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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

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Drug Name:	PG13-A2aB-1G4A-LY-3H10(anti-NY ES01 TCR) retroviral vector-transduced autologous peripheral blood lymphocytes (PBL)	NY ES0-1 ALVAC Vaccine
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Précis:

Background:

- We have constructed a single retroviral vector that contains both α and β chains of a T cell receptor (TCR) that recognizes the NY-ESO-1 (ESO) tumor antigen, which can be used to mediate genetic transfer of this TCR with high efficiency (> 30%) without the need to perform any selection.
- In co-cultures with HLA-A2 and ESO double positive tumors, anti-ESO TCR transduced T cells secreted significant amount of IFN-γ and additional secretion of cytokines with high specificity.
- Poxviruses encoding tumor antigens, similar to the ALVAC ESO-1 vaccine have been shown to successfully immunize patients against these antigens.

Objectives:

Primary objectives:

- Determine if the administration of anti-ESO –TCR engineered peripheral blood lymphocytes (PBL) and aldesleukin to patients following a nonmyeloablative but lymphoid depleting preparative regimen will result in clinical tumor regression in patients with metastatic cancer that expresses the ESO antigen.
- Determine if the administration of anti-ESO –TCR engineered PBL, aldesleukin, and ALVAC ESO-1 vaccine to patients following a nonmyeloablative but lymphoid depleting preparative regimen will result in clinical tumor regression in patients with metastatic cancer that expresses the ESO antigen.

Secondary objectives:

- Determine the in vivo survival of TCR gene-engineered cells.
- Determine the toxicity profile of this treatment regimen.

Eligibility:

Patients who are HLA-A*0201 positive and 18 years of age or older must have

- metastatic cancer whose tumors express the ESO antigen;
- Patients, other than those with metastatic melanoma, must have previously received and have been a non-responder to or recurred to standard care for metastatic disease;

Patients may not have:

- contraindications for high dose aldesleukin administration.

Design:

- PBMC obtained by leukapheresis (approximately 5×10^9 cells) will be cultured in the presence of anti-CD3 (OKT3) and aldesleukin in order to stimulate T-cell growth.
- Transduction is initiated by exposure of approximately 10^8 to 5×10^8 cells to retroviral vector supernatant containing the anti-ESO TCR genes.
- Patients will receive a nonmyeloablative but lymphocyte depleting preparative regimen consisting of cyclophosphamide and fludarabine followed by intravenous infusion of ex vivo tumor reactive, TCR gene-transduced PBMC plus IV aldesleukin (720,000 IU/kg q8h for a maximum of 15 doses) with or without ALVAC ESO-1 vaccine. Subcutaneous injection of ALVAC ESO-1 vaccine will be administered on day 0 approximately 2 hours prior to intravenous infusion of cells and a second dose of ALVAC ESO-1 vaccine is given on day 14 (+/- 2 days)..
- Patients will undergo complete evaluation of tumor with physical examination, CT of the chest, abdomen and pelvis and clinical laboratory evaluation four to six weeks after treatment. If the

patient has SD or tumor shrinkage, repeat complete evaluations will be performed every 1-3 months. After the first year, patients continuing to respond will continue to be followed with this evaluation every 3-4 months until off study criteria are met.

Cohorts 1 and 2:

- Patients will be entered into two cohorts based on histology: cohort 1 will include patients with metastatic melanoma or renal cell cancer; cohort 2 will include patients with other types of metastatic cancer.
- For each of the 2 strata evaluated, the study will be conducted using a phase II optimal design where initially 21 evaluable patients will be enrolled. For each of these two arms of the trial, if 0 or 1 of the 21 patients experiences a clinical response, then no further patients will be enrolled but if 2 or more of the first 21 evaluable patients enrolled have a clinical response, then accrual will continue until a total of 41 evaluable patients have been enrolled in that stratum.
- For both strata, the objective will be to determine if the combination of high dose aldesleukin, lymphocyte depleting chemotherapy, and anti-ESO TCR-gene engineered lymphocytes is able to be associated with a clinical response rate that can rule out 5% (p0=0.05) in favor of a modest 20% PR + CR rate (p1=0.20).

Cohorts 3 and 4:

- For patients receiving ALVAC ESO-1 vaccine, patients will also be entered into two cohorts based on histology: cohort 3 for patients with metastatic melanoma or renal cell cancer and cohort 4 for patients with other histologies and all patients will receive the treatment regimen including the ALVAC ESO-1 vaccine.
- For each of these 2 new strata, the study will be conducted using a phase II optimal design where initially 21 evaluable patients will be enrolled. For each of these two new cohorts of the trial, if 0 or 1 of the 21 patients experiences a clinical response, then no further patients will be enrolled but if 2 or more of the first 21 evaluable patients enrolled have a clinical response, then accrual will continue until a total of 41 evaluable patients have been enrolled in that stratum.
- For both strata, the objective will be to determine if the combination of high dose aldesleukin, lymphocyte depleting chemotherapy, anti-ESO TCR-gene engineered lymphocytes, and ALVAC ESO-1 vaccine is able to be associated with a clinical response rate that can rule out 5% (p0=0.05) in favor of a modest 20% PR + CR rate (p1=0.20).

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1.0 Introduction

1.1. Study Objectives:

1.1.1. Primary objective:

- In Cohorts 1 and 2, to determine if the administration of anti-ESO TCR-engineered peripheral blood lymphocytes and aldesleukin to patients following a nonmyeloablative but lymphoid depleting preparative regimen will result in clinical tumor regression in patients with metastatic cancer expressing ESO.
- In Cohorts 3 and 4, to determine if the administration of anti-ESO TCR-engineered PBL, aldesleukin, and ALVAC ESO-1 vaccine to patients following a nonmyeloablative but lymphoid depleting preparative regimen will result in clinical tumor regression in patients with metastatic cancer expressing ESO.

1.1.2. Secondary objectives:

- Determine the in vivo survival of TCR gene-engineered cells.
- Determine the toxicity profile of this treatment regimen.

1.2. Background and Rationale:

Studies in experimental animals have demonstrated that the cellular rather than the humoral arm of the immune response plays the major role in the elimination of murine tumors[1]. Much of this evidence was derived from studies in which the adoptive transfer of T lymphocytes from immune animals could transfer resistance to tumor challenge or in some experiments, the elimination of established cancer. Thus, most strategies for the immunotherapy of patients with cancer have been directed at stimulating strong T cell immune reactions against tumor-associated antigens.

In contrast to antibodies that recognize epitopes on intact proteins, T cells recognize short peptide fragments (8-18 amino acids) that are presented on surface class I or II major histocompatibility (MHC) molecules and it has been shown that tumor antigens are presented and recognized by T cells in this fashion. The molecule that recognizes these peptide fragments is the T-cell receptor (TCR). The TCR is analogous to the antibody immunoglobulin molecule in that, two separate proteins (the TCR alpha and beta chains) are brought together to form the functional TCR molecule. The goal of this protocol is to transfer tumor-associated antigen (TAA)-reactive TCR genes into normal peripheral blood lymphocytes (PBL) derived from cancer patients and to return these engineered cells to patients aimed at mediating regression of their tumors. This trial is similar to previous Surgery Branch TCR gene transfer adoptive immunotherapy protocols except that we will use a TCR that has the potential to treat HLA-A2 melanoma patients as well as patients with common epithelial malignancies that express ESO. Table 1.2 briefly summarizes the Surgery Branch protocols that demonstrate the progression of applicable adoptive cell therapies over time.

Table 1.2

Study #/ Disease	Cellular product administered	Arms (n)	Chemotherapy, Cytokines and Immunizations	# of Cells	Response	Reference
98-C-0095 in patients with metastatic melanoma	Cloned Peripheral Blood Lymphocytes (PBL)/ Tumor infiltrating lymphocytes (TIL)	1. Cloned PBL/TIL intravenously (12)	None	1.5 to 34.8 X 10^9 cells	NR[d]	Dudley, ME, *et al.* 2001
		2. IV Cloned PBL/TIL intravenously (6[a])	SQ IL-2 (125,000 IU/kg/d X 12 d)			
		3. IV Cloned PBL/TIL intravenously (6[b])	HD IL-2 (720,000 IU/kg 3X/d to tolerance (max 12)			
		4. IV Cloned PBL/TIL (3[c])	Gp100:209-217(210M)			
99-C-0158 in patients with metastatic melanoma	Tumor infiltrating lymphocytes (TIL)	1. *In vitro* expanded cloned T cells intravenously (3)	30 mg/kg cyclophosphamide X2 days, 25 mg/kg fludarabine X5 days	0.9 X 10^9 to 24.2 X 10^9 cells (ave. 10.4 X 10^9)	NR	Dudley, ME, *et al.* 2002
		2. *In vitro* expanded cloned T cells intravenously (3)	60 mg/kg cyclophosphamide X2 days, 25 mg/kg fludarabine X5 days		NR	
		3. *In vitro* expanded cloned T cells intravenously (3)	60 mg/kg cyclophosphamide X2 days, 25 mg/kg fludarabine X5 days plus IV IL-2 (72,000 IU/kg 3X/day X5 days)	0.9 X 10^9 to 24.2 X 10^9 cells (ave. 10.4 X 10^9)	NR	
		4. *In vitro* expanded cloned T cells intravenously (6)	[60 mg/kg cyclophosphamide X2 days, 25 mg/kg fludarabine X5 days][g] plus IV IL-2 (720,000 IU/kg 3X/day to tolerance (max 12)		NR	
		5. Autologous tumor reactive REP'd[e] bulk TIL cells intravenously (35)	Chemotherapy[g] plus IV HD IL-2 [(720,000 IU/kg 3X/day to tolerance (max 15)][h] with or without immunization with gp100:209-217(210M) or MART-1:26-35(27L) in Montanide ISA-51™ QD X5, then Qwk X3.	1.1 to 16.0 X 10^{10} cells (ave. 6.3 X 10^{10})	18/35 (51%)[f] 3 CRs 15 PRs	Dudley, ME, *et al.* 2003, Dudley, ME, *et al.* 2005
		6. Autologous tumor reactive REP'd[e] bulk TIL cells intravenously (5)	Chemotherapy[g] plus Low Dose IL-2[k] with or without immunization with gp100:209-217(210M) peptide or MART-1:26-35(27L) peptide in Montanide ISA-51™ QD X5, then Qwk X3.	19.7 X 10^9 to 52.9 X 10^9 cells (ave. 36.3 X 10^9)	1 PR 4 TE[j]	
04-C-0181 in patients with metastatic melanoma	Gp100 TCR engineered T cells (PBL or TIL)	1. Escalating doses of anti-gp100 TCR transduced PBL (8)	Chemotherapy[g] plus IV HD IL-2[h] with 1mg gp100:209-217(210M) in Montanide ISA-51™ QD X5, then Qwk X3	Up to 30 X 10^9	NR	NA
		2. Anti-gp100 TCR CD8+ enriched PBL (8)	Chemotherapy[g] plus IV HD IL-2[h] with 6X10^9 pfu rFgp100P209 IV; rFgp100P209 IV and IL-2 repeated 28 days later.	Up to 30 X 10^9	NR	
		3. Escalating doses of anti-gp100 TCR transduced TIL (3)	Chemotherapy[g] plus IV HD IL-2[h] with 6X10^9 pfu rFgp100P209 IV; rFgp100P209 IV and IL-2 repeated 28 days later.	Up to 30 X 10^9	1 PR[i]	
04-C-0251 in patients with metastatic melanoma	MART-1 TCR engineered T cells (PBL or TIL)	1. Escalating doses of anti-MART-1 TCR transduced PBL (18)	Chemotherapy[g] plus IV HD IL-2[h] with MART-1:26-35(27L) in Montanide ISA-51™ QD X5, then Qwk X3.	Up to 30 X 10^9	2PRs[i]	Morgan, *et al.* Science, 2006 Oct 6;314(5796): 126-9
		2. Escalating doses of anti-MART-1 CD8+ TCR transduced PBL (6)	Chemotherapy[g] plus IV HD IL-2[h] with MART-1:27-35 in Montanide ISA-51™ QD X5, then Qwk X3	Up to 30 X 10^9	1 PR[i]	
		3. Escalating doses of anti-MART-1 CD8+ TCR transduced PBL (8)	Chemotherapy[g] plus IV HD IL-2[h] with MART-1:26-35(27L) in Montanide ISA-51™ QD X5, then Qwk X3	Up to 30 X 10^9	1 PR[i]	
		4. Escalating doses of anti-MART-1 TCR transduced TIL (3)	Chemotherapy[g] plus IV HD IL-2[h] with MART-1:26-35(27L) in Montanide ISA-51™ QD X5, then Qwk X3	Up to 30 X 10^9	NR	

		5. Escalating doses of anti-MART-1 TCR transduced PBL plus 1200 TBI (4)	Chemotherapy[g] and 1200 TBI plus IV HD IL-2[h] with MART-1:26-35(27L) in Montanide ISA-51™ QD X5, then Qwk X3	Up to 30 X 10^9	NR	
07-C-0003 in melanoma/renal cell or other histologies	Anti-p53 TCR engineered T cells (PBL)	1. Melanoma or renal cell cancer: Up to 50 X 10^9 cells (PBL) (2)	Chemotherapy[g] plus IV HD IL-2[h]	Up to 50 X 10^9	NR	NA
		2. Other histologies: Up to 50 X 10^9 cells (PBL) (10)	Chemotherapy[g] plus IV HD IL-2[h]	Up to 50 X 10^9	1 PR[i]	
07-C-0174 in patients with metastatic melanoma	Anti-gp100(154) TCR engineered T cells (PBL)	Anti-gp100(154) TCR transduced PBL at a dose ranging from 0.5 X 10^9 cells up to 300 x10^9 (10)	Chemotherapy[g] plus IV HD IL-2[h]	1.7 X 10^9 to 19.4 X 10^9 cells (ave. 6.9 X 10^9)	2 NR 10 TE[j]	NA
07-C-0175 in patients with metastatic melanoma	Anti-MART-1 F5 TCR engineered T cells (PBL)	Anti-MART-1 F5 TCR transduced PBL at a dose ranging from 0.5 X 10^9 cells up to 300 x10^9 cells (11)	Chemotherapy[g] plus IV HD IL-2[h]	1.45 X 10^9 to 23.3 X 10^9 cells (ave. 12.4 X 10^9)	1 PR 2 NR 8 TE[j]	NA
07-C-0176 in patients with metastatic melanoma	Young TIL	Young TIL at a dose ranging from 1.0 X 10^9 cells up to 300 x10^9 cells (2)	Chemotherapy[g] plus IV HD IL-2[h]	54.5 X 10^9 to 83.2 X 10^9 cells (ave. 68.9 X 10^9)	2 TE[j]	NA
Data as of 10/29/07						

[a] Previously treated with cells alone
[b] Five previously treated with cells alone, one new patient for first cell treatment
[c] All three patients had previously been treated with cell alone and cells with IL-2
[d] NR: No Response
[e] Rapid Expansion Protocol
[f] Responses defined: CR (complete response) is disappearance of all clinical evidence of disease; PR (partial response) defined as > 50% reduction in the sum of the products of the perpendicular diameters for at least one month and no increase in any lesion and no new lesions.
[g] Chemotherapy at maximum dose: 60 mg/kg cyclophosphamide X2 days, 25 mg/kg fludarabine X5 days
[h] High dose (HD) IL-2: 720,000 IU/kg 3X/day to tolerance (max 15)
[i] Responses defined according to RECIST criteria
[j] TE: Too early to evaluate
[k] Low dose IL-2: 250,000 IU/kg subcutaneously daily for 5 days. After a two day rest, IL-2 will be administered at a dose of 125,000 IU/kg subcutaneously daily for 5 days for the next five weeks (2 days rest per week).

1.2.1. <u>Prior Surgery Branch Trials of Cell Transfer Therapy Using Cloned Lymphocytes in Patients with Metastatic Melanoma</u>

Studies in the Surgery Branch, National Cancer Institute, identified genes that encode melanoma tumor associated antigens (TAA) recognized by tumor infiltrating lymphocytes (TIL) in the context of multiple MHC class I molecules [2-4]. These TAA appeared to be clinically relevant antigens responsible for mediating tumor regression in patients with advanced melanoma since the TIL used to identify these antigens were often capable of mediating in vivo anti-tumor regression. Two antigens, which were present in virtually all fresh and cultured melanomas, were called MART-1 (Melanoma Antigen Recognized by T Cells - 1) and gp100 and the genes encoding them have been cloned and sequenced. The MART-1 gene encodes a 118 amino acid protein of 13 kd. The gp100 gene encodes a protein identical to that recognized by monoclonal antibody HMB-45. With the exception of melanocytes and retina no normal tissues express these antigens and no expression of these gene products has been seen on cancers other than melanoma. These antigens were thus the original targets of our initial cell transfer protocols using TIL in patients with metastatic melanoma.

Techniques for the cloning of human lymphocytes enabled the generation, in vitro, of greater than 10^9 cloned lymphocytes with a very high degree of reactivity to T-cell recognized antigens as measured by recognition of T2 cells pulsed with very low levels of peptide (10^{-4}M) or recognition of tumor cells. In Surgery Branch protocol 98-0095 we tested the feasibility of transferring large numbers of in vitro gp100 peptide stimulated PBL clones to melanoma patients. In this phase I study [5], 12 patients received multiple infusions of anti-melanoma T cell clones (average 1 X 10^{10} cells/cycle) with or without IL-2 administration. Peripheral blood samples were analyzed for persistence of transferred cells by T-cell receptor-specific PCR. Transferred cells reached a maximum level at 1 hr. post infusion but rapidly declined to undetectable levels by 1-2 weeks. No objective responses were observed in the trial arm that included IL-2 administration.

In the great majority of murine models demonstrating the therapeutic effectiveness of the adoptive transfer of lymphocytes mediating tumor regression, immunosuppression of the host prior to the adoptive transfer of lymphocytes was required. Although, the mechanism of the improved efficacy of adoptively transferred lymphocytes following immunosuppression was not clearly understood, the possible elimination of suppressor lymphocytes and elimination of cells that compete with the transferred cells for the homeostatic cytokines IL-7 and IL-15 were offered as explanations. The use of nonmyeloablative preparative regimens, popular for patients receiving allogeneic bone marrow transplants, appeared ideally suited to induce the transient immunosuppression required prior to the adoptive transfer of lymphocytes. This led to a Surgery Branch clinical trial (99-C-0158) in which 15 patients received cloned lymphocytes after receiving cyclophosphamide-fludarabine nonmyeloablative chemotherapy[6]. The regimen of cyclophosphamide and fludarabine used in this protocol was identical to that used in the bone marrow transplant unit of the National Heart, Lung and Blood Institute for patients receiving HLA matched allotransplants. Six of these 15 patients had the full-dose chemotherapy and high-dose IL-2.

No objective responses were observed in this cohort of 15 patients. One major factor limiting the effectiveness of these cloned lymphocytes appeared to be their shortened

survival upon adoptive transfer. Three of 6 patients who received the chemotherapy plus high dose IL-2 also received cloned T lymphocytes marked with neomycin phosphotransferase resistance gene (Neo^r). In none of these three patients, could Neo^r -marked cells be detected beyond one week after infusion. The cloned T lymphocytes from some of these 15 patients were also labeled with ^{111}Indium for trafficking studies: three received chemotherapy without IL-2 and two received chemotherapy with high-dose IL-2. No enhanced trafficking to the tumor sites of these labeled lymphocytes was observed after the chemotherapy.

No treatment related mortality was observed in this trial, suggesting that this approach of nonmyleoablative chemotherapy in combination with antitumor lymphocytes plus high-dose IL-2 was safe. Grade 3-4 toxicities are listed in Table 1.

The combination of cyclophosphamide and fludarabine was myelosuppressive. Neutrophils nadired on day 10 after chemotherapy at 6/mm^3 and recovered to above 500/mm^3 on day 14. Lymphocytes nadired at 6/mm^3 and recovered to above 500/mm^3. Platelets nadired at 5.5/mm^3 on day 8 and recovered to > 20,000/mm^3 on day 28 with support of filgrastrim (G-CSF). Patients were usually discharged between 2-3 weeks after the initiation of the chemotherapy, with neutrophil counts above 500/mm^3 and platelet counts above 20,000/mm^3. No patients needed a stem cell transfusion to rescue marrow function. However, CD4 counts remained persistently low (below 200), which is a known side effect of immunosuppression from fludarabine. Four patients developed herpes zoster. One patient had an RSV pneumonia requiring mechanical ventilatory support. All patients recovered after treatments. Thus we have shown that this approach was safe.

1.2.2. Prior Surgery Branch Trials of Cell Transfer Therapy Using Heterogeneous TIL plus High-Dose IL-2 Following Nonmyeloablative but Lymphodepleting Chemotherapy

In the Surgery Branch, NCI, we next added a cohort to 99-C-0158 to rapidly expand heterogeneous TILs for adoptive transfer. This protocol for growing TILs was different from the traditional one we developed earlier[7]. TILs were expanded using the rapid expansion protocol (REP) in the presence of OKT3, irradiated allogeneic feeder cells and IL-2. These REPed TILs retained highly specific in vitro anti-tumor activity, often contained reactivities against several antigenic epitopes and contained both CD8+ and CD4+ lymphocytes. These autologous bulk TIL were re-infused to patients following the same nonmyeloablative chemotherapy with cyclophosphamide and fludarabine used in the prior trial. These patients subsequently received high-dose IL-2 and some received peptide immunization when the TIL reactivity was against known MART-1 and gp100 peptides. A total of 35 patients received this type of the treatment [8].

This regimen using REPed TIL in protocol 99-C-0158 resulted in objective cancer regressions in 51% of patients (18 of 35) with metastatic melanoma (Table 2) patients achieved a clonal repopulation of anti-tumor lymphocytes that exceeded 80% of their circulating CD8+ lymphocytes that persisted for months after cell infusion. Immunohistochemistry studies revealed that specific clonotypes from the infused bulk TIL cells infiltrated the regressing tumor nodules. The toxicities in these 35 patients are shown in Table 3. Non-hematologic and hematologic toxicities were those expected from IL-2 and the myelosuppressive chemotherapy. In addition, autoimmunity was observed in the TIL therapy group. Some patients experienced vitiligo and one patient had an autoimmune uveitis easily controlled with steroid eye drops. One of the patients who had clonal

repopulation from infused TIL cells and a dramatic response of metastatic melanoma, developed Epstein-Barr virus (EBV)-associated B cell lymphoma. This patient was EBV-naïve prior to the treatments. The potential source of EBV was thought to be multiple blood product transfusions. This patient died several months later of disseminated lymphoma. One patient had an RSV pneumonia requiring transient mechanical ventilatory support and recovered completely. One patient developed polyneuropathy manifested by vision blindness, motor and sensory defects, approximately two months after chemotherapy. The etiology is unknown for this complication, but possibly related to the fludarabine. One patient who received the chemotherapy regimen plus high-dose IL-2 and bulk peripheral blood lymphocytes reactive to the melanoma, developed prolonged respiratory failure requiring mechanical ventilation and acute renal failure that required hemodialysis. Both toxicities have been reported with high-dose IL-2 therapy and all the patients recovered from the acute toxicities. The only treatment related death in this series was the patient who developed an EBV-associated lymphoma about 6 months after cell infusion. Responding patients survived significantly longer than non-responders (Figure 1) although caution should be exercised in interpreting this type of analysis.

1.2.3. Surgery Branch Trials of Cell Transfer Therapy Using Transduction of Anti-TAA TCR Genes into Non-reactive TIL or PBL

It is often not possible to isolate sufficient tumor samples from melanoma patients and even when tumor is available, only about 60 to 70% generate melanoma reactive TIL cultures. As a potential alternative to the requirement to establish TIL cultures from melanoma patients, we sought methods that could be used to easily obtain a polyclonal population of T cells with anti-TAA properties. Transfer of antigen specific TCR genes to PBL has recently been described as a potential method of generating large numbers of reactive T cells in infectious disease or as anti-cancer cells[9]. In a murine model of this approach, TCR gene transfer into murine peripheral T-cells was performed using a retroviral vector [10]. The engineered T cells were shown to expand in vivo upon viral challenge and efficiently homed to effector sites. In addition, small numbers of TCR-transduced T cells promoted the rejection of antigen-expressing tumors in the mice. Retroviral vector mediated gene transfer can be used to engineer human T cells with high efficiency. In published work, the Surgery Branch was among the first to demonstrate that retroviral vector-mediated transfer of TCR genes could endow human PBL with anti-tumor reactivity [11]. In this study, PBL were engineered with a retroviral vector expressing a TCR gene derived from a MART-1 reactive CTL. These engineered cells reacted with MART-1 expressing cells in an HLA-A201 restricted manner.

To expand on and potentially improve on these early results, we next isolated highly avid TCR genes from both gp100 and MART-1 reactive T cells. These studies have recently been published and are presented briefly here in detail [12;13]. As a source of anti-gp100 TCR genes, we used T cell clone R6C12. T cell line R6C12 is a highly avid CTL clone derived from the PBL of a melanoma patient whose PBL were subject to repeated in vitro stimulation on gp100 peptide-pulsed autologous PBMC. The anti-MART-1 TCR genes were derived from TIL clone M1F2. This T cell clone was isolated from the TIL of a patient that demonstrated a remarkable in vivo expansion of this clone (as described above). During the period of peak lymphocytosis, 63% of this patients' CD8+ PBL expressed the TCR associated Vβ12 protein from clone M1F2. This patient exhibited regression of more than

95% of his cutaneous and subcutaneous melanoma, ongoing for over 6 months, and developed vitiligo on his forearms.

