**NCI Protocol #:** CITN12-03

**Local Protocol #:** CITN12-03

**TITLE: A Phase 2 Study of recombinant glycosylated human interleukin-7 (CYT107) after completion of standard FDA approved therapy with sipuleucel-T (Provenge®) for patients with asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer (mCRPC)**

| | |
|---|---|
| **Coordinating Center:** | Cancer Immunotherapy Trials Network, Fred Hutchinson Cancer Research Center |
| **Principal Investigator:** | Lawrence Fong, M.D.<br>University of California San Francisco<br>Dept. of Medicine (Hematology/Oncology)<br>513 Parnassus Ave., Room S775, Box 0511<br>San Francisco, CA 94143-0511<br>(415) 514-3160<br>(415) 476-0459<br>lfong@medicine.ucsf.edu |
| **Participating Organizations:** | CITN – Cancer Immunotherapy Trials Network |

**Statistician:**
Bob Salim, PhD
Axio Research, LLC
2601, 4th Avenue, Suite 200
Seattle, WA 98121
(206) 577-0219
bobs@axioresearch.com

**Study Coordinator:** N/A

**Responsible Research Nurse:** N/A

**Responsible Data Manager:** N/A

**NCI Supplied Agent**: N/A

**Other Agents**: Recombinant Glycosylated Human Interleukin-7 (CYT107) NSC #767713

**IND Sponsor**: Martin A. "Mac" Cheever, M.D., IND# 124199

**Protocol Type / Version # / Version Date:** Amendment #7/Version 1.0/July 26, 2017

| **To submit site registration documents:** | **For patient enrollments:** | **Submit study data to:** |
|---|---|---|
| CTSU Regulatory Office<br>1818 Market Street,<br>Suite 1100<br>Philadelphia, PA 19103<br>Phone – 1-866-651-CTSU<br>Fax – 215-569-0206 | Please refer to the patient enrollment section for instructions on using the OPEN system or you may access the OPEN system at:<br>https://www.ctsu.org/OPEN_SYSTEM/<br>Or https://OPEN.ctsu.org<br><br>For problems using OPEN<br>https://www.ctsu.org/CTSUCOntact.aspx | Data collection for this study will be done via Medidata Rave (FHCRC/AXIO Research, Inc.) Please refer to the data submission section of the protocol for Medidata Rave instructions. |
| The current version of the **study protocol and all supporting documents** must be downloaded from the protocol-specific Web page of the CTSU Member Web site located at https://www.ctsu.org. Access to the CTSU members' website is managed through the Cancer Therapy and Evaluation Program - Identity and Access Management (CTEP-IAM) registration system and requires user log on with CTEP-IAM username and password. Permission to view and download this protocol and its supporting documents is restricted and is based on person and site roster assignment housed in the CTSU RSS. | | |
| **For patient eligibility or treatment-related questions:**<br>Contact the CITN Central Operations and Statistical Center at:<br>206-667-6609 or email mailto: citn@fhcrc.org | | |
| **For questions unrelated to patient eligibility, treatment, or data submission** contact the CTSU Help Desk by phone or e-mail:<br>CTSU General Information Line – 1-888-823-5923, or ctsucontact@westat.com. All calls and correspondence will be triaged to the appropriate CTSU representative. | | |
| **The CTSU Web site is located at** https://www.ctsu.org | | |

***Treatment Schema***

| Cohort | CYT107 Dose | Frequency |
|---|---|---|
| 1 - Observation | No CYT107 dose | -------- |
| 2 - CYT107 | 10 μg/kg | Days 0, 7, 14 and 21 |

**TABLE OF CONTENTS**

# 1. OBJECTIVES

The goal of the trial is to determine whether treatment with recombinant glycosylated human interleukin-7 (CYT107, RevImmune, Inc.) after standard therapy with sipuleucel-T (PROVENGE®, Dendreon Corporation) can substantially increase and prolong immune responses to the sipuleucel-T fusion protein vaccine construct PAP-GM-CSF (PA2024) and the prostatic acid phosphatase (PAP) component and thereby provide a novel, nontoxic regimen to prolong the survival of patients with metastatic castration-resistant prostate cancer (mCRPC) with no or minimal symptoms.

## 1.1 Primary Objectives

To determine whether CYT107 administration increases the vaccine-induced antigen-specific T-cell immune response to the sipuleucel-T fusion protein vaccine construct PAP-GM-CSF (PA2024)

## 1.2 Secondary Objectives

1. To determine whether CYT107 administration increases the vaccine-induced antigen-specific T-cell immune response to PAP.

2. To assess the character of the T-cell immune response to PAP and PA2024

3. To determine whether CYT107 administration increases the vaccine-induced antigen-specific antibody immune responses to PAP and PA2024

4. To quantify the effects of CYT107 on T-cell repertoire diversity

5. To assess the effects of CYT107 on the immune competence of patients with advanced prostate cancer

6. To assess the clinical efficacy and tolerability of sipuleucel-T plus CYT107 compared with sipuleucel-T alone

# 2. BACKGROUND

## 2.1 Study Disease

The proposed clinical trial is a phase II, open-label, multicenter, randomized study of the administration of CYT107 after the completion of standard FDA-approved therapy with sipuleucel-T for patients with asymptomatic or minimally symptomatic mCRPC.

Prostate cancer is the second leading cause of death from cancer in men [Cheever, 2011]. Localized prostate cancer may be cured with surgery or radiation therapy, but the disease recurs in approximately 20 to 30% of patients. Androgen-deprivation therapy, the most common treatment after recurrence, is effective, but the disease eventually progresses in most patients who receive such treatment [FDA.gov, 2010], termed castration-resistant prostate

cancer (mCRPC). For men with mCRPC, the median survival ranges from 12.2 to 21.7 months [Kantoff, 2010]. A number of drugs for mCRPC have been approved. Previous therapies like prednisone and mitoxantrone showed no additive impact on median overall survival beyond the expected survival of 10-14 months seen in mCRPC. Current therapeutics such as docetaxel [Tannock, 2004], abiraterone [Ryan, 2013], and enzalutamide [Beer, 2014] increase overall survival by 2 to 8 months. Unfortunately, all patients eventually progress after receiving these therapies. Therefore, there is a critical need for improved therapies for mCRPC that can treat metastatic disease, improve quality of life, decrease morbidity, and prolong overall patient survival.

Sipuleucel-T (PROVENGE®; Dendreon) is the first therapeutic cancer vaccine to be approved by the U.S. Food and Drug Administration. In men who have metastatic castration-resistant prostate cancer with no or minimal symptoms, sipuleucel-T prolongs median survival by 4.1 months compared with results in those treated with placebo [Kantoff, 2010].

IL-7 is a homeostatic growth factor for T cells and is capable of inducing proliferation, maintaining T-cell responsiveness, and preventing and reversing T-cell anergy [Mackall, 2011]. The hypothesis of the trial is that CYT107 will increase and prolong the sipuleucel-T induced immune response and thereby further prolong survival.

## 2.2 CTEP IND Agent: N/A

## 2.3 Other Agents

**Sipuleucel-T (PROVENGE®, Dendreon Corporation):**

Sipuleucel-T is not a direct component of this protocol. However, successful completion of standard sipuleucel-T therapy within 3-7 days is a mandatory entry criterion for the trial.

Sipuleucel-T is a therapeutic vaccine formulated to stimulate an immune response to prostate cancer cells [Cheever, 2011] by generating PAP-specific T cells capable of recognizing and killing prostate cancer cells that express PAP. In sipuleucel-T, the PAP vaccine immunogen is formulated as PA2024, a fusion protein combining recombinant PAP with recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF) [FDA.gov, 2010], [Kantoff, 2010], [Cheever, 2011].

Sipuleucel-T is constructed as an autologous cellular immunotherapy product consisting of peripheral blood mononuclear cells (PBMC), including antigen presenting cells (APC). To manufacture sipuleucel-T, a subject undergoes leukapheresis to collect PBMC. Mononuclear cells, including quiescent APC, are cultured ex vivo (serum and cytokine free conditions) at 37ºC in the presence of PAP-GM- CSF (PA2024) antigen. GM-CSF is used to activate APCs within the autologous PBMC product [FDA.gov 2010]. These activated APC are proficient at activating and inducing the replication of PAP-specific T cells. Three days after leukapheresis and 40 hours of subsequent incubation, the cells are washed to remove the fusion protein and suspended in Lactated Ringer's Injection, USP, shipped to the patient care site, and infused as the sipuleucel-T product into the patient. The standard FDA approved therapy is three doses. After infusing the first dose, increases in APC and T-cell activation

markers in PBMCs are detectable in treated patients as increased ex-vivo production of cytokines associated with T-cell activation. Thus, with each dose, increasing levels of activated APCs and T cells are infused into patients [Sheikh, 2010].

**Prior Sipuleucel-T Trials**: The phase III randomized IMPACT trial led to FDA approval because of improvements in overall survival (OS) for patients with mCRPC [Kantoff, 2010]. IMPACT was a double-blind, placebo-controlled study involving 512 patients with asymptomatic or minimally symptomatic mCRPC randomly assigned 2:1 to receive sipuleucel-T (n = 341) or placebo (n = 171). Sipuleucel-T was administered every 2 weeks for a total of 3 doses. Of the 330 patients who received sipuleucel-T, 92% received all 3 infusions. Median OS was 25.8 months in the treatment arm and 21.7 months in the control arm ($P = 0.032$). The adjusted hazard ratio for death was 0.78 (95% confidence interval, 0.61–0.98). The Kaplan-Meier estimate for OS for each group at 36 months was 31.7% for sipuleucel-T vs. 23.0% for placebo, indicating a 38% increase in survival at 3 years for patients in the sipuleucel-T arm [Kantoff, 2010]. The median OS predicted by the Halabi prognostic model was 20.3 months for the treatment arm vs. 21.2 months for the control arm [FDA.gov, 2010], supporting the efficacy of sipuleucel-T.

Vaccine-induced therapeutic responses sometimes appear to continue to function after the median time to progression. In the IMPACT trial, PSA reductions of at least 50% compared with baseline were confirmed at 4 weeks in 2.6% of treated patients vs. 1.3% of control patients [Kantoff, 2010]. However, in another double-blind, randomized, placebo-controlled study (P-11) of 176 men with rising PSA after prostatectomy receiving hormonal therapy followed by vaccine or control [Beer, 2007], sipuleucel-T did not significantly delay cancer progression (defined as PSA reaching 3.0 ng/mL); however, the rate of the PSA increase was slower in patients who received the vaccine.

The FDA-approved regimen is three vaccinations administered on days 0, 14, and 28. The initial IMPACT trial publication reported that at week 6 after the first vaccination (2 weeks after the third vaccination), T-cell proliferation responses (stimulation index >5) to PA2024 were observed in 73.0% of treated patients and 12.1% of control patients. In addition T-cell proliferation responses to PAP (stimulation index >5) were observed in 27.3% of treated patients compared with 8.0% of control patients [Kantoff, 2010]. Of note, the PA2024 fusion protein contains a joining-region segment that is not expressed by normal PAP or GM-CSF, which patient immune systems recognized as foreign. Eliciting an immune response to this foreign joining-region segment signals immune competence and is likely to facilitate epitope spreading to the PAP portion of the molecule. All patients with a T cell response to PAP also had a T cell response to PA2024.

In a prespecified analyses, patients in the sipuleucel-T group who had an antibody titer of more than 400 against PA2024 or PAP at any time after baseline lived longer than did those who had an antibody titer of 400 or less ($P < 0.001$ and $P = 0.08$, respectively, by log-rank test). PA2024-specific antibody responses that exceeded the designated low value of 400 at any time after baseline were observed in 66.2% of treated patients and only 2.9% of control patients. By contrast, titers of antibodies against PAP that exceeded 400 at any time after baseline were observed in 28.5% of treated patients compared with 1.4% of control patients. Although the IgG antibody responses correlated with survival and cognate recognition of antigen by CD4+ T cells is required for such antibody responses, the initial publication

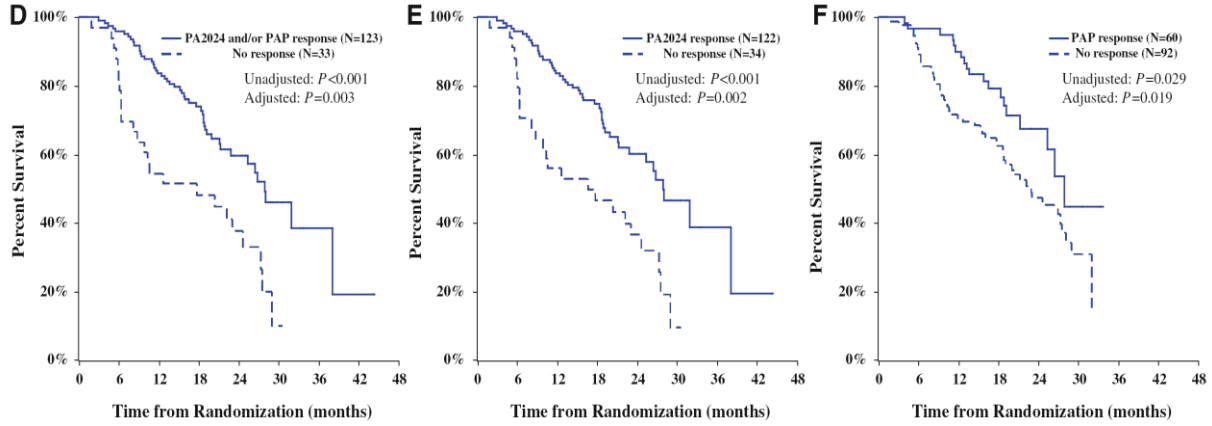
[Kantoff, 2010] reported that no survival difference could be detected between patients in the sipuleucel-T group who had T-cell proliferation responses to PA2024 or PAP at week 6 and those who did not [Kantoff, 2010]. The presumption is that the responses were present, but below the level of detection by the assay employed.

