ADAPTIMMUNE
transforming T cell therapy

A Phase I/IIa, dual-cohort, two-site, clinical trial evaluating the safety and activity of redirected autologous T cells expressing a high affinity TCR specific for NY-ESO-1 administered post ASCT in patients with advanced myeloma

Protocol Version: April 07, 2017

SPONSOR IND: IND14603
Adaptimmune LLC
351 Rouse Boulevard
Philadelphia, PA 19112
USA
IND Number: **14603**
Study Product: **NY-ESO-1^{c259}-T**
Study Number: **01411**
Protocol Version: **12.0**

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SIGNATURES AND AGREEMENT WITH THE PROTOCOL

I, the undersigned, have reviewed the original protocol, including the appendices, and I will conduct the clinical study as described and will adhere to International Conference on Harmonisation (ICH) guideline E6 (r2):Guideline for Clinical Practice (GCP) and all the ethical and regulatory considerations stated. I have read and understood the contents of the Investigator Brochure.

Principal Investigator Date

Protocol Amendment

Version:0.1	**22-Jul-2010**	Initial version
Version:0.2	**26-Oct-2010**	To incorporate feedback from the NIH RAC, FDA and general improvements
Version: 0.3	**30-Nov-2010**	To incorporate feedback from University of Maryland Greenebaum Cancer Center CRC
Version: 1.0	**06-Jan-2011**	Final version for IRB submission.
Version: 2.0	**22-Feb-2011**	Changes in response to IRB reviews at UMD and UPenn, and changes to harmonize the two protocol versions to each other.
Version: 3.0	**07-Jul-2011**	Changes to include additional antigen in the eligibility for each cohort, since they are also known to be targeted by the TCRs used in this study. Also, minor changes for clarification on study visit windows, and to explicitly state that frozen and previously collected apheresis product can be used in lieu of fresh.
Version: 4.0	**29-Nov-2011**	Changes made to include increased cardiac monitoring in response to an SAE on a parallel trial in the Mage arm, and to extend the NYESO arm now that sufficient safety data is available and we wish to discern more robustly the clinical effect of the NYESO TCR from the high dose melphalan by comparing to recently published controls. Amend SAE reporting requirements to FDA as requested.
Version: 5.0	**30-Apr-2012**	To allow for identical twin sibling stem cell transplant (SSCT) – Not submitted at University of Pennsylvania
Version: 6.0	**16-Jul-12**	To add optional second T-cell infusion and to clarify eligibility criteria for patients to undergo second infusion, and to correct T cell release criteria to be consistent with the IND 14603
Version: 7.0	**12-Mar-2013**	Removal of MAGE T cells conducted under IND 14604
Version: 8.0	**08-Jul-2013**	Changes to reflect changes in regulatory sponsorship from University of Pennsylvania to Adaptimmune. Changes to remove the MAGE cohort from the study design, changes to the long term follow up (LTFU) plan, changes on study stopping rules and changes on protocol deviation reporting, administrative changes.
Version: 9.0	**04-Oct-2013**	Administrative changes to comply with requests made by the CTSRMC at the University of Pennsylvania
Version: 10.0	**20-Nov-2013**	Administrative changes to comply with requests made by the CTSRMC and IRB at the University of Pennsylvania
Version: 11.0	**20-Jan-2015**	Administrative change under section 3.1 to correct misleading information on long term follow up for patients who disease progress. Administrative changes to reinforce long term follow up plan.
Version: 12.0	**07-Apr-2017**	Update of IMWG Disease Response Criteria [Rajkumar, 2011] to assess all disease responses up to Year 1 *a posteriori*. Clarification of efficacy objective and capture of disease progression dates for subjects who have porgressed beyond year 1 to assess duration of response and progression free survival. Once the Long-Term Follow-up (LTFU) protocol ADP-0000-002 is available at site, patients whose disease has progressed/relapsed will be considered having completed study ADP-01411 and be consented to the LTFU protocol ADP-0000-002.

		Patients who remain disease-free at the time of study completion (decided at sponsor's discretion) will also be enrolled in the LTFU protocol ADP-0000-002. Rewording of persistence and RCL risk sections to conform to current FDA approved LTFU procedures. Additional changes throughout the protocol amendment are to align assessments and wording in this amendment with those of the LTFU protocol (ADP-0000-002). Harmonization of protocol versions across sites.

CONFIDENTIALITY STATEMENT

DECLARATION

This study will be conducted in compliance with ICH Good Clinical Practice (GCP), all applicable subject privacy requirements, and the guiding principles of the current version of the Declaration of Helsinki (with amendments), and in accordance with local legal and regulatory requirements.

Clinical Trial Site - University of Maryland:

Principal Investigator:	Aaron P. Rapoport, MD	Protocol Chair

Clinical Trial Site - University of Pennsylvania (Coordinating Center):

Principal Investigator:	Edward Stadtmauer, MD	Clinical PI

RESPONSIBLE SPONSOR STUDY PHYSICIAN/SPONSOR INFORMATION PAGE

Sponsor Signatory

_____ ___ 07 April 2017___

Lini Pandite, MD Date
SVP, Clinical Development

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Sponsor Details

Adaptimmune LLC 351 Rouse Boulevard Philadelphia, PA 19112 USA

AGENT	NSC / NDC #	Supplier	IND
Autologous T-cells	NSC 727300	Investigator	**BB-IND014603**
Cyclophosphamide	NSC 26271	Commercial	
Melphalan	NSC 8806	Commercial	
Prevnar-13	NSC 731759	Commercial	
G-CSF (filgrastim)	NSC 614629	Commercial	
Plerixafor (Mozobil)	NDC 58468-0140-01	Commercial	
Lenalidomide	NSC 703813	Commercial	
Bortezomib	NSC 681239	Commercial	

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Table of Contents

Protocol Summary

Title	A Phase I/II, dual-cohort, two-site, clinical trial evaluating the safety and activity of redirected autologous T cells expressing a high affinity TCR specific for NY-ESO-1 administered post ASCT in patients with advanced myeloma
Sponsor	Adaptimmune
Study Centers	Two Centers: University of Maryland Greenebaum Cancer Center; Hospital of the University of Pennsylvania
Study Design	A Phase I/II, study. Patients with advanced myeloma and who are candidates for autologous stem cell transplants, or syngeneic stem cell transplants (SSCT), will be eligible. Prior to full screening on this study, patients will undergo prescreening to evaluate HLA-A type and presence of NYESO-1/LAGE antigen. 7-14 days after PCV (pneumococcal conjugate vaccine) immunization #1 all patients (or donors in the case of SSCT) will initially undergo a steady-state mononuclear cell apheresis for T cell collection, with an optional second collection. Once mononuclear cells have been collected, patients (or donors in the case of SSCT) will then undergo hematopoietic stem cell mobilization. All patients will then receive high-dose melphalan followed by hematopoietic stem cell transplant on day 0. On day +2, patients will receive a dose of >**0.1-1 x 10^{10}** anti-CD3/anti-CD28-costimulated autologous T cells which have been genetically modified to express high affinity NYESO-1 TCRs. A minimum dose of 0.1-1 x 10^{9} will be permitted, but patients receiving this low dose level will be evaluated separately for safety and efficacy. PCV immunizations #2, 3 and 4 will be given at days 14, 42, 90. At day 100, patients will start lenalidomide maintenance. Patients will undergo myeloma restaging at days +42, +100, 6 months, 9 months, 1 year post infusion and at least every 3 months or per local standard of care thereafter, until disease progression. In accordance with FDA Guidelines, all patients will be monitored at 3, 6, 9, and 12 months then biannually until 5 years post infusion then annually until 15 years post infusion for delayed gene therapy adverse events. From the time of their disease progression, patients will enter long-term follow-up (LTFU) phase and continue to be monitored for gene therapy delayed adverse events as per original schedule. Subjects who are still alive will be consented to the LTFU protocol ADP-0000-002.

Study Products to be Administered	• Activated/costimulated autologous genetically modified T-cells for expression of high affinity TCRs "NYESO-1-c259-T" infusions at day +2 • Pneumococcal Conjugate Vaccine (Prevnar-13®) • Lenalidomide
Primary Objective	To evaluate the safety and tolerability of autologous genetically modified T cells transduced to express the high affinity NYESO-1 TCR in HLA-A2 subjects.
Secondary Objective	To evaluate the clinical response as specified in the International Myeloma Workshop Group (IMWG) criteria published in 2011 [Rajkumar, 2011].
Number of Subjects	26 evaluable patients in the NYESO cohort (originally 6, but extended to 26).
Study Population and Main Inclusion Criteria	Study patients will have systemic or multifocal myeloma requiring autologous stem cell transplantation. Patients should have disease which has relapsed or incompletely responded to prior therapy or have high-risk features. Patients must also have measurable disease on study entry, as defined by quantifiable or detectable levels of serum or urine paraprotein or elevated serum free light chains with an abnormal ratio.
Primary Endpoints	The primary endpoint of the study is: 1) (SAFETY ENDPOINT) Occurrence of adverse events, per NCI CTC v4 guidelines, including $\geq$ grade 4 laboratory toxicities at any time from Day-40 until year 1. This will include infusional toxicity, and any toxicity probably or definitely related to NYESO-1-c259-T including but not limited to: a. Fevers b. Rash c. Neutropenia, thrombocytopenia, anemia, marrow aplasia d. Hepatic dysfunction e. Pulmonary infiltrates or other pulmonary toxicity f. Development of GVHD NOTE: transplant-related toxicities, typically occurring within a month post-transplant, are excluded as investigational study-related adverse events. Antitumor efficacy will be evaluated in depth through clinical, molecular and immunological secondary endpoints.
Secondary Endpoint	To evaluate the clinical response by measuring: a. objective response rate (ORR) at day 42, 100, 180, 270 and 360. b. best objective response (BOR) prior to initiating lenalidomide and at day 360. c. duration of response (DOR) and progression-free survival (PFS),

	d. overall survival (OS) will be followed in this interventional protocol and continue to be followed in the long-term follow-up protocol ADP-0000-002, once subjects have transferred. Initiation of lenalidomide as maintenance treatment will be addressed in a sensitivity analysis

1 OBJECTIVES

1.1 Primary Objectives

1. To evaluate the safety and tolerability of autologous genetically modified T cells transduced to express the high affinity NYESO-1 TCR in HLA-A2 subjects.
2. To measure the incidence of GVHD in patients following infusion of TCR modified autologous T cells.

1.2 Secondary Objectives

1. To demonstrate product bioactivity, and establish proof of concept and mechanism for the function of gene-modified cells in vivo by evaluating:
   Selective migration and engraftment of gene-modified infused cells to the marrow.
   a. Ex-vivo immune functionality and phenotype of infused cells in marrow and periphery
   b. Modulation of cytokine milieu in marrow and periphery
   c. Development of an expanded patient immune response against tumor via epitope spreading
   d. Expression levels of NY-ESO-1 in marrow samples obtained pre- and post-treatment from patients in each of the respective cohorts
2. To evaluate the effect of late (day+100) lenalidomide treatment on secondary objectives1a-e above.
3. To evaluate post-transplant cellular and antibody pneumococcal conjugate vaccine (PCV) responses following adoptive transfer of up to 1 x 10^{10}PCV-vaccine-primed and TCR-gene-transduced autologous Tcells.
4. To evaluate the clinical response in the NYESO cohort as specified in Rajkumar et al. on behalf of IMWG in 2011 [Rajkumar, 2011] by measuring:
   a. objective response rate (ORR) at day 42, 100, 180, 270 and 360.
   b. best objective response (BOR) prior to initiating lenalidomide and at day 360.
   c. duration of response (DOR) and progression-free survival (PFS),
   d. overall survival (OS) will be followed in this interventional protocol and continue to be followed in the long-term follow-up protocol, once subjects have transferred.

   Initiation of lenalidomide as maintenance treatment will be addressed in a sensitivity analysis

1.3 Definition of efficacy endpoints

- Objective Response Rate (ORR): percentage of subjects who have a positive response (PR, VGPR, CR, or sCR).
- Best Objective Response (BOR): best response experienced by the subject from first assessment of positive response ((PR, VGPR, CR, or sCR).
- Progression Free Survival (PFS): the time from T-cell infusion (Day 2) until the date on which disease is shown to have progressed or the date on which the patient dies, from any cause.
- Duration of Response (DOR): the time from first assessment of positive response (PR, VGPR, CR, or sCR) until the date on which disease is shown to have progressed or the date on which the subject dies, from any cause.
- Overall Survival (OS): survival time from T-cell infusion (Day 2)

2 INTRODUCTION AND RATIONALE FOR INVESTIGATIONAL AGENTS

2.1 Autologous Stem Cell Transplants (ASCT) for Myeloma

Although allogeneic (donor) stem cell transplants can eradicate myeloma through a T-cell mediated "graft vs. myeloma" (GVM) effect (Tricot et al., 1996; Lokhorst et al., 2004), toxicities can be prohibitive. High-dose chemotherapy followed by autologous stem cell rescue (ASCT) induces complete responses in about 20-40% of patients but cures are rare due in part to the lack of a GVM effect (Barlogie et al., 1997; Child et al., 2003). Retrospective studies suggest that better clinical outcomes from auto-transplantation for myeloma and other hematologic neoplasms may be associated with more rapid lymphocyte recovery during the early post-transplant period (Porrata et al., 2001; Porrataet al., 2004). In addition, myeloma-reactive T-cells have been detected at low frequencies in the marrow or blood of untreated myeloma patients (Dhodapkar et al., 2002; Noonan et al., 2005). Thus, strategies to augment the recovery and function of autologous T-cells post-transplant may be beneficial.

2.2 Post-transplant Infections

In addition to compromising the ability of auto-transplant patients to mount effective anti-tumor immune responses, post-transplant immune suppression including prolonged depletion of CD4+ T-cell numbers and function and altered dynamics of immune cell recovery (e.g. inversion of CD4/CD8 ratios) clearly increase the risk for serious infections with varicella-zoster virus (VZV), cytomegalovirus (CMV), and *S. pneumonia* (Hoyle et al., 1994; Ketter et al., 1999). Indeed, the 23-valent pneumococcal polysaccharide vaccine is recommended by the American Society for Blood and Marrow Transplantation for all auto-transplant recipients at 1 and 2 years post-transplant (Dykewicz et al., 2001). Unfortunately, immunogenicity for this vaccine is limited, with only 19% of patients achieving potentially protective antibody levels at

24 months post-transplant (Guinan et al., 1994). A protracted delay in immune reconstitution following autologous stem cell transplantation may help to explain this pattern of defective immune responses (Anderson et al., 1990; Guillaume et al., 1998).

2.3 Post-transplant Immunotherapy; clinical data supporting the rationale for the design of this study

2.3.1 Results of GCC-0065/UPCC-6401:

Randomized Study of Combination Immunotherapy using Costimulated Autologous T-cells and pre and post-transplant Immunizations using the Pneumococcal Conjugate Vaccine (PCV, Prevnar®)

We hypothesized that improved T-cell recovery through intravenous infusion of ex-vivo costimulated autologous T-cells might provide a platform for post-transplant immunotherapy of myeloma and enhance protection from post-transplant infections. Autologous T-cells were stimulated by coculture with immunomagnetic beads to which anti-CD3 and anti-CD28 monoclonal antibodies had been conjugated because signals through CD3 and CD28 can prevent T cell anergy (Li et al., 1999; Boussiotis et al., 2000).To test this hypothesis, a randomized clinical trial was conducted jointly at the University of Maryland Greenebaum Cancer Center and the University of Pennsylvania Abramson Cancer Center in which 54 patients with myeloma received infusions of costimulated autologous T-cells after autotransplantation along with immunizations using the pneumococcal conjugate vaccine (PCV) (Rapoport, 2005). The following observations were made from this trial:

1. Infusions of 5-10 x 10^9 costimulated T-cells on day 12 post-transplant led to significantly higher CD4 and CD8 T-cell counts at day 42 post-transplant.
2. Post-transplant infusions of costimulated T-cells were well-tolerated with patients experiencing the following common toxicities: grade I/II chills and rigors (~50% of patients affected); grade I-III fevers (27% of patients affected); grade I/II nausea (15% of patients affected). Uncommon toxicities included hypertension (8%, grade I-III), hypotension (4%, grade I), pain/headache (8%, grade I), and hypoxia (6%, grades I-III).
3. The group of patients who were randomly assigned to receive3 PCV immunizations [including one pre-transplant and two post-transplant (at days 30 & 90)] along with an "early" infusion of vaccine-primed costimulated T-cells, exhibited robust and sustained antibody responses to the pneumococcal saccharide antigens and robust T-cell responses to the vaccine carrier protein (CRM-197).
4. The group of CMV seropositive patients who were randomly assigned to receive costimulated T-cells "early" (day 12) after transplant exhibited enhanced recovery of CMV-directed CD8 T-cell responses.

In addition, a smaller multicenter study of costimulated T-cells after autotransplantation for myeloma showed that infusions of 5-10 x 10^{10} T-cells on day 3 of transplantation were well-

tolerated without grade 3 or 4 infusional toxicities (Vij et al., 2003). When compared to historical controls, these infusions led to significantly higher lymphocyte and CD4 counts as early as day 10 post-transplant.

The prior study of costimulated T-cells and PCV immunizations provided a basis for designing a new study that utilized T-cells and a candidate myeloma vaccine to determine if anti-tumor immune responses can be generated post-transplant through this strategy. Unfortunately, no myeloma vaccines have been licensed nor proven to be clinically effective. Efforts to develop a myeloma vaccine have focused on i) myeloma cell preparations, ii) myeloma-specific antigens (e.g. myeloma/idiotype protein), and iii) myeloma-associated antigens. Candidate whole cell vaccines include mixtures of irradiated autologous myeloma cells and GM-CSF-secreting K562 bystander cells (Borrello et al.,2004.) as well as fusions of autologous myeloma cells and autologous dendritic cells (Vasir et al.,2005; Raje et al.,2004; Rosenblatt et al, 2010). Other novel strategies for augmenting the immunogenicity of myeloma cells may include enzymatic synthesis of α-gal epitopes to permit invivo opsonization by naturally occurring IgG anti-Gal antibodies followed by internalization by antigen-presenting cells (Galili, 2004). Vaccines consisting ofidiotype myeloma protein or idiotype-pulsed dendritic cells have generated anti-idiotype T-cell and B-cell responses in about 20% of vaccinated patients and perhaps rare clinical responses (Reichardt et al., 1999; Titzer et al., 2000).While investigation into clinically effective myeloma vaccines continues, current data supports the merit for alternative approaches for augmenting tumor specific immunity, such as gene-modified T-cell therapy to provide better cellular targeting against myeloma tumor targets.

2.3.2 Results of Clinical Trial GCC-0610/UPCC-13406:

Phase I/II combination immunotherapy after ASCT for advanced myeloma to study hTERT vaccination followed by adoptive transfer of vaccine-primed autologous T cells.

In this second joint University of Maryland and University of Pennsylvania clinical trial which was recently completed in early 2009, 56 patients with advanced myeloma received auto-transplants in conjunction with post-transplant transfers of vaccine-primed and ex-vivo costimulated autologous T cells. In contrast to the earlier trial, this new trial incorporated the following modifications: i) The target dose of T cells was 5-fold higher (~5 x 10^{10} cells); ii) T cell transfers occurred at day +2 post-transplant to take greater advantage of endogenous homeostatic lymphocyte expansion mechanisms (e.g. increased IL-15, IL-7 levels); iii) HLA A2+ patients received pre- and post-transplant immunizations using a multi-peptide tumor antigen vaccine composed of HLA-A2 – restricted peptides derived from hTERT and survivin and CMV.

hTERTis an attractive target for immunotherapy because it is widely expressed in myeloma cells and immunologic escape through mutation or down-regulation may be incompatible with long-term survival of neoplastic cells. In addition, telomerase expression and function may be necessary for survival of clonogenic myeloma stem cells which may ordinarily escape ablation

by high-dose therapy alone. Nonetheless, the curative effect of allogeneic stem cell transplants for myeloma indicates that these neoplastic "stem cells" are susceptible to immunotherapy.

The multipeptide tumor antigen vaccine was comprised of three hTERT peptides including I540 (ILAKFLHWL) (Vonderheide et al.,1999), R572Y (YLFFYRKSV) and D988Y (YLQVNSLQTV) (Scardion et al.,2002) the Sur1M2 (LMLGEGLKL) peptide derived from the anti-apoptotic protein survivin which is overexpressed in a broad range of malignancies including myeloma and which may confer a poor prognosis (Anderson et al.,2001) and a CMV control peptide (Mclaughlin-Taylor et al.,2001). The multipeptide tumor antigen vaccine was given to HLA-A2+ patients (ARM A) at 4 timepoints: i) ~ 10 days before steady-state T cell collection; ii) day +14 post-transplant; iii) day +42 post-transplant; and iv) day +90 post-transplant. All patients (HLA-A2 positive and negative) received the Prevnar® pneumococcal conjugate vaccine at these same timepoints.

Among the observations from this trial was that rapid and robust T cell recovery (CD4+ & CD8+) occurred by day +14 post-transplant and this pattern of recovery was significantly improved compared to the earlier trial (Rapoport et al.,2005)in which costimulated T cells were transferred at day +12 post-transplant. Day +2 serum IL-15 levels correlated significantly and positively to day +14 T cell counts. Notably, a proportion of patients (~16%) developed an early and clinically significant form of autologous GVHD (graft-vs-host-disease) which required a short course of systemic steroids in most cases. This clinical syndrome may be due to a significant shift in the balance of T effector cells (Teff) and T regulatory cells (Treg) leading to an increase in the Teff/Treg ratio – a mechanism which may be necessary for effective cancer immunotherapy. These observations formed the basis for a recent paper (Rapoport et al., 2009).

The combination of enhanced immune cell recovery and reduced self-tolerance following high-dose therapy and adoptive transfer of activated autologous T cells may also promote the development of clinically significant immune responses to cancer vaccines. Immunoassays which were performed showed that of 28 total ARM A (HLA A2 positive) patients analyzed to date, 10 patients (36%) had positive tetramer responses at one or more time-points after immunization, defined as tetramer staining by flow cytometry > 0.1% (and >3-fold increase vs. enrollment/baseline). Using a larger number of patients (54) the relatively high frequency and robust anti-pneumococcal antibody and T-cell responses that were reported previously with early transfers of vaccine-primed and costimulated autologous T cells (Rapoport et al.,2005) have been confirmed. These data have been recently reported (Rapoport et al, 2010).

The mean and median numbers of T cells infused were 4.26×10^{10} and 4.54×10^{10} respectively, (range, 1.59 – 5.0). The mean CD4/CD8 ratio was 2.48 (range, 0.62-11.09). The day +2 T cell transfers were well-tolerated with common adverse effects being chills/rigors, nausea/vomiting and low grade fevers (see Table 2.3.2 below with events separated by ARM assignment, A=A2+, B=A2-). All early infusion-related toxicities were grade I/II. Later toxicities which were possibly, probably, or definitely considered to be related to the T cell infusions are also tabulated (see Table 1 below). These toxicities, also mainly grade I/II,

included mild maculopapular rashes (typically involving the face/scalp/neck/upper chest, fevers, arthralgias, myalgias and conjunctivitis which was readily responsive to glucocorticoid eyedrops. One patient had incomplete platelet recovery (grade III) (~ 40-50,000/mcl at 6 months post-transplant) considered to be *possibly* related to the T-cell infusion. There were no documented cases of autoantibody formation (e.g. ANA, thyroid antibodies) or autoimmune endocrinopathies.

Table 2.3.2: T-Cell Related Toxicities (Arm A vs. Arm B)

Event (0 to 48 hrs)	I		II		III		Totals		P Value
	A	B	A	B	A	B	A	B	
Rigors/Chills	7	6	11	7	0	0	18(64)	13(50)	NS
Nausea/Vomiting *	1	7	0	0	0	0	1(4)	7(27)	0.02
Fever	3	4	0	0	0	0	3(11)	4(15)	NS
Headache/Pain	0	3	0	0	0	0	0	3(12)	NS
Hypertension	0	0	0	1	0	0	0	1(4)	NS
Hypoxia/Pulmonary	0	1	0	0	0	0	0	1(4)	NS
Rash	0	0	1	0	0	0	1(4)	0	NS
Diarrhea	1	1	0	0	0	0	1(4)	1(4)	NS
Rash	19	19	4	4	0	0	23(82)	23(88)	NS
Fever	6	4	1	0	1	0	8(29)	4(15)	NS
Gut GVHD	1	0	3	2	0	1	4(14)	3(12)	NS
Arthralgias	3	4	0	1	0	0	3(11)	5(19)	NS
Myalgias	5	0	0	0	0	0	5(18)	0	0.05
Headache/Pain	4	1	3	0	0	0	7(25)	1(4)	0.05
Anorexia/Nausea *	6	2	2	0	1	0	9(32)	2(8)	0.04
Conjunctivitis	0	0	0	3	0	0	0	3(12)	NS
Fatigue	6	0	1	1	0	0	7(25)	1(4)	0.05
Cytopenias	1	2	0	0	0	0	1(4)	2(8)	NS
Neuropathy	1	2	0	0	0	0	1(4)	2(8)	NS
Hepatic(↑AST/ALT)	1	1	0	1	0	0	1(4)	2(8)	NS
Eosinophilia	0	1	1	0	0	0	1(4)	1(4)	NS
Renal/Edema	1	0	0	1	0	0	1(4)	1(4)	NS
Diarrhea *	10	3	6	1	2	1	18(64)	5(19)	0.001
Mucositis	1	2	2	1	1	1	4(14)	4(15)	NS
Mental Status Changes	0	1	0	0	0	0	0	1(4)	NS

To determine whether the early lymphocyte recovery which followed day +2 adoptive T cell transfer had an effect on hematopoietic recovery, we compared the times to absolute neutrophil counts (ANC) $\geq$ 500/mcl for 2 consecutive days for the current study patients with an historical control population of 102 myeloma patients who had standard autografts without additional T cells Gojo et al., 2006. The median number of days to neutrophil recovery in the current trial was 12 days [range 10-18] versus 12 days in the historical cohort [P = 0.49].

Similarly, the median days to an unsupported platelet count ≥ 20,000/mcl was similar for the two populations: 14 days [range 0-28] in the current trial versus 14.5 days in the historical cohort [P = 0.78].

Seven patients developed a post-transplant T cell "engraftment syndrome" characterized by watery diarrhea (up to 2000 cc/day), abdominal pain, and fever (in six of the patients). In six patients this syndrome developed at a median of 14 days post-transplant (range 9-17 days) while in one patient it occurred around day 60 post-transplant. Two patients (including one who developed GI GVHD) developed early bright facial rashes at about day +9 post-transplant. The mean day 14 CD4 count for the 7 patients who developed early GVHD-like T cell engraftment syndromes was 2443 (range 863 – 4049) while the mean day 14 CD8 count for this subset of patients was 5822 (range 671 – 11571). There were no significant differences in the CD4 and CD8 counts for the subset of patients who developed early "GVHD" versus the group of patients who did not.

All 7 patients with apparent "GI-GVHD" had colonoscopic biopsies at a median of 17 days post-transplant (range, 15-81): In 6 patients the biopsies were interpreted to show histopathologic grade II-III GVHD of the gut. The biopsy for the patient with late onset symptoms was considered to be non-specific, although intraepithelial lymphocytosis and rare apoptotic bodies were observed. All 7 patients were initially treated with 1-2 mg/kg of methylprednisolone and/or oral budesonide followed by a rapid taper over about 1 month and exhibited rapid and complete clinical responses.

