29, 36, 43) (±1 day) with administration of DUPILUMAB.

- performance status

- review of medication list
- review of toxicity/adverse events
- height/weight
- vital signs (before and 1 hour post subcutaneous injection (which is at the end of a 1 hour observation period)
- physical exam (focused)
- hematological laboratories and serum chemistry profile
- Administration of DUPILUMAB (see section 6.3 Study Drug Administration)

5.4 Radical Prostatectomy

The following must be performed on the day of radical prostatectomy, which itself should occur 56 (±3) days after the administration of first dose of DUPILUMAB.

- 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
- collection of prostatectomy tissue for analysis of study endpoints
- pathological processing of prostatectomy specimen according to standard procedures

5.5 Follow-Up Evaluations

Follow-up visit scheduled for 30 days and 60 days after radical prostatectomy may occur in the outpatient clinic or by telephone interview. Required evaluations during this follow-up visits are listed below. Patients should then continue to be followed by their treating urologist according to standard institutional practices.

- performance status
- review of medication list
- review of toxicity/adverse events

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.

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 DUPILUMAB . Patients receiving at least one dose of the study drug will be included in safety analyses, and those also undergoing prostatectomy will be used for the efficacy analyses.

6. STUDY TREATMENT

6.1 Description of Study Drug and Supplies

DUPILUMAB (dupilumab) Injection is supplied as a sterile, preservative-free, clear to slightly opalescent, colorless to pale yellow solution for subcutaneous injection. DUPILUMAB is provided as a single-dose pre-filled syringe with or without needle shield in a 2.25 mL siliconized Type-1 clear glass syringe. The needle cap is not made with natural rubber latex. Each pre-filled syringe delivers 300 mg dupilumab in 2 mL which also contains L-arginine hydrochloride (10.5 mg), L- histidine (6.2 mg), polysorbate 80 (4 mg), sodium acetate (2 mg), sucrose (100 mg), and water for injection, pH 5.9.

Under no circumstances is the Investigator allowed to release these clinical supplies for use by another physician not named on Form FDA 1572 or to administer study drug to a patient who is not enrolled in this study. Study drug must be dispensed at an institution specified on Form FDA 1572.

6.2 Drug Preparation

6.2.1 General Guidelines and Precautions

Men will be treated with dupilumab 600 mg s.q. on day 1, and then 300 mg s.q. on days 8,15, 22, 29, 36, 43.

Injection or allergic reactions may occur with the administration of monoclonal antibodies and other protein-based therapeutics. Precautions for anaphylaxis should be observed during DUPILUMAB administration. Supportive measures may include, but are not limited to: epinephrine, antihistamines, corticosteroids, IV fluids, vasopressors, oxygen, bronchodilators, diphenhydramine, and acetaminophen. Please refer to **Section 6.4.3** for specific guidelines regarding the management of drug reactions. Supportive care measures consistent with optimal patient care will be provided throughout the study according to institutional standards.

6.2.2 DUPILUMAB

- Inspect drug products visually for particulate matter and discoloration prior to administration. Discard vial if solution is cloudy, there is pronounced discoloration (solution may have pale-yellow color), or there is foreign particulate matter.
- The desired amount of DUPILUMAB should be withdrawn from the vial(s) and diluted to the appropriate final concentration with 0.9% Sodium Chloride Injection USP, according to the instructions provided.Discard partially used vials of DUPILUMAB
- Administration of study drug should begin immediately after preparation but no later than 6 hours after preparation. If there is a delay in administration of study drug such that it will not be administered on the day of preparation, the Principal Investigator should be notified immediately. Instructions on how to proceed will be provided.

6.2.3 Placebo or Control

There will be neither placebo nor active control drug for this study.

6.3 Study Drug Administration

- Do not mix DUPILUMAB with, or administer as an injection with, other medicinal products.

6.4 Potential Adverse Events and Supportive Care Measures

6.4.1 Grading of Administration Reactions

Injection reactions will be categorized as follows:

- Grade 1: mild reaction; intervention not indicated; (see **Section 6.4.3**);

- Grade 2: moderate reaction: responds promptly to symptomatic treatment [e.g., antihistamines, non-steroidal anti-inflammatory drugs (NSAIDS), narcotics, IV fluids]; prophylactic medications indicated for ≤ 24 hours;
- Grade 3: prolonged reaction: recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae (e.g., renal impairment, pulmonary infiltrates);
- Grade 4: life-threatening consequences; pressor or ventilatory support indicated;
- Grade 5: death.

