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Translational Program in Engineered Tumor Immunity

TITLE: Adoptive Transfer of MART-1 F5 TCR Engineered Peripheral Blood Mononuclear Cells (PBMC) after a Nonmyeloablative Conditioning Regimen, with Administration of MART-$1_{26\text{-}35}$-Pulsed Dendritic Cells and Interleukin-2, in Patients with Advanced Melanoma

UCLA IRB #:	**10-001212**
UCLA ISPRC/DSMB	**10-001212**
FDA IND #:	**13859**
NIH OBA/RAC #:	**0802-901**
UCLA IBC #:	**53.08.0-h/BUA-2016-005**
UCLA GCRC #	**1600**
Clinical Trial Registration #:	**NCT00910650**

VERSION DATE:	**01/10/2018, Amendment 15**
Study Agent:	**Retroviral Vector MART-1 F5 TCR (F5 TCR) Engineered PBMC**

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1. STUDY SYNOPSIS

1.1 Overview

This is a two-stage phase II clinical trial with the combined primary endpoints to determine the safety, feasibility and antitumor activity of adoptive transfer of peripheral blood mononuclear cells (PBMC) genetically engineered to express the alpha and beta chains of a high affinity T cell receptor (TCR) specific for the HLA-A*0201-restricted MART-1 melanoma tumor antigen to patients with locally advanced or metastatic melanoma. This gene transfer will be affected by a retroviral vector pseudotyped with a gibbon ape leukemia virus (GaLV) envelope. The two transgenes genes are linked by a picornavirus 2A sequence. Their expression is driven by the retroviral long terminal repeat (LTR).

Patients with MART-1-positive locally advanced or metastatic melanoma who are HLA-A*0201-positive, and HIV, hepatitis B and C seronegative, will receive a non-myeloablative but lymphocyte depleting chemotherapy conditioning regimen consisting of cyclophosphamide and fludarabine, and then receive the adoptive transfer of autologous PBMC transduced with the MSGV1-F5AfT2AB retroviral vector, which expresses a high affinity TCR for the MART-1 melanoma antigen (MART-1 F5 TCR). The cell dose will be up to 10^9 autologous PBMC transduced with the MSGV1-F5AfT2AB retroviral vector. The transgenic T cells will be infused fresh on the day of harvest as done in the last three patients within this protocol (since Amendment 8), prior to which, thawed cryopreserved cells were infused. Following adoptive cell transfer, patients will receive MART-1_{26-35} peptide-pulsed dendritic cell (DC) vaccines and low dose interlukin-2 (IL-2) under Amendment 11.

The MART-1 F5 TCR was provided by Dr. Stephen A. Rosenberg from the Surgery Branch, National Cancer Institute (NCI). The MART-1 F5 TCR is derived from the DMF5 tumor infiltrating lymphocyte (TIL) clone, and was selected from several MART-1-specific TCRs because of its high affinity and biological activity. This TCR delivered by the same retroviral vector is currently in clinical testing at the Surgery Branch/NCI. Both the NCI clinical trial and the trial at UCLA are based on the same retrovirus expressing the MART-1 F5 TCR used to transduce whole PBMC and re-infused to patients after a non-myeloablative but lymphodepleting chemotherapy conditioning regimen. Major differences between both clinical trials include the shorter *ex vivo* expansion of TCR transduced PBMC, the use of MART-1_{26-35} peptide pulsed DC and the use of positron imaging tomography (PET) for non-invasive imaging of adoptively transferred TCR transgenic cells in the UCLA clinical trial.

The primary endpoints will be safety, feasibility and objective tumor response. The phase II clinical trial design will have two treatment stages following a Simon optimal two-stage clinical phase II clinical trial design [1]. The clinical trial will have an initial stage with 8 patients followed by a second stage with up to 22 patients.

Safety will be determined in stage one, and if 3 out of 8 patients have MART-1 F5 TCR-induced dose limiting toxicities (DLT), then further accrual will not be warranted. Feasibility will be also determined in the first stage, and if 3 out of 8 patients cannot receive the intended cellular therapies, or if they result in suboptimal TCR transgenic cell *in vivo* persistence, further accrual will not be warranted to the protocol as currently designed. Objective tumor responses will be determined by RECIST objective response criteria with a design to rule out a 10% response rate as the null hypothesis, and a 35% response rate as the alternative hypothesis. With this statistical design, if 2 or more of 8 patients in stage one have an objective response, the study will proceed to stage two and accrue a total of 22 patients. If 5 or more patients in the overall study have a complete response (CR) or partial response (PR), which combined result in the objective response rate, the study will be declared positive.

Secondary study endpoints are transgenic T cell persistence in humans and their ability to home to MART-1 positive melanoma metastasis. Analysis will be performed by sampling of peripheral blood and tumor deposits for T cell persistence and by non-invasive metabolic imaging using PET scans.

1.1.1. Cell Dose Escalation within Amendment 6 after Meeting the Safety and Feasibility Criteria But Not Meeting the Initial Antitumor Criteria

Under the initial protocol 8 patients were accrued to stage 1 of this clinical trial. The criteria for safety and feasibility were met, with no dose limiting toxicities (DLTs) and feasibly delivering the intended cell therapies. However, the response rate was less than required to meet the minimal antitumor activity of 35% response rate, with less than 2 patients with an objective response at day 90 of the protocol among the first 8 patients. Therefore, the protocol as initially planned was halted and Amendment 6 was generated administering a cell dose escalation up to 10^{10} F5 TCR transgenic cells, or the maximum feasible for each patient, in a new start of this stage 2 clinical trial with an initial stage enrolling up to 8 patients following the same statistical design as the original protocol.

1.1.2. Cell Dose De-escalation and Change to Low Dose IL-2 with Amendment 11 After Exceeding the Safety Criteria Under Amendments 6-10

The safety of increasing the F5 TCR transgenic cell dose to 10^{10} within the basic F5 TCR ACT protocol from UCLA was assessed under Amendments 6 to 10. Dose escalation to up to 10^{10} cryopreserved cells was undertaken in 2 patients, and then the protocol was additionally modified to administer cells that had not undergone cryopreservation (Amendments 8 to 10) in three additional patients. However, two of these patients developed SAEs of respiratory distress requiring intubation. Thereafter, the total adoptively transferred cell dose was decreased back to a maximum of 10^9 cells and the regimen of administration of IL-2 was changed to LD IL-2 (Amendment 11).

1.2. Rationale and Hypothesis

Rationale for TCR Transgenic Cell Adoptive Transfer Therapy. The adoptive transfer of large numbers of clonally-expanded antigen-specific T cells into patients with melanoma that have received a conditioning regimen to deplete endogenous lymphocytes (non-myeloablative but lymphodepleting), together with high doses of interleukin-2 (IL-2), results in the highest rate of melanoma responses (50%) reported to date [2,3]. This approach provides a proof-of-principle for adoptive transfer immunotherapy for metastatic melanoma, but it is difficult to implement outside of pilot studies given its requirement for extensive *ex vivo* manipulations. The ability to generate large numbers of tumor antigen-specific T cells by a single *ex vivo* manipulation consisting of the transduction of melanoma-specific TCR genes may result in a more broadly applicable approach for patients with melanoma. In this project we will test the hypothesis that the engineering of human PBMC to express a high affinity TCR specific for the human melanoma antigen MART-1 is safe, feasible and can induce objective tumor responses in human subjects with advanced melanoma.

Rationale for the Clinical Trial Study Design and Primary Endpoints. This two-stage phase 2 clinical trial is designed to allow for the simultaneous testing of 3 primary endpoints. Safety and feasibility are being tested during stage one. The clinical trial will not proceed to stage two if one third or greater numbers of patients develop DLTs. This is the standard level for DLT assessment as safety criteria in phase 1 dose-escalation clinical trials. In addition, if the study drugs and procedures cannot be delivered as planned in over one third of patients, assessed by not meeting the study feasibility criteria (see below), then enrollment to stage two will not be warranted. The protocol would need to be amended to meet the feasibility criteria before further enrollment. Antitumor activity is assessed in the overall phase 2 clinical trial, but the study will only proceed to enroll in stage two if a minimal antitumor activity level is evident in stage one (see below).

1.3 Study Objectives:

Primary:

- SAFETY. This TCR and retroviral vector at a cell dose up to 10^{11} have been already in clinical testing within a reported phase 2 clinical trial at the Surgery Branch/NCI. We started with a maximum of up to 10^9 MART-1 F5 TCR transgenic cells for infusion. Since we did not meet the minimal hurdle of antitumor activity to proceed to stage 2 of this research after having entered the first 8 patients, we increased the dose to a maximum of up to 10^{10} (Amendment 6) which is still one log lower than the maximum cell dose within the NCI protocol. Therefore, there was no need for a standard phase1 dose escalation clinical trial design. After enrolling two patients under this increased dose, we further changed to infusion of freshly harvested transgenic cells in place of cryopreserved cells, keeping the same increased dose of up to 10^{10} (Amendment 8). Two out of three patients enrolled under this regimen required intubation for respiratory distress. Thereby, we propose to decrease the dose to 10^9 freshly harvested MART-1 F5 TCR transgenic cells and change to low dose IL2 in this amendment (Amendment 11). Safety continues to be a major concern and a clinical endpoint of this clinical trial. Therefore, the study is designed with an early stopping rule based on development of DLTs in one third or higher among patients enrolled in the initial stage of this clinical trial. Safety will be determined in stage one, and if 3 out of 8 patients have MART-1 F5 TCR-induced DLT, then further accrual will not be warranted.

- FEASIBILITY. This clinical trial intends to deliver two patient-specific cell therapies, the TCR transgenic PBMC and MART-$1_{26\text{-}35}$ peptide pulsed DC, within a study design that requires other significant interventions, like a lymphodepletive conditioning regimen and post-infusion high dose IL-2 (up to Amendment 10), and then low dose IL-2 (after Amendment 11). This protocol has significant technical challenges, making feasibility a primary endpoint and requiring a clinical trial design with a feasibility early stopping rule. Therefore, feasibility will be determined in stage one, and if 3 out of 8 patients cannot receive the intended cellular therapies further accrual will not be warranted.

- CLINICAL RESPONSE. The overall phase 2 clinical trial statistical design has objective tumor response as the main criteria for determining the number of study subjects. Objective tumor responses will be determined by RECIST objective response criteria, which are defined as adding the rate of complete response (CR) and partial response (PR) at restaging exams on day 90 of the protocol. To account for the frequently observed delayed responses with immunotherapy, patients with disease stabilization at day 90 who do not receive further therapy but go onto meet RECIST criteria for CR or PR will also be considered as having objective response on study. The clinical trial statistical design is aimed at ruling out a 10% response rate as the null hypothesis, and has a 35% response rate as the alternative hypothesis. The minimum hurdle to proceed from stage one to stage two of this clinical trial will be observing at least 2 objective responses out of the 8 initial patients. The study will be considered a positive study ruling out the null hypothesis if 5 patients have an objective response among 22 evaluable patients enrolled in the two stages of this phase II clinical trial.

Secondary:

- MART-1 F5 TCR TRANSGENIC CELL PERSISTENCE. To determine the persistence of MART-1 F5 TCR-engineered PBMC in serial peripheral blood samples and in biopsies of accessible metastatic melanoma lesions.

- *IN VIVO* MOLECULAR IMAGING. To explore the use of PET-based imaging using the PET tracer [^{18}F]FDG with the goal of determining if the adoptively transferred MART-1 F5 TCR-

engineered PBMC home and expand in secondary lymphoid organs and melanoma tumor deposits. Findings with non-invasive molecular imaging will be compared with results from immune monitoring assays in blood samples and tumor biopsies at different intervals after MART-1 F5 TCR PBMC adoptive transfer.

1.4 Study Population

Subjects must be HLA-A*0201, have locally advanced (stage IIIc) or metastatic melanoma (stage IV) with no standard therapeutic options with a curative intent, that is MART-1-positive, age greater than or equal to 18, clinical performance status of ECOG 0 or 1, life expectancy greater than 3 months, and be seronegative for HIV, Hepatitis B and C (seropositive subjects would be more likely to have toxic effects from the conditioning chemotherapy or may not tolerate it). Study subjects should have accessible lesions amenable to outpatient tumor biopsies that still allow an adequate assessment of the primary endpoint of antitumor activity and clinical response assessment.

1.5 Number of Patients

The phase 2 clinical trial design will have two treatment stages, an initial one with 8 patients, followed by a second stage with 14 additional patients (for a total of 22 patients). All patients will receive the same combined therapy of: 1) a lymphodepleting preparative regimen, 2) autologous PBMC retrovirally-transduced with a MART-1 F5 TCR, followed by 3) IL-2 and 4) MART-$1_{26\text{-}35}$ peptide–pulsed DC.

1.6 Study Duration:

Total duration of the study is estimated to be approximately 18-24 months, if recruitment proceeds optimally with 2 patients screened each month, resulting in a mean of 1 to 2 patients entered in the trial every month.

The initial screening period will be 30 days, but patients will be allowed to enroll after the 30-day initial screening period, for up to approximately 60 days, using the same screening procedure test results to avoid unnecessary assay duplication. A pregnancy test must be completed within 14 days prior to dosing, if applicable.

1.7 Study Schematic

Patients will undergo an initial leukapheresis to collect PBMC. The manufacture of both transgenic T cells and DC will be started on the day of leukapheresis. Immediately after processing the PBMC, $1.5\text{x}10^9$ PBMC will be put in activation media containing OKT3 (anti-CD3 antibody) and IL-2 for two days, $12\text{x}10^8$ PBMC will be set up for DC culture and the rest will be cryopreserved. The next day after leukapheresis, patient will start receiving the non-myeloablative conditioning chemotherapy. Following activation, cells are transduced twice on two consecutive days with the MSGV1-F5AfT2AB retrovirus in retronectin-coated plates. Transduced cells will be expanded *ex vivo* for 4 days after the first transduction and then put into an infusion bag to be infused i.v. fresh on the day of harvest as soon as the lot release is cleared. The following day, after a week of *ex vivo* differentiation culture in GM-CSF and IL-4, DC is harvested, peptide pulsed and administered i.d. Patient will also receive systemic low dose IL-2 for 7 days for up to 14 doses.

In the event the physician decides that the patient will not be clinically fit to receive the gene-modified MART-1 TCR CTL infusion within 24 hours of the scheduled day of cell harvest, the cells will be cryopreserved on the harvest day for later use.

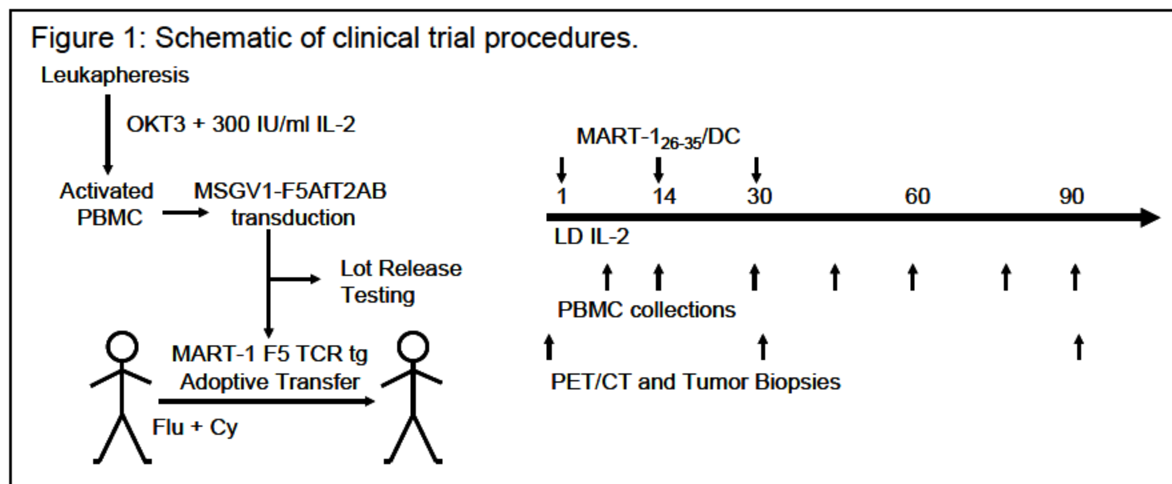

Figure 1: Schematic of clinical trial procedures.

Patients will receive two more MART-1$_{26-35}$ peptide pulsed DC vaccines at two weeks intervals that will be generated from cryopreserved PBMC following the same protocol of one week *ex vivo* differentiation culture in GM-CSF and IL-4 and peptide pulsing. Patients will undergo repeated peripheral blood sampling, PET CT scanning and biopsies of tumor deposits (Figure 1). Although evidence indicates that it is not directly related to the F5 MART-1 TCR transgenic T cells, considering the two recent SAE cases requiring intubation, 5 to 10 ml blood samples will be collected daily during the first week after adoptive cell transfer routinely and plasma derived from these samples will be cryopreserved and archived for cytokine analyses when necessary.

1.8 Experimental Study Agents

1.8.1 Retrovirus vector MSGV1-F5AfT2AB:

The retroviral vector, MSGV1-F5AfT2AB, was generated at the Surgery Branch/NCI by Dr. Steven A. Rosenberg and colleagues. It consists of 4,196 bps including the 5' LTR from the murine stem cell virus (promoter), packaging signal including the splicing donor and splicing acceptor sites, alpha chain and beta chain genes of the anti-MART-1 F5 TCR from TIL clone DMF5, and murine stem cell virus 3'LTR. The alpha and beta TCR chains are linked by a T2A self-cleaving sequence.

1.8.2 MART-1$_{26-35}$ Peptide Pulsed Dendritic Cells:

Autologous DC will be differentiated from adherent peripheral blood monocytes in a one-week *in vitro* culture in GM-CSF and IL-4 as we have previously described [4,5]. DC will be pulsed with the MART-1$_{26-35}$ anchor-modified immunodominant peptide in the HLA-A2*0201 haplotype [6] and administered i.d.

1.9 Timing of Events:

To account for the pilot investigational nature of the procedures in this protocol and anticipated problems in patient scheduling, timing of events are approximate and the study team will attempt to perform them on the dates included in this protocol, but variations on these dates will not be considered protocol violations.

2.0 Schedule of Events

	Screening	Day -6	Day –5 and -4	Day –4 to -1	Day 0	Day 1	Day 1 to 7	Day 14	Day 20 to 40	Day 30	Day 45	Day 60	Day 75	Day 75-90	Day 90	Q2-3 mo up to Year 2[a]	Q6 o up to Year 5[f]	Q12 mo up to Year 15[g]
Informed Consent	X																	
Visit and Physical	X	X								X	X	X	X		X	X	X	X
HLA Typing	X																	
PCR or IHC for MART-1	X																	
CBC[b]	X	X	X	X	X					X	X	X		X				
Metabolic Panel[b]	X	X	X	X	X					X	X	X		X				
LDH	X									X	X	X		X				
CRP	x													X				
Uric Acid	x	x																
Magnesium	x	x																
Phosphorous	x	x																
Lipase	x																	
Amylase	x																	
Coagulation Tests: PT/INR, PTT	x																	
Autoantibody Panel	X													X				
Thyroid Hormones	X													X				
Pregnancy Test[c]	X													X				
Infectious disease serologies	X																	
CT or MRI Scan of the Brain	X													X				
Otologic Visit	X													X				
Ophthalmologic Visit	X													X				
Leukapheresis		X							X									
Plasmapheresis		X							X									
Blood for plasma (cytokines)[d]							X	X										
Blood for cell analyses (immune monitoring)[e]	X							X		X	X	X	X		X	X	X	X
Biopsy	X								X									
ECHO or MUGA	X																	
ECG	X																	
PFT	X																	

	Screening	Day -6	Day –5 and -4	Day –4 to -1	Day 0	Day 1	Day 1 to 7	Day 14	Day 20 to 40	Day 30	Day 45	Day 60	Day 75	Day 75-90	Day 90	Q2-3 mo up to Year 2[a]	Q6 o up to Year 5[f]	Q12 mo up to Year 15[g]
PET/CT Scans	X								X					X				
Inpatient admission		X																
Cyclophosphamide			X															
Fludarabine				X														
MART-1 F5 TCR PBMC					X													
Low dose IL-2							X											
MART-1$_{26-35}$/Dendritic Cell Vaccine						X		X		X								

[a]Long term follow up is described in section 8.15. For the first 2 years, office visits and blood collection will be at least every 3 months.
[b]While patients are in the hospital, physical, CBC and metabolic panel will be per J-Medicine protocol.
[c]Females only.
[d]Additional blood for protein analyses in plasma or serum will only be collected after day 30 if there is a suspicion of a cytokine storm or an acute event requiring additional analyses in blood. The plasma or serum may be collected as 5-10 ml blood samples in anticoagulant or stored as the liquid phase of a Ficoll gradient when storing PBMCs.
[e]Blood draws on these days should be performed if considered safe due to the pancytopenia and hemodynamic state of the patient. Can be done +/- 6 days.
[f]After 2 years, office visits or phone follow-up at least every 6 months. Collection of blood for immune monitoring analysis and for RCR testing annually.
[g]After 5 years, office visits or phone follow-up at least annually. Collection of blood for immune monitoring analysis and for RCR testing annually.
In general, changes in the range of 7 days up or down from the scheduled event will not be considered significant deviations from the study timeline.

3.0 TABLE OF ABBREVIATIONS

ACT: Adoptive cell transfer
AE: Adverse event
ATG: Anti-Thymocite Globulin
CBC: Complete blood count
CFR: Code of federal regulations
CMV: Cytomegalovirus
CR: Complete response
CRF: Case report form
CRP: C reactive protein
CT: Computer tomography.
CTLs: Cytotoxic T lymphocytes
DC: Dendritic cells
DLT: Dose limiting toxicity
DSMB: Data Safety and Monitoring Board
EBV: Epstein-Barr virus
FDA: Food and Drug Administration
[^{18}F]FLT: [^{18}F] fluoro-L-thymidine
[^{18}F]FDG: [^{18}F]fluorodeoxy-glucose
GaLV: Gibbon ape leukemia virus
GCP: Good Clinical Practices
GCV: Ganciclovir
GM-CSF: Granulocyte-macrophage colony stimulating factor
GMP: Good Manufacturing Practices
GVHD: Graft-versus-host disease
GVL: Graft-versus-leukemia
HBV: Hepatitis B virus
HCV: Hepatitis C virus
HCT: Hematopoietic cell transplantation
H&E: Hematoxilin eosin staining
HEPA: High-efficiency particulate air
HGMP: Human Gene Medicine Program
HIV: Human immunodeficiency virus
HLA: Human leukocyte antigen
IBC: Institutional Biosafety Committee
ICF: Informed consent form
ICS: Intracellular Cytokine Staining
i.d.: Intradermal
IHC: Immunohistochemistry
IU VPF: Indiana University Virus Production Facility
i.v.: Intravenous
IQAC: Internal Quality Assurance Committee
IRB: Institutional Review Board
ISPRC: Internal Scientific Peer Review Committee
IL: Interleukin
JCCC: Jonsson Comprehensive Cancer Center
LDH: Lactate dehydrogenase
LTR: Long terminal repeat
MHC: Major histocompatibility complex
MLV: Molonely Leukemia Virus
MRI: Magnetic resonance imaging
MSCV: Murine stem cell virus
N.C.I.: National Cancer Institute
NGVL: National Gene Vector Laboratory

N.I.H.: National Institutes of Health
PBMC: Peripheral blood mononuclear cells
PCR: Polymerase chain reaction
PD: Progressive disease
PET: Positron emitting tomography
PR: Partial response
RAC: Recombinant Advisory Committee
RCR: Replication competent retrovirus
RECIST: Response Evaluation Criteria in Solid Tumors
SAE: Serious adverse event
S.C.: Subcutaneous
SD: Stable disease
SUV: Standardized uptake value
TSH: Thyroid stimulating hormone
TCR: T cell receptor
UCLA: University of California Los Angeles

4.0 STUDY OBJECTIVES

4.1 Primary:

4.1.1 Safety

The MART-1 F5 TCR and the retroviral vector MSGV1-F5AfT2AB have already been tested in two clinical experiences, including a completed and reported phase 2 clinical trial by investigators at the Surgery Branch/NCI [7], and the second experience being the 8 patients treated at UCLA under this same IND with the F5 TCR transgenic cell dose up to 10^9. Overall, the ACT of F5 TCR transgenic cells after a lymphodepletion protocol with high dose IL-2 and DC vaccination was well tolerated with no dose limiting toxicities (DLTs) developed in the first 8 patients. The safety of increasing the F5 TCR transgenic cell dose to 10^{10} within the basic F5 TCR ACT protocol from UCLA was assessed in stage 1 of the protocol described in Amendment 6. Dose escalation to up to 10^{10} cells was undertaken in the following 2 patients, and after that the protocol was additionally modified to administer cells that had not undergone cryopreservation (Amendment 8) in three additional patients. However, two of these patients had serious adverse events (SAE) of respiratory distress requiring intubation. Therefore, the total adoptively transferred cell dose was decreased back to a maximum of 10^9 cells and the regimen of administration of IL-2 was changed to LD IL-2 (Amendment 11).

Safety evaluations will continue into the second stage of the study and the same early stopping rules for DLTs will apply to the second stage of this study. Safety remains the priority in all phases and stage of the investigational studies.

4.1.2 Feasibility

This protocol attempts to administer two cell-based therapies that require laboratory manipulation within the setting of Good Manufacturing Practices (GMP). Each cell therapy, the MART-1 F5 TCR engineered T cells and the MART-1_{26-35} peptide pulsed DC, will require strict lot release criteria of the final product before administration. Feasibility will be determined in the first stage, and if 3 out of 8 patients cannot receive the intended cellular therapies, or there is suboptimal TCR transgenic cell persistence *in vivo*, further accrual will not be warranted. The feasibility assessment will be based on:

i) Potential problems in the manufacturing of MART-1 F5 TCR engineered PBMC or the MART-1_{26-35} peptide–pulsed DC vaccines.
ii) Suboptimal number of MART-1 F5 TCR engineered T cells recovered from patients at pre-specified time points.

4.1.3. Clinical Response

Objective tumor responses will be determined by RECIST objective response criteria. The rate of CR plus PR will be used to assess the primary endpoint of antitumor activity with a clinical trial design to rule out a 10% response rate as the null hypothesis, and 35% response rate as the alternative hypothesis. Response assessment will be performed by comparing standard CT imaging scans and photographs of target lesions from baseline with repeated imaging tests obtained at day +90 after the TCR transgenic PBMC adoptive transfer.

4.2 Secondary

4.2.1 MART-1 F5 TCR Transgenic T Cell Persistence.

The persistence of MART-1 F5 TCR-engineered cells will be determined by analyzing serial peripheral blood samples for the presence of T cells with the MART-1 F5 TCR by tetramer or dextramer analysis. In addition and when technically feasible, samples from metastatic melanoma

sites will be biopsied and assessed for the presence of the MART-1 F5 transgenic cells by IHC, PCR and/or immune monitoring assays in non-adherent TIL.

4.2.2 *In Vivo* Imaging.