Four patients, including 3 patients who also had GI GVHD and a fourth patient with no GI symptoms, developed early skin rashes including generalized erythroderma or bright facial rashes at about day +9 post-transplant. A biopsy of one of these rashes showed typical grade II skin GVHD with apoptotic keratinocytes, basal vacuolization and CD3+ T cell infiltration of the dermis and epidermis while the skin biopsy of a second patient with facial rash alone showed a follicular and eccrine duct-centric infiltration of lymphocytes in the superficial dermis. These rashes resolved in about 1 week with (1 patient) or without (3 patients) systemic glucocorticoids.

2.4 Cancer testes antigens in multiple myeloma

Cancer-testis antigens (CT antigens) are proteins with restricted expression in testicular germ cells and placenta, and are not normally expressed in other tissues of the body except in various cancers (Caballero and Chen, 2009). Many of these genes are encoded as multigene families on the X chromosome, including the MAGE-A and NY-ESO-1 antigens, and are accordingly called the "CT-X" antigens. CT antigens were first described in malignant melanoma as targets for cytotoxic T cells and humoral immunity. Specific members of the CTAg family including MAGE-A1, MAGE-A3, MAGE-A4, CT-7 and NY-ESO-1 have been shown to be overexpressed in myeloma as well (Dhodapkar et al.,2003). CT antigens are attractive targets for tumor vaccines or redirected T cell gene therapy due to their tumor-specific expression patterns.

2.4.1 NY-ESO-1 and relevance to myeloma

NY-ESO-1 was originally identified as a human tumor antigen by a method called serological expression cloning of recombinant cDNA libraries from human tumors (SEREX) (Chen, et al., 1997). The function of NY-ESO is unknown. NY-ESO-1expression is detected in testis, ovary and weakly in uterus specimens. No mRNA can be detected by RT-PCR in any other normal tissue (Chen et al., 1997). In multiple myeloma, expression figures vary widely depending on the study, with up to 80% of patients positive for the antigen, but the majority of reports quote figures of <50%. Expression increases in relapsed patients, and reaches 100% in patients with advanced disease and abnormal cytogenetics (approximately a third of the total patient population). A summary of the literature covering NY-ESO expression in myeloma is shown in Table 2.4.2. Of note, NY-ESO-1 (also known as LAGE-2) is very similar in sequence to LAGE-1, and both antigens contain the epitope (SLLMWITQC) recognized by the high affinity TCR evaluated in this protocol. Therefore, both NY-ESO and LAGE-1 expression levels are shown in the table.

In addition to myeloma, solid tumors express NY-ESO at rates of up to 50%: bladder, melanoma, lung, ovarian, uterine and esophageal cancer (Chen et al, 1997). Expression increases with tumor stage. Reported expression rates vary between different studies; RT-PCR is more sensitive than IHC, and tends to give higher figures for NY-ESO expression. Figures derived from IHC are more reliable, since this technique detects protein rather than RNA. CTL recognizing the A2 presented epitope NY-ESO$_{157-165}$, SLLMWITQC, have been grown from the blood and lymph nodes of myeloma patients by several different groups (van Rhee et al., 2005; Atanackovic et al., 2007). LAGE-1, a highly homologous TAA with an identical expression pattern, also shares the same epitope. T cell clones specific for this epitope kill tumor cells (van Rhee et al., 2005).A high affinity TCR recognizing the NY-ESO$_{157-165}$epitope binds to A2, NY-ESO +ve melanoma cell lines and fresh myeloma samples (but not to cells that lack either A2 or NY-ESO).

Table 2.4.2: Incidence of NY-ESO-1 or LAGE-1 expression in patients with myeloma

Method	Disease Stage	No. Myeloma Patients Analyzed	% NY-ESO-1 expression	% LAGE-1 expression	Citation
RNA[1] and IHC	Diagnosis	161	41	ND	Van Rhee *et al*, 2005
RNA[1] and IHC	Relapse	55	74	ND	Van Rhee *et al*, 2005
RNA[2]	Advanced disease[3]	39	33	49	Andrade *et al*, 2008
RNA[2]	Stage III	27	33	51	Van Baren *et al*, 1999
RNA[2]	Stage I or II	11	0	0	Van Baren *et al*, 1999
RNA[2]	Advanced disease	55	7	ND	Atanackovic *et al*, 2007
IHC	Stage III	13	23	ND	Dhodapkar *et al*, 2003
RNA[2]	Not reported	20	0	ND	Lim *et al*, 1999

1-Gene expression profiling

Table 2.4.2: Incidence of NY-ESO-1 or LAGE-1 expression in patients with myeloma

Method	Disease Stage	No. Myeloma Patients Analyzed	% NY-ESO-1 expression	% LAGE-1 expression	Citation

2-RT-PCR

3-95% of patients were classified as "advanced"

ND-Not Done

2.5 Redirected T cells for cancer immunotherapy

It is now well established that human tumors have antigens, and that natural immune responses are associated with improved survival in a variety of cancers (Smyth, Dunn, and Schreiber, 2006). The central problem is tolerance: the repertoire of TCRs is generally of too low avidity to efficiently recognize tumor antigens, and this explains the failure of most cancer vaccines (Greenberg and Riddell, 1999; Rosenberg, Yang and Restifo, 2004; Pardoll and Allison, 2004). To address this issue we have worked with Dr. B. Jakobsen at Adaptimmune Ltd to develop high affinity TCRs. Results recently published in Nature Medicine present our exciting results using high affinity TCRs to improve the immune response to HIV20, and these TCRs are currently under investigation in a clinical trial at UPenn. Higher affinity recognition allows T cells to respond to lower levels of antigen; this is critically important for tumor immunotherapy where the tumor microenvironment has adapted itself to reduce expression of antigen and also decrease expression of MHC class I molecules (Marincola *et al*, 2000; Barrett and Blazar, 2009).

To date at least 18 studies have been opened for TCR redirected T cells to date (**Table 2.5**), and are at various stages of completion. Of these, two have been published (Morgan et al, 2006 and Johnson et al, 2009). These studies evaluated three different TCRs isolated from tumor infiltrating lymphocytes, and chosen based on medium or high affinity binding to HLA-peptide complexes (Johnson et al, 2006). In the first trial evaluating medium affinity MART-1 DMF4 TCRs (identifier number: NCT00091104), 17 subjects were reported (to date, 31 have been enrolled). Subjects received as high as 6×10^{10} antigen specific redirected T cells. No toxicity related to T cell infusions was reported.

In two other studies, (identifier numbers: NCT0050946 and NCT00509288) the higher affinity MART-1 DMF5 and gp100 (154) TCRs were evaluated in 20 and 16 subjects, respectively (Johnson et al, 2009). In the case of these trials, 29 of 36 subjects developed an erythematous skin rash that subsided within 48 hours and was treated with local application of steroids. The reaction was an "on target" reaction of the TCRs against low levels of MART-1 and gp100 antigen that was present on normal melanocytes. The response rate was correspondingly higher in this study than in the previous MART-1 DMF4 study (30% and 19% compared to 13%). There was a correlation between the response rate and persistence of functional TCR redirected T cells.

Of note, the NY-ESO-1 receptor that is proposed for use in Cohort 2 patients in this trial has already been used in the clinic (clinicaltrials.gov identifier NCT00670748). Results from this trial were recently published (Robbins et al, 2011). Eleven patients with melanoma and 6 patients with synovial sarcoma were enrolled. Patients were HLA-A2 and the tumors expressed NY-ESO-1 as assessed by RT-PCR, immunohistochemistry, or serum reactive antibody. No adverse events were reported. Objective responses were found in 5/11 melanoma patients, and objective partial responses were observed in 4/6 synovial

sarcoma patients. Ongoing complete responses >1.5 year were found in 2 patients and at 9 months in one patient with melanoma and a 1.5 year partial response was observed in one patient with synovial cell carcinoma.

Table 2.5: Clinical trials using TCR redirected T cells open to date

Target Antigen	TCR Type	Indication	Protocol Title	Study Chair, Center	ClinicalTrials.gov Identifier
CEA	anti-CEA TCR	metastatic cancer expressing CEA antigen	Phase II study of metastatic cancer that expressed carcinoembryonic antigen (CEA) using lymphocepleting conditioning followed by infusion of anti-CEA TCR gene engineered lymphocytes	Steven A. Rosenberg, NCI - Surgery Branch	NCT00809978
CEA	anti-CEA TCR	metastatic cancer expressing CEA antigen	Phase II Study of Metastatic Cancer That Expresses Carcinoembryonic Antigen (CEA) Using Lymphodepleting Conditioning Followed by Infusion of Anti-CEA TCR-Gene Engineered Lymphocytes	Steven A. Rosenberg, NCI - Surgery Branch	NCT00923806
CEA	anti-CEA TCR	adenocarcinoma	Phase I Study of T Cells Modified With Chimeric Anti-CEA Immunoglobulin-T Cell Receptors (IgTCR) in Adenocarcinoma	Richard P. Junghans, BethIsraelDeaconessMedicalCenter	NCT00004178
gp100	TCR gene-engineered TILs	melanoma	Treatment of Patients With Metastatic Melanoma by Lymphodepleting Conditioning Followed by Infusion of TCR-Gene Engineered Lymphocytes and Subsequent Fowlpox gp100 Vaccination	Steven A. Rosenberg, NCI - Surgery Branch	NCT00085462

Table 2.5: Clinical trials using TCR redirected T cells open to date					
Target Antigen	**TCR Type**	**Indication**	**Protocol Title**	**Study Chair, Center**	**ClinicalTrials.gov Identifier**
gp100	anti-gp100:154-162 TCR	melanoma	Phase II Study of Metastatic Melanoma Using Lymphodepleting Conditioning Followed by Infusion of Anti-gp 100:154-162 TCR-Gene Engineered Lymphocytes	Steven A. Rosenberg, NCI - Surgery Branch	NCT00509496
gp100	anti-gp100:154-162 TCR, ALVAC virus	melanoma	Phase II Study of Metastatic Melanoma Using Lymphodepleting Conditioning Followed by Infusion of Anti-gp 100: 154-162 TCR-Gene Engineered Lymphocytes and ALVAC Virus Immunization	Steven A. Rosenberg, NCI - Surgery Branch	NCT00610311
gp100 MART-1	Anti-gp 100:154 TCR Anti-MART-1 F5 TCR	melanoma	Phase II Study of Metastatic Melanoma Using a Chemoradiation Lymphodepleting Conditioning Regimen Followed by Infusion of Anti-Mart-1 and Anti-gp100 TCR-Gene Engineered Lymphocytes and Peptide Vaccines	Steven A. Rosenberg, NCI - Surgery Branch	NCT00923195
MART-1	anti-MART-1 F5 TCR, ALVAC virus	melanoma	Phase II Study of Metastatic Melanoma Using Lymphodepleting Conditioning Followed by Infusion of Anti-MART-1 F5 TCR-Gene Engineered Lymphocytes and ALVAC Virus Immunization	Steven A. Rosenberg, NCI - Surgery Branch	NCT00612222

Table 2.5: Clinical trials using TCR redirected T cells open to date					
Target Antigen	**TCR Type**	**Indication**	**Protocol Title**	**Study Chair, Center**	**ClinicalTrials.gov Identifier**
MART-1	TCR anti-MART-1 F5	High-risk melanoma	Transfer of Autologous T Cells Transduced With the Anti-MART-1 F5 T Cell Receptor in High Risk Melanoma	Steven A. Rosenberg, NCI - Surgery Branch	NCT00706992
MART-1	CTL line expressing TCR ant-MART-1	melanoma	Open Label, Non-Randomized Phase I Dose Escalation Study of Adoptive Transfer of an anti-MART-1 TCR Expressing CTL Line Administered by Intra-Tumoral Injection in Patients with Melanoma	Hans von der Maase, Aarhus University Hospital	
MART-1 (DMF4)	anti- MART-1 TCR-engineered tumor-infiltrating lymphocytes or PBLs	melanoma	A Study in Metastatic Melanoma Using a Lymphodepleting Conditioning Followed by Infusion of Anti-MART-1 TCR-Gene Engineered Lymphocytes and Subsequent Peptide Immunization	Steven A. Rosenberg, NCI - Surgery Branch	NCT00091104
MART-1 (DMF5)	anti-MART-1 F5 TCR	melanoma	Phase II Study of Metastatic Melanoma Using Lymphodepleting Conditioning Followed by Infusion of Anti-MART-1 F5 TCR-Gene Engineered Lymphocytes	Steven A. Rosenberg, NCI - Surgery Branch	NCT00509288
MART-1 (DMF5)	anti-MART-1 F5 TCR	melanoma	Adoptive Transfer of MART-1 F5 TCR Engineered Peripheral Blood Mononuclear Cells (PBMC) After a Nonmyeloablative Conditioning Regimen, With Administration of MART-126•35-	Antoni Ribas, Bartosz Chmielowski, James S. Economou, John A Glaspy,UCLA	NCT00910650

Table 2.5: Clinical trials using TCR redirected T cells open to date					
Target Antigen	**TCR Type**	**Indication**	**Protocol Title**	**Study Chair, Center**	**ClinicalTrials.gov Identifier**
			Pulsed Dendritic Cells and Interleukin-2, in Patients With Advanced Melanoma		
MART-1 and gp100	anti-MART-1 and anti-gp100 TCR	melanoma	Phase II study of metastatic melanoma using a chemoradiation lymphodepleting conditioning regimen followed by infusion of anti-MART-1 and anti-gp100 TCR gene engineered lymphocytes and peptide vaccines	Steven A. Rosenberg, NCI - Surgery Branch	NCT00814684
NY ESO-1	anti-NY ESO-1 TCR	kidney cancer, melanoma, metastatic cancer expressing NY ESO-1	Phase II study of metastatic cancer that expresses NY ESO-1 using lymphodepleting conditioning followed by infusion of anti-NY ESO-1 TCR gene engineered lymphocytes	Steven A. Rosenberg, NCI - Surgery Branch	NCT00670748
NY-ESO-1	anti-NY ESO-1 TCR	Melanoma	Phase I/II study in melanoma expressing NY-ESO-1 antigen using lymphodepleting conditioning followed by infusion of anti-NY-ESO-1 gene engineered CD8 T cells, and administration of ipilumimab to evaluate its effects on persistence and activity of the gene modified cells	AudeChapuis-FredHutchinsonCancerResearchCenter	NCT00871481

Table 2.5: Clinical trials using TCR redirected T cells open to date					
Target Antigen	**TCR Type**	**Indication**	**Protocol Title**	**Study Chair, Center**	**ClinicalTrials.gov Identifier**
p53	anti-p53 TCR	metastatic cancer with p53 overexpression	Phase II Study of Metastatic Cancer That Overexpresses P53 Using Lymphodepleting Conditioning Followed by Infusion of Anti-P53 TCR-Gene Engineered Lymphocytes	Steven A. Rosenberg, NCI - Surgery Branch	NCT00393029
p53	anti-p53 TCR	kidney cancer, melanoma, metastatic cancer expressing p53	Phase II Study of Metastatic Cancer That Overexpresses p53 Using Lymphodepleting Conditioning Followed by Infusion of Anti-P53 TCR-Gene Engineered Lymphocytes and Dendritic Cell Vaccination	Steven A. Rosenberg, NCI - Surgery Branch	NCT00704938
TRAIL Bound to the DR4 Receptor	Anti-TRAIL TCR	Metastatic Renal Cancer	Phase 1/2 Study of Metastatic Renal Cancer Using T-Cells Transduced With a T-Cell Receptor Which Recognizes TRAIL Bound to the DR4 Receptor	James C. Yang NCI - Surgery Branch	NCT00923390

2.6 Generation and preclinical testing of high affinity TCRs targeting NY-ESO-1 antigen

2.6.1 Design of Study Drugs: NYESO-1 TCR

Isolation and validation of NYESO-1 specific TCR

TCR gene cDNA sequences were isolated from the NY-ESO *HLA-A*0201-SLLMWITQC* restricted T cell clone 1G4 (Jager E. et al., 1998). NOTE: the SLLMWITQC peptide sequence is the identical sequence as is expressed by the LAGE antigen, and therefore LAGE antigen positive tumors are also targeted by the 1G4 T cell clone. The cDNA coding sequences for the mature extracellular regions of the α and β chain TCR proteins were cloned into separate *E.coli* plasmid vectors and expressed as protein inclusion bodies. These inclusion bodies were purified and solubilized in protein denaturant then refolded as soluble α/β heterodimeric TCR protein (sTCR) by dialysis. Both TCR chains were genetically truncated at the C terminus immediately

before the native intra-chain cysteine residues. The refolded TCR chains were joined together by means of an artificial disulphide bond engineered between the α and β chain TCR constant regions. The 1G4 sTCR protein was purified by ion exchange and size exclusion chromatography. Its HLA peptide antigen binding kinetics were analyzed by Surface Plasmon Resonance (SPR) using a BIAcore 3000. The 1G4 gene sequences were then chosen for antigen binding affinity enhancement by selection/enrichment from gene libraries of bacteriophage displaying large numbers of mutated 1G4 TCR proteins.

Production of HLA-A*0201-SLLMWITQC antigen complex

The *HLA-A*0201-SLLMWITQC* peptide antigen complex was required for validation of the 1G4 T cell clone and sTCR validation, as well as for phage display based affinity enhancement. This complex was made in-house by cloning the heavy chain and β2m into *E.coli* expression vectors. These proteins were then expressed separately as inclusion bodies prior to solubilisation, mixing with the SLLMWITQC peptide and refolding by dialysis. Refolded antigen complex was then purified by ion exchange and size exclusion chromatography. Where required this complex was further modified by C-terminal enzymatic biotinylation.

NY-ESO sTCR affinity enhancement

A 1G4 sTCR phage display library was constructed with mutations covering the hypervariable complementarity determining region (CDR3 region) of the β chain Three rounds of selection/ enrichment for high affinity TCR clones were performed using streptavidin magnetic beads coated with biotinylated *HLA-A*0201-SLLMWITQC* complex. Competition ELISAs screens for high affinity mutant TCR phage identified several TCR β-chain CDR3 mutations. These high affinity β chain mutants then formed the basis of a library where the CDR3 α chain was also mutated. This complex library was then used to isolate still higher affinities. Further mutations were later introduced into the CDR2 regions of both chains and these libraries then re-selected (Li Y. et al., 2005). Further CDR2-only high affinity 1G4 TCRs were identified by phage display (Dunn S. et al., 2006)

Biochemical validation of the affinity enhanced NYESO-1 sTCR clones

High affinity mutant TCR chain genes were cloned separately into *E.coli* expression vectors. These mutant TCR chains were expressed and refolded in various paired combinations including with wild type chains. They were then purified and analyzed for binding to *HLA-A*0201-SLLMWITQC* antigen by SPR. Selected data showing the binding kinetics for several mutant TCRs is presented in Table 2.6.2(1) (see also Zhao et al., 2007).

Table 2.6.2(1): **Binding kinetics of the individual high affinity NYESO-1 sTCRs.**

Clone	Ka (1/Ms)	Kd (1/s)	$T_{1/2}$	KD
aWT/bWT	–	–	2.2s	9.30μM
a259/bWT	4.87e4	0.036	19s	730nM
a12/b2	9.00e3	4.00e-3	4 min	450 nM
aWT/b51	5.40e4	0.0014	8.3 min	25 nM
a10/b1	1.65e4	1.38e-3	12 min	84 nM
a5/b100	3.90e5	1.97e-4	98 min	5 nM
a58/b61	5.70e5	2.72e-5	>425 min	26 pM

Optimization of mutant NYESO-1 mTCR sequences to obtain affinities useful for adoptive T cell therapy.
Studies with T cells transfected with high affinity TCRs indicated that a range of TCR with intermediate affinity should be evaluated (Zhao et al., 2007). To do this, the high affinity mutant CDR3α chain TCR sequences of Li et al., (ibid) and mutant CDR2β chain TCR sequences of Dunn et al.,(ibid) were partially back-mutated to the wild-type 1G4 TCR sequence. A panel of these phage-derived 1G4 TCR mutants was then assessed in TCR-transfected T cells. Key mutant sTCR chains were then expressed and refolded in combination with wild type chains. They were then purified and analyzed for binding to *HLA-A*0201-SLLMWITQC* antigen by SPR (Robbins et al., 2008). From these data TCRs anticipated to have good cellular properties were selected for comparison in lentiviral T cell transduction studies (Table 2.6.2(2)).

Table 2.6.2(2): **Binding kinetics of 1G4 sTCRs**

Clone	$T_{1/2}$	KD
aWT/bWT ('wt')	2.2 sec	9.30μM
a259/bWT ('c259')	**19.0 sec**	**730nM**
a12/b2('c12c2')	**4 min**	**450 nM**

The clones wt, c259 and c12/c2 were then introduced into the transfer plasmid pELNS for lentivirus-based expression in human T cells for further testing.

2.6.2 In vitro studies showing the specificity and function of NYESO-1^{c259} TCRs

2.6.2.1 NYESO-1^{c259} TCR

Measurement of cytokine secretion by ELISpot. IFNγ secretion or granzyme B release by activated T cells at the single cell level in response to HLA/antigen was detected by ELISpot assay (BD Biosciences), according to the manufacturers' instructions. Briefly, TCR-transduced T cells (5,000 per well) were incubated with target cells (50,000 per well) in 96-well capture anti-cytokine antibody pre-coated ELISpot plates. The ELISpot plate was then incubated overnight at 37ºC/5% CO_2, followed by washing and incubation for 2 hours with biotinylated anti-cytokine detection antibody and 1 hour with streptavidin-substrate and subsequent development. As controls and for specificity testing, redirected T cells were also stimulated with normal cell lines that lack expression of NYESO-1 antigen. **Figure 2.6.2.1** demonstrates that IFNγ response was consistently higher for the high affinity c259 TCR transduced T cells than for the wt TCR transduced T cells. The c259- and c12c2-transduced T cells secrete IFNy in response to NYESO-1 positive tumor lines, and also with only a small response against the melanocytes cell line N9. A greater response to the primary melanocytes was observed with c12c2 than with c259 suggesting off-target activity.

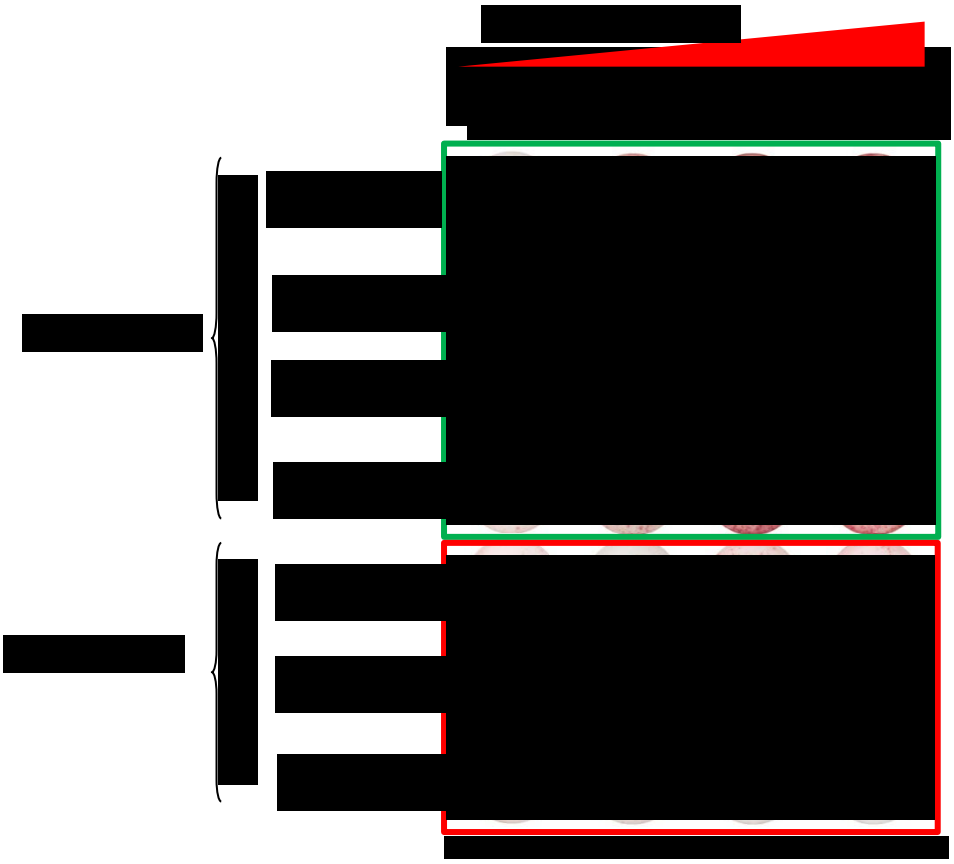


Figure 2.6.2.1: IFNγ ELISpot: IFNγ-release by TCR-transduced T cells in response to incubation with HLA-A2+ target cells. Mixed CD8 and CD4 T cells (mixed 1:1 at stimulation) were either non-transduced (ntd) or transduced with NY-ESO wt-, c259, or c12c2-TCRs and expanded until d10-13 prior to cryopreservation. IFNg ELISpot was carried out asdescribed previously. Data is representative of 3 independent experiments involving cells from 3 different T cell donors. The origin of these cell lines is shown in Tables 8.2.2 (1 and 2).

NY-ESO c259-transduced T cells exhibit enhanced functional responses to tumor cell lines but not normal cells. A summary of functional testing of NYESO1 TCR redirected T cells as determined by various functional assays mentioned above together with molecular analysis of NY-ESO expression by RT-PCR in target tumor and normal cell lines is presented in **Tables 2.6.2.1(1)** and **Table 2.6.2.2(2)**.

Table2.6.2.2(1) underscores the superiority of c12c2- and c259-transduced T cells showing greater levels of response than WT transduced T cells in collective assays**.** These representations of response levels correspond to the general ELISpot counts as tabulated as a reference in the earlier **Table 2.6.2.1(2).** Interestingly, c12c2 occasionally showed a reduced level of response to some target tumor cell lines compared to c259-transduced T cells. Reactivity of these redirected T cell against normal cell lines (**Table2.6.2.2(2)**)shows that WT and c259-transduced T cells do not respond to a variety of normal cells, with only minor, if

any, responses seen to some primary melanocytes lots (N2 and N9). However, c12c2-transduced cells showed a large response to renal epithelial cells suggesting than higher affinity TCR may have off-target effects.