As an initial test of the TCR vectors, we transduced the human T cell line SupT1. SupT1 is a human T-cell leukemia cell line with chromosomal translocations involving both the alpha and beta TCR genes and therefore, no functional endogenous TCR chains are expressed. SupT1 cells were transduced with the APB vector (expressing the gp100 TCR protein) and production of the Vβ8 chain (from the gp100 TCR) was confirmed by western blot analysis. Functional TCR expression was further tested by assaying for cell surface expression of the CD3 protein (without endogenous TCR expression there is no cell-surface expression of CD3). As expected from the lack of endogenous TCR beta gene expression, untransduced cells did not stain for CD3. When vector transduced cells were analyzed in parallel, significant CD3 expression was detected in both APB and AIB engineered cells, suggesting successful assembly of the full TCR complex. To determine if the transduced cells recognize tumor associated peptides, AIB transduced cells were stained with the MART-1 tetramer molecule. Cells positive for both CD3 protein and the MART-1 tetramer were readily detected by FACS analysis of transduced cells.

In patients where it is not possible to obtain viable TIL cultures, we explored whether this gene transfer technology could be used to engineer PBL to recognize cancer antigens. To transduce human PBL with the TCR vectors, PBMC from 2 melanoma patients were obtained by apheresis and stimulated with anti-CD3 antibody plus IL2 (to induce T cell proliferation). The cells were transduced on days 3 and 4, followed by expansion for 2-8 days post-transduction. Transduced T cells were stained for CD3 and Vβ8 (for the gp100 TCR) or Vβ12 (for the MART-1 TCR) and subjected to FACS analysis. The percentage of the transduced cell populations ranged from 30-50% Vβ positive cells without any selection for the transduced cells (background staining for Vβ8 or Vβ12 was approximately 4%). Gene transfer efficiency (as measured by Vβ8/12 + cells) was equally distributed into both CD4 and CD8 positive cells.

To determine if TCR vector transduced PBL could mediate the release of effector cytokines gamma-interferon and GM-CSF, co-culture experiments were setup with peptide pulsed T2 cells. T2 cells were pulsed with HLA-A2 specific peptides derived from the influenza virus (flu), the MART-1 TAA (Mart), the native gp100 epitope recognized by R6C12 (gp100) or an anchor residue modified version of the native 209 peptide (gp100-2M). As positive controls for cytokine release, both CTL clones and TIL cultures were used. The engineered PBL secreted very high levels of the cytokines gamma-interferon and GM-CSF (> 100,000 pg/ml and >10,000 pg/ml respectively). To determine the relative activity of the transduced PBL populations, serial dilutions of the gp100 peptide were incubated with T2 cells and peptide pulsed cells were co-cultured with APB engineered. The engineered PBL populations were capable of releasing cytokine at low dilutions of peptide (down to 0.2ng/ml).

To determine melanoma reactivity of the TCR gene-transduced PBL, two HLA-A2 positive melanoma cell lines and two non HLA-A2 melanoma cell lines were co-cultured with TCR and control vector transduced PBL cultures. HLA-A2 restricted gamma-interferon release was demonstrated in both engineered PBL cultures analyzed, with levels comparable to control TIL cultures. As a more stringent assay for effector function in the TCR engineered PBL, we determined if these cells were capable of lysing melanoma cell lines. The engineered PBL were incubated with 51Cr labeled melanoma cell lines (HLA-A2

positive and HLA-A2 negative line) at various effector to target cell ratios and the relative lysis then determined. The engineered cells readily lysed the HLA-A2 cell line but not the non HLA-A2 line.

These data demonstrated that using retroviral vector-mediated gene transfer we were able to transfer avid anti-gp100 and anti-MART-1 TCR genes into a bulk population of both TIL and PBL and these cells demonstrated HLA-A2 restricted effector functions including cytokine release and cell lysis.

Based on this technology the Surgery Branch currently has conducted five TCR gene transfer trials that have enrolled a total of 71 patients. In these protocols patients with metastatic cancer who are HLA-A2 positive received a nonmyeloablative but lymphocyte depleting preparative regimen consisting of cyclophosphamide and fludarabine, and then were treated with autologous peripheral blood lymphocytes or TIL that were genetically engineered to be reactive with melanoma tumor antigens gp100 or MART-1, or with p53. Following adoptive cell transfer, all patients received high-dose IL-2, and some patient received peptide vaccination. In four studies (04-C-0181, 04-C-0251, 07-C-0174, and 07-C-0175) patients with metastatic melanoma are being evaluated, and in one study (07-C-0003), patients with metastatic cancer whose tumors overexpress p53 are being evaluated.

To test the in vivo efficacy of the MART-1 TCR engineered T cells in 04-C-0251, 17 HLA-A*0201 patients with progressive metastatic melanoma (Table 4) were selected for treatment [14]. All patients were refractory to prior therapy with IL-2. T cell cultures from all 17 patients were biologically reactive, with specific secretion of interferon-β following co-culture with either MART-1 peptide pulsed T2 cells and or melanoma cell lines expressing the MART-1 antigen (Figure 2E). Gene transfer efficiencies measured by staining for Vβ12 expression in these lymphocytes ranged from 17% to 67% (mean value 42%) (Tables 4 and 5).

Patients received adoptive cell transfer (ACT) with MART-1 TCR transduced autologous PBL at a time of maximum lymphodepletion. An initial cohort of three patients was treated with cells following an extended culture period of 19 days, at which point they had cell doubling times ranging from 8.7 to 11.9 days (Table 4, cohort 1; patients 1, 2a, 3). In these patients, less than 10% of the transduced cells persisted across the time points tested during the first 30 days post-infusion and 2% or less persisted beyond 50 days (Figure 3A). These first three patients showed no delay in the progression of disease.

In an effort to administer gene-modified lymphocytes that were in their active growth phase, the culture conditions were modified to limit the ex vivo culture period to between 6 and 9 days after stimulation of cells with anti-CD3 antibody (Table 4; cohort 2, doubling times two days or less). In a further cohort, larger numbers of actively dividing cells for ACT were generated by performing a second rapid expansion protocol[15] after 8-9 days (Table 4, cohort 3; doubling time 0.9 to 3.3 days). In contrast to the lack of cell persistence seen in cohort 1 (Figure 3A), patients in cohorts 2 and 3 (Figure 3B, 3C, 3D), all exhibited persistence of the transduced cells at greater than 9% at one and four weeks post-treatment (range 9%-56%). All eight patients providing samples at greater than 50 days exhibited persistence at greater than 17%, and this was durable in the seven patients over a monitoring period of over 90 days. One patient (patient 14) had > 60% of circulating lymphocytes positive for the gene marked cells (Figure 3C).

In 14 patient samples tested at one month post-transfer, quantitative RT-PCR assays revealed the presence of vector derived RNA confirming that gene expression continued

(Table 6). All but one of 15 patients analyzed had increased levels of CD8+/Vβ12 cells at one week post-treatment and 11 of 15 were higher at one month compared to pretreatment levels (Figure 3E) All 13 patients examined had increased MART-1 tetramer-binding cells post-treatment (Figure 3F), and 11 of 14 had increased number of elispot positive cells (Table 7).

There was however, a discordance between the mean persistence of transduced cells at one month in cohorts 2 and 3 as measured by PCR (mean 26%), compared to the measurement of Vβ12 expressing cells (8.1%) and of MART-1 tetramer-binding cells (0.8%). This discordance is in part due to mispairing of the introduced TCR chains with the endogenous chains, as well as, the different sensitivities of the assays. Tetramer binding requires the aggregation of multiple receptor molecules on the lymphocyte surface and is the least sensitive of the assays. The reduced expression of the transgene in the persisting cells at one month and later is also a function of the described decrease [16]in the transcription of retrovially inserted trangenes and the decline in metabolic activity during the conversion of activated cells to memory cells.

Most importantly, four patients demonstrated a sustained objective regression of their metastatic melanoma assessed by standard RECIST criteria, two reported in Morgan, et al. [17]. Patient 4, a 52 year old male, had received prior treatment with alpha interferon, lymph node dissection, experimental vaccine, and high-dose IL-2. He then developed progressive disease in the liver (4.4 X 3.3 cm) and axilla (1.3 X 1.2 cm). Following treatment in the current protocol he experienced complete regression of an axillary mass, and an 89% reduction of a liver mass (Figure 4A, 4B) at which time it was removed. He experienced a sustained partial response of 23 months in duration. Patient 14, a 30 year old male, received prior treatment with lymph node dissection, alpha interferon, and high-dose IL-2. He developed an enlarging 4.0 X 2.5 cm mass in the lung hilum. Following treatment in the current protocol he underwent regression of the hilar mass and is now clinically disease free 30 months later (Figure 4C, 4D). In addition, patient 24, a 56 year old female, who had received prior treatment with alpha interferon, lymph node dissection, and high-dose IL-2 and received 36 X 10^9 CD8+ purified cells followed by immunization with the native MART-1 (MART-1:27-35). She has experienced a sustained partial response of retroperitoneal lymph node lesions. Also, patient 25, experienced a sustained partial response after treatment with 8.59 X 10^9 CD8+ purified cells followed by immunization with the modified MART-1 peptide (MART-1:27-35 (27L). This patient is a 49 year old male with history of metastatic melanoma from an unknown primary to the right groin/ iliac LN who previously received IL-2. Thus, four patients with rapidly progressive metastatic melanoma were successfully treated with genetically engineered autologous PBL.

In responding patients 4 and 14, gene marked cells in the circulation (assumed to be 1% of total body lymphocytes) expanded 1400 fold and 30 fold respectively compared to the infusion cell number. At one year post-infusion, both responding patients had sustained high levels (between 20%-70%) of circulating gene-transduced cells (Figure 4E). This high level of gene marked cells was confirmed in patient 4 by limiting dilution T cell cloning of circulating lymphocytes at one year post-treatment which revealed that 33 of 79 (42%) T cell clones contained the transgene as assessed by PCR assay. These two patients also displayed Vβ12 cells detectable by antibody staining between 12%-16% when followed out to >300 days post-treatment (Figure 4F). The responding patients 4 and 14 were also 2 of 4 patients who had greater than 1% circulating tetramer positive cells at greater than 15 days after cell

infusion (Figure 3F), and these patients demonstrated anti-TAA reactivity in ex vivo co-culture assays (Table 8). There were no toxicities in any patient attributed to the gene-marked cells.

We have thus demonstrated for the first time in humans, that normal autologous T lymphocytes, transduced ex vivo with anti-TAA TCR genes and reinfused in cancer patients can persist and express the transgene long-term in vivo and mediate the durable regression of large established tumors. Although the response rate of 2/15 (13%) seen in cohorts 2 and 3 is lower than the 50% response rate we achieved by the infusion of autologous TIL, this method can be used in patients for whom TIL are not available and in patients with common epithelial cancers (Figure 2A, Table 9). Two additional patients have recently been documented as PR in arm P2A (anti-MART1 TCR PBL, up to 3x10e9 with MART-1:27-35); and arm P1H (anti-MART-1 TCR CD8 PBL, up to 1x10e10 with MART-1:27-35 (27L) peptide for a total of 4/39 responses.

A similar study was conducted using gp100 TCR gene marked cells, however this retroviral vector had a low titer when produced under GMP conditions. Nineteen patients have been treated on this study, and one partial response was observed in a patient treated with TIL. To date, 12 patients have been treated on the anti-p53 TCR gene marked cells study. Eleven patient's tumors did not respond to treatment, and one partial response was observed in a patient with a parotid gland tumor metastatic to a hilar lymph node and multiple metastases throughout the lungs. There have been no grade 5 toxicities observed on this study, and all grade 3 and 4 toxicities observed, with one exception, are expected toxicities associated with the non-myeloabalative chemotherapy regimen or IL-2. One patient experienced grade 3 buttock pain which was unexpected, however this toxicity was due to progression of a gluteal lesion and therefore unrelated to the investigational agent.

Our two newest TCR studies investigate more potent TCRs which target gp100 and MART-1. The anti-gp100(154) TCR transduced PBL study (07-C-0174) has accrued 10 patients to date. At this time, 2 patients have been evaluated for response and these patients had progressive disease. Eight patients have been evaluated for toxicities, and most grade 3 and 4 toxicites are known toxicities of the research, and no toxicities have been attributed to the cells. One grade 3 toxicity (headache) was an unexpected event and was possibly related to the research. No grade 5 events have been observed on this study. The anti-MART-1 F5 TCR transduced PBL study (07-C-0175) has accrued 11 patients to date. At this time three patients have been evaluated for response with one patient experiencing a partial response. Nine patients have been evaluated for toxicities, and most grade 3 and 4 toxicities are known toxicities of the research, with no toxicities attributed to the cells. Two grade 3 toxicities (pain, headache) and one grade 4 toxicity (pulmonary embolism) were unexpected events but were not related to the research. No grade 5 events have been observed on this study.

1.2.4. NY ESO-1 as a Target for Cell Transfer Clinical Studies

The NY-ESO-1 molecule, which was initially identified by screening a cDNA expression library with an antiserum from a patient with esophageal squamous cell carcinoma, represents a tumor antigen that can be targeted in patients bearing a wide variety of malignancies [18]. Expression of NY-ESO-1 protein has been observed in approximately one third of melanoma, breast, prostate, lung ovarian, thyroid and bladder cancer, but is limited in normal tissues to germ cells and trophoblasts [19]. A related cancer/testis antigen, LAGE-1, has

also been identified and shown to possess 84% amino acid similarity to the NY-ESO-1 protein[20]. Further studies resulted in the identification of an identical peptide corresponding to amino acids 157 to 165 of the NY-ESO-1 and LAGE-1 proteins SLLMWITQC as a dominant epitope recognized by HLA-A2 restricted, NY-ESO-1 reactive T cells[21]. An HLA-A2 restricted epitope representing the first eleven amino acids of an alternative open reading frame of the NY-ESO-1 and LAGE-1 transcripts has also been described[22] and epitopes derived from the normal as well as alternative open reading frames of both gene products in the context of HLA-A31 have also been described[23]. In addition, NY-ESO-1 epitopes are recognized in the context of multiple HLA class II restriction elements [24-26]. Tumors may have heterogeneous expression of NY-ESO-1 as detected by immunohistochemistry though this is highly dependent on the affinity of the antibody. Studies in melanoma have shown that only 1-5 peptides on the surface of a cell can stimulate lymphocytes, this is far below the sensitivity of most antibodies.

The results of multiple studies indicate that relatively high levels of natural immunity directed against NY-ESO-1 may exist in cancer patient. Serum anti-NY-ESO-1 antibodies are prevalent in cancer patients, and in one study 10 out of 12 patients bearing NY-ESO-1 positive tumors possessed serum antibodies directed against this antigen [27]. This is in contrast to other tumor antigens for which antibody responses have been demonstrated in only 5% or less of patients [28]. Furthermore, the presence of high titers of anti-NY-ESO-1 antibodies in the serum of cancer patient has been associated with tumor burden [27] as well as the presence of T cell precursors reactive with the HLA class I restricted NY-ESO-1:157-165/HLA-A*02 epitope in the peripheral blood of cancer patients [29]. Clinical trials have been based on immunization with the dominant HLA-A2 restricted NY-ESO-1 epitope [30-32], a HLA class II restricted peptide [32], as well as recombinant NY-ESO-1 protein [33]. While evidence indicated that immunization was capable in some patients of enhancing the frequency of peptide or tumor reactive T cells, clinical responses were only observed in a small percentage of patients treated in these trials.

Active immunization strategies directed against a variety of additional tumor antigens have resulted in clinical responses in only a small minority of treated patients [34], whereas adoptive cell transfer strategies have resulted in response rates of up to 50 percent [8]. Another strategy now being employed is the adoptive transfer of T cells that have been genetically modified to express T cell receptors (TCR) directed against widely expressed tumor antigens. As previously discussed, in the first report of a clinical cancer TCR trial, objective clinical responses were observed in 17 percent of melanoma patients treated with T cells that were genetically modified to express a TCR directed against the immunodominant MART-1 HLA-A*02 restricted peptide epitope[14].

Current attempts to extend cancer therapies based on the genetic modification of T cells to patients bearing additional tumor types have focused on the identification of TCRs with high functional avidity directed against the dominant NY-ESO-1:157-165 T cell epitope. A comparison of two anti-NY-ESO-1 TCRs, designated 1G4 and ET-8F, for their ability to mediate peptide reactivity in gene modified $CD8^+$ T cells indicated that the 1G4 TCR was significantly more active than the ET-8F TCR [35]. The 1G4 was also more active than the ET-8F, and an additional NY-ESO-1 reactive TCR, when evaluated for their ability to mediate specific tumor cell recognition in gene modified $CD8^+$ T cells (unpublished results).

These results indicated that the 1G4 TCR may possess a relatively high functional avidity; nevertheless, this TCR, which was determined using a direct binding assay to possess an affinity of between 10 and 30 μM, was not able to confer $CD4^+$ T cells with the ability to recognize target cells expressing the endogenously processed NY-ESO-1 epitope. High affinity variants of the 1G4 TCR generated by bacteriophage display that contained multiple amino acid substitutions (AAS) in both the α and β chain TCR complementarity determining regions (CDR) [236] were then evaluated in an attempt to identify TCR variants that enhanced the function of gene modified $CD8^+$ as well as $CD4^+$ T cells [35]. The results demonstrated that high affinity variant TCRs possessing affinities of between 5 and 84 nM lead to the antigen-specific activation of TCR-gene modified $CD4^+$ T cells, but lead to the non-specific activation of TCR-gene modified $CD8^+$ T cells by HLA-A*02^+ NY-ESO-1^- target cells, whereas TCR variants with affinities nearer to that of the WT 1G4 TCR show enhanced reactivity in $CD4^+$T cells without any apparent loss of specificity in $CD8^+$ T cells. Further studies were carried out to evaluate the role of individual CDR substitutions on the function the 1G4 TCR resulted in the identification of variants containing single and dual CDR2β chain and CDR3α chain substitutions that enhanced the IFN-γ responses of $CD4^+$ as well as $CD8^+$ T cells (Robbins, PF et al., submitted for publication). The results indicated that substitutions of individual CDR2 β chain residues significantly enhanced the responses of gene modified $CD4^+$ T cells to NY-ESO-1^+/HLA-A*02^+ tumor target cells (Figure 5A) and modestly enhanced the responses of CD8+ T cells (Figure 5B). A TCR containing dual substitutions of alanine and isoleucine residues for the glycine and alanine residues present at positions 51 and 52 in the native CDR2 β chain, designated β51:AI, further enhanced the response of transfected $CD4^+$ T cells (Figure 5A). Individual substitutions for CDR3α residues did not lead to significant enhancement of the response of gene modified $CD4^+$ T cells; however, the analysis of TCRs containing dual substitutions for the threonine and serine residues present at positions 95 and 96 of the 1G4 α chain resulted in the identification of two variants, α95:LL and α95:LY, that dramatically enhanced the specific reactivity of transduced $CD4^+$ T cells (Figure 6A), and resulted in modest increases in the responses of transduced CD8+ T cells (Figure 6B). An increased affinity for the NY-ESO-1/HLA-A*02 complex appeared to be responsible for enhancing the responses of gene modified T cells, as the α95:LY plus WT β 1G4 TCR variant possesses an affinity of 730 nM and the WT α chain plus β51:AI variants possess an affinity of 280 nM.

These results clearly demonstrated that the responses of CD4+ T cells transduced with the 1G4 α95:LY and β51:AI variants to NY-ESO-1+/HLA-A*02+ tumor cells were dramatically enhanced relative to those of cells transduced with the WT TCR. An evaluation of the results of multiple experiments indicated that $CD8^+$ T cells transduced with the α95:LY/WT β TCR released higher levels of IFN-γ in response to the NY-ESO-1^+/HLA-A*02^+ tumor target cell lines than those expressing the WT α/β TCR (ratio of 2.2 ± 0.34, mean ± SEM), a statistically-significant level of enhancement ($p<0.01$ using a paired t test, 36 pairs tested). At the same time, the responses of $CD8^+$ T cells transduced with the WT α/β51:AI TCR pair did not differ significantly from the WT α/β TCR (ratio of 1.2 ± 0.24, $p>0.4$, 34 pairs tested). In addition, the responses of $CD8^+$ T cells transduced with the α95:LY/WT β TCR was significantly enhanced relative to those of cells transduced with the WT α /β51:AI variant (ratio of 3.1 ± 0.67, $p<0.01$, 34 pairs tested). Additional results

indicated that the CD8+ T cells produced low but significant levels of IFN-γ in response to NY-ESO-1^{-}/HLA-A*02^{+} tumor cells (Robbins, PF et al., submitted for publication).

The relative effectiveness of CD8^{+} or CD4^{+} T cells in mediating tumor regression is unknown; however, several observations suggest that the presence of CD4^{+} T cells can enhance the function of the CD8^{+} T cell population. The persistence of CMV-reactive CD4^{+} T cells is correlated with enhanced control of disease by CMV-reactive CD8^{+} T cells [37;38]. Co-operative interactions between CD8^{+} and CD4^{+} T cells expressing a high affinity CD8-independent TCR directed against a human p53 epitope have been observed, as assessed by their abilities both to secrete IFN-γ in response to cognate antigen complex expressing targets cell lines and to activate dendritic cells[39]. In addition, the adoptive transfer of CD4^{+} T cells expressing a CD8-independent TCR has been shown to enhance tumor protection mediated by CD8^{+} tumor reactive T cells [40]. Taken together, these results indicate that the α95:LY variant of the 1G4 TCR represents a potent TCR that results in high levels of specific anti-tumor activity in gene modified CD4^{+} as well as CD8^{+} T cells and provide evidence that this represents an attractive candidate for the further evaluation of TCR-based cancer adoptive immunotherapy.

As of October 1, 2009 in the current protocol using transfer of autologous cells transduced with the anti-NY-ESO-1 TCR, four patients with synovial cell sarcoma have been treated. Three of the four patients have experienced an objective partial response by RECIST criteria though the three responders recurred at 10 months, 6 months and 4 months respectively. An additional patient treated by compassionate exemption experienced an objective response and recurred at 3 months. Eight evaluable patients with metastatic melanoma were treated. One experienced a complete response ongoing at 10 months and 3 others experienced a partial response, one ongoing at 8 months and the other two recurred at 8 and 3 months respectively. Two patients with metastatic breast cancer were treated (one treated as a compassionate exemption) and one patient experienced a PR but recurred at 2 months.

Thus of the 15 treated patients evaluable as of October 1, 2009, 9 responded (60%) but 7 of the 9 have recurred at times varying from 2 to 10 months. This recurrence rate is unacceptably high. Our murine models of cell transfer have clearly indicated that the administration of a pox virus in conjunction with cell transfer can significantly increase the effectiveness of the cell transfer and we are thus amending the protocol to add immunization with an ALVAC virus encoding the NY-ESO-1 antigen to the treatment regimen.

In addition, recent studies have emphasized the impact of CD4+, CD25+ regulatory T-cells in inhibiting immune reactions. Our experimental animal data, as well as the studies of the PBL of melanoma patients, have revealed a significant incidence of these T regulatory cells that can impede anti-tumor responses. Eliminating T regulatory cells in our pmel murine models by eliminating CD4 cells significantly improved the impact of adoptively transferred anti-tumor CD8+ cells. It thus appears likely that the T regulatory cells present in peripheral blood lymphocytes may be impeding the effectiveness of the CD8+ anti-tumor lymphocytes. It is possible that removing the cells will improve the response rates in patients and may also reduce the toxicities seen by production of cytokines by CD4+ cells. Therefore, with the approval of amendment E, if after initial treatment with the unselected anti- ESO-1 TCR-transduced cells and ALVAC ESO-1 vaccine patients do not respond to treatment or progress after a response or have stable disease, they will be allowed retreatment with CD8-enriched anti ESO-1 TCR-transduced cells and ALVAC ESO-1 vaccine provided

they meet the original eligibility criteria. We plan to utilize the GMP quality, CliniMACS apparatus from Miltenyi Biotec that is in common use in the NCI and many other centers. The exact procedure for depletion utilized in protocol 07-C-0176 to eliminate CD4+ cells will be used in this current protocol. In our current study evaluating young TIL (07-C-0176), we have observed a 51% clinical response rate (CR and PR) in the 33 patients receiving the CD8+ young TIL cells compared to a 20% response rate in the 26 patients receiving young TIL containing CD4+ and CD8+ cells. In addition, patients receiving the CD8+ young TIL experienced less adverse events. With approval of amendment J, patients will not be retreated with the CD8-enriched cells. Data from our randomized study comparing CD8-enriched young TIL and bulk young TIL(containing CD4+ and CD8+ cells), indicates that there is no difference in clinical response nor toxicities in patients treated in these two randomized cohorts. Therefore, patients will be retreated with anti-ESO-1 TCR cells similar to their initial treatment which contained CD4+ and CD8+ cells. In addition, patients who are retreated will only receive the ALVAC vaccine if it was part of their initial treatment and it is available. In order to be retreated, patients must have a partial response to treatment that then stabilizes and have evaluable disease, or stable disease that subsequently progresses. They may be re-treated when progression by RECIST criteria is documented.

1.2.5. Rationale for the current protocol to add immunization with ALVAC pox viruses following adoptive transfer of cells.

In extensive studies utilizing the pmel murine melanoma model it has been clearly demonstrated that the adoptive transfer of tumor-reactive T cells into a lymphodepleted mouse can mediate the rejection of established melanoma and that the simultaneous administration of a pox virus vaccine encoding the antigen recognized by the T cells could greatly improve the activity of the T cell transfer [41, 42] Preclinical data has demonstrated that peptide vaccination is ineffective whereas vaccination with pox viruses can improve the effectiveness of the adoptively transferred cells and could mediate complete tumor regression[42].Thus, in this animal model, mice bearing large invasive B16 melanomas receive the adoptive transfer of transgenic murine T cells reactive with the gp100 epitope presented on the B16 melanoma. Following the adoptive transfer of these cells, mice received the administration of IL-2 with or without the simultaneous administration of the recombinant fowlpox virus encoding a modified form of the gp100 epitope reactive with the adoptively transferred cells. As shown in Figures 7, 8, and 9 the administration of this fowlpox virus substantially improved the effectiveness of the adoptively transferred cells and could mediate complete tumor regression of these large tumors in mice. These anti-tumor effects of the vaccine were highly reproducible and were substantially greater in mice that were immunosuppressed by whole body irradiation or in highly immunosuppressed RAC knockout mice. These studies of immunization with recombinant pox viruses after cell transfer in the immunosuppressed host provided strong evidence that suggests that adoptive transfer of lymphocytes in our clinical protocols in patients with melanoma will be substantially improved by the simultaneous administration of recombinant pox virus expressing the antigen recognized by the transferred T cells.