The adoptive transfer of MART-1 redirected TCR transgenic cells has the goal of targeting a tumor antigen expressed by melanoma cells. We plan to conduct serial PET molecular imaging using the tracer [^{18}F]FDG to determine changes in the metabolic rate in secondary lymphoid organs and tumor metastasis. Accumulation of immune cells in secondary lymphoid organs and tumor lesions will result in an increase in glucose uptake and retention, which will result in an increase in the standardized uptake value (SUV) of [^{18}F]FDG. The images obtained by whole-body PET will be matched with analysis of tumor biopsies to attempt to quantify the number of tumor-infiltrating MART-1 F5 TCR transgenic T cells.

5.0 BACKGROUND

5.1 Current Management of Surgically-incurable Melanoma

Locally advanced and metastatic melanoma is notoriously resistant to standard forms of therapy. Five agents are approved by the U. S. Food and Drug Administration (FDA) for the treatment of stage IV melanoma. The chemotherapy drugs dacarbazine (DTIC) and hydroxiurea, and the administration of high doses of IL-2 [8,9] were approved based on single arm studies. . These have response rates below 15%, and neither form of therapy has been shown to increase survival in a randomized trial. Two new agents have been approved for the treatment of metastatic melanoma based on a demonstrated impact on overall survival, the anti-CTLA4 antibody ipilimumab (Yervoy) and the BRAF inhibitor vemurafenib (Zelboraf). Ipilimumab induces low frequency of response rates (in the range of 10%) that tend to be durable and have translated into improved overall survival in two randomized clinical trials [10,11]. Vemurafenib gives a high response rate in BRAF V600 mutant metastatic melanoma, but median duration of response is 6-7 months [12,13]. In a randomized clinical trial it improved overall survival compared to dacarbazine [14].

5.2 Rationale for Immune-based Therapies for Metastatic Melanoma.

Progress in our understanding of how the immune system recognizes and kills cancer cells has led to the notion that high levels of circulating, tumor-antigen-specific T cells may be required for effective antitumor responses. Active immunotherapy with several forms of cancer vaccines has shown that antigen-specific T cells can be activated and occasionally lead to antitumor responses [15,16]. However, the circulating levels of antigen-specific T cells and tumor response rates are much lower than desirable [17].

Adoptive transfer of clonally-expanded, tumor antigen-specific lymphocytes to lymphopenic hosts after nonmyeloablative conditioning chemotherapy has resulted in cell proliferation and persistent clonal repopulation [2]. This approach has resulted in an objective response rate over 50% in patients with metastatic melanoma, that holds up in larger groups of patients [3]. The major limitation for the broad clinical applicability of this approach is the requirement for large-scale *ex vivo* lymphocyte culture expansion (up to 10^{11} cells) [2], which restricts this approach to a highly select group of patients.

5.3 MART-1 Melanoma Antigen as Target for Melanoma Immunotherapy

Multiple lines of evidence suggest that most tumor responses to immunotherapy involve the activation of tumor antigen-specific CD8+ cytotoxic T lymphocytes (CTL) [18]. These immune cells have a high affinity receptor, the TCR that specifically recognizes its ligand, a complex of MHC molecules with an 8 to 10 amino acid peptide that provides the antigen specificity. When the antigen specificity of T cell clones obtained from TIL was assessed, over 80% of them were restricted to the MART-1/Melan-A

melanoma antigen [19,20]. Over the past 10 years we have conducted prior experimental immunotherapy clinical trials attempting to induce melanoma regression by activating MART-1-specific T cells. These experiences demonstrated that a subset of patients have immune mediated tumor regressions to MART-1-based immunotherapy that are extremely long lived, lasting years without relapses [4,5].

5.4. Why Gene Therapy?

The adoptive transfer of large numbers of antigen-specific T cells leads to the highest percentage of tumor regressions reported in patients with melanoma, but this approach is only feasible in a minority of patient where TIL can be cloned and expanded from melanoma metastasis [2,3]. Genetic engineering of T cells with optimal TCRs would make this approach more broadly applicable. There is no other approach that would allow generating large numbers of T cells with uniform specificity for melanoma tumor antigens with a short (less than one week) *ex vivo* cell manipulation. Therefore, gene transfer techniques are the only approach that would allow testing this concept in human subjects, as supported by preliminary data from a published clinical trial at the Surgery Branch/NCI [21].

5.5 TCR Gene Therapy

The transfer of TCR genes is necessary and sufficient to endow recipient T cells with the specificity of donor T cells [22]. Genetically modified T cells carrying foreign TCRs respond to target antigen recognition through the transgenic TCR both *in vitro* and *in vivo*, leading to effective immune responses to viral and tumor challenges in murine adoptive transfer models [23]. T cells redirected by TCR gene transfer are fully functional after transfer into mice, and have been shown to expand dramatically (over three logs) after encounter with their cognate antigen *in vivo* [24]. Preliminary data in humans provides evidence that the same is true in patients with metastatic melanoma [21].

5.6 Retroviral Vector-based Human Gene Medicine

Retroviral-mediated gene transfer is an efficient means for the expression of transgene into actively dividing primary cells. Up to 50-60% of hematopoietic stem cells (HSC) and T lymphocytes can be transduced if cells are non-specifically activated, usually using a cytokine cocktail for HSC and either immobilized anti-CD3 and anti-CD28 or cytokines for T cells [25-27].

5.7 Safety and Regulatory Issues Regarding Retroviral-based Gene Transfer Approaches.

Ten infants with X-linked severe combined immune deficiency (SCID) resulting from a mutation in the γc gene (a surface cytokine receptor) were successfully treated with bone marrow transplantation of autologous HSC genetically modified to express the missing gene using retroviral vectors. Two of the infants developed a T cell leukemia approximately 3 years later. In both cases, the retrovirus carrying the γc gene had inserted near *LMO2*, an oncogene that is activated in some forms of acute leukemias [28,29]. This event highlights the risks of insertional mutagenesis induced by DNA-integrating viral vectors. However, multiple lines of evidence suggest that this may be an isolated event linked to the possible oncogenic capacity of the γc gene when under the control of a retroviral promoter, with a second hit being the activation of an endogenous oncogene like *LMO2* [28-30].

Retroviral vectors have a bias towards preferential integration in gene-rich regions, particularly near transcribed genes [31]. The theoretical concern of insertional mutagenesis had been recognized early in the use of retrovirus as gene therapy vectors, since retrovirus cause tumors in mice, and their ability to transactivate normally silent genes has been a useful technique for the description of multiple oncogenes [32]. However, retroviral vector oncogenesis from insertional mutagenesis has proven to be extremely rare in human subjects. Over 250 patients in over 40 clinical trials have received stem cells genetically modified with retroviral vectors [30], and the only cases of malignant transformation are the two infants in the SCID study [28,29]. Therefore, particularities of this study (the γc gene functioning as a first hit for leukemogenesis, a predilection for vector insertion near the *LMO2* oncogene as second hit,

the baseline immune deficiency of the patients not being able to control transformed clones) seem to have played a major role in these two cases [33].

5.8 Rationale for the Use of 2A Picornavirus Sequences

Problems with expressing multiple genes in a single vector become apparent with the simultaneous use of multiple promoters and IRES sequences. The different encoded proteins are usually not expressed at the same level. This is a critical issue for this protocol, since the correct surface expression of a functional transgenic MART-1 F5 TCR requires both proteins to pair, and in addition try to decrease the heterodimerization with other endogenous TCR chains. For example, a MART-1 F5 TCR α chain, produced in excess of the MART-1 TCR β chain, may pair with an endogenous TCR β chain, resulting in the generation of TCR with specificity other than MART-1 [34]. This problem has been circumvented by using picornavirus-derived "self-cleaving" 2A-like sequences. Using this approach, Szymczak *et al*. [35] have efficiently generated multicistronic retroviral vectors (with efficient translation of at least 4 genes) to restore TCR expression in CD3-deficient mice.

The original description of the 2A self-cleaving peptides was done by Martin Ryan and colleagues at the University of St. Andrews, U.K., who were working on the Foot and Mouth Disease Virus (FMDV) and other picornavirus [36]. The FMDV encores some of its proteins in a long single open reading frame (ORF) that is cleaved post-translationally by viral proteinases. At one position of this polyprotein there is a rapid co-translational self-processing due to the presence of an 18 amino acid peptide (termed 2A) that directed its own separation from the growing polyprotein [36-38]. The use of 2A-based vectors to express the two MART-1 TCR genes would be predicted to allow for the coordinated expression of the 2 transgenes.

5.9 PET-based Molecular Imaging

PET provides quantitative measurements of the 3D distribution of probes labeled with positron emitting radio-nuclides. PET scanners routinely measure radioactivity concentrations in the 10^{-12} M range, making PET the most sensitive imaging technology applicable to both preclinical and clinical studies. MicroPET scanners approach a spatial resolution of ~1 mm^3 while clinical scanners have an intrinsic resolution of ~4-5 mm^3 [39]. Currently, PET imaging of metabolic activity is extensively used in cancer diagnosis, with more than 3 million PET scans administered in clinical centers worldwide in 2006. A majority of these scans use the glucose analog 2-[^{18}F]-fluoro-2-deoxy-d-glucose ([^{18}F]FDG) to measure the rate of glycolysis, a metabolic process that is unregulated in the majority of cancers. [^{18}F]FDG PET allows early detection and staging of cancer, as well as evaluation of therapeutic interventions. In addition to cancer, [^{18}F]FDG also accumulates at sites of inflammation or active immune responses. Similar to malignant cells, T cells and other immune cells rapidly induce glycolysis following activation. Increased [^{18}F]FDG retention has been reported in several immune and inflammatory processes including autoimmune hepatitis, viral infections, systemic lupus erythematosus and rheumatoid arthritis.

The concept of PET measurements of metabolic processes exemplified by [^{18}F]FDG has been applied to the development of PET imaging probes sensitive to the rate of DNA synthesis and cell proliferation. The prototype for these probes is 3'-^{18}fluorothymidine ([^{18}F]FLT), a nucleoside analog developed by Shields and colleagues [40]. The accumulation of [^{18}F]FLT in proliferating cells reflects the enzymatic activity of thymidine kinase 1, a cytosolic deoxynucleoside kinase which catalyzes the transfer of a γ-phosphate group from a nucleoside triphosphate to the 5'-OH group of thymidine. TK1 expression is regulated during cell cycle: it starts increasing in late G1 cells, reaches a maximum in the S phase coinciding with DNA synthesis, and markedly decreases during mitosis.

5.10 MART-1 TCR-engineered T cell Experience at NCI Surgery Branch

5.10.1 Use of Other TCRs in Humans. The Surgery Branch/NCI has initiated two similar TCR gene transfer protocols using other TCRs, which as of the Fall of 2007 have enrolled a total of 54 patients:

i) NCI trial 04-C-0251 (OBA# 0308-599, IBC# RD-03-XII-01) has treated 35 patients using a similar retroviral vector backbone and packaging cell line proposed in the current study. This clinical trial used a TCR that had much lower avidity for MART-1, which was termed F4 [21]. There were no adverse events associated with these patient treatments that were attributed to the gene transfer therapy. There have been four clinical responses (PR) observed during the trial, and two were long-term PRs (>12 months).

ii) NCI trial 07-C-0175A (OBA# 0703-840) has treated 20 patients using the same retroviral vector backbone and packaging cell line proposed in the current study. The protocol was feasible and overall safe, with major toxicities attributed to the F5 TCR transgenic cells being anterior uveitis in 55% of patients and hearing loss in 50% of patients [7]. Antitumor activity was demonstrated with 6 partial responses (PR) out of 20 patients (30%), with 3 of the responses being durable beyond 1 year [7].

iii) NCI trial 07-C-0003 (OBA#0602-759, IBC # RD-06-II-10) has treated 7 patients using the same retroviral vector backbone and packaging cell line, the only difference being the TCR protein is specific for p53. There have been no adverse events associated with these patient treatments that were attributed to the gene transfer therapy. There has been no clinical response observed to date.

5.10.2 Clinical Use of the MART-1 F5 TCRs in Humans. NCI trial 07-C-0175A (OBA# 0703-840) uses the same MART-1 F5 TCR and retroviral vector construct proposed in the current protocol [7,41]. This clinical trial has initiated patient accrual and data is being generated currently. Below we list the major similarities and differences between both clinical trials:

i) Major similarities:

- TCR: Both clinical trials use the same MART-1 F5 TCR, derived from TIL clone DMF5, the highest affinity MART-1 TCR is a large screen at the Surgery Branch/NCI [7,41].
- Retrovirus: Both clinical trials use the same retrovirus vector backbone, construct and master cell bank, and produced at the same facility, the Indiana University Vector Production Facility (IU VPF).
- Target cells: Both experiences include the transduction of activated PBMC. Since a TCR can only be expressed and functional on the cell surface in cells that are CD3 positive, this allows the TCR engineering of both CD8+ CTL and CD4+ T helper cells. Therefore, CD4+ T cells acquire a TCR that redirects them to recognize antigen presented by MHC class I molecules.
- Clinical trial design: Both are phase 2 clinical trials with primary endpoints of safety and antitumor activity.
- Conditioning regimen: Both use the same non-myelodepleting but lymphodepleting chemotherapy conditioning regimen based on the administration of cyclophosphamide and fludarabine to allow homeostatic proliferation of the adoptively transferred TCR transgenic cells.

ii) Major differences:

- Rapid Expansion Protocol (REP): The F5 clinical trial at the Surgery Branch/NCI [7] included a 2-week *ex vivo* expansion after TCR engineering of lymphocytes following the REP protocol described by Riddell and colleagues [42]. The clinical trial at UCLA will not include

this forced *ex vivo* T cell expansion. The main reason is that several preclinical models suggest that extended *ex vivo* expansion of lymphocytes before adoptive transfer results in terminally differentiated cells with limited *in vivo* proliferation ability [43,44]. Therefore, at UCLA the TCR transduced PBMC will only be kept for up to 96 hours in culture after the first transduction before re-infusing into patients.

- Cell dose for adoptive transfer: Given that the UCLA study will not use REP, the target cell dose for adoptive transfer is one to two log lower at UCLA than in the clinical trial conducted at the Surgery Branch/NCI [7], where up to 10^{11} TCR transgenic cells were infused to patients.
- Dendritic cell vaccination: Provision of antigen in the form of a vaccine is required in some animal models to support the antitumor activity of adoptively transferred T cells, most notably with the pmel-1 adoptive transfer model [45,46]. This is because exposure to antigen while undergoing homeostatic proliferation stimulates further T cell expansion [47,48]. We propose to use MART-$1_{26\text{-}35}$ peptide-loaded GM-CSF/IL-4 differentiated DC, a vaccine that has resulted 2 durable complete responders out of 18 patients with metastatic melanoma treated, currently ongoing beyond 5 and 6 years, respectively [4,5].
- PET imaging: We include molecular imaging using the standard [^{18}F]FDG PET probe, which allows non-invasive imaging of a key feature of activated lymphocytes, their high uptake of glucose due to aerobic glycolysis.
- Low dose IL-2: The UCLA clinical trial initially used high dose IL-2 post-adoptive transfer as means to provide helper cytokines to support the proliferation of adoptively transferred TCR transgenic cells. After Amendment 11 this will change to low dose IL-2.

6.0 PRELIMINARY DATA

6.1 Origin and Functionality of the MART-1 F5 TCR.

The retroviral vector, MSGV1-F5AfT2AB, expressing the MART-1 F5 TCR, has been provided by Dr. Steven A. Rosenberg from the Surgery Branch/NCI. This high affinity TCR specific for the MART-$1_{27\text{-}35}$ epitope in the context of HLA-A*0201 has been selected based on its high affinity for its antigen and because it confers high functionality when genetically engineered into CD3 positive cells.

The low response rate in the prior MART-1 TCR gene transfer protocol at the Surgery Branch/NCI [21] led these investigators to attempt to identify MART-1 reactive TCR with higher avidity than the MART-1 F4 TCR used in the prior gene therapy clinical trial. They generated multiple MART-1 reactive clones from a variety of MART-1 reactive tumor infiltrating lymphocyte cultures. Twenty four clones were isolated and tested for reactivity against the MART-1 peptide and against A2^{+} MART-1 expressing melanoma lines as well as the 888 A2^{-} melanoma line [41,49].

The TCR utilized in their prior clinical trial came from the DMF4 TIL clone. When compared to many other MART-1 reactive clones this clone had only medium avidity for recognition of MART-1 peptide as well as MART-1 expressing tumors. The highest avidity T cell clone identified in this analysis was the DMF5 clone. In an overnight co-culture assay measuring interferon-gamma release the DMF5 clone had the highest recognition of T2 cells pulsed with the native MART-$1_{27\text{-}35}$ peptide. This clone secreted 17,161pgm/ml of interferon gamma in an overnight co-culture assay compared to 1,987pgm/ml secreted by the DMF4 clone used in prior trials. In addition, the recognition of the mel526 and the mel624 A2^{+} MART-1^{+} cell lines was also higher utilizing the DMF5 clone than the DMF4 clone (5806 and 10,865 pgm/ml compared to 1780 and 2397 pgm/ml). Neither clone had significant recognition of the control A2^{-} MART-1^{+} mel 888 line [41,49,50].

Investigators at the Surgery Branch/NCI next cloned the genes encoding the alpha and beta chains of the TCR from many of these cloned TIL populations. The genes encoding these alpha and beta chains were then electroporated into the same activated donor CD8^{+} PBMC. The DMF5 electroporated TCR was again the most reactive TCR when assessed against the MART-1 peptide

pulsed onto T2 cells as well as to $A2^+$ MART-1^+ melanoma cell lines. The reactivity of the TCRs recapitulated the reactivity seen in the original clones. They next checked lysis by these electroporated TCR in a 4-hour chromium release assay. Again the DMF5 TCR outperformed all of the other TCR and was approximately 10 times more avid compared to the DMF4 TCR used in their prior gene therapy clinical trial [41,51].

In summary, the anti-MART-1 F5 TCR appears to have excellent reactivity against both the MART-1 peptide as well as melanoma cell lines. Investigators at the Surgery Branch/NCI have developed a GMP quality retroviral vector encoding the alpha and beta chains of the MART-1 F5 TCR for use in an ongoing phase 2 clinical trial [51].

6.2 Data Using the MART-1 F5 TCR Retroviral Vector (MSGV1-F5AfT2AB) in Human Subjects at the Surgery Branch/NCI

Investigators at the Surgery Branch led by Dr. Steven A. Rosenberg have reported on 20 patients treated with the ACT of lymphocytes expressing the F5 TCR [7]. The Surgery Branch protocol differs with the UCLA protocol in that up to 10^{11} TCR transgenic cells were administered, that these cells are generated and infused fresh (without cryopreservation as at UCLA), and that no DC vaccine is included. The protocol was feasible and overall safe, with major toxicities attributed to the F5 TCR transgenic cells being anterior uveitis in 55% of patients and hearing loss in 50% of patients [7]. Antitumor activity was demonstrated with 6 partial responses (PR) out of 20 patients (30%), with 3 of the responses being durable beyond 1 year [7].

7.0 ELIGIBILITY CRITERIA

7.1 Inclusion Criteria

a) Histologically confirmed melanoma that is considered surgically incurable with either:
    a. Stage IIIc melanoma including locally relapsed, satellite, in-transit lesions or bulky draining node metastasis.
    b. Stage IV melanoma (M1a, M1b or M1c).
b) At least 1 lesion amenable for outpatient biopsies; this should be a cutaneous or palpable metastatic site or a deeper site accessible by image-guided biopsy that is deemed safe to access by the treating physicians and interventional radiologists. Patients without accessible lesions for biopsy but with prior tissue available from metastatic disease would be eligible at the investigator's discretion.
c) MART-1 positive melanoma by RT-PCR or IHC.
d) HLA-A*0201 (HLA-A2.1) positivity by molecular subtyping*.
e) Age greater than or equal to 18 years old.
f) Life expectancy greater than 3 months assessed by a study physician.
g) A minimum of one measurable lesion defined as:
    a. Meeting the criteria for measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST).
    b. Skin lesion(s) selected as non-completely biopsied target lesion(s) that can be accurately measured and recorded by color photography with a ruler to document the size of the target lesion(s).
h) No restriction based on prior treatments.
i) ECOG performance status (PS) 0 or 1.
j) Adequate bone marrow and hepatic function determined within 30-60 days prior to enrollment, defined as:
    a. Absolute neutrophil count $\geq 1.5 \times 10^9$ cells/L.
    b. Platelets $\geq 100 \times 10^9$/L.
    c. Hemoglobin $\geq$ 10 g/dL.

   d. Aspartate and alanine aminotransferases (AST, ALT) ≤ 2.5 x ULN (≤ 5 x ULN, if documented liver metastases are present).
   e. Total bilirubin ≤ 2 x ULN (except patients with documented Gilbert's syndrome).
   f. Creatinine < 2 mg/dl (or a glomerular filtration rate > 60).

k) Must be willing and able to accept at least two leukapheresis procedures.
l) Must be willing and able to accept at least two tumor biopsies.
m) Must be willing and able to provide written informed consent.

*Patients with HLA-A*0205 (HLA-A2.5) positivity by molecular subtyping may be eligible if there is demonstration that they can correctly present the MART-$1_{26\text{-}35}$ epitope as stimulators for IFN-gamma production by MART-1 F5 TCR transgenic cells.

7.2 Exclusion Criteria

a) Previously known hypersensitivity to any of the agents used in this study.
b) Received systemic treatment for cancer, including immunotherapy, within one month prior to initiation of dosing within this protocol. However, cell harvesting by leukapheresis may be performed before one month from prior therapy if the study investigators consider that it will not have a detrimental impact on the generate of the two cell therapies in this protocol.
c) History of, or significant evidence of risk for, chronic inflammatory or autoimmune disease (eg, Addison's disease, multiple sclerosis, Graves disease, Hashimoto's thyroiditis, inflammatory bowel disease, psoriasis, rheumatoid arthritis, systemic lupus erythematosus, hypophysitis, pituitary disorders, etc.). Patients will be eligible if prior autoimmune disease is not deemed to be active (e.g. fibrotic damage of the thyroid after thyroiditis or its treatment, with stable thyroid hormone replacement therapy). Vitiligo will not be a basis for exclusion.
d) History of inflammatory bowel disease, celiac disease, or other chronic gastrointestinal conditions associated with diarrhea or bleeding, or current acute colitis of any origin.
e) Potential requirement for systemic corticosteroids or concurrent immunosuppressive drugs based on prior history or received systemic steroids within the last 4 weeks prior to enrollment (inhaled or topical steroids at standard doses are allowed).
f) HIV seropositivity or other congenital or acquired immune deficiency state, which would increase the risk of opportunistic infections and other complications during chemotherapy-induced lymphodepletion. If there is a positive result in the infectious disease testing that was not previously known, the patient will be referred to their primary physician and/or infectious disease specialist.
g) Hepatitis B or C seropositivity with evidence of ongoing liver damage, which would increase the likelihood of hepatic toxicities from the chemotherapy conditioning regimen and supportive treatments. If there is a positive result in the infectious disease testing that was not previously known, the patient will be referred to their primary physician and/or infectious disease specialist.
h) Dementia or significantly altered mental status that would prohibit the understanding or rendering of informed consent and compliance with the requirements of this protocol.
i) Clinically active brain metastases. Radiological documentation of absence of active brain metastases at screening is required for all patients. Prior evidence of brain metastasis successfully treated with surgery or radiation therapy will not be exclusion for participation as long as they are deemed under control at the time of study enrollment.
j) Pregnancy or breast-feeding. Female patients must be surgically sterile or be postmenopausal for two years, or must agree to use effective contraception during the period of treatment and 6 months after. All female patients with reproductive potential must have a negative pregnancy test (serum/urine) within 14 days from starting the conditioning chemotherapy. The definition of effective contraception will be based on the judgment of the study investigators.
k) Since IL-2 is administered following cell infusion:
   a. Patients will be excluded if they have a history of clinically significant ECG abnormalities, symptoms of cardiac ischemia or arrhythmias and have a left ventricular ejection fraction

(LVEF) < 45% on a cardiac stress test (stress thallium, stress MUGA, dobutamine echocardiogram, or other stress test)

b. Similarly, patients who are 50 years old with a baseline LVEF < 45% will be excluded.
c. Patients with ECG results of any conduction delays (PR interval >200ms, QTC > 480ms), sinus bradycardia (resting heart rate <50 beats per minute), sinus tachycardia (HR>120 beats per minute) will be evaluated by a cardiologist prior to starting the trial. Patients with any arrhythmias, including atrial fibrillation/atrila flutter, excessive ectopy (defined as >20 PVCs per minute), ventricular tachycardia, 3rd degree heart block will be excluded from the study unless cleared by a cardiologist.
d. Patients with pulmonary function test abnormalities as evidenced by a FEV_1/FVC< 70% of predicted for normality will be excluded.

7.3. Definition of Study Enrollment Date

Enrollment will be considered the time when the eligibility criteria is being entered into the study eligibility form after the screening tests have been completed to declare if the patient is able or not able to be considered for this research.

8.0 STUDY PROCEDURES

This is a phase 2 study to determine the safety, feasibility and antitumor activity of retrovirus-transduced T cell immunotherapy in patients with metastatic melanoma. The retrovirus vector encodes the MART-1 F5 alpha and beta TCR chains. The retrovirus vector MSGV1-F5AfT2AB will be used to transduce autologous PBMC. Infusions of a target minimum cell dose of 10^8 TCR transgenic cells will be performed at day 0. Subjects will be followed for laboratory and clinical adverse events, for evidence of replication competent retrovirus (RCR), and for survival of transduced T cells. The most intensive monitoring of research volunteers will be made over 24 months, but subjects will be followed for life.

Given the pilot nature of this study, the multiple procedures involved and the use of two cell-based therapies manufactured from patients' blood samples, the timeline of the study is aimed at defining their approximate administration to patients. In general, changes in the range of 7 days up or down from the scheduled event will not be considered deviations from the study timeline. If there is a clear trend of procedures that need to be performed at different times in study subjects, the protocol will be amended to attempt to adjust the study timeline to feasible procedures in the clinical study schedule.

8.1 Baseline Screening Assessments

Patients will be screened to determine whether they meet full eligibility criteria after the informed consent form (ICF) is signed. Standard of care medical history, pertinent laboratory data and significant medical problems of the patient will form the basis for proceeding to evaluate the candidate in more detail. Following signing of the ICF and enrollment into the study, the remainder of screening procedures and tests will be completed.

An attempt will be made to complete all tests within 30 days of enrollment. However, given the multiple screening tests and the pre-study procedures to assess patient eligibility, tests older than 30 days may be acceptable and will limit unnecessary test repetition. Key tests will be repeated at the discretion of the treating physicians if older than 30 days.

Screening tests:

- Office visit, vital signs and physical exam. The physical exam will include weight, height, and vital signs. Weight measurement will only be obtained at subsequent physical exams if clinically

indicated. Determination of HLA-A*0201 (HLA-A2.1) or HLA-A*0205 (HLA-A2.5) positivity by subtyping using at least intermediate resolution techniques (this testing may already be available from prior analysis).