Table 2.6.2.2(1): Tumor cell lines analyzed in NY-ESO-1 efficacy studies

Cell line *	Origin	NY-ESO-1 expression	Reactivity to **wt**-TCR modified T cells**	Reactivity to **c259**-TCR modified T cells**	Reactivity to **c12c2**-TCR modified T cells**
IM-9	B cell (EBV)	+++	++	++++	+++
Mel624	Melanoma	+++	++	++++	+++
Mel526	Melanoma	+	+/-	+	+
SKMel37	Melanoma	+/-	+	+++	++
A375	Melanoma	+	+	++++	++
U266	Myeloma	++	++	+++	ND
OPM-2 (A2-)	Myeloma	+	-	-	ND
J82	Bladder carcinoma	-	-	-	-

* HLA-A2+ unless stated
** data generated by different assays (ND indicates sample not tested). Data represents 3 independent experiments (except n=1 for A375, U266, OPM-2 and J82)

Table 2.6.2.2(2): Normal cells (HLA-A2+) analyzed in NY-ESO-1 efficacy studies

Cell line	Origin	NY-ESO-1 expression	Reactivity to wt-TCR modified T cells**	Reactivity to c259-TCR modified T cells**	Reactivity to c12c2-TCR modified T cells**
HEP2	Hepatocyte	ND	-	-	-
HA2	Astrocyte	ND	-	-	-
HA10	Astrocyte	ND	-	-	-
N2	Melanocyte	ND	--/+	--/+	-/+
N9	Melanocyte	ND	-	--/+	-/+
N10	Melanocyte	-	-	-	-
REN2	Renal epithelial	-	-	-	+++
SMC3	Smooth muscle	-/+	-	-	-
PF5	Pulmonary fibroblast	ND	-	-	-
HBSMC2	Bronchial smooth muscle	ND	-	-	-
HPEpC1	Prostate epithelial	ND	-	-	-
CM5	Cardiac myocyte	ND	-	-	-
SkMC3	Skeletal muscle	ND	-	-	-

** data generated by different assays (ND indicates sample not tested). Data represents at least 3 independent experiments for HEP2, HA2 and N9 (n=1 for others)

2.6.3 In vivo studies showing the antigen specific efficacy of the NYESO-1^{c259} TCRs in tumor bearing mice

2.6.3.1 NALM-6 Leukemia Tumor Model

The effectiveness of NYESO-1 TCR transduced T cells was tested in the immunodeficient NSG (NOD/*scid*/γ_c^{null}) mouse model using the human B-cell precursor acute lymphoblastic leukemia cell line (NALM-6). The immunodeficient NOD/*scid*/γ_c^{null}(NOG) mouse is an excellent xenotransplantation model to measure the *in vivo* repopulation of human CD4 T cells (Ito et al,. 2009). Following engraftment, the human hematopoietic cells can be maintained in NSG mice for at least 2 months or until fatal xenogeneic graft-versus-host-disease (xGVHD). Injection (i.v.) of NALM-6 into NSG mice provides a systemic tumor model with rapid evolution toward animal death within 20-23 days. Parental NALM-6 cells express both HLA-A1 and HLA-A2 molecules and also low levels of MAGE A3 antigen, but no NYESO-1 antigen. For a higher expression of this antigen, we transduced NALM-6 cells with lentiviruses expressing NYESO-1 proteins in conjunction with the GFP protein (NALM6-GFP-MAGE A3 and NALM6-GFP-NYESO1). As a control cell line, we transduced NALM-6 cells with GFP only (NALM6-GFP). In a previous experiment we tested these three lines (NALM6-GFP-MAGE A3, NALM6-GFP-NYESO1 and NALM6-GFP) in the NSG mouse model and found that similarly to the parental NALM-6 cells, they induce mouse death within 23 days.

Our efficacy study used parental and transduced NALM-6 cell lines and evaluated the impact of CD4 and CD8 T cells, lentivirally transfected to express NYESO-1 TCR on animal survival. The cohorts are detailed in the **Table 2.6.3.1.** NSG mice were engrafted with 2 x 10^6 NALM-6 tumor alone, or expressing NYESO-1 antigens, via tail vein injection on day 0. On day 6, T cells were normalized for equivalent transduction percentages using untransduced cells and 5 x 10^6 transduced/control T cells were injected into animals via tail vein injection. Animals were followed weekly. The primary efficacy endpoint of the study was survival.

The infused study drug cell number is 5 x 10^6CD4 and CD8 T cells. This dose was chosen based on pilot data in the NALM-6 model which indicates that this is the effective dose required to observe an antitumor effect. A human is on average 3000-fold larger than an average mouse. Our maximum cell infusion dose in humans is anticipated to be 1 billion cells. 5 x 10^6cells in a mouse corresponds to a human dose of >10 billion cells (5,000,000 x 3000). Therefore, the dose evaluated in this experiment represents about 10 times greater than the maximum human dose that will be administered in Phase I trials.

Table 2.6.3.1: **Cohort summary for efficacy study in NSG-NALM6 mouse model**

Cohort	# mice	Tumor	T cells	Question
1	8	NALM-6/GFP	None	Control
2	8	NALM-6/GFP	Non-transduced	Control
3	8	NALM-6/GFP	NYESO-1 c259/wt	Negative control
4	8	NALM-6/NY-ESO-1	None	Control tumor growth
5	8	NALM-6/NY-ESO-1	Non-transduced	Control T cell effect on tumor
6	8	NALM-6/NY-ESO-1	MAGE-A3 WT	Control WT-TcR effect on tumor
7	8	NALM-6/NY-ESO-1	MAGE-A3 a3a	TcR effect on tumor
8	8	NALM-6/NY-ESO-1	NYESO-1 wt	Control WT-TcR effect on tumor
9	8	NALM-6/NY-ESO-1	NYESO-1 c259/wt	TcR effect on tumor
10	8	NALM-6/MAGE A3	Non-transduced	Control
11	8	NALM-6/MAGE A3	MAGE-A3 WT	Control WT-TcR effect on tumor
12	8	NALM-6/ MAGE A3	MAGE-A3 a3a	TcR effect on tumor

As expected, all the control mice (injected with saline and mock/untransduced T cells) died between day 19 and 23. Also, the high affinity NYESO1 TCR (c259) transduced T cells did not give the mice any survival advantage when mice were carrying the NALM6 tumor cells. However when mice given the NALM6/NYESO1 tumor were treated with NYESO1 TCR transduced T cells, a significant survival advantage was seen regardless of the TCR affinity (wt or c259) (**Figure 2.6.3.1(1)**). This data suggests that both NYESO-1 TCRs are effective against tumor cells expressing the antigen. A limitation of this system is that we did not have a control group testing the efficacy of the TCR transduced T cells against targets expressing low antigen levels.

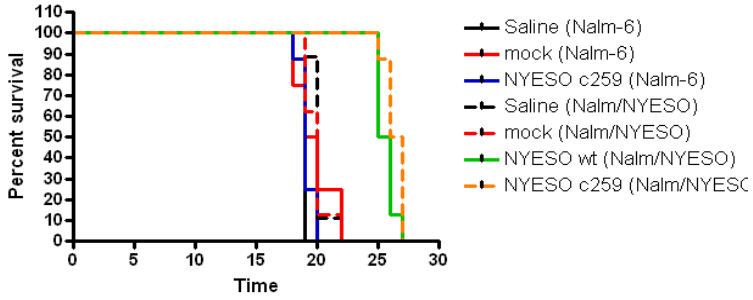


Figure 2.6.3.1(1). Efficacy of NYESO-1 TCR transduced T cells against parental and modified NALM-6 tumor line. NSG mice were injected with 2 x 10^6 NALM-6 tumor alone, or expressing NYESO-1 antigen, via tail vein injection on day 0. On day 6, 5 x 10^6 transduced/control T cells were injected (iv) into animals. Mice were followed weekly until death occurred.

2.7 Evaluation of pneumococcal conjugate vaccine (PCV, Prevnar-13®) responses

As a bridge to the prior studies involving post-transplant adoptive T-cell transfers and to assess whether the gene-modified T-cells would still be able to promote antibody and cellular responses to the Prevnar-13 vaccine, patients will receive pre and post-transplant immunizations using the same regimen that we have used previously. This intervention also offers the potential for clinical benefit since we have previously shown that pre-and post-transplant PCV immunizations plus early post-transplant transfer of vaccine-primed T-cells can lead to protective antibody responses against multiple pneumococcal serotypes.

2.8 Revlimid® (Lenalidomide) as Maintenance

Lenalidomide is an immunomodulatory agent which is derived from thalidomide. Lenalidomide with or without dexamethasone has potent activity against myeloma which has relapsed after prior therapy (Weber et. al, 2007; Richardson et. al., 2009). Recently, lenalidomide has also been used as a highly effective frontline treatment and in the maintenance setting. Unpublished data in a phase III multicenter, randomized, double-blind study conducted by the CALGB (#100104), has shown that Lenalidomide decreased the risk of disease progression by 58% vs placebo when Lenalidomide was given as maintenance after autotransplantation. Thus we plan to use lenalidomide maintenance at a dose of 10 mg per day, starting at day +100 post-transplant as a standard of care.

3 STUDY DESIGN (See Protocol Flow Diagram in Appendix B)

3.1 Overall Design

This study will be a Phase I/II study of auto-transplantation for patients with myeloma followed by infusion of autologous T cells which have been genetically modified to target tumor antigen. Approximately twenty six (26) subjects will be enrolled from two institutions, the University of Maryland and the University of Pennsylvania. Before enrollment, patients will be tested for HLA A status and for tumor expression of NY-ESO-1. Patients who are HLA-A2+ (and express NY-ESO-1 in the myeloma cells) will be eligible for enrollment. The outline of the protocol is as follows. First patients will receive an immunization using the pneumococcal conjugate vaccine (Prevnar-13 ®). About 7-14 days later, patients will initially undergo a steady-state mononuclear cell apheresis for T cell collection, with an optional second collection. Once mononuclear cells have been collected, patients will then undergo hematopoietic stem cell mobilization using cyclophosphamide and G-CSF (unless they have stem cells stored from a previous collection). For transplant, all patients will receive high-dose melphalan followed by hematopoietic stem cells on day 0. On day +2, patients with receive up to 1 x 10^{10}anti-CD3/anti-CD28-costimulated autologous T cells which have been genetically modified to express high affinity NYESO-1 TCRs.

Patients will be followed closely until disease progression or 1 year post T-cell infusion, whichever is sooner, for safety evaluations and correlative research, blood and marrow sampling, and then at least about every 6 months up to year 5 and every 12 months from year 5 up to year 15 post transfusion for delayed gene therapy adverse events in accordance with FDA Guidelines. The 2nd subject will not be infused until the 1st subject has completed the Day +21 visit.

Patients will undergo myeloma restaging at day 42, day 100, 6, 9 and 12 months post infusion, then per local standard of care, until disease progression. Shortly after the 3 month staging (day 100 post infusion), patients will start lenalidomide maintenance therapy.

After their disease progression, in accordance with FDA Guidelines, all patients will enter long term follow up (LTFU) and will continue to be monitored biannually for delayed gene therapy adverse events and collection of samples for persistence, and annually for collection of samples for RCL. From year 5 post-infusion, all patients will require annual visits for monitoring of delayed gene therapy adverse events, and assessment of persistence and RCL until 15 years after receiving the last infusion of genetically modified T cells. Patients whose disease progresses prior to year 1 will enter LTFU at time of progression; however these patients will be seen quarterly from progression until year 1 post infusion and then follow the LTFU schedule above mentioned.

Once the Long-Term-Follow-up protocol ADP-0000-002 will be available at site, subjects in LTFU will be considered having completed study ADP-01411 and be consented to the LTFU protocol ADP-0000-002.

3.1.1 Primary Objectives

1. To evaluate the safety and tolerability of autologous genetically modified T cells transduced to express the high affinity NY-ESO-1 TCR in HLA-A2 subjects.
2. To measure the incidence of GVHD in patients following infusion of TCR modified autologous T cells.

3.1.2 Secondary Objectives

1. To demonstrate product bioactivity, and establish proof of concept and mechanism for the function of gene modified cells in vivo by evaluating:
   Selective migration and engraftment of gene-modified infused cells to the marrow.
   a. Ex-vivo immune functionality and phenotype of infused cells in marrow and periphery
   b. Modulation of cytokine milieu in marrow and periphery
   c. Development of an expanded patient immune response against tumor via epitope spreading
   d. Expression levels of NY-ESO-1 in marrow samples obtained pre- and post-treatment from patients in each of the respective cohorts
2. To evaluate the effect of late (day+100) lenalidomide treatment on secondary objectives1a-e above.
3. To evaluate post-transplant cellular and antibody pneumococcal conjugate vaccine (PCV) responses following adoptive transfer of up to 1 x 10^{10}PCV-vaccine-primed and TCR-gene-transduced autologous Tcells.
4. To evaluate the clinical response in the NYESO cohort as specified in Rajkumar et al. on behalf of IMWG in 2011 [Rajkumar, 2011] by measuring:
   a. objective response rate (ORR) at day 42, 100, 180, 270 and 360.
   b. best objective response (BOR) prior to initiating lenalidomide and at day 360.
   c. duration of response (DOR) and progression-free survival (PFS).
   d. overall survival (OS) will be followed in this interventional protocol and continue to be followed in the long-term follow-up protocol, once subjects have transferred.

   Initiation of lenalidomide as maintenance treatment will be addressed in a sensitivity analysis.

3.2 Study Duration

Based on previous experience, it is anticipated that approximately 1 patient per month will be enrolled on the study. Therefore, it will take approximately twenty six (26) months to recruit all 26 evaluable patients into the study. There are frequent visits for the study during the first 6 months of participation, but then the visit frequency lengthens out to about every 3 months until year 1, unless a problem develops which requires closer monitoring. Patients will remain on study as long as they show no progression or relapse of myeloma or develop a complication which mandates that

they discontinue study treatment. The criteria for progression of myeloma will be as defined under Section 7.1. Patients whose disease has progressed/relapsed will enter the Long-Term Follow-up Phase of the study (LTFU). Patients will also be followed for survival until death from any cause or loss to follow up. Once the Long-Term Follow-up protocol ADP-0000-002 will be available at site, patients in LTFU will be considered having completed study ADP-01411 and be consented to the LTFU protocol ADP-0000-002.
Patients who remain disease-free at the time of study completion (timing at Sponsor's discretion) will also be consented to the LTFU protocol ADP-0000-002.

3.3 Study Evaluations

Patients will be monitored for myeloma activity throughout the study.

Absolute counts for leukocyte and lymphocyte subset at each sample draw will be determined as part of standard clinical care.

Bulk subset analysis (CD3/CD8+, CD3/CD4+, CD3+/CD4+/CD25+/CD127-, CD3-/CD16+, CD3-/CD19+, CD3-/CD14+ will be determined fresh on each sample using lyse-no-wash protocols.

Persistence and homing of infused cells will be evaluated using quantitative PCR (Q-PCR) on marrow and peripheral blood samples obtained pre-transplant and at multiple time-points post-infusion using transgene-specific primers. If persistence levels allow, persistence will be corroborated by flow-based analysis using Vb specific antibodies that correspond to the infused-cell clonotypes

Ex-vivo functionality of infused and persisting cells (a) and development of an expanded immune response against tumor (b) will be assessed on marrow samples and peripheral blood samples obtained pre-and post-T cell infusion and post lenalidomide using flow-cytometry- based immune effector assays, specifically degranulation, proliferation, and intracellular cytokine secretion of gene-modified (a) and bulk (b) cells in response to autologous tumor.

A decrease in target antigen expression in the marrow as a consequence of treatment (through immune-mediated tumor destruction or antigen-loss variant selection) will be evaluated on marrow samples obtained pre-and post-T cell infusion and post lenalidomide using Q-RT-PCR and primer/probe sets specific for NY-ESO-1.

The effect of lenalidomide administration on infused cell persistence and functionality will be assessed in marrow and peripheral blood samples collected just prior to and post initiation of lenalidomide administration as described above.

4 SUBJECT SELECTION AND WITHDRAWAL

4.1 Target Population

Study patients will have systemic or multifocal myeloma requiring autologous stem cell transplantation. All of these patients will have received initial treatment previously. Upon enrollment, patients will undergo marrow aspirations for staging purposes and to obtain myeloma cells for future immunoassays. In addition, newly diagnosed patients can have myeloma cells collected and stored under a separate IRB-approved specimen collection protocol. If during this initial assessment process, red top and lavender top tubes are collected as indicated in the schedule of study procedures, then additional marrow will not be required for collection during the screening phase of this protocol. These patients would then be treated with myeloma cytoreductive therapy as recommended in the STUDY PROCEDURES section and enrolled after completion of pre-transplant therapy.

4.2 Subject Compliance Monitoring

The study team will be able to completely monitor compliance with, vaccinations, T- cell apheresis, stem cell collection process, high-dose melphalan, and re-infusions of both stem cells and T-cells. Any steps in this sequence that are missed must be recorded as protocol violations. Written informed consent must be obtained from all patients before entry into the study.

4.3 Inclusion Criteria

Each subject must meet ALL of the following criteria during screening to be enrolled in the study. No exceptions to eligibility will be granted:

4.3.1 Written informed consent must be obtained from all patients before entry into the study

4.3.2 Patients must have a diagnosis of myeloma (see Appendix A for diagnostic criteria).

4.3.3 Patients must meet one of the following criteria:

- Myeloma has relapsed, progressed, or failed to respond after at least one prior course of therapy (consisting of at least 2 treatment cycles or months of therapy). Failure to respond would correspond to a reduction of less than or equal to 25% of the original, diagnostic serum or urine paraprotein measurement.
- Myeloma has responded partially to initial therapy but a complete response (immunofixation negative and normal serum free light chain studies) has NOT developed after a minimum of 3 cycles or months of initial therapy.
- Myeloma has high-risk features as defined by the presence of one or more cytogenetic abnormalities known to confer a poor outcome even after standard auto-transplants: complex karyotype (> or = to 3 abnormalities), t(4;14), t(14;16),

del (17) (p13.1), and/or chromosome 13 abnormalities. These patients may be enrolled even while in complete or near-complete remission. Extended disease-free survival after auto-transplantation would be unexpected for these patients and therefore especially meaningful.

4.3.4 Patients must have measurable disease on study entry. Measurable disease may include quantifiable or detectable levels of serum or urine paraprotein. For patients with minimally secretory disease or non-secretory myeloma on study entry, serum free λ or κ light chain levels or the serum free light chain ratio may be measured and used for disease monitoring if abnormal.

Patients who are in complete remission at the time of proposed study entry (serum and urine immunofixation consistently negative and serum free light chains normal) are not eligible unless their disease meets the criteria for high-risk as defined in section 4.3.4.

4.3.5 Patients must be between ages 18-80 (inclusive).

4.3.7 Patients should have adequate vital organ function as defined below:

- Serum creatinine ≤3.0 mg/dl and not on dialysis
- WBC at least 3000/mm^3, platelet count at least 100,000/mm^3 (See protocol section 5.9 for modified eligibility requirements pertaining to a second T-cell infusion)
- SGOT< or = to 2 x upper limit of normal and bilirubin < or = to 2.0 mg/dl (unless due to Gilbert's syndrome).
- Left ventricular ejection fraction (LVEF) ≥ 45**%.** A lower LVEF is permissible if a formal cardiologic evaluation reveals no evidence for clinically significant functional impairment.
- Adequate pulmonary function with mechanical parameters ≥ 40% predicted (FEV1, FVC, TLC, DLCO). Patients who are unable to complete PFTs due to bone pain or fracture must have a high resolution CT scan of the chest and must have acceptable arterial blood gases defined as a room air PO2 greater than 70 mmHg.
- Patients should have recovered from any toxicities related to prior therapy or at least returned to their baseline level of organ function.
- Patients should be off of glucocorticoids for at least 2 weeks and/or other therapies for at least 1 week prior to enrollment.

4.3.8 ECOG performance status 0-2 (unless due solely to bone pain. Also, see protocol section 5.9 for modified eligibility requirements pertaining to a second T-cell infusion.)

4.3.9 Prior to Lenalidomide maintenance phase, all study participants must be registered into the mandatory RevAssist® program, and be willing and able to comply with the requirements of RevAssist®.

4.3.10 Females of childbearing potential (FCBP) must have a negative serum or urine pregnancy test with a sensitivity of at least 50 mIU/mL within 10 – 14 days prior to and again within 24 hours of prescribing lenalidomide (prescriptions must be filled within 7 days) and must either commit to continued abstinence from heterosexual intercourse or begin TWO acceptable methods of birth control, one highly effective method and one additional effective method AT THE SAME TIME, at least 28 days before she starts taking lenalidomide. FCBP must also agree to ongoing pregnancy testing. Men must agree to use a latex condom during sexual contact with a FCBP even if they have had a successful vasectomy. See Appendix: Risks of Fetal Exposure, Pregnancy Testing Guidelines and Acceptable Birth Control Methods

4.3.11 Lenalidomide treatment phase: able to take aspirin (81 or 325 mg) daily as prophylactic anticoagulation (patients intolerant to ASA may use warfarin or low molecular weight heparin).

4.3.12 HLA-A201 patients must have confirmed expression of NY-ESO-1 and/or LAGE (these determinations will be under a pre-enrollment "screening" consent form using criteria listed below in study procedures). In addition HLA-A2 patients must have the A-201 allele

4.4 Exclusion criteria

Subjects who meet ANY of the following criteria cannot be enrolled in the study. No exceptions to eligibility will be granted:

4.4.1 Pregnant or nursing females

4.4.2 HIV or HTLV-1/2 seropositivity

4.4.3 Known history of myelodysplasia

4.4.4 Known history of chronic active hepatitis or liver cirrhosis (if suspected by laboratory studies, should be confirmed by liver biopsy).

4.4.5 Active Hepatitis B (as defined by + Hepatitis B surface antigen); + Hepatitis C virus (HCV) antibody is NOT an exclusion

4.4.6 Prior allogeneic transplant

4.4.7 History of severe autoimmune disease requiring steroids or other immunosuppressive treatments.

4.4.8 Active immune-mediated diseases including: connective tissue diseases, uveitis, sarcoidosis, inflammatory bowel disease, multiple sclerosis.

4.4.9 Evidence or history of other significant cardiac, hepatic, renal, ophthalmologic, psychiatric, or gastrointestinal disease which would likely increase the risks of

participating in the study. For any transplant patient over 60 and for patients with prior history of heart disease, a pre transplant cardiac stress test is recommended. The specific type of stress test will be selected at the PI's discretion.

4.4.10 Active bacterial, viral or fungal infections.

4.5 Subject Recruitment and Screening

Subjects will be recruited for this study from the clinical practices of the Hematology-Oncology division at the University of Pennsylvania and from the University of Maryland. Subjects will be required to give written informed consent to participate in the study before any screening tests or evaluations are conducted.

4.6 Early withdrawal of subjects

Subjects may withdraw from the study prior to the expected completion date for the following reasons:

- Unacceptable toxicity and other safety reasons
- Progression or disease relapse
- Subject consent withdrawal
- Decision by the investigators that withdrawing is in the patient's best interest
- Decision by the investigators because they determined the patient is unable to comply with the study procedures
- Decisions from the Sponsor to terminate the study
- Death

Standard supportive therapy may be maintained for subjects withdrawn from active treatment. Every effort will be made to collect toxicity information on withdrawn patients.

5 TREATMENT PLAN

***No study procedures may be performed prior to the patient signing informed consent. In addition, since the study is complex, simplified protocol flow diagrams should be used along with the consent document to enable the prospective study patient to fully understand the procedures and multiple steps that are involved in the study.**

In order to enroll, patients must have the following HLA/tumor antigen combinations:

1) HLA-A201+ and NY-ESO-1 and/or LAGE positive myeloma

To establish tumor antigen expression, the following procedures will be followed:

a) If the patient has measureable disease, fresh marrow will be collected and tested for tumor antigen expression by RT-PCR (in lab of Dr. Kalos at UPENN).

b) If the patient has insufficient myeloma cells in the marrow (due to prior therapy) then the diagnostic marrow or tissue sample will be retrieved for antigen expression analysis by IHC (immunohistochemistry).

c) If no prior tumor samples are available and a current marrow specimen is inadequate, then the patient will not be eligible since cognate tumor antigen expression cannot be proven.

5.1 Enrollment Procedures

The laboratory evaluation at enrollment will consist of the following studies:

- Complete medical history, physical examination, performance status
- CBC with differential
- Comprehensive metabolic panel
- Chest XRAY (Chest CT if clinically indicated)
- Myeloma markers (SPEP + immunofixation; quantitative IgG, IgM, IgA; UPEP + immunofixation based on a 24 hour urine collection; serum free κ and λ light chain levels and κ/λ ratio determination; Beta-2 microglobulin, CRP)
- Skeletal survey; MRI or PET scan if clinically indicated
- Serology for hepatitis B and C; HIV; HTLV1/2; CMV
- Pregnancy test if applicable
- CD3,CD4 and CD8 T-cell levels
- Staging marrow aspiration and biopsy (if not done within previous 2 months)
- HLA A/B/DR determination (low resolution is acceptable): in most cases this will have been done prior to enrollment.

5.2 Pre-transplant Therapy

Patients who are newly diagnosed should receive initial cytoreductive therapy with several cycles of a standard induction regimen (e.g. dexamethasone/thalidomide or dexamethasone/lenalidomide or bortezomib/dexamethasone or equivalent). Upon completion of initial therapy and in accordance with the eligibility criteria in section 4.0, patients may enroll as described in 5.1.

5.3 PCV (Prevnar-13) Immunization (day -40 [+/-4])

Patients will also receive an intramuscular (IM) injection of the pneumococcal conjugate vaccine (PCV, Prevnar-13) [0.5 ml] into the non-dominant deltoid.

5.4 Steady-State T-cell Harvesting (day -30 [+/-10])

After enrollment, patients will undergo a mononuclear cell apheresis procedure to collect steady-state T-cells. If a patient has previously undergone an apheresis for T cell collection, the frozen product may be used if, in the opinion of the investigator and the manufacturing facility these cells should be used in lieu of fresh product. The collection goal will be about 1 x 10^8 mononuclear cells/kg body weight. The minimum collection will be set a 0.5 x 10^8 mononuclear cells/kg. However, if the minimum collection is not reached, then a second apheresis procedure can be performed. The cells will be transferred by courier to the manufacturing facility where they will be cryopreserved for later expansion.

5.5 Stem Cell Mobilization (Cyclophosphamide +/- Bortezomib) (day -26 [+/-3])

After completion of the mononuclear cell apheresis procedure, patients will be offered low-dose cyclophosphamide chemotherapy for stem cell mobilization at a dose of 1500-3000mg/m2 daily. At the discretion of the treating physician and particularly for patients with higher myeloma burdens at the time of stem cell mobilization, bortezomib may be added at a dose of 1.3 mg/m2 on days 1, 4 and 8 (3 doses). Use of bortezomib is recommended for patients with ≥20% marrow plasmacytosis at study enrollment, prior to stem cell mobilization, to decrease tumor burden before stem cell collection.Addition of bortezomib to mobilization chemotherapy does not adversely affect stem cell yield or engraftment (Oakervee et al., 2007; Badros et al., 2006).

Patients should receive antibiotic prophylaxis upon completion of chemotherapy (e.g. moxifloxacin, acyclovir, mycelex troches or equivalent) and G-CSF daily, SQ, at a dose of 10 mcg/kg body weight (rounded to nearest multiple of 300 mcg and 480 mcg vials) starting on the day after completion of chemotherapy. An acceptable alternative mobilization regimen would be plerixafor with G-CSF.

The goal for stem cell collection will be ~8-10 x 10^6 CD34+ progenitors/ kg body weight with a minimum of 3 x 10^6/kg body weight needed to proceed with the transplant. An additional stem cell mobilization using another mobilization regimen could be performed at the discretion of the treating physician in order to reach the stem cell collection target.

Subject may receive treatment for myeloma prior to high dose therapy if there is a delay in stem cell transplant date due to scheduling, manufacturing delays, etc. Note, stem cell mobilization step may be skipped if the subject has stem cells already stored.