We have extensive experience in the administration of fowlpox viruses to patients with metastatic melanoma in the absence of cell transfer. Twelve patients with metastatic melanoma were treated with Fowlpox virus encoding MART-1[43]. In three consecutive clinical trials a total of 46 patients were immunized with recombinant fowlpox virus

encoding gp100[44]. In these studies viruses incorporating the native gp100 molecule as well as a "minigene" construct encoding a single modified epitope, gp100:209-217(210M) were used for immunization. We showed that these viruses could be administered safely in doses as high as 5-6x10^9/pfu intravenously or intramuscularly. Patients could be successfully immunized after inoculation with these recombinant viruses although clinical anti-tumor responses were only seen when these immunizations were administered in conjunction with IL-2. The current canary pox viruses (ALVAC) being supplied by Sanofi-Pasteur are very similar to the fowlpox viruses we used in the prior studies with the exception of the introduction of co-stimulatory molecules (TRICOM constructs) into the virus. We have now treated four metastatic melanoma patients with the current ALVAC viruses (including two who received the ALVAC MART-1 and two who received the ALVAC gp100 vaccine in conjunction with cell transfer) and have seen no toxicity attributable to the virus administration. We have not seen any added toxicities of this combination of cell transfer therapy along with the pox viruses and the number of viral particles being used for immunization is far lower than that we have previously administered.

1.2.6. NY-ESO-1 ALVAC Vaccine

Starting with Amendment E, the investigational vaccine to be used in the proposed clinical trial, is a viral vector-based vaccine that is comprised of a gene for a tumor-associated antigen (TAA). The vaccine was developed by Sanofi-Pasteur Ltd. who sponsors an IND for this agent. The NY-ESO-1 is one of five TAA chosen for inclusion in the ALVAC virus by Sanofi-Pasteur. The NY-ESO-1 gene used in ALVAC(2)-NY-ESO-1(M)/TRICOM [vCP2292] was altered by *in vitro* mutagenesis, changing the encoded amino acid at position 165 from cysteine to valine. This modification was designed to enhance the immunogenicity of the antigen

In addition, the vaccine contains genes that encode three co-stimulatory/adhesion molecules: B7.1, ICAM-1, and LFA-3. B7.1 is an immune response modulating signal, while the other two are cellular adhesion molecules. These molecules, which are collectively referred to as TRICOM, are intended to increase the immune response to the TAAs.

The vector is a modified canarypox virus, ALVAC(2), that induces both humoral and cellular immune responses to the inserted transgenes.

The genes for the TAA and the genes for the co-stimulatory molecules are included in the same construct. The complete vaccine therefore consists of the following construct:

- ALVAC(2)-NY-ESO-1(M)/TRICOM (also known as vCP2292 and herein called ALVAC ESO-1 vaccine)

The modified canarypox virus ALVAC(2) is used as the antigen-presentation platform. ALVAC is a plaque purified isolate of canarypox virus; ALVAC(2) was derived from ALVAC by inserting two coding sequences (E3L and K3L) from vaccinia virus. These additional sequences have been shown to enhance virus-specific gene expression. Previous studies suggest that ALVAC-based recombinants have significant advantages as vector-based vaccines, since they are safe for vaccination purposes in animals and humans and induce both humoral and cellular immune responses to inserted transgenes. The safety of ALVAC(2) has been established in a Canadian clinical trial conducted from February 2000 to April 2003,

titled “Recombinant Canarypox Virus Expressing Tumor Antigen gp100 (Modified) and Modified gp100 Peptide Combination”.

Poxviruses are strong stimulators of the cellular arm of the immune system, and are thus attractive vectors for use in immunotherapeutic strategies of stimulating T-cell responses to TAAs. Avian poxviruses, including ALVAC(2), provide additional safety advantages over other poxviruses as they do not productively replicate in human cells. Although they enter mammalian cells and express viral gene products from their cytoplasmic replication site, infectious progeny virus is not produced. Virally expressed gene products are available for processing and presentation by major histocompatibility complex (MHC) molecules. However, poxviruses have a limited life span in mammalian cells such that TAA expression is sustained for a limited time.

ALVAC-based vectors engineered to express antigens of choice have been developed for active vaccination of patients with infectious diseases or cancer. Several clinical trials using ALVAC-based vaccines have been conducted for melanoma (Canada), colorectal cancer (Canada and USA) and Human Immunodeficiency Virus (USA). In addition, there are ALVAC-based vaccines that have been licensed for veterinary applications, including canine distemper virus (USA), feline leukemia virus (EU), and rabies (USA).

TRICOM is composed of three co-stimulatory/adhesion molecules: B7.1, Intercellular Adhesion Molecule-1 (ICAM-1), and Lymphocyte Function Associated antigen-3 (LFA-3). These proteins are normally expressed at low levels on the surface of professional antigen-presenting cells. They are up-regulated and serve to augment immune responses through engagement of their counter-receptors on T-cells, CD28, CD11a/CD18, and CD2, respectively, after activation. There are a number of published reports using mouse, monkey, and human systems that demonstrate that the incorporation of TRICOM into poxviruses can enhance T-cell responses, and that the three components together are more effective than any one or two of the three. In addition, mice vaccinated with recombinant vaccinia and fowlpox viruses containing TRICOM and carcinoembryonic antigen (CEA) showed enhanced T-cell responses and tumor protection compared to similar vectors containing CEA alone. It is believed that ALVAC- encoded TRICOM vaccine will infect some antigen-presenting cells in the local vicinity after vaccination. This will result in expression of high levels of co-stimulatory molecules in such antigen-presenting cells that also express the melanoma tumor-associated antigens. After injection, these will be highly effective at activating both CD8 and CD4 T cells.

With approval of amendment G, cohorts 1 and 2 will be re-opened due to the shortage of the ALVAC ESO-1 vaccine at this time. We plan to leave cohorts 3 and 4 open as we would like to accrue to these arms if the ALVAC ESO-1 vaccine becomes available in the future. To date, we have seen remarkable clinical responses in patients treated on this study. In cohort 1, we have treated 13 patients with melanoma, and 5 of 11 patients with high expression of ESO-1 had clinical response including 3 partial responses (PRs) of 3, 8 and 14+ months, and 2 complete responses (CRs) of 24, and 25 + months. In cohort 2, we have treated 7 patients and 6 of these patients were heavily pretreated synovial cell sarcoma. Four patients with synovial cell sarcoma experienced PRs lasting 10, 14, 4 and 8 months. Therefore, the response rate in patients receiving non-myeloablative chemotherapy, anti-ESO-1 TCR PBL and high dose IL-2 but no vaccine is 45%. In cohort 3 (with vaccine), we have treated 6 patients with melanoma, and of the three evaluable patients, 1 patient has an ongoing PR of 6+ months. In cohort 4 (with vaccine), 5 patients have been treated, and out

of the 4 evaluable patients, 2 patients with synovial cell sarcoma have ongoing PRs of 7+ months. The preliminary data from cohorts 3 and 4 indicate that clinical response rates observed with vaccine in cohorts 3 and 4 are similar to those observed without vaccine in cohort 1 and 2. In addition, the adverse events observed in each arm are comparable with most toxicities attributed to the non-myeloablative chemotherapy regimen and high dose IL-2.

1.2.7. Safety Considerations

Several safety concerns regarding the infusion of large numbers of retrovirally modified tumor reactive T-cells have been addressed in our previous clinical studies. The non-myeloablative chemotherapy and the administration of high-dose IL-2 have expected toxicities discussed earlier. The immunomyeloablative chemotherapy used in this protocol has been administered to over 80 patients and all have reconstituted their hematopoietic systems.

In other protocols we have administered over 3×10^{11} TIL with widely heterogeneous reactivity including CD4, CD8, and NK cells without difficulty. As discussed above, the expansion of tumor reactive cells is a desirable outcome following the infusion of antigen reactive T-cells. Some patients receiving gp100 or MART-1 reactive cells have developed vitiligo or uveitis probably due to destruction of normal melanocytes though these toxicities have been manageable. We do not believe the transfer of these gene modified cells has a significant risk for malignant transformation in this patient population. While the risk of insertional mutagenesis is a known possibility using retroviral vectors, this has only been observed in the setting of infants treated for XSCID using retroviral vector-mediated gene transfer into CD34+ bone marrow cells. In the case of retroviral vector-mediated gene transfer into mature T-cells, there has been no evidence of long-term toxicities associated with these procedures since the first NCI sponsored gene transfer study in 1989. Although continued follow-up of all gene therapy patients will be required, data suggest that the introduction of retroviral vectors transduced into mature T-cells is a safe procedure. While we believe the risk of insertional mutagenesis is extremely low, the proposed protocol follows all current FDA guidelines regarding testing and follow up of patients receiving gene transduced cells.

2.0 Eligibility Assessment and Enrollment

2.1. Eligibility Criteria

2.1.1. Inclusion Criteria

a. Metastatic cancer that expresses ESO as assessed by one of the following methods: RT-PCR on tumor tissue, or by immunohistochemistry of resected tissue, or serum antibody reactive with ESO. Metastatic cancer diagnosis will be confirmed by the Laboratory of Pathology at the NCI.
b. Patients with histologies other than metastatic melanoma, must have previously received systemic standard care (or effective salvage chemotherapy regimens) for

metastatic disease, if known to be effective for that disease, and have been either non-responders (progressive disease) or have recurred.

c. Greater than or equal to 18 years of age and less than or equal to 66 years of age.
d. Willing to sign a durable power of attorney
e. Able to understand and sign the Informed Consent Document
f. Clinical performance status of ECOG 0 or 1.
g. Life expectancy of greater than three months.
h. Patients of both genders must be willing to practice birth control for four months after receiving the preparative regimen.
i. Patients must be HLA-A*0201 positive
j. Serology:
    1. Seronegative for HIV antibody. (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who are HIV seropositive can have decreased immune -competence and thus be less responsive to the experimental treatment and more susceptible to its toxicities.)
    2. Seronegative for hepatitis B antigen and hepatitis C antibody unless antigen negative. If hepatitis C antibody test is positive, then patients must be tested for the presence of antigen by RT-PCR and be HCV RNA negative.
k. Hematology:
    1. Absolute neutrophil count greater than $1000/mm^3$ without the support of filgrastim.
    2. WBC (> $3000/mm^3$).
    3. Platelet count greater than $100{,}000/mm^3$.
    4. Hemoglobin greater than 8.0 g/dl.
l. Chemistry:
    1. Serum ALT/AST less or equal to 2.5 times the upper limit of normal.
    2. Serum creatinine less than or equal to 1.6 mg/dl.
    3. Total bilirubin less than or equal to 1.5 mg/dl, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 3.0 mg/dl.
m. More than four weeks must have elapsed since any prior systemic therapy at the time the patient receives the preparative regimen, and patients' toxicities must have recovered to a grade 1 or less (except for toxicities such as alopecia or vitiligo).
n. Six weeks must have elapsed since prior ipilimumabtherapy to allow antibody levels to decline.

Note: Patients who have previously received ipilimumab or tremelimumab, anti- PD1 or anti-PD-L1 antibodies, and have documented GI toxicity must have a normal colonoscopy with normal colonic biopsies.

2.1.2. Exclusion Criteria

a. Prior vaccination with an ALVAC containing vaccine for patients who will receive the ALVAC ESO-1 vaccine (cohorts 3 or 4).
b. Women of child-bearing potential who are pregnant or breastfeeding because of the potentially dangerous effects of the preparative chemotherapy on the fetus or infant.

c. Active systemic infections, coagulation disorders or other major medical illnesses of the cardiovascular, respiratory or immune system, myocardial infarction, cardiac arrhythmias, obstructive or restrictive pulmonary disease.
d. Any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease).
e. Concurrent opportunistic infections (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who have decreased immune competence may be less responsive to the experimental treatment and more susceptible to its toxicities).
f. Concurrent Systemic steroid therapy
g. Known systemic hypersensitivity to any of the vaccine components, including egg products or Neomycin for patients who will receive the ALVAC ESO-1 vaccine (cohorts 3 or 4).
h. History of severe immediate hypersensitivity reaction to any of the agents used in this study.
i. History of coronary revascularization or ischemic symptoms
j. Any patient known to have an LVEF less than or equal to 45%.
k. Documented LVEF of less than or equal to 45% tested in patients with:
    1. History of ischemic heart disease, chest pain, or clinically significant atrial and/or ventricular arrhythmias including but not limited to: atrial fibrillation, ventricular tachycardia, second or third degree heart block
    2. Age greater than or equal to 60 years old
l. Documented FEV1 less than or equal to 60% predicted tested in patients with:
    1. A prolonged history of cigarette smoking (20 pk/year of smoking within the past 2 years).
    2. Symptoms of respiratory dysfunction

2.2. Research Eligibility Evaluation

Within **4 weeks** prior to starting the chemotherapy regimen:

a. Complete history and physical examination, including height, weight and vital signs, and eye exam noting in detail the exact size and location of any lesions that exist. (**Note**: patient history may be obtained within 8 weeks)
b. Chest x-ray
c. EKG
d. Baseline CT of the chest, abdomen and pelvis, and brain MRI to evaluate the status of disease. Additional scans and x-rays may be performed if clinically indicated based on patients' signs and symptoms.
e. Colonoscopy for patients meeting requirements noted in Section 2.1.1.o.
f. Pulmonary Function Testing for patients with a prolonged history of cigarette smoking or symptoms of respiratory dysfunction . Patients with an FEV1 of less than 60% predicted for age will not proceed to treatment. (Testing may be performed within 8 weeks of treatment)
g. Cardiac evaluation (stress thallium, echocardiogram, MUGA etc.,) in patients who are greater than or equal to age 60, or have a history of ischemic heart disease, chest pain, or clinically significant atrial and/or ventricular arrhythmias including but not limited to: atrial fibrillation, ventricular tachycardia, heart

block.. Patients with a LVEF of less than or equal to 45% will not proceed to treatment. (Testing may be performed within 8 weeks of treatment).

h. HIV antibody titer and HbsAG determination, anti HCV (may be performed within 3 months of chemotherapy start date)
i. Anti CMV antibody titer, HSV serology, and EBV panel. (**Note:** may be performed within 3 months of chemotherapy start date; patients who are known to be positive for any of the above do not need to be retested.)
j. Documentation of results HLA typing done at the NIH (testing is permitted to be conducted at any time prior to this point).
k. One of the following methods will be used to assess ESO expression: RT-PCR analysis of tumor biopsy, or immunohistochemistry, or analysis of serum to evaluate for expression of ESO1 antibody reactive with ESO. Immunohistochemistry verification of ESO expression will be carried out in a CLIA-approved pathology laboratory, while RT-PCR and ELISA assay for ESO1 antibody will be conducted in Dr. Rosenberg's laboratory using CLIA-approved methods. (Testing is permitted to be conducted at any time prior to this point).

Within **14 days** prior to starting the chemotherapy regimen:

l. Chem 20: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO^2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid)
m. Thyroid panel
n. CBC with differential and platelet count
o. PT/PTT
p. Urinalysis and culture, if indicated

Within **7 days** prior to starting the chemotherapy regimen:

q. β-HCG pregnancy test (serum or urine) on all women of child-bearing potential
r. ECOG performance status of 0 or 1

2.3. Patient Registration

Patients will be registered on protocol 03-C-0277 (Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols) prior to transduction of PBL cells (either fresh or cryopreserved samples), by the clinical fellow or research nurse within 24 hours of the patient signing the consent by faxing a completed Eligibility Checklist to the Central Registration Office (CRO) at 301-480-0757. Once cells exceed the potency requirement and are projected to exceed the minimum number specified in the Certificate of Analysis (COA), patients will sign the consent document for this protocol and the Eligibility Checklist will be completed and faxed to the Central Registration Office at 301-480-0757 within 24 hours of the patient signing the consent.

3.0 Study Implementation

3.1. Study Design:

3.1.1. Treatment Phase:

PBMC will be obtained by leukapheresis (approximately 1 X 10^{10} cells). Whole PBMC will be cultured in the presence of anti-CD3 (OKT3) and aldesleukin in order to stimulate T-cell growth. Transduction is initiated by exposure of approximately 1 X 10^7 to 5 X 10^8 cells to supernatant containing the anti-ESO TCR retroviral vector. These transduced cells will be expanded and tested for their anti-tumor activity. Successful TCR gene transfer will be determined by FACS analysis for the TCR protein and anti-tumor reactivity will be tested by cytokine release as measured on peptide pulsed T2 cells. Successful TCR gene transfer for each transduced PBL population will be defined as >10% TCR positive cells and for biological activity, gamma-interferon secretion must be at least 200 pg/ml. Patients will receive up to 1.0 X 10^{11} anti-ESO TCR engineered PBL. A minimum of approximately 1.0 X 10^8 cells will be given. The cells administered vary depending on their growth characteristic. In prior protocols over 3 X 10^{11} T cells have been safely infused to cancer patients.

Prior to receiving the engineered PBL cells, patients will receive a nonmyeloablative but lymphocyte depleting preparative regimen consisting of cyclophosphamide and fludarabine followed in one to four days by intravenous infusion of in vitro tumor reactive, TCR gene-transduced PBL plus IV aldesleukin (720,000 IU/kg q8h for a maximum of 15 doses). Patients will receive one course of treatment. The start date of the course will be the start date of the chemotherapy; the end date will be the day of the first post-treatment evaluation. Patients may undergo a second treatment as described in Section 3.4.

Patients will be entered into two cohorts based on histology: cohort 1 will include patients with metastatic melanoma or renal cell cancer; cohort 2 will include patients with other types of metastatic cancer. All cancers must be shown to express ESO.

Patients accrued to cohorts 3 and 4 will receive a nonmyeloablative but lymphocyte depleting preparative regimen consisting of cyclophosphamide and fludarabine followed by subcutaneous injection of ALVAC ESO-1 vaccine approximately 2 hours prior to intravenous infusion of ex vivo tumor reactive, TCR gene-transduced PBMC plus IV aldesleukin (720,000 IU/kg q8h for a maximum of 15 doses). A second vaccination with ALVAC ESO-1 vaccine will be given on day 14 (+/- 2 days). Cohort 3 will include patients with metastatic melanoma or renal cell cancer and cohort 4 will include patients with other types of metastatic cancer.

3.1.2. Protocol Stopping Rules:

The study will be halted pending discussions with the FDA and NCI IRB if the following conditions are met:

- Two or more patients develop a grade 3 or greater toxicity at any point in the study (≥ grade 3 toxicity) not attributable to the chemotherapy preparative regimen or aldesleukin therapy (or circumstances unrelated to this study).
- If 1 of the first 3 patients (OR 2 of the first 6 patients, OR 3 of the first 9 patients, OR 4 of the first 12 patients) develop grade 3 autoimmunity, that cannot be resolved to less than or equal to a grade 2 autoimmune toxicity within 10 days or any grade 4 or greater autoimmune toxicity.

- Development of EBV lymphoma or polyclonal lymphoproliferative disease (PLPD) in an EBV negative subject on this or any other SB adoptive cell therapy study. Accrual of EBV negative subjects will be halted only.

3.2. Drug Administration:

(see table 3.2)

3.2.1. Preparative Regimen with Cyclophosphamide and Fludarabine (all cohorts)

(*Times are offered as examples and may be changed as long as a similar time relationship between administration of the drugs is maintained. Study medication start times for drugs given once daily should be given within 2 hours of the scheduled time. All other medications should be given +/- one hour of the scheduled time; the length of administration is all +/- 15 minutes. Administration of diuretics, electrolyte replacement, and hydration and monitoring of electrolytes should all be performed as clinically indicated – the doses and times noted below are offered only as examples.)*

Day -7 and –6 1 am

Hydrate: Begin hydration with 0.9% Sodium Chloride Injection containing 10 meq/L of potassium chloride at 2.6 ml/kg/hr (starting 11 hours pre-cyclophosphamide and continue hydration until 24 hours after last cyclophosphamide infusion). At any time during the preparative regimen, if urine output <1.5 ml/kg/hr or if body weight >2 kg over pre-cyclophosphamide value, furosemide 10-20 mg IV maybe administered. Serum potassium should be monitored and treated as indicated following administration of furosemide.

11 am

Ondansetron (0.15 mg/kg/dose [rounded to the nearest even mg dose between 8 mg and 16 mg based on patient weight] IV every 8 hours X 3 days) will be given for nausea.

12 pm (NOON)

Cyclophosphamide 60 mg/kg/day X 2 days IV in 250 ml D5W with mesna 15 mg/kg/day X 2 days over 1 hr. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Table 10.

1 pm

Begin mesna infusion at 3 mg/kg/hour intravenously diluted in a suitable diluent (see pharmaceutical section) over 23 hours after each cyclophosphamide dose. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Table 10.

Day -5 to Day -1:

Fludarabine 25 mg/m2/day IVPB daily over 30 minutes for 5 days. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Table 10.

3.2.2. Cell Infusion and Aldesleukin Administration (cohorts 1 and 2)

Cells are delivered to the patient care unit by a staff member from the Tumor Immunology Cell Processing Laboratory. Prior to infusion, the cell product identity label is double-checked by two authorized staff (MD or RN), and identification of the product and documentation of administration are entered in the patient's chart, as is done for blood banking protocols. The cells are to be infused intravenously over 20-30 minutes via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping.

Day 0 (one to four days after the last dose of fludarabine):

- Cells will be infused intravenously (i.v.) on the Patient Care Unit over 20 to 30 minutes (between one and four days after the last dose of fludarabine). Note: for patients with miliary lung disease up to 5 x 10^{10} cells will be administered, split into two equal doses. The first dose will be administered on day 0, and if there are no infusion related toxicities, the second dose will be given on day +1. Aldesleukin will start after the 2nd dose of cells.
- Aldesleukin (based on total body weight) 720,000 IU/kg IV over 15 minute approximately every eight hours beginning within 24 hours of cell infusion and continuing for up to 5 days (maximum of 15 doses).

Day 1-4 (Day 0 is the day of cell infusion):

- Beginning on day 1 or 2, filgrastim will be administered subcutaneously at a dose of 5 mcg/kg/day (not to exceed 300 mcg/day). Filgrastim administration will continue daily until neutrophil count > 1.0 x10^9/L X 3 days or > 5.0 x10^9/L.
- Aldesleukin (based on total body weight) 720,000 IU/kg IV over 15 minute approximately every eight hours for up to 5 days

3.2.3. ALVAC Vaccine, Cell Infusion and Aldesleukin Administration (cohorts 3 and 4).

Cells are delivered to the patient care unit by a staff member from the Tumor Immunology Cell Processing Laboratory. Prior to infusion, the cell product identity label is double-checked by two authorized staff (MD or RN), an identification of the product and documentation of administration are entered in the patient's chart, as is done for blood banking protocols. The cells are to be infused intravenously over 20-30 minutes via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping.

Day 0 (one to four days after the last dose of fludarabine):

- ALVAC ESO -1 Vaccine: Approximately two hours prior to cell infusion, patients will receive 0.5 mL containing a target dose of 10^7 $CCID_{50}$ (with a range of approximately $10^{6.4}$ to $10^{7.9}$ / mL) of the ESO-1 ALVAC virus S.C. in each extremity (total of 4 x 10^7 $CCID_{50}$/2 mL. If a patient has a compromised extremity due to lymphedema, prior radiation or other causes, vaccination may be given on the abdominal wall or chest wall proximal to the extremity.
- Cells will be infused intravenously (i.v.) on the Patient Care Unit over 20 to 30 minutes (between one and four days after the last dose of fludarabine). Note: for patients with miliary lung disease up to 5 x 10^{10} cells will be administered, split

into two equal doses. The first dose will be administered on day 0, and if there are no infusion related toxicities, the second dose will be given on day +1. Aldesleukin will start after the 2nd dose of cells.

- Aldesleukin (based on total body weight) 720,000 IU/kg IV over 15 minute approximately every eight hours beginning within 24 hours of cell infusion and continuing for up to 5 days (maximum of 15 doses).

Day 1-4 (Day 0 is the day of cell infusion):

- Beginning on day 1 or 2, filgrastim will be administered subcutaneously at a dose of 5 mcg/kg/day (not to exceed 300 mcg/day). Filgrastim administration will continue daily until neutrophil count > 1.0 x10^9/L X 3 days or > 5.0 x10^9/L.
- Aldesleukin (based on total body weight) 720,000 IU/kg IV over 15 minute approximately every eight hours for up to 5 days

Day 14 (+/- 2 days):

- ALVAC ESO-1 Vaccine (see day 0 above)

Table 3.2

Day	**-7**	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0[1]**	**1**	**2**	**3**	**4**
Therapy												
Cyclophosphamide (60 mg/kg)	X	X										
Fludarabine (25 mg/m^2)			X	X	X	X	X					
Anti-ESO TCR PBL								X[1]				
ALVAC ESO-1 vaccine (cohort 3 and 4 only)								X[7]				
Aldesleukin								X[2]	X	X	X	X
Filgrastim[3] (5 mcg/kg/day)									X	X	X	X
TMP/SMX[4] 160mg/800mg (example)	X	X	X	X	X	X	X	X	X	X	X	X
Fluconazole[5] (400 mg po)								X	X	X	X	X
Valacyclovir po or Acyclovir IV[6]								X	X	X	X	X

[1]One to four days after the last dose of fludarabine
[2]Initiate within 24 hours after cell infusion
[3]Continue until neutrophils count > 1X10^9/L for 3 consecutive days or > 5x10^9/L.
[4]The TMP/SMX schedule should be adjusted to QD three times per week (Monday, Wednesday, Friday) and continue for at least six months and until CD4 > 200 X 2
[5]Continue until ANC > 1000/mm^3
[6]In patients positive for HSV continue until CD4 >200 X 2
[7]Administered to patients treated in cohorts 3 and 4 only. Vaccine is also administered on day 14 (not shown)

3.2.4. Infection Prophylaxis

Note: Other anti-infective agents may be substituted at the discretion of the treating investigator.