A subsequent analysis of immune function (Figure 1) [Sheikh, 2012] provided additional credence to the correlation between immune response and overall survival (OS). Antigen-specific peripheral cellular and humoral responses were assessed in a 237 patient subset of subjects enrolled in the IMPACT study. The IMPACT subset provided blood for immune response determination at baseline (week 0) as well as 6, 14, and 26 weeks following the first infusion. Antigen-specific immune responses were observed in 78.8% of monitored subjects and correlated with OS (p=0.003).

Overall survival correlated significantly with the development of at least one post-baseline peripheral immune response to PA2024 or PAP (HR=0.47 [95% CI: 0.29, 0.78] P=0.003; Figure 1D), to PA2024 (HR=0.46 [95% CI: 0.28, 0.76] P=0.002; Figure 1E), or to PAP (HR=0.53 [95% CI: 0.31, 0.90] P=0.019; Figure 1F). The strongest correlation between survival and the development of a post-baseline immune response to PA2024 at any timepoint was observed with antibody responses (HR=0.42 [95% CI: 0.26, 0.67] P<0.001), while the correlation with memory IFNγ ELISPOT at any time point approached statistical significance (HR=0.55 [95% CI: 0.28, 1.08] P=0.08); T cell proliferation response did not significantly correlate with overall survival.

Overall Survival is depicted in Figure 1 from Sheikh et al [Sheikh, 2012]. Data is presented as Kaplan-Meier survival plots for antigen-specific immune response for sipuleucel-T responders and non-responders for a subset of IMPACT subjects.

**Figure 1 [Sheikh, 2012]**




| Immune Response Status Post-Baseline By Antigen | Unadjusted *P* Value | *P* Value Adjusted for PSA and LDH |
|---|---|---|
| **PA2024 or PAP** | <0.001 | 0.003 |
| **PA2024** | <0.001 | 0.002 |
| **PAP** | 0.029 | 0.019 |

Figure 1. The hazard ratio and p-value are based on a Cox regression analysis with response

(0 = non responder, 1 = responder) in the model. Median-based analysis of cumulative product parameters from a Cox regression model, stratified by study, and adjusted for baseline PSA and LDH. P-values in the table are from analyses of each parameter as a continuous measure. Hazard ratios and 95% confidence intervals from a Cox regression model, adjusted for baseline PSA and LDH.
**D)** Response to PA2024 or PAP in at least one of the three immune response assays (HR=0.47 [95% CI: 0.29, 0.78] P=0.003)
**E)** Response to PA2024 in at least one of the three immune response assays (HR=0.46 [95% CI: 0.28, 0.76] P=0.002)
**F)** Response to PAP in at least one of the three immune response assays (HR=0.53 [95% CI: 0.31, 0.90] P=0.02).

The peripheral immune response parameters examined were log-transformed SI medians, ranked IFNγ ELISPOT medians, and ranked ELISA titers. Responder status was analyzed using a Cox regression model, with and without adjustment for baseline PSA and LDH [Sheikh, 2012]. All p-values reported are two-tailed. No adjustment for multiplicity of endpoints or time points was made. These correlations remained after adjusting for baseline prognostic factors (PSA and LDH) that are independently correlated with survival.

Positive thresholds for treatment-related immune responses were selected in order to ensure <5% of subjects would exceed the value at baseline. Thresholds were proliferation: SI > 12 for PA2024, > 8 for PAP; IFNγ ELISPOT (per $3x10^5$ PBMC): >10 spots for PA2024, >40 spots for PAP; ELISA titer: >400 for both anti-PA2024 and anti-PAP antibodies. Serum samples that gave a positive response to an initial ELISA were subsequently evaluated for IgM and IgG antibody isotypes.

Figure 1 presents the impact of any positive immune response parameter to OS survival. Below are the hazard ratios and associated 95% confidence intervals, as well as the p-values, for comparison of the survival curves in the IMPACT study for responders versus non-responders for the different immune response assays.

For the primary endpoint of the current trial, IFN ELISpot to PA2024, the hazard ratio estimate of 0.53 (0.27, 1.04) for responders to IFN ELISpot to PA2024 compared to non-responders.

**Table 1** (Data from Dendreon)

| Antigen | Assay | HR (95% CI) | p-value |
|---|---|---|---|
| PA2024 | Humoral | 0.45 (0.28, 0.71) | p=0.0007 |
| PA2024 | Proliferation | 0.87 (0.46, 1.67) | p=0.169 |
| PA2024 | IFN ELISpot | 0.53 (0.27, 1.04) | p=0.065 |
| | | | |
| PAP | Humoral | 0.60 (0.33, 1.08) | p=0.087 |
| PAP | Proliferation | 0.66 (0.27, 1.61) | p=0.364 |
| PAP | IFN ELISpot | 0.29 (0.04, 2.15) | p=0.227 |
| The hazard ratio and p-value are based on a Cox regression analysis with response (0 = non responder, 1 = responder) in the model. Similar results are obtained with models adjusting for baseline PSA and LDH. | | | |

Data from a neoadjuvant trial demonstrate that sipuleucel-T can modulate the presence of lymphocytes at the prostate tumor site giving further credence to the influence of the anti-tumor immune response to the effect of sipuleucel-T on OS. In the neoadjuvant trial [NeoACT (P07-1; NCT00715104)] [Fong, 2012] sipuleucel-T treatment has been shown to be associated with an increased frequency of T cells in prostate cancer tissue at the interface of the benign and malignant glands. Patients received the standard sipuleucel-T regimen of 3 infusions of sipuleucel-T at approximately 2-week intervals. At 6-7 weeks after start, patients underwent radical prostatectomy. By IHC there was a significant observed increase in CD3+ and CD4+ T cell populations (≥3 fold) at the tumor interface (where benign and malignant glands interface), compared with the pre-treatment biopsy, benign radical prostatectomy tissue, and tumor tissue (ANOVA post hoc Newman-Keuls test: $p<0.0001$ for each comparison). FoxP3+ CD4+ T cells were also increased ($p=0.0005$) at the tumor interface, but represented a small fraction of the observed CD4+ T cells.

**Recombinant Glycosylated Human Interleukin -7) (CYT107) NSC #767713, RevImmune, Inc.:**

IL-7 is a homeostatic growth factor for T cells and is capable of inducing proliferation, maintaining T-cell responsiveness, and preventing and reversing T-cell anergy [Mackall, 2011]. IL-7 signals through the IL-7 receptor (IL-7R) heterodimer composed of the common γ-chain (CD132) cytokine receptor and a unique IL-7Rα (CD127). During normal conditions, IL-7R expression is maintained on resting T cells [Mackall, 2011] and IL-7 is continuously available in secondary lymphoid organs as a result of stromal cell IL-7 production [Takada, 2009]. In general, IL-7 has more potent effects on conventional $CD8^+$ T cells and CD4+ cells than it does on $CD4^+$ regulatory T cells (Tregs). IL-7Rα expression is notably low on $FOXP3^+$ Treg cells compared with non-regulatory T-cell subsets [Seddiki, 2006], [Liu, 2006]. Its continuous signaling induces antiapoptotic and costimulatory responses that are essential for the survival of naïve T cells. After either T-cell activation, IL-7 signaling, or both, IL-7Rα is down-regulated on terminally differentiated, senescent T cells [Fry, 2003], [Park, 2004] but becomes re-expressed after several days and remains expressed during the later contraction phase. The effects of IL-7 are most potent on recent thymic emigrants in which IL-7–mediated signaling can induce proliferation in the absence of T-cell receptor signaling [Swainson, 2007]. IL-7Rα is also selectively expressed on a small minority of effector T cells that are destined to enter the central memory T-cell pool, thus implicating IL-7 as a modulator of the effector to memory cell transition [Kaech, 2003].

**Prior Trials**: Preclinical and clinical results support the rationale for developing trials testing IL-7 administration after sipuleucel-T therapy to increase or prolong the sipuleucel-T–induced T-cell response to PAP. In animal models and in humans, IL-7 has been shown to increase by several-fold the number of peripheral blood T cells with little toxicity and to increase T-cell receptor diversity (i.e., naïve T cells and conventional $CD4^+$ and $CD8^+$ T cells), with the expansion of conventional T cells at a greater proportion than the expansion of Tregs [Levy, 2012]. In animal models, IL-7 administration augments vaccine-induced cell responses (e.g., $CD8^+$ memory T-cell responses) and immune-mediated control of both tumors and viruses. In addition, IL-7 can maintain effector T-cell function and prevent T-cell exhaustion. Consistent with the preclinical work, human trials of IL-7 in chronic viral infections induce increased T-cell number and demonstrate beneficial effects. IL-7 has been

well tolerated in early phase clinical trials and can be administered on an outpatient basis. Furthermore, other animal studies show that IL-7 administration can augment immune reconstitution after lympholytic stem cell transplantation and chemotherapy [Mackall, 2001], [Morrissey, 1991], [Perales, 2011]. Important for the current protocol, IL-7 administration can augment vaccine-induced antigen-specific T-cell responses [Colombetti, 2009], [Melchionda, 2005]. In mice, transient administration of IL-7 around the time of vaccination or initial viral infection increases the number of antigen-specific effector and memory T-cell populations and has long-lasting effects on the memory T-cell pool [Colombetti, 2009], [Nanjappa, 2008], [Pellegrini, 2009], [Melchionda, 2005]. Therefore, administration of IL-7 after a vaccine such as sipuleucel-T may have enhanced efficacy and substantial improvements in vaccine-induced responses.

The effectiveness of IL-7 as a vaccine adjuvant depends on the timing of administration. Durable effects on the memory T-cell pool have been observed only when IL-7 was administered during the contraction phase of the $CD8^+$ T-cell response [Nanjappa, 2008]. IL-7 expanded the magnitude of the secondary $CD8^+$ response when administered during the contraction phase of the primary or the secondary response, which is relevant to this proposal.

Two IL-7 formulations have been successively developed by Cytheris. The technology for the second formulation, CYT107, as well as vialed material has been taken over by RevImmune, Inc. and is the agent used in this current protocol. Both had the same primary sequence similar to the sequence of the native protein. The IL-7 for this proposal (CYT107) is produced from recombined Chinese hamster ovary (CHO) mammalian cells and is highly glycosylated. Seven phase I/IIa dose escalation studies and two phase II studies have been initiated with CYT107 for the treatment of cancer and chronic viral infections (e.g., HIV, hepatitis B, hepatitis C) and for use after hematopoietic stem cell transplantation [Mackall, 2011]. In those studies, CYT107 has been administered subcutaneously in a dose range of 3–60 μg/kg with a schedule regimen of one weekly subcutaneous administration for 3 or 4 weeks (corresponding to one cycle). In general, CYT107 has been well tolerated as an outpatient regimen with mild to moderate constitutional symptoms and reversible spleen and lymph node enlargement. In addition, marked dose-dependent increases in peripheral $CD3^+$, $CD4^+$, and $CD8^+$ lymphocytes were seen, resulting in a rejuvenated circulating T-cell profile, resembling that seen earlier in life [Sportes, 2008]. The most common side effects have been low-grade fever, malaise, transient increases in liver enzyme levels, erythema and induration at the site of administration, and rash or allergic reaction with repeated cycles in patients with HIV. In marked contrast to IL-2 and IL-15, no significant capillary leak or acute toxicity have been observed following IL-7 therapy.

The trial regimen administers CYT107 subcutaneously once a week on days 0, 7, 14 and 21 and is expected to maintain increased T-cell number throughout this trial to at least week 12, although a sustained effect up to 1 year has been observed in an HIV trial (CLI-107-06) [Levy, 2012]. Although the measured half-life of CYT107 is 10–15 hours, the biologic effects persist well after circulating levels of IL-7 return to baseline. The biologic half-life is much longer possibly due to a depot effect from IL-7 binding to extracellular matrix and the slow release at lower than detectable levels [Mackall, 2011]. In a study of single-dose rhIL-7 (CYT 99 007) administered to patients with HIV infection, CYT 99 007 induced early

lymphopenia, followed by a significant increase in the rate of T-cell cycling on day 4 with increases in circulating $CD4^+$ and $CD8^+$ T-cell counts persisting for at least 14 days [Sereti, 2009]. The administration of CYT107 is also likely to transiently increase the size of the spleen and lymph nodes as well as peripheral blood T cells.

Specificities of IL-7/IL-7Rα pathway regulation and current data on CYT107 in humans favor injection once a week and short therapeutic cycles. According to monkey and human data, a 1-week interval between each injection of CYT107 should lead to full recovery of IL-7Rα expression (CD127) at the surface of T cells that responded to a prior dose of IL-7. Furthermore, a sustained response of T cells to IL-7 should be fully restored, as measured 1 week later by Ki-67 expression. Short cycles, limited to 3 doses, should avoid exhaustion of T-cell responsiveness to high doses of IL-7, which could occur despite the recovery of CD127 expression.

T-cell proliferation rates in response to IL-7 are correlated with IL-7Rα expression. At present, the kinetics of IL-7Rα after other cancer vaccines in general and sipuleucel-T specifically is not known, but critical. T-cell activation and IL-7 itself can down-regulate IL-7Rα expression. IL-7Rα expression is expected to be re-expressed at about the time of CYT107 administration and persist during the period of function of the administered IL-7. The once-weekly regimen beginning after sipuleucel-T is expected to increase proliferation and survival of PAP-specific T cells, possibly for an extended period, and increase the number of PAP-specific memory cells without equivalent expansion of Tregs to dampen the antitumor effect.

CYT107 will be instituted 3 to 7 days after the third and final administration of sipuleucel-T. IL-7 function requires upregulated IL-7 receptors and upregulation will occur after completion of PAP vaccination and during the contraction phase. Because sipuleucel-T is manufactured from leukapheresed patient PBMC, provisions of CYT107 before leukapheresis would change the character of cells placed into culture and thus change the manufacturing scheme and would be disallowed by the FDA. Many insurance programs, including Medicare, will not pay for unapproved agents or regimens in clinical trials. Sipuleucel-T would be considered an experimental agent by some insurance programs if administered concurrent with CYT107. Therefore, testing of CYT107 will begin only after completing standard therapy with sipuleucel-T.