- There is data that patients who are HLA-A*0205 (HLA-A2.5) positive by molecular subtyping can correctly present the MART-1_{26-35} epitope [52]. To enroll patients who are HLA-A*0205 positive, patient-derived PBMC pulsed with MART-1_{26-35} epitope will be used as stimulators for IFN-gamma production by MART-1 F5 TCR transgenic cells. If at least > 30,000 pg/ml/million cells of IFN-γ production upon MART-1_{26-35} peptide stimulation using MART-1_{26-35} peptide-pulsed HLA-A*0205 PBMC.
- Determination of MART-1 positivity in melanoma cells by immunohistochemistry (IHC) or PCR analysis of melanoma (this can be done in archived tissue, in a new tumor biopsy or may already be available from prior analysis). A new biopsy may not be required if an acceptable biopsy was performed within 60 days of prior to screening.
- Tumor Assessments (Imaging/Clinical): Documentation of baseline target and non-target lesions by imaging techniques or by measurement of clinical lesion(s) must be performed. Documentation of skin lesion(s) that can be clearly visualized must be established by color photography, including a ruler to document size.
- Radiological Assessment of the Brain: All patients are required to have a baseline CT scan with i.v. contrast or MRI scan of the brain. Patients found to have active brain metastases are excluded from enrollment until the brain metastases are under control after neurosurgery or radiation therapy.
- Collect blood for:
  - WBC with differential count and absolute neutrophil count (ANC), red blood cell (RBC) count, hemoglobin, hematocrit, and platelet count.
  - Blood Chemistries including: Calcium, chloride, total protein, potassium, random glucose, sodium, blood urea nitrogen (BUN), creatinine, AST (SGOT), ALT (SGPT), alkaline phosphatase (ALP), lactic acid dehydrogenase (LDH), total bilirubin, C-reactive protein, Magnesium, Phosphorous, Lipase, Amylase, Uric acid.
  - Coagulation tests: PT/INR, PTT.
  - Infectious disease serologies: HIV, CMV, EBV, HBV and HCV serotests (unless already tested within the past 1 year).
  - Thyroid Function: Thyroid stimulating hormone/TSH, T3, T4.
  - Autoantibody Panel: Anti-nuclear antibodies (ANA), antineutrophil cytoplasmic antibodies (ANCA), antimicrosomal, antithyroglobulin, anti liver-kidney microsomal (LKM), anti-islet cell, antibodies to Ro (SSA) and to La (SSB), and antiphospholipid antibodies.

A trial investigator or a clinical study staff member will complete an Eligibility Checklist based on these results. The checklist will be reviewed by the HGMP compliance officer. If eligible to proceed, the leukapheresis will be scheduled and the pertinent laboratory staff will be notified of the anticipated dates of cell processing.

8.2. Initial Protocol Procedures

- **Ophthalmologic Exam.** Patients will undergo a formal ophthalmologic exam by a qualified ophthalmologist. The minimally required procedures will be an ophthalmic history and a comprehensive ophthalmic examination.

- **Otological Exam.** Patients will undergo a formal otological exam by a qualified otorhynolaryngologist. The minimally required procedures will be an otological history and a comprehensive otologic examination.

- **Tumor Biopsy.** A biopsy of a melanoma lesion accessible to outpatient sampling will be collected whenever feasible. A target minimum of 5 x 5 x 5 mm tumor tissue will be collected if feasible. . A

new biopsy may not be required if an acceptable biopsy was performed within 60 days of prior to screening.

- **Baseline [^{18}F]FDG PET Scan.** A baseline PET scan using [^{18}F]FDG as tracer will be used to determine the background against which the assessment of MART-1 F5 TCR transgenic T cell accumulation will be compared to. An attempt will be made for the baseline PET to be performed in conjunction with the baseline tumor assessment by CT scan to minimize radiation exposure.

- **Cardiac Function Tests.** An electrocardiogram (ECG) will record potential pre-existing conduction abnormalities for future comparison if needed. A MUGA or echocardiogram will be performed to determine if the patient is fit to receive low dose IL-2. A LVEF lower than 45% would preclude participation.

- **Pregnancy Test.** All females of childbearing potential must have a negative serum or urine Pregnancy Test within 14 days from the initial dose of conditioning chemotherapy in order to be eligible for participation. Females who have undergone surgical sterilization or who have been postmenopausal for at least 2 years are not considered to be of childbearing potential.

- **Central Venous Access Catheter.** A central venous access catheter (CVC) will be placed during the screening period or upon hospital admission. This should ideally be a double lumen central catheter, but it may be a single lumen catheter at the discretion of the treating physicians. Examples may include double lumen Groshong or PICC catheters.

- **Pulmonary Function Tests (PFT)**: Tests to be performed in all patients, and if FEV_1/FVC< 70% of predicted patient will be excluded from participation.

8.3. Leukapheresis, Cell Cultures, and Admission to the Hospital

a. Day -6 in the Morning. Leukapheresis and Cell Cultures. In the morning of the day a leukapheresis will be performed to collect blood to produce the MART-1 TCR transgenic cells and the dendritic cell vaccines.

b. Day -6 in the Afternoon. Admission to the Hospital. Subject will be admitted to the Hematology/Oncology unit at the Ronald Regan UCLA Medical Center.

- Subject will be asked about illnesses, injuries, side effects, and any medications subject has taken or medical procedures that have been done to subject since the prior visit.
- Physical exam.
- A catheter will be placed in a vein through which subject will receive liquids in preparation for the chemotherapy regimen.
- Subject will have blood drawn to check health. Tests include complete blood counts; the ability of blood to clot; blood chemistries. Most of these blood tests will be repeated daily or every other day during subject’s stay in the hospital.
- Subject will start to receive one or two drugs to prevent complications with infections, the antibiotic Bactrim to decrease the risk of Pneumocistis carinii pneumonia (or PCP), and the drug ganciclovir to decrease the risk of cytomegalovirus (or CMV). Gancyclovir will only be given to subjects who have evidence of prior exposure to the CMV virus. Both of these drugs will be stopped before administering the gene-modified MART-1 TCR CTLs, since both drugs would interfere with their function in the body.

8.4 Days –5 to –1: Conditioning Chemotherapy Regimen with Cyclophosphamide and Fludarabine

The conditioning chemotherapy will be initiated the next day following leukapheresis.

Supportive Therapy During Chemotherapy Administration:

This therapy will be started on day –5, counting as day 0 the day of engineered TCR transgenic cell infusion. The i.v. infusions, procedures and medications will be modified as indicated based on the Attending physician and clinical team taking care of patients.

- Hydrate: Begin hydration with 0.9% Sodium Chloride Injection containing 10 meq/L of potassium chloride at 150 ml/hr, starting approximately 12 hours pre-cyclophosphamide and continuing hydration until 24 hours after last cyclophosphamide infusion.
- Daily CBC, and basic chemical panel as per standard procedures in the J-Medicine ward
- Ondansetron (Zofran) 8-16 mg i.v. on the days of chemotherapy (or similar antagonist of serotonin type 3 -5-HT3- receptors as per standard practices), lorazepam (Ativan) 1 mg i.v. q6h prn and prochlorperazine (Compazine) 10 mg. i.v. q6h prn on the days of chemotherapy conditioning, and po afterwards.
- Trimethoprim-Sulfamethoxazole (Bactrim) 1 tablet po tid from days –6 to day –2, restarted once the absolute neutrophil count (ANC) is above 1 x $10^3/\mu L$ until day +100.
- Ganciclovir (Cytovene) 6 mg/kg on days –6 to day –2 and then discontinued. Gancyclovir will only be given to patients that are CMV seropositive at baseline or following standard practices.
- Posaconazole 200 mg po tid (or similar prophylactic antifungal based on standard practice) starting on the day of reinfusion and continued until the ANC is above 3 x $10^3/\mu L$ for 3 consecutive days.
- MESNA at equidoses with cyclophosphamide, administered by continuous intravenous infusion for 24 hours on the days of administration of cyclophosphamide.
- Furosemide dosed as clinically indicated during the hydration period.

Dexamethasone 10 mg may be added as an adjunct to other anti-nausea medications, but it should be discontinued before day –1 to avoid its immune suppressive effect against MART-1 F5 TCR PBMC adoptive transfer, MART-$1_{26\text{-}35}$/DC administration and low dose IL-2.

Chemotherapy Administration:

Days –5 to –4:

- Cyclophosphamide 60 mg/kg/day x 2 days i.v. in 250 ml D5W with equidoses of mesna. Maximum dose calculated based on actual body weight should not exceed 140% of doses calculated based on ideal body weight.

Day -4 to Day -1:

- Fludarabine 25 mg/m^2/day i.v. daily over 30 minutes for 4 days. Maximum dose calculated based on actual body weight should not to exceed 140% of doses calculated based on ideal body weight.

Prophylactic Supportive Care Throughout the Pancytopenic Period.

In order to closely monitor patient care and decrease study-related toxicities, patients will receive prophylactic therapy throughout the pancytopenic period. The intravenous infusions, procedures and medications will be modified as indicated based on the attending physician and clinical team taking care of patients.

- During the preparative and pancytopenic period, patients will have a complete blood count and a comprehensive metabolic panel including a hepatic panel on a daily basis and periodic urine analysis (daily during cyclophosphamide administration days) following the standard J-Medicine protocol and the clinical judgment.
- G-CSF (Neupogen) 300 or 480 μg will be used following the standard J-Medicine protocol and the clinical judgment.
- Imipenem at 500 mg i.v. q6h. in case of fever > 38.2°C (other antibiotic in case of history of allergy to penicillin and derivates; exact antibiotic combination should be decided by the treatment team).
- Packed RBC transfusion to maintain a Hb > 8.0 g/dL, following the standard J-Medicine protocol and the clinical judgment.
- Platelet transfusion to attempt to maintain a platelet count of > 10 x 10^3/μL, > 20 x 10^3/μL if there is concomitant fever, and > 50 x 10^3/μL if there is evidence of bleeding, following the standard J-Medicine protocol and the clinical judgment.
- Norethindrone (Norlutate) 10mg PO daily will be used for menstruating women to prophylaxis against menorrhagia.

8.5 Day 0: MART-1 F5 TCR PBMC Infusion

Premedication

Side effects following T cell infusions include transient fever, chills, and/or nausea. For this reason, patients will receive pre-medication with:

- Approximately one hour prior to the infusion, an i.v. infusion of Dextrose 5%-Normal Saline, 250 ml/hr will begin and continue for 3 hours.
- Briefly (approximately 10 minutes) prior to the infusion, acetaminophen 650 mg po, 25 mg diphenhydramine and 12 g mannitol will be administered i.v. These medications may be repeated every six hours as needed.
- A course of non-steroidal anti-inflammatory medication may be prescribed if the patient continues to have fever not relieved by acetaminophen.
- It is recommended that patients not receive systemic corticosteroids such as hydrocortisone, prednisone, prednisolone, or dexamethasone, at any time, except in the case of a life-threatening emergency, since this may have an adverse effect on T cell survival. If corticosteroids are required for an acute infusion reaction, an initial dose of hydrocortisone 100 mg is recommended. Such subjects will not be removed from the study or replaced but will be followed to document the effects of such treatment.

Transgenic MART-1 F5 TCR PBMC Cell Infusion

The TCR genetically engineered PBMC product will be transported to the in-patient unit on ice in a rigid-walled container with a tight fitting lid (i.e. Tupperware; Nalgene container etc.) and labeled with a biohazard label. A target minimum of 10^8 and up to 10^9 transduced PBMC will be infused at a rate of ~10 ml/min through standard IV tubing without a filter.

Patient Monitoring After MART-1 F5 TCR PBMC Infusion

- Patients will receive the TCR genetically modified PBMC in an individual use room equipped with a high-efficiency particulate air (HEPA) filters that meets the precautions normally used for patients undergoing HSC transplantation.
- Patients will be in a monitored bed during cell infusion, with vital signs recorded.
- Sterile saline, acetaminophen and emergency drugs, including epinephrine [0.3-0.5 mg (0.3-0.5 ml of a 1:1000 solution), diphenhydramine (25-50 mg i.v.), and methylprednisolone (30-60 mg i.v.), should be readily available in the amount and formulation as per the standard treatment of a potential hypersensitivity/anaphylactic reaction

- If there is an adverse event (AE) characterized by life-threatening hypoxia or hypotension, or any other infusion reaction qualifying as a DLT as described in section 11.1.1.1, then further infusion of the TCR transgenic cells will be canceled and no other immune stimulating approaches, such as IL-2, or DC vaccines, will be administered under this protocol.

Febrile Reaction

In the event that a subject develops sepsis or systemic bacteriemia following PBMC infusion, appropriate cultures and medical management will be initiated. If a contaminated PBMC product is suspected, the product will be retested for sterility using archived samples that are stored in the UCLA HGMP/JCCC GMP Suite.

8.6 Days 1, 14 and 30: MART-1$_{26\text{-}35}$ Peptide Pulsed DC Administration

Patients will receive a target cell number of 10^7 MART-1$_{26\text{-}35}$ peptide pulsed DC administered intradermally (i.d.) close to a lymph node basin not known to be involved with melanoma.

8.7 Days 1-7: Low Dose IL-2

Patients will receive IL-2 (Prometheus) at 500,000 IU/m^2 s.c. twice daily for 7 days. Doses may be skipped depending on patient tolerance. Doses will be skipped if patients reach grade III or IV toxicity due to IL-2 except for the reversible grade III toxicities common to IL-2 such as diarrhea, nausea, vomiting, medically manageable hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes. Patients receiving IL-2 will have a CBC, electrolytes, BUN, creatinine, liver function tests, and serum chemistries evaluated at least once during the week.
IL-2 administration will be held per the parameters below:

A) Hypotension <80 systolic or hypertension >160 systolic
B) Sinus tachycardia > 120, sinus bradycardia <60, or any other arrythmias
C) Syncope
D) Chest pain
E) Seizures
F) Respirations of <10 or >30, or O2 Sat < 90% Room Air
G) Urine output of less than 100ml q 4 hr
H) Change in mental status
I) Temperature more than 103° C, unresponsive to medication

Fevers During IL-2 Administration

Fevers are a known side effect of IL-2 administration. Since patients at the time of IL-2 dosing will also be recovering from the conditioning chemotherapy and will likely be pancytopenic, the supportive care during this period includes empiric treatment for infections and IL-2-based toxicities without resulting in a definition of DLT.

Supportive Care During Low Dose IL-2 Administration

Concomitant medications to control side effects of therapy will be given following the standard UCLA practices for IL-2-based therapy. Meperidine (25-50 mg) will be given intravenously if severe chills/rigors develop. Other supportive therapy will be given as required and may include acetaminophen (650 mg q4h), indomethacin (50-75 mg q6h) and ranitidine (150 mg q12h). Ondansetron 0.15 mg/kg/dose i.v. every 8 hours (or an equivalent antiemetic) may be administered for nausea or vomiting. Additional antiemetics may be administered as needed for nausea or vomiting. Antibiotic coverage for central venous catheters may be provided at the discretion of the treating physicians. These side effects are more commonly seen with high doses of IL-2, not the low-doses

which is about 15 fold lower in total amount than the high dose IL-2 regimen, plus lasting over a longer period, that's used in this study.

Blood Collection for Immune Monitoring Assays

Collection of 100 ml of peripheral blood is scheduled on study days 0, 14, 30, 45, 60, 75, 90 and at each long term follow up visit as described in section 8.14. Blood draws on these days should be performed if considered safe, and may be skipped or delayed because patients are likely to have chemotherapy-induced pancytopenia and may be hemodynamically unstable after IL-2. These blood draws can be done +/- 7 days from the intended days.

Blood Collection for Plasma Cytokine Analyses

Although evidence indicates that it is not directly related to the F5 MART-1 TCR transgenic T cells, considering the two recent SAE cases requiring intubation, five to 10 ml of blood in anticoagulant will be collected daily for plasma during the first week after ACT routinely for cryopreservation/archiving and then as needed thereafter in case of an adverse event that warrants further collection of samples for cytokine/chemokine analyses.

8.8 Hospital Discharge

Patients will be discharged from the hospital when the following criteria are met and following the clinical judgment of the treating physicians:

- Absolute neutrophil count (ANC) > 500/μL.
- Platelet count > 20,000/μL.
- Hemodynamically stable.
- Creatinine on downward trend after L-2.
- Liver function tests stable.
- Not requiring daily blood product infusion.

8.9 Days 20 to 40: MART-1 F5 TCR Cell Tracking After Infusion

Between study days 20 and 40 (or when the treating physicians deem that the patient is stable enough to undergo these procedures), either as an inpatient or outpatient, the following will be repeated to attempt to detect the MART-1 F5 TCR transduced PBMC:

- [^{18}F]FDG PET scan with an attenuated restaging CT of the chest, abdomen and pelvis.
- Tumor biopsy whenever feasible.
- Leukapheresis and plasmapheresis: This will be a partial (5 liter) leukapheresis for PBMC collection and 100 ml of plasma.

8.10 Days 30, 45, 60 and 75: Follow Up Study Visits

On study days 30, 45, 60 and 75 patients will be seen as outpatients in the oncology clinic for office visit, vital sign determination, safety blood tests and collection of blood for immune monitoring analysis.

8.11 Permissible Systemic Therapies During the Protocol On-study Period

The on-study period is defined as one year from the date of the TCR engineered ACT infusion unless there is overt tumor progression or DLTs leading to patient discontinuation. Systemic immune suppressive therapies will be avoided during this time, but may be permissible in the following situations:

- Systemic corticosteroids: Used in case there is a suspicion or evidence of a DLT (as described in section 11.1.1.1) that may be related to autoimmune or inflammatory reactions, or cytokine storm. In this case, administration of IL-2 and DC vaccines will not be continued.

8.12 Days 75 to 90: Re-staging Exams and Procedures

Patients will undergo restaging CT, PET and/or MRI scans of the brain, chest, abdomen and pelvis between study days 75 and 90. Restaging exams every 2-3 months are standard of care in the management of patients with metastatic melanoma. In addition, a follow up ophthalmologic and otological tests will be scheduled. The results of this testing will be reviewed in a study visit on day 90, which will be the last visit during the most intensive part of the study protocol.

8.13 Day 90: Office Visit for Re-staging Evaluation

On study day 90 patients will be evaluated for antitumor activity after undergoing re-staging exams and laboratory tests. Patient with SD, PR or CR will be offered to continue on protocol after discussion of the study results. Patients with PD will be offered to terminate participation. However, given the phenomenon of delayed responses with tumor immunotherapy approaches, the final decision to continue or not on study will be at the discretion of the treating physician and the patient, regardless of the tumor response assessment.

8.14 Long-term Follow-up Plan

Regardless if followed on-study or off-study, patients will be asked to comply with the following long term plan:

- For the first 2 years, office visits at least every 3 months.
- After 2 years, office visits or phone follow-up at least every 6 months.
- After 5 years, office visits or phone follow-up at least annually.
- Collection of 60-100 ml of blood for immune monitoring analysis and for RCR testing at 3 and 6 months, and at one year post cell administration (as described in section 11.2.1.1).

9.0 STUDY AGENTS

9.1 PBMC Expressing MART-1 F5 TCR

Overview. A leukapheresis product obtained from study patients will be transferred to the HGMP/JCCC GMP Suite, on the 14th floor of the Factor Building. PBMC will be isolated by gradient centrifugation, and an aliquot of these cells will be cultured for 48 hours in the presence of anti-CD3 (OKT3) and IL-2 in order to stimulate T-cell growth to prepare for viral vector transduction. PBMC will be transduced with a clinical grade retrovirus vector expressing MART-1 F5 TCR (MSGV1-F5AfT2AB) using retronectin-coated plates in two consecutive days and kept in culture for 96 hours from the time of the first retroviral transduction. Transduced cells will be harvested and infused fresh as soon as the lot release criteria are cleared. On the infusion day, patients will have completed the non-myeloablative but lymphocyte depleting preparative regimen consisting of cyclophosphamide and fludarabine.

In the event the patient is not clinically fit to receive the gene-modified MART-1 TCR CTL infusion within 24 hours of the scheduled day of cell harvest, the cells will be cryopreserved on the harvest day for later use.

9.1.1 Retrovirus Vector MSGV1-F5AfT2AB

Product Description. The retroviral vector, MSGV1-F5AfT2AB, was generated at the Surgery Branch/NCI, and consists of 4,196 bps including the 5' LTR from the murine stem cell virus (promoter), packaging signal including the splicing donor and splicing acceptor sites, alpha chain and beta chain genes of the anti-MART-1 F5 TCR from TIL clone DMF5 [7,41], and murine stem cell virus 3'LTR. The alpha and beta TCR chains are linked by a T2A picornavirus peptide sequence.
Classification: Immunotherapeutic.
Active Ingredient. This retroviral vector has two active transgenes, the alpha and beta chains of a high affinity TCR that jointly recognizes the MART-$1_{27\text{-}35}$ epitope in the context of HLA-A*0201.

- **MART-1 F5 TCR:** This high affinity TCR pair specific for the melanoma antigen peptide MART-$1_{27\text{-}35}$ presented by HLA-A*0201 was cloned from a patient with metastatic melanoma. The TCR chain genes have not been modified to alter their amino acid sequence, and the affinity for the MART-$1_{26\text{-}35}$ peptide presented by MHC tetramers is identical between the original cells obtained from this patient and the affinity of the MART-1 F5 TCR when expressed as a transgene in primary human T cells [7,41].

Mode of Action: One functional complex will be generated by the active transgenes:

- **MART-1 F5 TCR:** The MART-1 F5 TCR is expected to redirect the specificity of the TCR transgenic T cells to the MART-$1_{27\text{-}35}$ peptide presented by HLA-A2*0201 on the surface of melanoma cells.

Manufacturing Information for the Clinical Grade Vector: The clinical grade MSGV1-F5AfT2AB was generated at the Indiana University Vector Production Facility (IU VPF). The retroviral vector supernatant (PG13/F5af2aB C162D1) was prepared and preserved following cGMP conditions at IU VPF. The physical titer was determined by RNA dot blot according to the manufacturer’s certificate. The supernatant was stored upon the completion of production at least at –70° C at IU VPF. Upon shipment on dry ice, the supernatant is stored at –80° C at the HGMP/JCCC GMP suite. This facility is equipped with around-the-clock temperature monitoring. Supernatant is thawed and used for *in vitro* transduction of activated PBMC. There will be no re-use of the same unit of supernatant for different patients. The retroviral titer has been shown to be stable after immediate thawing and immediate administration (tissue culture wells previously coated with retronectin). Handling of the vector should follow the guidelines of Biosafety Level-2 (BSL-2).
How Supplied: The clinical grade vector is supplied by IU VPF following their Standard Operating Procedures (SOP) for retroviral vector suspension, labeling and cryopreservation.
Storage: Single use aliquots are stored in a viral bank established in a dedicated –80°C freezer with central, computerized monitoring system, with alarm and recording of all GMP storage systems located at the restricted access JCCC GMP Suite, 14th floor of the Factor Building at UCLA.
Stability: Under the conditions of continuous storage at –80°C without freeze-thawing, it is expected that the retroviral vector is stable for at least 5 years. Aliquots of this vector will be recertified annually throughout the study period with selected assays for purity, identity and potency.
Dose and Schedule: For *ex vivo* use only.
Route of Administration. All use of this retrovirus vector is *ex vivo*, and no direct injection will be done in human subjects.
Prior Human Experience. This same retrovirus vector expressing the MART-1 F5 TCR and similarly used to transduce human PBMC has already been administered to humans within a phase 2 clinical trial at the Surgery Branch/NCI [7]. The prior human experience is as follows:

- **Retrovirus Vectors:** Retroviral vectors have undergone extensive testing in humans. Their main safety caveat is the development of insertional mutagenesis, which seems to be a rare event and may be related to the expressed transgene and the immune competency of the host. Over 250 patients in over 40 clinical trials have received stem cells genetically modified with retroviral vectors, and the only cases of malignant transformation are the two infants in a clinical trial inserting the gammaC gene (a surface cytokine receptor) to HSC of infants with X-linked severe combined immune deficiency (SCID) [28,29].

- **MART-1 F5 TCR:** The same MART-1 F5 TCR alpha and beta gene pair is being tested at the Surgery Branch/N.C.I. (Steven A. Rosenberg, P.I.) for its ability to redirect antigen specificity of human PBMC adoptively transferred to lymphopenic hosts in a protocol design similar to the one proposed herein [7]. If we gain knowledge of the development of serious adverse events (SAE) related to the MART-1 TCR transgenic T cell administration, our protocol procedures and informed consent form will be modified accordingly.

Reported Adverse Events and Potential Risks: The potential adverse events are related to the viral vector, transgenes and genetically modified cells, as discussed in the following section.

9.1.2 PBMC Transgenic for MART-1 F5 TCR

Product Description. PBMC obtained from leukapheresis and activated for 48 hours with OKT3 (anti-CD3 antibody) and IL-2, infected by the clinical grade MSGV1-F5AfT2AB retroviral vector supernatant in two consecutive days, maintained in culture for 4 days from the start of transduction in IL-2. Aliquots of these cells will be used to fulfill the lot release criteria.
Classification: Immunotherapeutic.
Active Ingredient. As described for the retrovirus vector MSGV1-F5AfT2AB.
Mode of Action: The genetically modified PBMC expressing a transgenic surface MART-1 F5 TCR will be expected to be redirected to the MART-1 antigen expressed by HLA-A2*0201 and MART-1 positive melanoma cells. Recognition of the cognate antigen will result in the release of effector molecules from T cells, like the cytotoxic granules perforin and granzyme B, and activating cytokines like interferon-gamma, IL-2 and TNF-alpha.
Manufacturing Information: Genetically modified PBMC expressing transgenic MART-1 F5 TCR will be manufactured by transduction of previously activated PBMC using the clinical grade retroviral vector MSGV1-F5AfT2AB.
Storage: PBMC transgenic for MART-1 F5 TCR will be harvested within 96 hours from the first transduction and infused fresh. If cryopreserved, they will be stored in a centrally-monitored liquid nitrogen freezer in the JCCC GMP suite located on the 14th floor of Factor Building, UCLA.
Stability: These genetically modified PBMC will be infused fresh. When stored in liquid nitrogen, PBMC are viable for over 2 years with minimal viability loss if not subject to freeze-thawing. Cells will be used within 6 months of manufacture.
Dose and Schedule: Administered once on day 0 of this protocol, receiving a minimum target cell dose of 10^8 and up to 10^9 PBMC transgenic for MART-1 F5 TCR.
Administration: Transgenic cells will be transported from the GMP suite to the hospital bed in an infusion bag with over-wrap, on ice in a rigid-walled container with a tight fitting lid (i.e. Tupperware; Nalgene container etc.) labeled with a biohazard label. A detailed explanation for the change of PBMC protocol from Amendment 7 is provided at the end of the protocol in the Appendix: Amendment 8.
Unused Transgenic Cells. Transgenic cells generated but not administered to patients will be cryopreserved and banked for up to 5 years. Reinfusion of cells has to be done within 6 months of their cryopreservation. Cells may be used for additional *in vitro* testing beyond this period of time. Disposal of transgenic cells remaining in the bag and i.v. tubing after administration to patients will be done in a biohazard container that will be autoclaved before disposal.
Prior Human Experience: As described above, PBMC transduced with the same retrovirus vector MSGV1-F5AfT2AB, is currently in clinical testing [7].
Reported Adverse Events and Potential Risks: Potential risks are derived from the viral vector, the transgenes it will express and the infusion of genetically modified cells:

- **Retroviral Vectors:** There are two potential serious anticipated risks associated with retroviral vectors. One is related to its ability to randomly and permanently integrate in the genome of target cells, which may lead to altered expression of genes in these cells potentially inducing oncogenic changes. The second one is related to the possibility of its recombination and generation of replication competent virus that could potentially lead to a HIV-like disease.
    - **Risk of Insertional Mutagenesis with Retroviral Vectors.** The risks of insertional oncogenesis with integrating gene delivery vectors is likely a complex function of several

factors, related to the type of vector used, the promoter and other transcriptional control elements of the vector, the transgene *per se*, as well as properties of the target cells. Moloney Leukemia Virus (MLV)-based gamma-retroviral vectors have been shown to have a high predilection for insertion into the 5' region of actively transcribed genes, which may increase the risks for trans-activation of transcription from the cellular gene promoter [55]. Genes that modulate cell proliferation, such as the gammaC gene used to treat X-linked SCID may provide one component of a proliferative signal that plays a cooperative role in cellular transformation with cellular genes involved in cellular proliferation, such as LMO-2, when activated by insertional trans-activation [28]. In contrast, effector proteins such as TCR would not, *a priori*, be expected to confer an unregulated proliferative stimulus, although they may play a role in clonal expansion upon encounter with the cognate antigen.