3.2.4.1 Pneumocystis Jirovecii Pneumonia

All patients will receive the fixed combination of trimethoprim and sulfamethoxazole [SMX] as double strength (DS) tab (DS tabs = TMP 160 mg/tab, and SMX 800 mg/tab)

P.O. daily three times a week on non-consecutive days, beginning between days -5 and -8.

Pentamidine will be substituted for TMP/SMX-DS in patients with sulfa allergies. It will be administered aerosolized at 300 mg per nebulizer within one week of chemotherapy start date and monthly thereafter.

3.2.4.2 Herpes Virus Prophylaxis

Patients with positive HSV serology will be given valacyclovir orally at a dose of 500 mg daily the day after chemotherapy ends, or acyclovir, 250 mg/m^2 IV q 12 hrs if the patient is not able to take medication by mouth. Reversible renal insufficiency has been reported with IV but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs have been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made or the drug will be discontinued. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

Prophylaxis for Pneumocystis and Herpes will continue for 6 months post chemotherapy. If the CD4 count is less than 200 at 6 months post chemotherapy, prophylaxis will continue until the CD4 count is greater than 200 for 2 consecutive measures.

3.2.4.3 Fungal Prophylaxis

Patients will start Fluconazole 400 mg p.o. the day after chemotherapy concludes and continue until the absolute neutrophil count is greater than 1000/mm^3. The drug may be given IV at a dose of 400 mg in 0.9% sodium chloride USP daily in patients unable to take it orally.

3.2.4.4 Empiric Antibiotics

Patients will start on broad-spectrum antibiotics, either a 3rd or 4th generation cephalosporin or a quinolone for fever of 38.3°C once or two temperatures of 38.0°C or above at least one hour apart, AND an ANC <500/mm^3. Aminoglycosides should be avoided unless clear evidence of sepsis. Infectious disease consultation will be obtained for all patients with unexplained fever or any infectious complications.

3.2.5. Blood Product Support

Using daily CBC's as a guide, the patient will receive platelets and packed red blood cells (PRBC's) as needed. Attempts will be made to keep Hb >8.0 gm/dl, and plts >20,000/mm^3. All blood products with the exception of the stem cell product will be irradiated. Leukocyte filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused WBC's and decrease the risk of CMV infection.

3.2.6. Interleukin-2 (aldesleukin): Intravenous Administration

Aldesleukin (NSC #373364) (based on total body weight) will be administered at a dose of 720,000 IU/kg as an intravenous bolus over a 15 minute period approximately every eight hours (+/- 1hr) beginning within 24 hours of cell infusion and continuing for up to 5 days (maximum 15 doses). Doses may be skipped depending on patient tolerance. Doses will be skipped if patients reach Grade 3 or 4

toxicity due to aldesleukin except for the reversible Grade 3 toxicities common to aldesleukin such as diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes as detailed in Appendix1. Toxicities will be managed as outlined in Appendix 2. If these toxicities can be easily reversed within 24 hours by supportive measures then additional doses may be given. If greater than 2 doses of aldesleukin are skipped, aldesleukin administration will be stopped. In addition, dosing may be held or stopped at the discretion of the treating investigator (Appendix 3 lists the toxicities seen in patients treated with aldesleukin at the NIH Clinical Center). Because confusion is a possible side effect of aldesleukin administration, a Durable Power of Attorney will be signed by the patient to identify a surrogate to make decisions if a patient becomes unable to make decisions.

3.3. On-Study Evaluation

3.3.1. Prior to starting the preparative regimen

- Apheresis as indicated
- Within 14 days prior to starting the preparative regimen, patients will have a complete blood count, serum chemistries performed including electrolytes, BUN, creatinine, and liver function tests. If any results are beyond the criteria established for eligibility, the patient will not proceed until the abnormalities can be resolved.

3.3.2. During the preparative regimen: DAILY

- Complete Blood Count
- Chem 20: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO^2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid)
- Urinalysis

3.3.3. Prior to cell infusion:

- Blood samples for analysis for detection of RCR by PCR

3.3.4. After Cell Infusion:

- Vital signs will be monitored hourly (+/- 15 minutes) for four hours and then routinely (every 4-6 hours) unless otherwise clinically indicated.
- CMV PCR assay will be assessed if clinically indicated (e.g. unexplained fevers, pulmonary changes). **Every 1-2 days (until values return to normal limits/baseline)**
    - A review of systems and physical exam as clinically indicated.
    - CBC
    - Chem 20 (Sodium (Na), Potassium (K), Chloride (Cl), Total CO^2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid)
    - Other tests will be performed as clinically indicated.

3.3.5. Additional research evaluations:

3.3.5.1 Post cell infusion evaluations:

- The amount of blood that may be drawn from adult patients for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight week period.
- Once total lymphocyte count is greater than 200/mm^3, the following samples will be drawn and sent to the TIL lab on Monday, Wednesday and Friday X 5, then weekly (while the patient is hospitalized):
  - 5 CPT tubes (8 ml each)
  - 1 SST tube (8 ml)
  - 1 SST tube (4 ml) daily starting on the day of the chemotherapy
- At other time points, patient peripheral blood lymphocytes (PBL) will be obtained from whole blood by purification using centrifugation on a Ficoll cushion. Aliquots of these PBMC will be 1) cryopreserved for immunological monitoring of cell function and 2) subjected to DNA and RNA extraction for PCR analysis of TCR and vector copy number estimation.

3.3.5.2 Biopsies

- Biopsies of tumor tissue or lymph nodes may be performed but are not required during the course of therapy. These biopsies will only be performed if minimal morbidity is expected based on the procedure performed and the granulocyte and platelet count. Biopsy tissue will be processed in the Surgery Branch Cell Production Facility in the presence of a Pathology Laboratory pathologist and part of all biopsy tissue will go to the Laboratory of Pathology. Studies will be performed to evaluate the antigen expression by the tumor and to evaluate the reactivity of lymphocytes grown from these biopsies. In addition the presence of transduced cells will be quantitated using RT-PCR for vector sequences.

3.3.5.3 Immunological Testing:

- Apheresis may be performed, prior to and 4-6 weeks after the treatment. At other time points, patient peripheral blood lymphocytes (PBL) will be obtained from whole blood by purification using centrifugation on a Ficoll cushion. Aliquots of these PBMC will be:
  1. Cryopreserved for immunological monitoring of cell function,
  2. Subjected to DNA and RNA extraction for PCR analysis of TCR and vector copy number estimation
- Lymphocytes will be tested directly and following in vitro culture. Direct immunological monitoring will consist of quantifying T cells reactive with ESO by FACS analysis using tetramer staining. Ex vivo immunological assays will consist of cytokine release by bulk PBL (+/- peptide stimulation) and by other experimental studies such as cytolysis if sufficient cells are available. If cell numbers are limiting, preference will be given to the direct analysis of immunological activity. Immunological assays will be standardized by the inclusion of 1) pre-infusion PBMC and 2) an aliquot of the engineered PBL cryopreserved at the time of infusion. In general, differences of 2 to 3 fold in these assays are indicative of true biologic differences. Foxp3 levels will be analyzed by semiquantitative RT-PCR to evaluate for mRNA on PBL samples obtained prior to cell infusion and at the follow up time point.

3.3.5.4 Monitoring Gene Therapy Trials: Persistence and RCR:

- Engineered cell survival. TCR and vector presence will be quantitated in PBMC samples using established PCR techniques. Immunological monitoring using both tetramer analysis and staining for the TCR will be used to augment PCR-based analysis. This will provide data to estimate the in vivo survival of lymphocytes derived from the infused cells. In addition, measurement of CD4 and CD8 T-cells will be conducted and studies of these T-cell subsets in the circulation will be determined by using specific PCR assays capable of detecting the unique DNA sequence for each retroviral vector engineered T-cell.
- Patients blood samples will be obtained and undergo analysis for detection of RCR by PCR prior to cell infusion and RCR PCR will be performed at 3 and 6 months, and at one year post cell administration. Blood samples will be archived annually thereafter if all previous testing has been negative with a brief clinical history. If a patient dies or develops neoplasms during this trial, efforts will be made to assay a biopsy sample for RCR. If any post-treatment samples are positive, further analysis of the RCR and more extensive patient follow-up will be undertaken, in consultation with the FDA. RCR PCR assays detect the GaLV envelop gene and are performed under contract by the Indiana University Vector Production Facility. The results of these tests are maintained by the contractor performing the RCR tests and by the Surgery Branch research team.
- Due to nature of these studies, it is possible that expansion of specific T-cell clones will be observed as tumor reactive T-cell proliferate in response to tumor antigens. Therefore, care will be taken to track T-cell persistence both immunologically and molecularly. Blood samples (5-10 mL) for persistence of TCR transduced cells will be obtained 1 month after cell infusion, then at 3, 6, 12 months, and then annually thereafter. If any patient shows a high level of persistence of TCR gene transduced cells at month 6 (by semi quantitative DNA-PCR using primers specific for vector sequences) the previously archived samples will be subjected to techniques that would allow the identification of clonality of persisting TCR gene transduced cells. Such techniques may include T cell cloning or LAM-PCR. If a predominant or monoclonal T cell clone derived from TCR gene transduced cells is identified during the follow-up, the integration site and sequence will be identified and subsequently analyzed against human genome database to determine whether the sequences are associated with any known human cancers. If a predominant integration site is observed, the T cell cloning or LAM-PCR test will be used at an interval of no more than three months after the first observation to see if the clone persists or is transient. In all instances where monoclonality is persistent and particularly in instances where there is expansion of the clone, regardless of whether or not the sequence is known to be associated with a known human cancer, the subject should be monitored closely for signs of malignancy, so that treatment, if available, may be initiated early.

- Blood and tissue specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study. However, this research may only be done if the risks of the new questions

were covered in the consent document. If new risks are associated with the research (e.g., analysis of germ line genetic mutations) the principal investigator must amend the protocol and obtain informed consent from all research subjects. Any new use of samples will require prospective IRB review and approval.

3.4. Re-Treatment

Patients will be evaluated 4 to 6 weeks after the initial treatment regimen (defined as the end of the last aldesleukin dose). If patients have a partial response to treatment and then stabilizes and have evaluable disease, or stable disease that subsequently progresses, they may be re-treated when progression by RECIST criteria is documented after evaluation by principal investigator with the same schedule that they had been given safely. Retreatment will consist of the non-myeloablative chemotherapy regimen, ALVAC ESO-1 vaccine (if available for cohorts 3 and 4), cell infusion, and aldesleukin (See table 3.4 below). Patients who develop grade 3 toxicity due to cell infusion which is reversible within 24 hours with supportive measures may be retreated, but patients who develop grade 4 toxicity due to cell infusion will not be retreated. Patients must continue to meet the original eligibility criteria to be considered for retreatment. Toxicity related to cyclophosphamide or fludarabine should be stable and resolved to less than grade 1 prior to retreatment. Re-treatment benefits and risks will be carefully explained to the patient. Patients must be reconsented prior to retreatment. Retreatment would not begin prior to 6 to 8 weeks after the last aldesleukin dose for each of the cohorts. A maximum of 2 retreatment courses may occur.

Table 3.4: Retreatment with anti-ES0-1 PBL

Day	-7	-6	-5	-4	-3	-2	-1	0[1]	1	2	3	4
Therapy												
Cyclophosphamide (60 mg/kg)	X	X										
Fludarabine (25 mg/m^2)			X	X	X	X	X					
anti-ESO TCR PBL								X[1]				
ALVAC ESO-1 vaccine (cohort 3 and 4 only, if available)								X[7]				
Aldesleukin								X[2]	X	X	X	X
Filgrastim[3] (5 mcg/kg/day)									X	X	X	X
TMP/SMX[4] 160mg/800mg (example)	X	X	X	X	X	X	X	X	X	X	X	X
Fluconazole[5] (400 mg po)								X	X	X	X	X
Valacyclovir po or Acyclovir IV[6]								X	X	X	X	X

[1]One to four days after the last dose of fludarabine
[2]Initiate within 24 hours after cell infusion
[3]Continue until neutrophils count > $1X10^9$/L for 3 consecutive days or > $5x10^9$/L.
[4]The TMP/SMX schedule should be adjusted to QD three times per week (Monday, Wednesday, Friday) and continue for at least six months and until CD4 > 200 X 2
[5]Continue until ANC > 1000/mm^3
[6]In patients positive for HSV continue until CD4 > 200 X 2
[7]Administered to patients treated in cohorts 3 and 4 only, if available. Vaccine is also administered on day 14 (not shown)

3.5. Post Study Evaluation (Follow-up):

3.5.1 All patients return to the NIH Clinical Center for evaluation 6 weeks (+/- 2 weeks) following the administration of the cell product.

3.5.2 Patients who experience stable disease, a partial response, or a complete response or have unresolved toxicities will be evaluated as noted below:

- Week 12 (+/- 2 weeks)
- Every 3 months (+/- 1 month) x 3
- Every 6 months (+/- 1 month) x 2
- As per PI discretion for subsequent years
  Note: Patients may be seen more frequently as clinically indicated.

3.5.3 At each scheduled evaluation, patients will undergo:

- Physical examination
- Chem 20: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO^2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid)
- Complete blood count
- Thyroid panel as clinically indicated.
- Toxicity assessment, including a review of systems.
- CT of the chest, abdomen and pelvis. This end of course evaluation will be used to determine tumor response. If clinically indicated, other scans or x-rays may be performed, e.g. brain MRI, bone scan.
- Visual symptoms will be evaluated and if changes have occurred from baseline, i.e. changes in visual acuity, an ophthalmologic consult will be performed.
- A 5 liter apheresis may be performed. If the patient is unable to undergo pheresis, approximately 96 ml of blood may be obtained. Subsequently, approximately 60 ml of blood will be obtained at follow up visits (approximately monthly) for at least 3 months. Peripheral blood mononuclear cells will be cryopreserved so that immunologic testing may be performed.
- .

3.5.4 Detection of RCR and persistence of TCR gene transduced cells: (see section 3.3.7.4)

3.5.5 Long-term follow up of patients receiving gene transfer: Physical examinations will be performed and documented annually for 5 years following cell infusion to evaluate long-term safety. After 5 years, health status data will be obtained from surviving patients via telephone contact or mailed questionnaires. The long term follow up period for retroviral vectors is 15 years.

3.5.6 Patients who are unable or unwilling to return for follow up evaluations will be followed via phone or e-mail contact. Patients may be asked to send laboratory , imaging, and physician exam reports performed by their treating physician.

3.6. Off Treatment Criteria:

Patients will be taken off treatment (and followed until progression of disease) for the following

- Grade 3 autoimmunity that involves vital organs (heart, kidneys, brain, eye, liver, colon, adrenal gland, lungs).

- Grade 3 or greater toxicity attributable to the ALVAC ESO-1 vaccine, except for known toxicities such as local injection site reactions, skin rash, pruritis, transient fatigue, transient fever, transient laboratory changes or local adenopathy.
- If a patient experiences a grade 3 or 4 toxicity due to cell infusion (reaction to cellular product or infusion reaction) the patient will receive no further cells but may continue to receive aldesleukin if the reaction resolves to grade 2 or less within 24 hours.
- Completion of treatment period.

3.7. <u>Off Study Criteria:</u>

Patients will be taken off study for the following:

- The patient voluntarily withdraws
- There is significant patient noncompliance
- Radiographic or clinical disease progression, unless eligible for second treatment
- General or specific changes in the patient's condition render the patient unacceptable for further treatment on this study in the judgment of the investigator.
- Death

Note: patients who are taken off study for progressive disease or study closure maybe followed on Protocol 09-C-0161 *Follow up Protocol for subjects Previously Enrolled in Surgery Branch Studies*.
Note: Patients must be followed until all adverse events have resolved to grade 2 or less with the exception of lymphopenia and alopecia. If an adverse event is not expected to resolve to grade 2 or less this will be noted in the patient medical record and the patient may be taken off study.

4.0 Supportive Care

Concomitant medications to control side effects of therapy will be given. Meperidine (25-50 mg) will be given intravenously if severe chilling develops. Other supportive therapy will be given as required and may include acetaminophen (650 mg q4h), indomethacin (50-75 mg g6h) and ranitidine (150 mg g12h). If patients require steroid therapy they will be taken off treatment. Patients who require transfusions will receive irradiated blood products. Ondansetron 0.15 mg/kg/dose IV every 8 hours will be administered for nausea and vomiting. Additional antiemetics will be administered as needed for nausea and vomiting uncontrolled by ondansetron. Antibiotic coverage for central venous catheters may be provided at the discretion of the investigator.

5.0 Data Collection and Evaluation

5.1. <u>Data Collection</u>

The investigators will be responsible for the collection, maintenance, quality control of the study data. Clinical data will be entered in to the NCI CCR C3D database.

5.1.1. Routine Adverse Event Reporting

Following registration through 30 days after the last treatment, adverse events will be recorded in the source documents, reviewed by the designated data manager or research nurse or principle investigator and captured in C3D. Events occurring during this period must be followed until resolution to grade 2 or baseline. Events that occur after this period will only be captured in C3D if they are related to investigational agent.

5.1.2. Exclusions to Routine Adverse Event Reporting

Patients will be receiving multiple agents, which include commercially available agents (fludarabine, cyclophosphamide and supportive medications) in combination with the investigational agents. Therefore, Grade 2 adverse events ‘unrelated’ or ‘unlikely related’ to the investigational agent, and ‘possibly’, ‘probably’ or ‘definitely’ related to the commercially available agents as specified in the package inserts do not require reporting/recording. In addition, all grade 1 events and all expected grade 2 events not related to the cell product will not be reported/recorded.

5.1.3. Reporting of Laboratory Events

Laboratory results will be uploaded in C3D however only those grade 3 and 4 events that support the diagnosis of a reportable adverse event or that reflect major organ function will be considered adverse events. For example grade 3 and 4, creatinine, liver function tests, hemoglobin, ANC, ALC, platelets, and lipase and amylase as indicated will be captured as adverse events; electrolytes, BUN, albumin, total protein, uric acid etc, and the remainder of the CBC differential will not be captured as adverse events. For hematological toxicities, the event will not be considered resolved until it reaches grade 2 without the support of transfusions or growth factors.

5.1.4. Reporting of non-Laboratory Events

For reportable expected events: the adverse event start date will be the date the event reaches a grade 3; the event will be considered resolved once it reaches grade 2. The highest grade the event reaches in that period will be considered the grade of the event. For unexpected adverse events, the adverse event start date will be the date the event reaches grade 2; the event will be considered resolved once it returns to grade 1 or baseline. The highest grade the event reaches in that period will be considered the grade of the event.

5.2. Response Criteria

5.2.1 Evaluation of target lesions[1]

- Complete Response (CR): Disappearance of all target lesions
- Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions taking as reference the baseline sum LD.
- Progression (PD): At least a 20% increase in the sum of LD of target lesions taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.
- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD.

5.2.2 Evaluation of non-target lesions[2]

- Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level.

- Non-Complete Response: Persistence of one or more non-target lesions
- Progression (PD): Appearance of one or more new lesions. Unequivocal progression of existing non-target lesions

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[1] All measurable lesions up to a maximum of 10 lesions representative of all involved organs should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

[2] All other lesions (or sites of disease) should be identified as **non-target lesions** and should also be recorded at baseline. Measurements are not required, and these lesions should be followed as "present" or "absent."

5.2.3 Evaluation of best overall response

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

5.2.4 Confirmatory Measurement/Duration of Response

Confirmation

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat studies that should be performed at least 4 weeks after the criteria for response are first met. In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 6-8 weeks.

Duration of Overall Response

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

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

Duration of Stable Disease

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

5.3. Toxicity Criteria

This study will utilize the CTC version 3.0 for toxicity and adverse event reporting. A copy of the CTC v3.0 can be downloaded from the CTEP home page (http://ctep.cancer.gov).

Over 100 patients have been treated in the Surgery Branch, NCI with tumor infiltrating lymphocytes. Early toxicities related specifically to the infusion of the cells (those which are seen immediately following cell infusion and prior to aldesleukin administration) are generally mild and include fevers, chills, headache, and malaise. Toxicities which occur following administration of aldesleukin but are thought to be related to the cells include immune mediated events such as vitiligo, transient uveitis, hearing loss and vestibular dysfunction. The use of the non-myeloablative regimen prior to cell administration increases the toxicity of this treatment as profound myelosuppression occurs in all patients. In 93 patients treated with TIL using the non-myeloablative chemotherapy regimen with or without total body irradiation, there was one treatment related death (NMA + 200 cGY TBI) due to an unexpected but preexisting diverticular abscess.

The standard approach to the administration of high-dose aldesleukin in all studies is to continue dosing until grade 3 or 4 events occur. The most commonly seen grade 4 events are pulmonary, and renal impairment, and mental status changes. These toxicities may sometimes require intubation for protection of the patient's airway. It is important to note that although these patients require significant supportive measures during this period, all toxicities are reversible and the overwhelming majority of patients have suffered no long term sequelae following this treatment regimen. However, fatal complications are possible and it is therefore only appropriate to carry out this experimental treatment in the context of life threatening metastatic cancer.

To ensure safety using this treatment, the NCI SB will review safety data on all protocols semi-annually at the time of continuing review. Data will be presented for both the recent 6 month period and for the entire length of time the protocol has been open. The toxicity data for review will include all toxicities captured on the protocol and will be presented in individual tables as follows:

- all toxicities attributed to the cells,
- all incidences of intubation including the duration of and reason for intubation,
- all grade 2 unexpected adverse events, and all grade 3 or greater events regardless of attribution.

Toxicities seen on protocols using this non-myeloablative regimen and aldesleukin, that occur during the follow up period are rare but have included EBV lymphoma following prolonged lymphopenia, herpes zoster infection, and sensory neuropathy likely related to fludarabine.

The major discomforts of the research are those of nausea, mucositis, anorexia, diarrhea, fever and malaise. Side effects of common drugs used in this nonmyeloablative regimen include:

Cyclophosphamide: Marrow suppression, nausea, mucositis, rash, hemorrhagic cystitis, myocardial damage, alopecia, infertility, nausea and vomiting, SIADH.

Fludarabine: Myelosuppression, fever and chills, nausea and vomiting, malaise, fatigue, anorexia, weakness, neurologic toxicity including sensory neuropathies and blindness, and interstitial pneumonitis. Serious opportunistic infections have occurred in CLL patients treated with fludarabine.

Antimicrobials in general: Allergic reactions, renal impairment, nausea, vomiting, hepatic damage, marrow suppression.

A variety of side effects have been associated with high-dose aldesleukin administration in our experience and a listing of these side effects in 652 patients who received 1,039 treatment courses are listed in Appendix 1 and Appendix 2.

5.4. Statistical Section

For cohorts 1 and 2, the primary objective of this trial is to determine whether the combination of high dose aldesleukin, lymphocyte-depleting chemotherapy, and an infusion of anti-ESO TCR-gene engineered lymphocytes is able to be associated with a modest fraction of patients that can experience a clinical response (PR +CR) to therapy. For cohorts 3 and 4, the primary objective is to determine whether the combination of high dose aldesleukin, lymphocyte-depleting chemotherapy, and an infusion of anti-ESO TCR-gene engineered lymphocytes and ALVAC ESO-1 vaccine is able to be associated with a modest fraction of patients that can experience a clinical response (PR +CR) to therapy. A secondary objective is to have sufficient patients in order to do exploratory evaluations of survival of cells.

Patients who express ESO will be enrolled into individual strata depending on their specific histology. This stratification is being used to separate patients who have been shown historically to be IL-2 sensitive (melanoma and renal cell cancer) from those who have not. In each case, the ESO expression is expected to be so dominant in regulating potential for response that differences in clinical response according to histology will not be expected but will be evaluated in an exploratory manner to help assess this, in a pilot sense.

As of March 2011, the ALVAC ESO-1 is not currently available, therefore with approval of amendment G cohorts 1 and 2 will be reopened. We plan to leave cohorts 3 and 4 open as we would like to accrue to these arms if the ALVAC ESO-1 vaccine becomes available in the future. To date, we have seen remarkable clinical responses in patients treated on this study. In cohort 1, we have treated 13 patients with melanoma, and 5 of 11 patients with high expression of ESO-1 had clinical response including 3 partial responses (PRs) of 3, 8 and 14+ months, and 2 complete responses (CRs) of 24, and 25 + months. In cohort 2, we have treated 7 patients and 6 of these patients were heavily pretreated synovial cell sarcoma. Four patients with synovial cell sarcoma experienced PRs lasting 10, 14, 4 and 8 months. Therefore, the response rate in patients receiving non-myeloablative chemotherapy, anti-ESO-1 TCR PBL and high dose IL-2 but no vaccine is 45%. In cohort 3 (with vaccine), we

have treated 6 patients with melanoma, and of the three evaluable patients, 1 patient has an ongoing PR of 6+ months. In cohort 4 (with vaccine), 5 patients have been treated, and out of the 4 evaluable patients, 2 patients with synovial cell sarcoma have ongoing PRs of 7+ months. The preliminary data from cohorts 3 and 4 indicate that clinical response rates observed with vaccine in cohorts 3 and 4 are similar to those observed without vaccine in cohort 1 and 2. In addition, the adverse events observed in each arm are comparable with most toxicities attributed to the non-myeloablative chemotherapy regimen and high dose IL-2..

For each of the 2 strata, the study will be conducted using a phase II optimal design (Simon R, Controlled Clinical Trials 10:1-10, 1989). For both strata, the objective will be to determine if the combination of high dose aldesleukin, lymphocyte depleting chemotherapy, ALVAC ESO-1 vaccine and anti-ESO TCR-gene engineered lymphocytes is able to be associated with a clinical response rate that can rule out 5% (p0=0.05) in favor of a modest 20% PR + CR rate (p1=0.20).