The above grading scale is the CTCAE v 4.03 grading scale for CRS, which is nearly identical to the CTCAE v 4.03 grading scale for injection reaction and allergic reaction, and therefore considered appropriate for grading all injection reactions in this study, irrespective of the underlying mechanism of the reaction. The Sponsor's Medical Monitor or designee should be contacted immediately if questions arise concerning the grade of the reaction.

6.4.2 Premedications and Prophylaxis

For DUPILUMAB, the following suggested guidelines (which may be modified by the investigator according to the institutional guidelines for management of anaphylaxis reactions) are measures to be followed if a drug reaction is noted after s.c. administration to avoid potential reactions with future administration.

Treatment (guidelines to be followed):

- Acetaminophen 650 mg
- Diphenhydramine 50 mg or appropriate dose of equivalent H1 antagonist
- Ranitidine 300 mg or appropriate dose of equivalent H2 antagonist at the discretion of the investigator
- Hydrocortisone 20 to 40 mg (dose selected at the discretion of the Investigator)

Prior to next administration (guidelines to be followed):

- Acetaminophen 650 mg
- Diphenhydramine 50 mg or appropriate dose of equivalent H1 antagonist
- Ranitidine 300 mg or appropriate dose of equivalent H2 antagonist at the discretion of the investigator
- Hydrocortisone 20 to 40 mg (dose selected at the discretion of the Investigator)

6.4.3 Dose Management for Adverse Events considered related to DUPILUMAB

Temporary interruptions of DUPILUMAB may be required in the event of treatment-related toxicity. General guidelines for specific toxicity regarding dosing and treatment are provided below. All toxicities will be graded according to NCI CTCAE v4.03 (see **Section 7.1.2**).

6.4.3.1 Grade 1 or 2 AEs

Study drug administration may continue despite observation of drug-related low grade adverse events (CTCAE grade 1 or 2). If the investigator in his/her medical judgment considers that a low grade AE is of clinical significance or inordinately prolonged, the Investigator may, at his/her discretion, delay treatment to allow for resolution. Necessity to delay for more than two consecutive doses will require the patient to permanently discontinue study drug. No dose reductions are allowed.

6.4.3.2 Grade 3 or 4 AEs

Drug administration should be held upon observation of AEs of ≥ Grade 3 severity to enable patient management, monitoring of the resolution of the event, and assessment of the relatedness of the event. An AE of ≥ Grade 3 that is considered related to DUPILUMAB will result in discontinuation of therapy unless other specified in the protocol. For AEs associated with liver function test abnormalities, drug administration may resume upon resolution of the AE to ≤ Grade 1 severity unless more than seven days elapse before return to ≤ Grade 2 severity or fourteen days have elapsed from the last dose. In this case, the AE is considered dose limiting, two consecutive doses will have been missed, and study drug administration should be discontinued. Following a single missed dose, the treatment schedule should resume as if no delay had occurred and the patient receive all intended DUPILUMAB doses.

6.5 Concomitant Therapy

DUPILUMAB is the only cancer drug to be administered routinely in this study. No concomitant anti-cancer therapy will be given.

All concomitant medications and blood products administered during the patient's participation in the study until the post treatment follow-up visit must be recorded in the source document and on the electronic Case Report Form (eCRF). All changes in infusions, including interruptions and their duration as well as reductions in rate and duration must be recorded.

The following rules concerning concurrent treatment(s) will apply in this study:

Any other anti-neoplastic therapies including but not limited to chemotherapy or other small molecules, biologics, or radiotherapy are not allowed.

- Patients may not receive other investigational drugs during the period of study participation.
- Because DUPILUMAB has a mechanism of action dependent upon inhibiting macrophages, the use of corticosteroids should be limited to the extent possible. Chronic doses of corticosteroids in excess of 10 mg daily of prednisone or equivalent is prohibited other than for the management of drug-related adverse experiences. Steroids may be employed in the treatment of suspected DUPILUMAB-associated immune-inflammatory or autoimmune AEs in consultation with the Sponsor.
- The use of other immuno-suppressive agents is prohibited, unless they are being used to treat an adverse event.
- Use of granulocyte colony stimulating factor, granulocyte-macrophage colony stimulating factor or other growth factors is prohibited.

Patients may receive the following concurrent therapy:

- Antiemetics, antidiarrheals, anticholinergics, antispasmodics, antipyretics, antihistamines, analgesics, antibiotics and other antimicrobials, histamine receptor antagonists or proton pump inhibitors, and other medications intended to treat symptoms or signs of disease.
- Transfusions such as red blood cells and platelets are permitted to treat symptoms or signs of anemia or thrombocytopenia and should be documented on the concomitant medication form.