## 2.4 Rationale

Sipuleucel-T is the first FDA-approved cancer vaccine, designed to elicit therapeutic immune responses to PA2024 and subsequently PAP. Sipuleucel-T prolongs patient survival by 4.1 months on average. IL-7 is a homeostatic growth factor for T cells. IL-7 induces substantial proliferation of antigen-specific T cells that express a unique IL-7 receptor, IL-7Rα with very little toxicity. IL-7 can maintain T-cell responsiveness and prevent and reverse T-cell anergy. IL-7 is also thymopoïetic and can expand recent thymic immigrants and thus the T-cell repertoire, which is known to be low in individuals the age of most patients with advanced prostate cancer.

We expect that CYT107 will increase and prolong T-cell and antibody immune responses to PA2024 and PAP and provide a novel, nontoxic regimen to prolong survival. We also expect that CYT107 will expand T-cell repertoire and reset the homeostatic total T-cell level potentially mitigating the immune compromise observed in patients with mCRPC because of age and prior treatment.

Because clinical development of sipuleucel-T for FDA approval began in 1997, the formulation and regimen of sipuleucel-T have remained unchanged for almost 15 years. Given the advances made in immunology and immunotherapy in the last decade and a half, it is axiomatic that regimens based on current immunologic science would provide higher and longer lasting immune responses (i.e., greater areas under the curve) and, in the case of sipuleucel-T, enhanced patient benefit. The question remains "Which strategies are most likely to be effective and the most ripe for testing?"

In answer to this question, a strong rationale supports therapy with IL-7. IL-7 is a homeostatic T-cell growth factor responsible for the expansion of T cells during lymphopenia [Mackall, 2011]. In human clinical trials, IL-7 induces dramatic expansions in peripheral T-cell numbers and in total body $CD4^+$ and $CD8^+$ T cells without substantial toxicity. Yet, if an immunotherapy is to enhance the effects of an approved regimen, the agent must address many of the reasons an immunotherapy fails. Such reasons include (1) insufficient T-cell number, (2) insufficient $CD8^+$ or $CD4^+$ T-cell subsets, (3) a preponderance of regulatory T cells (Treg), (4) short-lived T cells unable to provide long-term tumor control, (5) a narrow repertoire of response (sometimes monoclonal), (6) little or no physical access of T cells to the tumor, and (7) strong local immunosuppressive effects of TGFß, one of the most potent tumor immunosuppressors.

IL-7 can mitigate many of these problems. Regarding insufficient numbers and types of T cells, IL-7 administration increases the number of $CD4^+$ and $CD8^+$ T cells, an effect that is both potent and sustained [Mackall, 2011], [Fry, 2003], [Levy, 2009], [Levy, 2012]. This boost of $CD4^+$ T cells provides efficient help to $CD8^+$ T cells and warrants their functionality. IL-7 favors the homing of T cells to lymph nodes, gut, mucosa, and tumors [Kaech, 2003], [Beq, 2009], [Sereti, 2012] and thus potentially the ability of T cells to infiltrate tumors. With IL-7, the expansion of Treg is proportionally less than conventional $CD8^+$ and $CD4^+$ T cells and the produced T cells are long-lived because of various antiapoptotic effects and the boosting of central memory T cells [Kaech, 2003], [Seddiki, 2006], [Liu, 2006]. In addition, IL-7 broadens the repertoire of T cells because IL-7 supports thymopoïetic activity that produces naïve T cells [Colombetti, 2009], [Nanjappa, 2008], [Levy 2012]. Finally, IL-7 antagonizes the production and signaling of TGFß [Pellegrini, 2009].

## 2.5 Correlative Studies Background

The primary aim is to determine whether CYT107 administration increases the vaccine-induced antigen-specific T-cell immune response to PA2024, as immune response to PA2024 best correlates with survival. The level and character of T-cell immune response to PA2024 as well as PAP will be assessed at day 0 (week 1) [before CYT107 administration], at day 35 (week 6) [two weeks after last CYT107 to assess the presumed peak immune response], at

day 70 (week 11) [to assess persistence of immune response and to compare to Dendreon data] and, at day 154 (week 23),and day 365 (week 53) [to assess persistence of immune response]. Both T cell responses and antibody responses will be assessed. The primary endpoint will be quantification of T-cell responses to PA2024 measured at day 70 (week 11), 7 weeks after the last CYT107 injection. T cell responses will be measured by interferon-gamma ELISPOT as the most quantifiable and reproducible assay. In the IMPACT study, 2 weeks after last sipuleucel-T administration, the mean PA2024 ELISPOT was 40 with a standard deviation of 69 based on n = 63. At 10 weeks after the last administration the mean PA2024 ELISPOT was 23 with a standard deviation of 36 based on n = 42. This data is based on Dendreon performing the ELISPOT assay on PBMC. This assay has been performed in the UCSF neo-adjuvant program study and was shown to be robust. Dendreon is supplying reagents to the UW Central lab (Immune Monitoring Lab) for the development of assays needed to test for response to specific proteins. The UW Central Lab is validating and establishing standards for the assay.

Longitudinal responses to PA2024 and PAP will be considered in the primary analysis; however power calculations are based on estimations of the magnitude of T cell responses at week 11. To meet the primary aim and determine whether CYT107 administration increases the vaccine-induced antigen-specific T-cell immune response to PA2024, the level and character of T-cell immune response to PA2024 will be determined at day 0 (week 1), day 35 (week 6), day 70 (week 11), day 154 (week 23), and day 365 (week 53) evaluating T cell responses. T-cell responses to PA2024 will be measured by interferon-gamma ELISPOT and T-cell proliferative responses to PAP as a confirmatory assay. The effect on antigen-specific T-cell immune responses to PAP will be measured using the same assays and time points. The immune response to other ongoing and nascent antitumor responses (e.g., PRAME, NY-ESO-1 and p53), additional tumor antigens specific to prostate cancer (e.g., PSA and PSMA), memory viral responses (influenza A), and chronic viral responses (cytomegalovirus) will also be assessed. Assays will be performed in the Cancer Immunotherapy Trials Network (CITN) Central Laboratory, IML (Immunologic Monitoring Laboratory), of the Tumor Vaccine Group led by Nora Disis, MD.

A number of other (secondary) correlative studies will be conducted to evaluate the effects of IL-7 on T and B cell immunity. Certain blood and serum for secondary correlative studies will be collected and stored for future analysis pending funding availability.

The effect on T lymphocyte number and phenotype (i.e., CD4+ and $CD8^+$ subsets of naïve, effector memory, central memory cells, $CD4^+$ Treg and activated T cells) will be assessed. The frequency and percentage of these and other PBMC subsets (i.e., T cells, NK cells, NKT cells, B cells, monocytes and dendritic cells) will be determined using multiparameter flow cytometric analysis on whole blood.

IgM and IgG responses to PA2024 and PAP will be measured by standard ELISA.

The T-cell repertoire will be characterized by T-cell receptor (TCR) deep sequencing and the analysis will be done with the Immunoseq Analyzer software (Harlan Robins, PhD, with Adaptive Biotechnologies, Seattle).

Thymic function will be assessed by measuring levels of T-cell receptor rearrangement DNA excision circles (TRECs).

Adverse events will be graded and reported using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. Therapeutic response will be assessed by consensus PSA response criteria for PSA blood tests, immune-related response criteria for computed tomography and bone scan (only as clinically indicated), circulating tumor cells, and patient survival.

## 3. PATIENT SELECTION

### 3.1 Eligibility Criteria

3.1.1 Asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer (mCRPC)

3.1.2 Patients must have successfully completed therapy with sipuleucel-T within 3-7 days of planned CYT107 study drug treatment

3.1.3 Assessable disease with a positive bone scan and/or measurable disease on CT scan and/or MRI of the abdomen and pelvis

3.1.4 Prior orchiectomy or must be on ongoing LHRH agonist or antagonist (e.g., degarelix) therapy

3.1.5 No ongoing anti-androgen therapy

- Patients must be off anti-androgen therapy for at least 30 days

3.1.6 Patients receiving any other hormonal therapy, including any dose of megestrol acetate (Megace), Proscar (finasteride), any herbal product known to decrease PSA levels (e.g. Saw Palmetto, PC-SPES), or any systemic corticosteroid, must discontinue the agent for at least 30 days prior to study treatment

3.1.7 Laboratory requirements (assays within 7 days of first CYT107 injection)

- Absolute neutrophil count (ANC) $\geq$ 1500/µL
- Bilirubin < 1.5 x ULN
- Hemoglobin $\geq$ 10 g/dL
- Platelets $\geq$ 100,000/mcL
- AST and ALT $\leq$ 2.5 x ULN
- Creatinine clearance $\geq$ 60mL/min by the Cockcroft-Gault equation (see Appendix C)

3.1.8 Testosterone ≤ 50 ng/dL (documented at any time while on LHRH agonist or antagonists or s/p orchiectomy)

3.1.9 ECOG performance status of 0-1 or a Karnofsky performance status of ≥ 80%

3.1.10 Life expectancy of at least 6 months

3.1.11 At least 18 years of age or older

3.1.12 Prior local radiation therapy must be completed at least 30 days prior to enrollment and the patient must have recovered from all toxicity

3.1.13 Prior "systemic" radiopharmaceuticals (strontium, samarium, radium 223 dichloride) must be completed ≥ 8 weeks prior to enrollment

3.1.14 Patients must agree to use 2 methods of adequate contraception for the duration of study participation, and for four months after discontinuing therapy, because of the unknown potential risk to a gamete and/or developing embryo from this investigational therapy.

3.1.15 Ability to understand and the willingness to sign a written informed consent document.

## 3.2 Exclusion Criteria

3.2.1 Prior chemotherapy for castration resistant prostate cancer. Neoadjuvant chemotherapy and chemotherapy given for hormone sensitive prostate cancer are allowed.

3.2.2 Prior investigational immunotherapy

3.2.3 Prostate cancer pain requiring regularly scheduled narcotics

3.2.4 Pathologic long-bone fractures, imminent pathologic long-bone fracture (cortical erosion on radiography > 50%) or spinal cord compression

3.2.5 Current treatment with systemic steroid therapy (inhaled/topical steroids are acceptable)

- Systemic corticosteroids must be discontinued for at least 30 days prior to first CYT107 injection.

3.2.6 Known central nervous system metastases

3.2.7 Documented cirrhosis or documented acute hepatitis

**Note**: A positive hepatitis B serology indicative of previous immunization (i.e., HBsAb positive and HBcAb negative), or a fully resolved acute HBV infection is not an exclusion criterion.

3.2.8 History of severe asthma, as defined by prior or current use of systemic corticosteroids for disease control, with the exception of physiological replacement doses of cortisone acetate or equivalent, as defined by a dose of 10 mg or less

3.2.9 Medical or psychiatric illness that would, in the opinion of the investigator, preclude participation in the study or the ability of patients to provide informed consent for themselves

3.2.10 Cardiovascular disease that meets one of the following: congestive heart failure (New York Heart Association Class III or IV), active angina pectoris, or recent myocardial infarction (within the last 6 months)

3.2.11 Concurrent or prior malignancy except for the following:

- Adequately treated basal or squamous cell skin cancer
- Adequately treated stage I or II cancer from which the patient is currently in complete remission
- Any other cancer from which the patient has been disease-free for 5 years

3.2.12 Known HIV or other history of immunodeficiency disorder. HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interaction with CYT107. Other trials are examining the effect of CYT107 in patients with HIV infection.

3.2.13 Prisoners or subjects who are compulsorily detained (involuntarily incarcerated) for treatment of either a psychiatric or medical (e.g. infectious) illness

3.2.14 Any underlying medical or psychiatric condition, which in the opinion of the investigator will make the administration of CYT107 hazardous or obscure the interpretation of AEs, such as a condition associated with frequent diarrhea

3.2.15 History of allergic reactions attributed to compounds of similar chemical or biologic composition to CYT107

3.2.16 Patients who have received prior immunosuppressive therapy within 30 days prior to enrollment

3.2.17 Active (as defined by requiring immunosuppressive therapy) or *history of clinically significant* autoimmune disease (as defined by previously requiring immunosuppressive therapy)

3.2.18 Patients who have received hepatotoxic drugs less than 7 days prior to enrollment

3.2.19 Patients who have received prior biologic agents less than 30 days prior to enrollment

3.2.20 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements

3.2.21 Patients who have a history of any hematopoietic malignancy

3.2.22 History of pulmonary disease such as emphysema or COPD, (FEV > 60% of predicted for height and age required in patients with prolonged smoking history or symptoms of respiratory dysfunction).

### 3.3 Inclusion of Minorities

Men of all races and ethnic groups are eligible for this trial. Prostate cancer does not occur in women.

### 3.4 Subject Recruitment

Subjects will be identified through the clinical practices of the investigator or sub-investigators at participating research sites and through referrals from outside hospitals and physicians. No direct-to-patient advertising will be performed.

## 4. REGISTRATION PROCEDURES

### 4.1 General Guidelines

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

Prior to the recruitment of a patient for this study, investigators must be registered and have a current CTEP-IAM account. Each investigator must have an NCI investigator number and must maintain an "active" investigator registration status through the annual submission of a complete investigator registration packet (FDA Form 1572 with original signature, current CV, Supplemental Investigator Data Form with signature, and Financial Disclosure Form with original signature) to the Pharmaceutical Management Branch, CTEP, DCTD, and NCI. These forms are available on the CTSU Web site (enter credentials at https://www.ctsu.org; then click on the Register tab) or by calling the PMB at (240) 276-6575 Monday through Friday between 8:30 a.m. and 4:30 p.m. Eastern time.

Each investigator or group of investigators at a CITN member site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can enroll patients. CITN member sites can check the status of their registration packets by querying the Regulatory Support System (RSS) site registration status page of the CTSU member web site by entering credentials at https://www.ctsu.org.

Requirements for CITN12-03 site registration:

- CTSU IRB Certification
- CTSU IRB/Regulatory Approval Transmittal Sheet
- IRB-approved consent form

### 4.2 Registration Process

Patient registration can occur only after pre-treatment evaluation is complete, eligibility criteria have been met, and the study site is listed as 'approved' in the CTSU RSS (Regulatory Support System). Patients must have signed and dated all applicable consents and authorization forms.