In patients in each of the two strata (melanoma or renal carcinoma vs. other histologies), the following design will be used. For each strata, with alpha=0.05 (5% probability of accepting a poor therapy) and beta=0.10 (10% probability of rejecting a good therapy,), initially 21 evaluable patients will be enrolled. If 0 or 1 of the 21 patients experiences a clinical response, then no further patients will be enrolled. If 2 or more of the first 21 evaluable patients enrolled have a clinical response, then accrual will continue until a total of 41 evaluable patients have been enrolled. As it may take several weeks to determine if a patient has experienced a clinical response, a temporary pause of up to 6 months in the accrual to the trial may be necessary to ensure that enrollment to the second stage is warranted. If 2 to 4 of the 41 have a clinical response, then this will be considered inadequate for further investigation. If 5 or more of 41 patients have a clinical response, then this will indicate that this strategy provides a new approach that may be worthy of further consideration. Under the null hypothesis (5% response rate), the probability of early termination is 72%.

Further, to help ensure that maldistribution of patients who are either particularly responsive or unresponsive in the first stage does not materially interfere with the intended use of the two-stage design, we will aim to enroll 4-5 patients of each allowed histology among the first 21 enrolled in the ‘other histology’ arm. Although this has its own inherent issues due to limited sample size, since we believe that these ‘other histologies’ will behave the same clinically, it will permit us to evaluate the different response rates in a limited number of subjects and determine if they differ markedly or not. Since this would merely be an exploratory analysis, we will also look at minor response as well to help evaluate for hints of efficacy. If the response rates do seem to potentially differ markedly by histology, despite our hypothesis that this will not happen, we may consider amending the protocol when appropriate to try to restrict enrollment to those histologies with stronger evidence of responsiveness. For patients with breast cancer or other chemotherapy-sensitive tumors (i.e. sarcoma), only responses seen at day 28 and maintained at 4 months will be considered a positive response for accrual to the second phase of this study.

To complete cohorts 3 and 4, a total of up to 164 patients may be required (2 with a maximum of 41 apiece). It is not expected that more than 20 patients will be accrued in cohorts 1 and 2 prior to approval of amendment E, therefore, the maximum

accrual for this study will be 102 patients. Provided that about 4-5 patients per month will be able to be enrolled onto this trial, approximately 2.5 years may be needed to accrual the maximum number of required patients. However, as adequate responses to proceed to the second stage of accrual may not occur, the trial may end up accruing as few as 62 patients if no arm demonstrates sufficient patients to accrue a second stage.

5.5. Data and Safety Monitoring Plan

Careful evaluation to ascertain the toxicity, immunologic effects and anti-tumor efficacy of cell infusions will be performed. Due to the nature of these studies, it is possible that expansion of specific T-cell clones will be observed as tumor reactive T-cell proliferate in response to tumor antigens. Therefore, care will be taken to track T-cell persistence both immunologically and molecularly according to plan specified in Section 3.3.7.

The principal investigator will review all serious adverse events and will monitor the data and toxicities to identify trends monthly. The principal investigator will be responsible for revising the protocol as needed to maintain safety. The NCI IRB will review submitted adverse events monthly to also evaluate trends and will require a follow up plan from the principal investigator whenever a trend is identified. A CCR Safety Monitoring Committee will monitor toxicity trends on this study on at least an annual basis and report any trends to the NCI IRB and Principal Investigator.

5.6. Clinical Trial Monitoring Plan

A detailed description of the clinical trial monitoring plan has been included in the initial IND submission as required. This trial will be monitored by personnel employed by Harris Technical Services on contract to the NCI, NIH. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

At least 25% of enrolled patients' will be randomly selected and monitored at least quarterly, base on accrual rate. The patients selected will have 100% source document verification done. Additional monitoring activities will include: adherence to protocol specified study eligibility, treatment plans, data collection for safety and efficacy, reporting and time frames of adverse events to the NCI IRB and FDA, and informed consent requirements. Written reports will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

5.7. Handling of Tissue Specimens for Research Purposes

Blood and tissue collected during the course of this study will follow the Cell Tracking and Labeling System established by the Tumor Immunology Cell Processing Laboratory. The Cell Tracking and Labeling System is designed to unambiguously ensure that patient/data verification is consistent. The patients' cell samples (blood or tissue) are tracked by distinct identification labels that include a unique patient identifier and date of specimen collection. Cryopreserved blood and tissue samples also bear the date the sample was frozen. All cryopreserved samples

are tracked for freezer location and storage criteria. All samples are stored in monitored freezers/refrigerators in 3NW Surgery Branch Laboratories at specified temperatures with alarm systems in place. All samples (blood or tissue) are entered into a central computer database with identification and storage location, and this database is backed up every night.

At the conclusion of this protocol, if additional studies are to be performed on any samples obtained during the conduct of this trial, a Request to Conduct Research for Stored Human Samples Specimens, or Data Collected in a Terminated NCI-IRB Protocol will be submitted. Otherwise, specimens will be disposed of in accordance with the environmental protection laws, regulations and guidelines of the Federal Government and the State of Maryland.

Any loss or unintentional destruction of the samples will be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

6.0 Human Subjects Protections

6.1. Rationale for Patient Selection

The patients to be entered in this protocol have metastatic cancer which is refractory to standard therapy, and limited life expectancies.

Subjects from both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one group compared to another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on the one hand and the need to explore gender and ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to gender or to ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

6.2. Participation of Children

The use of the nonmyeloablative regimen in this protocol is a major procedure which entails serious discomforts and hazards for the patient, such that fatal complications are possible. It is therefore only appropriate to carry out this experimental procedure in the context of life threatening metastatic cancer. Since the efficacy of this experimental procedure is unknown, it does not seem reasonable to expose children to this risk without further evidence of benefit. Should results of this study indicate efficacy in treating metastatic cancer, which is not responsive to other standard forms of therapy, future research can be conducted in the pediatric population to evaluate potential benefit in that patient population.

6.3. Evaluation of Benefits and Risks

The experimental treatment has a chance to provide clinical benefit though this is unknown. The risks in this treatment are detailed in section 5.2. The goal of this study is to improve upon the number of patients who may benefit from adoptive cell therapy by using patients' own transduced T-cells without the need to identify

anti-tumor T-cells uniquely from each patient as was required in several prior protocols. The success of this effort cannot be predicted at this time. Because all patients in this protocol have melanoma cancer and limited life expectancies the potential benefit is thought to outweigh the potential risks.

6.4. Consent Document

Patients initially sign a consent when they agree to have PBMC obtained for study and growth on 03-C-0277, Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols. If the lymphocytes can be generated for infusion and the patient meets the thorough screening for eligibility, the patient, with family members or friends at the request of the patient, will be presented with a detailed description of the protocol treatment. The specific requirements, objectives, and potential advantages and disadvantages will be presented. The Informed Consent document is given to the patient, who is requested to review it and to ask questions prior to agreeing to participate in the treatment portion of this protocol. The patient is reassured that participation on trial is entirely voluntary and that he/she can withdraw or decide against treatment at any time without adverse consequences. The research nurse, principal investigator, associate investigator, or clinical associate is responsible for obtaining written consent from the patient.

7.0 Data Reporting

This study will utilize the CTCAE 3.0 for toxicity and Adverse Event reporting. A copy of the CTCAE 3.0 can be downloaded from the CTEP home page (http://ctep.cancer.gov). All appropriate treatment areas should have access to a copy of the CTCAE 3.0.

This study will be monitored by the Internal Monitoring System conducted by Harris Technical Services Corporation, with reporting to the QA specialist of the CCR, NCI.

7.1 Definitions

7.1.1 Adverse Event

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactory resolution. AEs should be reported up to 30 days following the last dose of study drug. AEs that are considered treatment related, expected, continuing, but not resolvable by 30 days after treatment completion (e.g., alopecia) will not be followed after the 30-day period.

7.1.2 Suspected Adverse Reaction

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. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 Unexpected Adverse Reaction

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. "Unexpected”, 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.

7.1.4 Serious

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.1.5 Disability

A substantial disruption of a person’s ability to conduct normal life functions.

7.1.6 Life threatening Adverse Drug Reaction

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.1.7 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved study procedures in a research protocol that **does not** have a major impact on the subjects’ rights, safety or well-being, or the completeness, accuracy and reliability of the data.

7.1.8 Protocol Violation (NIH Definition)

Any change, divergence, or departure from the study procedures from the IRB-approved study procedures in a research protocol that **does** have a major impact on the subject’s rights, safety, or well-being and/or the completeness, accuracy or reliability of the study data.

7.1.9 <u>Unanticipated Problems</u>

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and
  (b) the characteristics of the subject population being studied; **AND**

- Is related or possibly related to participation in the research; **AND**

- Places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized

7.2 NCI-IRB Reporting

7.2.1 NCI-IRB Expedited Reporting of Adverse Events, Unanticipated Problems, and Deaths

The Protocol PI will report to the NCI-IRB:

- All unexpected serious adverse events that are possibly, probably, or definitely related to the research
- All deaths, except deaths due to progressive disease
- All Protocol Violations or Deviations
- All Unanticipated Problems

Reports must be received by the NCI-IRB within 7 working days of PI awareness via iRIS.

7.2.2 NCI-IRB Requirements for PI Reporting of Adverse Events at Continuing Review

The PI will provide a summary report of adverse events that have occurred on the protocol since the previous continuing review and in aggregate, and interpreted in relation to the risk:benefit of study participants in the narrative.

The protocol PI will report to the NCI-IRB:

1. All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
2. All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
3. All Grade 5 events regardless of attribution;
4. All Serious Events regardless of attribution.

NOTE: Grade 1 events are not required to be reported.

7.2.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that require a sponsor recommended change to the protocol or the consent form or in the opinion of the PI increases risks to study participants will need to be reported to the NCI IRB.

7.3 NIH Office of Biotechnology Activities (OBA)/Institutional Biosafety Committee (IBC) Reporting Criteria

7.3.1 Serious Adverse Event Reports to OBA/IBC

The Principal Investigator will notify OBA/IBC via email of any unexpected fatal or life-threatening experience associated with the use of anti-NY-ESO1 TCR-transduced PBL as soon as possible but in no event later than 7 calendar days of initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the anti-NY-ESO1 TCR-transduced PBL, but are no fatal or life-threatening, much be reported to NIH OBA/IBC as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information.

7.3.2 Annual Reports to OBA/IBC

The study Principal Investigator will submit a brief report annually of the progress of the trial within 60 days of the anniversary date that the IND went into effect unless the IND sponsor has been authorized to submit this report, as per NIH Guidelines, Appendix M.

7.4 <u>Serious Adverse Event Reporting Requirements to the FDA, OBA and IBC:</u>

During the duration of this trial, the principal investigator will assume responsibility for reporting any serious and unexpected adverse events to the U.S. Food and Drug Administration (FDA) with appropriate deadlines defined by the regulations:

The FDA, OBA and IBC will be informed by phone or fax of any unexpected fatal or life threatening suspected adverse reaction as soon as possible but in no event, later than 7 calendar days after the sponsor's initial receipt of the information;

The FDA, OBA and IBC will be informed of any other unexpected and serious suspected adverse reaction by written notification within 15 calendar days after the sponsor's initial receipt of the information.

All adverse events, including serious adverse events, will be reported in the annual report to the FDA, OBA, and IBC as indicated in 21CFR312.33.

8.0 Pharmaceutical Information

8.1. <u>Aldesleukin (Interleukin-2, Proleukin, Recombinant Human Interleukin 2)</u>

<u>How Supplied:</u> Aldesleukin is obtained from commercial sources by the NIH Clinical Pharmacy Department.

<u>Formulation/Reconstitution:</u> Aldesleukin, NSC #373364, is provided as single-use vials containing 22 million IU (□1.3 mg) aldes off-white lyophilized cake plus 50 mg mannitol and 0.18 mg sodium dodecyl sulfate, buffered with approximately 0.17 mg monobasic and 0.89 mg dibasic sodium phosphate to a pH of 7.5 (range 7.2 to 7.8). The vial is reconstituted with 1.2 mL of Sterile Water for Injection, USP, and the resultant concentration is 18 million IU/ml or 1.1 mg/mL. Diluent should be directed against the side of the vial to avoid excess foaming. Swirl contents gently until completely dissolved. Do not shake. Since vials contain no preservative, reconstituted solution should be used with 24 hours.

<u>Storage:</u> Intact vials are stored in the refrigerator (2 – 8 °C) protected from light. Each vial bears an expiration date.

<u>Dilution/Stability:</u> Reconstituted aldesleukin should be further diluted with 50 mL of 5% Human Serum Albumin (HSA). The HSA should be added to the diluent

prior to the addition of aldesleukin. Dilutions of the reconstituted solution over a 1000-fold range (i.e., 1 mg/mL to 1 mcg/mL) are acceptable in either glass bottles or polyvinyl chloride bags. Aldesleukin is chemically stable for 48 hours at refrigerated and room temperatures, 20 – 30°C.

Administration: The final dilution of aldesleukin will be infused over 15 minutes. Aldesleukin will be administered as an inpatient.

Toxicities: Expected toxicities of aldesleukin are listed in the product label and in Appendix 1 and 2. Grade 3 toxicities common to aldesleukin include diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes as detailed in Appendix 1. Additional grade 3 and 4 toxicities seen with aldesleukin are detailed in Appendix 2.

8.2. Fludarabine

Description: (Please refer to package insert for complete product Information) Fludarabine phosphate is a synthetic purine nucleoside that differs from physiologic nucleosides in that the sugar moiety is arabinose instead of ribose or deoxyribose. Fludarabine is a purine antagonist antimetabolite.

How Supplied: It will be purchased by the NIH Clinical Pharmacy Department from commercial sources. Fludarabine is supplied in a 50 mg vial as a fludarabine phosphate powder in the form of a white, lyophilized solid cake.

Stability: Following reconstitution with 2 mL of sterile water for injection to a concentration of 25 mg/ml, the solution has a pH of 7.7. The fludarabine powder is stable for at least 18 months at 2-8°C; when reconstituted, fludarabine is stable for at least 16 days at room temperature. Because no preservative is present, reconstituted fludarabine will typically be administered within 8 hours. Specialized references should be consulted for specific compatibility information. Fludarabine is dephosphorylated in serum, transported intracellularly and converted to the nucleotide fludarabine triphosphate; this 2-fluoro-ara-ATP molecule is thought to be required for the drug's cytotoxic effects. Fludarabine inhibits DNA polymerase, ribnucleotide reductase, DNA primase, and may interfere with chain elongation, and RNA and protein synthesis.

Storage: Intact vials should be stored refrigerated (2-8°C).
Administration: Fludarabine is administered as an IV infusion in 100 ml 0.9% sodium chloride, USP over 15 to 30 minutes. The doses will be based on body surface area (BSA). If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Table 10.

Toxicities: At doses of 25 mg/m2/day for 5 days, the primary side effect is myelosuppression; however, thrombocytopenia is responsible for most cases of severe and life-threatening hematologic toxicity. Serious opportunistic infections have occurred in CLL patients treated with fludarabine. Hemolytic anemia has been reported after one or more courses of fludarabine with or without a prior history of a positive Coomb's test; fatal hemolytic anemia has been reported. In addition, bone marrow fibrosis has been observed after fludarabine therapy. Other common adverse effects include malaise, fever, chills, fatigue, anorexia, nausea and vomiting, and weakness. Irreversible and potentially fatal central nervous system toxicity in the form of progressive encephalopathy, blindness, and coma is only rarely observed at the currently administered doses of fludarabine. More common neurologic side

effects at the current doses of fludarabine include weakness, pain, malaise, fatigue, paresthesia, visual or hearing disturbances, and sleep disorders. Adverse respiratory effects of fludarabine include cough, dyspnea, allergic or idiopathic interstitial pneumonitis. Tumor lysis syndrome has been rarely observed in fludarabine treatment of CLL. Treatment on previous adoptive cell therapy protocols in the Surgery Branch have caused persistently low (below 200) CD4 counts, and one patient developed polyneuropathy manifested by vision blindness, and motor and sensory defects.

8.3. Cyclophosphamide

(Refer to FDA-approved package insert for complete product information)

Description: Cyclophosphamide is a nitrogen mustard-derivative alkylating agent. Following conversion to active metabolites in the liver, cyclophosphamide functions as an alkyating agent; the drug also possesses potent immunosuppressive activity. The serum half-life after IV administration ranges from 3-12 hours; the drug and/or its metabolites can be detected in the serum for up to 72 hours after administration.

How Supplied: Cyclophosphamide will be obtained from commercially available sources by the Clinical Center Pharmacy Department.

Stability: Following reconstitution as directed with sterile water for injection, cyclophosphamide is stable for 24 hours at room temperature or 6 days when kept at 2-8°C.

Administration: It will be diluted in 250 ml D5W and infused over one hour. The dose will be based on the patient's body weight. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Table 10.

Toxicities: Hematologic toxicity occurring with cyclophosphamide usually includes leukopenia and thrombocytopenia. Anorexia, nausea and vomiting, rash and alopecia occur, especially after high-dose cyclophosphamide; diarrhea, hemorrhagic colitis, infertility, and mucosal and oral ulceration have been reported. Sterile hemorrhagic cystitis occurs in about 20% of patients; severity can range from microscopic hematuria to extensive cystitis with bladder fibrosis. Although the incidence of hemorrhagic cystitis associated with cyclophosphamide appears to be lower than that associated with ifosfamide, mesna (sodium 2-mercaptoethanesulfonate) has been used prophylactically as a uroprotective agent in patients receiving cyclophosphamide. Prophylactic mesna is not effective in preventing hemorrhagic cystitis in all patients. Patients who receive high dose cyclophosphamide may develop interstitial pulmonary fibrosis, which can be fatal. Hyperuricemia due to rapid cellular destruction may occur, particularly in patients with hematologic malignancy. Hyperuricemia may be minimized by adequate hydration, alkalinization of the urine, and/or administration of allopurinol. If allopurinol is administered, patients should be watched closely for cyclophosphamide toxicity (due to allopurinol induction of hepatic microsomal enzymes). At high doses, cyclophosphamide can result in a syndrome of inappropriate antidiuretic hormone secretion; hyponatremia with progressive weight gain without edema occurs. At high doses, cyclophosphamide can result in cardiotoxicity. Deaths have occurred from diffuse hemorrhagic myocardial necrosis and from a syndrome of

acute myopericarditis; in such cases, congestive heart failure may occur within a few days of the first dose. Other consequences of cyclophosphamide cardiotoxicity include arrhythmias, potentially irreversible cardiomyopathy, and pericarditis. Other reported adverse effects of cyclophosphamide include headache, dizziness, and myxedema; faintness, facial flushing, and diaphoresis have occurred following IV administration. Mesna (sodium 2-mercaptoethanesulphonate; given by IV injection) is a synthetic sulfhydryl compound that can chemically interact with urotoxic metabolites of cyclophosphamide (acrolein and 4-hydroxycyclophosphamide) to decrease the incidence and severity of hemorrhagic cystitis.

8.4. Mesna (Sodium 2-mercaptoethanesulfonate, Mesnum, Mesnex, NSC-113891) (Please refer to the FDA-approved package insert for complete product information)

Description: Mesna will be obtained commercially by the Clinical Center Pharmacy Department and is supplied as a 100 mg/ml solution.
Storage: Intact ampoules are stored at room temperature.

Stability: Diluted solutions (1 to 20 mg/mL) are physically and chemically stable for at least 24 hours under refrigeration. Mesna is chemically stable at room temperature for 48-72 hours in D5W, 48-72 hour in D5W/0.45% NaCl, or 24 hours in 0.9% NaCl.

Administration: Dilute to concentrations less than or equal to 20 mg mesna/ml fluid in D5W or 0.9% NaCl and to be administered intravenously as a continuous infusion. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Table 10. Toxicities include nausea, vomiting and diarrhea.

8.5. Filgrastim (Granulocyte Colony-Stimulating Factor, G-CSF, Filgrastim, Neupogen)

Filgrastim will be obtained commercially by the Clinical Center Pharmacy Department and is supplied in 300 ug/ml and 480 ug/1.6 ml vials. G-CSF should be refrigerated and not allowed to freeze. The product bears the expiration date. The product should not be shaken. It is generally stable for at least 10 months when refrigerated. The appropriate dose is drawn up into a syringe. G-CSF will be given as a daily subcutaneous injection. The side effects of G-CSF are skin rash, myalgia and bone pain, an increase of preexisting inflammatory conditions, enlarged spleen with occasional associated low platelet counts, alopecia (with prolonged use) elevated blood chemistry levels.

8.6. Trimethoprim and Sulfamethoxazole Double Strength (TMP / SMX DS)

TMP/SMX DS will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used for the prevention of PCP pneumonia. The oral dose is 1 tablet PO daily three times a week (MUST be on non-consecutive days) beginning on day -7 and continuing for at least 6 months and until the CD4 count is greater than 200 on 2 consecutive lab studies. Like other sulfa drugs, TMP/SMX DS can cause allergies, fever, photosensitivity, nausea, and vomiting. Allergies typically develop as a widespread itchy red rash with fever eight to fourteen days after

beginning the standard dose. Neutropenia, a reduction in the number of neutrophils, can also occur.

8.6.1. Aerosolized Pentamidine in Place of TMP/SMX DS:

Patients with sulfa allergies will receive aerosolized Pentamidine 300 mg per nebulizer with one week prior to admission and continued monthly until the CD4 count is above 200 on two consecutive follow up lab studies and for at least 6 months post chemotherapy. Pentamidine Isethionate will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prevent the occurrence of PCP infections. It is supplied in 300 mg vials of lyophilized powder and will be administered via nebulizer. Toxicities reported with the use of Pentamidine include metallic taste, coughing, bronchospasm in heavy smokers and asthmatics; increased incidence of spontaneous pneumothorax in patients with previous PCP infection or pneumatoceles, or hypoglycemia.

8.7. Herpes Virus Prophylaxis

8.7.1. Valacyclovir (Valtrex)

Valacyclovir will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used orally to prevent the occurrence of herpes virus infections in patients with positive HSV serology. It is supplied in 500 mg tablets. Valcyclovir will be started the day after the last dose of fludarabine at a dose of 500 mg orally daily if the patient is able to tolerate oral intake. See package insert for dosing adjustments in patients with renal impairment. Common side effects include headache, upset stomach, nausea, vomiting, diarrhea or constipation. Rare serious side effects include hemolytic uremic syndrome and thrombotic thrombocytopenic purpura.

8.7.2. Acyclovir

Acyclovir will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prevent the occurrence of herpes virus infections in patients who cannot take oral medications. It is supplied as powder for injection in 500 mg/vials. Reconstitute in 10 mL of sterile water for injection to a concentration of 50 mg/mL. Reconstituted solutions should be used within 12 hours. IV solutions should be diluted to a concentration of 7mg/mL or less and infused over 1 hour to avoid renal damage. Reversible renal insufficiency has been reported with IV but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs have been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made or the drug will be discontinued. Stomach upset, headache or nausea, rash or hives; peripheral edema; pain, elevated liver function tests; and leukopenia, diarrhea, lymphadenopathy, myalgias, visual abnormalities and elevated creatinine have been reported. Hair loss from prolonged use has been reported. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

8.8. Fluconazole

Fluconazole will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prophylax against fungal infections. It is available in 200 mg tablets. It can cause headache, nausea, vomiting, diarrhea or abdominal pain, and liver damage which may be irreversible. It can cause rashes and itching, which in rare cases has caused Stevens Johnson Syndrome. It has several significant drug interactions. The package insert should be consulted prior to prescribing. For IV administration in patients who cannot tolerate the oral preparation, Fluconazole comes in 2 MG/ML solution for injection, and prepared according to Clinical Center Pharmacy standard procedures. It should be administered at a maximum IV rate of 200 mg/hr.

8.9. Anti-ESO TCR transduced PBL

The procedure for expanding the human PBL and the Certificate of Analysis are similar to those approved by the Food and Drug Administration, and used at the NCI in ongoing protocols evaluating cell therapy in the Surgery Branch (most recently 07-C-0003, 07-C-0174, 07-C-0175). The CoA is included in Appendix 4. The PBL will be transduced with retroviral supernatant containing the alpha chain and beta chain genes of the anti-ESO TCR.

8.9.1. Retroviral Vector Containing the anti-ESO TCR Gene

The retroviral vector supernatant (PG13-A2aB-IG4A-LY3H10) encoding a T cell receptor directed against NY-ESO-1, was prepared and preserved following cGMP conditions in the Indiana University Vector Production Facility (IUVPF). The retroviral vector, MSGV1- A2aB-IG4A-LY3H10, consists of 7,417 bps including the 5' LTR from the murine stem cell virus (promoter), packaging signal including the splicing donor (SD) and splicing acceptor sites, alpha chain and beta chain genes of the anti-NY-ESO-1 TCR cloned from the peripheral blood lymphocytes of a patient with melanoma, and murine stem cell virus 3'LTR. The alpha and beta chains are linked by a T2A peptide. The physical titer will be determined by RNA dot blot according to sponsor certificate. The supernate has been stored in IUVPF upon the completion of production at –80 °C at IUVPF. Upon shipment on dry ice, the supernatant will be stored at –80 °C in Cryonix, Rockville, MD. This storage facility is equipped with around-the-clock temperature monitoring. Upon request, supernatant will be delivered on dry ice to be used in *in vitro* transduction. There will be no re-use of the same unit of supernate for different patients. Retroviral titer has been shown to be stable after immediate thawing and immediate administration (coating the tissue culture wells previously coated with Retronectin). Handling of the vector should follow the guidelines of Biosafety Level-2 (BSL-2). The specific guidelines for Biosafety Level-2 (BSL-2) can be viewed at http://bmbl.od.nih.gov/sect3bsl2.htm.

8.9.2 OKT3

OKT3 will be obtained by the Surgery Branch Laboratory from commercial sources.

Formulation: Muromonab-CD3 (Ortho), NSC #618843, is provided as a sterile, clear, colorless solution at a concentration of 1 mg/ml in 5 ml ampoules. The solution may contain a few fine, translucent protein particles. The antibody is dissolved in a buffered solution at pH of 6.5 to 7.5. The solution contains 2.25 mg of

monobasic sodium phosphate, 9 mg of dibasic sodium phosphate, 43 mg of sodium chloride and 1 mg of polysorbate 80 per 5 ml of water for injection.

Storage/Stability: Ampules should be stored in a refrigerator at 2-8 °C. Solution should not be frozen or shaken. Each ampule bears an expiration date.