6.6 Restrictions

6.6.1 Prior Therapy

Prior therapy restrictions are described in the inclusion/exclusion criteria specified in Section 3.

6.6.2 Fluid and Food Intake

There are no requirements for fasting and no restrictions for fluid and food intake by the patients during the study.

6.6.3 Patient Activity Restrictions

There are no restrictions on patient activities and no requirement for patient confinement during the study.

6.7 Treatment Compliance

DUPILUMAB will be administered by healthcare professionals under the supervision of the Investigators. Records of DUPILUMAB dose calculation, administration, and dosing regimen will be accurately maintained by site staff. The monitor will review dose calculation, administration and regimen as well as medication accountability during investigational site visits and at the completion of the study.

6.8 Packaging and Labeling

DUPILUMAB will be supplied in bulk, open-label, single-use vials. All investigational product will be labelled with a minimum of the protocol number, directions for use,

storage conditions, expiry date (if applicable), batch number, the statements “For clinical trial use only,” and/or “CAUTION: New Drug – Limited by Federal (United States) Law to Investigational Use,” and the Sponsor's name and address. Please see the Pharmacy Manual for detailed information about the packaging of the study drug.

6.9 Storage and Accountability

The vials containing study drug should be stored at 2°– 8° C (36°– 46° F) and must not be frozen or shaken. Protect from sunlight. To ensure compliance with storage conditions, temperature logs will be maintained.

Because there is no preservative and drug loss occurs over time, administration of study drug should begin immediately after preparation but no later than 6 hours after preparation (see Pharmacy Manual). If there is a delay in administration of study drug such that it will not be administered on the day of preparation, the Clinical Project Manager should be notified immediately. Instructions on how to proceed will be provided.

The Investigator or his/her designee is required to maintain accurate drug accountability records. A binder containing instructions and the required accountability documentation will be provided to the Investigator or his designee. When the study is completed, copies of study drug accountability records must be sent to the Sponsor. The original drug accountability records must be maintained with the rest of the documentation in accordance with Section 9.3 of the protocol.

Additional details regarding storage, handling, and accountability can be found in the Pharmacy Manual.

6.10 Investigational Product Disposition at End of Study

Upon completion or termination of the study, all unopened vials of study medication must be returned to MacroGenics or its representative, unless the site has received written authorization from MacroGenics to destroy study drug at the site. All drug returns to MacroGenics or its representative must be accompanied by the appropriate documentation and be clearly identified by protocol number and study site number on the outermost shipping container. If MacroGenics approves the destruction of drug at the site, the Investigator must ensure arrangements are made for proper disposal and that appropriate records of disposal are documented and maintained and copies provided to the Sponsor.

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 DUPILUMAB is provided in this Protocol and in the Consent form. The study agent may 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 within 24 hours.

The lead study coordinator, Alice Jordan should be contacted when reporting an SAE or death at 410-955-1239 and ajorda30@jhmi.edu If this person cannot be reached within 24 hours, the PI should be contacted at 410-502-3137 or at kpienta1@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 study coordinator 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). 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 for as long as required to comply with national and international regulations (generally at least 3 years after study completion).

7.5.3 Research Involving).

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 Endpoint

8.2.1 Analysis of M2-TAM infiltration

The primary measure of treatment effect in this study will be achieved by quantification of M2-TAMS in prostate tumor specimens of treated patients. Tissue microarrays (TMAs) from formalin-fixed tumor samples will be analyzed for the degree of TAM infiltration using immunohistochemical staining for CD206 (28, 29). Analyses will be performed in the laboratory of Dr Angelo DeMarzo. 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

8.3 Secondary Endpoints

8.3.1 Rate of adverse events

All subjects receiving at least one dose of the study drug 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.

8.3.2 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 (30, 31), 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 8.3.3. This endpoint will be expressed as the mean staining percentage in tumor tissue. Assays will be performed in the laboratory of Dr DeMarzo.

8.3.3 $CD8^+$ T cell infiltration

To assess the immune response to DUPILUMAB, we will quantify the extent of $CD8^+$ T cell infiltration into the prostate from harvested prostate glands. This will be done using immunohistochemical staining methods. This endpoint will be expressed as the mean $CD8^+$ T cell staining percentage in harvested tumor

tissue. 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.