5.6 High-dose Therapy (day -2)

High-dose therapy will consist of melphalan at a dose of 200 mg/m^2 given IV over about 20 minutes on approximately day -2. For patients who are ≥70 years of age, the dose of melphalan will be reduced to 140 mg/m^2. All patients will receive standard anti-emetic prophylaxis and standard antibiotic prophylaxis. Stem cell infusion will take place on day 0, at least 18 hours after

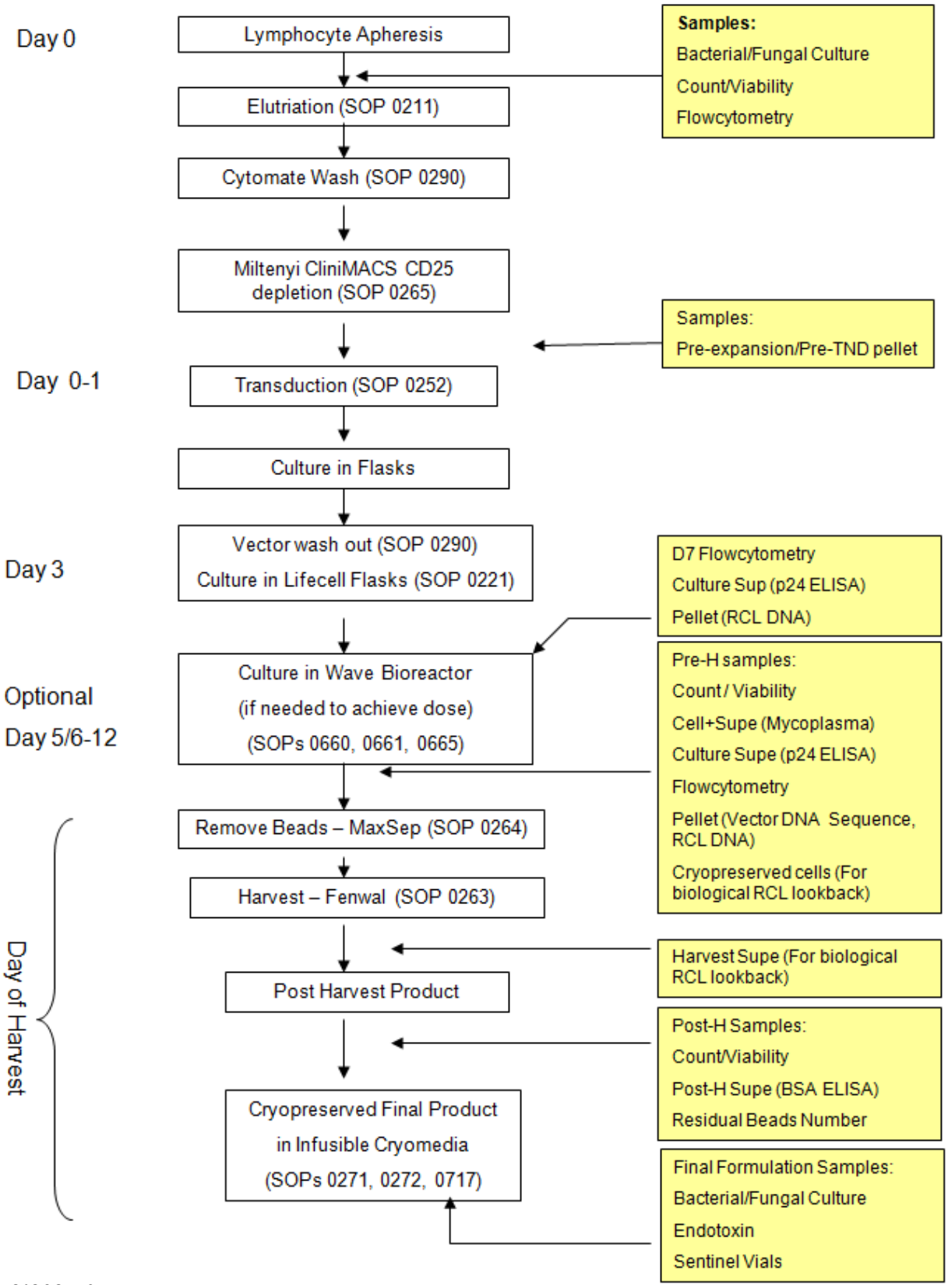
the administration of the high-dose melphalan. Stem cells will be infused intravenously over about 20-60 minutes following premedication with acetaminophen (650 mg) and diphenhydramine (25 mg) with or without 1mg/kg of hydrocortisone, depending on institutional preference. G-CSF should be administered SQ beginning on day +5 at a dose of 480 mcg/day (or 300 mcg/day if body weight is < 60 kg). Other supportive care measures such as transfusional support will be done in accordance with standard institutional guidelines. At least 2 x 10^6 CD34 + progenitors/kg body weight should be infused after high-dose therapy. In addition, at least 1 x 10^6 CD34 + progenitors/kg body weight should be available as a backup stem cell product to be infused in the event of delayed engraftment or late graft failure.

5.7 Autologous T-cell Expansion and Infusion (day +2)

5.7.1 Mononuclear Cell Transfer and transport to the manufacturing facility

The manufacturing facility will prepare the expanded T cells for patients enrolled at both sites. The pre-expansion mononuclear cells will be transferred by express courier (same day delivery) in accordance with FDA-approved guidelines to the manufacturing facility. Cell products will be packed and shipped according to (International Air Transport Association) IATA regulations, in specially designed Saf-T-Pak insulated shippers. For patients enrolled at each site, the cells will be washed and cryopreserved until approximately 12 days prior to the planned infusion and then thawed for expansion. The cells will be processed and expanded in a Class 100,000 clean room according to Standard Operating Procedures that are written to comply with Good Manufacturing Practices. The flow diagram below outlines the manufacturing steps that will be followed:

TCR-redirected T Cell Manufacturing Process



5.7.2 Apheresis Wash:

The apheresis product is first washed before further processing in order to remove plasma or cell suspension solution, and reduce platelets and red blood cell contamination in the apheresis product using the Baxter CytoMate Cell Washer with X-VIVO 15 based media. Alternatively, cells may be washed on the COBE 2991 Blood Cell Processor.

5.7.3 Monocyte Depletion:

Monocytes present in mononuclear cells collected during the apheresis may inhibit lymphocyte proliferation. Therefore, monocytes will depleted via adherence to Dynal M-450 Epoxy beads or by counter flow centrifugal elutriation (Gambro Elutra™ Cell Separation System). The bead adherence method can be performed on fresh or cryopreserved apheresis products, and is usually done on cell suspensions with ≥20% monocyte content. About 2-4 beads per monocyte are added to the cell suspension to be processed, followed by an incubation period. The beads are then removed by magnetic separation by the MaxSep Magnetic Separator, and the numbers of recovered (monocyte-depleted) cells are determined. An entire leukaphereis product can be monocyte depleted in 1-2 hours by either method.

5.7.4 Ex-vivo Costimulation of T-cells

In the first trial (Rapoport et al.,2005), the cells were cultured using Dynal microbeads coated with anti-CD3 (OKT3)/anti-CD28 (9.3) monoclonal antibodies and were manufactured at UPenn under IND #6675. Since then, we have evaluated commercially available clinical grade microbeads produced by Dynal that are coated with anti-CD3/CD28, but with different hybridoma clones. These Dynal microbeads are functionally equivalent to the beads produced at UPenn. We anticipate using beads manufactured at UPenn or the commercial beads produced by Dynal for the expansion of T cells for this study.

The culture process in the phase I trial was done using Baxter Lifecell® flasks (Hami et al.,2005). We are currently culturing cells in the first 3-5 days in a Baxter Lifecell® or equivalent gas permeable bag such as the Origen PermaLife™. Once cells are in log phase expansion, they are seeded into the WAVE® bioreactor system, which has been adapted for T cell expansion cultures by Xcyte Therapies (Levine et al., 1998; Rapoport et al.,2009) and subsequently validated by the CVPF. Because this system is more efficient in terms of cell yield and media requirements, we have switched from Lifecell® flasks to the WAVE bioreactor process. Cells will be grown in X-VIVO™ media supplemented with 5% commercial pooled human AB serum. The microbeads will be washed and added at a 3:1 ratio of beads per cell. The cultures will be maintained for up to 12 days prior to harvesting and preparation for reinfusion. The cells will be counted and fresh medium added daily and then perfused in the WAVE Bioreactor.

5.7.5 Expansion and transduction of T cells

The procedures for T-cell stimulation, TCR transduction, static cell culture, and Wave bioreactor cell culture are shown in the above figure. Briefly, enriched lymphocytes (via elutriation) are stimulated with Dynabeads conjugated with mouse anti-human CD3 and CD28 in static tissue culture flasks at an approximate range of 5 x 10^8-1 x 10^9 cells in a supplemented XVIVO-15 media (Modified X-VIVO 15 Media). Beads are added at a 3:1 bead to cell ratio. This optimal bead: cell ratio was previously determined (IND#6675 and Levine et al., 1997). Transduction is performed on day 1 of culture with a predetermined MOIby addition of the TCR lentiviral vector.

Table 5.7.5: T cell product release criteria

Test	Method	SOP	Criteria
Cell Viability on Sentinel Tube	Trypan Blue Exclusion	0331	≥70%
% CD3 positive T cells	Flow Cytometry	0501	≥80%
Residual beads number	Visual	0263	≤100 beads/3x10^6 cells
Endotoxin	Gel Clot	0365	≤ 3.5 EU/mL
Mycoplasma	MycoAlert Assay	0367	Negative
VSV-G (RCL)	PCR	TBD	< 50 copies/micrograms of DNA
Transduction efficiency (TCR expression)	Flow Cytometry	TBD	≥10% positive
Transduction efficiency (copy number)	PCR	TBD	0.1≤ x ≤5 average copies per CD8 cell**
BSA	ELISA	0368	≤1 µg/ml
Bacterial culture	Culture	0361	No growth
Fungal culture	Culture	0358	No growth

On day 3, the vector is washed away in a Baxter CytoMate Cell Processing System. The T cell culture with beads are seeded back into gas permeable tissue culture flasks at a cell density of 5x10^5 cells/mL in fresh Modified X-VIVO 15 Media and placed at a 37ºC incubator with 5% CO_2 and >90% of humidity for cultivation and further expansion. The cell culture is maintained in a closed system. Tubing leads on the tissue culture flasks are connected or disconnected through a variety of sterile tubing connecting devices and heat sealers (e.g. spike connectors, tubing welds from the Terumo Sterile Connecting Device, heat seal from the Sebra Heat Sealer) to reduce the risk of contamination.

The Principal Investigator and Sponsor will be notified if any of these test results are positive or if any criterion is not met.

5.7.6 T cell quality control

The manufacturing facility ensures that all operations and clinical trials conducted comply with GLP, GMP, and GCP.

5.7.7 Infusion of Autologous T-cells

The cryopreserved costimulated ("activated") T-cells will be thawed at the bedside using standard procedures and infused over about 20 minutes **without** a leukocyte filter on about day +2 of transplant. As mentioned above, the cells will be re-suspended in 100-500 ml of Plasma-lyte A or other appropriate infusion solution containing 0.5%-1% human serum albumin. Patients should be pre-medicated with acetaminophen and diphenhydramine (benadryl). Corticosteroids will be available at the bedside in the event of an allergic-type reaction but should not be administered on a routine basis. **The target dose of total T-cells infused after gene transfer and expansion will be >0.1-1 x 10^{10}**since this was the dose of cells that was employed for the first study (Rapoport et al., 2005) and proved to very safe and did not appear to induce any cases of clinically significant autologous GVHD. A minimum of 0.1-1 x 10^9cells in the final product will be permitted. Since other gene-modified adoptive T-cell transfer studies have demonstrated biological activity at doses below 0.1 x 10^9, lower doses of T-cells will generally be permitted after assent of the PI, DSMC and Sponsor but this "low-dose" cohort will be analyzed separately for safety and immunoreactivity.

Patients will be monitored for infusion related AE, which is facilitated by the fact that they are inpatients. Blood samples will be taken for cytokine monitoring prior to infusion, and at 1, 2, 4, 8, and 12 hours post infusion, and then at each visit thereafter as described in the table of study procedures. Cytokine monitoring will be batched and done retrospectively and at a minimum IFNγ, GM-CSF, and IL-6 will be evaluated. If toxicity is observed following infusion, review of the cytokine levels will be undertaken to assess risk to subsequent patients. Cytokine levels will also be evaluated retrospectively for correlation with antitumor effects. It is acknowledged that the high dose chemotherapy and stem cell infusion may contribute to changes in cytokine levels.

Cytokine release syndrome

Cytokine release syndrome is caused by a release of inflammatory cytokines such as IL2, IFN-γ, and TNF typically by macrophages, tumor cells and T cells. This type of reaction is common in cancer immunotherapy where antibodies bind T cells that release large amounts of cytokines, or cause rapid destruction of tumor/target cells. This causes a systemic inflammatory response similar to sepsis and includes fever, nausea, chills, rash

or flushing, rigors, hypotension, tachycardia, headache, rash, throat tightness, and dyspnea. Capillary leak with fluid retention is worsened by hydration commonly given to treat hypotension. The syndrome can be associated with pulmonary infiltrates, pulmonary edema, arrhythmias and cardiac arrest. The syndrome is also associated with an elevation in LFTs, d-dimers, LDH, CR, uric acid, and phosphorous from immune-mediated cytolysis of targeted cells, with release of intracellular contents as well as a possible bystander effect (on neighboring, nontargeted cells). The potential of NYESO-1^{c259}-T to cause cytokine release syndrome is unknown but is thought to be low because 1) the NYESO-1^{c259}-T has been tested in a trial at the NCI has not caused CRS in patients with melanoma or sarcoma and 2) cytokine release syndrome has not occurred in any TCR trial to date.

Premedication with anti-histamine, acetaminophen can help prevent cytokine release syndrome. Antihistamines and acetaminophen can be administered prior to infusion, while steroids cannot be administered because they are cytotoxic or inhibitory for the study drug. If a severe cytokine release syndrome develops, the patient will be administered a one-time dose of Decadron 20-40 mg IV to prevent further cytokine activation and release, anti-histamines, and supportive care (fluids, pressors, intubation if needed). Management of the cytokine release syndrome is described on below.

Cytokine release syndrome (CRS). CRS is managed in the same fashion as septic shock following infection with gram negative bacteria, with the exception that antibiotics are not required. Capillary leak with fluid retention is worsened by hydration commonly given to treat hypotension. The syndrome can be associated with pulmonary infiltrates, pulmonary edema, arrhythmias and cardiac arrest. The syndrome is also associated with an elevation in LFTs, d-dimers, LDH, CR, uric acid, and phosphorous from immune-mediated cytolysis of targeted cells, with release of intracellular contents as well as a possible bystander effect (on neighboring, nontargeted cells). Prophylactic measures to prevent CRS include correction of overhydration/volume overload before infusion with diuresis if necessary and pre-medication. Patients are pre-medicated with acetaminophen and an antihistamine before CIR infusion.

As a routine, after infusion the vital signs (including pulse oximetry) will be monitored every 15 minutes for 4 h. In the event of CRS, patients with rigors will be treated symptomatically with hot pack, blankets, Demerol 12.5-50 mg IVP. An MD will be called immediately if subject experiences a decrease in blood pressure. Vital signs will be monitored 15 minutes before infusion, while infusing and after infusion. If a subject experiences hypotension, this will be managed by administering intravenous fluids in boluses as needed to maintain an adequate blood pressure. Should the hypotension not respond to intravenous fluid boluses, admission to the CTRC or inpatient hospital will be performed to provide the appropriate level of care. Should hypoxia or dyspnea develop that results in oxygen desaturation as detected by standard pulse oximetry, the patient will be administered nasal cannula oxygen and have a chest X-ray performed as deemed appropriate by the investigator. The use of ACE inhibitors and angiotensin-II receptor blockade may have benefit for CRS (Das et al, 2005). Corticosteroids have been reported

to block systemic effects of T cell toxicity (Lamers et al, 2006). See Wang et al, 2008 and Stebbings et al, 2009 for experimental management of CRS.

5.8 PCV (Pneumococcal Conjugate Vaccine, Prevnar-13®) Boosters

At days 14, 42 and 90 post-transplant, patients will also receive booster intramuscular (IM) injections of the pneumococcal conjugate vaccine (PCV, Prevnar-13) [0.5 ml] into the non-dominant deltoid.

5.9 Second Infusion

Patients enrolled at University of Pennsylvania are not eligible for receiving a second infusion.

In patients who have progressive disease following initial infusion but whose tumors continue to express the appropriate HLA and antigen target, these patients will be eligible for a second infusion with redirected T cells. Either the previously manufactured cell product will be used, or remaining apheresis product from collections prior to receipt of the gene modified T cells will be utilized for a new product manufacture. Patients will not be re-apheresed for cells unless there is no detection of gene modified cells and approval to do so has been received by the FDA prior to the procedure.

Following intensive animal and clinical research over the past decade, it is now generally accepted that the incorporation of lymphodepletion prior to adoptive T cell therapy enhances immune reconstitution by the transferred cells, and increases tumor specific responses. Immune reconstitution is enhanced through homeostatic proliferation of T cells which is different from antigen driven T cell expansion in that it occurs in the absence of costimulation (Ernst, Lee et al. 1999; Prlic, Blazar et al. 2001). Homeostatic proliferation is promoted by an increase in availability of γ-chain cytokines IL-7, IL-15, and IL-21 (Wrzesinski and Restifo 2005; Rapoport, Stadtmauer et al. 2009; Wallen, Thompson et al. 2009), and also by MHC-peptide interactions against self or tumor antigens (Ernst, Lee et al. 1999; Wrzesinski and Restifo 2005). Lymphodepletion also enhances the activity of the adoptively transferred cells via the removal of inhibitory factors and activation of antigen presenting cells.

Since patients receiving a second infusion will most likely not be undergoing another ASCT, an alternate conditioning regimen will be used comprised of Cytoxan 1.5 gm/m2 (+/- bortezomib x 2 doses. If bortezomib is given, the first dose should be given ~ 72 hours before the Cytoxan and the second dose can be given on the same day as the Cytoxan). The engineered T cells will be administered as previously described in this protocol, on day 2 following the Cytoxan. For example, if Cytoxan is administered on Monday, the T cells can be given on Wednesday. A longer timeframe between the Cytoxan administration and the T cell infusion is allowable provided the patient is shown to have a white blood count (WBC) of ≤ 0.5(500 cells/mcl) within 24 hours prior to the planned T cell infusion. This can be an outpatient procedure.

If there was no serious toxicity attributed to the initial infusion, the second infusion may be increased up to 5 x 10e10 total cells (5-fold higher than the first infusion dose target). The second infusion must be a minimum of 1 e 8 total cells.

Patients must continue to meet the eligibility requirements for this study in order to remain eligible to receive a second infusion, with the exception that 1) blood counts lower than that described in the inclusion criterion are acceptable if they are believed by the Investigator to be due to disease activity, and 2) a performance status of up to an ECOG=3 is acceptable. The rationale for these exceptions is that patients with relapsing disease are expected to have rapidly advancing disease, and this population will not be required to undergo the high intensity conditioning required for auto-HSCT. Documentation of laboratory tests performed within the 60 days of the planned second T cell infusion may be used to confirm eligibility of other eligibility criteria.

In cases where a second infusion is given, lenalidomide (see next section) will be held until day 100 post the second infusion, unless otherwise recommended by the PI.

For follow-up post second infusion, the Cytoxan administration will be day 0, and the T cell infusion will be day 2. The patient will be followed in accordance with the schedule of procedures provided in Section 14 as shown. Long term follow-up will be calculated from the second infusion date.

5.10 Maintenance Therapy (Lenalidomide)

At about day 100 post-transplant, after completion of post-transplant immunological assessments and myeloma restaging studies, patients will be eligible to receive low-dose lenalidomide for maintenance therapy (10 mg/day) until progression of myeloma or development of intolerance. This dose level and schedule is identical to the one that was used in a recently completed cooperative group randomized study of post-transplant maintenance therapy with lenalidomide which was conducted by the CALGB (CALGB Protocol # 100104). Because of the increased risk of thrombo-embolic events, patients will also receive low-dose aspirin (81 mg/day or 325 mg/day). Preparations for lenalidomide maintenance therapy should be made at least 7-10 days before the day 100 visit in order to minimize delay in starting it. In addition, at 1year post-transplant, patients may receive a standard 23- valent pneumococcal polysaccharide vaccine in accordance with the recommendations of the American Society for Blood and Marrow Transplantation.

Prior to starting the lenalidomide, patients should have adequate bone marrow function defined as: PLT $\geq$ 50,000/mcl and ANC (absolute neutrophil count) $\geq$ 1000). If patients develop excessive myelosuppression with the dose of 10 mg daily (PLT $\leq$ 25,000/mcl or ANC $\leq$ 500/mcl), then the schedule should be adjusted to 21 days of treatment per month (dose reduction #1) or 14 days of treatment per month (dose reduction #2) once the blood counts rebound to the levels of PLT $\geq$ 50,000/mcl and ANC (absolute neutrophil count) $\geq$ 1000. If patients develop excessive myelosuppression with the dose of 10 mg for 14 days per month (PLT $\leq$ 25,000/mcl or ANC $\leq$ 500/mcl), then the dose level should be decreased to 5 mg daily for 21 days per month (dose reduction #3) and 5 mg daily for 14 days per month (dose reduction #4) (See table below).

Patients can be re-escalated by 1 dose level after 3 months of Lenalidomide if ANC ≥1000 and platelets are ≥75,000. See following tables for dose modifications. Guidelines for Lenalidomide dose modification and interruptions are provided below and in Appendix I.

Lenalidomide Dose Reduction Levels		
* At physician discretion, lenalidomide dose may be increased, if CR not achieved and no grade II or higher myelosuppression develops during 3 months of therapy.	Dose Level 2 (only after 3 months of 10mg)	15mg daily days 1 – 28 of a 28 day cycle
	Dose Level 1	10mg daily days 1 – 28 of a 28 day cycle
Dose Reduction #1	Dose Level -1	10mg days 1 – 21 of a 28 day cycle
Dose Reduction #2	Dose Level -2	10mg days 1 – 14 of a 28 day cycle
Dose Reduction #3	Dose Level -3	5mg days 1 – 21 of a 28 day cycle
Dose Reduction #4	Dose Level -4	5mg days 1 – 14 of a 28 day cycle

5.11 Protocol Modifications for Syngeneic Stem Cell Transplant (SSCT)

If available, a patient may receive stem cells and gene-modified T cells from an identical twin sibling. This is a rare event, and is not expected to occur more than once or twice on this protocol.

In the event that an identical donor sibling is identified, the following things must occur:

a) Submission to UPenn regulatory committees (for UPenn subjects only):
   i) For UPenn subjects an exception request must be submitted to the UPenn regulatory committees for review and approval prior to consenting the subjects (twin donor and donor recipient)
b) Submission to the FDA regarding the following aspects of donor identity and screening:
   i) Confirmation that donor screening must adhere to the requirements outlined in 21 CFR 1271
   ii) A twin donor test must be performed by an accredited tissue typing laboratory to confirm that the donor and patient are indeed identical twins; this information must be submitted to the FDA by the regulatory sponsor
c) Consent
   i) Unique consent forms for patient screening and for the patient and donor participation on the study have been developed. These consent forms outline the study procedures that are specific to an SSCT, which are slightly different than for an ASCT due to the donor undergoing certain procedures.
d) Modification in study procedures
   i) SSCT requires that the sibling donor perform the following procedures in lieu of the patient:
   (1) Receives initial prevnar vaccination at approximately day -40

(2) Undergoes initial apheresis for T cell collection at approximately day 30
(3) Undergoes stem cell mobilization using G-CSF +/- plerixafor/Mozobil® starting at or around day -26
(4) Undergoes stem cell collection by apheresis at or around day -20

Allowing a patient with an identical twin donor to receive an SSCT on this protocol is an ethical way to proceed since overall outcomes following twin transplants may be more favorable than ASCT due to potential contamination with residual myeloma in autologous grafts (Bashey et al, 2008) which is lacking in syngeneic grafts. However, whether an improvement in response rates and survival is truly associated with SSCT over ASCT does remain an active area of investigation in the field and is not firmly established.

Since graft-vs-host-disease (GVHD) does not usually occur after identical twin/syngeneic transplants, it is expected that the primary endpoint of this study which is assessment of safety, can be equally assessed using twin donor cells or autologous T cells. Therefore, patients receiving an SSCT will be considered evaluable under primary study endpoints

5.12 Toxicity Management

5.12.1 Monitoring for and management of RCL

RCL testing will be performed on the vector material, each cell lot, and as a patient monitoring assay. RCL testing on the cell product will be performed by a DNA based assay. For ongoing patient monitoring for RCL, PBMC samples obtained from subjects at 3, 6, 9, and 12 months, and annually from year 2-15 post treatment will be tested for VSV-G DNA sequences. If these tests are negative for 3 consecutive assessments, PBMC samples will be collected and archived yearly until 15 years post infusion.

If a positive VSV-G DNA signal is obtained, the PI will be informed and the patient scheduled for a retest as soon as possible and no later than one month after the initial positive result was reported to the Sponsor. A review by Adaptimmune's Safety Review Team and Safety Governance Board will take place. If the second test is positive, infusions for all patients receiving cells modified with the same vector lot will be postponed. The patient with the confirmed positive VSV-G signal will be scheduled for apheresis and a biological RCL performed on the apheresed product. If the biological RCL is positive, all infusions using NY-ESO-1^{c259}T in the interventional protocol(s) will be halted. An action plan will be discussed with FDA and other regulatory authorities and experts as appropriate. Additional subjects will not be treated with NY-ESO-1^{c259}T cells until such time as a plan is completed, reviewed, and agreed upon.. If the test is negative, infusions for all patients can resume.

A replication-competent lentivirus (RCL) may be generated during the production phase or subsequently after introduction of vector transduced cells into the patient. However, an RCL resulting from the production phase is highly unlikely since elements are incorporated in the design of the vector system that minimize vector recombination and generation of RCL. Furthermore, the vector used to transduce the product undergoes

sensitive assays for detection of RCL before it can be released for use in cell manufacture. Nevertheless, while an RCL has never before been reported, generation of an RCL following infusion of the vector product remains a theoretical possibility. The consequences of such recombination events could be neutral, could reduce the replication rate or pathogenicity of the subject's virus, or could increase the replication rate or pathogenicity of the subject's virus. Since the development of a strain with increased pathogenicity would pose greater risk to both the patient and their close contact(s), periodic monitoring for RCL is conducted during the course of the trial.

Regulatory agencies and the gene therapy community have previously discussed measures to be taken should an RCL be confirmed in a patient. However, because the probability and characteristics of an RCL are unknown, no concrete plans have been put in place.
At the current time, it is agreed that the subject must be isolated and no additional subjects treated with NY-ESO-1^{c259}T cells until a plan is agreed upon.
The following approaches have been discussed for subject management:

1. Intensive follow up of subject in consultation with FDA, and other regulatory authorities, NIH, gene therapy experts, study investigators, and HIV physicians.
2. Provide targeted antiretroviral therapies based on genotyping of the RCL.

5.12.2 Monitoring for and management of insertional oncogenesis

Monitoring for insertional oncogenesis will be performed in accordance with FDA recommendation. The PBMC sample is used as the "surrogate sample" for testing for insertional oncogenesis.

The current FDA Guidance (Guidance for Industry, Gene Therapy Clinical Trials-Observing Subjects for Delayed Adverse Events; November 2006) recommends that if at least 1% of the cells in the surrogate sample are positive for vector sequences by PCR for a sustained period of three consecutive weeks, then cells will be analyzed for clonality (by TCR Vβ repertoire for example). In this protocol, the cytoreductive conditioning followed by infusion of T cell transduced at >20% with the transgene is guaranteed to result in >1% marking in the "surrogate sample".

Therefore, clonality analysis will be performed if greater than 1% of the PBMC is expressing NYESO TCR at 1 year or later post infusion.
DNA from the subject's PBMCs will be sent for Next-Gen Sequencing for integration site analysis by the University of Pennsylvania. Integration site analysis assesses clonality and the possibility of insertional oncogenesis. This integration site analysis method, used in previous clinical trials [Hacein-Bey Abina 2015], uses sonic length quantitation, an accurate method of measuring relative clonal abundance [Berry, 2012].
Clonality is defined as follows: 1) monoclonality is 1 predominant clone at ≥5% of transduced T cells; 2) oligoclonality is defined as 2-5 predominant clones, each at ≥5% of transduced T cells; and 3) polyclonality is defined as no single predominant clone of ≥5% of transduced T cells.

If there is clonal dominance in the genetically modified T cell population (either monoclonality or oligoclonality) the persistence assessment will be repeated within 3 months on a new sample. If the repeated analyses demonstrates: 1) persistent monoclonality, 2) other evidence of insertional oncogenesis (for example, integration of the vector in the promoter region of a known oncogene or tumor suppressor gene), or 3) clonal expansion (an increase in percent predominance of a clone), there will be a review by Adaptimmune's Safety Review Team and Safety Governance Board to develop a monitoring plan specific to the health care risk and strategies to inform appropriate subjects, investigators, FDA and other regulators of the findings.
If the integration site analysis indicates polyclonality of the genetically modified T cell population then screening for persistence continues as scheduled.