8.10. ALVAC ESO-1 Vaccine

The vaccine consists of the following construct:

- ALVAC(2)-NY-ESO-1(M)/TRICOM (vCP2292)

The construct is provided in liquid formulation, and is administered by the subcutaneous route.

Identity of the Investigational Product: NY-ESO-1 antigen therapeutic vaccine.

Composition: The investigational vaccine uses ALVAC(2), a modified canarypox virus, as a vector for carrying the gene for the NY-ESO-1 tumor-associated antigen. The vaccine also contains the genes for three co-stimulatory molecules: B7.1, ICAM-1, and LFA-3, collectively referred to as TRICOM.

The genes for NY-ESO-1 and the co-stimulatory molecules are contained in the same construct. Each vial contains a target dose of 10^7 $CCID_{50}$ (with a range of approximately $10^{6.4}$ to $10^{7.9}$ /mL) of the ALVAC(2) virus.

Other ingredients: The only excipient used in the final formulation of the drug product is 10 mM Tris pH 9.0 with 0.9% NaCl, which may be added if necessary to reduce titer. This type of titer adjustment has not been required for any of the clinical lots to date.

Production Site: Sanofi Pasteur Ltd., BB-IND 13450

Batch/lot numbers: TBD

Formulation: Liquid; 1 vial contains 0.72 mL of vaccine construct, with a target dose of 10^7 $CCID_{50}$ (with a range of approximately $10^{6.4}$ to $10^{7.9}$ /mL) of the ALVAC(2) virus in a withdrawable volume of 0.5 mL.

Appearance: Clear to opalescent solution with white to pink tint. Some settling may occur, which can be resuspended with shaking

Storage: 2–8°C

Injection route: Subcutaneous

Preparation and Administration: Patients will receive 0.5 mL of the construct in each of the four extremities. If a patient has a compromised extremity due to lymphedema, prior radiation or other causes, vaccination may be given on the abdominal wall or chest wall proximal to the extremity. As the construct is provided in a 0.5 mL vial, one vial is therefore required for each injection. Each construct must be administered at the same injection site throughout the treatment. All injections will be subcutaneous, using a needle not more than 5/8 of an inch in length.

In the event of overdose: Due to the nature of the product, there is no suggested treatment currently available.

Precautions for Use: Prior to administration, all study products must be inspected visually for cracks, broken seals, correct label content and extraneous particulate matter and/or discoloration, whenever solution and container permit. If

any of these conditions exists, the vaccine should not be administered and a replacement dose may be used.

As after any vaccination, subjects must be kept under observation for 30 minutes after each injection to ensure their safety. Appropriate equipment must be available on site in the event of immediate allergic reactions.

Dose Selection and Timing: The vaccine construct contains a target dose of 10^7 $CCID_{50}$ (with a range of approximately $10^{6.4}$ to $10^{7.9}$ /mL) of the ALVAC(2) virus. Earlier studies with ALVAC vaccines have found lower doses to not yield satisfactory immune responses, while higher doses present a manufacturing challenge, so cannot be used at this stage of development. Patients will receive two sets of vaccinations, two weeks apart (+/- 2 days).

8.11. Support Medications

Ondansetron hydrochloride

Ondansetron hydrochloride will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to control nausea and vomiting during the chemotherapy preparative regimen. It can cause headache, dizziness, myalgias, drowsiness, malaise, and weakness. Less common side effects include chest pain, hypotension, pruritis, constipation and urinary retention. Consult the package insert for specific dosing instructions.

Furosemide

Furosemide will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to enhance urine output during the chemotherapy preparative regimen with cyclophosphamide. Adverse effects include dizziness, vertigo, paresthesias, weakness, orthostatic hypotension, photosensitivity, rash and pruritis. Consult the package insert for a complete list of all side effects.

9.0 References

1. Wunderlich, J. R. and R. J. Hodes. 1991. Principles of tumor immunity:Biology of cellur immune responses. *Biologic Therapy of Cancer, DeVita, Hellman and Rosenberg, eds.*3-21.

2. Kawakami, Y., S. Eliyahu, C. H. Delgado, P. F. Robbins, L. Rivoltini, S. L. Topalian, T. Miki, and S. A. Rosenberg. 1994. Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. *Proc Natl Acad Sci USA* 91:3515-3519.

3. Kawakami, Y., S. Eliyahu, K. Sakaguchi, P. F. Robbins, L. Rivoltini, J. R. Yannelli, E. Appella, and S. A. Rosenberg. 1994. Identification of the immunodominant peptides of the MART-1 human melanoma antigen recognized by the majority of HLA-A2 restricted tumor infiltrating lymphocytes. *J.Exp.Med* 180:347-352.

4. Kawakami, Y., S. Eliyahu, C. H. Delgado, P. F. Robbins, K. Sakaguchi, E. Appella, J. R. Yannelli, Adema, GJ, T. Miki, and Rosenberg SA. 1994. Identification of a human melanoma antigen recognized by tumor infiltrating lymphocytes from a patient responding to immunotherapy. *Proc.Natl.Acad.Sci.* 54:3124-3126.

5. Dudley, M. E., J. Wunderlich, M. I. Nishimura, D. Yu, J. C. Yang, S. L. Topalian, D. J. Schwarztentruber, P. Hwu, F. M. Marincola, R. Sherry, S. F. Leitman, and S. A. Rosenberg. 2001. Adoptive transfer of cloned melanoma-reactive T lymphocytes for the treament of patients with metastatic melanoma. *J.Immunotherapy* 24:363-373.

6. Dudley, M., J. Wunderlich, J. C. Yang, P. Hwu, D. J. Schwartzentruber, S. L. Topalian, R. Sherry, F. M. Marincola, S. F. Leitman, C. A. Seipp, L. Rogers-Freezer, K. E. Morton, S. A. Mavroukakis, D. E. White, and Rosenberg SA. 2002. A Phase I study of non-myeloablative chemotherapy and adoptive transfer of autologous tumor antigen-specific T lymphocytes in patients with metastatic melanoma. *J Immunother* 25:243-251.

7. Dudley, M. E., J. R. Wunderlich, T. E. Shelton, J. Even, and S. A. Rosenberg. 2003. Generation of tumor-infiltrating lymphocyte cultures for use in adoptive transfer therapy for melanoma patients. *J.Immunother* 26:332-342.

8. Dudley, M. E., J. R. Wunderlich, J. C. Yang, R. M. Sherry, S. L. Topalian, N. P. Restifo, R. E. Royal, U. Kammula, D. E. White, S. A. Mavroukakis, L. J. Rogers, G. J. Gracia, S. A. Jones, D. P. Mangiameli, M. M. Pelletier, J. Gea-Banacloche, M. R. Robinson, D. M. Berman, A. C. Filie, A. Abati, and S. A. Rosenberg. 2005. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. *J.Clin Oncol* 23:2346-2357.

9. Sun, J. C. and M. J. Bevan. 2003. Defective CD8 T cell memory following acute infection without CD4 T cell help. *Science* 300:339-342.

10. Khong, H. T. and N. P. Restifo. 2002. Natural selection of tumor variants in the generation of "tumor escape" phenotypes. *Nature Immunology* 3:999-1005.

11. Clay, T. M., M. C. Custer, J. Sachs, P. Hwu, S. A. Rosenberg, and M. I. Nishimura. 1999. Efficient transfer of a tumor antigen-reactive TCR to human peripheral blood lymphocytes confers anti-tumor reactivity. *J.Immunol.* 163:507-513.

12. Hughes, M. S., Y. Y. L. Yu, M. E. Dudley, Z. Zheng, P. F. Robbins, Y. Li, J. Wunderlich, R. G. Hawley, M. Moayeri, S. A. Rosenberg, and R. A. Morgan. 2005. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. *Hum.Gene.Ther.* 16:457-472.

13. Morgan, R. A., M. E. Dudley, Y. Y. L. Yu, Z. Zheng, P. F. Robbins, M. R. Theoret, J. R. Wunderlich, M. S. Hughes, N. P. Restifo, and S. A. Rosenberg. 2003. High efficiency TCR gene transfer into primary human lymphocytes affords avid recognition of melanoma tumor antigen glycoprotein 100 and does not alter the recognition of autologous melanoma antigens. *J.Immunol.* 171:3287-3295.

14. Morgan, R. A., M. E. Dudley, J. R. Wunderlich, M. S. Hughes, J. C. Yang, R. M. Sherry, R. E. Royal, S. L. Topalian, U. S. Kammula, N. P. Restifo, Z. Zheng, A. Nahvi, C. R. de Vries, L. J. Rogers-Freezer, S. A. Mavroukakis, and S. A. Rosenberg. 2006. Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science* 314:126-129.

15. Riddell, S. R. and P. D. Greenberg. 1990. The use of anti-CD3 and anti-CD28 monoclonal antibodies to clone and expand human antigen-specific T cells. *J Immunol Meth* 128:189-201.

16. Kohn, D. B., M. S. Hershfield, D. Carbonaro, A. Shigeoka, J. Brooks, E. M. Smogorzewska, L. W. Barsky, R. Chan, F. Burotto, G. Annett, J. A. Nolta, G. Crooks, N. Kapoor, M. Elder, D. Wara, T. Bowen, E. Madsen, F. F. Snyder, J. Bastian, L. Muul, R. M. Blaese, K. Weinberg, and R. Parkman. - T lymphocytes with a normal ADA gene accumulate after transplantation of transduced autologous umbilical cord blood CD34+ cells in ADA-deficient SCID neonates.-80.

17. Therasse, P., S. G. Arbuck, E. A. Eisenhauer, J. Wanders, R. S. Kaplan, L. Rubinstein, J. Verweij, M. Van Glabbeke, A. T. van Oosterom, M. C. Christian, and S. G. Gwyther. 2000. New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 92:205-216.

18. Chen, Y. T., M. J. Scanlan, and U. Sahin. 1997. A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. *Proc Natl Acad Sci USA* 94:1914-1918.

19. Scanlan, M. J., A. O. Gure, A. A. Jungbluth, L. J. Old, and Y. T. Chen. 2002. Cancer/testis antigens: an expanding family of targets for cancer immunotherapy. *Immunol.Rev.* 188:22-32.

20. Lethe, B., S. Lucas, and L. Michaux. 1998. A New gene with tumor specificity. *Int J Cancer* 76:903-908.

21. Jager, E., Y. T. Chen, and J. W. Drijfhout. 1998. Simultaneous humoral and cellular immune response against cancer-testis antigen NY-ESO-1:definition of human histocompatibility leukocyte antigen (HLA)-A2-binding peptide epitopes. *J Exp Med* 187:265-270.

22. Aarnoudse, C. A., P. B. van den Doel, B. Heemskerk, and P. I. Schrier. 1999. Interleukin-2-induced, melanoma-specific T cells recognize CAMEL, an unexpected translation product of LAGE-1. *Int.J.Cancer* 82:442-448.

23. Wang, R.-F., S. L. Johnston, G. Zeng, S. L. Topalian, D. J. Schwartzentruber, and S. A. Rosenberg. 1998. A breast and melanoma-shared tumor antigen: T cell responses to antigenic peptides translated from different open reading frames. *J Immunol* 161:3596-3606.

24. Zeng, G., C. E. Touloukian, X. Wang, N. P. Restifo, S. A. Rosenberg, and R.-F. Wang. 2000. Identification of CD4+ T cell epitopes from NY-ESO-1 presented by HLA-DR molecules. *J.Immunol.* 165:1153-1159.

25. Jager, E., D. Jager, J. Karbach, Y. T. Chen, G. Ritter, Y. Nagata, S. Gnjatic, E. Stockert, M. Arand, L. J. Old, and A. Knuth. 2000. Identification of NY-ESO-1 epitopes presented by human histocompatibility antigen (HLA)-DRB4*0101-0103 and recognized by CD4(+) T lymphocytes of patients with NY-ESO-1-expressing melanoma. *J.Exp.Med.* 191:625-630.

26. Chen, Q., H. Jackson, P. Parente, T. Luke, M. Rizkalla, T. Y. Tai, H. C. Zhu, N. A. Mifsud, N. Dimopoulos, K. A. Masterman, W. Hopkins, H. Goldie, E. Maraskovsky, S. Green, L. Miloradovic, J. McCluskey, L. J. Old, I. D. Davis, J. Cebon, and W. Chen. 2004. Immunodominant CD4+ responses identified in a patient vaccinated with full-length NY-ESO-1 formulated with ISCOMATRIX adjuvant. *Proc.Natl.Acad.Sci.U.S.A* 101:9363-9368.

27. Jager, E., E. Stockert, Z. Zidianakis, Y. T. Chen, J. Karbach, D. Jager, M. Arand, G. Ritter, L. J. Old, and A. Knuth. 1999. Humoral immune responses of cancer patients against "Cancer-Testis" antigen NY-ESO-1: correlation with clinical events. *Int.J.Cancer* 84:506-510.

28. Stockert, E., E. Jager, and Y. T. Chen. 1998. A survey of the humoral immune response of cancer patients to a panel of human tumor antigens. *J Exp Med* 187:1349-1354.

29. Valmori, D., V. Dutoit, D. Lienard, D. Rimoldi, M. J. Pittet, P. Champagne, K. Ellefsen, U. Sahin, D. Speiser, F. Lejeune, J. C. Cerottini, and P. Romero. 2000. Naturally occurring human lymphocyte antigen-A2 restricted CD8+ T-cell response to the cancer testis antigen NY-ESO-1 in melanoma patients. *Cancer Res.* 60:4499-4506.

30. Jager, E., S. Gnjatic, Y. Nagata, E. Stockert, D. Jager, J. Karbach, A. Neumann, J. Rieckenberg, Y.-T. Chen, G. Ritter, E. Hoffman, M. Arand, L. J. Old, and A. Knuth. 2000. Induction of primary NY-ESO-1 immunity: CD8+ T lymphocyte and antibody responses in peptide-vaccinated patients with NY-ESO-1+ cancers. *Proc Natl Acad Sci* 97:12198-12203.

31. Shackleton, M., I. D. Davis, W. Hopkins, H. Jackson, N. Dimopoulos, T. Tai, Q. Chen, P. Parente, M. Jefford, K. A. Masterman, D. Caron, W. Chen, E. Maraskovsky, and J. Cebon. 2004. The impact of imiquimod, a Toll-like receptor-7 ligand (TLR7L), on the immunogenicity of melanoma peptide vaccination with adjuvant Flt3 ligand. *Cancer Immun.* 4:9.

32. Khong, H. T., J. C. Yang, S. L. Topalian, R. M. Sherry, S. A. Mavroukakis, D. E. White, and S. A. Rosenberg. 2004. Immunization of HLA-A*0201 and/or HLA-DPB1*04 patients with metastatic melanoma using epitopes from the NY-ESO-1 antigen. *J.Immunother.* 27:472-477.

33. Davis, I. D., W. Chen, H. Jackson, P. Parente, M. Shackleton, W. Hopkins, Q. Chen, N. Dimopoulos, T. Luke, R. Murphy, A. M. Scott, E. Maraskovsky, G. McArthur, D. MacGregor, S. Sturrock, T. Y. Tai, S. Green, A. Cuthbertson, D. Maher, L. Miloradovic, S. V. Mitchell, G. Ritter, A. A. Jungbluth, Y. T. Chen, S. Gnjatic, E. W. Hoffman, L. J. Old, and J. S. Cebon. 2004. Recombinant NY-ESO-1 protein with ISCOMATRIX adjuvant induces broad integrated antibody and CD4(+) and CD8(+) T cell responses in humans. *Proc.Natl.Acad.Sci.U.S.A* 101:10697-10702.

34. Rosenberg, S. A., J. C. Yang, and N. P. Restifo. 2004. Cancer immunotherapy: moving beyond current vaccines. *Nat Med* 10:909-915.

35. Zhao, Y., A. D. Bennett, Z. Zheng, Q. J. Wang, P. F. Robbins, L. Y. Yu, Y. Li, P. E. Molloy, S. M. Dunn, B. K. Jakobsen, S. A. Rosenberg, and R. A. Morgan. 2007. High-Affinity TCRs Generated by Phage Display Provide CD4+ T Cells with the Ability to Recognize and Kill Tumor Cell Lines. *J.Immunol.* 179:5845-5854.

36. Li, Y., R. Moysey, P. E. Molloy, A. L. Vuidepot, T. Mahon, E. Baston, S. Dunn, N. Liddy, J. Jacob, B. K. Jakobsen, and J. M. Boulter. 2005. Directed evoluation of human T-cell receptors with picomolar affinities by phage display. *Nat.Biotechnol.* 23:349-354.

37. Peggs, K. S., S. Verfuerth, A. Pizzey, N. Khan, M. Guiver, P. A. Moss, and S. Mackinnon. 2003. Adoptive cellular therapy for early cytomegalovirus infection after allogeneic stem-cell transplantation with virus-specific T-cell lines. *Lancet* 362:1375-1377.

38. Walter, E. A., P. D. Greenberg, M. J. Gilbert, R. J. Finch, K. S. Watanabe, E. D. Thomas, and S. R. Riddell. 1995. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. *N.Engl.J.Med.* 333:1038-1044.

39. Kuball, J., F. W. Schmitz, and R. H. Voss. 2005. Cooperation of human tumor-reactive $CD4^+$ and $CD8^+$ T cells after redirection of their specificity by a high-affinity p53A2.1-specific TCR. *Immunity* 22:117-129.

40. Morris, E. C., A. Tsallios, G. M. Bendle, S. A. Xue, and H. J. Stauss. 2005. A critical role of T cell antigen receptor-transduced MHC class I-restricted helper T cells in tumor protection. *Proc.Natl.Acad.Sci.U.S.A* 102:7934-7939.

41. Overwijk, W. W., M. R. Theoret, S. E. Finkelstein, D. R. Surman, L. A. de Jong, F. A. Vyth-Dreese, T. A. Dellemijn, P. A. Antony, P. J. Spiess, D. C. Palmer, D. M. Heimann, C. A. Klebanoff, Z. Yu, L. N. Hwang, L. Feigenbaum, A. M. Kruisbeek, S. A. Rosenberg, and N. P. Restifo. 2003. Tumor regression and autoimmunity after reversal of a functionally tolerant state of self-reactive CD8+ T cells. *J.Exp.Med* 198:569-580.

42. Gattinoni, L., D. J. Powell, S. A. Rosenberg, and N. P. Restifo. 2006. Adoptive immunotherapy for cancer: building on success. *Nat Rev Immunol* 6:383-393.

43. Eglitis, M. A. and W. F. Anderson. 1988. Retroviral vectors for introduction of genes into mammalian cells. *Biotechniques* 6:608-614.

44. Rosenberg, S. A., J. C. Yang, D. J. Schwartzentruber, P. Hwu, S. L. Topalian, R. M. Sherry, N. P. Restifo, J. R. Wunderlich, C. A. Seipp, L. Rogers-Freezer, K. E. Morton, S. A. Mavroukakis, L. Gritz, D. L. Panicali, and White D.E. 2003. Recombinant fowlpox viruses encoding the anchor-modified gp100 melanoma antigen can generate antitumor immune responses in patients with metastatic melanoma. *Clin Cancer Res* 9:2973-2980.

10.0 Tables, Figures, and Appendices:

Table 1. Grade 3-4 non-hematologic toxicities, transfusion requirements, and episodes of febrile neutropenia during the first cycle of treatment.

	Number of occurrences[1]				
Study group[2]	**Arm 1**	**Arm 2**	**Arm 3**	**Arm 4**	**Total**
Toxicity					
Hypotension	0	0	0	1	**1**
Malaise	0	0	0	0	**0**
Nausea	2	0	0	2	**4**
Neurologic	0	0	0	0	**0**
Febrile neutropenia	0	2	1	3	**6**
RBC transfusion	1	2	3	6	**12**
Platelet transfusion	1	3	1	6	**11**

[1]Counted once for each patient at the highest grade

[2] Arms defined:
Arm 1: 3 patients received up to 3 X 10^{11} cloned and/or uncloned lymphocytes plus cyclophosphamide 30 mg/kg d-7 & -6 and fludarabine 25 mg/m^2 d-5 to -1, without IL-2.
Arm 2: 3 patients received up to 3 X 10^{11} cloned and/or uncloned lymphocytes plus cyclophosphamide 60 mg/kg d-7 & -6 and fludarabine 25 mg/m^2 d-5 to -1, without IL-2.
Arm 3: 3 patients received up to 3 X 10^{11} cloned and/or uncloned lymphocytes plus cyclophosphamide 60 mg/kg d-7 & -6 and fludarabine 25 mg/m^2 d-5 to -1 + IV IL-2 (72,000 IU/kg q8h for a maximum of 15 doses)
Arm 4: 6 patients received up to 3 X 10^{11} cloned and/or uncloned lymphocytes plus cyclophosphamide 60 mg/kg d-7 & -6 and fludarabine 25 mg/m^2 d-5 to -1+ IV IL-2 (720,000 IU/kg q8h for a maximum of 12 doses)

Table 2: Clinical Responses to treatment

11/27/06

Patient[1]	Sites of Disease	Response	Duration (months)[2]
1	Axillary, Mesenteric, and Pelvic Lymph Nodes	PR	29
2	Subcutaneous and Skin	PR	8
4	Iliac and Inguinal Lymph Nodes, Skin	PR	2
6	Intraperitoneal Lymph Nodes, Lungs, Subcutaneous	PR	28
9	Subcutaneous and Skin	PR	12
10	Inguinal Lymph Nodes, Subcutaneous and Skin	PR	14
16	Subcutaneous	CR	52+
17	Bone, Liver, Lung, Subcutaneous	PR	38+
19	Intramuscular	PR	13
21	Lung, Subcutaneous	CR	45+
25	Lung, Subcutaneous	PR	2
26	Inguinal Lymph Nodes, Liver, Subcutaneous	PR	8
28	Axillary Lymph Nodes, Brain	PR	4
30	Axillary and Inguinal Lymph Nodes, Intramuscular, Subcutaneous	PR	7
31	Liver, Lung	CR	35+
32	Skin	CR	34+
33	Lung, Subcutaneous	PR	4
34	Intramuscular, Pelvis	PR	5

[1]Patients 2, 6, and 17 had minor (Patient 6) or mixed (Patient 2 and Patient 17) responses after a first course of treatment, and were treated with a second course of treatment consisting of ablation, cell transfer and high dose IL-2 therapy prior to achieving an objective clinic response.
[2]Measured from treatment date to time of first recurrence. Patients 16, 17, 21, 31 and 32 had ongoing responses at the time of this writing.

Table 3

Time in Hospital and Non-hematological Grade 3 and 4 Toxicities Related to Lymphodepleting Chemotherapy and Cell Transfer

Attribute measured	Duration, Number or Type	Number of Patients (%)
Days in Hospital[1]	6-10	6 (17%)
	11-15	18 (51%)
	16-20	4 (11%)
	21-25	7 (20%)
pRBC Transfusions	0	2 (6%)
	1-5	18 (51%)
	6-10	13 (37%)
	11-15	2(6%)
Platelet Transfusions	0	6 (17%)
	1-5	21 (60%)
	6-10	5 (14%)
	11-15	2 (6%)
	16-20	1 (3%)
Autoimmunity	Uveitis	5 (14%)
	Vitiligo	13 (37%)
Opportunistic Infections	Herpes zoster	3 (9%)
	Pneumocystis pneumonia	2 (6%)
	EBV-B cell lymphoma	1 (3%)
	RSV pneumonia	1 (3%)
Other	Febrile neutropenia	13 (37%)
	Intubated for dyspnea	3 (9%)
	Cortical blindness	1 (3%)

[1]Measured from the day of cell administration to discharge

Table 4

Patient demographics, treatments received and clinical outcome

Cohort	Patient	Age/Sex	Total Cells infused ($x10^{-9}$)	%CD4/ %CD8	%VB12	MART-1 cells infused ($x10^{-9}$)#	Days in culture	Doubling time (days)**	IL-2 doses[+]	Sites of evaluable disease	Response (duration in months)[@]
1	1	28/M	11.0	27/73	67	7.4	19	8.7	7	lymph node, cutaneous	NR
	2a*	44/F	13.0	3/95	64	8.3	19	11.9	10	lymph node, cutaneous	NR
	3	58/M	14.0	17/82	35	4.9	19	10.0	11	cutaneous, subcutaneous	NR
2	4	52/M	1.0	50/50	42	0.5	6	1.4	9	liver, subcutaneous	PR (16+)
	5	50/M	12.0	18/82	17	2.2	8	1.0	7	lung, lymph node, subcutaneous	NR
	6	55/F	7.0	37/72	51	3.6	7	1.3	8	lung, lymph node	NR
	7	56/M	9.0	75/21	40	3.6	7	1.0	5	lung, lymph node	NR
	8	37/M	6.1	68/40	32	1.9	7	1.3	12	lung, lymph node	NR
	9	53/M	4.2	72/24	41	1.7	7	2.0	9	lymph node, adrenal, subcutaneous	MR
	10	45/M	8.6	53/30	34	2.9	6	0.6	5	lymph node, subcutaneous	
	11	45/M	6.3	7/92	45	2.8	6	0.8	5	lung, pancreas, lymph node	NR
	12	32/F	4.7	30/60	61	2.9	6	0.7	5	brain, subcutaneous	NR
	13	41/M	7.7	40/67	42	3.2	6	0.9	7	lung, subcutaneous	NR
	2b*	44/F	2.1	30/59	53	1.1	6	1.9	14	lymph node, cutaneous	NR
3	14	30/M	86	11/60	40	34.4	18+9	0.9	5	hilum	PR (15+)
	15	51/M	38	16/82	45	17.1	18+9	3.3	8	lung	NR
	16	25/F	33	13/76	21	6.9	18+9	1.2	2	lung, liver, subcutaneous	NR
	17	20/F	23	17/78	30	6.9	17+8	1.1	3	lung, lymph node, subcutaneous	NR

*This patient was treated twice, separated by seven months
**Determined based on cell counts in the two days prior to infusion
[#]Total cells infused multiplied by %Vβ12
[+]720,000 IU/kg every eight hours. All patients were previously refractory to treatment with IL-2 alone
[@]Based on RECIST criteria

Table 5: Gene transfer efficiency at time of infusion.