Analysis of this endpoint will be achieved by preparing tissue microarrays (TMAs) using the highest-grade/largest tumor per patient and sampling it with 100-fold redundancy (28-31). 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 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).

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).

8.3.4 Regulatory T cell (T_{reg}) infiltration

The method for quantifying T_{reg} density from harvested prostate tissue is as described in Section 8.3.3. This endpoint will be expressed as the mean staining percentage in tumor tissue.

8.3.5 $CD4^+$ T cell infiltration

The method for quantifying $CD4^+$ T cell density from harvested prostate tissue is as described in Section 8.3.3. This endpoint will be expressed as the mean staining percentage in tumor tissue.

8.3.6 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 endpoint will be expressed as the proportion of men achieving a pCR.

8.3.7 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 endpoint will be expressed as the proportion of men achieving a PSA response.

8.3.8 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 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), (Name) 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, (Name), 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 (Name) 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

Data and safety monitoring will follow Level Medium under the SKCCC Data and Safety Monitoring Plan. *The SKCCC Compliance Monitoring Program will provide external monitoring for JHU-affiliated sites in accordance with SKCCC DSMP (Version 6.0, 02/21/2019). The SMC Subcommittee will determine the level of patient safety risk and level/frequency of monitoring.* Additionally, scheduled meetings will take place monthly and will include the protocol principal investigator, research nurse, data manager, and, when appropriate, the collaborators, sub-investigators, and biostatistician involved with the conduct of the protocol.

During these meetings the investigators will discuss matters related to: safety of protocol participants, validity and integrity of the data, enrollment rate relative to expectation, characteristics of participants, retention of participants, adherence to protocol (potential or real protocol violations), data completeness, and progress of data for secondary objectives.

10. STATISTICAL CONSIDERATIONS

10.1 Study Design, Sample Size, and Study Endpoints

For the primary outcome: pre- and post-treatment M2-TAM infiltration will be compared using a paired t-test, or nonparametric signed rank test if data don't conform to a Gaussian distribution. Power is based on the paired t-test. With 20 patients, we have the power ($\geq$ 81%) to detect a decrease of $\geq$0.67 standard deviation unit (effect size = 0.67) (14). This is likely to be conservative as we observed a much larger difference in M2-TAM levels between primary and

metastatic tumors in our data. Power calculated using PASS v. 11 (NCSS Software, Kaysville, UT).

For the secondary analysis of safety, we will use a continuous toxicity monitoring approach with sequential dose-limiting toxicity boundaries (13). Patients will be evaluated for AEs after neoadjuvant dupilumab. We consider a 15% rate of Grade ≥3 AEs to be unacceptable and indication to halt the trial. Accrual will continue until the number of Grade ≥3 AEs equals or exceeds the boundary for a given number of patients, or the completion of 20 patients without crossing any toxicity boundaries. The table below shows the schedule of accrual and associated toxicity boundaries.

Number of patients:	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Toxicity Boundary:	3	3	4	4	4	4	5	5	5	6	6	6	6	7	7	7	7	7

The AE rate and 95% confidence interval will be calculated, either at trial halt or completion of accrual.

Secondary analyses of other immune infiltrate cells will be compared using a paired t-test, or nonparametric signed rank test if data don't conform to a Gaussian distribution. Other secondary analyses will include calculating the rate and 95% confidence interval (CI) for complete response rates, and undetectable post-treatment PSA, and paired t-tests for the secondary outcome biomarkers listed above. In exploratory analyses we will evaluate whether # of biopsy cores evaluated, patient, or clinical factors correlate with extent of decrease in infiltration, although the small sample size limits these analyses purely to hypothesis generating.

Power. For the primary outcome of pre- vs. post-treatment decrease in M2-TAM infiltration, power is based on the paired t-test. With 20 patients, we have the power (≥ 81%) to detect a decrease of ≥0.67 standard deviation unit (effect size = 0.67) (14). This is likely to be conservative as we observed a much larger difference in M2-TAM levels between primary and metastatic tumors in our data. Power calculated using PASS v. 11 (NCSS Software, Kaysville, UT). The continuous toxicity monitoring design with n=20 above has probability = 0.048 of early stopping if the Grade ≥3 AE rate is <15%. Toxicity boundaries calculated using software developed by Ivanova (13) (http://cancer.unc.edu/biostatistics/program/ivanova/ContinuosMonitoringForToxicity.aspx)

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 Dupilumab until the 60th postoperative day.