A summary of all integration site analyses will be presented in the annual report to the FDA. If oligo or monoclonality is observed, this data will be entered as an information amendment to the IND with best efforts made to submit this amendment within 30 days of data confirmation.

5.12.3 Risk of on target activity of NYESO-1^{c259}against normal tissue

As described in the background section of this protocol, cancer testes (CT) antigens have restricted expression on normal tissue, and have only been found in testicular germ cells and placenta. This protocol has been designed to incorporate birth control procedures and pregnancy monitoring in order to protect any potential toxicity during pregnancy. Toxicity against testicular germ cells in men enrolled in this study is a theoretical risk. This risk is mitigated in part because the testes are an immune privileged site primarily due to their immune suppressive environment (induced by hormonal, cell based and cytokine based factors), and perhaps also in part by the physiologic barriers provided by the blood testes barrier (Fijak, Bhushan and Meinhardt, 2011; Fijak and Meinhardt, 2006). This reduces the opportunity for NYESO-1^{c259}-T to enter into and to activate against the germ line cells. Nonetheless, immune reactivity is possible in testes, which is exemplified by the fact that >10% of male infertility can be attributable to immunopathology, and therefore this remains a risk that is noted in the informed consent document.

A previous clinical study where T cells expressing the CT antigen specific NYESO-1^{c259} TCR (the same TCR as proposed for cohort 1 in this study) were administered to patients with melanoma or synovial sarcoma was conducted and recently published (Robbins et al, 2011). This study enrolled 11 men, who received a range of 1.6-13 x 10^{10} total cells transduced at >50%. No toxicity was reported in this study, which supports the safety of our proposed study.

5.12.4 Management of rash and diarrhea

A common side effect of the melphalan conditioning and T cell infusion is a transient skin rash with lymphocytosis (favoring lower extremities) and a diarrheal syndrome that is later occurring than most melphalan mucositis not particularly full of lymphocytes that is taking

longer to recover (but eventually does) after the neutrophil recovery. The diarrhea can be confounded by c-diff colitis infection and melphalan mucositis and so is probably multifactorial.

A non-mandatory guideline for treatment of patients with rash and diarrhea is as follows, and specifically the recommendation for treatment of diarrhea is taken from the published management guideline for diarrhea associated with Ipilumimab treatment (O'Day et al, 2011). It is recommended to take 3-5 days to monitor and evaluate, with biopsy of skin and gut if indicated, and rule out infection or reaction to NYESO-1-T. If grade 1, institute and continue conservative and symptom management (change meds, treat infection, anti diarrheals). If no sign can't improvement and/or if grade 2, institute topical steroids (hydrocortisone for skin, budesinide for gut) and if no significant improvement in 3-5 days start systemic steroids (solumedrol 1-2 mg per kg).

5.12.5 Risks to patients with preexisting cardiac conditions

Two patients treated with the Mage TCR died of cardiogenic shock following infusion. The first death occurred in the first patient to be treated with the Mage TCR. This patient was on a separate melanoma trial. At the time of the death, all Mage cohorts in our studies were paused until the outcome of the investigation. Upon autopsy, the patient was found to have severe underlying cardiovascular disease which had not previously been clinically detectable. After an extensive scientific and clinical investigation showing absence of correlation to the infused cells, and subsequent review of the data and this conclusion by the NIH RAC and FDA, the cohort pause was removed once: 1) the informed consents were updated to report the death to patients, 2) additional cardiac enzyme monitoring was added to all trials with the Mage arm, and 3) a cardiac stress test was added as a screening procedure to capture patients with underlying heart conditions which otherwise may not be picked up in routine medical exams. The second patient treated with the Mage TCR was on this study. This patient passed cardiac screening tests, and was informed that he was the first patient to be treated following a prior death, and he signed the updated informed consent document. Data from this patient, including the absence of underlying heart disease, and pathologic evidence of acute rejection of the heart, indicated that the Mage TCR appeared to be targeting heart tissue. Consequently extensive additional studies were carried out to investigate the specificity of the Mage TCR and we discovered that the Mage TCR recognized a protein that is expressed in heart tissue. This was not picked up in preclinical screens that were performed because the protein is only expressed in beating cardiac tissue, and not single cell cardiomyocytes. As a result of these findings, which are to be submitted this week for publication, we have withdrawn the IND for the Mage TCR and no further patients will be treated. The NY-ESO IND remains open, and is unaffected by these events. As a result of this SAE, additional cardiac monitoring has been implemented in this protocol for the first week post infusion in order to capture such events, consisting of daily troponins and EKG. In addition, a recommended cardiac stress test for screening of patients upon enrollment was added for patients 60 years and older.

5.12.6 Autologous Graft Versus Host Disease (GVHD) Syndrome

Autologous Graft Versus Host Disease (GVHD) could occur after receiving a routine autologous stem cell transplant (ASCT). GVHD develops when the transplanted immune cells mistakenly attack the patient's normal cells. Subjects could develop a skin rash, develop diarrhea and sometimes belly pain, jaundice (yellowing of the skin and white of the eyes). In a previous study of investigational T cells infused following ASCT, 1 out of 6 subjects developed mild to moderate GVHD. In the more severe cases, some patients had as much as 1-2 liters (quarts) of watery diarrhea per day. All patients got completely better after taking steroid medicines given IV or orally (pill) for a few weeks. No patients became seriously ill nor died from this side effect. However, it should be noted that GVHD is only rarely observed. In the current study, several patients have developed diarrhea, nausea, and/or abdominal bloating that was worse and lasted longer than we would typically expect from the transplant chemotherapy. Prophylactic non-systemic steroids may be used for these symptoms as per institutional standards. It is possible that these symptoms could be related to the investigational "redirected" T cells. All of these cases either improved on their own or appeared to respond to treatment with steroids, though sometimes several weeks to months of steroids were required, which can cause additional side effects, like upset stomach, stomach ulcers, fluid retention, irritability, high blood sugar levels, fat redistribution, facial flushing, increased appetite and weight gain, and insomnia."

6 SCHEDULE OF EVALUATIONS (Including Correlative Research Studies)

6.1 Clinical Assessments (STANDARD OF CARE)

These will occur at:

	Enrollment	Admit Stem Cell Mobilization	Admit High Dose Melphalan	~D0	~D2,3,5,7,14	~D21	~D28	~D42	~D100	~D130	~D180	~Q3 mo until disease progression
Recent History and Physical Examination	X	X	X	X	X	X	X	X	X		X	X
CBC, differential	X	X	X	X	X	X	X	X	X	X	X	X
Comprehensive Metabolic Panel	X	X	X	X	X	X	X	X	X	X	X	X
CD3, CD4, CD8 counts	X				X[1]			X	X		X	

[1]-Day 14 only

After year 1, only delayed gene therapy adverse events will be recorded.

6.2 Myeloma Assessments (STANDARD OF CARE)

These will occur at:

	Enrollment	~D42	~D100	~D180	~Q3 mo up to year 1	~Q3 mo or per local standard of care after year 1
Myeloma markers	X	X	X	X	X	X
Bone Marrow Aspirate +/- Core Bx	X		X	X	X[0]	X[0]
Skeletal Survey	X		X	X	X[0]	X[0]

[0]-Optional

The myeloma assessments will be performed and recorded in the eCRF per schedule above until year 1 or disease progression, whichever is first. Myeloma assessments beyond year 1 will be performed per local standard of care and only the date of progression will be recorded in the eCRF. These myeloma assessments will include the following:

- Myeloma markers (SPEP with immunofixation; quantitative IgG, IgA, IgM,; Beta 2 microglobulin; 24 hour urine for UPEP and immunofixation; serum free light chain analysis)
- Bone marrow aspirate +/- core biopsy
- Skeletal surveys

6.3 Immunological Assessments (RESEARCH STUDIES)

For molecular studies (Q-PCR and Q-RT-PCR), immune phenotyping and functional assays, peripheral blood and marrow samples will be collected in Lavender top tubes. For cytokine analyses peripheral blood and marrow samples will be collected in red top (no additive) tubes. Samples will be collected according to the sample collection schedule (Appendix C). Samples will be processed according to a single standard operating Protocol (SOP) at both the University of Pennsylvania and University of Maryland sites. Samples should be delivered, processed within 2 hours of collection, and frozen as per SOP to the Translational and Correlative Studies Laboratory (TCSL) (University of Pennsylvania). Samples will be stored in the TCSL at the University of Pennsylvania for storage and bulk analyses,
University of Maryland samples will be shipped to

Dr. Michael Kalos
Translational and Correlative Studies Laboratory
University of PennsylvaniaSchool of Medicine
Stellar-Chance Laboratories, room 410
422 Curie Boulevard,
Philadelphia, PA19104

- All research analyses will be performed based on principles of good laboratory practice, with assay-specific SOP using qualified and if possible validated assays. Documentation

for sample -receipt, -processing, and storage and primary data from the research analyses will be collected and stored in the TCSL.

6.4 Long term follow-up for observation of delayed adverse events for gene therapy

All subjects will be followed for 15 years from time of T-cell infusion for observation of delayed adverse events in accordance with FDA and EMA requirements for gene therapy clinical trials [FDA, 2006b; FDA, 2010; EMA, 2009]. If a subject receives a second T cell infusion, the clock restarts with the second infusion. These assessments will be collected in the Interventional Phase of the study until disease progression and thereafter in the long term follow up (LTFU) phase. Subjects will be rolled over to the LTFU protocol ADP-0000-002, when available at the site. If the protocol ADP-0000-002 is not available, subjects will continue to be followed as described in Appendix C.
Reporting criteria for delayed AEs related to gene therapy during LTFU are described below. Please refer to the table of procedures for Long Term Follow-up provided in Appendix C of the protocol.

The most recently released (November 2006) Guidance on Monitoring for Delayed Adverse Events states that for the first five years, all subjects should undergo monitoring of vector sequences every 6 months, and a full physical examination including a medical history, concomitant medications and examination of appropriate organ systems and a hemogram annually. For the final 10 years, if vector sequences are no longer detected in PBMCs, a one page questionnaire or post card may be sufficient for reporting of any adverse events and questions relating to the patient's status.

From year 1 to year 5, patients will return to the clinic at biannual intervals for a medical history, physical exam, and blood tests for disease monitoring, CBC, chemistries, monitoring for persistence of vector modified cells and annually for RCL testing/archive. The physical exam and medical history (including concomitant medications and adverse events) will be conducted with careful attention to features possibly related to oncoretroviral diseases including: 1) New malignancies, 2) New incidence or exacerbation of a pre-existing neurologic disorder, 3) New incidence of exacerbation of a prior rheumatologic or other autoimmune disorder, 4) New incidence of a hematologic disorder, 5) Opportunistic and/or serious infections, and 6) Unanticipated illness or hospitalization deemed related to gene modified cell therapy.

For the next 10 years (up to year 15 post-infusion), subjects will be asked to return annually for a physical exam, collection of blood and testing for persistence (if vector modified cells were detected in any of the 3 previous assessments), and RCL testing or archive.

7 RESPONSE DEFINITIONS AND STATISTICAL TREATMENT

7.1 Clinical Myeloma Responses

The following criteria will be used to evaluate myeloma response and progression. The baseline for evaluation will be the myeloma parameters that are measured at screening (Day-50).

Response	*IMWG criteria (Rajkumar et al., Blood 2011)*
sCR	CR as defined below plus normal FLC ratio and absence of clonal cells in bone marrow[a] by immunohistochemistry or immunofluorescence[b]
CR	Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and < 5% plasma cells in bone marrow[a]
VGPR	Serum and urine M-protein detectable by immunofixation but not on electrophoresis or ≥ 90% reduction in serum M-protein plus urine M-protein level < 100 mg/24 h
PR	≥ 50% reduction of serum M-protein and reduction in 24 hours urinary M-protein by ≥ 90% or to < 200 mg/24 h If the serum and urine M-protein are unmeasurable,[c] a > 50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria If serum and urine M-protein are not measurable, and serum free light assay is also not measurable, ≥ 50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow plasma cell percentage was ≥ 30% In addition to the above listed criteria, if present at baseline, a ≥ 50% reduction in the size of soft tissue plasmacytomas is also required
MR	Not Applicable
No change/Stable disease	Not meeting criteria for CR, VGPR, PR, or progressive disease
Plateau	Not Applicable

Response	*IMWG criteria (Rajkumar et al., Blood 2011) - continued*
Progressive disease[c]	Increase of 25% from lowest response value in any one or more of the following: • Serum M-component and/or (the absolute increase must be > 0.5 g/dL)[d] • Urine M-component and/or (the absolute increase must be > 200 mg/24 h) • Only in patients without measurable serum and urine M-protein levels; the difference between involved and uninvolved FLC levels. The absolute increase must be > 10 mg/dL • Bone marrow plasma cell percentage; the absolute percentage must be > 10%[e] • Definite development of new bone lesions or soft tissue plasmacytomas or definite increase in the size of existing bone lesions or soft tissue plasmacytomas • Development of hypercalcaemia (corrected serum calcium > 11.5 mg/dL or 2.65 mmol/L) that can be attributed solely to the plasma cell proliferative disorder
Relapse	• Clinical relapse requires one or more of: Direct indicators of increasing disease and/or end organ dysfunction (CRAB features).[d] It is not used in calculation of time to progression or progression-free survival but is listed here as something that can be reported optionally or for use in clinical practice • Development of new soft tissue plasmacytomas or bone lesions • Definite increase in the size of existing plasmacytomas or bone lesions. A definite increase is defined as a 50% (and at least 1 cm) increase as measured serially by the sum of the products of the cross-diameters of the measurable lesion • Hypercalcemia (> 11.5 mg/dL) [2.65 mmol/L] • Decrease in haemoglobin of > 2 g/dL [1.25 mmol/L] • Rise in serum creatinine by 2 mg/dL or more [177 mmol/L or more]
Relapse from CR[c] (To be used only if the end point studied is DFS)[f]	Any one or more of the following: • Reappearance of serum or urine M-protein by immunofixation or electrophoresis • Development of > 5% plasma cells in the bone marrow[e] • Appearance of any other sign of progression (i.e., new plasmacytoma, lytic bone lesion, or hypercalcaemia)

Source: Adapted from Durie BGM, et al. Leukemia 2006; 20: 1467-1473; and Kyle RA, Rajkumar SV. Leukemia 2008;23:3-9.
Note: A clarification to IMWG criteria for coding CR and VGPR in patients in whom the only measurable disease is by serum FLC levels: CR in such patients is defined as a normal FLC ratio of 0.26–1.65 in addition to CR criteria listed above. VGPR in such patients is defined as a >90% decrease in the difference between involved and uninvolved free light chain (FLC) levels.
[a] Confirmation with repeat bone marrow biopsy not needed.
[b] Presence/absence of clonal cells is based upon the kappa/lambda ratio. An abnormal kappa/lambda ratio by immunohistochemistry and/or immunofluorescence requires a minimum of 100 plasma cells for analysis. An abnormal ratio reflecting presence of an abnormal clone is kappa/lambda of > 4:1 or < 1:2.
[c] All relapse categories require two consecutive assessments made at anytime before classification as relapse or disease progression and/or the institution of any new therapy. In the IMWG criteria, CR patients must also meet the criteria for progressive disease shown here to be classified as progressive disease for the purposes of calculating time to progression and progression-free survival. The definitions of relapse, clinical relapse and relapse from CR are not to be used in calculation of time to progression or progression-free survival.
[d] For progressive disease, serum M-component increases of ≥1 gm/dL are sufficient to define relapse if starting M-component is≥5 g/dL.
[e] Relapse from CR has the 5% cut-off versus 10% for other categories of relapse.
[f] For purposes of calculating time to progression and progression-free survival, CR patients should also be evaluated using criteria listed above for progressive disease.

Abbreviations: CR, complete response; FLC, free light chain; M, monoclonal; MR, minimal response; PD, progressive disease; PR, partial response; sCR, stringent complete response; SD, stable disease; VGPR, very good partial response.

7.2 Study Endpoints

Primary Endpoint

Occurrence of adverse events, per NCI CTC v4 guidelines, including ≥ grade 4 laboratory toxicities at any time from day-40 until year 1. This will include infusional toxicity, and any toxicity probably or definitely related to NYESO-1-c259-T including but not limited to:

a. Fevers
b. Rash
c. Neutropenia, thrombocytopenia, anemia, marrow aplasia
d. Hepatic dysfunction
e. Pulmonary infiltrates or other pulmonary toxicity
f. Development of GVHD

NOTE: transplant-related toxicities, typically occurring within a month post-transplant, are excluded as study related adverse events.

Secondary Endpoints

Secondary endpoints will evaluate correlates of treatment efficacy by measuring clinical response rates to treatment:

a) progression-free survival (PFS) and duration of response (DOR),
b) objective response rate (ORR) at day 42, 100, 180, 270 and 360.
c) best objective response (BOR) prior to initiating lenalidomide and at day 360.
d) overall survival (OS) will be followed in this interventional protocol and continue to be followed in the long-term follow-up protocol, once subjects have transferred.

Initiation of lenalidomide as maintenance treatment will be addressed in a sensitivity analysis.

Exploratory Endpoints

Exploratory endpoints will evaluate correlates of treatment efficacy by measuring (i) the appearance of target antigen/MHC loss variants upon disease recurrence, as well as (ii) immunological parameters associated with product persistence, bioactivity and functionality.

(i). Appearance of target antigen/MHC loss variants upon disease recurrence will be evaluated by quantifying expression of targeted antigens and MHC in marrow samples obtained on disease recurrence, and comparing those values to the pre-treatment (diagnosis) samples. NY-ESO-1 and MHC expression will be evaluated by RNA and/or protein-based assays on tumor samples.

(ii) Immunological parameters associated with product bioactivity and functionality will measure selective migration and engraftment of gene-modified infused cells to the marrow post infusion, the ex-vivo immune functionality and phenotype of infused cells in marrow and periphery, the modulation of cytokine milieu in marrow and periphery at baseline, and post T cell infusion and post lenalidomide treatment as well as the development of an expanded patient immune response against tumor via epitope spreading

7.3 Statistical Analyses

7.3.1 Sample size determination

Since the primary endpoint is the occurrence of study related adverse events, the sample size determination is based on testing the null hypothesis that the rate of study related adverse events is ≥40% against the alternative that this rate is <40%. Here a significant level of 25% is chosen because this study is an early stage feasibility trial meant to be tested further in future studies (Stallard et al., 2001). Hence, by using the two-stage procedure by Simon (Simon, 1989) with such a significance level and a power of 85% to reject the null when the true rate of study related adverse events is 10%, a maximum of 6 patients for each cohort are needed. At the first stage, 3 patients per cohort will be entered. If 2 or more patients experience study related adverse events, we will conclude that the treatment is not worth pursuing. Otherwise, an additional 3 patients will be entered for the second stage. If there are 3 or more patients experiencing study related adverse events for the full two stages of 6 patients, we will conclude that the treatment is not worth pursuing. If there are 2 or less patients experiencing study related adverse effects out of a total of 6 patients, the treatment will be considered worthy of further study.

If a patient is withdrawn from the study **before** infusion of the gene-modified T-cells then an additional patient may be enrolled so that, safety-permitting, up to 26 patients can be evaluated. Patients receiving the low dose (1-10 x 10e8) will be replaced with another patient so that a total of 26 patients will receive the target cell dose of >1-10e9.

7.3.2 Data analysis plan and statistical power

Two sided paired t-tests will be used to evaluate within-subject difference for measurements of interest (not including binary or time to event endpoints) as mentioned in the Secondary Objectives. Some differences are between marrow and periphery at each time point and some are between pre- and post-treatment time points. Linear mixed effects model will be employed to estimate the trends of measurements of interest over time as appropriate. Thus an evaluable sample size of 6 will provide 80% power at a significance level of 5% to detect an effect size of 1.5 for normally distributed measurements using a two-sided paired t-test. We expect the preliminary results from 6 patients will provide sufficient data to support the design of an adequately powered follow-on Phase II clinical trial.

For evaluation of the response rate of the extension arm in comparison to the Krishnan et al published controls, at the time of the extension phase for the NYESO-1 arm, responses of 4 patients (out of the initial 6 patients) are pending. Therefore, taking a conservative approach, with an additional 20 patients we will have 24 patients for analysis. Based on the prior studies (Krishnan et al., 2011), the complete response (CR) rate after autologous stem cell transplant in patients is estimated to be 36%. We expect that the CR rate in this trial will be 66%. Using the one-sided Chi-square test, we have 91.6% power to detect this meaningful difference at a significant level of 5%. With only 20 patients, we have 85.8% power to detect this meaningful difference at a significant level of 5%.

7.3.3 Study Populations

Intent-to-Treat (ITT) population: all subjects who are enrolled in the study.
Modified Intent-to-Treat (mITT) population: all ITT subjects who receive NY-ESO-1c259T cell infusion.
The mITT population is the primary analysis population for efficacy and safety evaluations.
If the ITT and mITT populations are identical, only analyses associated to the mITT population will be reported.

A per-protocol population (PP) may be included if there are subjects in the mITT population who have protocol violations that are expected to affect efficacy assessments (e.g., subjects enrolled who do not meet key eligibility criteria) during the trial. Protocol violators resulting in exclusion from the per-protocol population will be identified and documented prior to database lock.

7.3.4 Statistical Methods for Efficacy Endpoints

Efficacy endpoints are defined in sections 1.2 and 1.3.

Binary endpoints such as ORR, BOR will be analyzed as follows using Investigator assessments of responses in accordance with [Rajkumar, 2011].
Subjects with unknown or missing response will be treated as non-responders, i.e. they will be included in the denominator when calculating the proportion.
These endpoints will be summarized using Clopper-Pearson 95% (exact) confidence interval (CI). In addition, 95% confidence intervals will also be computed using the Wilson method.

Time to event endpoints are defined as:

- Progression Free Survival (PFS) is the interval between T-cell infusion (Day 2) and the earliest date of disease progression or death due to any cause.
- Overall Survival (OS), is the interval between T-cell infusion (Day 2) and death.
- Duration of Response (DOR), defined as the time from the initial date of the confirmed response to the date of progressive disease or death.

No hypothesis testing is planned for these secondary endpoints.

Time to event endpoints will be summarized and displayed graphically using Kaplan-Meier (K-M) methodology to estimate the median, and the 25th and 75th percentiles. Two-sided 95% confidence intervals will be produced.

Censoring rules: The following censoring rules will be applied in the analyses.

- For PFS, subjects who do not have a documented date of disease progression or death will be censored at the date of the last assessment.
- For OS, subjects who are lost to follow-up or still alive will be censored at the date of last contact.
- For DOR, subjects who are still alive and who do not have a documented disease progression will be censored at the date of the last assessment.

The proportion of censored observations will be summarized.

7.3.5 Statistical Methods for Safety Endpoints

Descriptive statistics will be provided for disposition, demography, exposure, safety and laboratory assessments.

The safety profile will be based on adverse events including serious adverse events

Descriptive statistics on continuous data will include means, medians, standard deviations, and ranges, while categorical data will be summarized using frequency counts and percentages. Graphical summaries of the data may be presented.

All adverse events including SAEs will be listed and coded by appropriate version of MedDRA. The number and percent of subjects reporting any adverse events will be tabulated by system organ class and preferred term. Adverse events will be further classified by severity, relationship to treatment and seriousness in tabulation. Tables and/or narratives of any death, or serious or significant adverse event, including early withdrawals because of adverse events, will be provided should they occur.

Laboratory tests will be summarized using appropriate statistics.

No hypothesis tests of these data will be done.

7.4 Stopping Rules for Treatment Toxicities

The trial will be suspended (and/or resumed), pending discussion with Sponsor and Investigators and to allow protocol review with institutional IRBs, as well as the DSMC if any of the following occurs among patients who receive the study drug:

- Any death deemed to be probably or definitely related to the investigational medication/cell product by the principal investigator and study sponsor;
- Any grade 4 events deemed to be probably or definitely related to the investigational medication/cell product by the principal investigator and study sponsor. Please note: this criterion excludes autoimmune adverse events. For autoimmune adverse events, two (2) or more grade 4 adverse events judged to be probably, or definitely related to the investigational medication/cell product by the principal investigator and study sponsor must occur prior to accrual to study be suspended;
- Two (2) or more grade 4 autoimmune events deemed to be probably, or definitely related to the investigational medication/cell product by the principal investigator and study sponsor;

Or

- An apheresis confirmed positive biological RCL occurs and no additional lot of vector is available (see section on Monitoring for and Management of RCL)

7.5 TOXICITY ASSESSMENTS AND DATA REPORTING

7.5.1 Toxicities Related to Investigational Therapies

The adverse event reporting period starts at day -40 visit and ends at 1 year after T cells infusion.

During long term follow-up (years 1 - 15), patients will only be monitored for delayed adverse events related to the gene transfer aspect of the protocol (See section on long term follow up).

Toxicities or adverse events, which are defined as any symptom, sign, illness or experience that develops or worsens in severity over the course of the study, will be primarily assessed in relation to the investigational parts of this trial. The investigational parts include i) infusions of the gene-modified T-cells. These toxicities will be graded in accordance with the NIH Common Toxicity Criteria (CTC) (version 4.0). A copy of the CTCAE Version 4.0 can be downloaded from the CTEP homepage (HTTP://CTEP.INFO.NIH.GOV). All appropriate treatment areas should have access to a copy of the CTCAE Version 4.0. All adverse clinical experiences, whether observed by the investigator or reported by the patient, must be recorded, with details about the duration and intensity of each episode, the action taken with respect to the test drug, and the patient's outcome. The investigator must evaluate each adverse experience for its relationship to the test agents and for its seriousness.

The investigator must appraise all abnormal laboratory results for their clinical significance. If any abnormal laboratory result is considered clinically significant, the investigator must provide details about the action taken with respect to the test agents and about the patient's outcome.

A pre-existing condition, defined as one that is present at the start of the study, should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period. Worsening of a pre-existing condition after enrollment in the study but prior to the initiation of study treatment will be recorded in the medical record but not reported as an adverse event.

Post-Study Adverse Event

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

7.5.2 Toxicity Causation

All adverse experience reports must include the patient number, age, sex, weight, severity of reaction (mild, moderate, severe), relationship to drug (probably related, unknown relationship, definitely not related), date and time of administration of test medications and all concomitant medications, and medical treatment provided. The investigator is responsible for evaluating all adverse events to determine whether criteria for "serious" and as defined above are present. The investigator is responsible for reporting lenalidomide adverse events to Celgene as described below.

Each toxic event will be considered for relationship to the T-cell infusions and to the peptide immunizations and lenalidomide as follows:

7.5.2.1 Unrelated

This category applies to those adverse events which after careful medical consideration are clearly felt to be due to extraneous causes. Operationally, this may be inferred from reversal of the adverse event following treatment of the putative extraneous cause.

7.5.2.2 Unlikely (must satisfy 1 and 2 and 3)

This category applies to those adverse events which, after careful medical and sponsor consideration, are felt unlikely to be related to the investigational agent based on the following criteria:

- It does not follow a reasonable temporal sequence from administration of the investigational agent.
- It could readily have been a result of the patient's condition, environmental or toxic factors, or other therapies administered to the patient.
- No causative mechanism for the investigational agent can be identified.

7.5.2.3 Possible (must satisfy 1,2 and 3)

This category applies to those adverse events which, after careful medical and sponsor consideration, are felt unlikely to be related to the investigational agent, but the possibility cannot be ruled out with certainty. An adverse event can be considered to be possibly related if:

- It follows a reasonable temporal sequence from administration of the investigational agent.
- It could reasonably have been a result of the patient's clinical condition, environmental or toxic factors, or other therapies administered to the patients
- A possible causative mechanism for the investigational agent can be identified.