- **Risk of Replication Competent Retrovirus (RCR) with Retroviral Vectors.** Another risk is that genetic recombination within components of the vector system could lead to the generation of RCR. This event would have the potential of generating infective retrovirus that may lead to oncogenesis or immune deficiency. Our patients will be screened at baseline to be HIV seronegative. Since there will be no wild type HIV virus present, the possibility of providing the missing genes for the generation of infective retrovirus *in vivo* would be lower than when retroviral vectors are administered to HIV positive subjects.

- **MART-1 TCR:** The main potential toxicities from the transgenic alpha and beta MART-1 TCR genes expressed in PBMC are derived from the MART-1 specificity of the TCR, or the mispairing with endogenous TCR genes leading to cells with autoimmune specificity.
  - **Autoimmune Toxicity against MART-1 Positive Cells.** Toxic events could be potentially derived from cytotoxic activity of the transgenic PBMC against MART-1 positive cells [7]. This could result in disappearance of pigmented cells in the skin and development of vitiligo, pigmented cells in the uvea of the eye inducing uveitis, in the retina with the potential development of blindness, and in the inner ear resulting in vestibular problems. In particular, in the NCI Surgery Branch experience, anterior uveitis was noted in 55% of patients and hearing loss in 50% of patients receiving F5 TCR transgenic cells. These cases responded to local injection of corticosteroids [7]. These toxicities were not noted in the original UCLA experience with this F5 TCR with cell doses up to 10^9. With the F5 infusion cell number going back down to 10^9, We will keep prospectively monitoring ophthalmologic toxicity following standard eye exam evaluation for patients receiving tumor immunotherapy [56,57], and potential otological toxicities by serial otological exams.
  - **Autoimmune Toxicity from Mispairing of TCR Chains.** The alpha and beta chains of the MART-1 TCR could undergo heterologous pairing with endogenous alpha and beta chains of TCR expressed by the transduced T cells, leading to misdirected T cells with newly acquired TCR specificities. These would be unpredictable and not subjected to the thymic selection process, potentially resulting in T cells with autoreactivities. If evidence of autoimmunity developed, patients would receive immune suppressive therapy as clinically indicated based on the severity of symptoms, using medications like corticosteroids, cyclosporin-A, mycophenolate mofetil, anti-TNF-alpha antibodies or anti-thymocite globulin (ATG).
- **MART-1 F5 TCR Transgenic T Cells.** The adoptive transfer of an *ex vivo* modified autologous cellular product may lead to potential toxicities related to the cell manipulation.
  - **Allergic Reaction and Cytokine Release Syndrome.** The culture of cells *ex vivo* in media products to maintain lymphocyte viability, the use of reagents to activate cells, the retroviral transduction process, and the expression of foreign genes may lead to alterations in the cell product resulting in immediate untoward effects. The most serious would be infusion reactions leading to severe allergic reactions, or the triggering of a cytokine release syndrome. Therefore, the genetically modified cells will be re-infused in an inpatient setting, with continuous monitoring of vital signs and by experienced personnel within the Hematology-Oncology HSC unit.

- **Contaminated Cell Product.** *Ex vivo* culture and manipulation of cells may result in a cell product contaminated by bacteria, fungus, mycoplasma or virus. With the goal of detecting potential contaminants, the genetically modified cells will undergo intensive lot release testing prior to infusion.
- **Autoimmune Toxicity from Mispairing of TCRs**. The chains of the MART-1 TCR could pair with chains of TCR previously expressed by the T cells, leading to T cells that could then attack normal organs inducing a condition similar to autoimmune diseases. If evidence of autoimmunity developed, patients would receive immune suppressive therapy as clinically indicated based on the severity of symptoms, using medications like corticosteroids, cyclosporin-A, mycophenolate mofetil, anti-TNF-alpha antibodies or anti-thymocite globulin (ATG).

9.2 MART-1$_{26-35}$ Peptide Pulsed Dendritic Cells

9.2.1 MART-1$_{26-35}$ Peptide

Product Description: MART-1$_{26-35}$ is an anchor-modified decamer derived from the immunodominant epitope derived from the well-characterized MART-1 melanoma lineage antigen in the context of HLA-A*0201 [20,58-60]. This HLA haplotype corresponds to greater than 30% of the general population in major ethnicities. This decamer anchor-modified peptide derived from the native MART-1$_{27-35}$ peptide with the amino-acid sequence ELAGIGILTV (MART-1$_{26-35}$) has more favorable HLA-A*0201 binding kinetics, with a longer off-rate resulting in greater ability to interact with T cells specific for MART-1$_{27-35}$ [58-60]. This peptide has shown ability to activate MART-1$_{27-35}$-specific T cells in human subjects with a very good toxicity profile [6,61].
Amino Acid Sequence: ELAGIGILTV (H-Glu-Leu-Ala-Gly-Ile-Gly-Ile-Leu-Thr-Val-OH).
Other Names: Melan-A.
Classification: Antitumor vaccine.
MART-1$_{26-35}$ Peptide Molecular Weight: 985.2.
Chemical Formulation: $C_{45}H_{80}N_{10}O_{14}$.
Active Ingredient: MART-1$_{26-35}$ Peptide: An immunodominant anchor-modified peptide in the HLA-A*0201 haplotype derived from the melanoma tumor antigen MART-1.
Mode of Action: MART-1$_{26-35}$ is recognized *in vivo* by the immune system and induces tumor-specific CTL, by initiation of a MHC class I-restricted cytotoxic response against tumor cells bearing this antigen.
MART-1$_{26-35}$ Clinical Grade Peptide Manufacture: Clinical grade peptides will be purchased from Biosynthesis, and provided with a Certificate of Analysis (COA).
How Supplied: MART-1$_{26-35}$ peptide is supplied as a lyophilized powder by Biosynthesis (Lewisville, TX) and provided with a COA.
Storage: MART-1$_{26-35}$ peptide for injection 10 mg per vial: Store intact vials refrigerated at -20° C. Working stocks of peptide solubilized in DMSO can be stored at – 80°C.
Stability: The peptide is stable at the recommended storage temperature for at least one year.
Dose and Schedule: For *ex vivo* use only.
Administration: The peptide will be used *ex vivo* to pulse DC, will not be directly administered to human subjects.
Prior Human Experience: Administration of DC pulsed with the anchor-modified MART-1$_{26-35}$ peptide has shown to be safe and able to activate T cells specific for the native MART-1$_{27-35}$ peptide in human subjects [6,61].
Reported Adverse Events and Potential Risks: Multiple clinical trials of tumor antigen-based peptide immunization have been conducted and published, either using biochemical adjuvants or pulsing DC [62]. Immunization with the native MART-1$_{27-35}$ peptide pulsed onto DC has proven to be a generally safe approach able to stimulate antigen-specific T cells in human subjects [4,5]. A recently completed phase I clinical trial at UCLA administered DC pulsed with this same modified MART-1$_{26-35}$ peptide together with the anti-CTLA4 antibody tremelimumab (CP-675,206, Pfizer Inc.) to 16 patients with metastatic melanoma, with no untoward adverse events attributed to the modified MART-1$_{26-35}$ peptide. Four patients in this series have ongoing objective responses and are melanoma-free at 9+ to 42+ months.

9.2.2 MART-1$_{26-35}$ Peptide Pulsed Dendritic Cells (DC):

Product Description: Dendritic cells will be differentiated from autologous adherent PBMC in cultures containing GM-CSF and IL-4. After one week, loosely-adherent cells are harvested and pulsed with the anchor-modified MART-1$_{26-35}$ peptide, and then prepared for i.d. administration to patients as fresh cells.
Active Ingredient: The biologically active ingredient is the MART-1$_{26-35}$ peptide, which is presented by HLA-A2*0201 complexes on the cell surface of DC together with costimulatory molecules and activating cytokines.
Mode of Action: Dendritic cells are the central cells for initiating a cellular immune response. They are the most efficient cells for processing and presenting antigen in a MHC class I and II-restricted fashion. Antigenic epitopes are presented together with a high level of cell surface costimulatory (B7.1, B7.2, CD40) molecule expression.
Tissue Source: Autologous adherent PBMC differentiated *in vitro* in GM-CSF (Genzyme) and IL-4 (Cell Genix or the current supplier of clinical grade IL-4) to generate DC.
Manufacturing Information: Dendritic cells are generated from PBMC obtained from a single, unmobilized leukapheresis procedure by *in vitro* culture for 7 days in media containing 5% autologous heat-inactivated plasma, supplemented with GM-CSF and IL-4. The *in vitro* generation of DC at the JCCC GMP Facility for MART-1 peptide DC-based clinical trials is covered by INDs BB # 7122 and 11579 [4,5]. The DC manufacture in the current study will be conducted following the same procedures.
Storage: MART-1$_{26-35}$ peptide pulsed DC will not be stored; they will be administered fresh after lot release testing. During the time between peptide pulsing and injection to patients after lot release testing, the cells will be kept loaded in the syringe used for the injection in the final formulation, and kept at 4°C or on ice in an ice bucket.
Stability: MART-1$_{26-35}$ peptide pulsed DC are stable and viable up to 24 hours from the time they are loaded onto a syringe for injection.
Dose and Schedule: A target cell number of 10^7 MART-1$_{26-35}$ peptide pulsed DC i.d. days 1, 14 and 30.
Route of Administration: Intradermal, close to a lymph node basin not known to be infiltrated by melanoma.
Prior Human Experience: Our completed phase I/II clinical trial (IND #7122) [4,5], where patients with melanoma were treated with DC pulsed with the native MART-1$_{27-35}$ immunodominant peptide, has allowed us to gain ample experience in this cell-based immunization strategy. This experience has been incorporated to an ongoing clinical trial administering the DC pulsed with the anchor-modified MART-1$_{26-35}$ anchor-modified peptide together with the anti-CTLA4 antibody tremelimumab (formerly CP-675,206) manufactured by Pfizer Inc. (Groton-New London, CT) (IND # 11579). We have optimized our laboratory technique to minimize the contamination risk and allow detection of any adventitious component in the vaccine before injection into patients. Pharmacokinetics of DC-based vaccines in humans have been conducted by others. When DC are injected i.d., as proposed in the current trial, the cells can be detected in the draining lymph nodes, supposedly at the site where they are able to stimulate the host immune system [63,64]. The pharmacodynamic activity of i.d.-injected DC can be demonstrated by the generation of antigen-specific immune responses detected in PBMC [4,5].
Reported Adverse Events and Potential Risks: Several phase I/II clinical trials have been published where DC have been administered to human subjects [62,65]. This has proven to be a generally safe treatment with significant activity in previously treated low grade lymphomas, metastatic melanomas, renal cell carcinoma, prostate cancer and colon cancer, among other malignancies. A potential adverse event induced by the administration of *ex vivo* cultured DC is the administration of a product contaminated by pathogens. In an attempt to avoid this event, DC will undergo strict lot release criteria of the final product before administration to human subjects, and the administration route has been chosen to minimize the possibility of systemic toxicities. Otherwise, in our experience of 63 patients (25 with MART-1$_{27-35}$/DC, 22 with AdVMART1/DC and 16 with MART-1$_{26-35}$/DC and concomitant anti-CTLA4 antibodies), we have not observed SAE attributable to the MART-1-expressing DC.

9.3 Interleukin-2 (aldesleukin, Proleukin, recombinant human Interleukin 2)

Description: Please refer to package insert for complete product Information. Human recombinant interleukin-2 is a highly purified protein with a molecular weight of approximately 15,300 daltons. It is a lymphokine produced by recombinant DNA technology using a genetically engineered E. coli strain containing an analog of the human interleukin-2 gene.
Mode of Action: Immunotherapeutic agent which stimulates T and NK cell responses.
How Supplied: Interleukin-2 (IL-2) is manufactured by Prometheus.
Formulation/Reconstitution: IL-2 is provided as single-use vials containing 22 million IU (~1.3 mg) IL-2 as a sterile, white to off-white lyophilized cake plus 50 mg mannitol and 0.18 mg sodium dodecyl sulfate, buffered with approximately 0.17 mg monobasic and 0.89 mg dibasic sodium phosphate to a pH of 7.5 (range 7.2 to 7.8). The vial is reconstituted with 1.2 mL of Sterile Water for Injection, USP, and the resultant concentration is 18 million IU/ml or 1.1 mg/mL. Diluent should be directed against the side of the vial to avoid excess foaming. Swirl contents gently until completely dissolved. Do not shake. Since vials contain no preservative, reconstituted solution should be used within 24 hours.
Storage: Intact vials are stored in the refrigerator (2° – 8°C) protected from light. Each vial bears an expiration date.
Dilution/Stability: Reconstituted IL-2 should be further diluted with 50 mL of 5% Human Serum Albumin (HSA). The HSA should be added to the diluent prior to the addition of recombinant IL-2. Dilutions of the reconstituted solution over a 1000-fold range (i.e., 1 mg/mL to 1 mcg/mL) are acceptable in either glass bottles or polyvinyl chloride bags. IL-2 is chemically stable for 48 hours at refrigerated and room temperatures, 2° to 30°C.
Dose and Schedule: 500,000 IU/m^2 q12 hours for a maximum of 14 doses.
Administration: The final dilution of IL-2 will be given subcutaneously.
Toxicities: Expected toxicities of IL-2 are listed in the product label. Grade III toxicities common to IL-2 include diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes.

9.4 Fludarabine

Description: Please refer to package insert for complete product Information. Fludarabine phosphate is a synthetic purine nucleoside that differs from physiologic nucleosides in that the sugar moiety is arabinose instead of ribose or deoxyribose. Fludarabine is a purine antagonist antimetabolite.
Mode of Action: Chemotherapy agent.
How Supplied: Fludarabine is supplied by Bayer in a 50 mg vial as a fludarabine phosphate powder in the form of a white, lyophilized solid cake.
Stability: Following reconstitution with 2 mL of sterile water for injection at a concentration of 25 mg/ml, the solution has a pH of 7.7. The fludarabine powder is stable for at least 18 months at 2-8°C; when reconstituted, fludarabine is stable for at least 16 days at room temperature. Because no preservative is present, reconstituted fludarabine will typically be administered within 8 hours. Specialized references should be consulted for specific compatibility information. Fludarabine is dephosphorylated in serum, transported intracellularly and converted to the nucleotide fludarabine triphosphate; this 2-fluoro-ara-ATP molecule is thought to be required for the drug's cytotoxic effects. Fludarabine inhibits DNA polymerase, ribnucleotide reductase, DNA primase, and may interfere with chain elongation, and RNA and protein synthesis.
Storage: Intact vials should be stored refrigerated (2-8°C).
Dose and Schedule: 25 mg/m^2/day for 4 days. The dose of fludarabine will be adjusted based on estimation of the creatinine clearance (CrCl). For CrCl 30-70 the dose should be reduced by 20%, and for CrCl <30 – not given.
Administration: Fludarabine is administered as an i.v. infusion in 100 ml 0.9% sodium chloride, USP over 15 to 30 minutes. To prevent undue toxicity the dose will be based on body surface area (BSA), but will not exceed a dose calculated on surface areas based on body weights greater than 140% of the maximum ideal body weight per height and weight.

Toxicities: At doses of 25 mg/m^2/day for 5 days, the primary side effect is myelosuppression; however, thrombocytopenia is responsible for most cases of severe and life-threatening hematologic toxicity. Serious opportunistic infections have occurred in patients with chronic lymphocytic leukemia (CLL) treated with fludarabine. Hemolytic anemia has been reported after one or more courses of fludarabine with or without a prior history of a positive Coomb's test; fatal hemolytic anemia has been reported. In addition, bone marrow fibrosis has been observed after fludarabine therapy. Other common adverse effects include malaise, fever, chills, fatigue, anorexia, nausea and vomiting, and weakness. Irreversible and potentially fatal central nervous system toxicity in the form of progressive encephalopathy, blindness, and coma is only rarely observed at the currently administered doses of fludarabine. More common neurologic side effects at the current doses of fludarabine include weakness, pain, malaise, fatigue, paresthesia, visual or hearing disturbances, and sleep disorders. Adverse respiratory effects of fludarabine include cough, dyspnea, allergic or idiopathic interstitial pneumonitis. Tumor lysis syndrome has been rarely observed in fludarabine treatment of CLL. Treatment on previous adoptive cell therapy protocols in the Surgery Branch/NCI have caused persistently low (below 200) CD4 counts, and one patient developed polyneuropathy manifested by vision blindness, and motor and sensory defects [3].

9.5 Cyclophosphamide

Description: Please refer to package insert for complete product Information. Cyclophosphamide is a nitrogen mustard-derivative alkylating agent. Following conversion to active metabolites in the liver, cyclophosphamide functions as an alkyating agent; the drug also possesses potent immunosuppressive activity. The serum half-life after i.v. administration ranges from 3-12 hours; the drug and/or its metabolites can be detected in the serum for up to 72 hours after administration.
Mode of Action: Chemotherapy agent.
How Supplied: Cyclophosphamide will be obtained from commercially available sources.
Stability: Following reconstitution as directed with sterile water for injection, cyclophosphamide is stable for 24 hours at room temperature or 6 days when kept at 2-8^{0}C.
Dose and Schedule: 60 mg/kg/day x 2 days i.v.
Administration: It will be diluted in 250 ml D5W and infused i.v. over one hour. The dose will be based on the patient's body weight, but to prevent undue toxicity, it will not exceed a dose greater than 140% of the maximum ideal body weight per height and weight.
Toxicities: Hematologic toxicity occurring with cyclophosphamide usually includes leukopenia and thrombocytopenia. Anorexia, nausea and vomiting, rash and alopecia occur, especially after high-dose cyclophosphamide; diarrhea, hemorrhagic colitis, infertility, and mucosal and oral ulceration have been reported. Sterile hemorrhagic cystitis occurs in about 20% of patients; severity can range from microscopic hematuria to extensive cystitis with bladder fibrosis. Although the incidence of hemorrhagic cystitis associated with cyclophosphamide appears to be lower than that associated with ifosfamide, mesna (sodium 2-mercaptoethanesulfonate) has been used prophylactically as a uroprotective agent in patients receiving cyclophosphamide. Prophylactic mesna is not effective in preventing hemorrhagic cystitis in all patients. Patients who receive high dose cyclophosphamide may develop interstitial pulmonary fibrosis, which can be fatal. Hyperuricemia due to rapid cellular destruction may occur, particularly in patients with hematologic malignancy. Hyperuricemia may be minimized by adequate hydration, alkalinization of the urine, and/or administration of allopurinol. If allopurinol is administered, patients should be watched closely for cyclophosphamide toxicity (due to allopurinal induction of hepatic microsomal enzymes). At high doses, cyclophosphamide can result in a syndrome of inappropriate antidiuretic hormone (SIADH) secretion; hyponatremia with progressive weight gain without edema occurs. At high doses, cyclophophamide can result in cardiotoxicity. Deaths have occurred from diffuse hemorrhagic myocardial necrosis and from a syndrome of acute myopericarditis; in such cases, congestive heart failure may occur within a few days of the first dose. Other consequences of cyclophosphamide cardiotoxicity include arrhythmias, potentially irreversible cardiomyopathy, and pericarditis. Other reported adverse effects of cyclophosphamide include headache, dizziness, and myxedema; faintness, facial flushing, and diaphoresis have occurred following IV administration. Mesna (sodium 2-mercaptoethanesulphonate; given by IV injection) is a

synthetic sulfhydryl compound that can chemically interact with urotoxic metabolites of cyclophosphamide (acrolein and 4-hydroxycyclophosphamide) to decrease the incidence and severity of hemorrhagic cystitis.

9.6 Laboratory Reagents

9.6.1 OKT3. OKT3 (Miltenyi Biotec or other supplier of clinical grade OKT3), is an anti-CD3 antibody used to activate T cells *in vitro* by engaging the CD3 complex. In the current clinical trial, OKT3 will be strictly used *ex vivo* for the activation of PBMC for retroviral transduction. A clinical grade OKT3 (Miltenyi) is a therapeutic monoclonal antibody approved by the FDA to treat rejection of transplanted organs, including the heart, kidneys and liver. However, this antibody is currently not being produced by the clinical grade manufacture. When used *in vivo*, OKT3 antibodies eliminate CD3 positive lymphocytes which is beneficial to treat graft rejections. It can induce anaphylactic or anaphylactoid reactions, cytokine release syndrome and a variety of constitutional symptoms when administered systemically. The source of OKT3 antibody for the current studies is obtained with a certificate of analysis providing its manufacturing and product characterization. In addition, after aliquoting, the batches used within this study are further characterized by performing the following tests: Gram stain, fungal stain, bacterial culture, fungal culture, mycoplasma culture, endotoxin assay.

9.6.2. Interleukin-2. The IL-2 used for *in vitro* stimulation of PBMC will be the same as described for systemic IL-2 treatment in patients. All pertinent information is described in that section.

9.6.3 GM-CSF. Leukine® (Genzyme) is a recombinant granulocyte-macrophage colony-stimulating factor (rhu GM-CSF) approved by the FDA for use following induction chemotherapy to shorten time to neutrophil recovery. When used *in vivo*, it can induce constitutional symptoms and bone pain. When used *in vitro*, it can sustain the differentiation of monocytes to DC. In the current clinical trial, GM-CSF will be strictly used *ex vivo* for the generation of DC.

9.6.4 Interleukin-4. Clinical grade recombinant human IL-4 is purchased from Miltenyi Biotec or the current supplier of clinical grade IL-4. This cytokine has been previously tested systemically in human subjects [66], but has not been licensed by the FDA for human use. When used *in vitro*, IL-4 increases the differentiation of monocytes to DC. This same cytokine source has been used in prior DC-based clinical trials at UCLA (IND # 11579 and 11053) and was safe and biologically active when used for DC generation [67].

9.6.5. Retronectin. RetroNectin® (CH-296) is purchased from Clontech Laboratories or the current supplier of clinical grade RetroNectin®. RetroNectin® is a chimeric peptide of recombinant human fibronectin fragments produced in *E. coli*, consisting of three functional domains: a central cell-binding domain (type III repeat, 8-10), heparin-binding domain II (type III repeat, 12-14), and a CS1 site within the alternatively-spliced IIICS region. It is a 574 amino acid protein with a molecular weight of 63 kDa. When coated on the surface of containers such as culture dishes, petri dishes, flasks or bags, retronectin significantly enhances retrovirus-mediated gene transduction into mammalian cells. This enhancement is hypothetically due to co-localization of retroviral particles and target cells on the molecules of retronectin. Virus particles bind retronectin via interaction with heparin-binding domain II, and target cells bind mainly through the interaction of the cell surface integrin receptor VLA-4 with the fibronectin CS1 site. Additionally, cells may also bind through the interaction of another fibronectin ligand (RGDS in repeat 10) within the central cell-binding domain with a corresponding integrin receptor VLA-5 on the cell surface.

10.0 GENERATION OF EXPERIMENTAL CELL THERAPIES: MART-1-TCR TRANSDUCED CD8 CELLS AND MART-1$_{26-35}$ PEPTIDE PULSED DENDRITIC CELLS

Feasibility is one of the primary endpoints of this protocol. Repetitive deviations to the procedures described in this section will be recorded in the SOPs and the protocol modified accordingly and highlighted in the next submission to the IRB and FDA.

10.1 Generation of MART-1 F5 TCR Transduced PBMC

10.1.1 Leukapheresis Procedures

Patients will undergo a 12 liter leukapheresis procedure to obtain PBMC for retroviral transduction and monocytes for DC generation. Any signs and symptoms of citrate toxicity due to apheresis (such as perioral paresthesia and muscle cramps) will be treated according to the UCLA Hemapheresis center standard procedures. The leukapheresis product will be transferred to the UCLA HGMP/JCCC GMP Suite for cell processing.

10.1.2 PBMC Isolation

Following leukapheresis, the product will be separated into mononuclear fraction for PBMC isolation using Ficoll and layering the leukapheresis product over this liquid and centrifuging at 500g for 30 minutes.

10.1.3 PBMC Activation

An aliquot of PBMC obtained from leukapheresis will be cultured in AIMV with 5% heat-inactivated AB serum at 10^6 cells/ml and activated for 48 hours in the presence of OKT3 (50 ng/ml, anti-human CD3 antibody) and human IL-2 (300 IU/ml, aldesleukin) in order to stimulate T-cell growth to prepare for viral transduction.

10.1.4 Clinical Grade Retrovirus MSGV1-F5AfT2AB Lot Release Testing

Prior to use, the clinical grade retroviral vector will require fulfilling the lot release criteria included in Table 1.

Table 1. Clinical Grade MSGV1-F5AfT2AB Retrovirus Lot Release Criteria:

Table 1a. Certificate of Analysis of the MSGV1-F5AfT2AB Master Cell Bank (MCB).