10.1.2: **Early stopping rule for feasibility:** We do not anticipate an increase in the surgical difficulty with the use of neoadjuvant DUPILUMAB. 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 DUPILUMAB would not pose a substantial increase in surgical difficulties. However, in addition to safety, feasibility will be monitored separately. 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 drug. 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 have surgical complications.
- Not feasible if two of the first 3 patients have complications.
- Not feasible if 2 out of the first 4 patients have complications.
- Not feasible if 3 out of the first 6 patients have complications.
- Not feasible if 4 out of the first 8 patients have complications.
- Not feasible if 5 out of the first 10 patients have complications.
- Not feasible if 6 out of the first 12 patients have complications.
- Not feasible if 7 out of the first 14 patients have complications.
- Not feasible if 8 out of 16 patients 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.

- **Apoptotic markers.** The primary efficacy objective of this study is to observe an anti-tumor effect consistent with the agent's proposed mechanism of action (MOA), antibody-dependent cellular cytotoxicity (ADCC). Tumor cell death will be quantified by TUNEL staining, Caspase 3 staining, and FcGamma staining and will be expressed as the mean staining percentage in tumor samples. Standard deviations, 95% confidence intervals, and ranges will also be reported where appropriate. Means will be compared pre-treatment (from pre-treatment biopsies) vs. post-treatment using two-way analysis of variance (ANOVA).
- **Proliferation markers.** Ki-67 staining will be expressed as the mean staining percentage in tumor samples. Standard deviations, 95% confidence intervals, and ranges will also be reported where appropriate. Means will be compared pre-treatment vs. post-treatment using two-way ANOVA.
- **$CD8^+$ T cell infiltration.** Mean $CD8^+$ T cell staining percentage in harvested prostate tissues will be reported. 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 outcome. The mean will be used to pool multiple spot measurements for each patient..

- **Regulatory T cell (T_{reg}) density**. The methods for analyzing T_{reg} infiltration will be similar to those described for $CD8^+$ T cell infiltration. Descriptive statistics and graphical summaries will be provided. In addition, the $CD8^+/T_{reg}$ ratio and the $CD4^+/T_{reg}$ ratio will be computed, and reported using descriptive statistics.
- **$CD4^+$ T cell density.** The methods for quantifying and analyzing $CD4^+$ T cell infiltration will be similar to those described for $CD8^+$ T cell infiltration. Descriptive statistics and graphical summaries will be provided.
- **Pathological complete responses (pCR)**. This will be defined as an absence of tumor identification on standard histological analysis of the resected prostate specimens.
- **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 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 secondary endpoints (e.g., CD206, apoptosis/proliferation marker analysis, tissue $CD8^+$ T cell 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.

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

For the primary outcome: pre- and post-treatment M2-TAM infiltration will be compared using a paired t-test, or nonparametric signed rank test if data don't conform to a Gaussian distribution. Power is based on the paired t-test. With 20 patients, we have the power ($\geq$ 81%) to detect a decrease of $\geq$0.67 standard deviation unit (effect size = 0.67) (14). This is likely to be conservative as we observed a much larger difference in M2-TAM levels between primary and metastatic tumors in our data. Power calculated using PASS v. 11 (NCSS Software, Kaysville, UT).

For the safety analysis, we will use a continuous toxicity monitoring approach with sequential dose-limiting toxicity boundaries (14). Patients will be evaluated for AEs after neoadjuvant dupilumab. We consider a 15% rate of Grade >3 AEs to be unacceptable and indication to halt the trial. Accrual will continue until the number of Grade >3 AEs equals or exceeds the boundary for a given number of patients, or the completion of 20 patients without crossing any toxicity

boundaries. The table below shows the schedule of accrual and associated toxicity boundaries.

Number of patients:	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Toxicity Boundary:	3	3	4	4	4	4	5	5	5	6	6	6	6	7	7	7	7	7

The AE rate and 95% confidence interval will be calculated, either at trial halt or completion of accrual.

10.5.3 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 and MacroGenics 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.

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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

Protocol Title:	Neoadjuvant Anti-B7-H3 (DUPILUMAB) in Men with Localized High-Risk Prostate Cancer		
Protocol Number:	**Principal Investigator:**	**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 DUPILUMAB Dose:**	**Action taken regarding the study drugs:** ☐ **None** ☐ **Interrupted** ☐ **Discontinued**	
Event Onset Date:		**Event End Date:**	

Relationship to:	**DUPILUMAB**	**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

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
ºC	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
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
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
MTD	maximum tolerated dose
NA	not applicable
N/A	not available
NCI	National Cancer Institute
OR	operating room

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)
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)