Patient Number

		1	2a	3	4	5	6	7	8	9	10	11	12	13	2b	14	15	16	17	**Ave**
Vβ12 (%CD4)		58	63	21	n/d	18	36	45	38	43	52	51	66	43	66	58	47	18	41	**45**
Tetramer (%CD4)		8	15	1	n/d	2	10	15	8	15	21	22	33	15	15	7	4	10	7	**12**
Vβ12 (%CD8)		49	65	63	24	17	32	23	29	43	42	43	59	44	72	51	51	36	24	**42**
Tetramer (%CD8)		16	28	3	23	3	10	6	5	13	15	19	30	16	33	17	19	11	15	**17**

Samples were taken from transduced T lymphocytes cultures at or near time of infusion. FACS analysis for CD4, CD8, Vβ12, and MART-1 Tetramer (M27L) was as described in methods. n/d, not determined.; Ave, average value.

Table 6: Q-RT PCR Analysis

Patient Number

	4	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Infusion	5.8	2.2	14.5	10.7	21.5	20.8	4.0	10.3	9.6	11.2	4.0	5.1	24	11.1
Post Infusion	1.2	0.8	3.5	1.9	0.6	2.8	0.9	1.4	0.6	2.9	2.8	1.1	1.9	2.2

Amount of TCR vector-derived RNA normalized to cellular actin mRNA. Using RNA derived from a stable transduced human T cell line (Sup T1) as reference; total RNA from infused T cells or patient PBMC post-infusion were subjected to Q-RT PCR and then normalized to the amount of actin mRNA (values are vector/1 x 10^6 actin). Samples were 2-5 weeks post-infusion, with exception of patients 8, 9, and 11, which were 2 months, 3 months, and 2 weeks respectively.

Table 7: Elispot Analysis

Patient Number

	1	2a	3	4	5	6	8	9	13	2b	14	15	16	17
Infusion	1523	1783	215	4527	1367	1203	1352	460	3380	4877	2023	2938	1393	190
Pre treatment	22	21	1	14	0	1	0	0	0	0	20	1	6	0
Post infusion	13	45	0	41	7	1	215	20	700	13	530	35	92	108

Number of interferon-γ positive Eispots/100,000 cells. Post-infusion values derived from PBL taken at one to four weeks post-infusion and rested overnight in medium (without cytokine) prior to assay.

Table 8: Antigen reactivity of *ex vivo* cultured PBL.

Patient 4:

		Melanoma Cell Line				Peptide pulsed T2 cells				
		A2-		A2+						
		888	938	526	624	None	gp100	MART-1		
	None	A1,24	A1,24	A2,3	A2,3		1.0 μM	10 μM	1.0 μM	0.1 μM
Controls										
None	0	0	0	0	0	0	0	0	0	0
AK 1700-3	181	66	9	37	10	46	36	32	35	32
JKF6	0	22	28	**10250**	**10680**	1	0	**>16910**	**>15430**	**7770**
JR6C12	0	41	0	**4190**	**4640**	0	**8000**	0	**8520**	143
Patient 4										
Pre-treatment	130	204	117	98	133	521	421	422	462	520
Day 7	40	65	44	**131**	**165**	104	94	**>1255**	**>1480**	**851**
Day 8	87	110	75	92	78	122	190	**957**	**893**	**537**
Day 18	42	27	23	43	55	184	63	108	160	113

Anti-melanoma properties of genetically engineered lymphocytes determined for patient 4 PBL following overnight culture in IL-2 (300 IU/ml). The production of interferon-γ (pg/ml) following co-culture with peptide pulsed T2 cells (peptide reactivity) and anti-melanoma activity (tumor reactivity) for HLA-A2 + lines (526, 624) and HLA-A2- lines (888, 938). Controls included; non-peptide reactive TIL AK 1700-3, MART-1 reactive TIL JKF6, and gp100 reactive TIL JR6C12. JR6C12 recognition of 1.0μM MART-1 peptide was not reproducible in repeated assays. Values demonstrating specific release of cytokine are in bold.

Table 8 (con't)

Patient 14:

		Melanoma Cell Line				Peptide pulsed T2 cells				
		A2-		A2+						
		888	938	526	624	None	gp100	MART-1		
	None	A1,24	A1,24	A2,3	A2,3		1.0 μM	10 μM	1.0 μM	0.1 μM
Controls										
None	0	0	43	0	0	0	23	70	0	18
AK 1700-3	35	53	33	21	26	52	43	94	73	105
JKF6	15	11	66	**6750**	**9160**	37	337	**>2168**	**>2168**	**>1828**
JR6C12	0	0	0	**4410**	**3760**	0	**>1510**	23	15	61
Patient 14										
Pre-treatment	368	410	352	284	344	639	645	874	614	471
Day 5	66	53	67	110	**170**	134	96	**918**	**716**	**634**
Day 7	69	52	45	41	55	33	57	**192**	**221**	109
Day 10	79	91	0	80	46	33	52	**245**	**214**	108
Day 27	52	76	35	33	55	67	103	**282**	**232**	158
Day 88	26	50	31	0	52	75	41	52	43	31
Day 257	105	52	93	53	46	111	83	**597**	**492**	**399**

Anti-melanoma properties of genetically engineered lymphocytes determined for patient 14 PBL following overnight culture in IL-2 (300 IU/ml). The production of interferon-γ (pg/ml) following co-culture with peptide pulsed T2 cells (peptide reactivity) and anti-melanoma activity (tumor reactivity) for HLA-A2 + lines (526, 624) and HLA-A2- lines (888, 938). Controls included; non-peptide reactive TIL AK 1700-3, MART-1 reactive TIL JKF6, and gp100 reactive TIL JR6C12. Values demonstrating specific release of cytokine are in bold

Table 9 **RNA electroporation of tumor antigen specific TCR.**

Tumor	Effector TCR RNA				
	GFP	MART-1	gp100	NY-ESO-1	p53
938mel	23	68	35	47	38
526mel	61	**1146**	**317**	163	**306**
H2087	62	224	96	116	**>1654**
MDA 453S-A2	96	175	125	**342**	127
MDA 453S	130	65	73	75	65

CD8+ PBLs were electroporated with IVT GFP RNA or tumor antigen specific TCRs (MART-1, gp100, NY-ESO-1, p53). Two hours post electroporation, the cells were co-cultured with tumor cell lines and after overnight co-culture, the supernatants were collected and subjected to ELISA detection of interferon-γ secretion (in pg/ml). The known phenotypes of these tumor cell line were; melanoma 938mel (HLA-A2-/MART-1+/gp100+), melanoma 526mel (HLA-A2+/MART-1+/gp100+/p53+), non-small cell lung cancer H2087 (HLA-A2+/NY-ESO-1-/p53+), breast cancer MDA 453S-A2 (HLA-A2+/NY-ESO-1+/p53-), and breast cancer MDA 453S (HLA-A2-/NY-ESO-1+/p53-). Values demonstrating specific release of cytokine are in bold.

<u>TABLE 10: Modification of Dose Calculations* in Patients whose BMI is greater than 35</u>

Unless otherwise specified in this protocol, actual body weight is used for dose calculations of treatment agents. In patients who are determined to be obese (BMI > 35), the **practical weight** (see 3 below) will be used.

1. BMI Determination:

   BMI = weight (kg) / [height (m)]2

2. Calculation of ideal body weight

   Male = 50 kg + 2.3 (number of inches over 60 inches)
   Example: ideal body weight of 5'10'' male
   50 + 2.3 (10) = 73 kg

   Female = 45.5 kg + 2.3 (number of inches over 60 inches)
   Example: ideal body weight of 5'3'' female
   45.5 + 2.3 (3) = 57 kg

3. Calculation of "practical weight"

   Calculate the average of the actual and the ideal body weights. This is the practical weight to be used in calculating the doses of chemotherapy and associated agents designated in the protocol.

*Practical weight will NOT be used in the calculation of dose for aldesleukin.

Figure 1

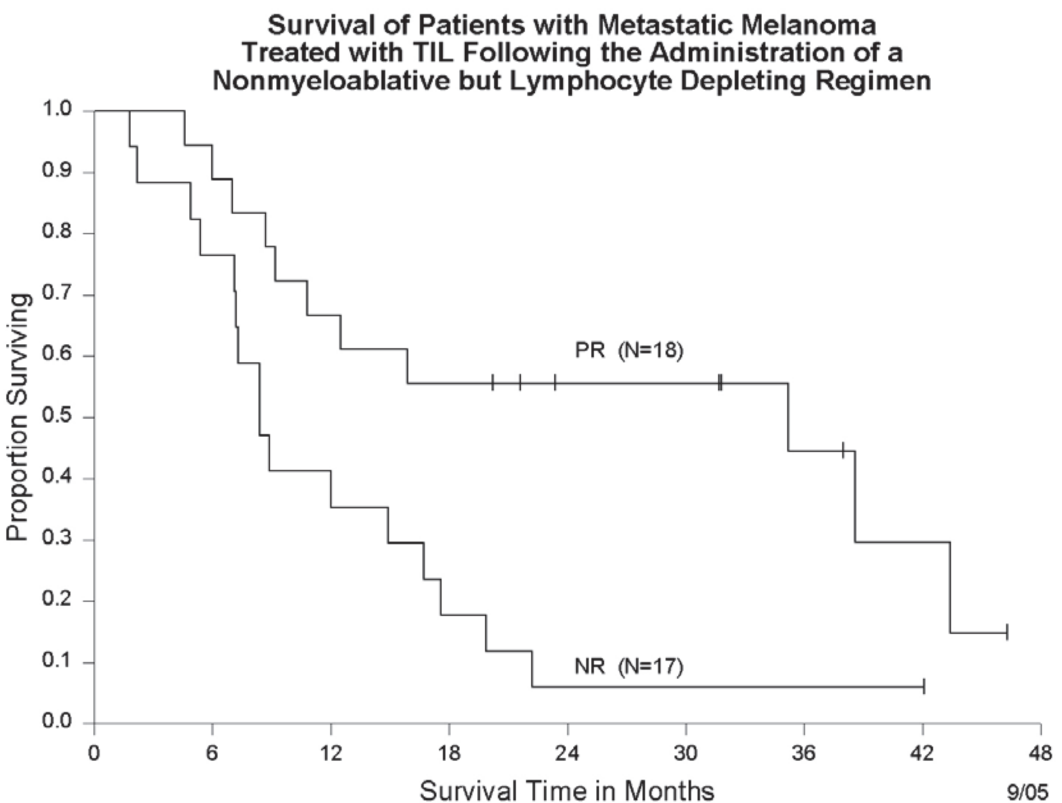
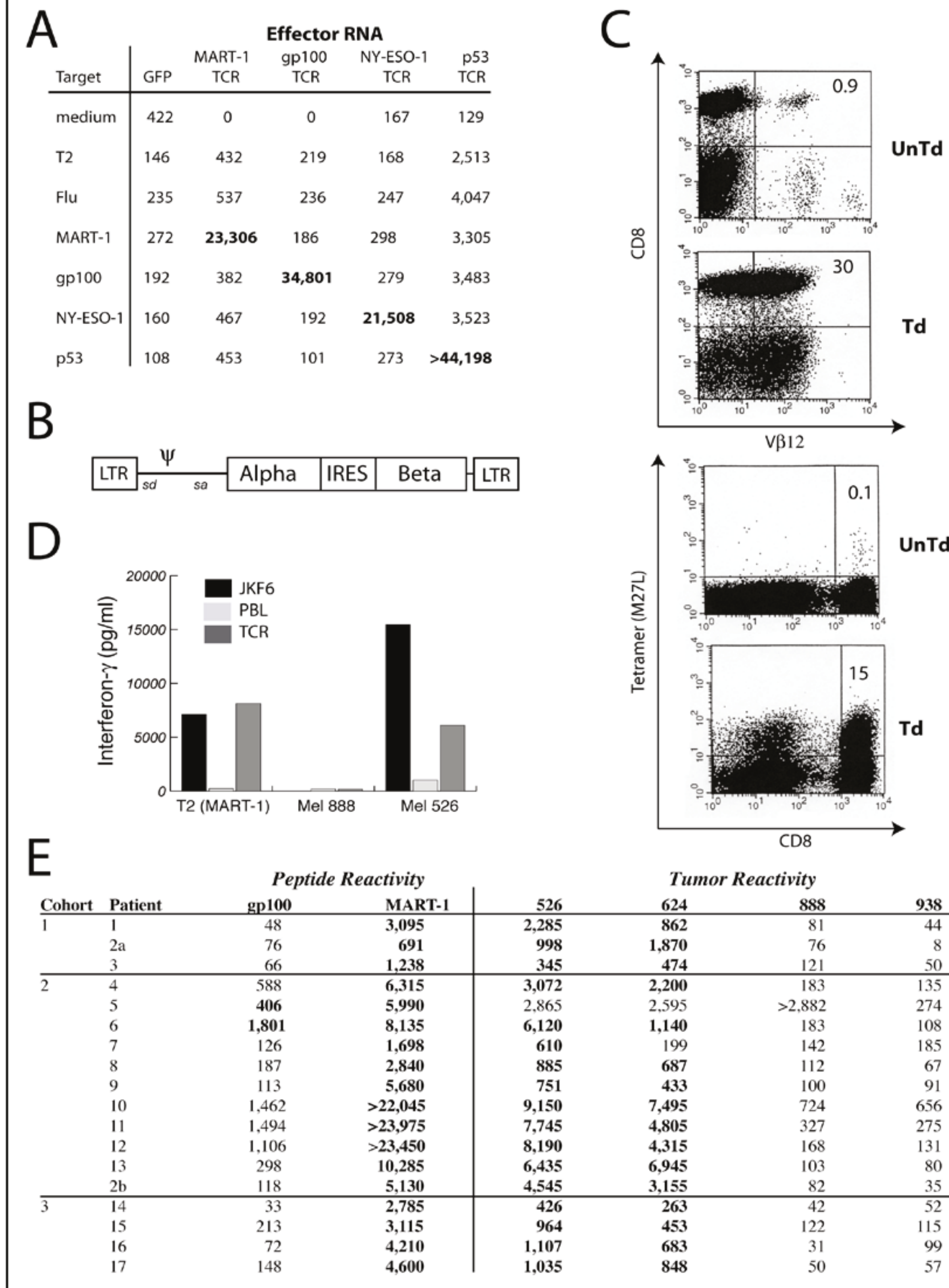


Figure 2



A

Target	GFP	MART-1 TCR	gp100 TCR	NY-ESO-1 TCR	p53 TCR
	Effector RNA				
medium	422	0	0	167	129
T2	146	432	219	168	2,513
Flu	235	537	236	247	4,047
MART-1	272	**23,306**	186	298	3,305
gp100	192	382	**34,801**	279	3,483
NY-ESO-1	160	467	192	**21,508**	3,523
p53	108	453	101	273	**>44,198**

E

Cohort	Patient	*Peptide Reactivity* gp100	MART-1	*Tumor Reactivity* 526	624	888	938
1	1	48	**3,095**	**2,285**	**862**	81	44
	2a	76	**691**	**998**	**1,870**	76	8
	3	66	**1,238**	**345**	**474**	121	50
2	4	588	**6,315**	**3,072**	**2,200**	183	135
	5	**406**	**5,990**	2,865	2,595	>2,882	274
	6	**1,801**	**8,135**	**6,120**	**1,140**	183	108
	7	126	**1,698**	**610**	199	142	185
	8	187	**2,840**	**885**	**687**	112	67
	9	113	**5,680**	**751**	**433**	100	91
	10	1,462	**>22,045**	**9,150**	**7,495**	724	656
	11	1,494	**>23,975**	**7,745**	**4,805**	327	275
	12	1,106	**>23,450**	**8,190**	**4,315**	168	131
	13	298	**10,285**	**6,435**	**6,945**	103	80
	2b	118	**5,130**	**4,545**	**3,155**	82	35
3	14	33	**2,785**	**426**	**263**	42	52
	15	213	**3,115**	**964**	**453**	122	115
	16	72	**4,210**	**1,107**	**683**	31	99
	17	148	**4,600**	**1,035**	**848**	50	57

Figure 2. Transduction and analysis of TCR engineered cells. **A**. CD8 + human lymphocytes were electroporated with RNA encoding control (GFP) or cloned TCRs reactive with HLA-A2 restricted epitopes from human tumor antigens MART-1, gp100, NY-ESO-1, and p53. Effector T-cells were co-cultured with T2 cells pulsed with 1μM of the indicated peptide (values are interferon-α, pg/ml). **B**. Diagram of recombinant retroviral vector MSGV1AIB used to engineer human lymphocytes. **C**. Transduced (Td) lymphocytes were analyzed five days post-transduction for the expression of Vβ12 and MART-1 tetramer in CD8+ cells in comparison to untransduced (UnTd) cells. Numbers are the percent positive cells in that region. **D**. TCR vector-engineered cells from patient 6 (TCR) were co-cultured with MART-1 peptide pulsed T2 cells, HLA-A2- melanoma line (Mel 888), or HLA-A2+ melanoma line (Mel 526) and the amount of interferon-α produced determined. Control effectors were untransduced cells (PBL) and MART-1 reactive TIL JKF6 (JKF6). **E**. Anti-melanoma properties of genetically engineered lymphocytes were determined for all patients prior to infusion. The production of interferon-α (pg/ml) following co-culture with peptide pulsed T2 cells (Peptide Reactivity) and anti-melanoma activity (Tumor Reactivity) for HLA-A2 + lines (526, 624) and HLA-A2- lines (888, 938). Values demonstrating specific release of cytokine are in bold.

Figure 3

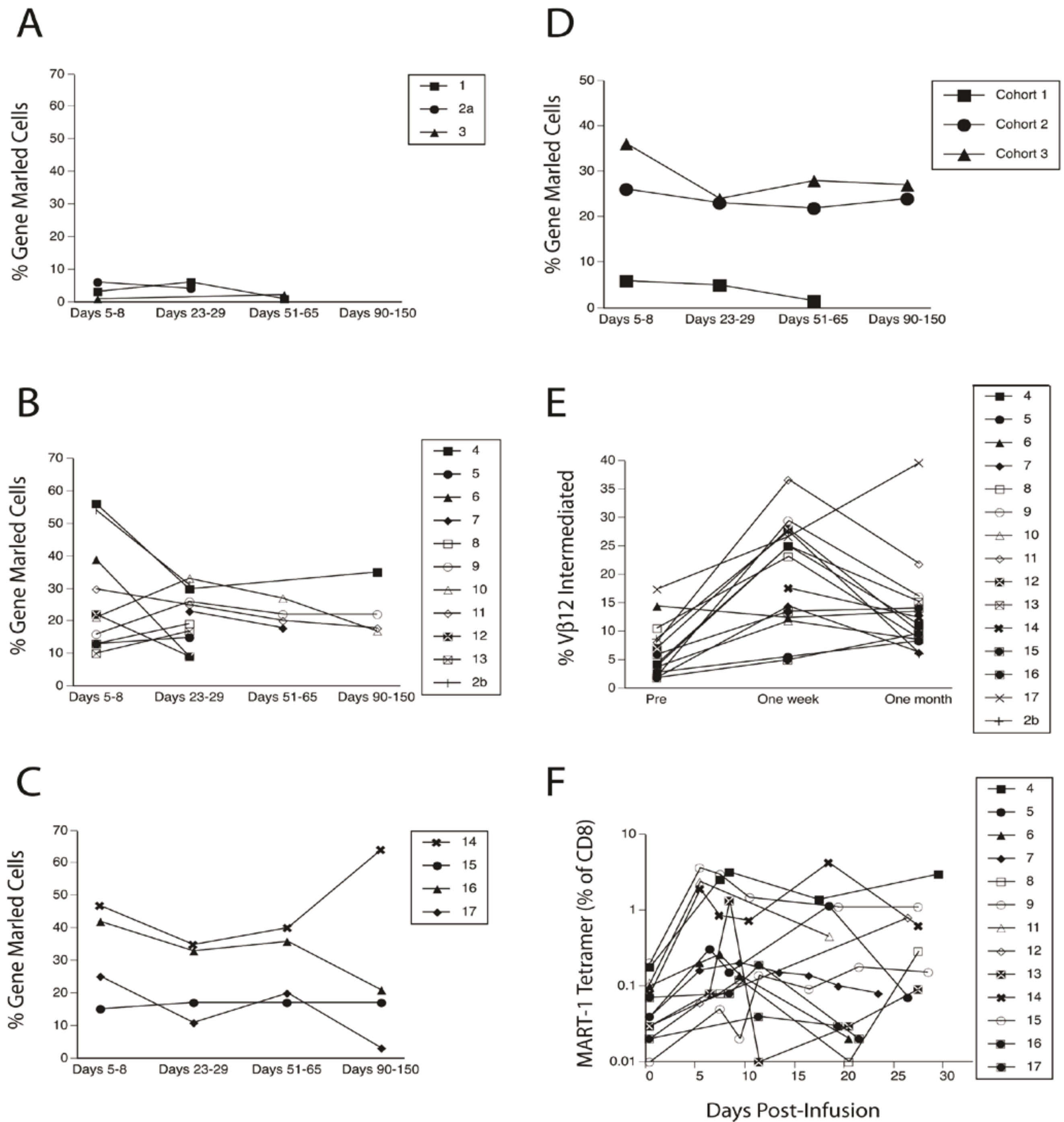


Figure 3:.
Persistence of gene marked cells. DNA extracted from PBMC was subjected to real-time quantitative PCR to determine the percent of vector-transduced cells in patient circulation at various times post-infusion. Each line represents data from a separate patient. **A**, cohort 1; **B**, cohort 2; **C**, cohort 3. **D**. Mean value of the % of gene marked cells for all patients in each cohort at the given time interval post-treatment. **E.** The percentage of CD8+/ Vβ12+ cells in the intermediate gate for patients in cohorts 2 and 3 are shown. **F.** The percentage of CD8+/MART-1 tetramer+ cells was determined for patients in cohorts 2 and 3 at the times shown. Pre-treatment values for each patient are plotted as day 0 post-infusion.

Figure 4

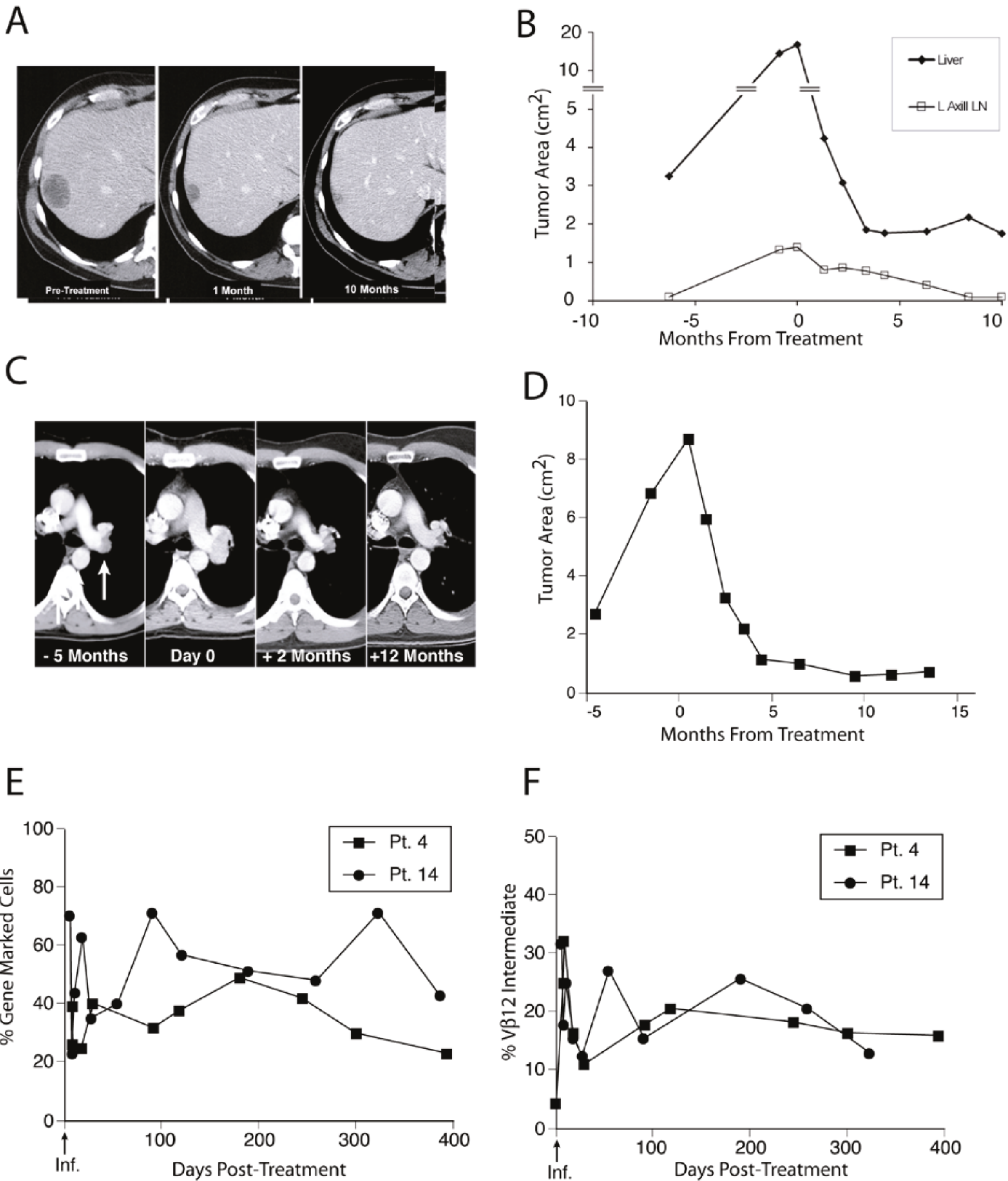


Figure 4.:Cancer regression in two patients. A. CT images of patient 4 liver metastasis; pre-treatment, one month and 10 months post-treatment with TCR engineered T-cells. B. Size of liver and axillary tumors and tempo of regression of tumor sites in patient 4 (treatment time = day 0). C. CT images of patient 14 hilar lymph node metastasis; pre-treatment, beginning of treatment (Day 0), and two months and 12 months post-treatment. D. Size of tumor and tempo of regression in patient 14. E. Quantitation of gene marked cells in patients 4 and 14 PBMC was determined by real-time quantitative PCR. Day of infusion (Inf.) indicated by arrow. F. The percentage of CD8+/Vβ12 cells in the intermediate gate in the circulation of

Figure 5:

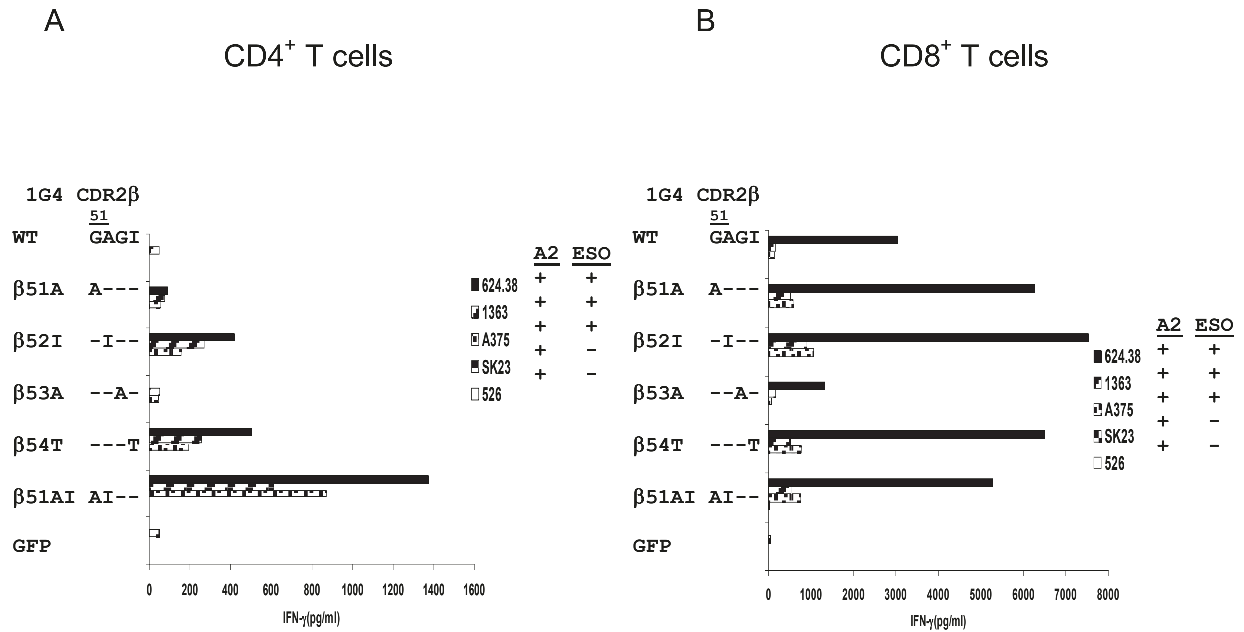


Figure 5. **Rapid screening of NY-ESO-1/HLA-A*02-reactive TCR 1G4 CDR2β variants in CD4+ and CD8+ T cells.** T cells were activated with anti-CD3 antibody and isolated CD4+ (**A**) or CD8+ (**B**) T cells were co-transfected with RNA 1G4 TCR β chain constructs encoding AASs within the CDR2 along with a WT 1G4 TCR α chain RNA construct. Transfected T cells were incubated overnight with HLA-A*02+ melanoma target cell lines that did or did not also express NY-ESO-1 transcript and the release of IFN-γ measured the following day.