Test	Methods	Limits	Results	Date
Contaminants	Aerobic and anaerobic culture for bacteria and fungus	No growth within 14 days	No growth within 14 days	Apr-10-2007
	Mycoplasma culture and vero indicator cells	Negative for the presence of mycoplasma	Negative	Apr-04-2007
	In-vitro viral assay utilizing 3T3, MRC-5 and Vero cells	No CPE or hermadsorption	No CPE or hermadsorption	Apr-10-2007
	Bovine viral assay*	Negative for cyto-pathic effect, hermadsorption and the presence of 7 specific bovine viruses using IFA	Negative for cyto-pathic effect, hermadsorption and the presence of 7 specific bovine viruses using IFA	Dec-11-2006

	Porcine viral assay*	Negative for cytopathic effect, hemadsorption and the Presence of porcine Viruses, BVDV Reovirus and rabies	Negative for cytopathic effect, hemadsorption and the Presence of porcine Viruses, BVDV Reovirus and rabies	Dec-06-2006
	In-viro viral assay*	No evidence of contamination with adventitious viral agents	No evidence of contamination with adventitious viral agents	Dec-21-2006
	MAP/LCM*	No evidence of viral contamination	No evidence of viral contamination	Dec-21-2006
	Transmission E.M.*	No Vital particles other than retro-virus like particles and no other identifiable microbial agents.	No Vital particles other than retro-virus like particles and no other identifiable microbial agents	Nov-15-2006
	SV40Tag (PCR)*	Negative	Negative	Nov-14-2006
	E1a (PCR)*	Negative	Negative	Jan-22-2007
Human Vital Contaminants	HTLV-1/11 (PCR)*	Negative	Negative	Nov-16-2006
	HIV-1/2 (PCR)*	Negative	Negative	Nov-16-2006
	HEP B (PCR)*	Negative	Negative	Nov-14-2006
	HEP C (PCR)*	Negative	Negative	Nov-08-2006
	HHV 6 (PCR)*	Negative	Negative	Nov-15-2006
	HHV 7 (PCR)*	Negative	Negative	Nov-14-2006
	HHV 8 (PCR)*	Negative	Negative	Nov-15-2006
	CMV (PCR)*	Negative	Negative	Nov-15-2006
	EBV (PCR)*	Negative	Negative	Nov-14-2006
	Human parvovirus B19	Negative	Negative	Apr-14-2006
Replication Competent Retrovirus: GAL-V	S+L- (PG-4/293) amplification) 5% of MCB supernatant	No Evidence of RCR	No Evidence of RCR	Apr-10-2007
Replication Competent Retrovirus: GAL-V	S+L- (PG-4/293 co-culture) 1% of MBC	No Evidence of RCR	No Evidence of RCR	Apr-10-2007
Replication Competent Retrovirus: ECO	Marker rescue	No Evidence of RCR	No Evidence of RCR	Apr-10-2007
Identity	ADA Isoenzyme	Cells confirmed to be Of murine origin	Cells confirmed to of murine origin	Apr-10-2007
	Vector insert stability	Vector size/sequence consistent with expectant fragment size	Vector size/sequence consistent with expectant fragment size	Apr-10-2007
Vector Sequencing	Sequencing of the insert in the MCB	> 98% homology with the expected sequence	> 99.9% homology	Sep-26-2008

Table 1b. Certificate of Analysis of the MSGV1-F5AfT2AB Final Product.

Test	Methods	Limits	Results	Date
Contaminants	Aerobic and anaerobic culture for Bacteria and	No growth 14 days	No growth 14 days	March-18-2008

	Fungus			
	Mycoplasma culture and Vero indicator cells	Negative for the presence of Mycoplasma	Negative for the presence of Mycoplasma	Apr-04-2008
	In-Vitro viral assay utilizing 3T3, MRC-5 And Vero cells	No CPE or hermadsortion	No CPE or hermadsortion	Mar-18-2008
Endotoxin	Limulus amebocyte lysate	Less than 0.33 EU/mL	Harvest 1-7: less than 0.06 EU/mL	Mar-18-2008
Replication competent retrovirus: GAL-V	S+L-(PG-4) (293 infection) 5% of vector supernatant	No Evidence of RCR	No Evidence of RCR	Mar-18-2008
	S+L-(PG-4) (293 co-cuture) 10^8 cells from production run	No Evidence of RCR	No Evidence of RCR	Mar-18-2008
Transgene expression	MART-1_{26-35} tetramer or dextramer staining in activated PBMC	> 10% MART-1_{26-35} tetramer or dextramer positive cells among CD3+ T lymphocytes	> 75% MART-1_{26-35} tetramer positive cells among CD3+ T lymphocytes	Sept-02-2008
Potency	MART-1_{26-35} peptide and antigen-specific IFN-γ production by ELISA	> 30,000 pg/ml of IFN-γ production upon MART-1_{26-35} peptide stimulation using MART-1_{26-35} peptide-pulsed K562/A2.1 cells	> 100,000 pg/ml of IFN-γ production upon MART-1_{26-35} peptide stimulation using MART-1_{26-35} peptide-pulsed K562/A2.1 cells	Sept-05-2008

10.1.5 Clinical Grade Retrovirus MSGV1-F5AfT2AB Annual Recertification

Single-use aliquots of the clinical grade retrovirus vector will be subjected to annual recertification, and the results of this testing will be included in the annual progress report for the FDA (Table 2).

Table 2a. MSGV1-F5AfT2AB Retrovirus Annual Recertification:

Test Item	Method	Limit of Detection	Acceptable Criteria
Physical viral vector titer	qRT-PCR	5 x 10^3 copies/μl	Report value
Infectious viral vector titer (Transgene expression)	MART-1_{26-35} tetramer or dextramer staining in activated PBMC	0.03% MART-1_{26-35} tetramer or dextramer positive cells among CD3+ T lymphocytes	> 10% MART-1_{26-35} tetramer or dextramer positive cells among CD3+ T lymphocytes
Sterility	Aerobic and anaerobic culture for bacteria and fungus	No growth within 14 days	No growth within 14 days
Mycoplasma	MycoAlert test	20 cfu/ml	Negative (<1 ratio)
	PCR/ELISA mycoplasma assay if the MycoAlert results are equivocal or positive	10 cfu.ml	Negative by PCR
Endotoxin	Limulus amebocyte lysate or Endosafe - MCS System	0.1 EU/ml	<0.33 EU/ml for each sample

Legend: EU: endotoxin units.

Table 2b. MSGV1-F5AfT2AB Retrovirus Annual Recertification 2017:

TEST	METHOD	ACCEPTABILITY CRITERIA	RESULTS	DATE
Physical viral vector titer	qRT-PCR	5 x 10^3 viral particles/ ml	2.06 x 10^5 viral particles/ml	11/30/2017
Infectious viral vector titer (Transgene expression)	MART-$1_{26\text{-}35}$ tetramer staining in transduced PBMC	>10% MART-$1_{26\text{-}35}$ tetramer positive cells among CD3+ T lymphocytes	54.7% MART-$1_{26\text{-}35}$ tetramer positive cells among CD3+ T lymphocytes	11/15/2017
Sterility	Aerobic and anaerobic culture for bacteria and fungus	No growth within 14 days	No growth within 14 days	11/29/2017
Mycoplasma	MycoAlert test	Negative (<1 ratio)	Negative (<1)	10/27/2017
Endotoxin	Limulus amebocyte lysate or Endosafe - PTS System	<0.33 EU/ml	<0.25 EU/ml	10/27/2017

Legend: EU: endotoxin units.

10.1.6 Retrovirus Transduction Method

The MSGV1-F5AfT2AB retrovirus vector lot will be stored in a monitored and locked < = -70ºC freezer in the UCLA HGMP/JCCC GMP Suite, 14th floor, Factor Building (Viral Bank). Transductions will be performed in retronectin-coated 6-well plates.

RetroNectin Coating. Wells will be pre-coated with retronectin (Takara Bio Inc., Japan), a recombinant chimeric fibronectin molecule. 1 ml of 10 μg/ml retronectin in sterile PBS is placed per well in 6-well plates and incubated overnight at 4ºC (alternatively at room temperature for 2 hours). Wells are blocked with 1.5 ml of HBSS with 2.5% human serum albumin at room temperature for 30 minutes and washed with HBSS containing 2.5% HEPES.

Retroviral Transduction. Two to four ml of thawed and 1:1 diluted MSGV1-F5AfT2AB retrovirus viral supernatant in TCR medium is applied to each retronectin-coated well of Transduction #1 plates and centrifuged at 2000g for 2 hours at 32ºC. Retroviral supernatant is removed from transduction #1 plates (leaving behind one ml), 2 x 10^6 activated PBMC per well in AIMV plus 5% heat inactivated human AB serum supplemented by 300 IU/ml of IL-2 are added and centrifuged at 1000g for 10 minutes at 32 ºC. Plates are then incubated at 37ºC overnight at 5% CO_2. On the following day, after blocking and washing, two to four ml of thawed and 1:1 diluted MSGV1-F5AfT2AB retrovirus viral supernatant in TCR medium is applied to each retronectin-coated well of Transduction #2 plates and centrifuged at 2000g for 2 hours at 32ºC. Then, viral supernatant is removed from each well. PBMC are transferred from transduction #1 plates to transduction #2 plates and centrifuged at 1000g for 10 minutes at 32 ºC. Plates are incubated at 37ºC overnight at 5% CO_2.

Post-transduction Expansion. At the end of the transduction, cells are washed and maintained at 37ºC, 5% CO_2 in AIMV plus 5% heat inactivated human AB serum supplemented by 300 IU/ml of IL-2 at a density of 0.7 x 10^6 cells/ml for up to 96 hours from the initial transduction.

10.1.7 Transgenic PBMC Product Washing and Bagging

After completion of the cell transduction procedure and short term *ex vivo* expansion, the MART-1 F5 TCR transduced PBMC product is subjected to two washings, resuspended in saline containing 1%

HSA, put into an infusion bag and kept in a 4 degrees refrigerator until lot release clearance and i.v. infusion. Lot release tests are performed on aliquots of this final product. In the event patient is not able to receive the infusion within 24 hours of the scheduled harvest, cells will be re-suspended in cryopreservation medium and put into an infusion bag. Cryopreservation will be at < -130ºC in a monitored liquid nitrogen freezer at the JCCC GMP suite.

10.1.8 Product Labeling

The final MART-1 F5 TCR transgenic PBMC will be labeled using a preprinted study label with the following information:

- Patient UCLA ID. Number.
- Patient's initials.
- Subject laboratory clinical trial code.
- Date of cell preparation, with an expiration date of 24 hours if fresh and 6 months if cryopreserved.
- Initials of person who prepared the vaccine.
- Labeled with "For autologous use only".
- Labeled as "Not Tested for Biohazards" (due to no specific testing for HIV, HepC, HepB and other adventitious viruses in the final product).

10.1.9 In-process Testing and Final Product Lot Release Testing

In Process Testing. We will conduct the following in-process testings (Table 3a and 3b).

Table 3a. Activated PBMC (Day 2) In-process Testing:

Test Item	Acceptability Criteria	Results
Gram Stain	Negative	Negative
Fungal Stain	Negative	Negative
Bacterial Culture	No growth	No growth x 14 days
Fungal Culture	No growth	No growth x 14 days
Mycoplasma Culture	No growth	No growth x 14 days

Table 3b. Transduced PBMC (Day 5) In-process Testing:

Test Item	Acceptable Criteria
MycoAlert test	Negative ;ratio<1 (readingB/readingA)
PCR/ELISA mycoplasma assay if the MycoAlert results are equivocal or positive	Negative by PCR
TCR transgene expression	>10% of T cells by MART-1_{26-35} tetramer or dextramer staining
TCR transgene functionality	> 30,000 pg/ml/million cells of MART-1 specific IFN-γ production by ELISA

Final Product Testing and Lot Release Criteria. In Table 4a the results that will be required before administering the cells to the patients are described, and in Table 4b the results that need to be in process at that time but results may not be available at the time of administration to patients are described.

Table 4a. MART-1 F5 TCR Transgenic PBMC Lot Release Testing with Results Available Before Administration to Patients:

Test Item	Acceptable Criteria
Viability	$\geq$70%
Gram and fungal stains[a]	Negative
Endotoxin Assay [b]	≤ 5 EU/ kg body weight per dose
MycoAlert test[c]	Negative ;ratio<1 (readingB/readingA)
PCR/ELISA mycoplasma assay if the MycoAlert results are equivocal or positive	Negative by PCR
TCR transgene expression	>10% of T cells by MART-1_{26-35} tetramer or dextramer staining

a. Performed on the final product by the UCLA Clinical Microbiology Laboratory. All sterility cultures and mycoplasma culture will be followed up to 14 days. If the cultures become positive after the patient has received the cells, the patient will be started on empiric antibiotics.

b. Performed on the final product using the Limulus amebocyte lysate read using the Quantitative Chromogenic Procedure analyzed using an OCL-1000 (Bio Whittaker), or the Endosafe - PTS System (Charles River) assays.

c. If first line MycoAlert test becomes positive, it will be repeated with a second sample from the final product. Moreover, we will perform PCR/ELISA mycoplasma assay if the MycoAlert results are equivocal or positive. The release specifications for the mycoplasma test should be "negative by PCR".

Table 4b. MART-1 F5 TCR Transgenic PBMC Lot Release Testing with Results Available After Administration to Patients:

Test Item	Acceptable Criteria
Bacterial and fungal culture	No growth x 14 days
Mycoplasma culture	No growth x 14 days
GalV S^+ /L^- RCR assay	Sample archived for later use if required. Not tested prospectively in all samples.

Plan in Case of Positive Results of Tests Not Available at the Time of TCR Transgenic Cell Administration to Patients. The following will be our plan in case of results different from our proposed acceptable criteria:

- Bacterial or Fungal culture: An antibiogram will be obtained on the culture to typify the contaminant. The patient will be contacted, two peripheral blood draws will be collected for culture, and the patient will be started on therapy, first with broad spectrum antibiotics or antifungals, and then adapted to the antibiogram of the cultures.
- Mycoplasma culture: If the culture is positive after the rapid mycoplasma test had been negative and the cells were released for administration to patients, then the patient will be contacted, two peripheral blood draws will be collected for mycoplasma culture, and the patient will be started on therapy with erythromycin, clarithromycin or azithromycin.
- RCR: Since the TCR transgenic cell manufacture process will include *ex vivo* culture for up to 4 days from the first transduction, RCR will not be tested prospectively in the final product following the Guidance for Industry document by CBER, FDA from November 2006. If a sample from a patient tested at 3, 6 or 12 months or later is positive for RCR by PCR test, the stored sample of the final product will be tested. The patient will be contacted, two peripheral blood draws will be

collected for confirmation, and the patient will be started on therapy with combination antiretrovirals used to treat HIV infection.

Notification of Regulatory Agencies for Positive Results of TCR Transgenic Cell Administration to Patients. If a preparation with bacterial, fungal, mycoplasma or RCR contamination has been administered to a patient, this event should be reported to the following agencies and committees within 48 hours of first knowledge:

- Human Gene Medicine Program compliance officer.
- UCLA IRB.
- UCLA ISPRC.
- Study DSMB.
- RAC.
- FDA.

Additional Optional Testing of TCR Transgenic Cells. The release tests will be used to determine whether the final cell product can be released for infusion (see Tables above). In addition, the cell product may undergo further characterization apart from the release testing. The vector-specific proviral copy number per cell may be determined. We will perform additional testing for the number of vector copies and if higher than 5 we will propose a new dilution of the vector supernatant before using it for cell transduction. The sites of integration may be defined as the structure of the integrated transgene. Detailed flow cytometry and TCR chain usage analysis may be performed. The product may be further characterized for measurable transgene products (Table 5).

Table 5. MART-1 F5 TCR Transgenic PBMC Optional Additional Testing:

Characterization Assays	Comments
Vector proviral copy per transduced cell	Quantification of the copy number of provirus by qRT-PCR
Final product phenotyping	CD3, CD4, CD8, CD27, CD28, CD45RA/RO, CD62L, CCR7, PD-1
Transgene integration site analysis	Determination of integration site(s)
Structure of integrated transgene	Stability of integrated transgene
Detailed flow cytometry	Phenotype of cells
TCR transgene functionality	> 30,000 pg/ml/million cells of MART-1 specific IFN-γ production by ELISA
Other characterizations	Telomere loss, telomerase expression, Th1 vs Th2 pattern, FoxP3 expression in pre/post expansion T cells

10.2 Generation of MART-1$_{26\text{-}35}$ Peptide Pulsed Dendritic Cells

10.2.1 MART-1$_{26\text{-}35}$ Peptide:

MART-1$_{26\text{-}35}$ is an anchor-modified immunodominant epitope [58-60] derived from the well-characterized MART-1 melanoma lineage antigen in the context of HLA-A*0201 [20]. This HLA haplotype present in greater than 30% of the general population in major ethnicities.

10.2.2 Peptide Manufacturing

MART-1 peptide was purchased from Biosynthesis (Lewisville, TX) and was provided with a COA. Additional tests were performed to make it clinical grade. The aliquoted peptide for clinical use was lot release tested as defined in Table 6.

10.2.3 Peptide Aliquoting and Storage:

Peptides are solubilized in GMP-grade DMSO and stored aliquoted in single-use vials at -80ºC. Storage site is the UCLA Gene and Cell Therapy Core Facility, 14th floor Factor Building, in a monitored freezer with backup energy system containing material for experimental clinical trial use only.

10.2.4 MART-1$_{26\text{-}35}$ Peptide Working Stock Lot Release Testing:

This peptide working stock will undergo the lot release testing described in Table 6.

Table 6. MART-1$_{26\text{-}35}$ Peptide Working Stock Lot Release Testing:

Test Item	Acceptability Criteria	**Results**	**Date**
Gram Stain	Negative	Negative	8/25/2008
Fungal Stain	Negative	Negative	8/25/2008
Bacterial Culture	No growth	No growth x 14 days	09/10/2008
Fungal Culture	No growth	No growth x 14 days	09/10/2008
Endotoxin level	< 0.5 EU/ml	0.4 EU/ml	09/24/2008
Potency: IFN-γ ELISA upon MART-1$_{26\text{-}35}$ peptide stimulation	> 30,000 pg/ml of MART-1 specific IFN-γ production by ELISA using 1 x 10^6 MART-1 TCR transduced cells	> 100,000 pg/ml of MART-1 specific IFN-γ production	09/05/2008

10.2.5 MART-1$_{26\text{-}35}$ Peptide Annual Recertification:

The stored peptide working stock aliquots will undergo the annual recertification testing described in Table 7.

Table 7. MART-1$_{26\text{-}35}$ Peptide Working Stock Annual Recertification:

Test	**Method**	**Acceptability Criteria**	**Results**	**Date**
Microbiological studies	Bacterial culture	No growth	Negative	11/29/2017
	Fungal culture	No growth	Negative	11/29/2017
Endotoxin Assay	Endosafe – MCS or PTS System	<0.1 EU/µg peptide	<0.005 EU/µg peptide	10/31/2017
Mycoplasma test	Mycoalert/ PCR based assay	Negative (<1 ratio)	Negative (0.12)	10/31/2017

Legend: EU: endotoxin units.

10.2.6 Leukapheresis and Plasma Collection:

Patients will undergo a leukapheresis to obtain PBMC for MART-1 F5 TCR transduced cells and DC manufacture. Autologous plasma (100 ml) will be obtained by plasmapheresis on the day of leukapheresis. Autologous plasma is heat-inactivated at 56ºC for 1/2 hour and kept at -20ºC in sterile conical tubes labeled with the patient's assigned trial identification. Each batch of complete medium is filtered (0.20□ μm) prior to use.

10.2.7 Leukapheresis Lot Release Criteria:

Before adding DMSO-containing freezing media to the PBMC obtained from the leukapheresis product, an aliquot of these cells is sent for gram stain, sterility cultures for bacteria, fungi and mycoplasma (Table 8). All cultures are kept for at least 14 days, but the product can be used after negative stains are assessed and before final results of the cultures.

Table 8. Leukapheresis Lot Release Criteria:

Test Item	Acceptability Criteria
Gram Stain, Fungal Stain	Negative
Bacterial Culture	No growth
Fungal Culture	No growth
Mycoplasma culture	No growth

10.2.8 Plasma Lot Release Criteria:

Each batch of autologous plasma will be tested for gram stain, sterility cultures for bacteria, fungi and mycoplasma after heat inactivation (Table 9). All cultures are kept for at least 14 days, but the product can be used after negative stains are assessed and before final results of the cultures.

Table 9. Plasma Lot Release Criteria:

Test Item	Acceptability Criteria
Gram Stain, Fungal Stain	Negative
Bacterial Culture	No growth
Fungal Culture	No growth
Mycoplasma Culture	No growth

10.2.9 Dendritic Cell Manufacture:

DC are cultured at the UCLA Cell and Gene Therapy Core Facility, 14th floor Factor Building, a cell culture facility meeting cGMP standards, specifically designated for the conduct of clinical trials requiring the production of biological cell-based products. DC are prepared from adherent, autologous peripheral blood mononuclear cells exposed for 1 week in tissue culture to GM-CSF and IL-4.

Mononuclear cells are isolated from a leukapheresis procedure by Ficoll-Hypaque centrifugation, and used fresh or cryporeserved in at least 20% human AB serum, and 10% DMSO. DC Vaccine #1 will be cultured from fresh PBMC and the subsequent DC vaccines will be cultured by thawing cryopreserved PBMC. One week before the planned DC vaccination, cells are washed in saline and plated at 2.5 – 5 x 10^6 viable cells/ml in RPMI 1640 + 1-5% heat-inactivated autologous plasma (DC culture medium). The culture can also be done in clinical grade human AB serum instead of autologous plasma, which may be done if the amount of autologous plasma is limiting, or if there is failure to generate good DC preparations with them and the problem is assigned to the autologous product. After allowing adherence for 2 hr at 37ºC, non-adherent cells are gently removed by washing

with sterile saline solution. Adherent cells are cultured in DC culture medium for 7 days at 37ºC in 5% $C0_2$ in the presence of rhGM-CSF (768 IU/ml) and rhIL-4 (480 IU/ml).

10.2.10 In-Process Testing

In-Process Bacterial and Fungal Testing. On day 4 of DC culture, an aliquot of 0.5 ml of spent culture medium is removed from each tissue culture flask containing DC cultures, and is subjected to gram stain, fungal stain and sterility cultures for bacteria, fungi and mycoplasma. All cultures are kept for at least 14 days.

Mycoplasma Testing. On day 6 of DC culture, a sample is taken out from each flask for mycoplasma detection using a commercial PCR-ELISA kit from Roche (catalog No. 1 663 925) or the MycoAlert Mycoplasma Detection Kit (Lonza Inc, Allendale, NJ), which is based on detecting selective activity of mycoplasmal enzymes in a biochemical test, with the readout by bioluminescence (or another rapid assay with similar sensitivity to detect Mycoplasma contamination). Results will be reported before vaccine injection into the patient. Criteria for acceptance of the DC preparation as mycoplasma-free will be < 1 ratio and 0.2 based on the bioluminescent assay and PCR-ELISA assay, respectively.

10.2.11 Preparation of MART-1_{26-35} Peptide Pulsed DC for Immunization:

MART-1_{26-35} Peptide Pulsing. On the day of immunization, DC are harvested, washed in sterile saline and resuspended at a concentration of 10^7 in 1 ml serum-free RPMI 1640 and 10 □µg/ml of MART-1_{26-35} peptide. After 1-2 hour of incubation at room temperature, MART-1_{26-35}/DC are prepared for immunization.

Final MART-1_{26-35}/DC Vaccine Preparation. After one hour of incubation, MART-1_{26-35}/DC are pelleted and washed three times in sterile saline solution. Cells are counted in trypan blue and up to 1 x 10^7 DC are resuspended in sterile saline (optionally supplemented with 1-5% of heat-inactivated autologous plasma), loaded onto the syringe and kept in a sealed bag on ice or at 4ºC in a refrigerator, waiting final testing for lot release. Up to 1 x 10^7 DC will be administered, as feasible based on the number of DC in each preparation. If a batch contains less than the maximum allowed cells, it will still be administered to patients and the final number of DC administered recorded in the SOP and CRFs.

10.2.12 Final MART-1_{26-35}/DC Lot Release Testing:

Flow Cytometry. After the DC are peptide-pulsed, an aliquot of 0.6 x 10^5 to 1.2 x 10^6 cells will be used for phenotype check. No single marker can successfully recognize DC, and thus a panel of markers will be used to determine the DC composition. Cells will be stained with an anti-MHC class II antibody and anti-CD86 costimulatory molecule antibody and assayed by FACS analysis to demonstrate that the cells are equipped to function as antigen presenting cells. Cells will also be stained with an anti-CD14 antibody to assess the DC maturation level. After gating the large and granular cells in the population, the percentage of DC strongly double positive for MHC class II and CD86 and weakly positive for CD14 is determined. Acceptable DC content defined as cells double positive for MHC class II and CD86 > 10% from the total cell population (> 30% of the gated large and granular cells).

Testing of the Final Product. After the MART-1_{26-35}/DC have been loaded onto a syringe and placed in a sealed bag on ice, an aliquot of the final product containing approximately a minimum of 1 x 10^5 DC for each study is subjected to the following testing:

a. Viability determination by trypan blue exclusion. Limit of acceptance: > 70% viability of the large granular cells by trypan blue exclusion.

b. Gram and fungal stains, with negative results to be confirmed before administration of the vaccine to the patient. The sensitivity of the Gram stain is 10^5 cells/ml.

c. Repeated culture for sterility, maintained in culture for a total of 14 days. Culture is done by inoculating the sample on Thioglycollate and Tryptic soy broths and Sheep Blood agar plates for each sample, and the cultures are examined for 14 days for evidence of growth. The Thioglycollate broth will detect aerobic, facultative anaerobic and anaerobic bacteria, and the Tryptic soy broth and Blood agar plates detect aerobic and facultative anaerobic bacteria and fungi. If results of this or the in process cultures become positive at any time, the patient will be immediately contacted and started on empirical broad spectrum intravenous antibiotic treatment with Imipenem and Vancomycin, with adjustment of the antibiotic treatment according to the sensitivities obtained from the cultures.

d. Mycoplasma Culture. A sample from the DC final product is sent for mycoplasma culture. If the mycoplasma culture becomes positive after the patient has been injected with MART-$1_{26\text{-}35}$/DC, the patient will be contacted and started on Azithromycin.

e. Evaluation of endotoxin contamination by Limulus Amebocyte Lysate (LAL, Quantitative Chromogenic Procedure) or the Endosafe - PTS System (Charles River). The assays have a sensitivity of 0.01 and 0.05 endotoxin units (EU)/ml. Acceptance Criteria for Test Article: <5.0 EU/Kg weight of the study subject.

Additionally, a 0.05-0.1 ml sample of the final MART-$1_{26\text{-}35}$/DC preparation from each vaccination time will be sterily cryopreserved and stored at < –130ºC in a monitored liquid nitrogen tank for possible ancillary testing in the future. These samples will be stored a minimum of one year and a day after vaccine administration.

10.2.13 MART-$1_{26\text{-}35}$/DC Lot Release Criteria:

The criteria for lot release with all results available at the time of injection to the patient is described in Table 10.

Table 10. MART-$1_{26\text{-}35}$/DC Lot Release Criteria:

Culture Day	Testing	Lot Release Criteria
Leukapheresis	Gram stain Sterility cultures	Negative Negative to date*
Plasma	Gram stain Sterility cultures	Negative Negative to date*
0	Fresh/ Thawed PBMC	> 70% Viability
+4	Gram stain Sterility cultures	Negative Negative to date
+6	Mycoplasma Rapid Test	Negative (Ratio < 1)
+7	DC Viability DC Phenotype Gram stain Fungal stain Sterility cultures Mycoplasma culture Endotoxin Label	> 70% Viability CD86 + MHC Class II > 30% LGC Negative Negative Negative to date* Negative to date* < 5 EU/Kg body weight/dose “Not Tested for Biohazards”

*All sterility cultures and mycoplasma culture will be followed up to 14 days and the final results will not be available at the time of vaccination. After injection of the MART-$1_{26\text{-}35}$/DC into the patient, if positive culture is reported at any time, the patient will be started on empiric antibiotics.