7.5.2.4 Probable (must satisfy 1,2 and 3)

This category applies to those adverse events which, after careful medical and sponsor consideration, are felt to be related to the investigational agent with a high degree of certainty. Adverse event can be considered probably related if:

- It follows a reasonable temporal sequence from administration of the investigational agent.

- It could not be reasonably explained by other clinical conditions or factors.
- A probable causative mechanism for the investigational agent can be identified.

7.5.2.1 Definite (must satisfy 1 and 2 and 3)

This category applies to those adverse events which, after careful medical and sponsor consideration, are felt to be related to the investigational agent. An adverse event can be considered to be definitely related if:

- It follows a reasonable temporal sequence from administration of the investigational agent.
- There are no confounding clinical conditions or factors which could explain the event.
- A probable causative mechanism for the investigational agent can be identified.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others

Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e., not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc).
- Related or possibly related to participation in the research (i.e., possibly related means there is a reasonable possibility that the incident experience or outcome may have been caused by the procedures involved in the research).
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

Adverse Event

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal.
- is associated with a serious adverse event.
- is associated with clinical signs or symptoms.
- leads to additional treatment or to further diagnostic tests.
- is considered by the investigator to be of clinical significance.

Suspected Adverse Reaction(21 CFR 312.32(a))
Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Unexpected Suspected Adverse Reaction (21 CFR 312.32(a))

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Serious Adverse Events

A Serious Adverse Event (SAE) is any AE that is:

- Fatal
- Life-threatening[1]
- Requires or prolongs hospital stay
- Results in persistent or significant disability or incapacity[2]
- A congenital anomaly or birth defect
- An important medical event[3]

[1]"Life-threatening" means that the subject was at immediate risk of death at the time of the serious adverse event; it does not refer to a serious adverse event that hypothetically might have caused death if it were more severe.

[2]"Persistent or significant disability or incapacity" means that there is a substantial disruption of a person's ability to carry out normal life functions.

[3]Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in situations where none of the outcomes listed above occurred. Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above should also usually be considered serious. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse. A new diagnosis of cancer during the course of a treatment should be considered as medically important.

All Serious Adverse Events will be recorded and reported to the Sponsor, to IRBs, DSMCs and IBCs of both collaborating institutions (University of Maryland and University of Pennsylvania) in

accordance with standard reporting procedures. SAEs will be reported to the FDA and NIH OBA by the sponsor in accordance with established guidelines. .The following table also shows the expedited reporting guidelines for the University of Maryland (unless otherwise specified) of AEs to the IRBs and IBCs. If not listed then the event need not be reported in an expedited fashion (also see "Pregnancies" section and Lenalidomide next page).

Attribution	Grade II	Grade III	Grade III	Grade IV-V
	Unexpected	Expected	Unexpected	Expected & Unexpected
Unrelated Unlikely	Not required	10 days (if pt hospitalized only)	10 calendar days (if hospitalized)	5 calendar days
Possible Probable Definite	10 calendar days	10 days (if pt hospitalized only)	5 calendar days	5 calendar days

8.2 Recording of Adverse Events

Adverse events will be assessed using CTC V4. At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded (section 8.3). The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

8.3 Adverse Event Reporting Period and Adverse Event Reporting

Patients will be monitored for the duration of the intervention protocol (Day -40 visit until relapse/progression or until 1 year, whichever comes first). For patients who remain disease progression-free beyond Year 1 patients will only be monitored for delayed adverse events related to the gene transfer aspect of the protocol (see Section 6.4 for definition) until disease progression or until transferred into the long-term follow-up protocol. Post disease progression, patients will be rolled over to the LTFU protocol ADP-0000-002, when available at the site. If the protocol ADP-0000-002 is not available, patients will continue to be followed as described in Appendix C.

All serious adverse events must be reported to the study sponsor. Any study related unanticipated problem posing risk of harm to subjects or others and is considered serious. Any study-related

unanticipated problem posing risk of harm to subjects or others, and any type of serious adverse event, must be reported to the study sponsor within 24 hours of knowledge of the event.

For specific instructions for reporting SAEs to the Sponsor, please see the Manual of Procedures for the study, or contact the study manager at Adaptimmune.

8.3.1 Investigator Reporting: Notifying the IRB

Investigators at each site are responsible for safety reporting to their local IRB. Investigators are responsible for complying with their local IRB's reporting requirements, though must submit the required reports to their IRB no later than 10 working days. Copies of each report and documentation of IRB notification and receipt will be kept in the investigator's study file.
Sponsor Responsibility for Reporting Adverse Events

All adverse events will be reported by the Sponsor to regulatory agencies, and investigators in accordance with all applicable laws and regulations.

8.3.2 Investigator reporting: notifying the Penn IRB and IBC

This section describes the requirements for safety reporting by investigators who are Penn faculty, affiliated with a Penn research site, or otherwise responsible for safety reporting to the Penn IRB and IBC. The University of Pennsylvania IRB and IBC (Penn IRB/IBC) requires expedited reporting of those events related to study participation that are unforeseen and indicate that participants or others are at increased risk of harm. The Penn IRB will not acknowledge safety reports or bulk adverse event submissions that do not meet the criteria outlined below. The Penn IRB requires researchers to submit reports of the following problems within 10 working days from the time the investigator becomes aware of the event:

- Any adverse event (regardless of whether the event is serious or non-serious, on-site or off-site) that occurs any time during or after the research study, which in the opinion of the principal investigator is:

  Unexpected (An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.)

  AND

  Related to the research procedures (An event is "related to the research procedures" if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.)

Reporting Process

Unanticipated problems posing risks to subjects or others as noted above will be reported to the Penn IRB using the form: "Unanticipated Problems Posing Risks to Subjects or Others Including Reportable Adverse Events" or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria,

follow-up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

Reporting Deaths: more rapid reporting requirements

Concerning deaths that occur during the course of a research study, the following describes the more rapid reporting requirement of the Penn IRB for specific situations:

- Report the event within 24 hours when the death is unforeseen (unexpected) and indicates participants or others are at increased risk of harm.
- Report the event within 72 hours, for all other deaths, regardless of whether the death is related to study participation.

For reportable deaths, the initial submission to the Penn IRB may be made by contacting the IRB Director or Associate Director. The AE/Unanticipated Problem Form is required as a follow up to the initial submission.

Other Reportable events:

For clinical drug trials, the following events are also reportable to the Penn IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
  - An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
  - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
  - A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.

- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

8.3.3 Investigator reporting: notifying the Penn DSMC

All events meeting the DSMC reporting requirements must be entered into the mandatory Velos AE/SAE form. A study CRF does not replace the ACC central reporting form.

Once an event is entered, please send an email alert to Doris Shank doris.shank@uphs.upenn.edu. If your study has prospective DOCM monitoring (as determined by the CTSRMC) you should notify your study monitor.

Every effort should be made to report an as a diagnosis, not as list of symptoms. Symptoms that led to the diagnosis should be included in the event description, but should not be the actual event.

Do not leave any of the fields blank.

Once an event is reported, you must keep the information accurate and current in Velos. If new/updated information is learned about the event, the event should be amended or corrected promptly.

Off-site (external sponsors)
• All **SAEs** (on the specific protocol opened here only) defined as reportable in the protocol for Phase I and II studies must be reported to the DSMC.
• SAEs on cooperative or industry sponsored **Phase III** studies do not have to be reported.
• SAEs on Other Externally Peer Reviewed Phase III have to be reported if the protocol defines them as reportable.

Note: The DSMC reserves the right to modify the reporting window for certain types of studies i.e. gene therapy, adoptive therapies, Penn manufactured vaccines etc.

8.3.4 Investigator reporting: Notifying a non-Penn IRB (See table on page 67 for UMD IRB reporting guidelines)

Investigators who are not Penn faculty or affiliated with a Penn research site are responsible for safety reporting to their local IRB.

8.3.5 Sponsor Reporting: Notifying the FDA

The study sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND safety reports. The sponsor must report an IND safety reports as described in:

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM227351.pdf

The following describes the safety reporting requirements by timeline for reporting and associated type of event:

- Within 7 Calendar Days
  Any study event that is:
  - Unexpected fatal or life-threatening suspected adverse reaction. Including all deaths and grade 4 non-hematological events irrespective of attribution occurring within 30 days of study drug administration.
- Within 15 Calendar Days
  Any study event that is:
  - unexpected
  - Suspected adverse reaction that is serious, but not fatal or life-threatening

  -or-
  - a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

Any finding from tests in laboratory animals that:

- suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity or reports of significant organ toxicity at or near the expected human exposure.

Increase in rate of occurrence of serious suspected adverse reactions:

- any clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol or investigator brochure.

Additional Reporting Requirements
Sponsor is also required to review all adverse events to make a causality determination on the basis of information from investigators and report these findings to the FDA in accordance with 21 CFR 312.32.

If the adverse event does not meet expedited reporting requirements, the Sponsor will report the SAE as in the IND Annual Report.

8.4 Investigator Reporting Responsibilities

The conduct of the study will comply with all FDA safety reporting requirements.

IND Annual Reports

If the FDA has granted an IND number, it is a requirement of 21 CFR 312.33, that an annual report is provided to the FDA within 60-days of the IND anniversary date. 21 CRF 312.33 provides the data elements that are to be submitted in the report. The Annual Report should be filed in the study's Regulatory Binder.

In the event of a grade 4 or 5 unexpected event the PI and the Research Coordinator will convene a meeting with the study team or have a teleconference to thoroughly discuss the adverse event. This meeting will be convened within 24 business hours of knowledge of the grade 4 or 5 unexpected event and will not occur via email nor will the sponsor be involved in discussions about attribution. Meeting minutes capturing the review of and ongoing investigations of the grade 4 or 5 unexpected event, including next steps in the management of the subject and any proposed changes to the protocol will be forwarded to the DSMC.

8.5 Coordination of Patient Registration/Reporting

To register a patient, the following documents should be completed by the research nurse or data manager and sent to the sponsor:

1) Copies of screening documents (laboratory tests, radiology, PE, History, Concomitant Medications)
2) Copy of signed patient consent form
3) Copy of HIPAA authorization form
4) Eligibility checklist
5) Enrollment Form

The research nurse or data manager at each site will then call or email the Monitor at the Sponsor to confirm eligibility. Afterwards, the patient will be assigned a study number and will formally register on the study.

Clinical trial event scheduling and data collection and storage will be supported by Velos software. CRFs (case report forms) for each study event will be completed and submitted to Velos on-line or within time specified by study contract.

8.6 Protocol Deviations

Exception

A one time, **intentional** action or process that departs from the IRB and CTSRMC approved study protocol, intended for **one** occurrence. If the action disrupts the study progress, such that the study design or outcome (endpoints) may be compromised, or the action compromises the safety and welfare of study subjects, **advance** documented IRB and DSMC approval is required.

For this study, there will be no exceptions to eligibility granted for this study.

Deviation

Protocol deviations are defined as any variation from the requirements and procedures described in the study protocol and protocol amendments that occurs during the conduct of the study including deviations from ICH, GCP and local applicable regulatory requirements and laws.

Any departure from the protocol that meets the following criteria should be submitted to the Sponsor:

- Investigational Product
- Concomitant Medications
- Laboratory Procedures
- Study visit schedules (safety / endpoints)
- Serious adverse events reporting
- Stopping criteria
- Protocol specific discontinuation criteria

9 THERAPEUTIC AND INVESTIGATIONAL AGENTS

This section will describe all the therapeutic and investigational agents to be administered to the patient, including dose, route of administration, and possible adverse reactions that may be observed.

9.1 Lenalidomide: Drug information

PHARMACOLOGICCATEGORY
Antiangiogenesis Agent Tumor Necrosis Factor (TNF) Blocking Agent.

The mechanism of action of lenalidomide remains to be fully characterized. Lenalidomide possesses immunomodulatory and antiangiogenic properties. Lenalidomide inhibited the secretion of pro-inflammatory cytokines and increased the secretion of anti-inflammatory cytokines from peripheral blood mononuclear cells. Lenalidomide inhibited cell proliferation with varying effectiveness (IC50s) in some but not all cell lines. Of cell lines tested, lenalidomide was effective in inhibiting growth of Namalwa cells (a human B cell lymphoma cell line with a deletion of one chromosome 5) but was much less effective in inhibiting growth of KG-1 cells (human myeloblastic cell line, also with a deletion of one chromosome 5) and other cell lines without chromosome 5 deletions. Lenalidomide inhibited the expression of cyclooxygenase-2 (COX-2) but not COX-1 in vitro.

PHARMACOKINETICS AND DRUG METABOLISM OF LENALIDOMIDE:

Absorption:Lenalidomide, in healthy volunteers, is rapidly absorbed following oral administration with maximum plasma concentrations occurring between 0.625 and 1.5 hours post-dose. Co administration with food does not alter the extent of absorption (AUC) but does reduce the maximal plasma concentration (Cmax) by 36%. The pharmacokinetic disposition of lenalidomide is linear. Cmax and AUC increase proportionately with increases in dose. Multiple dosing at the recommended dose-regimen does not result in drug accumulation.

Pharmacokinetic analyses were performed on 15 multiple myeloma patients treated in the phase I studies. Absorption was found to be rapid on both Day 1 and Day 28 with time to maximum blood levels ranging from 0.7 to 2.0 hours at all dose levels (5mg, 10mg, 25mg, and 50mg). No plasma accumulation was observed with multiple daily dosing. Plasma lenalidomide declined in a monophasic manner with elimination half-life ranging from 2.8 to 6.1 hours on both Day 1 and 28 at all 4 doses. Peak and overall plasma concentrations were dose proportional over the dosing range of 5mg to 50mg. Exposure (AUC) in multiple myeloma patients is 57% higher than in healthy male volunteers.

Pharmacokinetic Parameters:

Distribution: In vitro (14C)-lenalidomide binding to plasma proteins is approximately 30%.

Metabolism and Excretion: The metabolic profile of lenalidomide in humans has not been studied. In healthy volunteers, approximately two-thirds of lenalidomide is eliminated unchanged through urinary excretion. The process exceeds the glomerular filtration rate and therefore is partially or entirely active. Half-life of elimination is approximately 3 hours.

ADMINISTRATION

Administer with water. Swallow capsule whole; do not break, open, or chew. If a dose of lenalidomide is missed, it should be taken as soon as possible on the same day. If it is missed for the entire day, it should not be made up.

Patients who take more than the prescribed dose of lenalidomide should be instructed to seek emergency medical care if needed and contact study staff immediately.

SUPPLIER

Celgene Corporation will supply Revlimid® (lenalidomide) to study participants through the RevAssist® program.The RevAssist program is a restricted distribution program under which lenalidomide is provided by Celgene. The reason for this program is to track and control the use of lenalidomide since it is a derivative of thalidomide and therefore there are serious reproductive safety concerns. The purpose of the program is to ensure that patients receiving, and physicians administering, lenalidomide are aware of these concerns and take precautions to avoid prescribing lenalidomide while pregnant, and to take necessary precautions to avoid pregnancy while on lenalidomide.

The program requires any prescriber of lenalidomide to be registered with the program. Patients must also be enrolled in the program and agree to comply with the requirements of the program as described in the informed consent. In addition, only RevAssist contract pharmacies are able to provide lenalidomide. Additional details and information packets regarding lenalidomide and the RevAssist program can be found at http://www.revlimid.com.

PACKAGING

Lenalidomide will be shipped directly to patients. Bottles will contain a sufficient number of capsules for one cycle of dosing.

STORAGE

Lenalidomide should be stored at room temperature away from direct sunlight and protected from excessive heat and cold.

PRESCRIBING INFORMATION

Lenalidomide will be provided in accordance with the RevAssist® program of Celgene Corporation. Patient insurance will be charged for the lenalidomide prescription. Per standard RevAssist® requirements all physicians who prescribe lenalidomide for research subjects enrolled into this trial, and all research subjects enrolled into this trial, must be registered in and must comply with all requirements of the RevAssist® program. Prescriptions must be filled within 7 days. Only enough lenalidomide for one cycle of therapy will be supplied to the patient each cycle.

ADVERSE REACTIONS SIGNIFICANT

>10%:

Cardiovascular: Peripheral edema (8% to 21%). **Central nervous system:** Fatigue (31% to 38%), insomnia (10% to 32%), pyrexia (21% to 23%), dizziness (20% to 21%), headache (20%). **Dermatologic:** Pruritus (42%), rash (16% to 36%), dry skin (14%). **Endocrine & metabolic:** Hyperglycemia (15%), hypokalemia (11%). **Gastrointestinal:** Diarrhea (29% to 49%), constipation (24% to 39%), nausea (22% to 24%), weight loss (18%), dyspepsia (14%), anorexia (10% to 14%), taste perversion (6% to 13%), abdominal pain (8% to 12%). **Genitourinary:** Urinary tract infection (11%). **Hematologic:** Thrombocytopenia (17% to 62%; grades 3/4: 10% to 50%), neutropenia (28% to 59%; grades 3/4: 21% to 53%), anemia (12% to 24%; grades 3/4: 6% to 9%); myelosuppression is dose-dependent and reversible with treatment interruption and/or dose reduction. **Neuromuscular & skeletal:** Muscle cramp (18% to 30%), weakness (15% to 23%), arthralgia (10% to 22%), back pain (15% to 21%), tremor (20%), paresthesia (12%), limb pain (11%). **Ocular:** Blurred vision (15%). **Respiratory:** Nasopharyngitis (23%), cough (15% to 20%), dyspnea (7% to 20%), pharyngitis (16%), epistaxis (15%), upper respiratory infection (14% to 15%), pneumonia (11% to 12%)

1% to 10%:

Cardiovascular: Edema (10%), deep vein thrombosis (≤8%; grades 3/4: 7%), hypertension (6%), chest pain (5%), palpitation (5%), atrial fibrillation (grades 3/4: 3%), syncope (grade 3: 2%). **Central nervous system:** Hypoesthesia (7%), pain (7%), depression (5%). Dermatologic: Bruising (5% to 8%), cellulitis (5%), erythema (5%). **Endocrine & metabolic:** Hypothyroidism (7%), hypomagnesemia (6%), hypocalcemia (grades 3/4: 4%). **Gastrointestinal:** Vomiting (10%), xerostomia (7%), loose stools (6%). **Genitourinary:** Dysuria (7%). **Hematologic:** Leukopenia (8%; grade 3: 4%), febrile neutropenia (5%), lymphopenia (grade 3: 2%). **Hepatic:** ALT increased (8%). **Neuromuscular & skeletal:** Myalgia (9%), rigors (6%), neuropathy (peripheral 5%). **Respiratory:** Sinusitis (8%), rhinitis (7%), bronchitis (6%), pulmonary embolism (≤3%; grades 3/4: 3%). **Miscellaneous:** Night sweats (8%), diaphoresis increased (7%)

<1% (Limited to important or life-threatening):

Acute febrile neutrophilic dermatosis, acute leukemia, acute myeloid leukemia (AML), adrenal insufficiency, angina, angioedema, aortic disorder, aphasia, azotemia, bacteremia, Basedow's disease, biliary obstruction, blindness, bone marrow depression, bradycardia, brain edema, C-reactive protein decreased, cardiac arrest, cardiac failure, cardiogenic shock, cardiomyopathy, cardiopulmonary arrest, cellulitis, cerebellar infarction, cerebral infarction, cerebrovascular accident, CHF, cholecystitis, chondrocalcinosis, chronic obstructive airway disease, circulatory collapse, coagulopathy, colonic polyp, dehydration, delirium, delusion, diabetes mellitus, diabetic ketoacidosis, diverticulitis, dysphagia, encephalitis, erythema multiforme, gait abnormal, gastritis, gastroenteritis, gastrointestinal hemorrhage, gout, hematuria, hemoglobin decreased, hemolysis, hemolytic anemia, hemorrhage, hepatitis, herpesvirus infection, hyperbilirubinemia, hypernatremia, hypersensitivity, hypoglycemia, hypotension, hypoxia, infection, INR increased, interstitial lung disease, intestinal perforation, intracranial hemorrhage, intracranial venous sinus thrombosis, irritable bowel syndrome, ischemia, ischemic colitis, leukoencephalopathy, liver failure, liver function tests

abnormal, lung cancer, lung infiltration, lymphoma, melena, MI, migraine, myocardial ischemia, myopathy, neutropenic sepsis, orthostatic hypotension, pancreatitis, pancytopenia, performance status decreased, peripheral ischemia, phlebitis, post procedural hemorrhage, pseudomembraneous colitis, pulmonary edema, rectal hemorrhage, refractory anemia, renal calculus, renal failure, renal mass, renal tubular necrosis, respiratory failure, septic shock, sepsis, serum creatinine increased, skin desquamation, small bowel obstruction, somnolence, spinal cord compression, splenic infarction, Stevens-Johnson syndrome, stomatitis, subarachnoid hemorrhage, supraventricular arrhythmia, tachyarrhythmia, thrombophlebitis, thrombosis, toxic epidermal necrolysis, transient ischemic attack, troponin I increased, urinary retention, urosepsis, urticaria, ventricular dysfunction, wheezing

CONTRAINDICATIONS
Hypersensitivity to lenalidomide or any component of the formulation; pregnancy or women capable of becoming pregnant; patients unable to comply with the RevAssist^{SM} program

WARNINGS / PRECAUTIONS
Boxed warnings: Hematologic toxicity: See "Concerns related to adverse effects" below.
Pregnancy: See "Special populations" below. Thrombosis/embolism: See "Concerns related to adverse effects" below.

Special handling: Hazardous agent: Use appropriate precautions for handling and disposal.

Concerns related to adverse effects:
CNS effects: May cause dizziness or fatigue; caution patients about performing tasks which require mental alertness (eg, operating machinery or driving).

Hematologic toxicity: [U.S. Boxed Warning]: Hematologic toxicity (neutropenia and thrombocytopenia) occurs in a majority of patients (grade 3/4: 80%) and may require dose reductions and/or delays; the use of blood product support and/or growth factors may be needed.
Thromboembolic events: [U.S. Boxed Warning]: Lenalidomide has been associated with a significant increase in risk for thrombosis and embolism in multiple myeloma patients treated with combination therapy. Deep vein thrombosis (DVT) and pulmonary embolism (PE) have occurred; monitor for signs and symptoms of thromboembolism (shortness of breath, chest pain, or arm or leg swelling) and seek prompt medical attention with development of these symptoms.

Disease-related concerns:
Renal impairment: Use with caution in patients with renal impairment; may experience an increased rate of toxicities.

Special populations:
Pediatrics: Safety and efficacy have not been established in children.

Pregnancy: [U.S. Boxed Warning]: Lenalidomide is an analogue of thalidomide (a human teratogen) and could potentially cause birth defects in humans. Distribution is restricted; physicians, pharmacists, and patients must be registered with the RevAssist^{SM} program.

Other warnings/precautions:
Blood donation: Patients should be advised not to donate blood during therapy and for 4 weeks following completion of therapy.

Lactose intolerance: Product may contain lactose; avoid use in patients with Lapp lactase deficiency, glucose-galactose malabsorption, or glucose intolerance.

RESTRICTIONS

Lenalidomide is approved for marketing in the U.S. only under a Food and Drug Administration (FDA) approved, restricted distribution program called RevAssist^{SM} (www.REVLIMID.com or 1-888-423-5436). In Canada, distribution is restricted through RevAid^{SM} (www.RevAid.ca or 1-888-738-2431). Physicians, pharmacies, and patients must be registered; a maximum 28-day supply may be dispensed; a new prescription is required each time it is filled; pregnancy testing is required for females of childbearing potential.

An FDA-approved medication guide must be distributed when dispensing an outpatient prescription (new or refill) where this medication is to be used without direct supervision of a healthcare provider. Medication guides are available at http://www.fda.gov/cder/Offices/ODS/medication_guides.htm.

DRUG INTERACTIONS

Abatacept: Anti-TNF Agents may enhance the adverse/toxic effect of Abatacept. An increased risk of serious infection during concomitant use has been reported. Risk D: Consider therapy modification

Anakinra: Anti-TNF Agents may enhance the adverse/toxic effect of Anakinra. An increased risk of serious infection during concomitant use has been reported. Risk X: Avoid combination

Dexamethasone: May enhance the thrombogenic effect of Lenalidomide. Risk D: Consider therapy modification

Echinacea: May diminish the therapeutic effect of Immunosuppressants. Risk D: Consider therapy modification

Natalizumab: Immunosuppressants may enhance the adverse/toxic effect of Natalizumab. Specifically, the risk of concurrent infection may be increased. Risk X: Avoid combination

Rilonacept: Anti-TNF Agents may enhance the adverse/toxic effect of Rilonacept. Risk X: Avoid combination

Trastuzumab: May enhance the neutropenic effect of Immunosuppressants. Risk C: Monitor therapy

Vaccines (Inactivated): Immunosuppressants may diminish the therapeutic effect of Vaccines (Inactivated). Risk C: Monitor therapy

Vaccines (Live): Immunosuppressants may enhance the adverse/toxic effect of Vaccines (Live). Vaccinal infections may develop. Immunosuppressants may also decrease therapeutic response to vaccines. Risk X: Avoid combination

PREGNANCY IMPLICATIONS

[U.S. Boxed Warning]: Lenalidomide is an analogue of thalidomide (a human teratogen) and could potentially cause birth defects in humans. Animal studies with lenalidomide are ongoing; there are no adequate and well-controlled studies in pregnant women. Women of childbearing potential should be treated only if they are able to comply with the conditions of the RevAssist^{SM} program. Two forms of effective contraception are required beginning 4 weeks prior to, during, and for 4 weeks after therapy and during therapy interruptions. Pregnancy tests (sensitivity of at least 50 mIU/mL) should be performed 10-14 days and 24 hours prior to beginning therapy; weekly for the first 4 weeks and every 4 weeks (every 2 weeks if menstrual cycle irregular) thereafter and during therapy interruptions. Lenalidomide must be immediately discontinued and the patient referred to a reproductive toxicity specialist if pregnancy occurs during treatment. Males (even those vasectomized) should use a latex condom during any sexual contact with women of childbearing age. Risk to the fetus from semen of male patients is unknown. The parent or legal guardian for patients between 12 and 18 years of age must agree to ensure compliance with the required guidelines. Any suspected fetal exposure should be reported to the FDA via the MedWatch program (1-800-FDA-1088) and to Celgene Corporation (1-888-423-5436)

LACTATION

Excretion in breast milk unknown/not recommended

DIETARY CONSIDERATIONS

Should be taken at least 1 hour after the evening meal.

MONITORING PARAMETERS

WBC with differential; signs of neuropathy monthly for the first 3 months, then periodically during treatment; consider monitoring of sensory nerve application potential amplitudes (at baseline and every 6 months) to detect asymptomatic neuropathy. In HIV-seropositive patients: viral load after 1 and 3 months, then every 3 months. Pregnancy testing is required within 24 hours of initiation of therapy, weekly during the first 4 weeks, then every 4 weeks in women with regular menstrual cycles or every 2 weeks in women with irregular menstrual cycles.

PHARMACODYNAMICS / KINETICS

Absorption: Rapid

Protein binding: ~30%

Half-life elimination: ~3 hours

Time, to peak, plasma: Healthy volunteers: 0.6-1.5 hours; Myeloma patients: 0.5-4 hours

Excretion: Urine (~67% as unchanged drug)

PATIENT EDUCATION

Patients will be given oral and written instructions regarding the special distribution program for Lenalidomide called RevAssist®.
Lenalidomide is commercially available. Additional information may be obtained from the FDA-approved package insert.

