Statistical Analysis Plan
CITN12-03 NCT01881867

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Protocol: 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)

STATISTICAL CONSIDERATIONS

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.

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)

Stratification Factors

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

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.