10.2.14 Product Labeling.

The final MART-$1_{26\text{-}35}$/DC vaccine will be labeled using a preprinted study label with the following information:

- Patient UCLA ID. Number.
- Patient's initials.
- Subject laboratory clinical trial code.
- Date of cell preparation, with an expiration time of 24 hours (since it is administered fresh).
- Initials of person who prepared the vaccine.
- Labeled with "For autologous use only".
- Labeled as "Not Tested for Biohazards" (due to no specific testing for HIV, HepC, HepB and other adventitious viruses in the final product).

10.2.15 MART-$1_{26\text{-}35}$/DC Administration.

Patients will receive up to 1 x 10^7 MART-$1_{26\text{-}35}$/DC that meet the lot release criteria injected intradermally (i.d.) in 0.1-0.5 ml normal saline close to a lymph node basin not known to be involved with melanoma.

10.3 Samples for Transgenic TCR Cell Persistence and Immune Monitoring

Samples of peripheral blood are collected for the secondary endpoints of MART-1 F5 TCR transgenic T cell persistence, RCR analysis, retrovirus insertion sites in dominant clones and immunological monitoring assays. These PBMC will be obtained from the following:

- PBMC obtained from the baseline leukapheresis.
- A second leukapheresis scheduled between study days + 20-40 processing 5 liters of blood (partial leukapheresis).
- Peripheral blood collections in EDTA (from which PBMC are separated through Ficoll gradient centrifugation.

11.0 EVALUATION OF STUDY ENDPOINTS

11.1.1 Safety and Definitions of DLT.

Criteria for safety evaluation. Safety will be assessed by monitoring and recording potential adverse effects of the treatment using the Common Toxicity Criteria at each study visit. Subjects will be monitored by medical histories, physical examinations, ophthalmologic exams, and blood studies to detect potential toxicities from the treatment.

11.1.1.1 Definition of Dose Limiting Toxicities (DLT).

If 3 patients in the initial 8 patients experience DLTs, the study will not proceed to the next phase.

Adverse events are defined following NCI CTCAE v3.0. DLTs are defined as:

- Grade 2 or greater allergic reaction/hypersensitivity (except for fever or rash).
- Grade 2 or greater autoimmune toxicity not associated with anti-tumor response or which threatens vital organ (heart, lung, kidney, bowel, bone marrow, musculoskeletal and central nervous system,) function with continued treatment.
- Clinical evidence of grade 3 or higher autoimmune reaction in the eye:
  - New subjective patient symptoms in comparison to the data obtained on day 1 of the study protocol:
    - Nyctalopia.
    - Loss of peripheral visual field.
    - Photopsias.
    - New floaters.
    - Color desaturation.
    - Blurry vision.
  - New ophthalmologic findings in comparison to the data obtained on day 1 of the study protocol:
    - Loss of greater than the equivalent of 1 line of visual acuity.
    - Reproducible decreases in visual field of greater than 3 MD deviation.
    - New onset defined scotoma, depigmentation of the retinal pigment epithelium.
    - Appearance of subretinal fluid.
    - Onset of uveitis not controlled with corticosteroid eye drops.
- Clinical evidence of grade 2 or higher autoimmune reaction in the ear:
  - New subjective patient symptoms in comparison to the data obtained on day 1 of the study protocol:
    - Deafness.
    - Oscilopia.
  - New otological findings in comparison to the data obtained on day 1 of the study protocol:
    - Loss of greater than the equivalent of 1 line of ear acuity.
- Grade 3 non-hematologic toxicity due to cell infusion, not easily reversed by standard measures to less than a grade 2 within 48 hours.
- Grade 3 skin toxicity at the DC injection site (pain or swelling with inflammation or phlebitis) will not be a reason for therapy withholding, as long as it has improved to grade 1 by the time of retreatment. However, grade 4 local skin toxicity (ulceration or necrosis) will be a reason for treatment withholding.
- Any Grade of skin rashes related to Toxic Epidermal Necrolysis/ Steven Johnson's Syndrome
- Hypersensitivity reaction, Grade 3.
- Hepatitis, Grade 3.
- Unexpected Grade 3 or greater skin ulceration and skin rashes with desquamation.
- Unexpected ≥ Grade 3 toxicity due to cytokines that does not decrease to ≤ Grade 2 in 96 hours.
- Grade 4 non-laboratory toxicity due to cell infusion
- Intubation due to respiratory distress after receiving the TCR transgenic ACT therapy.

The known toxicities and side effects of the chemotherapy preparative regimen, as listed in the protocol or package insert, or IL-2 (Proleukin) or G-CSF (Neupogen) cytokine administration adverse event, as listed in the protocol or package insert, will not be considered for the assessment of DLTs.

11.1.1.2 Ocular Examinations.

Since MART-1 positive pigmented cells are present in the uvea and retina, careful evaluation of the eye will be conducted throughout the study period.

Qualifications of the Examiners: The ophthalmologic evaluation will be performed by a qualified ophthalmologist with a faculty appointment at UCLA. The primary ophthalmologists performing these examinations will be the study co-investigators Dr. Bradley R. Straatsma, M.D., J.D., Professor of

Ophthalmology and Director Emeritus of the Jules Stein Eye Institute, and Dr. Tara McCannel, M.D., Ph.D., Assistant Professor of Ophthalmology.

Procedures: The following will be the procedures performed in the comprehensive ophthalmologic visits at screening and post treatment Day 75-90: ophthalmic history, comprehensive ophthalmic examination, psychophysical visual function assessment and fundus photography. If any abnormality is detected, subjects will undergo an additional fluorescein angiography and electrophysiological visual function assessment. For other ophthalmologic visits, the minimally required procedures will be an ophthalmic history and a comprehensive ophthalmic examination.

Ocular DLTs: Ocular DLTs consist of new ophthalmologic findings or subjective patient symptoms in comparison to the data obtained during screening. The new symptoms of concern include nyctalopia, loss of peripheral visual field, photopsias, new floaters, color desaturation, or blurry vision. On examination, several findings would consist of an ocular DLT. These include loss of greater than the equivalent of 1 line of visual acuity, reproducible decreases in visual field of greater than 3 MD deviation or new onset defined scotoma, depigmentation of the retinal pigment epithelium, appearance of subretinal fluid, or onset of uveitis.

Procedures to follow if an ocular DLT is determined: Subjects with findings of an ocular DLT as defined above will receive either topical or systemic prednisone (100 mg qday for 5 days) as recommended by the ophthalmologist, with repeated ophthalmologic assessment. If a favorable course is noted, then patients will be followed every week for one month, and monthly afterwards until complete resolution of the findings. If the patient does not have a favorable course after the initial prednisone treatment, then the patient will be admitted for intravenous corticosteroids therapy with the possibility of adding cyclosporin A, OKT3, micophenolate or other immune suppressant.

11.1.1.3 Otologic Examinations.

Since MART-1 positive pigmented cells are present in the inner ear, careful evaluation of the ear function will be conducted throughout the study period.

Qualifications of the Examiners: The otologic visit will be performed by a qualified otorhinolaryngologist with a faculty appointment at UCLA. The primary otorhinolaryngologist performing these visits will be the study co-investigator Dr. Akira Ishiyama.

Procedures: The following will be the procedures performed in the comprehensive ophthalmologic visits on day 1 and 75-90: otologic history, comprehensive otologic examination. If any abnormality is detected, subjects will undergo additional targeted examinations.

Otologic DLTs: Otologic DLTs consist of new ophthalmologic findings or subjective patient symptoms in comparison to the data obtained on day 1 of the study protocol.

Procedures to follow if an otologic DLT is determined: Subjects with findings of an otologic DLT as defined above will receive either topical or systemic prednisone (100 mg qday for 5 days) as recommended by the otorhynolaryngologist, with repeated otologic assessment. If a favorable course is noted, then patients will be followed every week for one month, and monthly afterwards until complete resolution of the findings. If the patient does not have a favorable course after the initial prednisone treatment, then the patient will be admitted for intravenous corticosteroids therapy with the possibility of adding cyclosporin A, OKT3, micophenolate or other immune suppressant.

11.1.2 Feasibility

After entering 8 patients to the first stage of this study, followed up for a minimum of 3 months after the last subject has received the infusion of the MART-1 F5 TCR transgenic cells, an assessment of protocol feasibility will be done by the study investigators (Table 9). The feasibility assessment will be based on: i) the number of MART-1 F5 TCR engineered cells that can be recovered from patients at pre-specified time points, and ii) potential problems in the manufacturing of MART-1 F5 TCR engineered cells and/or the MART-$1_{26\text{-}35}$ peptide–pulsed DC vaccines (Table 11).

Table 11. Criteria to Declare the Study Unfeasible.

Parameter	Criteria for Unfeasibility
Feasibility of generating MART-1 transgenic cells that meet the lot release criteria.	3 or more preparations not meeting the lot release criteria in stage 1.
Feasibility of generating MART-$1_{26\text{-}35}$/DC that meet the lot release criteria.	3 or more preparations not meeting the lot release criteria in stage 1.

11.1.3 Antitumor Activity

11.1.3.1 Malignancy status

Objective tumor responses are aimed at ruling out a 10% response rate as the null hypothesis, and 35% response rate as the alternative hypothesis within a phase II two-stage clinical trial design. Therefore, if 2 out of 8 patients in the first stage have objective responses and the criteria for safety and feasibility are met, the study will proceed to a second stage entering 14 additional patients for a total of 22 patients in the clinical trial.

Potential objective responses to this combinatorial immunotherapy will be recorded following the RECIST (Response Evaluation Criteria in Solid Tumors) criteria [68]. Appropriate evaluations, including physical exam, pictures of visible lesions and imaging exams, will be evaluated at screening and then between study days 75-90, or at discontinuation of study. The duration of patient evaluation may be extended if additional outpatient visits are required to assess duration of tumor response or time to progression.

All patients who have received an infusion of MART-1 F5 TCR transgenic T cells and have tumor assessments at baseline and during the study follow up will be considered evaluable for tumor response.

11.1.3.2 Definition of Measurable and Non-measurable Lesions

Measurements of all lesions should be recorded in metric units. All baseline evaluations must be performed as close as possible to the first day of study 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.

a. Measurable Lesions:

- Lesions that can be accurately measured in at least 1 dimension (longest diameter to be recorded) as ≥2.0 cm with conventional imaging techniques, ≥1.0 cm with spiral CT scan with slices of 5 mm thickness, or no less than double the slice thickness using modern CT scanners with thinner slices.
- Skin nodules that can be clearly documented by color photography, including a ruler, to document the maximum diameter of the target lesion(s), regardless of their size.

- If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology, or by positivity by [^{18}F]FDG PET scan if the lesion is not easily accessible for tissue sampling.

b. Non-measurable Lesions:

- All other melanoma lesions: Previously irradiated lesions are non-measurable except in cases of documented progression of the lesion since the completion of radiation therapy.

11.1.3.3 Definition of Target and Non-Target Lesions

Patients must have at least one measurable lesion at baseline to be included in the analysis of this endpoint. Baseline documentation of tumor sites may include imaging assessment of disease in the chest, abdomen and pelvis. A baseline imaging study of the brain is required.

a. Target Lesions:

- Up to 10 total lesions, a maximum of 5 lesions per organ, that are representative of all involved organs may be selected and recorded as target lesions at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically). The sum of the longest diameter for *all target lesions* will be calculated and reported as the baseline sum longest diameter. The baseline sum longest diameter will be used as reference to further characterize the objective tumor response of the disease.
- Since at least 2 biopsies will be performed, at least one of the baseline target lesions should not be completely excised throughout the study period.

b. Non-Target Lesions:

- All other lesions (or sites of disease) should be identified as non-target lesions and recorded as non-target lesions at baseline. Measurement of non-target lesions at baseline is not required and should be recorded as "present." Each non-target lesion should be documented as either present, absent or new in each subsequent evaluation.

11.1.3.4 Objective Response Classifications

The following RECIST criteria will be the primary method utilized in this study for the assessment and reporting of tumor response data:

- Complete Response (CR): Disappearance of all evidence of melanoma in target and non-target lesions. CR must be confirmed by repeat assessments performed no less than 4 weeks after the criteria for response are first met to qualify as CR. This definition includes a pathological CR when hyperpigmented lesions visible by exam are biopsied and demonstrate no viable measurable melanoma and pigment accumulated in phagocytic cells.
- Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions taking as reference the baseline sum LD. Non-target lesions may persist provided there is no unequivocal progression in these lesions. PR must be confirmed by repeat assessments performed no less than 4 weeks after the criteria for response are first met to qualify as PR;
- Progressive Disease (PD): At least a 20% increase in the sum LD of the target lesions from the smallest sum LD recorded since the beginning of therapy or the appearance of one or more new lesions or unequivocal progression of existing non-target lesions; and

- Stable Disease (SD): Measurements demonstrating neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify as PD after the start of treatment taking as reference the smallest sum LD since the treatment started. During this time, non-target lesions may persist provided there is no unequivocal progression in these lesions.

11.1.3.5 Frequency of Tumor Measurements

Tumor assessments for response will be performed starting at the end of 3 months (Day 90) and every 2-3 months subsequently. Tumor assessment will also be performed at discontinuation from the study, unless these have been performed within the last 4 weeks prior to discontinuation. CR and PR must be confirmed by repeat assessments performed no less than 4 weeks after the criteria for response are first met to qualify as such response. Additional tumor measurements may be completed as needed.

11.1.3.6 Assessment of Response for In-Transit Metastasis

In patients with in-transit metastasis only, at least one in-transit metastatic lesion should not be excised. If there is only one measurable lesion, biopsies should not interfere with the measurement of the largest diameter of the lesion. Response will be assessed as above, taking the measurement from pictures with a built-in ruler.

11.1.3.7 Duration of Response:

The duration of overall complete response will be measured from the time measurement criteria has been first met for CR until the first date that recurrent or progressive disease is objectively documented.

The duration of overall response will be measured from the time measurement criteria is met for CR/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).

11.1.3.8 Time to Disease Progression

Defined as the length of time from the date of enrollment to the date of progressive disease first documented, death, or the start of secondary antitumor therapy, whichever occurs first. Subjects lost to follow-up will be censored at their last available follow-up date.

11.1.4 Definition of Study Failure Due to Failure of Meeting the Primary Study Endpoints.

Study failure according to safety and feasibility considerations is defined as follows:

1) Unexpected SAEs that are related to the transduction process or research reagent and required that the study be stopped.
2) 3 or more DLT in the first stage with 8 patients.
3) Considered to be an unfeasible approach as defined above.
4) 1 or less patients with CR or PR among 8 patients in stage 1.
5) 4 or less patients with CR or PR among 22 patients in the completed study combining patients in stage 1 and 2.

11.2 Secondary Study Endpoints:

11.2.1 MART-1 TCR/sr39 Transgenic Cell Persistence

11.2.1.1 Transgenic Cell Persistence.

TCR and vector presence will be quantitated in PBMC samples, obtained before adoptive transfer and at 7, 14, 30, 45, 60, 75 and 90 days after transgenic cell adoptive transfer. Thereafter, sampling will be every 3 months during the first 2 years, and then every 6 to 12 months. Analysis will be performed both using immune monitoring and molecular techniques. Detection of surface expression of the MART-1 TCR transgenic protein will be analyzed both by MHC tetramer or dextramer analysis and staining for the specific region. Molecular analysis of the persistence of cells bearing the MART-1 F5 TCR cDNA will be done by real time PCR techniques using primers specific for the transgenes and retroviral vector sequences. This testing will provide data to estimate the *in vivo* survival of lymphocytes derived from the infused cells.

11.2.1.2 Long Term Monitoring for Replication Competent Retrovirus (RCR).

Analysis for detection of RCR by PCR will be performed at the National Gene Vector Biorepository (NGVB) at the Indiana University Viral Production Facility (IU VPF) under the supervision of Dr. Kenneth G. Cornetta. Samples taken from cells prior to infusion will be archived and blood samples obtained from study patients at 3 and 6 months, and at one year post cell administration will be tested. Blood samples will be archived annually thereafter if all previous testing has been negative.

If a patient dies or develops a neoplasm (other than melanoma recurrence) during this clinical trial, efforts will be made to assay a biopsy sample for RCR. If any post-treatment samples are positive, then the baseline final product will be tested for RCR. Further analysis of the RCR and more extensive patient follow-up will be undertaken, in consultation with the UCLA IRB, RAC and FDA.

11.2.1.3 Analysis of Retroviral Insertion Sites in Long Term Persisting MART-1 F5 TCR Clones

It is possible that expansion of specific T-cell clones will be observed as tumor reactive T cells proliferate in response to tumor antigens. Therefore, care will be taken to track T cell persistence both immunologically and molecularly. Blood samples for persistence of TCR gene transduced cells will be obtained at 3, 6, 12 months, and then annually thereafter. If any patient shows a high level of persistence of TCR gene transduced cells at month 6 (by semi quantitative DNA-PCR using primers specific for vector sequences) the previously archived samples will be subjected to techniques that would allow the identification of clonality of persisting TCR gene transduced cells. Such techniques may include T cell cloning or LAM-PCR. Clonality analysis is available through NGVL [69].

If a predominant or monoclonal T cell clone derived from TCR gene transduced cells is identified during the follow-up, the integration site and sequence will be identified and subsequently analyzed against the human genome database to determine whether the sequences are associated with any known human cancers. If a predominant integration site is observed, the T cell cloning or LAM-PCR test will be used at an interval of no more than three months after the first observation to see if the clone persists or is transient. In all instances where monoclonality is persistent and particularly in instances where there is expansion of the clone, regardless of whether or not the sequence is known to be associated with a known human cancer, the subject should be monitored closely for signs of malignancy.

11.2.1.4 Immunological Monitoring.

Immunological monitoring will consist of quantifying T cells bearing surface MART-1 F5 TCR by MART-$1_{26\text{-}35}$ tetramer or dextramer analysis. The functionality of these cells will be tested in ELISA

and/or multicytokine array assays, intracellular cytokine staining (ICS). Immunological assays will be compared between 1) pre-infusion PBMC, 2) an aliquot of the engineered PBL at the time of infusion, 3) cells recovered from patients' peripheral blood after adoptive transfer.

Our published definitions for a positive or negative immunological response using standardized MHC tetramer assays will be used for these two assays [70]. These definitions are based on the assay performance specifications by defining changes that are beyond the assay variability with a 95% confidence level. For the other assays, differences of 1 fold will be considered indicative of true biologic differences.

Additional testing may be performed with other assays, for example intracellular phosphoprotein staining for signaling networks, multiplexed microfluidic and nanotechnology-based immune monitoring assays [71,72].

11.2.2 Evaluation of MART-1 TCR Transgenic Cell Tumor Trafficking

11.2.2.1 *In Vivo* Imaging

The ability to non-invasively image T cell responses to cancer hinges upon the detection of metabolic changes related to TCR transgenic cell proliferation *in vivo* detectable with PET probes.

Patients will be injected with 200 MBq [^{18}F]FDG at baseline and approximately 20-40 days after ACT, with allowed variability of scheduling for 2-4 weeks of the intended dates. One hour post injection, a PET/CT scan covering the area from the base of the skull to the upper abdomen will be acquired. Scans of the extremities will be performed in patients with metastatic lesions in the arms or legs. The CT scan will be performed in a "low dose mode" (110 kVp, 30 mAs anode current) resulting in an effective dose of less than 1.3 mSv per scan [73]. These doses are comparable with typical diagnostic CT or PET scans and below the limits set by federal regulations for research studies involving radioactive imaging agents (http://www.access.gpo.gov/nara/cfr/waisidx_01/21cfr361_01.html).

Regional uptake of [^{18}F]FDG within metastatic tumor sites and secondary lymphoid organs will be quantified by standardized uptake values (SUV) normalized to the body weight of the patient. As an internal quality control, SUVs will also be determined for several normal organs, such as muscle, liver and lungs. These measurements will allow us to identify technical problems in the SUV calculations, such as partially paravenous tracer administration [74]. An attempt will be made to have all imaging studies will be performed on the same PET/CT system in order to eliminate confounding effects of differences in scanner sensitivity, spatial resolution or image processing.

11.2.2.2 Tumor Biopsies

To correlate the [^{18}F]FDG signal with the numbers and functional phenotype of MART-1 F5 TCR transgenic T cells accumulating at the tumor site, percutaneous or image-guided biopsies of tumor lesions between study days 20-40, with allowed variability in this range, will be performed. The biopsies will be analyzed by H&E, IHC to quantify the numbers of T lymphocytes. If sufficient quantity of tissue is available, the study investigators will attempt to monitor the phenotype of the TIL obtained from tumor biopsy samples by MHC tetramer or dextramer, multicolor flow cytometry (FACS), and other immune monitoring assays.

12.0 ADVERSE EVENT REPORTING

12.1 Definition of Serious Adverse Events (SAE)

A serious adverse event or serious adverse drug reaction is defined as any untoward medical occurrence at any dose that:

- Results in death.
- Is life-threatening (immediate risk of death).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Results in congenital anomaly/birth defect.

Progression of the malignancy under trial should not be reported as an adverse event. However, if the malignancy has a fatal outcome during the trial or within the safety-reporting period, then disease progression must be recorded as a Grade 5 serious adverse event.

Medical and scientific judgment should be exercised in determining whether an event is an important medical event. An important medical event may not be immediately life threatening and/or result in death or hospitalization. However, if it is determined that the event may jeopardize the patient and may require intervention to prevent one of the other outcomes listed in the definition above, the important medical event should be reported as serious.

SAE Reporting Period

Events that meet the SAE definition will be reported within the following timelines based on the time of first knowledge of the SAE by a study investigator:

- SAE other than resulting in unexpected death:
    - P.I.: Within 24 hours.
    - IRB, ISPRC, DSMB, IBC, NIH/RAC: Within 7 days.
    - FDA: Within 15 days.
- Unexpected death:
    - P.I.: Within 24 hours.
    - IRB, ISPRC, DSMB, IBC, NIH/RAC: Within 24 hours.
    - FDA: Within 7 days.

In general, any SAE will be reported as soon as it is known by the study investigators and enough data is gathered for report filing. All SAE reports will be managed through the UCLA SAE Compliance Officer, working under the JCCC Regulatory Affairs Office. Initial reports may be completed or amended with any new relevant additional information.

Copies of all reports will be maintained in the study regulatory file. Copies of any report sent to the FDA will also be sent to the study sponsor.

12.2 Definition of an Adverse Event (AE)

An adverse event is any untoward medical occurrence in a clinical investigation patient administered a product or medical device; the event need not necessarily have a causal relationship with the treatment or usage.

Abnormal Test Findings

The criteria for determining whether an abnormal objective test finding should be reported as an adverse event are as follows:

- Test result is associated with accompanying symptoms, and/or
- Test result requires additional diagnostic testing or medical/surgical intervention, and/or

- Test result leads to a change in trial schedule or discontinuation from the trial, significant additional concomitant drug treatment, or other therapy, and/or
- Test result is considered to be an adverse event by the investigator or sponsor.

Merely repeating an abnormal test, in the absence of any of the above conditions, does not constitute an adverse event. Any abnormal test result that is determined to be an error does not require reporting as an adverse event.

12.3 Causality Assessment

The investigator's assessment of causality must be provided for all adverse events (serious and non-serious). An investigator's causality assessment is the determination of whether there exists a reasonable possibility that the investigational product caused or contributed to an adverse event. If the investigator's final determination of causality is unknown and the investigator does not know whether or not investigational product caused the event, then the event will be handled as "related to investigational product" for reporting purposes. If the investigator's causality assessment is "unknown but not related to investigational product", this should be clearly documented on the study records. In addition, if the investigator determines a serious adverse event is associated with trial procedures, the investigator must record this causal relationship in the source documents and CRF, as appropriate, and report such an assessment in accordance with the serious adverse event reporting requirements, if applicable.

12.4 Withdrawal Due to Adverse Events

Withdrawal due to adverse event should be distinguished from withdrawal due to insufficient response, according to the definition of adverse event noted earlier, and recorded on the appropriate adverse event CRF page. When a patient withdraws due to a serious adverse event, the serious adverse event must be reported in accordance with the reporting requirements defined below.

13.0 COMPLIANCE WITH GOOD CLINICAL PRACTICE, ETHICAL CONSIDERATIONS, INFORMED CONSENT

13.1 Compliance with Good Clinical Practice and Ethical Considerations

This study must be conducted in compliance with all local and federal regulatory requirements, in particular those which afford greater protection to the safety of the trial participants. This study will be conducted according to the current revision of the Declaration of Helsinki and with local and federal laws and regulations relevant to the use of new therapeutic agents. The ICF must contain a language readily understood by the potential study subjects.

13.2 Regulatory Approvals

The Protocol and ICF must be approved by the following committees before initiation of patient enrollment:

Local Committees:

- Institutional Review Board (IRB).
- Institutional Biosafety Committee (IBC).
- Institutional Scientific Peer Review Committee (ISPRC).

Federal Committees:

- Recombinant DNA Advisory Committee (RAC).
- Food and Drug Administration (FDA).

13.3 Subject Recruitment

Study subjects will be invited to participate by the study investigators as part of the discussion of standard and experimental therapy for locally advanced or metastatic melanoma. Patients may access the study investigators through:

a) Subjects may be referred from UCLA or outside clinics. No patient will be approached without a referral or agreement from the patient's physician in order to uphold patient confidentiality.
b) Subjects may be identified by the study investigators from their own patient pools while discussing standard and experimental options for advanced or metastatic melanoma.
c) Study flyers will be posted in public places, including the oncology clinic waiting room, for subject self-referral.
d) Subjects may self-refer to this study when inquiring about clinical trial options at UCLA.
e) This study will be listed at the NIH web site listing active clinical trials, which also assures trial registration at a public site.

13.4 Gender and Minorities

There will be no discrimination based upon sex or race. Subjects of any gender and ethnicity with melanoma have been and will be considered in the proposed clinical trials. There has been and will certainly be no discrimination due to gender or ethnicity, but the epidemiology of melanoma and HLA haplotypes may result in uneven minority population distribution. Since melanoma is slightly more prevalent in males (2/3), we expect that approximately 30-35% of the study population will be females.

Most subjects referred to the UCLA Melanoma Program come from the UCLA Oncology Network, with over 50 offices throughout the Los Angeles County and Western US, including areas where there is a majority of subjects of Asian, Hispanic and African American descent. Cutaneous malignant melanoma occurs less frequently among non-white populations than among whites. Data from the California Cancer Registry reveal that, between 1988 and 1993, 95.5% of subjects with melanoma in California were non-Hispanic whites, 3.5% were Hispanic, 0.5% Asian, and 0.5% non-Hispanic blacks [75]. The patients treated in previous melanoma clinical trials conducted over the past 5 years at the UCLA Melanoma Program mirror the gender and race characteristics of the malignant melanoma patient population in the Southern California area. Table 12 reflects the gender and minority distribution of subjects accrued to clinical trials in the UCLA Melanoma Program over the past 5 years. 93% were white and 33% were female, which is in accordance with the incidence of melanoma in our population.