Figure 6:

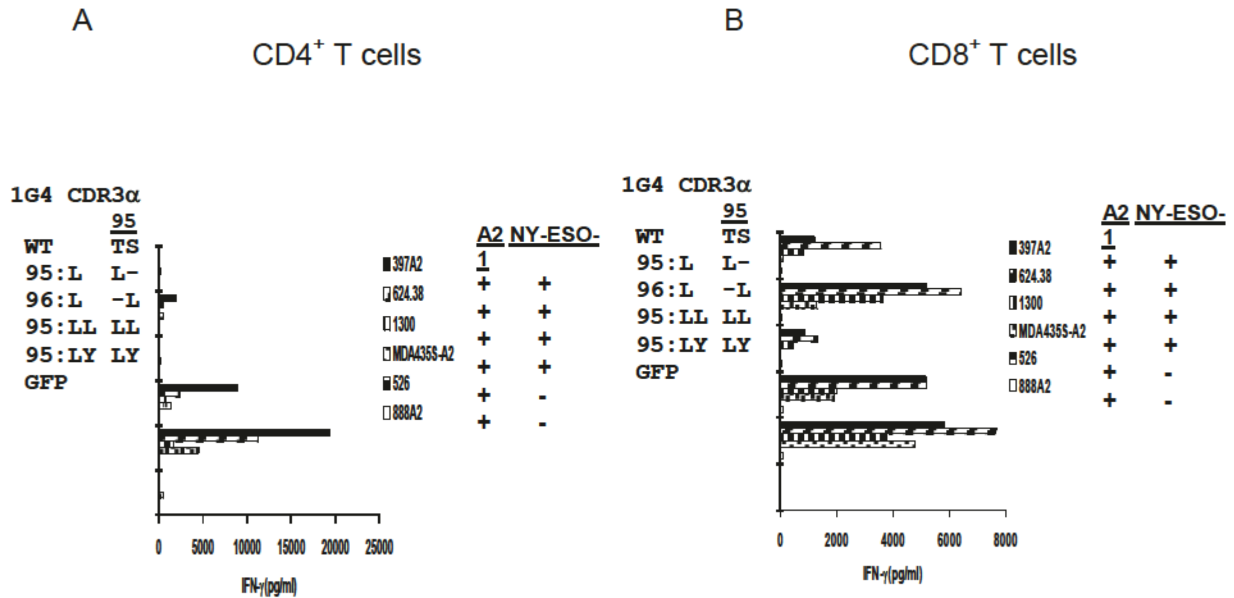


Figure 6. **Rapid screening of NY-ESO-1/HLA-A*02-reactive TCR 1G4 CDR3α variants in CD8$^+$ and CD4$^+$ T cells.** T cells were activated with anti-CD3 antibody and isolated CD4$^+$ (**A**) or CD8$^+$ (**C**) T cells were co-transfected with RNA 1G4 TCR α □chain constructs encoding AASs within the CDR3 region along with a WT 1G4 TCR α chain RNA construct. Transfected T cells were incubated overnight with HLA-A*02$^+$ melanoma (397-A2, 624.38, 1300, 526 and 888-A2) or breast cancer (MDA435S-A2) target cell lines that did or did not also express NY-ESO-1 transcript and the release of IFN-γ was measured the following day.

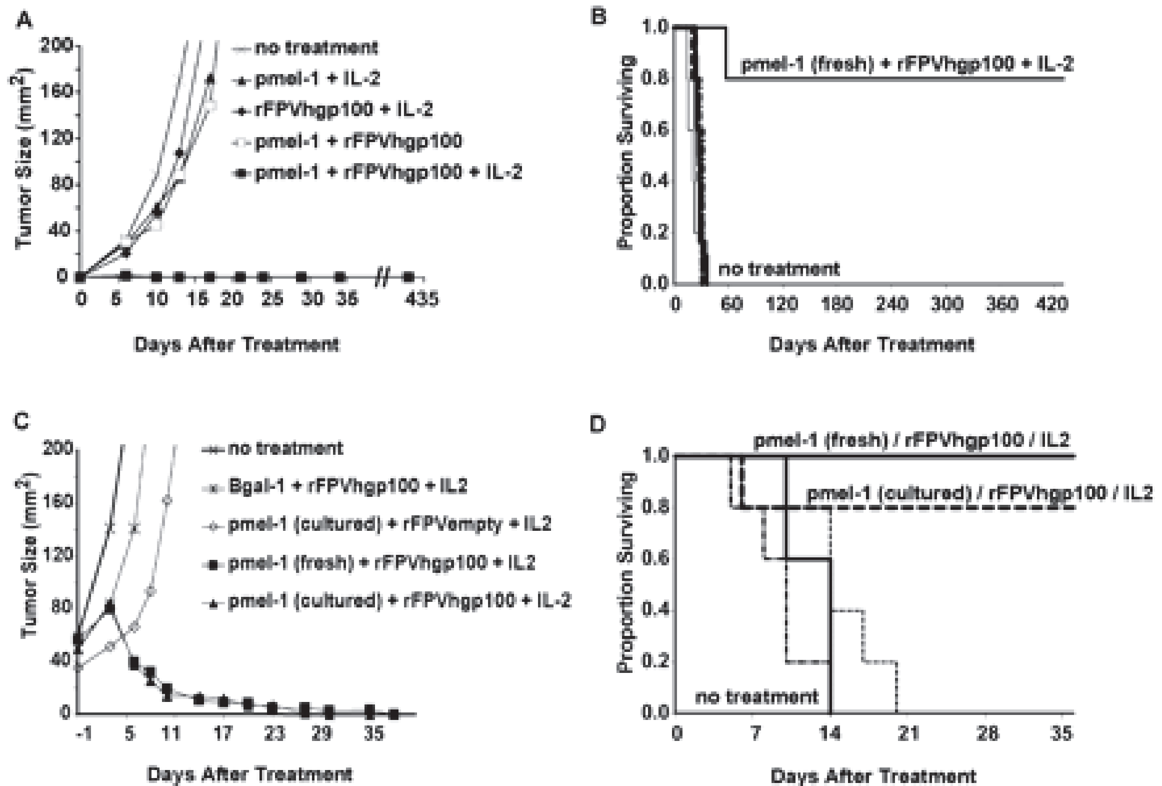


Figure 7.

Adoptive transfer of tumor-specific T cells combined with vaccination and IL-2 causes regression and cure of large, established tumors. B16 tumor was implanted subcutaneously into C57BL/6 mice were treated by adoptive transfer of fresh pmel-1 splenocytes +/- vaccination with recombinant fowlpox virus (rFPV)hgp100 either seven (A,B) or fourteen days (C,D) after inoculation with B16 melanoma. IL-2 was administered twice daily for 6 doses. Fresh or cultured splenocytes were effective I the treatment of large, established tumor. Splenocytes derived from an identically constructed TCR transgenic mouse with specificity for beta–galactosidase were used as a control in some experiments, (a representative of which is shown in panels C,D) were not therapeutic. Statistically significant tumor regression was seen in mice treated with pmel-1 cells given in combination with rFPVhgp100 and IL-2 in over 20 independently performed experiments. There were at least 5 mice/group in all experiments. Mouse survival consistently correlated with tumor growth reduction.

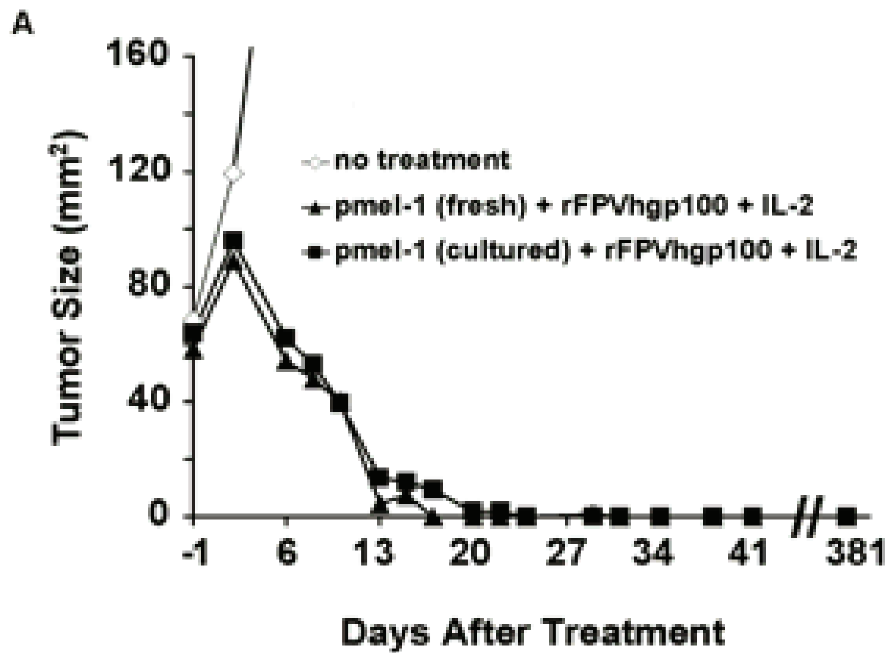


Figure 8.
Long –term (>1 yr) survival of mice bearing large, established B16 tumors after treatment with adoptive transfer of tumor-specific T cells combined with vaccination and IL-2 is associated with the development of vitiligo. C57BL/6 mice were treated with adoptive transfer of fresh or cultured pmel-1 transgenic splenocytes fourteen days after inoculation with B16 melanoma, then vaccinated with rFPVhgp100. IL-2 was administered twice daily for 6 doses. Mice treated with fresh naïve or cultured transgenic T cells were cured of B16 and vitiligo was seen at the former tumor site. At more than 1-year post therapy, these mice remain tumor-free.

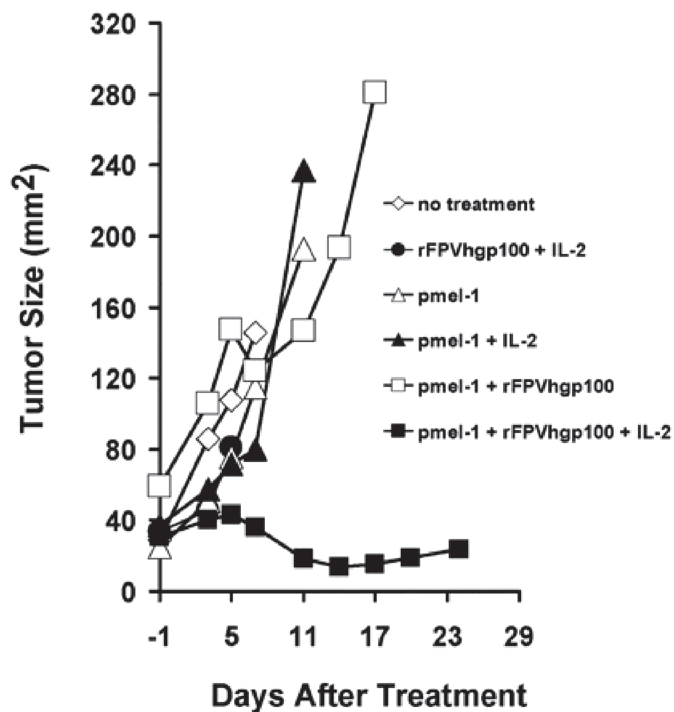


Figure 9.
Endogenous host B or T lymphocytes are not required for the treatment of established B16 tumors. C57BL/6 Rag-1$^{-/-}$ knockout mice bearing subcutaneous B16 tumors established for 14 days were treated with 1 x 10^5 cultured pmel-1 cells given cells given in combination with rFPVhgp100 and IL-2 as described previously. Similar results were obtained in five independently completed experiments.

Appendix 1

Toxicity of Treatment with Interleukin-2

Interleukin-2 Plus	Alone	TNF	a-IFN	MoAB	CYT	LAK	TIL	Total
Number of Patients	155	38	128	32	19	214	66	652*
Number of Courses	236	85	210	35	30	348	95	1039
Chills	75	16	68	8	8	191	33	399
Pruritus	53	9	26	2	2	82	6	180
Necrosis	3	—	2	—	—	—	—	5
Anaphylaxis	—	—	—	1	—	—	—	1
Mucositis (requiring liquid diet)	6	1	7	—	2	12	2	30
Alimentation not possible	1	—	1	—	—	2	—	4
Nausea and vomiting	162	42	117	14	20	263	48	666
Diarrhea	144	38	98	15	13	250	38	596
Hyperbilirubinemia (maximum/mg %)								
2.1–6.0	126	49	97	21	18	190	46	547
6.1–10.0	49	3	12	8	9	72	26	179
10.1+	26	1	4	3	1	40	8	83
Oliguria								
<80 ml/8 hours	81	37	67	14	9	114	25	347
<240 ml/24 hours	19		2	3	1	12	5	42
Weight gain (% body weight)								
0.0–5.0	106	23	65	8	9	117	49	377
5.1–10.0	78	41	111	22	10	148	26	436
10.1–15.0	43	17	26	3	9	62	15	175
15.1–20.0	7	3	8	1	1	15	3	38
20.1+	2	1		1	1	6	2	13
Elevated creatinine (maximum/mg %)								
2.1–6.0	148	43	121	20	14	237	54	637
6.1–10.0	21	1	14	3	—	34	12	85
10.1+	5	—	1	1	—	2	1	10
Hematuria (gross)	—	—	—	—	—	2	—	2
Edema (symptomatic nerve or vessel compression)	4	—	6	—	—	7	—	17
Tissue ischemia	—	—	—	—	1	1	—	2
Resp. distress:								
not intubated	17	1	9	4	1	28	7	67
intubated	15	—	6	3	—	12	5	41
Bronchospasm	2	—	2	—	1	4	—	9
Pleural effusion (requiring thoracentesis)	4	1	—	1	2	8	1	17
Somnolence	29	2	22	6	2	45	8	114
Coma	9	1	8	—	2	8	5	33
Disorientation	52	3	50	7	4	89	10	215
Hypotension (requiring pressors)	119	16	40	17	12	259	45	508
Angina	5	1	8	—	—	8	—	22
Myocardial infarction	4	—	1	—	—	1	—	6
Arrythmias	15	2	13	3	—	39	6	78
Anemia requiring transfusion (number units transfused)								
1–15	77	16	53	9	6	176	40	377
6–10	22	1	5	3	2	53	9	95
11–15	4	—	1	—	—	15	4	24
16+	1	—	1	—	—	11	1	14
Thrombocytopenia (minimum/mm^3)								
<20,000	28	1	2	4	6	71	19	131
20,001–60,000	82	11	62	14	12	150	30	361
60,001–100,000	53	36	76	11	8	79	22	285
Central line sepsis	13	—	7	1	4	36	2	63
Death	4	—	1	—	—	3	2	10

* Eleven patients are in two protocols.

Appendix 2

Expected IL-2 Toxicities and their Management

Expected toxicity	Expected grade	Supportive Measures	Stop Cycle*	Stop Treatment **
Chills	3	IV Meperidine 25-50 mg, IV q1h, prn,	No	No
Fever	3	Acetaminophen 650 mg, po, q4h; Indomethicin 50-75 mg, po, q8h	No	No
Pruritis	3	Hydroxyzine HCL 10-20 mg po q6h, prn; Diphenhydramine HCL25-50 mg, po, q4h, prn	No	No
Nausea/ Vomiting/ Anorexia	3	Ondansetron 10 mg, IV, q8h, prn; Granisetron 0.01 mg/kg IV daily prn; Droperidol 1 mg, IV q4-6h, prn; Prochlorperazine 25 mg pr, prn or 10 mg IV q6h prn	No	No
Diarrhea	3	Loperamide 2mg, po, q3h, prn; Diphenoxylate HCl 2.5 mg and atropine sulfate 25 mcg, po, q3h, prn; codeine sulfate 30-60 mg, po, q4h, prn	If uncontrolled after 24 hours despite all supportive measures	No
Malaise	3 or 4	Bedrest	If other toxicities occur simultaneously	No
Hyperbilirubinemia	3 or 4	Observation	If other toxicities occur simultaneously	No
Anemia	3 or 4	Transfusion with PRBCs	If uncontrolled despite all supportive measures	No
Thrombocytopenia	3 or 4	Transfusion with platelets	If uncontrolled despite all supportive measures	No
Edema/Weight gain	3	Diuretics prn	No	No
Hypotension	3	Fluid resuscitation Vasopressor support	If uncontrolled despite all supportive measures	No

Dyspnea	3 or 4	Oxygen or ventilatory support	If requires ventilatory support	No
Oliguria	3 or 4	Fluid boluses or dopamine at renal doses	If uncontrolled despite all supportive measures	No
Increased creatinine	3 or 4	Observation	Yes (grade 4)	No
Renal failure	3 or 4	Dialysis	Yes	Yes
Pleural effusion	3	Thoracentesis	If uncontrolled despite all supportive measures	No
Bowel perforation	3	Surgical intervention	Yes	Yes
Confusion	3	Observation	Yes	No
Somnolence	3 or 4	Intubation for airway protection	Yes	Yes
Arrhythmia	3	Correction of fluid and electrolyte imbalances; chemical conversion or electrical conversion therapy	If uncontrolled despite all supportive measures	No
Elevated Troponin levels	3 or 4	Observation	Yes	If changes in LV function have not improved to baseline by next dose
Myocardial Infarction	4	Supportive care	Yes	Yes
Elevated transaminases	3 or 4	Observation	For grade 4 without liver metastases	If changes have not improved to baseline by next dose
Hyperbilirubinemia	3 or 4	Observation	For grade 4 without liver metastases	If changes have not improved to baseline by next dose
Electrolyte imbalances	3 or 4	Electrolyte replacement	If uncontrolled despite all supportive measures	No
Neutropenia	4	Observation	No	No

*Unless the toxicity is not reversed within 12 hours

** Unless the toxicity is not reversed to grade 2 or less by next treatment.

Appendix 3: Interleukin-2 toxicities observed in patients treated at the NIH Clinical Center

TABLE 8. *Toxicity of Treatment with Interleukin-2*

Interleukin-2 Plus	Alone	TNF	a-IFN	MoAB	CYT	LAK	TIL	Total
Number of Patients	155	38	128	32	19	214	66	652*
Number of Courses	236	85	210	35	30	348	95	1039
Chills	75	16	68	8	8	191	33	399
Pruritus	53	9	26	2	2	82	6	180
Necrosis	3	—	2	—	—	—	—	5
Anaphylaxis	—	—	—	1	—	—	—	1
Mucositis (requiring liquid diet)	6	1	7	—	2	12	2	30
Alimentation not possible	1	—	1	—	—	2	—	4
Nausea and vomiting	162	42	117	14	20	263	48	666
Diarrhea	144	38	98	15	13	250	38	596
Hyperbilirubinemia (maximum/mg %)								
2.1–6.0	126	49	97	21	18	190	46	547
6.1–10.0	49	3	12	8	9	72	26	179
10.1+	26	1	4	3	1	40	8	83
Oliguria								
<80 ml/8 hours	81	37	67	14	9	114	25	347
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Weight gain (% body weight)								
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10.1–15.0	43	17	26	3	9	62	15	175
15.1–20.0	7	3	8	1	1	15	3	38
20.1+	2	1		1	1	6	2	13
Elevated creatinine (maximum/mg %)								
2.1–6.0	148	43	121	20	14	237	54	637
6.1–10.0	21	1	14	3	—	34	12	85
10.1+	5	—	1	1	—	2	1	10
Hematuria (gross)	—	—	—	—	—	2	—	2
Edema (symptomatic nerve or vessel compression)	4	—	6	—	—	7	—	17
Tissue ischemia	—	—	—	—	1	1	—	2
Resp. distress:								
not intubated	17	1	9	4	1	28	7	67
intubated	15	—	6	3	—	12	5	41
Bronchospasm	2	—	2	—	1	4	—	9
Pleural effusion (requiring thoracentesis)	4	1	—	1	2	8	1	17
Somnolence	29	2	22	6	2	45	8	114
Coma	9	1	8	—	2	8	5	33
Disorientation	52	3	50	7	4	89	10	215
Hypotension (requiring pressors)	119	16	40	17	12	259	45	508
Angina	5	1	8	—	—	8	—	22
Myocardial infarction	4	—	1	—	—	1	—	6
Arrythmias	15	2	13	3	—	39	6	78
Anemia requiring transfusion (number units transfused)								
1–15	77	16	53	9	6	176	40	377
6–10	22	1	5	3	2	53	9	95
11–15	4	—	1	—	—	15	4	24
16+	1	—	1	—	—	11	1	14
Thrombocytopenia (minimum/mm^3)								
<20,000	28	1	2	4	6	71	19	131
20,001–60,000	82	11	62	14	12	150	30	361
60,001–100,000	53	36	76	11	8	79	22	285
Central line sepsis	13	—	7	1	4	36	2	63
Death	4	—	1	—	—	3	2	10

* Eleven patients are in two protocols.

Appendix 4

Certificate of Analysis:
Anti-ESO-1 TCR transduced T cells, protocol 08-C-0121

Date of preparation of final product:
Patient:

Allogeneic PBMC
Donor Name:
Pheresis date:

Tests performed on final product:

Test	*Method*	*Limits*	*Result*	*Initials/ Date*
Cell viability[1]	trypan blue exclusion	>70%		
Total viable cell number[1]	visual microscopic count	>1 x10^8		
Tumor reactivity[2]	γ-IFN release vs. peptide pulsed T2 cells	>200 pg/ml		
TCR expression[2]	FACS analysis of the transduced cells	PBL, >10%		
Microbiological studies	gram stain[1,3,]	no micro-organisms seen		
	aerobic culture[3,4]	no growth		
	fungal culture[3,4]	no growth		
	anaerobic culture[3,4]	no growth		
	mycoplasma test[5]	negative		
Endotoxin	limulus assay[1]	<5 E.U./kg		
RCR	S+L- Assay[4] RCR-PCR[6]	negative		

[1] Performed on sample of the final product immediately prior to infusion. Results are available at the time of infusion.
[2] Performed 2-10 post transduction. Results are available at the time of infusion.
[3] Performed 2-4 days prior to infusion. Results are available at the time of infusion but may not be definitive.
[4] Sample collected from the final product prior to infusion. Results will not be available before cells are infused into the patient.
[5] Performed 2-10 days prior to infusion. Results are available at the time of infusion.
[6] Performed on sample approximately 1-4 days prior to infusion. Results are available at the time of infusion.

Prepared by: ______________________________ Date: ________________

QC sign-off: ______________________________ Date: ________________
James C. Yang, M.D. or designee