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available on a 24/7 basis.

All CITN member site staff will use OPEN to enroll patients to this study. OPEN is integrated with the CTSU Enterprise System for regulatory and roster data and, upon enrollment, initializes the patient position in the RAVE database. OPEN can be accessed at https://open.ctsu.org or from the OPEN tab on the CTSU members' side of the website at https://www.ctsu.org.

Prior to accessing OPEN CITN member site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes. Site staff should use the registration forms provided on the group or CTSU web site as a tool to verify eligibility.
- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

Access requirements for OPEN:

- CITN member site staff will need to be registered with CTEP and have a valid and active CTEP-IAM account. This is the same account (user id and password) used for the CTSU members' web site.
- To perform registrations, the site user must have been assigned the 'Registrar' role on the CITN roster.

**Note**: The OPEN system will provide the site with a printable confirmation of registration information and treatment information. Please print this confirmation for your records.

Further instructional information is provided on the OPEN tab of the CTSU members' side of the CTSU website at https://www.ctsu.org or at https://open.ctsu.org. For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

## 5. TREATMENT PLAN

### 5.1 Agent Administration

The patients will receive 10 μg/kg of CYT107 every week for 4 weeks, beginning between 3 to 7 days (3 days preferred) after completion of standard sipuleucel-T therapy (i.e., after the

third and final dose of sipuleucel-T). At 10-20 µg/kg, some specific immune response or antiviral activity has been documented.

CYT107 will be administered subcutaneously, which according to studies in monkeys, yields satisfactory bioavailability (> 75%), with an extended kinetic profile that may favor prolonged CYT107 activity.

| ***REGIMEN DESCRIPTION*** | | | | |
|---|---|---|---|---|
| ***Agent*** | ***Dose*** | ***Route*** | ***Schedule*** | ***Treatment Phase*** |
| CYT107 (cohort 1) | No therapy (observation) | | | 28 days (4 weeks) |
| CYT107 (cohort 2) | 10 µg/kg | Subcutaneous | Dose 1: (day 0) 3-7 days post sipuleucel-T<br>Dose 2: (day 7) 10-14 days post sipuleucel-T<br>Dose 3: (day 14) 17-21 days post sipuleucel-T<br>Dose 4 (day 21) 24-28 days post sipuleucel-T | |

Patients assigned to observation (cohort 1) and patients assigned to receive CYT107 (cohort 2) will follow the same study procedures and blood collection schedule. However, the study visits on Week 2 (Day 7) and Week 4 (Day 21) are optional for patients assigned to observation (cohort 1).

All patients randomly assigned to receive CYT107 (cohort 2) will remain under observation for 4 hours after each injection of CYT107 (Day 0, Day 7, Day 14 and Day 21). The vital signs will be monitored every 2 hours and patients will be observed for the development of any allergic reaction to the CYT107, such as hives, bronchospasm, rash, etc.

The first six patients randomly assigned to receive CYT107 will be followed for an additional 4 weeks prior to the study expanding past six patients, as a lead-in phase to ensure safety and tolerability of the selected CYT107 dose. In order to continue enrollment, only one grade 3 or 4 Adverse Events related to CYT107 is allowed with the exception of transient lymphopenia (See Section 6). For the purpose of evaluating the first 6 patients, a Grade 3 or 4 'lymphocyte count decrease' will be counted as an Adverse Event towards stopping enrollment, only if the count fails to return to a minimum of 500 cells/mm3 by the time of the scheduled start of the next cycle.

Patients must complete standard sipuleucel-T therapy before receiving the CYT107 doses. The standard, FDA approved, sipuleucel-T therapy is three vaccinations administered on days 0, 14 and 28. CYT107 must be administered within 3 to 7 days of the patient receiving their last sipuleucel-T (Provenge®) vaccination.

Patients may **not** use any of the following therapies during the administration of CYT107:

- Concomitant systemic or local anti-cancer medications or treatments (investigational or non-investigational) are prohibited in this study while receiving CYT107 treatments.
- Treatment with hormones or other chemotherapeutic agents may not be administered except for hormones administered for non-disease-related conditions (e.g., insulin for

diabetes mellitus). Neoadjuvant chemotherapy and chemotherapy given for hormone sensitive prostate cancer are allowed.

- Therapy with anti-androgens must be stopped for at least 30 days prior to first CYT107 injection.
- Palliative radiation therapy may not be administered during protocol treatment.
- Immunosuppressive agents including chronic systemic corticosteroids, except for those directed by the protocol or for toxicities such as hypersensitivity or other immune events.
- Any non-oncology vaccine therapies used for the prevention of infectious diseases (for at least 30 days prior to or after any dose of study drug).

Patients may continue to receive the following:

- Continued LHRH agonist or antagonist therapy is allowed and is mandatory, except for patients with prior orchiectomy.
- Continued bone targeted agents (e.g. zolendronate, denosumab) are allowed
- Patients should receive full supportive care, including transfusions of blood and blood products, erythropoietin, antibiotics, antiemetics, etc. when appropriate.

After the period of CYT107 administration, patients should not receive additional prostate cancer therapies until or unless substantial progression occurs. Therapies should be held until after day 70 if medically appropriate in the view of the primary treating physician. Adverse events noted following subsequent therapies within the 53 weeks duration of follow-up will be chronicled and attribution assessed.

5.1.1 CTEP IND Agent: N/A

5.1.2 Other Agent(s): N/A

5.1.3 Other Modality(ies) or Procedures: N/A

5.1.4 Investigational Imaging Agent Administration: N/A

## 5.2 Definition of Dose Limiting Toxicity: N/A

## 5.3 General Concomitant Medication and Supportive Care Guidelines

Studies performed to investigate the potential of CYT107 to inhibit or to act as a time dependent inhibitor of hepatic microsomal cytochrome P450 isozmyes (see Investigator Brochure) concluded that at the concentration investigated, it is unlikely that CYT107 will be involved in drug-drug interactions and CYT107 is not expected to cause clinically significant P450 inhibition or induction. However, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies including vitamins and nutritional supplements.

## 5.4 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue for one cycle (4 doses total) or until one of the following criteria applies:

- Unacceptable adverse event(s)
- Intercurrent illness that prevents further administration of treatment
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Disease progression
- Patient decides to withdraw from the study

## 5.5 Duration of Follow Up

Patients will be followed for 53 weeks or until death, whichever occurs first. Patients, in whom therapy is stopped due to unacceptable adverse event(s) will continue to be followed for 53 weeks or until resolution or stabilization of the adverse event if that takes more than 53 weeks.

Many patients will receive additional therapy for progression within the 53 weeks duration of follow-up. Additional therapy will be chronicled.

Patients will be asked to be followed by phone, once a year, after completion of Week 53. Overall Survival data will be collected and reported (see Section 11.1) until the end of the study.

## 5.6 Criteria for Removal from Study

Patients will not be removed from the study; however the patient may decide to withdraw from the study at any time. Patients with Adverse Events or progression will continue to be followed for a total of 53 weeks. If the patient elects to leave the study, notify the Principal Investigator, and document the reason for study removal and the date the patient was removed in the Case Report Form. The patient should otherwise be followed per protocol requirements.

## 5.7 Laboratory Specimen collection

The Study Calendar in section 10 outlines the timing of procedures and follow up visits.

The CITN12-03 Laboratory Manual outlines the timing of blood draws as well as the maximum total amount of the blood draws.

Quantity of Blood Draws: The maximum total blood draw on weeks 1, 6, 11, 23, and 53 is between 276 and 286 mL. Due to the large quantity of blood scheduled to be drawn at these visits, assessment for anemia is necessary. Based on the safety laboratories the volume of blood drawn at weeks 1, 6, 11, 23, and 53 will be determined.

Inclusion criteria demand a hemoglobin (Hgb) of ≥10 g/dL for entry into the study. Thus, profound anemia is not likely to be an issue. If a substantial drop in Hgb is observed, patients should be assessed for cryptic bleeding and hemolysis. If Hgb is <10g/dL prior to the Week 1 Day 0, Week 6 Day 35, Week 11 Day 70, Week 23 Day 154 or Week 53 Day 365 visits, less than the maximum total volume of blood should be drawn as follows:

- On Week 1 Day 0, if the Hgb is ≥9 g/dL, up to 9.9 g/dL, then blood draw amounts may be decreased to 226 mL (from 286 mL), on Week 1 Day 0.
- On Week 6 Day 35 and Week 11 Day 70, blood draw amount may be decreased to 216 mL (from 276 mL).
- On Week 23, blood draw amount may be decreased to 220 mL (from 270 mL)
- On Week 53 Day 365, blood draw amount may be decreased to 226 mL (from 286 mL). If the Hgb is less than 9 g/dL, drawing blood only for local safety labs is recommended.

If a site is unable to collect all specimens due to low hemoglobin or other issues such as vein access, specimens should be collected in the following order if required at the visit:

1) Toxicity/safety specimens and local safety labs
2) ELISA
3) ELISPOT and other cellular assays
4) Other assays

If the Hgb is ≥ 10 all labs will be drawn.

## 6. DOSING DELAYS/DOSE MODIFICATIONS

The NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 will be used to grade adverse events. Patients enrolled in this study will be evaluated clinically and with standard laboratory tests before and at regular intervals during their participation in this study as specified in this section.

Patients will be evaluated for adverse events (all grades), serious adverse events, and adverse events requiring study drug interruption or discontinuation at each study visit for the duration of their participation in the study.

Delays in dosing will be allowed for delays such as weather factors resulting in delays getting to the clinic or hospital, travel delays, clinic schedules or emergencies. A delay of up to 72 hours will be allowed on one occasion during the course of a treatment cycle.

Removing patients from protocol therapy for Adverse Events: Patients will come off protocol therapy if any grade 3 or 4 Adverse Events by CTCAE Version 4.0 criteria occur with the exception of transient lymphopenia. Three cases of Grade 3 lymphopenia have been documented with CYT107. For purposes of this trial, since peripheral lymphopenia is a transient event, a Grade 3 'lymphocyte count decrease' to <500-200 cells/mm3 and a Grade 4 'lymphocyte count decrease' to <200 cells/mm3 will not require patients coming off protocol provided the lymphocyte count returns to a minimum of 500 cells/mm3 by the time of the scheduled start of the next weekly dose within the treatment phase. A Grade 3 or 4 'lymphocyte count decrease'

will require patients coming off protocol if the count fails to return to a minimum of 500 cells/mm3 by the time of the scheduled start of the next cycle.
Dose modifications for Adverse Events: Substantial adverse events are not expected and thus dose modifications are not planned or recommended with the exception of fatigue (see below). In initial human trials with CYT107 adverse events were frequent, but transient and grade 2 or less (as noted in the Adverse Event list, section 7.1.2.). These include erythema or local reaction at site injection, peripheral adenopathies next to the site injection, rash, general reactions of fever and flu-like symptoms.

Fatigue was infrequent; dose modification for this event is noted below:

| **Event Name** | **Nausea** |
|---|---|
| **Grade of Event** | **Management/Next Dose of CYT107** |
| ≤ Grade 1 | No change in dosing schedule |
| Grade 2 | No change in dosing schedule |
| Grade 3 | Off protocol therapy if with adequate/maximal medical intervention symptoms persist |
| Grade 4 | Off protocol therapy |
| Patients requiring a delay of >72 hours should go off protocol therapy. | |
| Recommended management: Anti-emetics | |

| **Event Name** | **Vomiting** |
|---|---|
| **Grade of Event** | **Management/Next Dose of CYT107** |
| ≤ Grade 1 | No change in dosing schedule |
| Grade 2 | No change in dosing schedule |
| Grade 3 | Off protocol therapy if with adequate/maximal medical intervention symptoms persist |
| Grade 4 | Off protocol therapy |
| Patients requiring a delay of >72 hours should go off protocol therapy. | |
| Recommended management: Anti-emetics | |

| **Event Name** | **Diarrhea** |
|---|---|
| **Grade of Event** | **Management/Next Dose of CYT107** |
| ≤ Grade 1 | No change in dosing schedule |
| Grade 2 | No change in dosing schedule |
| Grade 3 | Off protocol therapy if with adequate/maximal medical intervention symptoms persist |
| Grade 4 | Off protocol therapy |
| Patients requiring a delay of >72 hours should go off protocol therapy. | |
| Recommended management: Physician discretion | |

| **Event Name** | **Neutropenia** |
|---|---|
| **Grade of Event** | **Management/Next Dose of CYT107** |
| ≤ Grade 1 | No change in dosing schedule |
| Grade 2 | No change in dosing schedule |
| Grade 3 | Hold* until ≤Grade 2. |

| | |
|---|---|
| Grade 4 | Off protocol therapy |
| *Patients requiring a delay of >72 hours should go off protocol therapy. | |
| Recommended management: Treatment of infection as appropriate | |

| **Event Name** | **Thrombocytopenia** |
|---|---|
| **Grade of Event** | **Management/Next Dose of CYT107** |
| ≤ Grade 1 | No change in dosing schedule |
| Grade 2 | No change in dosing schedule |
| Grade 3 | Off protocol therapy |
| Grade 4 | Off protocol therapy |
| Patients requiring a delay of >72 hours should go off protocol therapy. | |
| Recommended management: Platelet transfusions if indicated | |

| **Event Name** | **Fatigue** |
|---|---|
| **Grade of Event** | **Management/Next Dose of CYT107** |
| ≤ Grade 1 | No change in dosing schedule |
| Grade 2 | No change in dosing schedule |
| Grade 3 | Hold* until ≤Grade 2. |
| Grade 4 | Off protocol therapy |
| *Patients requiring a delay of >72 hours should go off protocol therapy. | |
| Recommended management: Physician discretion | |

| **Event Name** | **Hepatic damage** |
|---|---|
| **Grade of Event** | **Management/Next Dose of CYT107** |
| ≤ Grade 1 | No change in dosing schedule |
| Grade 2 | No change in dosing schedule[1] |
| Grade 3 | Off protocol therapy |
| Grade 4 | Off protocol therapy |
| *Patients requiring a delay of >72 hours should go off protocol therapy. | |
| Recommended management: Physician discretion | |

**Note**: [1]CYT107 will be discontinued if patients have a concurrent elevation of ALT >3 x upper limit of normal (ULN) and total bilirubin > 2 x ULN in whom there is no evidence of biliary obstruction or other causes that can reasonably explain the concurrent elevation.