9.2 Cyclophosphamide: Drug information

U.S. BRAND NAMES — Cytoxan®

PHARMACOLOGIC CATEGORY
Antineoplastic Agent, Alkylating Agent

ADMINISTRATION: I.V. infusions may be administered over 1-24 hours. Doses >500 mg to approximately 2 g may be administered over 20-30 minutes. To minimize bladder toxicity, increase normal fluid intake during and for 1-2 days after cyclophosphamide dose. Most adult patients will require a fluid intake of at least 2 L/day. High-dose regimens should be accompanied by vigorous hydration with or without mesna therapy.

ADVERSE REACTIONS SIGNIFICANT
>10%:
Dermatologic: Alopecia (40% to 60%) but hair will usually regrow although it may be a different color and/or texture. Hair loss usually begins 3-6 weeks after the start of therapy. **Endocrine & metabolic: Fertility:** May cause sterility; interferes with oogenesis and spermatogenesis; may be irreversible in some patients; gonadal suppression (amenorrhea) **Gastrointestinal:** Nausea and vomiting occur more frequently with larger doses, usually beginning 6-10 hours after administration; anorexia, diarrhea, mucositis, and stomatitis are also seen. **Genitourinary:** Severe, potentially fatal acute hemorrhagic cystitis, believed to be a result of chemical irritation of the bladder by acrolein, a cyclophosphamide metabolite, occurs in 7% to 12% of patients and has been reported in up to 40% of patients in some series. Patients should be encouraged to drink plenty of fluids during therapy (most adults will require at least 2 L/day), void frequently, and avoid taking the drug at night. With large I.V. doses, I.V. hydration is usually recommended. The use of mesna and/or continuous bladder irrigation is rarely needed for doses <2 g/m2. **Hematologic**: Thrombocytopenia and anemia are less common than leukopenia Onset: 7 days Nadir: 10-14 days Recovery: 21 days.

1% to 10%:

Cardiovascular: Facial flushing. **Central nervous system:** Headache. **Dermatologic:** Skin rash. **Renal:** SIADH may occur, usually with doses >50 mg/kg (or 1 g/m2); renal tubular necrosis, which usually resolves with discontinuation of the drug, is also reported. **Respiratory:** Nasal congestion occurs when I.V. doses are administered too rapidly (large doses via 30-60 minute infusion);

patients experience runny eyes, rhinorrhea, sinus congestion, and sneezing during or immediately after the infusion. If needed, a decongestant or decongestant/antihistamine (eg, pseudoephedrine or pseudoephedrine/triprolidine) can be used to prevent or relieve these symptoms.

<1% (Limited to important or life-threatening):

High-dose therapy may cause cardiac dysfunction manifested as CHF; cardiac necrosis or hemorrhagic myocarditis has occurred rarely, but may be fatal. Cyclophosphamide may also potentiate the cardiac toxicity of anthracyclines. Other adverse reactions include anaphylactic reactions, darkening of skin/fingernails, dizziness, hemorrhagic colitis, hemorrhagic ureteritis, hepatotoxicity, hyperuricemia, hypokalemia, jaundice, neutrophilic eccrine hidradenitis, radiation recall, renal tubular necrosis, secondary malignancy (eg, bladder carcinoma), Stevens-Johnson syndrome, toxic epidermal necrolysis; interstitial pneumonitis and pulmonary fibrosis are occasionally seen with high doses.

BMT: **Cardiovascular:** Heart failure, cardiac necrosis, pericardial tamponade. **Endocrine & metabolic:** Hyponatremia. **Hematologic:** Methemoglobinemia. **Gastrointestinal:** Severe nausea and vomiting. **Miscellaneous:** Hemorrhagic cystitis, secondary malignancy

DRUG INTERACTIONS

Substrate of CYP2A6 (minor), 2B6 (major), 2C8/9 (minor), 2C19 (minor), 3A4 (major); Inhibits CYP3A4 (weak); Induces CYP2B6 (weak), 2C8/9 (weak)

Allopurinol may cause increase in bone marrow depression and may result in significant elevations of cyclophosphamide cytotoxic metabolites.

Anesthetic agents: Cyclophosphamide reduces serum pseudocholinesterase concentrations and may prolong the neuromuscular blocking activity of succinylcholine; use with caution with halothane, nitrous oxide, and succinylcholine.

Chloramphenicol results in prolonged cyclophosphamide half-life to increase toxicity.

CYP2B6 inducers: May increase the levels/effects of acrolein (the active metabolite of cyclophosphamide). Example inducers include carbamazepine, nevirapine, phenobarbital, phenytoin, and rifampin.

CYP2B6 inhibitors: May decrease the levels/effects of acrolein (the active metabolite of cyclophosphamide). Example inhibitors include desipramine, paroxetine, and sertraline.

CYP3A4 inducers: CYP3A4 inducers may increase the levels/effects of acrolein (the active metabolite of cyclophosphamide). Example inducers include aminoglutethimide, carbamazepine, nafcillin, nevirapine, phenobarbital, phenytoin, and rifamycins.

CYP3A4 inhibitors: May decrease the levels/effects of acrolein (the active metabolite of cyclophosphamide). Example inhibitors include azole antifungals, ciprofloxacin, clarithromycin, diclofenac, doxycycline, erythromycin, imatinib, isoniazid, nefazodone, nicardipine, propofol, protease inhibitors, quinidine, and verapamil.

Digoxin: Cyclophosphamide may decrease digoxin serum levels.

Doxorubicin: Cyclophosphamide may enhance cardiac toxicity of anthracyclines.

Tetrahydrocannabinol results in enhanced immunosuppression in animal studies.

Thiazide diuretics: Leukopenia may be prolonged.

MECHANISM OF ACTION

Cyclophosphamide is an alkylating agent that prevents cell division by cross-linking DNA strands and decreasing DNA synthesis. It is a cell cycle phase nonspecific agent. Cyclophosphamide also possesses potent immunosuppressive activity. Cyclophosphamide is a prodrug that must be metabolized to active metabolites in the liver.

PHARMACODYNAMICS / KINETICS

Distribution: Vd: 0.48-0.71 L/kg; crosses placenta; crosses into CSF (not in high enough concentrations to treat meningeal leukemia)

Protein binding: 10% to 56%

Metabolism: Hepatic to active metabolites acrolein, 4-aldophosphamide, 4-hydroperoxycyclophosphamide, and nor-nitrogen mustard

Bioavailability: >75%

Half-life elimination: 4-8 hours

Time to peak, serum: Oral: ~1 hour

Excretion: Urine (<30% as unchanged drug, 85% to 90% as metabolites)

Cyclophosphamide is commercially available. Additional information may be obtained from the FDA-approved package insert.

9.3 Melphalan: Drug Information

U.S. BRAND NAMES — Alkeran®

PHARMACOLOGIC CATEGORY
Antineoplastic Agent, Alkylating Agent

DOSING: ADULTS
Refer to individual protocols; dose should always be adjusted to patient response and weekly blood counts. High dose BMT: I.V.: 140-240 mg/m2 as a single dose or divided into 2-5 daily doses. Infuse over 20-60 minutes.

ADMINISTRATION
Parenteral: Due to limited stability, complete administration of I.V. dose should occur within 60 minutes of reconstitution. I.V. infusion: I.V. dose is FDA-approved for administration as a single infusion over 15-20 minutes. I.V. bolus: I.V. may be administered via central line and via peripheral vein as a rapid I.V. bolus; there have not been any unexpected or serious adverse events specifically

related to rapid I.V. bolus administration; the most common adverse events were transient mild symptoms of hot flush and tingling sensation over the body. Central line: I.V. bolus doses of 17-200 mg/m2 (reconstituted and not diluted) have been infused over 2-20 minutes. Peripheral line: I.V. bolus doses of 2-23 mg/m2 (reconstituted and not diluted) have been infused over 1-4 minutes

ADVERSE REACTIONS SIGNIFICANT
>10%:
Hematologic: Myelosuppressive: Leukopenia and thrombocytopenia are the most common effects of melphalan. Irreversible bone marrow failure has been reported. WBC: Moderate Platelets: Moderate. Onset (days): 7. Nadir (days): 8-10 and 27-32. Recovery (days): 42-50.

1% to 10%:
Cardiovascular: Vasculitis. **Dermatologic:** Vesiculation of skin, alopecia, pruritus, rash. **Endocrine & metabolic:** SIADH, sterility and amenorrhea. **Gastrointestinal:** Nausea and vomiting are mild; stomatitis and diarrhea are infrequent. **Emetic potential:** Low (<10%): <100 mg/m2; high (>90%): >100 mg/m2. **Genitourinary:** Bladder irritation, hemorrhagic cystitis. **Hematologic:** Anemia, agranulocytosis, hemolytic anemia. **Hepatic:** Transaminases increased (hepatitis, jaundice have been reported). **Respiratory:** Pulmonary fibrosis, interstitial pneumonitis. **Miscellaneous:** Hypersensitivity, secondary malignancies

BMT: **Dermatologic:** Alopecia. **Gastrointestinal:** Mucositis (severity increases with Clcr ≤40 mL/minute), nausea and vomiting (moderate), diarrhea. **Hematologic:** Myelosuppression, secondary leukemia. **Renal:** Increased serum creatinine and azotemia possible without adequate hydration. Rare side effects: Abnormal LFTs, interstitial pneumonitis, secondary leukemia, SIADH, vasculitis

DRUG INTERACTIONS

Cyclosporine: Risk of nephrotoxicity is increased by melphalan. Nalidixic acid: Concomitant use of I.V. melphalan with nalidixic acid may increase risk of necrotic enterocolitis (reported with pediatric patients).

MECHANISM OF ACTION

Alkylating agent which is a derivative of mechlorethamine that inhibits DNA and RNA synthesis via formation of carbonium ions; cross-links strands of DNA

PHARMACODYNAMICS / KINETICS

Distribution: Vd: 0.5-0.6 L/kg throughout total body water
Bioavailability: Unpredictable, decreasing from 85% to 58% with repeated doses

Half-life elimination: Terminal: 1.5 hours

Time to peak, serum: ~2 hours

Melphalan is commercially available. Additional information may be obtained from the FDA-approved package insert.

Additional risks of BMT may include some or all of the following side effects (this information is taken from the standard of care consent form used for Bone Marrow Transplants at UPENN):

Chemotherapy:

HAIR: partial or total hair loss, which is usually temporary
SKIN: darkening and/or peeling of the skin, nail changes, rashes, flushing
EARS: decreased hearing, loss of balance
EYES: watery eyes, discomfort, blindness
MOUTH: ulcers, unusual taste, sore throat, mouth pain, difficulty swallowing
LUNGS: lung damage, difficulty breathing
HEART: changes in heart beat, heart failure or damage, changes in blood pressure
GI: ulcers, nausea, vomiting, constipation or diarrhea, weight loss, liver damage, abdominal pain
URINARY SYSTEM: burning with urination, bloody urine, kidney damage
ENDOCRINE: elevation of blood sugar, impairment or loss of reproductive function
NERVOUS SYSTEM: tingling in the fingers/toes/arms/legs, jaw pain, muscle weakness, irritability, depression, confusion, seizures, dizziness, loss of coordination
BONE MARROW: destroys bone marrow which may increase weakness, infections and Bleeding
VEINS: hardening of the veins in and around the area of intravenous medication injection; ulcer formation, tissue damage or discomfort in 6 and around the area of intravenous medication injection

Stem Cell Infusion:

• During infusion: o Shortness of breath, chest tightness, abdominal tightness, slowing of heart rate

• Following infusion: o Red urine following infusion due to damage to red bloods cells during the thawing process
o Breath odor for up to 24 hours following infusion due to DMSO used to protect stem cells while frozen
o Engraftment failure (harvested stem cells may fail to grow normally to restore blood cell production or may be very delayed in re-growth, leading to complications, primarily infection and/or bleeding, which could be fatal)

G-CSF:

• Side effects are usually mild and short-lived and may include bone or joint pain, low-grade fever, and mild nausea.

9.4 G-CSF: Drug Information

U.S. BRAND NAMES — Neupogen®

PHARMACOLOGIC CATEGORY
Colony Stimulating Factor

ADMINISTRATION
May be administered undiluted by SubQ or by I.V. infusion over 15-60 minutes in D5W; incompatible with sodium chloride solutions.

ADVERSE REACTIONS SIGNIFICANT

Effects are generally mild and dose related

>10%:

Central nervous system: Neutropenic fever, fever. **Dermatologic:** Alopecia. **Gastrointestinal:** Nausea, vomiting, diarrhea, mucositis, splenomegaly (more common in patients who prolonged (>14 days) treatment, usually subclinical). **Neuromuscular & skeletal:** Medullary bone pain (24%)

1% to 10%:
Cardiovascular: Chest pain, fluid retention. **Central nervous system:** Headache. **Dermatologic:** Skin rash. **Gastrointestinal:** Anorexia, stomatitis, constipation. **Hematologic:** Leukocytosis. Local: Pain at injection site. **Neuromuscular & skeletal:** Weakness. **Respiratory:** Dyspnea, cough, sore throat

<1% (Limited to important or life-threatening):

Pericarditis, thrombophlebitis, transient supraventricular arrhythmia

DRUG INTERACTIONS

Drugs which may potentiate the release of neutrophils (eg, lithium) should be used with caution

MONITORING PARAMETERS

CBC and platelet count should be obtained twice weekly. Leukocytosis (white blood cell counts ≥ 100,000/mm3) has been observed in ~2% of patients receiving G-CSF at doses >5 mcg/kg/day. Monitor platelets and hematocrit regularly.

MECHANISM OF ACTION

Stimulates the production, maturation, and activation of neutrophils, G-CSF activates neutrophils to increase both their migration and cytotoxicity.

PHARMACODYNAMICS / KINETICS

Onset of action: ~24 hours; plateaus in 3-5 days
Duration: ANC decreases by 50% within 2 days after discontinuing G-CSF; white counts return to the normal range in 4-7 days; peak plasma levels can be maintained for up to 12 hours
Absorption: SubQ: 100%
Distribution: Vd: 150 mL/kg; no evidence of drug accumulation over a 11- to 20-day period
Metabolism: Systemically degraded
Half-life elimination: 1.8-3.5 hours
Time to peak, serum: SubQ: 2-6 hours

G-CSF is commercially available. Additional information may be obtained from the FDA-approved package insert.

9.5 Plerixafor: Drug Information

U.S. BRAND NAMES — Mozobil®

PHARMACOLOGIC CATEGORY
Hematopoietic Stem Cell Mobilizer

ADMINISTRATION
Dosing is based on actual body weight. Begin plerixafor after patient has received filgrastim 10 mcg/kg once daily for 4 days. Filgrastim (G-CSF), Perixafor and apheresis should be continued daily until a sufficient cell collection. Administered undiluted by SubQ at dose of 0.24 mg/kg once daily ~ 11 hours prior to apheresis for up to 4 consecutive days; maximum dose = 40 mg/day.

ADVERSE REACTIONS SIGNIFICANT
Effects are generally mild and dose related.
>10%:
Central nervous system: Fatigue (27%), headache (22%), dizziness (11%). **Gastrointestinal:** Nausea (34%), diarrhea (37%). Local: Injection site reactions (34% including erythemia, hematoma, hemorrhage, induration, inflammation, irritation, pain, paresthesias, pruritis, rash, swelling urticaria. Neuromuscular & skeletal: arthralgias (13%)

5% to 10%:
Central nervous system: insomnia (7%). **Gastrointestinal:** vomiting (10%), flatulence (7%)

<5% (Limited to important or life-threatening):
Abdominal discomfort, abdominal distension, abdominal pain, constipation, diaphoresis, dyspepsia, dyspnea, erythema, hypesthesia, hypoxia, increased leukocytes, malaise, musculoskeletal pain, orthostatic hypotension, periorbital swelling, syncope, thrombocytopenia, urticaria, vasovagal reaction , xerostomia

DRUG INTERACTIONS — None known

MONITORING PARAMETERS — CBC with differential and platelets

MECHANISM OF ACTION
Reversibly inhibits binding of stromal cell-derived factor-1-alpha (SDF-1α), expressed on bone marrow stromal cells, to the CXC chemokine receptor 4 (CXCR4), resulting in mobilizationof hematopoietic stem and progenitor cells from the bone marrow into peripheral blood. Plerixafor used in combination with Filgrastim results in syngergistic increase in CD34+ cell mobilization. Mobilized CD34+ cells are capable of engrafting with extended repopulating capacity.

PHARMACODYNAMICS / KINETICS
Onset of action:monotherapy: 6-9 hours after administration; with G-CSF: 10-14 hours. Duration: white counts return to baseline at ~ 24 hours after administration. Absorption: SubQ: Rapid. Distribution: Vd: 0.3 L/kg, primarily to extravascular fluid space. Metabolism: not metabolized Half-life elimination: Terminal: 3-6 hours. Time to peak, serum: SubQ: 30-60 minutes. Excretion: Urine (~70% as parent drug).

Mozobil® is commercially available. Additional information may be obtained from the FDA-approved package insert.

9.6 Bortezomib: Drug Information

MEDICATION SAFETY ISSUES
High alert medication: The Institute for Safe Medication Practices (ISMP) includes this medication among its list of drug classes which have a heightened risk of causing significant patient harm when used in error.

U.S. BRAND NAMES — Velcade®

PHARMACOLOGIC CATEGORY
Antineoplastic Agent. Proteasome Inhibitor.

DOSING: ADULTS

Details concerning dosing in combination regimens should also be consulted. Multiple myeloma (first-line therapy; in combination with melphalan and prednisone): I.V.: 1.3 mg/m^2 days 1, 4, 8, 11, 22, 25, 29, and 32 of a 42-day treatment cycle for 4 cycles, followed by 1.3 mg/m^2 days 1, 8, 22 and 29 of a 42-day treatment cycle for 5 cycles. Relapsed multiple myeloma and mantle cell lymphoma: I.V.: 1.3 mg/m^2 twice weekly for 2 weeks on days 1, 4, 8, and 11 of a 21-day treatment cycle. Consecutive doses should be separated by at least 72 hours. Therapy extending beyond 8 cycles may be given once weekly for 4 weeks (days 1, 8, 15, and 22), followed by a 13-day rest (days 23 through 35). Non-Hodgkin's lymphoma, other than mantle cell (unlabeled use): I.V.: 1.3-1.5 mg/m^2 twice weekly for 2 weeks on days 1, 4, 8, and 11 of a 21-day treatment cycle. DOSING: ELDERLY — Refer to adult dosing. DOSING: RENAL IMPAIRMENT — No dosage adjustment necessary. Note: Dialysis may reduce bortezomib concentrations; administer postdialysis. DOSING: HEPATIC IMPAIRMENT — Specific guidelines are not available; clearance may be decreased; monitor closely for toxicity. DOSING: ADJUSTMENT FOR TOXICITY Myeloma (first-line therapy): Platelets should be ≥70,000/mm^3, ANC should be ≥1000/mm^3, and nonhematologic toxicities should resolve to grade 1 or baseline prior to therapy initiation. Platelets ≤30,000/mm^3 or ANC <750/mm^3 on bortezomib day(s): Withhold bortezomib; if several consecutive bortezomib doses are withheld, reduce dose 1 level (1.3 mg/m^2/dose reduced to 1 mg/m^2/dose; 1 mg/m^2/dose reduced to 0.7 mg/m^2/dose). Grade ≥3 nonhematological toxicity (other than neuropathy): Withhold bortezomib until toxicity resolves to grade 1 or baseline. May reinitiate bortezomib at 1 dose level reduction (1.3 mg/m^2/dose reduced to 1 mg/m^2/dose; 1 mg/m^2/dose reduced to 0.7 mg/m^2/dose). Neuropathic pain and/or peripheral sensory neuropathy: See "Neuropathic pain and/or peripheral sensory neuropathy" toxicity adjustment guidelines below. Relapsed multiple myeloma and mantle cell lymphoma: Grade 3 nonhematological (excluding neuropathy) or Grade 4 hematological toxicity: Withhold until toxicity resolved; may reinitiate with a 25% dose reduction (1.3 mg/m^2/dose reduced to 1 mg/m^2/dose; 1 mg/m^2/dose reduced to 0.7 mg/m^2/dose). Neuropathic pain and/or peripheral sensory neuropathy: Grade 1 without pain or loss of function: No action needed Grade 1 with pain or Grade 2 interfering with function but not activities of daily living: Reduce dose to 1 mg/m^2. Grade 2 with pain or Grade 3 interfering with activities of daily living: Withhold until toxicity resolved, may reinitiate at 0.7 mg/m^2 once weekly. Grade 4: Discontinue therapy.

DOSAGE FORMS
Excipient information presented when available (limited, particularly for generics); consult specific product labeling. Injection, powder for reconstitution [preservative free]: Velcade®: 3.5 mg [contains mannitol 35 mg]

DOSAGE FORMS: CONCISE
Injection, powder for reconstitution [preservative free]: Velcade®: 3.5 mg

GENERIC EQUIVALENT AVAILABLE — No

ADMINISTRATION — Administer via rapid I.V. push (3-5 seconds)

USE — Treatment of multiple myeloma; treatment of relapsed or refractory mantle cell lymphoma
USE - UNLABELED / INVESTIGATIONAL — Treatment of non-Hodgkin's lymphomas (other than mantle cell lymphoma)

ADVERSE REACTIONS SIGNIFICANT
>10%:
Cardiovascular: Edema (11% to 28%), hypotension (12% to 15%; grades 3/4: 3%). **Central nervous system:** Fever (19% to 37%), psychiatric disturbance (35%), headache (17% to 26%), dysesthesia (9% to 27%), insomnia (18% to 21%), dizziness (14% to 23%; excludes vertigo), anxiety (5% to 11%). **Dermatologic:** Rash (17% to 28%), pruritus (11%). **Endocrine & metabolic:** Dehydration (7% to 11%). **Gastrointestinal:** Diarrhea (47% to 57%), nausea (44% to 57%), constipation (40% to 50%), anorexia (34% to 39%), vomiting (27% to 35%), abdominal pain (14% to 16%), abnormal taste (13%), dyspepsia (13%). **Hematologic:** Thrombocytopenia (21% to 38%; grade 4: 4% to 5%; nadir: day 11; recovery: by day 21), anemia (17% to 30%; grade 4: <1%), neutropenia (6% to 19%; grade 4: 2% to 3%; nadir: day 11; recovery: by day 21). **Neuromuscular & skeletal:** Weakness (61% to 72%; grades 3/4: 12% to 19%), peripheral neuropathy (36% to 55%; grade 3: 7% to 11%; grade 4: <1%), paresthesia (9% to 27%), arthralgia (13% to 18%), limb pain (5% to 17%), bone pain (2% to 16%), back pain (<1 % to 15%), myalgia (10% to 12%), muscle cramps (5% to 12%), rigors (11%). **Ocular:** Blurred vision (11%). **Respiratory:** Dyspnea (20% to 23%), cough (19% to 21%), lower respiratory infection (15%), upper respiratory tract infection (11% to 15%), nasopharyngitis (8% to 14%), pneumonia (9% to 12%). **Miscellaneous:** Herpesvirus infections (7% to 13%).

1% to 10%:
Cardiovascular: Syncope (2%). **Endocrine & metabolic:** Hypercalcemia (grade 4: 2%). Frequency not defined (limited to important or life-threatening): Acute diffuse infiltrative pulmonary disease, acute respiratory distress syndrome, alkaline phosphatase increased, allergic reaction, amyloidosis, anaphylaxis, angina, angioedema, ascites, aspergillosis, atelectasis, atrial fibrillation, atrial flutter, AV block, bacteremia, bradycardia, cardiac amyloidosis, cardiac arrest, cardiac tamponade, cardiogenic shock, cardiopulmonary arrest, cerebral hemorrhage, cerebrovascular accident, CHF, cholestasis, coma, complete heart block, confusion, cranial palsy, deafness, deep venous thrombosis, diplopia, disseminated intravascular coagulation (DIC), duodenitis (hemorrhagic), dysautonomia, dysphagia, edema (facial), encephalopathy, embolism, epistaxis, fecal impaction, fracture, gastritis (hemorrhagic), gastroenteritis, GGT increased, glomerular nephritis, hematemesis, hematuria, hemoptysis, hemorrhagic cystitis, hepatic failure, hepatic hemorrhage, hepatitis, hepatocellular damage, herpes meningoencephalitis, hyperbilirubinemia, hyper-/hypoglycemia, hyper-/hypokalemia, hyper-/hyponatremia, hypersensitivity, hyperuricemia, hypocalcemia, hypoxia, immune complex hypersensitivity, injection site reaction, intestinal obstruction, intestinal perforation, intracerebral hemorrhage, ischemic colitis, ischemic stroke, laryngeal edema, leukocytoclastic vasculitis, leukopenia, listeriosis, lymphopenia, melena, mental status change, MI, myocardial ischemia, neuralgia, neutropenic fever, ophthalmic herpes, oral candidiasis, pancreatitis, paralytic ileus, paraplegia, pericardial effusion, pericarditis, peritonitis, pleural effusion, pneumonia, pneumonitis, portal vein thrombosis, proliferative glomerular nephritis, psychosis, pulmonary alveolar hemorrhage, pulmonary edema, pulmonary embolism, pulmonary hypertension, psychosis, QT_c prolongation, renal calculus, renal failure, respiratory failure, respiratory insufficiency, reversible posterior leukoencephalopathy syndrome (RPLS), seizure, septic shock, sepsis, sinus arrest, spinal cord compression, stomatitis, stroke (hemorrhagic), stroke, subarachnoid hemorrhage, subdural

hematoma, suicidal ideation, tachycardia, torsade de pointes, toxic epidermal necrolysis, toxoplasmosis, transaminases increased, transient ischemic attack, tumor lysis syndrome, urinary incontinence, urinary retention, urinary tract infection, urticaria, ventricular tachycardia.

CONTRAINDICATIONS
Hypersensitivity to bortezomib, boron, mannitol, or any component of the formulation

WARNINGS / PRECAUTIONS
Special handling: Hazardous agent: Use appropriate precautions for handling and disposal.
Concerns related to adverse effects: Bone marrow suppression: Hematologic toxicity, including neutropenia and severe thrombocytopenia may occur; risk is increased in patients with pretreatment platelet counts <75,000/µL; frequent monitoring is required throughout treatment; may require dosage adjustments; withhold treatment for platelets <25,000/µL. Hemorrhage (gastrointestinal and intracerebral) due to low platelet count has been observed.

Cardiovascular effects: May cause hypotension (including postural and orthostatic); use caution with dehydration, history of syncope, or medications associated with hypotension. Has been associated with the development or exacerbation of congestive heart failure and decreased left ventricular ejection fraction; use caution in patients with risk factors or existing heart disease. Has also been associated with QT_c prolongation.

Hepatotoxicity: Acute liver failure has been reported (rarely) in patients receiving multiple concomitant medications; hepatitis, transaminase increases, and hyperbilirubinemia have also been reported. Use caution in patients with hepatic dysfunction; toxicities may be increased.

Herpes reactivation: Herpes (zoster and simplex) reactivation has been reported with bortezomib; consider antiviral prophylaxis during therapy.

Peripheral neuropathy: May cause peripheral neuropathy (usually sensory but may be mixed sensorimotor); risk may be increased with previous use of neurotoxic agents or pre-existing peripheral neuropathy; adjustment of dose and schedule may be required.

Pulmonary toxicity: Pulmonary disorders including pneumonitis, interstitial pneumonia, lung infiltrates, and acute respiratory distress syndrome (ARDS) have been reported. Pulmonary hypertension (without left heart failure or significant pulmonary disease) has been reported rarely.

Reversible posterior leukoencephalopathy syndrome (RPLS): Has been reported (rarely). Symptoms of RPLS include confusion, headache, hypertension, lethargy, seizure, blindness and/or other vision, or neurologic disturbances; discontinue if RPLS occurs. The safety of reinitiating bortezomib in patients previously experiencing RPLS is unknown.