Table 12. Gender and Minorities Accrued to Prior Melanoma Clinical Trials at the UCLA Melanoma Program (2002-2007):

	American Indian or Alaskan Native	Asian or Pacific Islander	Black, not of Hispanic Origin	Hispanic	White, not of Hispanic Origin	Other or Unknown	Total
Female	-	1	1	5	52	1	60
Male	-	2	-	2	117	1	122
Unknown	-	-	-	-	-	-	-
Total	-	3	1	7	164	2	182

13.5 Children

Metastatic melanoma is very rare in subjects younger than 18 years old. The use of the non-myeloablative regimen in this protocol is a major procedure that entails serious discomforts and hazards for the patient, such that fatal complications are possible. It is therefore only appropriate to carry out this experimental procedure in the context of life threatening metastatic cancer. Since the efficacy of this experimental procedure is unknown, it does not seem reasonable to expose children to this risk without further evidence of benefit. Should results of this study indicate efficacy in treating metastatic melanoma, which is not responsive to other standard forms of therapy, future research will be conducted in the pediatric population to evaluate potential benefit in that patient population. However, patients with age less than 18 will be considered on a case-by-case basis, and a specific pediatric consent will be developed if necessary.

13.6 Informed Consent Procedure

13.6.1 Informed Consent Form (ICF). This study must be conducted in compliance with UCLA IRB, IBC, ISPRC, the RAC and the FDA, and relevant informed consent regulations. The ICF must contain a language readily understood by the potential study subjects. The original consent form, signed and dated by the patient and by the person who conducted the informed consent discussion, will be included in the patient's UCLA medical chart. A copy of the signed ICF will be provided to the patient.

There will be one consent form describing all of the study procedures, including the administration of genetically modified cells and the procedures for gene transfer. The rationale for including gene transfer and non-gene transfer procedures in the same consent form is that their separation would result in unnecessary duplication of text and non-linear explanation of the study procedures.

13.6.2 Procedure of Consent. Subjects will be seen by one of the study investigators who will explain the study in detail including procedures, potential risks and side effects. Subjects will be encouraged to take additional time to consider the study and will be given the opportunity to take home the ICF for review and consideration. They will then be invited to meet with one of the investigators at another time. Subjects will be encouraged to have significant others or relatives with them at the time of the interviews. Questions from subjects, significant others and relatives will be encouraged. If the subject is non-English speaking, the study investigators will have the informed consent translated in the subject's native language. Ample time will be allowed for discussions and clarification of the goals of the research project with the trial investigators. Potential subjects will be asked if they wish to participate and told that it is voluntary and refusal will not affect their relationship with their physician or the care given to them at UCLA. No coercion will be used.

13.7 Subject Enrollment

Subjects will be enrolled at the time the ICF is signed. Fulfillment of the eligibility criteria for each new subject enrolled will be prospectively and independently monitored by the UCLA Human Gene Medicine Program (HGMP) Compliance Officer.

14.0 QUALIFICATIONS OF PERSONNEL AND STUDY FACILITIES

14.1 Qualifications of Investigators

The UCLA Human Gene Medicine and Melanoma Programs: Antoni Ribas, James S. Economou, John A. Glaspy, William H. McBride, Bartosz Chmielowski, Arun Singh, Deborah Wong, Begonya Comin-Anduix, Richard C. Koya, Thinle Chodon, Donald Kohn. The first human gene transfer clinical trial conducted by Drs. Economou, Glaspy and McBride was initiated in 1992. This was a double retroviral vector gene marking of tumor infiltrating lymphocytes (TIL)[76]. Two adenovirus vector gene transfer-based clinical trials were conducted by Drs. Economou, Glaspy,

McBride and Ribas at UCLA. These clinical trials involved the generation of genetically manipulated DC-based cellular vaccines using adenoviral vectors for melanoma and hepatocellular carcinoma (INDs 9908 and 11299).

- **Antoni Ribas, M.D.**, Professor of Medicine and Surgery, Director of the Tumor Immunology Program Area at the JCCC,. Dr. Ribas is a translational medical oncologist and tumor immunologist, conducting experimental clinical trials in patients with melanoma.

- **James S. Economou, M.D. Ph.D.**, Professor of Surgery and Microbiology, Immunology, and Molecular Genetics, Chief of the Division of Surgical Oncology, Deputy Director of the JCCC and the Director of the Human Gene Medicine Program (HGMP), and Vice Chancellor for Research at UCLA. Dr. Economou is an accomplished translational tumor immunologist and pioneer in clinical trials with retrovirus-modified tumor infiltrating lymphocytes (TIL).

- **John A. Glaspy, M.D., M.P.H.**, Professor of Medicine, Vice-Chair of the Division of Hematology-Oncology, Director of the JCCC Clinical Research Unit Shared Resource, and the Associate Chief, UCLA 100 Medical Plaza Community Oncology Practice. Dr. Glaspy is a leading expert in clinical research, with a focus on melanoma and the development of cytokines and growth factors for human use.

- **William H. McBride, D.Sc**. Professor and Vice Chair for Research, Director, Experimental Radiation Oncology. Dr. McBride is a recognized expert in animal models of tumor immunotherapy and radiation therapy.

- **Bartosz Chmielowski, M.D., Ph.D.** Assistant Professor of Medicine in the Division of Hematology-Oncology. An expert in thymic development [77-79] and in the care of patients with malignant melanoma.

- **Arun Singh, M.D**., Clinical Instructor in Medicine at the Division of Hematology-Oncology. Dr. Singh is a trained hematology-oncologist with expertise in high dose chemotherapy and IL-2 administration, and an expert in the use of molecular imaging to study immune responses to cancer.

- **Deborah Wong, M.D., Ph.D.** Clinical Instructor, Division of Hematology and Oncology. Dr. Wong is a translational medical oncologist whose research and clinical interest is in improving the understanding and the treatment of patients with melanoma.

- **Siwen Hu-Lieskovan, M.D, Ph.D.** Clinical Instructor, Division of Hematology and Oncology at UCLA. Dr. Hu-Lieskovan is a translational medical oncologist whose research and clinical expertise is in development of immune and targeted therapy for melanoma, lung cancer and other solid malignancies.

- **Daniel Shin, M.D.** Fellow, Division of Hematology and Oncology at UCLA. Dr. Shin is a translational medical oncologist.

- **Begoña Comin-Anduix, Ph.D.** Adjunct Associate Professor, Division of Surgical Oncology. An expert in immune monitoring and culture of human lymphocytes [70]. Will lead the laboratory analysis of patient-derived samples.

- **Beata Berent-Maoz, Ph.D.** Assistant Project Scientist, Division of Hematology and Oncology at UCLA. An expert in retroviral and lentiviral vector manufacture and transduction of target cells. Will be responsible for the GMP manufacture of TCR genetically modified cells and DC.

- **Paula Kaplan-Lefko, Ph.D.** Project Scientist, Division of Hematology-oncology at UCLA. Experienced in writing industry-sponsored and investigator-initiated regulatory documents. Will coordinate the regulatory activities during the conduct of this clinical trial.

- **Richard C. Koya, M.D., Ph.D**. Associate Professor of Oncology, Vector Development and Production Facility and Associate Director, Center for Immunotherapy at the Roswell Park Cancer Institute.,. An experienced molecular biologist and expert in gene therapy vectors, including retrovirus and lentiviral vectors.

- **Thinle Chodon, M.D., Ph.D.** Assistant Professor of Oncology, Center for Immunotherapy and Facility Director of CFI Translational Research Operations at the Roswell Park Cancer Institute, an expert in retroviral and lentiviral vector manufacture and transduction of target cells. Will be responsible for the GMP manufacture of TCR genetically modified cells and peptide pulsed DC.

- **Donald Kohn, M.D.**, Professor of Pediatrics and Molecular Microbiology and Immunology at UCLA. Dr. Kohn is an international leader in clinical trials of HSC genetically modified using retroviral vectors.

The UCLA AIDS Institute Gene Therapy Program: Jerome A. Zack and Zoran Galic. Two clinical trials of HSC gene therapy in patients with HIV infection and AIDS, have been led by Dr. Zack (INDs 7328 and 10183). These trials involve introduction of an anti-HIV ribozyme into autologous CD34+ hematopoietic progenitor cells from HIV+ adults.

- **Jerome A. Zack, Ph.D.** Professor of Medicine and Vice Chair of Microbiology, Immunology, and Molecular Genetics. Dr. Zack is a thymocyte development, embryonic stem cells and HIV immunity expert.

- **Zoran Galic, Ph.D.** Assistant Professor of Medicine. Dr. Galic is an embryonic stem cell and thymocyte development expert.

The Caltech/USC Engineering Immunology Program: David Baltimore, Pin Wang, Lili Yang.

- **David Baltimore Ph.D.**, President of Caltech and Nobel Prize Laureate for the description of reverse transcriptase. Dr. Baltimore has a longstanding interest in the development of immunotherapy for cancer and infectious diseases, and has unique expertise in retroviral vectors for gene transfer.

- **Pin Wang, Ph.D.** Associate Professor in Chemical Engineering and Material Sciences at USC. Dr. Wang is an expert in the genetic engineering of retroviral and lentiviral vectors.

- **Lili Yang, Ph.D.**, Project Leader at the Engineering Immunology Program at Caltech. Dr. Yang is an expert in TCR genetic cloning and the genetic engineering of HSC.

The University of Connecticut Immunotherapy Program: Bijay Mukherji.

- **Bijay Mukherji, M.D.** Professor of Medicine at the University of Connecticut. Dr. Mukherji is a pioneer in cloning human T cells specific for tumor antigens and in the study of their immunobiology. Dr. Mukherji conducted the first dendritic cell-based clinical trial in human subjects.

The UCLA *In Vivo* Cellular and Molecular Imaging Center: Caius Radu, Johannes Czernin, Michael Phelps.

- **Caius G. Radu, M.D.**, Associate Professor of Molecular and Medical Pharmacology. Dr. Radu is an expert in molecular immunology and *in vivo* imaging of immune responses in murine models.

- **Owen N. Witte, M.D.** Howard Hughes Medical Institute Investigator at UCLA, Professor of Microbiology, Immunology, and Molecular Genetics, President's Chair in Developmental Immunology, Professor of Molecular and Medical Pharmacology, and Director of the Institute of Stem Cell Biology and Medicine at UCLA. Dr. Witte is a recognized molecular biologist who has pioneered an approach to non-invasively image antitumor T cell responses in alive animals.

- **Johannes Czernin, M.D.**, Professor of Molecular and Medical Pharmacology. Dr. Czernin is a leading expert in the use of metabolic imaging for assessment of tumor response in patients.

- **Michael E. Phelps, Ph.D.**, Norton Simon Professor and Chairman of Molecular and Medical Pharmacology, Chief, Division of Nuclear Medicine. Dr. Phelps pioneered PET imaging to examine molecular and cellular functions in humans.

NanoSystems Biology Cancer Center (NSBCC): James R. Heath.

- **James R. Heath, Ph.D.**, Elizabeth W. Gilloon Professor of Chemistry at Caltech and Director of the Caltech-UCLA-Institute for Systems Biology (ISB) NSB Cancer Center. Dr. Heath is a nanotechnology and microfluidics expert who has developed novel and highly multiplexed immune monitoring assays able to analyze limiting samples from tumor biopsies.

Biostatistics, Analytical Support & Evaluation (BASE) Unit

- **David Gjertson, Ph.D.** Professor of Biomathematics at UCLA. Dr. Gjertson is an experienced biostatistician who will provide overall statistical oversight.

- **Xiaoyan Wang, Ph.D.** Assistant Professor of Medicine at UCLA. Dr. Wang is an experienced biostatistician who will work with Dr. Gjertson.

The Jules Stein Eye Institute Ophthalmology Program: Bradley Straatsma, Tara McCannel.

- **Tara McCannel, M.D., Ph.D.**, Assistant Professor of Ophthalmology. Dr. McCannel is an expert in ocular melanoma who has been involved in the monitoring of potential ocular toxicities of experimental immunotherapies tested in clinical trials at UCLA.

Otological Exams: Akira Ishiyama.

- **Akira Ishiyama, M.D.** Professor of Head and Neck Surgery at UCLA. He is an expert in otological analysis, in particular in the study of autoimmune phenomena in the middle and inner ear.

In conclusion, the study investigators are very familiar with the use of retroviral vectors to genetically modify primary lymphocytes, the requirements of GMP standards for *ex vivo* cell culture and genetic manipulation, the requirements for GCP standards for conducting human experimental clinical trials, and have fully equipped clinical facilities that have been successfully reviewed by regulatory agencies for the conduct of cellular therapy and gene transfer studies.

14.2 Personnel Training

a. **Clinical Personnel**: The following will be required courses, training sessions or certificates required for clinical study personnel:

- UCLA IRB Human subjects certificate.
- UCLA IRB HIPPA certificate.
- Certificate in Good Clinical Practice.
- UCLA Human Gene Medicine Program Educational Course.

b. **Laboratory Personnel**: The following courses, training sessions or certificates will be required for study laboratory personnel involved in TCR transgenic cell manufacturing:

- UCLA IRB Human subjects certificate.
- UCLA IRB HIPPA certificate.
- UCLA JCCC current Good Manufacturing Practice training.
- UCLA Human Gene Medicine Program Educational Course.
- UCLA IBC training on Biosafety Level 2.
- UCLA IBC certificate in the use of biosafety cabinets.
- UCLA IBC shipping of Biological Material
- UCLA IBC training in biological waste management.
- UCLA IBC training on Bloodborne Pathogens.

14.3 HGMP/JCCC Cell and Gene Therapy GMP Suite at UCLA

UCLA has a dedicated, fully equipped and functional GMP suite specifically designed for cellular and gene transfer clinical trials. This facility is located on the 14th floor of the JCCC Factor Building and is jointly managed by the JCCC and the HGMP. It consists of a 2395 square feet BioSafety level 2 (BSL2) suite with 7 HEPA-filtered rooms. This secured area has centralized monitoring of air and equipment linked to the study investigators' pagers. Access is restricted to investigators with certified training and is supervised by the JCCC Regulatory Affairs Office. The 4 positive pressure laboratories are designed for *ex vivo* cell manipulation of cells, since it minimizes the possibility of outside pathogen contamination. The 3 negative pressure laboratories are designed for gene transfer procedures, since it ensures that the recombinant vectors will not contaminate the surrounding environment. There have been 18 investigator-initiated clinical trials in which the cell or gene therapy product has been manufactured by the investigator team in the JCCC GMP suite within RAC and FDA compliance.

14.4. Gene Therapy Viral Bank

The clinical grade MSGV1-F5AfT2AB will be stored in a dedicated –80°C freezer with central, computerized monitoring system, with alarm and recording of all GMP storage systems (refrigerators, freezers and incubators). This freezer is located at the JCCC GMP Suite. This freezer is designed for exclusive use as a viral bank for ongoing gene therapy trials at UCLA, and is located in a secure area for containment to ensure proper storage. Access to the facility is guarded by two security doors with restricted access only to adequately trained and authorized individuals. Completion of a five-course training program is required to gain access to this GMP suite. This training program is provided by the JCCC Regulatory Affairs Office and the UCLA IBC.

Under these conditions, retroviral vectors retain activity for a period exceeding 5 years if not subjected to freeze-thawing. Each aliquot of the final retroviral vector is calculated to be for a single use, therefore avoiding freeze-thawing cycles. All activities related to the GMP retroviral vector stored in the viral bank will be recorded in appropriate SOPs. This includes initial lot shipment, viral vector storage, viral vector dispensing, remaining samples after use to generate retrovirus gene-modified cell therapies, and yearly recertification.

14.5. Clinical Facilities

Chemotherapy conditioning and MART-1 F5 TCR cell adoptive transfer will be delivered in an isolation hospital bed in the Hematology/Oncology Hematopoietic Stem Cell (HSC) transplantation ward. This is a fully staffed inpatient ward with experienced nurses, house officers, clinical consultants and attending physicians. This ensures that, in the event of an adverse reaction or any other adverse event, treatment will be promptly available. Patients will be placed in individual use rooms equipped with a high-efficiency particulate air (HEPA) filters that meet the precautions normally used for patients undergoing hematopoietic stem cell transplantation. While receiving the TCR transgenic PBMC adoptive transfer, patients will be continuously monitored.

This inpatient stay will be under the administrative responsibility of the UCLA General Clinical Research Center (Isidro B. Salusky, M.D., G-CRC Director, telephone: 310-206-6987). This will provide an additional level of administrative and scientific oversight. The G-CRC staff provides expert care for research subjects and collaborates with investigators for the coordination and performance of clinical research. The G-CRC includes a Computer Center under the supervision of the G-CRC Biostatistician Dr. Robert M. Elashoff, Ph.D. (telephone: 310-825-9421) to assist in data analysis.

The UCLA Melanoma Program medical oncology outpatient clinic is located at the UCLA 100 Medical Plaza, Suite 550. This facility is staffed with an office manager, 3 front desk assistants, 6 nurses, 2 phlebotomists, 2 insurance specialists and one nurse practitioner.

The UCLA Hemapheresis Unit is located at the 6th floor of the 200 Medical Plaza building, Suite 660. This unit has 6 staff nurses on a one-to-one basis, a supervisor, a coordinator, and a Hematology/Oncology fellow and attending doctors.

14.6 Compliance with Protocol Procedures and Rights and Welfare of Study Participants

Human Gene Medicine Program (HGMP) Compliance Officer. Kit Shaw (contact phone: 310-267-0584), Ph.D. Dr. Shaw worked on engineering and testing retroviral gene-transfer vectors in primary cells and small animal models. She has been the study director for IND study of gene transfer to hematopoietic stem cells (HSCs) for adenosine deaminase (ADA)-deficient severe combined immunodeficiency (SCID), where she had to ensure trial compliance with the regulatory bodies (FDA, DSMB, RAC, IRB) overseeing the trial. She also has experience with IND submissions and cGMP for cell-based products. She is familiar with clinical laboratory operations in addition to research laboratory operations. Dr. Shaw will oversee activities involving gene transfer in the UCLA GMP suite and will prospectively review adherence to the protocol, RAC and FDA procedures. Dr. Shaw also serves on the Jonsson Comprehensive Cancer Center (JCCC) DSMB. For our investigator-initiated gene therapy trial, Dr. Shaw will perform real time monitoring and auditing of the study.

JCCC Office of Regulatory Compliance. The manager of this office is Terra Hughes. Ms. Terra Hughes recently joined the JCCC from the UCLA IRB where she managed the Medical IRB committee 2. She has a bachelor's degree from the University of Southern California (USC) in Biology and a Masters degree from CSULA in Criminalistics. Ms. Hughes will oversee the adequate conduct of this clinical trial.

Clinical Research Compliance Officer. Sujna Raval-Fernandes, Ph.D. (contact phone 310-794-8693). Dr. Raval-Fernandes has a strong background in cancer research with more than 10 years of basic research experience. She has been in the Jonsson Comprehensive Cancer Center (JCCC)'s Office of regulatory Compliance for the past four and half years serving as the compliance officer and as a resource to investigators and clinical study staff in resolving quality assurance issues related to

research procedures and documentation. She is responsible for monitoring and auditing of studies to ensure regulatory compliance. She also serves on the JCCC Data and Safety Monitoring Board (DSMB). Her responsibilities also include timely review of all serious adverse events submitted to the DSMB. She ensures that the investigators provide the JCCC DSMB with periodic summary reports that include accrual, safety and efficacy data. For our investigator-initiated gene medicine study, she will serve to ensure compliance to the JCCC DSMB.

Office of Research Participant Advocacy (ORPA). The ORPA was established to assure the safety and well-being of all participants in clinical research studies conducted at the UCLA G- CRC while maximally facilitating the research process. Stanley Korenman, M.D. (telephone: 310-794-1818), Associate Dean of Ethics and Professor of Endocrinology, serves as the Clinical Director of the office. Laurie Shaker-Irwin, Ph.D. (telephone: 310-794-7504) serves as the Research Subject Advocate and manager of the office. ORPA fosters policies for the ethical conduct of clinical research, assuring autonomy and respect in the informed consent process, justice in the selection of participants, and beneficence in the initial and ongoing review and evaluation of risks and benefits. The office enhances patient safety by providing guidance and education to investigators and research personnel in the areas of ethics, research integrity, data and safety monitoring, adverse event reporting, and Good Clinical Practices (GCP).

15.0 DATA SAFETY MONITORING BOARD, STUDY OVERSIGHT, DATA COLLECTION AND ANALYSIS

15.1 Data Safety Monitoring Board (DSMB)

The Jonsson Comprehensive Cancer Center (JCCC) Data and Safety Monitoring Board (DSMB) was constituted in January 2001. The DSMB membership consists of representatives of the Cancer Center clinical research community. The Director of the Cancer Center appoints members to a term of 2 years and each member is selected for their professional expertise in oncology practice and research.

The JCCC DSMB meets monthly to review all serious adverse event reports for JCCC institutional clinical trials at UCLA and those encountered in the UCLA TORI network where the JCCC DSMB has oversight. All serious adverse event reports, which have been filed since the previous meeting, are presented to the committee for review.

For all JCCC and TORI studies, where the JCCC DSMB has primarily oversight for AE review, all AEs occurring within these studies shall be reported to the JCCC DSMB in a timely manner consistent with the UCLA IRB time requirements [ten days, two days for a death] regardless of relationship or seriousness. The JCCC Office of Regulatory Compliance (ORC) will review all submissions and staff will enter the information into the JCCC Clinical Trials database. Reports are generated for full JCCC DSMB review of those SAEs that have some component of relatedness to the study drug and may, at the discretion of the JCCC compliance officers, include SAE reports that may be incorrectly assessed by the PI. For institutional trials, where the JCCC DSMB has primary DSMB review responsibility, the office will request that the PI generate cumulative adverse event reports for biannual or annual review.

The DSMB reviews each Serious Adverse Event reported and determines whether or not protocol modifications are warranted to ensure patient safety. In this review, prior occurrences of similar toxicity with the therapy under study are taken into consideration, as well as the seriousness of the event and the likelihood that it was related to a study drug. The DSMB may recommend no changes to the study if the event is expected or related to other causes such as the patient's underlying condition. The DSMB may request an expert's advice of other non-UCLA Principal Investigator with national experience to support their deliberations and decisions. The JCCC DSMB has the authority to recommend to the UCLA IRB the immediate halt to the study (i.e., discontinuation of any further treatment of enrolled patients and discontinuation of enrollment of new patients) should there be any serious unexpected toxicity that warrants further investigation. JCCC DSMB correspondences are

addressed to the Principal Investigator and copied to the UCLA IRB. Minutes of the DSMB meetings are recorded and processed into the computer file.

15.2 JCCC DSMB Internal Monitoring Plan. The NIH and NCI policy statements allow for variable monitoring and reporting plans, commensurate with the potential risks and with the size and complexity of the trial. The monitoring plan must be sufficiently rigorous and effective to ensure subject safety and to ensure protocol compliance and data validity and integrity.

15.2.1 Level of Risk of a Study. For trials overseen by the JCCC DSMB, the JCCC DSMB will determine the degree of risk of the study and will ensure that there are procedures in place to ensure the safety of the subjects that are enrolled in the trial. The intensity level of the monitoring is determined by the risk category. Some of the factors that must be considered when assigning the Level of Risk category include:

1. A biostatistical design and appropriate procedures for proper data management so that the information collected can be properly validated.
2. Appropriate Serious Adverse Event reporting procedures must be in place.
3. The study duration must be appropriate and must be based on a realistic rate of enrollment.
4. Data collection and data management must be adequate to verify and ensure subject safety.

15.2.2 Assignment of risk. Assigning risk ensures that the data and safety monitoring is based on the level of risk (low, medium, or high) to ensure that the data and safety monitoring activities are appropriate. Below are some of the criteria used to make a decision regarding the assignment of risk:

- Expected duration of the study based upon the estimated rate of one.
- Type of study population (e.g., children, geriatric)
- The procedures used in the trial are commensurate with the degree of risk.
- Adequate data management systems in place and appropriate case report forms
- Proper serious adverse event reporting procedures in place
- Proper biostatistical design and data analysis procedures in place.

15.3 Monitoring/Auditing Activities.

The compliance officer of the JCCC Office of Regulatory Compliance [ORC] will monitor the clinical records for all human subjects enrolled onto JCCC institutional trials overseen by the JCCC DSMB. The JCCC compliance officer will perform real time review of informed consent form processes and the meeting of all inclusion and exclusion criteria at study entry. Active monitoring will offer the JCCC study teams prospective information that can be used to enhance the quality of research being performed contemporaneously. Auditing is a review of historic performance of the research effort and is performed on case report forms, regulatory files and source documents to measure the quality of the research effort in a retrospective manner.

15.4 Detailed Reporting Mechanism for Adverse Events

Each protocol is required to have a detailed description of the Adverse Event Reporting method. The DSMB expects each protocol to abide by the reporting time line and definitions consistent with the NCI and FDA guidelines.

An adverse event is any undesirable experience associated with the use of a medical product in a patient (any unfavorable and unintended sign, symptom or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure). Any clinical adverse event must be recorded on the case report form during the course of the study. The investigator must evaluate and document the adverse event for severity,

grade it according to the NCI Common Toxicity Guideline, causal relationship to the study drug under study, take appropriate action to care for the patient and to document the outcome. All information recorded in the case report form must be verifiably documented in the source (i.e., medical record or physician office chart).

When an adverse event occurs, it is the responsibility of the investigator to evaluate and record into the source documents, the nature of the symptom, prescribe the appropriate remedy and to report the event. The adverse event must be reported to the sponsor, the IRB, FDA and any other appropriate agency (e.g., IBC and NIH for gene medicine trials.) The assessment and reporting must occur in writing within 10 days, 48 hours for death.

If the event is **Serious and Unexpected**, the event should be reported immediately (in writing within 10 days, 48 hours for death) by the Principal Investigator to the IND sponsor, UCLA IRB, JCCC DSMB and to any appropriate agency; the NIH and the UCLA IBC for gene medicine trial; directly to the FDA if it is an institutional IND trial; and to the NCI if it is an NCI sponsored trial. For institutional trials and for gene medicine trials, the JCCC ORC and Gene Medicine Compliance Officers will assist the investigator to ensure that all serious adverse events are properly documented and reported in accordance to federal and institutional requirements.

All adverse events that occur in a research study overseen by the JCCC DSMB must be submitted, regardless of relationship, expectedness, or seriousness.

15.5 Data Recording and Retention of Study Data

Data recording will be done in compliance with Good Clinical Practice. Medical records and notes should be clearly marked and permit easy identification of participation by an individual in the specified clinical trial. Medical records and source data should be completed within a reasonable amount of time for purposes of timely data collection and entry.

The investigators are to record all data with respect to protocol procedures, drug administration, laboratory data, safety data and efficacy ratings on the case report forms (CRF). The CRF may be a printed, optical or electronic document. All corrections on a CRF and on source documents must be made in a way that does not obscure the original entry. The correct data must be inserted, dated and initialed/authorized by study site personnel. If the change is not obvious provide a reason.