## 7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.1.2) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting (via CTEP-AERS) **in addition** to routine reporting.

### 7.1 Comprehensive Adverse Events and Potential Risks List(s) (CAEPRs): N/A

#### 7.1.1 CAEPRs for CTEP IND Agent(s): N/A

7.1.2 Adverse Event List for CYT107

IL-7 Tabular summary of adverse drug reactions for all clinical trials (as of May 2012)
(See Investigator Brochure)

| CYT107 (N = 256) Events | Grade | Frequency |
|---|---|---|
| **Transient events observed whatever the dose** | | |
| Erythema or local reaction at site injection | Grade <1, 1 and 2 | Frequent: approximately 2/3 of patients, mostly less than Grade 1 or Grade 1 |
| Peripheral adenopathies next to the injection site | Grade 1<br>Grade 2 | Frequent, but transient<br>Rare (1 case) |
| **Transient events observed dependent on the dose administered** | | |
| General reactions:<br>Fever, Flu-like symptoms | Grade 1 or 2 | Uncommon |
| Rash | Grade 1 or 2<br>Grade 3 | Rare<br>2 cases |
| Fatigue (PT in MedDRA), reported as "Asthenia" by investigative team | Grade 1 or 2<br>Grade 3 | Uncommon<br>Rare (1 case) |
| Hepatic damage (cytolysis and/or cholestasis) ALT and/or AST elevation | Grade 1 or 2<br>Grade 3<br>Grade 4 | Uncommon<br>2 cases<br>Rare (1 case) |
| Peripheral lymphopenia | Grade 1 or 2<br>Grade 3 | Uncommon<br>3 cases |
| Anaphylactic Reaction | Grade 3 | Uncommon |
| **Other events** | | |
| Chest pressure | Grade 2 | Rare (1 case) |
| Immunogenicity | Non applicable | Frequent: about 25% |
| Isolated transient HIV RNA VL "blips" (in patients Known to be HIV positive) | Mostly Grade 1 | Uncommon (31 events/590 measurements or 0.05%) |
| Phlebitis | Grade 3 | Rare (1 case) |
| Henoch-Schoenlein Purpura | Grade 2 | Rare (1 case) |
| Lichen Planus | Grade 3 | Rare (1 case) |

7.1.3 Adverse Event List(s) for Commercial Agent(s): N/A

7.1.4 Adverse Event List(s) for CIP (*e.g.* Study-Specific) Commercial Imaging Agents: N/A

## 7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE

version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **Attribution** of the AE:
- Definite – The AE *is clearly related* to the study treatment.
- Probable – The AE *is likely related* to the study treatment.
- Possible – The AE *may be related* to the study treatment.
- Unlikely – The AE *is doubtfully related* to the study treatment.
- Unrelated – The AE *is clearly NOT related* to the study treatment.

## 7.3 Expedited Adverse Event Reporting

7.3.1 Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP Web site (http://ctep.cancer.gov). The reporting procedures to be followed are presented in the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" which can be downloaded from the CTEP Web site (http://ctep.cancer.gov). These requirements are briefly outlined in the tables below (Section 7.3.3).

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

7.3.2 CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Study Coordinator of the Lead Organization, Principal Investigator, and the local treating physician. CTEP-AERS provides a copy feature for other e-mail recipients.

7.3.3 Expedited Reporting Guidelines

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

**Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.**

Death due to progressive disease should be reported as **Grade 5 "Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

**Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1, 2]**

**FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)**
**NOTE:** Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

**ALL SERIOUS** adverse events that meet the above criteria MUST be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

| Hospitalization | Grade 1 and Grade 2 Timeframes | Grade 3-5 Timeframes |
|---|---|---|
| Resulting in Hospitalization ≥ 24 hrs | 10 Calendar Days | 24-Hour 5 Calendar Days |
| Not resulting in Hospitalization ≥ 24 hrs | Not required | |

**Expedited AE reporting timelines are defined as:**

- "24-Hour; 5 Calendar Days" - The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
**Expedited 24-hour notification followed by complete report within 5 calendar days for:**

- All Grade 3, 4, and Grade 5 AEs

**Expedited 10 calendar day reports for:**

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[2]For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

### 7.3.4 Additional Protocol-Specific Expedited Adverse Event Reporting Exclusions: N/A

### 7.3.5 Serious Adverse Event Reporting to the Industry Partners

The Sponsor will provide the industry partners with SAE Reports that are sent to the FDA. All SAE reports will include the investigator's assessment of causality.

### 7.4 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported through** CTEP-AERS **must <u>also</u> be reported in routine study data submissions.**

### 7.5 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.
CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

### 7.6 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine reporting via CDUS unless otherwise specified.

## 8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational agent administered in this study can be found in Section 8.1.

### 8.1 CTEP IND Agent(s): N/A

### 8.2 Other Investigational Agent

**Recombinant Glycosylated Human Interleukin-7, (CYT107) rhIL-7**

**Product description**:
Interleukin-7 (IL-7) is a multifunctional cytokine, mainly produced by non-hematopoietic cells, which is active on T cell development, expansion, response and protection from apoptosis. IL-7 has a critical and, at some steps, a non-redundant stimulating effect on T lymphocyte development, notably on thymopoiesis and downstream from the thymus, on homeostasis expansion of peripheral T cells.

CYT107, developed by Cytheris SA and owned by RevImmune, Inc., is a glycosylated recombinant human IL-7 produced from a recombinant Chinese Hamster Ovary (CHO) cell line (CHO S).

**Chemical Name or Amino Acid Sequence:**
CYT107 (rhIL-7) Drug Substance is a glycosylated 152 amino acid recombinant protein (same as the native protein) which comprises three putative N-glycosylation sites and one putative O-glycosylation site. The composition of CYT107 includes a majority of rhIL-7 polypeptides which are glycosylated on at least three distinct amino acid residues (2 to 3 N-glycans and one O-glycan) and a minority of di-glycosylated rhIL-7 polypeptide (one N-glycan and one O-glycan). CYT107 has 3 disulfide bridges: Cys2- Cys 92, Cys 34- Cys 129, and Cys 47- Cys 141.

**Molecular Weight:**
CYT107 has an average molecular mass, as determined by mass spectrometry, of 22kDa; corresponding to an average apparent molecular weight of 27kDa as determined by SDS gel electrophoresis.

**Solution preparation**:
CYT107 (rhIL-7) Drug Product is supplied in 2 cc vials as a colorless solution suitable for subcutaneous administration, formulated at a concentration of 2 mg/ml in the following buffer: 10mM sodium acetate, 100 mM NaCl, 50mM glutamic acid, and 50mM arginine. The pH of the solution is in the range of 4.8 and 5.2. Vials are filled to deliver 1 mg/0.5 mL of CYT107.

**Storage requirements:**
CYT107 should be stored at -20°C. Store vials of CYT107 according to instructions on the label.

**Stability:**
Stability studies are ongoing and will be continued throughout the clinical study. Regular updates are submitted to the FDA through quality amendments. Stability information will be periodically communicated to the Hospital Pharmacy, to guarantee the stability of the drug product. The vial is for 1 injection only and any unused portion should not be re-used for a subject or for other purpose.

**Consideration for safe handling and administration:**
There are no specific guidelines for the safe handling of CYT107 Drug Product. Institutional guidelines for safe handling of proteins in general should be followed.

**Do not shake vials before injection**.

CYT107 should be administered **slowly** and **strictly in the subcutaneous space**.

**Route of administration:**
CYT107 will be administered subcutaneously, which according to studies in monkeys, yields satisfactory bioavailability (> 75%), with an extended kinetic profile that may favor

prolonged IL-7 activity.

**Dose adjustment:**
The appropriate quantity of CYT107 to be given at each injection will depend on the patient's weight and dose level. For obese patients, a corrected weight will be used to calculate the final dose the patient will receive. Carefully check dose and dosing regimens before CYT107 administration (See Appendix D).

**Agent Ordering and Agent Accountability:**
CYT107 is produced in the USA by Laureate Biopharmaceuticals now operating as Patheon®. After manufacturing, the product is stored at CSM in Fargo, ND for clinical supply, packaging, and labeling. The label indicates the product name, strength, manufacturing date, and the study requirement information. CYT107 will be shipped from CSM to each participating site. (See the Study Procedures Manual for instructions on how to order CYT107).

**Agent Inventory Records:**
The investigator, or a responsible party designated by the investigator (e.g. institutional investigational pharmacy), must maintain a careful record of the inventory and disposition of all agents received from CSM in Fargo, ND using the Study Agent Drug Accountability Record. (See the Study Procedures Manual for the form to use and instructions on how to complete it).

## 8.3 Commercial Agent: N/A

# 9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

## 9.1 Biomarker Studies

The major focus will be to determine the relative level and persistence of vaccine induced immune response to PA2024 and PAP in patients receiving sipuleucel-T with and without CYT107.

## 9.2 Laboratory Correlative Studies

### 9.2.1 T-cell interferon-gamma responses to PA2024 – *Laboratory Correlative Study #1*

The primary endpoint will be the T-cell response to PA2024 measured at day 70 (week 11), 7 weeks after last CYT107 injection. T-cell responses to PA2024 and PAP will be measured by a direct 48 hour interferon-gamma ELISPOT assay utilizing recombinant proteins PA2024 and PAP (provided by Dendreon Corp.). Cellular immune responses to other ongoing and nascent antitumor responses (PRAME, NY-ESO-1 and/or p53), additional tumor antigens specific to prostate cancer (e.g., PSA and/or PSMA), and memory viral responses (influenza A, CEF) will also be assessed. Longitudinal responses to PA2024 and PAP will be considered in the primary analysis; however power calculations are based on the magnitude of T cell ELISPOT responses measured at week 11.

9.2.1.1 Collection of Specimen(s): Whole blood will be collected into heparinized (green top) tubes.

9.2.1.2 Handling of Specimens(s): Samples will be collected at room temperature and shipped to the CITN Central Laboratory the same day as the blood draw, so that the sample is received at the central laboratory within 24 hours of blood draw. PBMC will be isolated and cryopreserved prior to testing.

9.2.1.3 Shipping of Specimen(s): Samples will be shipped at ambient temperature for overnight FEDEX delivery. Clinical research sites will utilize a web-based specimen tracking system (BSI-II) to communicate with the CITN Central Laboratory.

9.2.1.4 Site(s) Performing Correlative Study: The interferon-gamma ELISPOT assay will be conducted at the CITN Central Laboratory at the University of Washington (UW) Immunologic Monitoring Laboratory (IML), Seattle, WA.

9.2.2 T-cell proliferative responses – *Laboratory Correlative Study #2*

Peripheral blood lymphocytes will be analyzed directly *ex vivo* for the presence of proliferating T cells using a flow cytometric Ki-67 assay. Other surface markers will include CD3, CD4, CD8, CD45RA and CCR7 (among others) to identify T cell memory subsets. In addition, T cell proliferative responses to protein antigens PA2024 and PAP (from Dendreon Corp.) will also be tested *in vitro*. Results from this latter assay should corroborate the antigen-specific data obtained from Correlative Study #1. Longitudinal responses to PA2024 and PAP will also be evaluated. Cellular immune responses to other ongoing and nascent antitumor responses (PRAME, NY-ESO-1 and p53), additional tumor antigens specific to prostate cancer (e.g., PSA and PSMA), memory viral responses (influenza A and CEF) may also be tested.

9.2.2.1 Collection of Specimen(s): Whole blood will be collected into heparinized tubes.

9.2.2.2 Handling of Specimen(s): Samples will be collected at room temperature and shipped to the CITN Central Laboratory the same day as the blood draw, so that the sample is received at the central laboratory within 24 hours of blood draw. PBMC will be isolated and cryopreserved prior to testing.

9.2.2.3 Shipping of Specimen(s): Samples will be shipped at ambient temperature for overnight FEDEX delivery. Clinical research sites will utilize a web-based specimen tracking system (BSI-II) to communicate with the CITN Central Laboratory.

9.2.2.4 Site(s) Performing Correlative Study: The proliferation assays will be conducted at the CITN Central Laboratory at the University of Washington Immunologic Monitoring Laboratory, Seattle, WA.

9.2.3 Quantification of PBMC and T cell subsets – *Laboratory Correlative Study #3*

The effect on the number and percentage of PBMC subsets and T lymphocyte subsets (i.e., $CD4^+$ and CD8+ T cell naïve, effector memory, and central memory subsets, $CD4^+$ Treg and activated CD4+ and CD8+ T cells) will be assessed using multiparameter flow cytometric analysis of whole blood. The PBMC immunophenotyping assay will quantify the absolute number and proportion of T cells (both CD8+ and CD4+), NK cells (CD56+CD3-), NKT cells (CD56+CD3+), B cells (CD19+), monocytes (CD16+), dendritic cells (HLA-DR+ CD123+ or CD11c+), and CD122 (IL15R-beta)-expressing cells.

An additional panel will quantify the absolute number and proportion of memory and naïve T cell subsets using antibodies to CD45RA, CCR7, CD28, of regulatory T cells using antibody to CD25 (IL2R-alpha), the activation state of T cells using antibodies to PD-1, ICOS, and HLA-DR, among others and CD-127 (IL7R-alpha). The absolute change in each parameter as well as variance in change over time for each patient (mean, median, and SE/SD) will be evaluated.

9.2.3.1 Collection of Specimen(s): Whole blood will be collected into heparinized tubes.

9.2.3.2 Handling of Specimen(s): Samples will be collected at room temperature and shipped to the CITN Central Laboratory the same day as the blood draw, so that the sample is received at the Central Laboratory within 24 hours of blood draw.

9.2.3.3 Shipping of Specimen(s): Samples will be shipped at ambient temperature for overnight FEDEX delivery. Clinical research sites will utilize a web-based specimen tracking system (BSI-II) to communicate with the CITN Central Laboratory.