Tumor lysis syndrome: May cause tumor lysis syndrome; risk is increased in patients with high tumor burden prior to treatment

Disease-related concerns: Diabetes: Hyper-and hypoglycemia may occur in diabetic patients receiving oral hypoglycemics; monitor; may require adjustment of diabetes medications. Hepatic impairment: Use with caution in patients with hepatic impairment; clearance may be reduced; monitor for toxicity.

Special populations:
Pediatrics: Safety and efficacy have not been established in children.

METABOLISM / TRANSPORT EFFECTS
Substrate of CYP1A2 (minor), 2C9 (minor), 2C19 (major), 2D6 (minor), 3A4 (major); Inhibits CYP1A2 (weak), 2C9 (weak), 2C19 (moderate), 2D6 (weak), 3A4 (weak)

DRUG INTERACTIONS
(For additional information: Launch Lexi-Interact™ Drug Interactions Program)

CYP2C19 Inducers (Strong): May increase the metabolism of CYP2C19 Substrates. Risk C: Monitor therapy

CYP2C19 Inhibitors (Moderate): May decrease the metabolism of CYP2C19 Substrates. Risk C: Monitor therapy

CYP2C19 Inhibitors (Strong): May decrease the metabolism of CYP2C19 Substrates. Risk D: Consider therapy modification

CYP2C19 Substrates: CYP2C19 Inhibitors (Moderate) may decrease the metabolism of CYP2C19 Substrates. Risk C: Monitor therapy
CYP3A4 Inducers (Strong): May increase the metabolism of CYP3A4 Substrates. Risk C: Monitor therapy

CYP3A4 Inhibitors (Moderate): May decrease the metabolism of CYP3A4 Substrates. Risk C: Monitor therapy

CYP3A4 Inhibitors (Strong): May decrease the metabolism of CYP3A4 Substrates. Risk D: Consider therapy modification

Dasatinib: May increase the serum concentration of CYP3A4 Substrates. Risk C: Monitor therapy

Deferasirox: May decrease the serum concentration of CYP3A4 Substrates. Risk C: Monitor therapy

Herbs (CYP3A4 Inducers): May increase the metabolism of CYP3A4 Substrates. Risk C: Monitor therapy

ETHANOL / NUTRITION / HERB INTERACTIONS
Herb/Nutraceutical: Avoid St John's wort (may decrease bortezomib levels).

PREGNANCY RISK FACTOR — D (show table)

PREGNANCY IMPLICATIONS
Adverse effects (fetal loss and decreased fetal weight) were observed in animal studies. There are no adequate and well-controlled studies in pregnant women. Effective contraception is recommended for women of childbearing potential.
LACTATION
Excretion in breast milk unknown/not recommended
BREAST-FEEDING CONSIDERATIONS
Due to the potential for serious adverse reactions in the nursing infant, breast-feeding is not recommended.

MONITORING PARAMETERS
Signs/symptoms of peripheral neuropathy, dehydration, or hypotension; CBC with differential and platelets (monitor frequently throughout therapy); renal function, pulmonary function (with new or worsening pulmonary symptoms), liver function tests (in patients with existing hepatic impairment)

CANADIAN BRAND NAMES — Velcade®

INTERNATIONAL BRAND NAMES — Velcade (AR, AT, AU, BE, BG, CH, CL, CN, CO, CR, CZ, DE, DK, DO, EC, EE, ES, FI, FR, GB, GR, GT, HK, HN, ID, IE, IL, IT, MX, MY, NL, NO, PA, PH, PT, PY, RU, SE, TH, TR, TW, VE)

MECHANISM OF ACTION
Bortezomib inhibits proteasomes, enzyme complexes which regulate protein homeostasis within the cell. Specifically, it reversibly inhibits chymotrypsin-like activity at the 26S proteasome, leading to activation of signaling cascades, cell-cycle arrest, and apoptosis.

PHARMACODYNAMICS / KINETICS
Distribution: 498-1884 L/m^2
Protein binding: ~83%
Metabolism: Hepatic primarily via CYP2C19 and 3A4 and to a lesser extent CYP1A2; forms metabolites (inactive) via deboronization followed by hydroxylation
Half-life elimination: Single dose: 9-15 hours; multiple dosing: 1 mg/m^2: 40-193 hours; 1.3 mg/m^2: 76-108 hours

9.7 Prevnar-13 (Pneumococcal Conjugate Vaccine): Drug Information

U.S. BRAND NAMES — Prevnar-13®

PHARMACOLOGIC CATEGORY
Vaccine

DOSING: ADULTS

Dosing established for infants and toddlers

DOSAGE FORMS —Injection, suspension: Prevnar 13™: 2 mcg of each capsular saccharide for serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F, and 23F, and 4 mcg of serotype 6B [bound to diphtheria CRM_{197} protein ~34 mcg] per 0.5 mL (0.5 mL) [contains aluminum, polysorbate 80, soy, and yeast]

ADMINISTRATION
Do not inject I.V.; avoid intradermal route; administer I.M. (deltoid muscle or lateral mid-thigh)
For patients at risk of hemorrhage following intramuscular injection, the ACIP recommends "it should be administered intramuscularly if, in the opinion of the physician familiar with the patients bleeding risk, the vaccine can be administered with reasonable safety by this route. If the patient receives antihemophilia or other similar therapy, intramuscular vaccination can be scheduled shortly after such therapy is administered. A fine needle (23 gauge or smaller) can be used for the vaccination and firm pressure applied to the site (without rubbing) for at least 2 minutes. The patient should be instructed concerning the risk of hematoma from the injection."

ADVERSE REACTIONS SIGNIFICANT
All serious adverse reactions must be reported to the U.S. Department of Health and Human Services (DHHS) Vaccine Adverse Event Reporting System (VAERS) 1-800-822-7967.

>10%:
Central nervous system: Drowsiness, fever, insomnia, irritability. **Gastrointestinal:** Appetite decreased. **Local:** Erythema, swelling, tenderness
1% to 10%:
Dermatologic: Rash. Gastrointestinal: Diarrhea, vomiting
<1% (Limited to important or life-threatening):
Abnormal crying, breath holding, febrile seizures, hypersensitivity reaction (bronchospasm dyspnea, facial edema), seizure, urticaria, urticaria-like rash. Adverse reactions observed with PCV7 which may also be seen with PCV-13: Anaphylactic reaction, angioneurotic edema, apnea, breath holding, edema, erythema multiforme, hypotonic hyporesponsive episode, injection site reaction (dermatitis, pruritus), lymphadenopathy, shock

WARNINGS / PRECAUTIONS
Caution in latex sensitivity. Children with impaired immune responsiveness may have a reduced response to active immunization. Safety and efficacy have not been established in children <6 weeks of age. Not for I.V. use.

DRUG INTERACTIONS
Immunosuppressants: May decrease response to active immunizations

MECHANISM OF ACTION
Promotes active immunization against invasive disease caused by *S. pneumoniae* capsular serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F, all which are individually conjugated to

CRM197 protein. Prevnar-13 is commercially available. Additional information may be obtained from the FDA-approved package insert.

10 Data Handling and Record Keeping

10.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

Celgene, study supporter, affirms the subject's right to protection against invasion of privacy. In compliance with United States federal regulations, Celgene requires the Investigator to permit representatives of Celgene Corporation and, when necessary, representatives of the FDA or other regulatory authorities to review and/or copy any medical records relevant to the study in accordance with local laws.

Should direct access to medical records require a waiver or authorization separate from the subject's statement of informed consent, it is the responsibility of the Investigator to obtain such permission in writing from the appropriate individual.

In the event that a subject revokes authorization to collect or use PHI, the investigator and Sponsor by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

10.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

10.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF/eCRF must be recorded. All missing data must be explained. If a space on the CRF/eCRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

10.4 Records Retention

Since this is an FDA-regulated study, it is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

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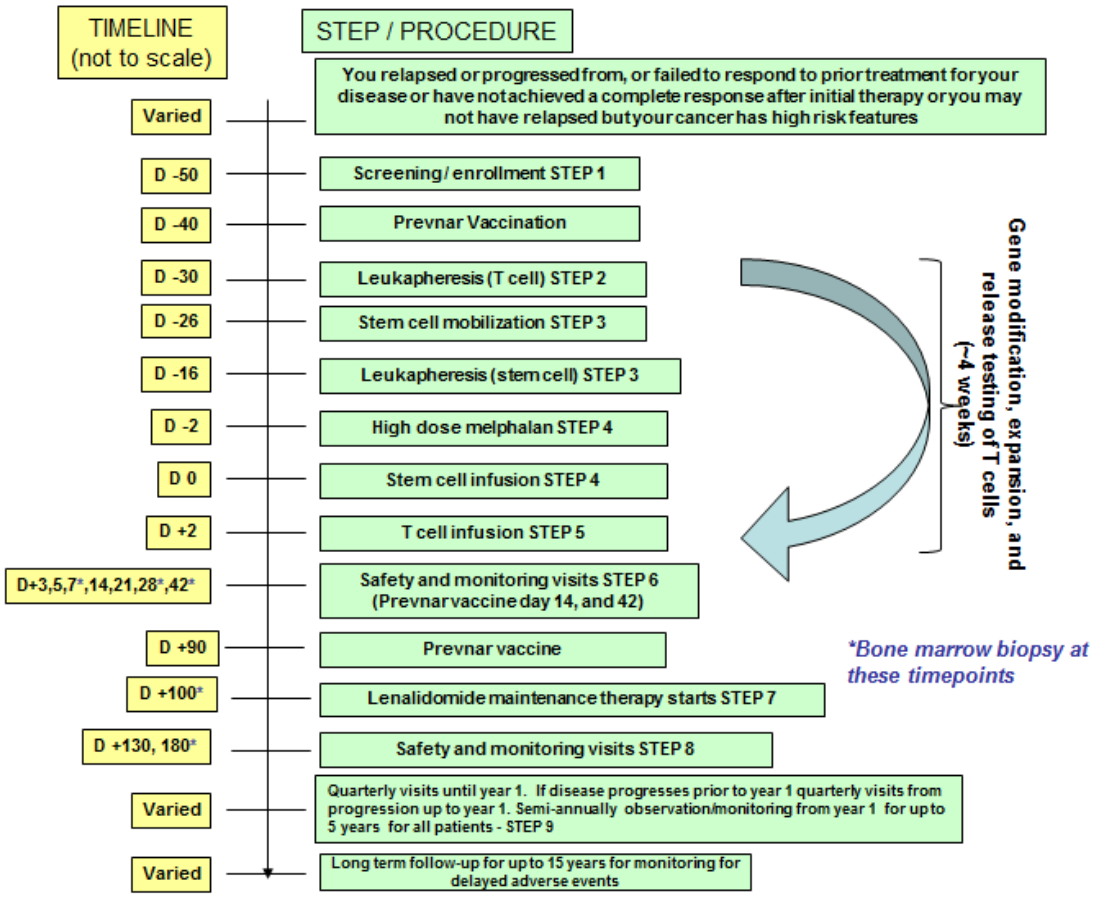
12 Appendix A: Criteria for Diagnosis of Plasma Cell Myeloma*

Major criteria
Plasmacytomas on tissue biopsy
Marrow plasmacytosis with > 30% plasma cells
Monoclonal globulin spike on serum electrophoresis > 3.5 g/dl for IgG or > 2.0 g/dl for IgA; ≥ 1.0 g/24 h of κ or λ light-chain excretion on urine electrophoresis in the absence of amyloidosis

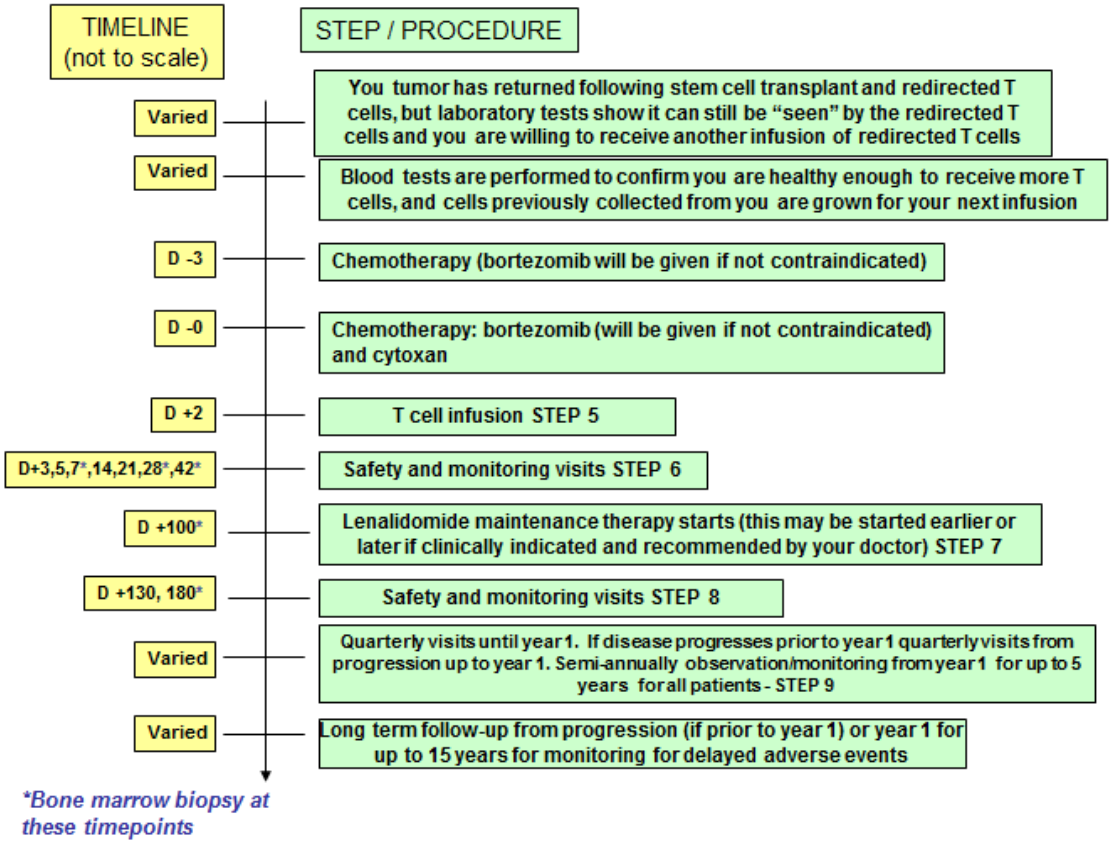
Minor criteria
Marrow plasmacytosis 10-30%
Monoclonal globulin spike present, but less than the levels defined above
Lytic bone lesions
Normal IgM < 0.05 g/dl, IgA < 0.1 g/dl, or IgG < 0.6 g/dl

*The diagnosis of plasma cell myeloma is confirmed when at least one major and one minor criteria or at least three minor criteria are documented in *symptomatic* patients with *progressive* disease. The presence of features not specific for the disease may support the diagnosis, particularly if of recent onset: anemia, hypercalcemia, azotemia, bone demineralization, or hypoalbulinemia.

13 APPENDIX B: Study Schema



Study Schema for patients receiving a second infusion. For patients enrolled at University of Maryland only. Patients enrolled at Universtiy of Pennsylvania are not receiving a sencond infusion:



14 APPENDIX C: SCHEDULE OF EVALUATIONS*

Note: for patients receiving a second infusion (Protocol Section 5. 9), cytoxan administration will be day 0, and T cells will be given on day +2 and all other follow-up will continue on as shown. Long term follow-up for observation of AEs potentially related to gene therapy will be calculated from the second infusion date.

Intervention Protocol																				
	Enrollment D -50 (+/-10)	**PVC immunization Day -40 (+/-10)**	**T cell harvest D -30 (+/-10)**	**SC mobilization D -26**	**SC collection D -16 (+/-3)**	**High dose melphalan D -2**	**D 0**	**D+2**	**D+3**	**D+5 +/- 1**	**D+7 +/- 1**	**D+14** (+/-1)	**D+21** (+/-3)	**D+28** (+/-3)	**D+42** (+/-3)	**D +90** (+/-3)	**D+100** (+/-3)	**D+130** (+/-3)	**D+180** (+/-3)	**[A]Day +270 and year 1** (+/-7)
Procedures																				
Chemotherapy				X		X														
SC Infusion							X													
Apheresis**			X		X															
T cell infusion								X												
Lenalidomide																	X[B]			
PCV Immunization		X										X			X	X				
Clinical Assessments																				
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Consent	X																			
Medical Hx, PE, PS	X			X		X	X	X	X	X	X	X	X	X	X		X		X	X
CBC, differential	X			X		X	X	X	X	X	X	X	X	X	X		X	X	X	X
Comprehensivemetabolic[L]	X			X		X	X	X	X	X	X	X	X	X	X		X	X	X	X
Troponin								X[M]	X	X	X									
EKG								X[M]	X	X	X									
CD3, CD4, CD8	X											X			X		X		X	
HLA-A2/A1 test	X																			
Tumor antigen testing[K]																				
Pregnancy test[D,E]	X					X									X		X	X	X	X
HIV, HTLV 1/2, Hep B, Hep C, CMV	X																			
Register patient into RevAssist® program															X[F]					
PFT	X																			
MUGA/Echo	X																			

Intervention Protocol																				
	Enrollment D -50 (+/-10)	**PVC immunization Day -40 (+/-10)**	**T cell harvest D -30 (+/-10)**	**SC mobilization D -26**	**SC collection D -16 (+/-3)**	**High dose melphalan D -2**	**D 0**	**D+2**	**D+3**	**D+5 +/- 1**	**D+7 +/- 1**	**D+14** (+/-1)	**D+21** (+/-3)	**D+28** (+/-3)	**D+42** (+/-3)	**D +90** (+/-3)	**D+100** (+/-3)	**D+130** (+/-3)	**D+180** (+/-3)	**[A]Day +270 and year 1** (+/-7)
CXR (Chest CT if indicated)	X																			
Cardiac Stress Test[N]																				
RCL monitoring	X															X			X	X[G]
Myeloma Assessments																				
Myeloma Markers[C]	X														X		X		X	X
Bone Marrow Biopsy[H]	X										X		X		X		X		X	X
Bone surveys[I]	X																X		X	X
Research Assesments																				
Peripheral blood (25 cc)	X							X[J]	X	x	x	x	x	x	x		x	x	x	x
DNA (persistence)	X							x	x	x	x	x	x	x	x		x	x	x	x
PBMC (functional assays, immunophenotyping)	X							x	x	x	x	x	x	x	x		x	x	x	x
serum (2 cc)	x							X[J]	x	x	x	x	x	x	x		x	x	x	x
multiplex cytokine	x							x	x	x	x	x	x	x	x		x	x	x	x
Marrow (20 cc)	x										x		x		x		x		x	x***
DNA (homing)	x										x		x		x		x		x	x
MDMC (functional assays, immunophenotyping)	x										x		x		x		x		x	x
Marrow serum (2 cc)	x										x		x		x		x		x	x***
multiplex cytokine	x										x		x		x		x		x	x
Total Research blood draws																				
Lavender top	45 cc							20 cc	25 cc	25 cc	45 cc	25 cc	45 cc	25 cc	25 cc		45 cc	25 cc	45 cc	25-45*** cc

Intervention Protocol																				
	Enrollment D -50 (+/-10)	**PVC immunization Day -40 (+/-10)**	**T cell harvest D -30 (+/-10)**	**SC mobilization D -26**	**SC collection D -16 (+/-3)**	**High dose melphalan D -2**	**D 0**	**D+2**	**D+3**	**D+5 +/- 1**	**D+7 +/- 1**	**D+14** (+/-1)	**D+21** (+/-3)	**D+28** (+/-3)	**D+42** (+/-3)	**D +90** (+/-3)	**D+100** (+/-3)	**D+130** (+/-3)	**D+180** (+/-3)	**[A]Day +270 and year 1** (+/-7)
red top	4 cc							4 cc	2 cc	2 cc	4 cc	2 cc	4 cc	2 cc	2 cc		4 cc	2 cc	4 cc	2-4*** cc
Total	49 cc							24 cc	27 cc	27 cc	49 cc	27 cc	49 cc	27 cc	27 cc		49 cc	27 cc	49 cc	27-49*** cc

A-Visits ≥Year 1 will occur at least every 3 months or per local standard of care until disease progression.
B-Start lenalidomide 10 mg PO daily
C-SPEP + immunofixation; quantitative IgG, IgM, IgA; UPEP + immunofixation based on a 24 hour urine collection; serum free κ and λ light chain levels and κ/λ ratio determination; Beta-2 microglobulin, CRP.
D-Pregnancy tests for females of childbearing potential. A female of childbearing potential (FCBP) is a sexually mature female who: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).
E-Pregnancy tests must occur within 10 – 14 days and again within 24 hours prior to prescribing lenalidomide (prescriptions must be filled within 7 days). FCBP with regular or no menstruation must have a pregnancy test weekly for the first 28 days and then every 28 days while on therapy (including breaks in therapy), at discontinuation of lenalidomide and at Day 28 post the last dose of lenalidomide. Females with irregular menstruation must have a pregnancy test weekly for the first 28 days and then every 14 days while on therapy (including breaks in therapy), at discontinuation of lenalidomide and at Day 14 and Day 28 post the last dose of lenalidomide (see Appendix: Risks of Fetal Exposure, Pregnancy Testing Guidelines and Acceptable Birth Control Methods).
F-If patient is not registered in the RevAssist program, patient must be registered. Patient can be registered approximately 1-2 weeks prior to day +100 as well.
G-Procedure optional at these points, except for RCL sample which is collected on an annual basis
H-a bone marrow biopsy will be requested on days 7, 21, 42 (optional); BM Bx will also be taken at disease progression (not indicated in schedule above)
I-MRI or PET scan if clinically indicated
J-2cc serum and 10cc lavender pre infusion and 2cc serum 1, 2, 4, 8, and 12 hours post infusion, and 10 cc lavender 2 hours post infusion.
K-Tumor antigen testing will be performed on the marrow sample collected at this timepoint. Testing will be performed by RT-PCR in the TSCL at UPenn. Marrow for this timepoint only will be shipped fresh same day via courier if not collectected at UPenn.
L-Glucose, Bun, Cr, NA, K, Cl, CO2, Ca, T. Protein, Albumin, T.Bili, Alk Phos, AST, ALT
M-Performed prior to infusion
N-Recommended for any transplant patient over 60 and for patients with a prior history of heart disease. The specific type of stress test will be at the PI's discretion.
P-Window refers to enrollment of subject with Sponsor
Q-The synergistic twin will undergo these visits in lieu of the subject for a synergistic twin transplant (stem cell collection will be around day -20)

**if available frozen apheresis product for the T-cell and/or stem cell product may be used in lieu of fresh apheresis as indicated in this table
*** Marrow sample to be collected for research purposes semi-annually or whenever follow-up marrow aspiration collected at physician discretion

Long-Term Follow-up Schedule for Year 2-15

	Time post-infusion								
	Year 2		**Year 3**		**Year 4**		**Year 5**		**Years 6-15**
Months	18	24	30	36	42	48	54	60	Annually
Visit window	± 3 month								± 6 months
Safety Assessments									
Medical History and Physical Exam[1]	X	X	X	X	X	X	X	X	X
Mutagenic agents, other investigational agents or anti-cancer therapies[1]	X	X	X	X	X	X	X	X	X
Adverse Events[2]	X	X	X	X	X	X	X	X	X
Hematology		X		X		X		X	X[3]
Serum chemistry		X		X		X		X	X[3]
Central Lab									
VSV-G DNA (RCL) for safety		X		X		X		X	X
Vector Copies (Persistence) for safety	X	X	X	X	X	X	X	X	X[3]
Other Assessments									
Overall Survival	X	X	X	X	X	X	X	X	X

1. New medical history/medications/chemotherapies
2. Adverse event collection is limited to:
   - New malignancies
   - New incidence or exacerbation of a pre-existing neurologic disorder
   - New incidence or exacerbation of a prior rheumatologic or other autoimmune disorder
   - New incidence of a hematologic disorder
   - Opportunistic and or serious infections
   - Unanticipated illness and/or hospitalization deemed related to gene modified cell therapy
3. In year 6-15, laboratory assessments are performed for as long as persistence is analyzed. If persistence samples are discontinued then laboratory assessments are discontinued.

15 APPENDIX D: LENALIDOMIDE DOSE MODIFICATIONS

Lenalidomide Dose Modifications	
NCI CTC Toxicity Grade	**Dose Modification Instructions for Lenalidomide**
Grade 3 neutropenia associated with fever (temperature ≥ 38.5° C) or Grade 4 neutropenia	• Hold (interrupt) lenalidomide dose. • Follow CBC weekly. • If neutropenia has resolved to ≤ grade 2 prior to Day 21, restart lenalidomide at next lower dose level and continue through the scheduled end of the cycle. Otherwise, omit for remainder of cycle and reduce the dose of lenalidomide by 1 dose level at the start of the next cycle. Omitted doses are not made up.If neutropenia is the only toxicity for which a dose reduction is required, G-CSF may be used and the lenalidomide dose maintained at the discretion of the investigator.
Thrombocytopenia ≥Grade 4 (platelet count < 25,000/mm³)	• Hold (interrupt) lenalidomide dose. • Follow CBC weekly. • **NOTE**: Consider holding and then resuming prophylactic anti-coagulation, if applicable. • If thrombocytopenia resolves to ≥30,000 prior to day 21 restart lenalidomide at next lower dose level and continue through the scheduled end of the cycle. Otherwise, omit for remainder of cycle and reduce the dose of lenalidomide by 1 dose level at the start of the next cycle. Omitted doses are not made up.

Lenalidomide Dose Modifications (continued)	
NCI CTC Toxicity Grade	**Dose Modification Instructions for Lenalidomide**
Non-blistering rash **Grade 3** **Grade 4**	• If Grade 3, hold (interrupt) lenalidomide dose. Follow weekly. • If the toxicity resolves to ≤ grade 1 prior to Day 21, restart lenalidomide at next lower dose level and continue through the scheduled end of the cycle. Otherwise, omit for remainder of cycle and reduce the dose of lenalidomide by 1 dose level at the start of the next cycle. Omitted doses are not made up. • If Grade 4, discontinue lenalidomide.
Desquamating (blistering) rash- any Grade	• Discontinue lenalidomide.
Neuropathy **Grade 3** **Grade 4**	• If Grade 3, hold (interrupt) lenalidomide dose. Follow at least weekly. • If the toxicity resolves to ≤ grade 1 prior to Day 21, restart lenalidomide at next lower dose level and continue through the scheduled end of the cycle. Otherwise, omit for remainder of cycle and reduce the dose of lenalidomide by 1 dose level at the start of the next cycle. Omitted doses are not made up. • If Grade 4, discontinue lenalidomide.

Lenalidomide Dose Modifications (continued)	
NCI CTC Toxicity Grade	**Dose Modification Instructions for Lenalidomide**
Venous thrombosis/embolism **≥ Grade 3**	• Hold (interrupt) lenalidomide and start anticoagulation; restart lenalidomide at investigator's discretion (maintain dose level). • Omit lenalidomide for remainder of cycle. See Anticoagulation Consideration (Section 5.6.1.2)
Hyperthyroidism or hypothyroidism	• Omit lenalidomide for remainder of cycle, evaluate etiology, and initiate appropriate therapy. • See Instructions for Initiation of a New Cycle and reduce the dose of lenalidomide by 1 dose level.
other non-hematologic toxicity ≥ Grade 3	• Hold (interrupt) lenalidomide dose. Follow at least weekly. • If the toxicity resolves to ≤ grade 2 prior to Day 21, restart lenalidomide and continue through the scheduled end of the cycle. Otherwise, omit for remainder of cycle. Omitted doses are not made up. For toxicity attributed to lenalidomide, reduce the lenalidomide dose by 1 dose level when restarting lenalidomide.