16.0 STATISTICAL METHODS

This is a two-stage phase II clinical trial aimed at determining the safety, feasibility and antitumor activity of gene transfer using retrovirus MSGV1-F5AfT2AB transduced PBMC. UCLA will be responsible for registering patients and for maintaining a complete database of the study information excluding laboratory assay data. The clinical laboratory assay data will be obtained from source documents from the UCLA medical records (including both paper and electronic files and laboratory results) and transcribed into CRFs. A shadow chart may be kept with copies of the source documents in a locked room at the UCLA Clinical Research Unit or under direct control of the study P.I. Failure to complete the study due to the stopping rules being invoked will be the main basis for determining safety and feasibility of this study. This study is intended to provide data that might allow the investigators to conduct a preliminary assessment of safety and feasibility.

16.1 Sample Size Determination

The Simon optimal two-stage design[1] is used to determine the sample size, using the co-primary endpoint of response rate as the criterion for the clinical trial statistical design.

This clinical trial is set up to rule out the null hypothesis that $p_0 \leq 0.10$ (i.e. to rule out that this combined therapy has a response beyond 10%, since several current treatment approaches achieve response rates of 10% in patients with advanced melanoma) versus the alternative that the effect-size = $p_1 - p_0 > 0.25$ has an expected sample size (*Ave n*) of 10.6 and a probability of early termination (*PET*) of 0.81. The alternative hypothesis of a response rate of 35% (and thus a difference between the null and alternative hypothesis of 25% or effect-size = $p_1 - p_0 > 0.25$) is chosen since it is felt to represent a clinically-meaningful difference and results in a study sample size that is feasible to be conducted within a pilot single-institution study. If the drug is actually not effective, there is $\alpha \leq 0.05$ probability of concluding that it is. If the drug is actually effective, there is a $\beta \leq 0.20$ probability of concluding that it is not (Table 13).

After testing the drug on 8 patients in the first stage, the trial terminates if only 1 patient responds. If the trial goes on to the second stage, a total of 22 patients will be studied. If the total number responding is less than or equal to 4, the combined immunotherapy approach of lymphodepletion plus MART-1 F5 TCR transgenic cell adoptive transfer, together with high dose IL-2 (up to Amendment 10) or low dose IL-2 (starting in Amendment 11) and MART-1/DC vaccines, is rejected.

Table 13. Calculations of Simon's Optimal Two-Stage Designs for Phase II Clinical Trials. Clinical trials with $p_1 - p_0$ varying from 0.15 to 0.50 (α=0.05, β=0.20), calculated using the PASS 2005 software (Kaysville, Utah).

p_0	Key	***Effect size $p_1 - p_0$***					
		0.15	**0.20**	**0.25**	**0.30**	**0.40**	**0.50**
0.05	n_1 =	10	9	5	4	3	3
	r_1 =	0	0	0	0	0	0
	n =	29	17	18	16	8	7
	r =	3	2	2	2	1	1
	PET	0.60	0.63	0.77	0.82	0.86	0.86
	Ave n	17.6[3]	12.0	7.9	6.2	3.7	3.6
0.10	n_1 =	18	10	8	4	3	2
	r_1 =	2	1	1	0	0	0
	n =	43	29	22	15	9	8
	r =	7	5	4	3	2	2
	PET	0.73	0.74	0.81	0.66	0.73	0.81
	Ave n	24.7	15.0	10.6	7.8	4.6	3.1
0.15	n_1 =	19	9	7	6	3	2
	r_1 =	3	1	1	1	0	0
	n =	55	34	25	19	10	6
	r =	12	8	6	5	3	2
	PET	0.68	0.60	0.72	0.78	0.61	0.72
	Ave n	30.4	19.0	12.1	8.9	5.7	3.1

Legend: n1: required number of patients in stage 1; r1: number of patients with an objective response that need to be exceeded to proceed with the study; n: total number of required patients combining stage 1 and 2; r: number of patients with an objective response that are needed to be exceeded to conclude that the clinical trial had successfully ruled out the null hypothesis; Ave n: expected sample size; PET: probability of early termination.

16.2 Definition of Evaluable Patients

Patients evaluable for the primary analysis are those fully enrolled in the study after meeting the inclusion/exclusion criteria. Other patients will be considered un-evaluable and excluded from the primary analysis.

16.3 Efficacy Analysis

The toxicities observed after each TCR transgenic cell infusion will be summarized in terms of type (organ affected or laboratory determination such as absolute neutrophil count), severity (by Toxicity Table) and nadir or maximum values for the laboratory measures, time of onset (i.e. course number), duration, and reversibility or outcome. Tables will be created to summarize these toxicities and side effects by dose and by course. Baseline information (e.g. the extent of prior therapy) and demographic information will be presented, as well, to describe the patients treated in this pilot study. All responses will be reported.

16.4 Study Stopping and Non-stopping Rules.

It is recognized that AEs can occur frequently in this population based on the underlying metastatic melanoma and these can be SAEs. The review of SAEs will form the basis for potential early stopping of the study. Only unexpected SAEs that are related to the transduction process/research reagent would define a stopping rule. The review of these adverse events, and any decision to prematurely stop subject enrollment, will be determined by the UCLA DSMB and reviewed by the IRB.

Absolute stopping rules for this clinical trial will be:

1. Any death that is possibly related to the investigational agents namely MART-1 F5 TCR PBMC or MART-1_{26-35} peptide pulsed DC.
2. Two or more grade 4 events that are possibly related to MART-1 F5 TCR PBMC or MART-1_{26-35} peptide pulsed DC.
3. Any event of hematological malignancy must be considered study stopping criteria until oncogenesis related to retroviral mutagenesis, EBV lymphoma and Post Transplant Lymphoproliferative Disorder (PTLD) can be excluded.

Premature termination of the clinical trial may occur because of a regulatory authority decision, change in opinion of the FDA, RAC, IRB, the DSMB, or determination that there are problems in the cell product generation or the safety of their administration as descried in the assessment of primary study endpoints. Additionally, recruitment may be stopped for reasons of particularly low recruitment, protocol violations, or inadequate data recording.

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Appendix: Summary of Protocol Amendments

Amendment 1

Date: March 16, 2009

Main protocol and consent modifications;

a) Protocol:

While generating the forms to fill in the product lot release criteria we noted that the approved version of the study protocol did not specify which final product lot release tests should be received before administration of the cells to patients. The lot release criteria were detailed in Table 4 of the protocol, but both tests with immediate results and with delayed results were listed together. The key issues were the low likelihood of obtaining results of the copy number of viral insertions in the target cells, and the testing for replication competent retrovirus (RCR), which Dr. Reiser, the FDA product reviewer for this IND, had asked to be done at the Indiana University Viral Production Facility (IU VPF) under the direction of Dr. Ken Cornetta.

Dr. Reiser at phone number 301-827-3420 who agreed that final results of the copy number and RCR were not required to be in hand before administering the TCR transgenic cells to patients. Dr. Reiser said that a plan should be in place in case the results were positive, including the contacting and treatment of study subjects and the reporting of the event to the FDA.

With this clarification, we discussed that we would amend the protocol to clearly state which results would be in hand before TCR transgenic cell administration to patients, and which results would be available at a later time point. We also discussed that this protocol amendment would include a plan for patient and FDA notification.

The lot release criteria have been broken down into two tables in the amended version of the protocol dated 03/16/09. Table 4a (page 37) details the results that need to be in hand before the release of the TCR transgenic cells for patient administration. Table 4b (page 37-38) details the results that may be available afterwards. After this table there is a specification of the plans to contact and treat the patients and how the local and federal regulatory agencies should be notified.

b) Consent Form:

No changes.

c) HS-1 Form:

No changes.

Amendment 2

Date: April 13, 2009

Main protocol and consent modifications;

a) Protocol:

- After discussion with the FDA reviewers in a series of teleconferences and email exchanges on April 7 and 8 (see attached minutes of these information exchanges), we deleted the requirement for quantitation of the number of copy inserts of retroviral vector in the final product. The FDA reviewers agreed with us that the quantitation of surface TCR expression in transduced cells was a good quantitative assessment of transduction efficiency. In fact, the previously IRB approved protocol had this test listed twice, as lot release criteria and as optional additional testing in the TCR transduced cells.
- We clarified the intent of the schedule of events as the intent to perform the protocol procedures on those days. However, since this is a pilot study and needs to account for variation on patient outcome and scheduling issues, we clarify in this second amendment that the dates are approximate.
- The use of antiementics and prophylactic antifungals was updated to match the standard practice in the stem cell transplantation ward at UCLA.
- The administration of MESNA was corrected to continuous intravenous infusion for 24 hours.

b) Consent Form:

- We received the NIH Certificate of Confidentiality (CoC). Therefore, we added back the CoC language to the consent form.
- We corrected a typographical error in page 7.

c) HS-1 Form:

No changes.

Amendment 3

Date: May 26, 2009

Main protocol and consent modifications;

a) Protocol:

- After new discussion with the FDA reviewers on April 27 (see attached minutes), we slightly modified our *ex vivo* culture plan after transduction to limit it to 4 days, which following the Guidance for Industry for Replication Competent Retrovirus (RCR) testing (November 2006) allows us to bank but not prospectively test for RCR in the final product. The FDA reviewers agreed with us that a reasonable plan would be to test samples from peripheral blood obtained at 3, 6 and 12 months after TCR transgenic adoptive transfer and only test the final product sample if one of the patient-derived samples is positive for RCR. The proposed S+/L- assay is a one-month assay with high costs that would not be able to provide results in time for patient safety. Therefore, we discussed with the FDA and went onto amend our protocol following the Guidance for Industry plan for studies where gene-modified cell therapies are cultured for less than 4 days. This was included in Table 4b and Sections 10.1.9 and 11.2.1.2.
- The timing of sampling of blood of patients for RCR was modified to follow the Guidance for Industry document to samples at 3, 6 and 12 months, and stored samples annually thereafter (Section 11.2.1.2).
- The reporting of the results of the mycoplasma PCR-based rapid test was changed, and we changed it in the protocol accordingly (Tables 4a and 10, and Section 10.2.10).
- We deleted a 100 ml blood draw for immune monitoring analysis that was planned on day +1 since in the first two patients this blood draw provided very few lymphocytes since the blood draw happens at a time when the patient is pancytopenic. Analysis of these lymphocytes by immune monitoring assays did not provide much information.
- We added a note in the schedule of events (Section 2) and in the protocol description in Section 8.6 text to note that 100 ml blood draws for immune monitoring analysis being planned for days 7 and 14 after TCR transgenic adoptive transfer can be done with a wider window since in our first 2 patients we have noted the recovering pancytopenia and hemodynamic state after high dose IL-2 may not make it clinically wise to obtain these blood draws on these particular days.
- We added statements to the supportive care plan for patients to follow the clinical judgment and the standard practices in J-Medicine (Section 8.3).
- Minor typographical errors were corrected throughout and marked in tracking mode in one of the protocol copies.

b) Informed Consent Form

- Upon review of the ICF we noted that there was no good explanation of the amount and timing of blood draws for immune monitoring analysis. Therefore, we added a paragraph in Section 4 of the Study Procedures to explain to patients that repeated blood draws are planned to obtain cells for immune monitoring analysis.
- After a correspondence with the IRB, an additional change was made to the consent form to take out the explanation and checkbox for the request for autopsy.

c) HS-1 Form:

- A new grant from our collaborator Dr. Bijay Mukherji from the University of Connecticut, with a UCLA subcontract, was added as funding source for this research project.
- The timing of immune monitoring assays was added to the experimental procedures.
- The tables of cell therapy lot release criteria were updated to match the tables included in the protocol after amendments 1, 2 and 3.

Amendment 4

Date: July 16, 2009

Main protocol and consent modifications;

a) Protocol:

- A new section (8.12.) was added to allow the administration of booster MART-1 peptide pulsed DC vaccinations when the levels of MART-1-specific T cells would fall lower than 5% of total T lymphocytes. The schedule of events was included in the new section 2.1.
- The requirement for paired tumor biopsies as inclusion criteria was changed to performing biopsies when felt feasible by the study investigators.
- An appendix at the end of the protocol detailing the sequential protocol amendments was added.
- Minor typographical errors were corrected throughout and marked in tracking mode in one of the protocol copies.

b) Informed Consent Form

- The ICF was updated to include the administration of 3 delayed DC booster vaccinations.
- The details on how the study was funded were updated in the ICF.
- The change in the requirement for tumor biopsies was added, where patients without easily accessible lesions for surgical biopsy would still be eligible for this protocol either with no biopsy, or with an image-guided biopsy.
- The contact information for study investigators was updated.

c) HS-1 Form:

- A new grant from our collaborator Dr. David Baltimore from the California Institute of Technology, with a UCLA subcontract, was added as funding source for this research project.
- The changes in the eligibility not strictly requiring paired tumor biopsies were included.
- The addition of booster DC vaccinations was included.

Amendment 5

Date: October 13, 2009

Main protocol and consent modifications;

a) Protocol:

- Correction of the schedule of events to reflect the follow up of patients who are in response or without progression after 3 months.

- Added subjects who are HLA-A*0205 (HLA-A2.5) positive as potential participants in this research, provided that we demonstrate that MART-1 F5 TCR transgenic cells produce > 30,000 pg/ml/million cells of IFN-γ production upon MART-$1_{26\text{-}35}$ peptide stimulation using MART-$1_{26\text{-}35}$ peptide-pulsed PBMC from the potential HLA-A*0205 (HLA-A2.5) subject candidate.
- Added the Endosafe - MCS System as an alternative method for testing for endotoxin levels in TCR transgenic cells and dendritic cells.
- Added to the pre-study procedures the placement of a double lumen central venous access catheter.
- Added the possibility of using clinical grade human AB serum for the DC manufacture.

b) Informed Consent Form

- The dendritic cell vaccine booster part of the dendritic cell addendum consent was corrected to monthly vaccines.
- The table at the end of the main consent form was updated to add the follow up visits, blood work and scans every 3 months for at least 2 years in patients who have no progression after the first 90 days of study participation.

c) HS-1 Form:

- The changes in eligibility and procedures included in the protocol were made in the HS-1 form.

Amendment 6

Date: April 6, 2010

Main protocol and consent modifications;

a) Protocol:

- The major modification in Amendment 6 was the increasing of the F5 TCR transgenic cell dose to 10^{10} and restarting the stage 1 of this protocol since with the prior version administering a cell dose up to 10^9 we did not meet the minimal hurdle of antitumor activity to proceed to stage 2 of this research after having entered the first 8 patients.
- The description and references to the NCI Surgery Branch F5 TCR adoptive cell transfer protocol have been updated based on the published data included in the manuscript by Johnson *et al*. in Blood 2009.
- C reactive protein (CRP) testing was added to the baseline exams since there is emerging evidence that it may provide information about the immune permissively of the tumor microenviroment.
- The timing of procedures related to prior therapy was clarified.
- The eligibility/exclusion for patients with prior brain metastasis was clarified.
- The sources of clinical grade IL-2 and GM-CSF were updated since they have recently changed due to pharmaceutical company agreements and cross-licensing.
- We added a description of Retronectin to the detailed explanation of reagents used in this study.
- We added the possibility of samples being tested with novel immune monitoring assays that have become available in the past several months.
- We added Dr. James Heath from Caltech as a non-clinical investigator.
- Several typographic errors and inconsistencies with the laboratory procedures were corrected and updated.

b) Informed Consent Form

- No modifications were made to the ICF.

c) HS-1 Form:

- The changes in eligibility and procedures included in the protocol were made in the HS-1 form.

Amendment 7

Date: October 5, 2010

Main protocol and consent modifications;

a) Protocol:

- Deletion of Dr. Erika von Euw from the protocol roster since she is no longer involved in this research project.
- Clarification of the enrollment of patients with a prior diagnosis of brain metastasis.
- Updated information on the source of OKT3 for this protocol.
- Updated details on the procedure of retroviral transduction of activated lymphocytes..
- The inclusion of the testing for C reactive protein (CRP) in the Schedule of Events.

b) Informed Consent Form

- Several typographical errors have been corrected in the ICF, and wording has been slightly modified to improve it.

c) HS-1 Form:

- The relevant changes in the main study protocol were made in the HS-1 form.

Amendment 8

Date: December 17, 2010

Main protocol and consent modifications

a) Protocol: The following is a description of the major changes in Amendment 8 and the reasoning behind them:

1. Four days of Fludarabine at 25 mg/m^2/day instead of 5 days:

   The available information based on the course, correlative studies and outcome of the SAE on patient F5-10 strongly suggest that it was due to direct marrow toxicity from the conditioning regimen as the most likely cause (see full description of the SAE). The most likely cause of a delayed marrow aplasia is fludarabine. Therefore, we have decreased the number of fludarabine doses at 25 mg/m^2/day i.v. daily from 5 days to 4 days.

2. Capping of the administration of high dose IL-2 to up to 9 doses instead of 14 doses:

A potential adverse contributor to the marrow aplasia in patient F5-10 is the 14 doses of IL-2 that this patient received, which is higher than other patients treated in this protocol. The prior versions of the protocol included dosing IL-2 at 600,000 IU/kg every 8 hours for up to 14 doses based on tolerance. This patient tolerated all doses within the expected parameters and received all of them. In conversations with our colleagues at the NCI Surgery Branch, who have administered the same conditioning regimen followed by high dose IL-2 to over 200 patients, they feel that the dosing of IL-2 may be an adverse contributor. In addition, review of the literature shows that higher doses of IL-2 up to 15 (720,000 IU/kg, 8 hourly for 5 days) lead to grade 3/4 toxicities whereas decreasing it to a maximum of 9 (720,000 IU/kg, 8 hourly for 3 days) results in fewer toxicities and still have the anti-tumor activities in conjunction with the adoptive cell transfer (ACT) [7,21]. In fact, there was a trend wherein less doses of IL-2 correlated with higher clinical responses. Therefore, we plan to cap the number of IL-2 doses to a maximum of 9 doses.

3. Using freshly harvested F5 MART-1 TCR transgenic T cells:

   The major problem for patients treated within this protocol has been relapse of melanoma after an initial tumor response. Our ongoing experiments analyzing the functionality of the TCR transgenic cells recovered from patients demonstrate a progressive loss of ability to secrete multiple cytokines upon MART-1 antigen exposure. This is reminiscent of preclinical studies of T cell exhaustion and lack of secondary response to antigen. Therefore, improving the *in vivo* functionality of the TCR transgenic cells continues to be a major goal of our research plans.

   A recent experiment in a murine model of ACT performed by our collaborators Drs. Lili Yang and David Baltimore from the California Institute of Technology (Caltech) indicated that cryopreservation has a profound detrimental effect on the *in vivo* long-term survival of the adoptively transferred T cells and their ability to have a secondary response to antigen exposure. We noted a similar effect within our clinical trial, since in patient F5-3 received a round of three delayed MART-1 peptide pulsed dendritic cell vaccines and had no secondary expansion of MART-1-specific T cells.

   These results have prompted us to modify our protocol to avoid cryopreservation of the TCR transgenic cells administered to patients, which is included in Amendment 8.

b) Informed Consent Form

- The use of freshly harvested F5 MART-1 TCR transgenic T cells for infusion in place of cryopreserved cells as depicted in Amendment 8

- The use of high dose IL-2 every 8 hours following transgenic T cell infusion for a maximum 9 doses over 3 days has been adopted to replace the previous regimen of maximum 14 doses in Amendment 8

c) HS-1 Form

- As IRB submission at UCLA was converted to webIRB which is entirely done online, HS-1 form was no longer required.

Amendment 9

Date: April 20, 2011

Main protocol and consent modifications;

a) Protocol:

- Addition of a PCR/ELISA mycoplasma assay if the MycoAlert results are equivocal or positive both during in-process testing and before cells are administered to the patient.
- Lowering of the lot release criteria to 10% of tetramer positive cells for MART-1(26-35) as long as all the other lot release criteria are met.
- Indication of performing additional testing for the number of vector copies. If higher than 5 we will propose a new dilution of the vector supernatant before using it for cell transduction.
- Cardiac stress test on Day 75-90 has been removed.
- Addition of potential risks associated with autoimmune toxicity from mispaired TCRs
- The use of Trimethoprim-Sulfamethoxazole and Ganciclovir will start from Day -6
- Replacement of William Quan by Kit Shaw as the Human Gene Medicine Program (HGMP) Compliance Officer
- More detailed description of Day -6 events
- Minor formatting and wording modification

b) Informed Consent Form

- Addition of potential risks associated with autoimmune toxicitiy from mispairing TCRs
- Cardiac stress test on Day 75-90 has been removed
- Addition of Day -6 events to the schedule table in the end of the consent
- Addition of risk to include life-threatening infection and/or respiratory failure requiring mechanical ventilation

Amendment 10

Date: August 22, 2011

Main protocol and consent modifications;

a) Protocol

- Addition of Sujna Raval-Fernandes as the compliance officer for the DSMB.
- Addition of Arun Singh as a study investigator with patient contact.
- Addition of Phyllis Wu as study coordinator and regulatory coordinator.
- Change of manufacturer of OKT3 to Miltenyi: As per FDA recommendation on July 28, 2011, we replaced eBioscience OKT3 with Miltenyi OKT3.
- Change of GM-CSF manufacturer to Berlex and IL-2 manufacturer to Prometheus only in accordance to FDA product reviewer recommendation on July 22, 2011. The sources of clinical grade IL-2 and GM-CSF were changed due to pharmaceutical company agreements and cross-licensing.
- Added dextramer as an alternative option for tetramer for the purpose of quantification of F5 TCR transgenic cells. We had tested by staining the same sample side-by-side at least three times and obtained comparable results repeatedly. Test results attached.
- Corrected “Blood for Plasma/serum” procedure in the schedule of events to “Plasmapheresis”. Plasmapheresis is carried out at the time of leukapheresis for every patient on Day 0 and Day 30.
- Correction of minor typographic errors.

b) Informed Consent Form

- Addition of Arun Singh as a study investigator.
- Description of a day 20-40 ECHO/MUGA was deleted.

Amendment 11

Date: November 7 , 2011

Main protocol and consent modifications;

a) Protocol

- Decrease the maximum cell dose for ACT to 10^9 total cells.
- Changing the IL-2 administration from high dose (HD) intravenously to low dose (LD) subcutaneously for up to 14 days.
- Require having pulmonary function tests in all patients and change the patient exclusion criteria to exclude patients with a FEV_1/FCV< 70% of predicted for normality.
- Electrocardiogram (ECG) is added as a heart test for patients.
- List pancytopenia caused by bone marrow infiltration by melanoma cells as a risk factor.
- Added to exclusion criteria the following:
    - History of, or significant evidence of risk for, chronic inflammatory or autoimmune disease (eg, Addison's disease, multiple sclerosis, Graves disease, Hashimoto's thyroiditis, inflammatory bowel disease, psoriasis, rheumatoid arthritis, systemic lupus erythematosus, hypophysitis, pituitary disorders, etc.). Patients will be eligible if prior autoimmune disease is not deemed to be active (e.x. fibrotic damage of the thyroid after thyroiditis or its treatment, with stable thyroid hormone replacement therapy). Vitiligo will not be a basis for exclusion.
    - History of inflammatory bowel disease, celiac disease, or other chronic gastrointestinal conditions associated with diarrhea or bleeding, or current acute colitis of any origin.
- Added "Must be willing to perform two biopsies at least" to inclusion criteria.
- Revised screening blood test details, reflected in the schedule table and section 8.1
- In section 8.4, added "Norethindrone (Norlutate) 10mg PO daily will be used for menstruating women for prophylaxis against menorrhagia" under "Prophylactic Supportive Care Throughout the Pancytopenic Period".
- Added 8.11 Permissible Systemic Therapies During the Protocol On-study Period
- Change GM-CSF source to Genzyme
- Revisions in response to FDA inquires regarding cross-referenced IND 14760.
- Correction of minor typographic errors.

b) Informed Consent Form

- Description of the LD IL-2 administration
- Description of the SAE of intubation in a second patient.
- Description of pancytopenia caused by bone marrow infiltration by melanoma cells as a risk factor.
- Requirement of having pulmonary function tests in all patients and change the patient exclusion criteria to exclude patients with a FEV_1/FCV< 70% of predicted for normality.
- Electrocardiogram (ECG) is added as a heart test for patients.
- Added MART-1 testing in the screening procedures.

Amendment 12

Date: July 25, 2013

Main Protocol and Consent Modifications:

a) Protocol

- No changes were made to the protocol

b) Informed Consent Form

- Description of an SAE in which the patient died due to melanoma progression with massive intraabdominal bleeding.

Amendment 13

Date: September 9, 2014

Main Protocol and Consent Modifications:

a) Protocol

- Changed contact information for several co-investigators on page 1
- Added Paula Kaplan-Lefko, Beata Berent-Maoz, Daniel Shin, Siwen Hu-Lieskovan and Xiaoyan Wang
- Removed Bradley Straatsma and Phyllis Wu
- Blood tests (Mg, Phos and uric acid) in table in Section 2.0 were changed from Day -5 through Day 0 to only on Day -6.
- Modified days of blood collection and physical exams in table in Section 2.0 and 8.0.
- Added to section 8.0 and section 2.0 that changes in the range of 7 days up or down from the scheduled event will not be considered deviations from the study timeline. Removed the word "significant" referring to deviations.
- On page 25, ECOG has been deleted from the list of tests to be performed in the hospital.
- On page 26, "mineral panel" has been deleted from the blood tests to be monitored.
- Clarification that physical exam at baseline will include weight, height, and vital signs and that weight measurement will only be obtained at subsequent physical exams if clinically indicated (Section 8.1)
- Deleted that physical exam would include weight, height and vital signs on Day -6 (Section 8.3) since weight measurement is not standard inpatient practice.
- In section 8.1 and 8.2, added that a new biopsy may not be required if an acceptable biopsy was performed within 60 days of prior to screening per coverage analysis for other trials.
- In section 8.2, modified cardiac function test details.
- Reduced IL-2 administration from twice daily for 14 days to twice daily for 7 days per IRB due to SAEs in IRB #12-000153 and 13-001624.
- In section 8.4, changed dose of Ativan from 2 mg to 1 mg
- Added holding parameters for IL-2 and fevers due to IL-2 to section 8.7
- Changed section 8.9 to indicate that CT will be attenuated.
- In section 8.15, removed optional repeated dendritic cell vaccines.
- In sections 9.6.4 and 9.6.5, updated information regarding IL-4 and retronectin.
- In section 10.1.5, Table 2b Annual Recertification was updated.

b) Informed Consent Form

- Reduced IL-2 administration from twice daily for 14 days to twice daily for 7 days.
- Removed optional repeated dendritic cell vaccines

Amendment 14

Date: August 1, 2016

a) Protocol

- Modified annual recertification tests required (Section 10.1.5)

b) Informed Consent Form

- No changes

Amendment 15

a) Protocol

- In section 10.1.5, Table 2b Annual Recertification was updated
- In section 10.2.5 MART-$1_{26\text{-}35}$, Table 7 Peptide Annual Recertification was updated, and modified annual recertification tests required.