9.2.3.4 Site(s) Performing Correlative Study: The immunophenotyping assays will be conducted at the CITN Central Laboratory at the University of Washington Immunologic Monitoring Laboratory, Seattle, WA.

9.2.4 PA2024 and PAP-specific Antibody Responses – *Laboratory Correlative Study #4*

IgM and IgG responses to PAP and PA2024 will be quantified using standard ELISA. The level of antibody responses to these antigens will be compared between the groups. The absolute change in each parameter as well as variance in change over time for each patient (mean, median, and SE/SD) will also be evaluated.

9.2.4.1 Collection of Specimen(s): Whole blood will be collected into red top (serum) tubes.

9.2.4.2 Handling of Specimen(s): Samples will be centrifuged, and serum aliquoted at each collection site within 2-4 hours of blood draw. Samples will be frozen at -80C and stored for batch shipment to the CITN Central Laboratory.

9.2.4.3 Shipping of Specimen(s): Batched samples will be shipped on dry ice for overnight FEDEX delivery. Clinical research sites will utilize a web-based specimen tracking system (BSI-II) to communicate with the CITN Central Laboratory. The Central Laboratory will ship coded, batched specimens to Dendreon Corp. for testing.

9.2.4.4 Site(s) Performing Correlative Study: The ELISA assays will be conducted at Dendreon Corp., Seattle, WA.

9.2.5 T-cell receptor rearrangement DNA excision circles (TRECs) – *Laboratory Correlative Study #5*

Thymic function will be assessed by measuring serum levels of T-cell receptor rearrangement DNA excision circles (TRECs), in order to determine the contribution of new thymic emigrants to the T cell receptor repertoire. TREC levels will be compared between the two arms. The absolute change in each parameter as well as variance in change over time for each patient (mean, median, and SE/SD) will be evaluated.

9.2.5.1 Collection of Specimen(s): Whole blood will be collected into red top (serum) tubes.

9.2.5.2 Handling of Specimen(s): Samples will be centrifuged, and serum aliquoted at each collection site within 2-4 hours of blood draw. Samples will be frozen at -80C and stored for batch shipment to the CITN Central Laboratory.

9.2.5.3 Shipping of Specimen(s): Batched samples will be shipped on dry ice for overnight FEDEX delivery. Clinical research sites will utilize a web-based specimen tracking system (BSI-II) to communicate with the CITN Central Laboratory.

9.2.5.4 Site(s) Performing Correlative Study: The TREC assays will be conducted at the laboratory of Dr. Bruce Blazar, CITN site at the University of Minnesota.

9.2.6 T-cell receptor deep sequencing – *Laboratory Correlative Study #6*

The T-cell repertoire will be characterized by T-cell receptor (TCR) deep sequencing (Harlan Robins, PhD, with Adaptive Biotechnologies, Seattle, WA) and compared between the two arms. These assays will evaluate the extent to which polyclonal or oligoclonal expansion of T cells has occurred with CYT107 therapy.

9.2.6.1 Collection of Specimen(s): Whole blood will be collected into ACD tubes.

9.2.6.2 Handling of Specimen(s): Samples will be collected at room temperature and shipped to the CITN Central Laboratory the same day as the blood draw, so that the sample is received at the Central Laboratory within 24 hours of blood draw. PBMC will be isolated and cryopreserved prior to isolation of genomic DNA.

9.2.6.3 Shipping of Specimen(s): Samples will be shipped at ambient temperature for overnight FEDEX delivery. Clinical research sites will utilize a web-based specimen tracking system (BSI-II) to communicate with the CITN Central Laboratory. Processed samples will be batch-shipped by the Central Laboratory to Adaptive Biotechnologies for testing.

9.2.6.4 Site(s) Performing Correlative Study: The TCR deep sequencing assay will be conducted at the Adaptive Biotechnologies, Seattle, WA.

9.2.7 Peripheral Blood Kyn/Trp Ratios – *Laboratory Correlative Study #7*

Kyn/Trp ratios will be assayed in peripheral blood at baseline and at specified intervals throughout the trial. IDO catabolizes the conversion of Tryptophan to Kynurenine, which has potent immunosuppressant properties. An increase in Kynurenine levels over time, relative to Tryptophan levels, in patients who are failing therapy would indicate that IDO expression may be leading to treatment failure. This mechanism of resistance could potentially be overcome with an IDO inhibitor.

9.2.7.1 Collection of Specimen(s): Whole blood will be collected into heparinized tubes.

9.2.7.2 Handling of Specimen(s): Samples will be collected at room temperature and shipped to the CITN Central Laboratory the same day as the blood draw, so that the sample is received at the Central Laboratory within 24 hours of blood draw.

9.2.7.3 Shipping of Specimen(s): Samples will be shipped at ambient temperature for overnight FEDEX delivery. Clinical research sites will utilize a web-based specimen tracking system (BSI-II) to communicate with the CITN Central Laboratory. The Central Laboratory will subsequently ship batched plasma samples to the assay site.

9.2.7.4 Site(s) Performing Correlative Study:
The Kyn/Trp analysis will be conducted at Incyte Corporation, Wilmington, Delaware.

## 9.3 Special Studies: Immunogenicity Testing

The formation of anti-drug antibodies (ADA) and neutralizing anti-drug antibodies (NADA) to glyco-rhIL-7 (CYT107) will be evaluated at: Pre-treatment with IL-7 (CYT107), and Week 11 (Day 70). If positive at Week 11 (Day 70), immunogenicity testing will be repeated at the end of the study.

9.3.1 Collection of specimen(s): Whole blood will be collected into a Lithium Heparin tube. Samples will be centrifuged within 15 minutes and the plasma aliquoted into at least two vials. Please refer to the CITN-12-03 Laboratory Manual.

9.3.2 Handling of specimen(s): Plasma will be frozen at -80C and stored until shipped to the CITN Central Laboratory, Seattle, WA.

9.3.3 Shipping of Specimen(s): Samples will be packed on dry ice and shipped for overnight FEDEX delivery to the CITN Central Laboratory, Seattle, WA.

9.3.4 Site(s) Performing Immunogenicity Testing: RevImmune, Inc., Bethesda, Maryland will perform both ELISA Binding (non-neutralizing) and neutralizing antibody assays according to their Standard Operating Procedure.

## 9.4 Sample Storage

PBMC, serum, and plasma that remain after conducting the above-described correlative assays will be saved for future ancillary studies (e.g. soluble IL-7 receptor levels). Residual cell pellets from whole blood processing (that would otherwise be discarded) will be stored for the possibility of isolating genomic DNA and conducting IL-7 receptor polymorphism analyses, should funding become available.

## 10. STUDY CALENDAR

Baseline evaluations are to be conducted following the last dose of sipuleucel-T, but prior to the first CYT107 injection. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next CYT107 administration.

| | Pre-study | Pre-IL7 | Week 1 | Week 2 *(f) | Week 3 | Week 4 *(f) | Week 6 | Week 11 | Week 23 | Week 53 | Off Study *(d) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Within 7 days of receiving CYT107 | Day 0 | Day 7 ± 2 days | Day 14 ± 2 days | Day 21 ± 2 days | Day 35 ± 2 days | Day 70 ± 7 days | Day 154 ±14 days | Day 365 ±14 days | |
| CYT107 *(A) | | | X | X | X | X | | | | | |
| Sipuleucel-T (Provenge®) *(B) | Standard Sip-T | X | | | | | | | | | |
| Informed consent | | X | | | | | | | | | |
| Demographics | | X | | | | | | | | | |
| Medical history | | X | | | | | | | | | |
| Concomitant medications | | X | X | X | X | X | X | X | X | X | X |
| Physical exam | | X | X | X | X | X | X | X | X | X | X |
| Vital signs | | X | X*(e) | X*(e) | X*(e) | X*(e) | X | X | X | X | X |
| Height | | X | | | | | | | | | |
| Weight | | X | X | X | X | X | X | X | X | X | X |
| Performance status | | X | X | X | X | X | X | X | X | X | X |
| CBC w/diff, plts | | X | X | X | X | X | X | X | X | X | |
| Serum chemistry *(a) | | X | X | X | X | X | X | X | X | X | |
| Anti-nuclear antibody (ANA) | | X | | | | | | | | | X |
| CYT107 immunogenicity (ADA) assay *(b) | | X | | | | | | X | | X | |
| Adverse event evaluation | | X | X | X | X | X | X | X | X | X | X |
| Tumor measurements | | Within 45 days | | Tumor measurements are repeated every 12 weeks. Documentation (radiologic) must be provided for patients removed from study for progressive disease. | | | | | | | X |
| CT, MRI or PET of chest, abdomen, pelvis *(c) | | Within 45 days | | Documentation (radiologic) must be provided for patients removed from study for progressive disease. | | | | | | | X |

| | Pre-study | Pre-IL7 | Week 1 | Week 2 *(f) | Week 3 | Week 4 *(f) | Week 6 | Week 11 | Week 23 | Week 53 | Off Study *(d) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Within 7 days of receiving CYT107 | Day 0 | Day 7 ± 2 days | Day 14 ± 2 days | Day 21 ± 2 days | Day 35 ± 2 days | Day 70 ± 7 days | Day 154 ±14 days | Day 365 ±14 days | |
| CT or MRI Brain (as indicated) *(c) | | | | Documentation (radiologic) must be provided for patients with symptoms | | | | | | | X |
| Bone scan *(c) | | Within 45 days | | Radiologic measurements should be performed every 12 weeks | | | | | | | X |
| EBV serostatus | | X | | | | | | | | | X |
| CMV serostatus | | X | | | | | | | | | X |
| PSA measurement | | | X | | | | X | X | X | X | |
| CTC | | | X | | | | | | | X | |
| IFNg ELISPOT | | | X | | | | X | X | X | X | |
| Proliferation assay | | | X | | | | X | X | X | X | |
| PBMC and T cell subset quantitation | | | X | | | | X | X | X | X | |
| PAP Ab, PA2024 ELISA | | | X | | | | X | X | X | X | |
| TREC detection | | | X | | | | X | X | X | | |
| TCR deep sequencing | | | X | | | | X | | | | |
| Kyn/Trp ratio | | | X | | | | X | X | X | X | X |

*(A): CYT107; Dose as assigned; administration schedule.
*(B): Sipuleucel-T (Provenge®); Dose as assigned; administration schedule. Standard Provenge® therapy (3 vaccinations) required prior to study entry.
*(a): Serum chemistries include: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
*(b): CYT107 ADA testing will be repeated if positive at week 11 (Day 70 ± 7 days). **Frozen plasma is required.**
*(c): CT, MRI or PET within 45 days (14 days preferred) is acceptable for imaging assessment purposes
*(d): Off-study evaluation
*(e): Vital signs will be repeated every 2 hours during the 4 hours observation period required after each injection of CYT107 (cohort 2 only).
*(f): Optional study visit for patients assigned to observation (cohort 1)

# 11. MEASUREMENT OF EFFECT

The primary endpoint for sizing the trial will be quantification of T-cell responses to PA2024 measured at day 70 (week 11), 7 weeks after the last CYT107 injection. Assessment of the primary endpoint is described in Sections 2.5 and 9.2.

One secondary endpoint is an assessment of the clinical efficacy of sipuleucel-T plus CYT107 compared to sipuleucel-T alone. To assess clinical efficacy the protocol will assess differences in (1) overall survival, (2) PSA kinetics (3) progression free survival and, (4) circulating tumor cells (CTC).

## 11.1 Overall Survival

Sipuleucel-T was approved by the FDA in men who have mCRPC with no or minimal symptoms due to a prolongation of median overall survival by 4.1 months compared with results in those treated with placebo. The protocol is not powered to discern modest effects on overall survival. However, data on overall survival will be collected and analyzed. Patients will not be formally followed on protocol after week 53; however, data on OS will be collected and reported.

## 11.2 PSA Kinetics

In the pivotal trials, sipuleucel-T induced no substantial differences in PSA reductions. However, the rate of the PSA increase was slower in patients who received the vaccine in another double-blind, randomized, placebo-controlled study (P-11) of 176 men with rising PSA after prostatectomy receiving hormonal therapy followed by vaccine or control [Beer, 2007]. The PSADT was calculated from day 90 after vaccine to biochemical recurrence.

Patients will have PSA measurements on weeks 1, 6, 11, 23, and 53. The presumption is that immune response will not have immediate effects but will require a period of time to induce an effect on PSADT, if it occurs. Thus, for trial analysis PSADT will arbitrarily be calculated from week 11 to week 23, and 53 or until new therapeutic intervention is initiated, whichever comes first. The PSADT will be evaluated according to the recommendations from PSA Working Group (PSAWG) [Arlen, 2008].

## 11.3 Progression free survival

There was no discernable prolongation of progression free survival in the pivotal trial. However, if the addition of CYT107 substantially increases the immune response to PAP, it is possible that differences in progression free survival will be observed.

PFS is defined as the duration of time from start of treatment to time of radiographic progression or death, whichever occurs first. PFS will consider both measurable disease and unmeasurable bony metastasis. Measurable disease will be measured by RECIST 1.1 criteria [Eisenhauer, 2009]. Unmeasurable bony metastasis will be measured by radiographic criteria [Scher, 2008]. PFS is not the primary endpoint. Thus, responses will not *pro forma* be reviewed by an expert(s) independent of the study at the study's completion.

## 11.4 Circulating Tumor Cells

Circulating tumor cells (CTC) will be enumerated by the approved Veridex assay as exploratory analysis.

## 11.5 Measurement of Disease

For the purposes of this study, baseline scans must be done within 45 days prior to beginning treatment (14 days preferred). Scans can be done before or after sipuleucel-T given that sipuleucel-T is not likely to have induced remissions. In addition to the patient's baseline scan, tumor measurements are repeated every 12 weeks post therapy *or* per institutional standard of care.

Response and progression for purpose of reporting data and publication will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [Eisenhauer, 2009].

**Duration of Response**

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm by chest x-ray or as ≥10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

**Note:** Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable.

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

**Note**: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts. 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions: All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions: All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

11.5.1 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed within 7 days from the beginning of treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions**:** Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray**:** Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition

protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT: At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers: PSA alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Cytology, Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (*e.g.*, residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

FDG-PET: FDG-PET usually not used in prostate cancer. However, rarely it might be reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up

corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

**Note:** A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

11.5.2 Response Criteria: Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

## 11.6 Radiologic Progression-Free Survival of Bony Metastasis

Radiographic progression-free survival is based on parameters suggested by Prostate Cancer Working Group 2 [Scher, 2008]. A patient is considered to have progressed by bone scan if one of the following occurs after randomization:

11.6.1 The first bone scan with ≥2 new lesions compared to baseline is observed <12 weeks from randomization and is confirmed by a second bone scan taken ≥6 weeks later showing ≥2 additional new lesions (a total of ≥4 new lesions compared to baseline).

11.6.2 The first bone scan with ≥2 new lesions compared to baseline is observed ≥12 weeks from randomization and the new lesions are verified on the next bone scan ≥6 weeks later (a total of ≥2 new lesions compared to baseline).

## 11.7 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

**For Patients with Measurable Disease (*i.e.*, Target Disease)**

| Target Lesions | Non-Target Lesions | New Lesions | Overall Response | Best Overall Response when Confirmation is Required* |
|---|---|---|---|---|
| CR | CR | No | CR | ≥4 wks. Confirmation** |
| CR | Non-CR/Non-PD | No | PR | ≥4 wks. Confirmation** |
| CR | Not evaluated | No | PR | |
| PR | Non-CR/Non-PD/not evaluated | No | PR | |
| SD | Non-CR/Non-PD/not evaluated | No | SD | Documented at least once ≥4 wks. from baseline** |
| PD | Any | Yes or No | PD | no prior SD, PR or CR |
| Any | PD*** | Yes or No | PD | |
| Any | Any | Yes | PD | |

* See RECIST 1.1 manuscript [16] for further details on what is evidence of a new lesion.

** Only for non-randomized trials with response as primary endpoint.

*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

**For Patients with Non-Measurable Disease (*i.e.*, Non-Target Disease)**

| Non-Target Lesions | New Lesions | Overall Response |
|---|---|---|
| CR | No | CR |
| Non-CR/non-PD | No | Non-CR/non-PD* |
| Not all evaluated | No | not evaluated |
| Unequivocal PD | Yes or No | PD |
| Any | Yes | PD |
| * 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised | | |

## 12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 8.0 (Adverse Events: List and Reporting Requirements).

### 12.1 Data Reporting

12.1.1 Method

Data collection for this study will be done exclusively through Medidata Rave. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate Rave roles in the CTSU Regulatory Support System (RSS). To access iMedidata/Rave the site user must have an active CTEP IAM account (https://eapps-ctep.nci.nih.gov/iam). In addition, site users that are members of the CITN must have the appropriate Rave roles (Rave CRA, Site PI, or Site co-PI) in RSS at the enrolling site.

Upon initial site registration approval for the study in RSS, all persons with Rave roles assigned on the CITN roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site users must log into the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM user name and password, and click on the "accept" link in the upper right corner of the iMedidata page. Please note, site users will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen.

Users who have not previously activated their iMedidata/Rave accounts at the time of initial site registration approval for the study in RSS will also receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website, Rave tab under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU website under the Rave tab at www.ctsu.org/RAVE/ or by contacting the CTSU Help Desk at 1-888-823-5923 or by e-mail at ctsucontact@westat.com.

12.1.2 Responsibility for Data Submission

This study will be monitored by the Clinical Data Update System (CDUS) Version 3.0. Cumulative protocol- and patient-specific CDUS data will be submitted electronically to CTEP on a quarterly basis, either by FTP burst of data or via the CDS web application. This is the responsibility of the CITN COSC (Central Operating and Statistical Center). Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be found on the CTEP Web site (http://ctep.cancer.gov/reporting/cdus.html).

The Coordinating Center is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing the data to the Principal Investigator for review.

When setting the dates, allow time for Coordinating Center compilation, Principal Investigator review, and timely submission to CTEP by the quarterly deadlines (see Section 13.1.1).

## 12.2 CTEP Multicenter Guidelines

This protocol will adhere to the policies and requirements of the CTEP Multicenter Guidelines. The specific responsibilities of the Principal Investigator and the Coordinating Center and the procedures for auditing are presented in Appendix B.

- The Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports received from CTEP to all participating institutions for submission to their individual IRBs for action as required.

## 12.3 Collaborative Agreements Language: N/A

# 13. STATISTICAL CONSIDERATIONS

## 13.1 Study Design/Endpoints

The proposed clinical trial is a phase II, open-label, multicenter, randomized study of the administration of CYT107 after the completion of standard FDA-approved therapy with sipuleucel-T for patients with asymptomatic or minimally symptomatic mCRPC. Patients who have completed a standard course of sipuleucel-T will be randomly assigned to either observation or 10 μg/kg of subcutaneous CYT107 every week for 4 doses beginning 3 to 7 days after completing sipuleucel-T therapy. Day 0 is the day of the first injection of CYT107.

The first six patients randomly assigned to receive CYT107 will be followed for an additional 4 weeks prior to the study expanding past six patients, as a lead-in phase to ensure safety and tolerability of the selected CYT107 dose.

***Treatment Schema***

| Cohort | CYT107 Dose | Frequency |
|---|---|---|
| 1 - Observation | No CYT107 dose | -------- |
| 2 - CYT107 | 10 μg/kg | Days 0, 7, 14 and 21 |

IL-7 is expected to increase the peak and the persistence of the immune response. The persistence might be the most important for effective immunotherapy, i.e. increasing the "area under the curve". Thus, the primary endpoint for sizing the trial will be quantification of T-cell responses to PA2024 measured at day 70 (week 11), 7 weeks after the last CYT107 injection.

T cell responses will be measured by interferon-gamma ELISPOT as the most quantifiably and reproducible assay. In the IMPACT study, 2 weeks after last sipuleucel-T administration, the background-subtracted mean PA2024 ELISPOT was 40 SFC/ $3x10^5$ PBMC with a standard deviation of 69 based on n = 63. At 10 weeks after the last administration the background-subtracted mean PA2024 ELISPOT was 23 SFC/ $3x10^5$
PBMC with a standard deviation of 36 based on n = 42. Participant leukapheresis samples from pre- and post-Provenge® vaccine will be used when possible to allow planned a more comprehensive evaluation of longitudinal vaccine responses.

**Primary Endpoint:**
With 38 patients per group with evaluable data (40 patients per group, assuming 5% missing immunogenicity data), there is 80% power to detect a difference between a mean of 47 in the sipuleucel-T plus CYT107 group and a mean of 23 in the sipuleucel-T alone group, assuming the same standard deviation of 36 in both groups. This calculation is based on a two-sided t-test with a significance level of 0.05. The presumption is that the effect of CYT107 will be positive on all of the immune parameters and that the effect on each assay will track with positive effects on the other immune assays such that there should be similar power to detect differences in the other endpoints. These, however, are not considered primary endpoints, and hence no multiplicity adjustment will be made. The Mann-Whitney-Wilcoxon (MWW) Test will be used as part of the statistical analysis; the power is roughly equivalent to that based on the t-test. The effect of CYT107 will also be positive on clinical activity, but the results are likely to be more variable.

## 13.2 Sample Size/Accrual Rate

80 patients total will be enrolled in this study, 6-8 patients per month accrual total at approximately 12 CITN sites. Patients without adequate sampling for immune responses will be replaced.

| Accrual Targets | | | | |
|---|---|---|---|---|
| **Ethnic Category** | **Sex/Gender** | | | |
| | | **Males** | | **Total** |
| Hispanic or Latino | + | 15 | = | 15 |
| Not Hispanic or Latino | + | 65 | = | 65 |
| **Ethnic Category: Total of all subjects** | + | 80 (B1) | = | 80 (C1) |
| **Racial Category** | | | | |
| American Indian or Alaskan Native | + | 12 | = | 12 |
| Asian | + | 8 | = | 8 |
| Black or African American | + | 31 | = | 31 |
| Native Hawaiian or other Pacific Islander | + | 8 | = | 8 |
| White | + | 21 | = | 21 |
| **Racial Category: Total of all subjects** | + | 80 (B2) | = | 80 (C2) |

### 13.3 Stratification Factors

Patients will be stratified for prior abiraterone or neo-adjuvant chemotherapy with or without abiraterone in order to balance arms.

### 13.4 Analysis of Secondary Endpoints

1. To determine whether CYT107 administration increases the vaccine-induced antigen-specific T-cell immune response to PAP

   The effect on antigen-specific T-cell immune responses to PAP will be measured using the same assays and time points as for the primary endpoint, PA2024.

2. To assess the character of the T-cell immune response to PAP and PA2024

   The effect on the number and percentage of PBMC subsets and T lymphocyte subsets (i.e., $CD4^+$ and CD8+ T cell naïve, effector memory, and central memory subsets, $CD4^+$ Treg and activated CD4+ and CD8+ T cells) will be assessed using multiparameter flow cytometric analysis of whole blood. The PBMC immunophenotyping assay will quantify the absolute number and proportion of T cells (both CD8+ and CD4+), NK cells (CD56+CD3-), NKT cells (CD56+CD3+), B cells (CD19+), monocytes (CD16+), dendritic cells (HLA-DR+ CD123+ or CD11c+), and CD122 (IL15R-beta)-expressing cells.

   An additional panel will quantify the absolute number and proportion of memory and naïve T cell subsets using antibodies to CD45RA, CCR7 and CD28 of regulatory T cells using antibodies to CD127 and CD25, and the activation state of T cells using antibodies to PD-1, ICOS, and HLA-DR, among others. The absolute change in each parameter as well as variance in change over time for each patient (mean, median, and SE/SD) will be evaluated.

3. To determine whether CYT107 administration increases the vaccine-induced antigen-specific antibody immune responses to PAP and PA2024

   IgM and IgG response to PAP and PA2024 will be measured by standard ELISA.

4. To quantify the effects of CYT107 on T-cell diversity

   The T-cell repertoire will be characterized by T-cell receptor (TCR) deep sequencing and the analysis will be done with the Immunoseq Analyzer software (Harlan Robins, PhD, with Adaptive Biotechnologies, Seattle).

5. To assess the effects of CYT107 on the immune competence of patients with advanced prostate cancer

   Bystander antigen specific immune responses will be assessed to other ongoing and nascent antitumor responses (e.g., PRAME, NY-ESO-1 and/or p53), additional tumor antigens specific to prostate cancer (e.g., PSA and/or PSMA), and memory viral responses (influenza A and CEF) using the IFNg ELISPOT assay.

   Thymic function will be assessed by measuring levels of T-cell receptor rearrangement DNA excision circles (TRECs).

6. To assess the clinical efficacy and tolerability of sipuleucel-T plus CYT107 compared with sipuleucel-T alone

   Adverse events will be graded and reported using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. Clinical efficacy will be assess differences in (1) overall survival, (2) PSA kinetics (3) progression free survival and, (4) circulating tumor cells (CTC). Progression free survival will be assessed by RECIST criteria for measureable disease by bone scans and radiographic criteria for non-measurable bony metastasis.

## 13.5 Reporting and Exclusions

### 13.5.1 Evaluation of Toxicity

All patients will be evaluable for toxicity from the time of their first treatment with CYT107.

### 13.5.2 Evaluation of Response

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.]

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

All conclusions should be based on all eligible patients. Sub analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (*e.g.*, early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these sub analyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

**REFERENCES**

Arlen PM, Bianco F, Dahut WL, D'Amico A, Figg WD, Freedland SJ, Gulley JL, Kantoff PW, Kattan MW, Lee A, Regan MM, Sartor O. Prostate-Specific Antigen Working Group's Guidelines on PSA Doubling Time. J. Urol. 2008 June; 179(6): 2181-2186.

Beer TM, Bernstein GT, Corman JM, Glode LM, Hall S, Poll WL. Randomized trial of active cellular immunotherapy with sipuleucel-T in androgen dependent prostate cancer (ADPC) [abstract]. J Clin Oncol 25(June 20 Suppl), 5059 (2007).

Beer TM, Armstrong AJ, Rathkopf DE, Loriot Y, Sternberg CN, Higano CS, et al. Enzalutamide in metastatic prostate cancer before chemotherapy. N Engl J Med. 371:424-33 (2014).

Beq S, Rozlan S, Gautier D, Parker R, Mersseman V, Schilte C, Assouline B, Rancé I, Lavedan P, Morre M, Cheynier R. Injection of glycosylated recombinant simian IL-7 provokes rapid and massive T-cell homing in rhesus macaques. Blood 114(4):816-25 (2009).

Cheever MA, Higano CS. PROVENGE (Sipuleucel-T) in prostate cancer: the first FDA-approved therapeutic cancer vaccine. Clin Cancer Res 17, 3520-3527 (2011).

Colombetti S, Levy F, Chapatte L. IL-7 adjuvant treatment enhances long-term tumor-antigen-specific $CD8^+$ T-cell responses after immunization with recombinant lentivector. Blood 113, 6629–6637 (2009).

Eisenhauer EA, Therasse P, Bogaerts J, et al: New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 45:228-47, 2009

FDA.gov. [Homepage]. Clinical review—PROVENGE. Rockville, MD: US Food and Drug Administration Consumer Affairs Branch (CBER); 2010 [updated 2010 May 28; cited 2010 April]. Available from: http://www.fda.gov/BiologicsBloodVaccines/default.htm.

Fong L, Weinberg VK, Chan SE, Corman JM, Amling CL, Stephenson RA, Formaker C, Simko J, Brownell Sims R, Carroll P, Small EJ. Neoadjuvant sipuleucel-T in localized prostate cancer: Effects on immune cells within the prostate tumor microenvironment. J Clin Oncol 30, 2012 (suppl; abstr 2564)

Fry TJ, Moniuszko M, Creekmore S, Donohue SJ, Douek DC, Giardina S, *et al*. IL-7 therapy dramatically alters peripheral T-cell homeostasis in normal and SIV-infected nonhuman primates. Blood 101, 2294–2299 (2003).

Kaech SM, Tan JT, Wherry EJ, Konieczny BT, Surh CD, Ahmed R. Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. Nature Immunol 4, 1191–1198 (2003).

Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, *et al*. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 363,411–22 (2010).

Levy Y, Lacabaratz C, Weiss L, Viard JP, Goujard C, Lelièvre JD, *et al*. Enhanced T cell recovery in HIV-1-infected adults through IL-7 treatment. J Clin Invest 119, 997–1007 (2009).

Levy Y, Sereti I, Tambussi G, Routy JP, Lelievre JD, Delfraissy JF, Molina JM, Fischl M, Goujard C, Rodriguez B, Rouzioux C, Avettand-Fenoel V, Croughs T, Beq S, Morre M, Poulin JF, Sekaly RP, Thiebaut R, Lederman MM. Effects of rhIL-7 on T Cell Recovery and Thymic Output in HIV-infected Patients receiving antiretroviral therapy: results of a Phase I/IIa Randomized, Placebo Controlled, Multicenter Study. Clin Infect Dis. 2012 May 1. [Epub ahead of print]

Liu W, Putnam AL, Xu-Yu Z, Szot GL, Lee MR, Zhu S, *et al*. CD127 expression inversely correlates with FoxP3 and suppressive function of human $CD4^+$ $T_{Reg}$ cells. J Exp Med 203, 1701–1711 (2006).

Mackall CL, Fry TJ, Bare C, Morgan P, Galbraith A, Gress RE. IL-7 increases both thymic-dependent and thymic-independent T-cell regeneration after bone marrow transplantation. Blood 97, 1491–1497 (2001).

Mackall Cl, Fry TJ, Gress RE. Harnessing the biology of IL-7 for therapeutic application. Nature Rev Immunol 11, 330-342 (2011).

Melchionda F, Fry TJ, Milliron MJ, McKirdy MA, Tagaya Y, Mackall CL. Adjuvant IL-7 or IL-15 overcomes immunodominance and improves survival of the $CD8^+$ memory cell pool. J Clin Invest 115, 1177–1187 (2005).

Morrissey PJ, Conlon P, Braddy S, Williams DE, Namen AE, Mochizuki DY. Administration of IL-7 to mice with cyclophosphamide-induced lymphopenia accelerates lymphocyte repopulation. J Immunol 146, 1547–1552 (1991).

Nanjappa SG, Walent JH, Morre M, Suresh M. Effects of IL-7 on memory CD8 T cell homeostasis are influenced by the timing of therapy in mice. J Clin Invest 118, 1027–1039 (2008).

Park JH, Yu Q, Erman B, Appelbaum JS, Montoya-Durango D, Grimes HL, *et al*. Suppression of IL-7Rα transcription by IL-7 and other prosurvival cytokines: a novel mechanism for maximizing IL-7-dependent T cell survival. Immunity 21, 289–302 (2004).

Pellegrini M, Calzascia T, Elford AR, Shahinian A, Lin AE, Dissanayake D, *et al*. Adjuvant IL-7 antagonizes multiple cellular and molecular inhibitory networks to enhance immunotherapies. Nature Med 15, 528–536 (2009).

Perales M-A, Goldberg JD, Lechner L, Koehne G, Yuan J, Papadopoulos E, Young J, Jakubowski A, Zaidi B, Gallardo H, Kendle R, Liu C, Rasalan T, Xu Y, Wolchok J, Croughs T, Morre M, Maloy M, Heller G, Van den Brink, M. Recombinant human interleukin-7 (CYT107) enhances T-cell recovery without causing graft-versus-host disease following T-cell depleted allogeneic haematopoietic stem cell transplantation. Oral Session 5: Immunotherapy/Immune reconstitution, Annual Meeting of the European Group for Blood and Marrow Transplantation, Paris, France (April 2011).

Ryan CJ, Smith MR, de Bono JS, Molina A, Logothetis CJ, de Souza P, et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. N Engl J Med. 368:138-48 (2013).

Seddiki N, Santner-Nanan B, Martinson J, Zaunders J, Sasson S, Landay A, *et al*. Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. J Exp Med 203, 1693–1700 (2006).

Scher HI, Halabi S, Tannock I, Morris M, Sternberg CN, Carducci MA, et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. J Clin Oncol 2008; 26:1148-59.

Sereti I, Dunham RM, Spritzler J, Aga E, Proschan MA, Medvik K, *et al*. IL-7 administration drives T cell-cycle entry and expansion in HIV-1 infection. Blood 113, 6304–6314 (2009).

Sereti I, Estes J, Thompson W, Fischl M, Croughs T, Beq S, Yao M, Boulassel MR, Lederman MM, Routy J-P. Gut Mucosa T Lymphocyte Restoration in Chronically HIV-Infected Patients Treated with Recombinant Huma Interleukin-7 (rhIL-7). Conference on Retrovirus and Opportunistic Infections, Seattle, WA (March 2012).

Sheikh NA, De La Rosa CP, Kuan L, Stewart FP, Frohlich MW, Urdal DL, *et al*. Sipuleucel-T treatment results in sequential ex vivo activation of APCs and T cells during the culture step—evidence for in vivo immunological priming [abstract]. In: Proceedings of the 101st Annual Meeting of the American Association for Cancer Research; 2010 Apr 17–21; Washington, DC. Philadelphia (PA): AACR Abstract nr. 5608 (2010).

Sheikh NA, Petrylak D, De La Rosa CP, Stewart FP, Kuan LY, Whitmore JB, Trager JB, Poehlein CH, Frohlich MW, Urdal DL. Sipuleucel-T immune parameters correlate with survival: an integrated analysis of randomized Phase 3 clinical trials in men with castrate-resistant prostate cancer (2012).

Sportes C, Hakim FT, Memon SA, Zhang H, Chua KS, Brown MR, *et al*. Administration of rhIL-7 in humans increases in vivo TCR repertoire diversity by preferential expansion of naive T cell subsets. J Exp Med 205, 1701–1714 (2008).

Swainson L, Kinet S, Mongellaz C, Sourisseau M, Henriques T, Taylor N. IL-7-induced proliferation of recent thymic emigrants requires activation of the PI3K pathway. Blood 109, 1034–1042 (2007).

Takada K, Jameson SC. Naive T cell homeostasis: from awareness of space to a sense of place. Nature Rev Immunol 9, 823–832 (2009).

Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. N Engl J Med. 351:1502-12 (2004).

## APPENDIX A PERFORMANCE STATUS CRITERIA

| ECOG Performance Status Scale | | Karnofsky Performance Scale | |
|---|---|---|---|
| Grade | Descriptions | Percent | Description |
| 0 | Normal activity. Fully active, able to carry on 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 of his/her 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 more than 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 CTEP MULTICENTER GUIDELINES

If an institution wishes to collaborate with other participating institutions in performing a CTEP sponsored research protocol, then the following guidelines must be followed.

Responsibility of the Protocol Chair

- The Protocol Chair will be the single liaison with the CTEP Protocol and Information Office (PIO). The Protocol Chair is responsible for the coordination, development, submission, and approval of the protocol as well as its subsequent amendments. The protocol must not be rewritten or modified by anyone other than the Protocol Chair. There will be only one version of the protocol, and each participating institution will use that document. The Protocol Chair is responsible for assuring that all participating institutions are using the correct version of the protocol.
- The Protocol Chair is responsible for the overall conduct of the study at all participating institutions and for monitoring its progress. All reporting requirements to CTEP are the responsibility of the Protocol Chair.
- The Protocol Chair is responsible for the timely review of Adverse Events (AE) to assure safety of the patients.
- The Protocol Chair will be responsible for the review of and timely submission of data for study analysis.

Responsibilities of the Coordinating Center

- Each participating institution will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH. The Coordinating Center is responsible for assuring that each participating institution has an OHRP assurance and must maintain copies of IRB approvals from each participating site.
- Prior to the activation of the protocol at each participating institution, an OHRP form 310 (documentation of IRB approval) must be submitted to the CTEP PIO.
- The Coordinating Center is responsible for central patient registration. The Coordinating Center is responsible for assuring that IRB approval has been obtained at each participating site prior to the first patient registration from that site.
- The Coordinating Center is responsible for the preparation of all submitted data for review by the Protocol Chair.
- The Coordinating Center will maintain documentation of AE reports. There are two options for AE reporting: (1) participating institutions may report directly to CTEP with a copy to the Coordinating Center, or (2) participating institutions report to the Coordinating Center who in turn report to CTEP. The Coordinating Center will submit AE reports to the Protocol Chair for timely review.
- Audits may be accomplished in one of two ways: (1) source documents and research records for selected patients are brought from participating sites to the Coordinating Center for audit, or (2) selected patient records may be audited on-site at participating sites. If the NCI chooses to have an audit at the Coordinating Center, then the Coordinating Center is responsible for having all source documents, research records, all IRB approval documents, NCI Drug Accountability Record forms, patient registration lists, response assessments scans, x-rays, etc. available for the audit.

Inclusion of Multicenter Guidelines in the Protocol

- The protocol must include the following minimum information:
  - The title page must include the name and address of each participating institution and the name, telephone number and e-mail address of the responsible investigator at each participating institution.
  - The Coordinating Center must be designated on the title page.
  - Central registration of patients is required. The procedures for registration must be stated in the protocol.
  - Data collection forms should be of a common format. Sample forms should be submitted with the protocol. The frequency and timing of data submission forms to the Coordinating Center should be stated.
  - Describe how AEs will be reported from the participating institutions, either directly to CTEP or through the Coordinating Center.
  - Describe how Safety Reports and Action Letters from CTEP will be distributed to participating institutions.

Agent Ordering

- Except in very unusual circumstances, each participating institution will order DCTD-supplied investigational agents directly from CTEP. Investigational agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO.

## APPENDIX C COCKCROFT-GAULT FORMULA FOR CALCULATED CREATININE CLEARANCE

This formula expects weight to be measured in kilograms and creatinine to be measured in mg/dL, as is standard in the USA.

$$eC_{Cr} = \frac{(140 \text{ - Age}) \times \text{Mass (in kilograms)} \times [0.85 \; if \; Female]}{72 \times \text{Serum Creatinine (in mg/dL)}}$$

## APPENDIX D DOSE ADJUSTMENT FOR OBESE PATIENTS

Obesity is defined as a BMI > 30 (NHLBI Class 1 Obesity.)

In order to determine the proper dose for patients classified as obese, an adjusted body weight is calculated. The following steps are provided to assist with the process:

### BMI Chart

| BMI | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 35 | 40 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ht | Wt (lbs) | | | | | | | | | | | | | |
| 4'10" | 91 | 96 | 100 | 105 | 110 | 115 | 119 | 124 | 129 | 134 | 138 | 143 | 167 | 191 |
| 4'11" | 94 | 99 | 104 | 109 | 114 | 119 | 124 | 128 | 133 | 138 | 143 | 148 | 173 | 198 |
| 5'0" | 97 | 102 | 107 | 112 | 118 | 123 | 128 | 133 | 138 | 143 | 148 | 153 | 179 | 204 |
| 5'1" | 100 | 106 | 111 | 116 | 122 | 127 | 132 | 137 | 143 | 148 | 153 | 158 | 185 | 211 |
| 5'2" | 104 | 109 | 115 | 120 | 126 | 131 | 136 | 142 | 147 | 153 | 158 | 164 | 191 | 218 |
| 5'3" | 107 | 113 | 118 | 124 | 130 | 135 | 141 | 146 | 152 | 158 | 163 | 169 | 197 | 225 |
| 5'4" | 110 | 116 | 122 | 128 | 134 | 140 | 145 | 151 | 157 | 163 | 169 | 174 | 204 | 232 |
| 5'5" | 114 | 120 | 126 | 132 | 138 | 144 | 150 | 156 | 162 | 168 | 174 | 180 | 210 | 240 |
| 5'6" | 118 | 124 | 130 | 136 | 142 | 148 | 155 | 161 | 167 | 173 | 179 | 186 | 216 | 247 |
| 5'7" | 121 | 127 | 134 | 140 | 146 | 153 | 159 | 166 | 172 | 178 | 185 | 191 | 223 | 255 |
| 5'8" | 125 | 131 | 138 | 144 | 151 | 158 | 164 | 171 | 177 | 184 | 190 | 197 | 230 | 262 |
| 5'9" | 128 | 135 | 142 | 149 | 155 | 162 | 169 | 176 | 182 | 189 | 196 | 203 | 236 | 270 |
| 5'10" | 132 | 139 | 146 | 153 | 160 | 167 | 174 | 181 | 188 | 195 | 202 | 207 | 243 | 278 |
| 5'11" | 136 | 143 | 150 | 157 | 165 | 172 | 179 | 186 | 193 | 200 | 208 | 215 | 250 | 286 |
| 6'0" | 140 | 147 | 154 | 162 | 169 | 177 | 184 | 191 | 199 | 206 | 213 | 221 | 258 | 294 |
| 6'1" | 144 | 151 | 159 | 166 | 174 | 182 | 189 | 197 | 204 | 212 | 219 | 227 | 265 | 302 |
| 6'2" | 148 | 155 | 163 | 171 | 179 | 186 | 194 | 202 | 210 | 218 | 225 | 233 | 272 | 311 |
| 6'3" | 152 | 160 | 168 | 176 | 184 | 192 | 200 | 208 | 216 | 224 | 232 | 240 | 279 | 319 |
| 6.4” | 156 | 164 | 172 | 180 | 189 | 197 | 205 | 213 | 221 | 230 | 238 | 246 | 287 | 328 |

If BMI > 30, proceed with the following steps:

1. **Determine Ideal Weight (1 kg = 2.2 lbs):**
   **Males:** 50 kg + (2.3 kg/inch over 5 feet)
   **Females:** 45.5 kg + (2.3 kg/inch over 5 feet)
   (Patients less than 5 feet: subtract 2.3 kg/inch)

2. **Determine Adjusted Body Weight:**

Ideal Weight + 0.25 (actual weight – ideal weight) = Adjusted Body Weight

Dose of CYT107 is 10 µg/kg. Vial is 1mg/0.5cc.