Abbreviated Title: Anti-NY-ESO-1 mTCR PBL
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PROTOCOL TITLE

A Phase II Study of Metastatic Cancer that Expresses NY-ESO-1 Using Lymphodepleting Conditioning Followed by Infusion of Anti-NY-ESO-1 Murine TCR-Gene Engineered Lymphocytes

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Investigational Agent:

Drug Name:	Anti-ESO murine TCR transduced PBL
IND Number:	15698
Sponsor:	Center for Cancer Research
Manufacturer	Surgery Branch Cell Production Facility

Commercial Agents: Cyclophosphamide, Fludarabine, and Aldesleukin

PRÉCIS

Background:

- We have constructed a single retroviral vector that contains both α and β chains of a murine T-cell receptor (mTCR) that recognizes the NY-ESO-1 (ESO) tumor antigen, which can be used to mediate genetic transfer of this TCR with high efficiency.
- In co-cultures with HLA-A2 and ESO double positive tumors, anti-ESO mTCR transduced T-cells secreted significant amounts of IFN-γ with high specificity.

Objective:

- Primary objective:
  - To determine whether the administration of anti-ESO mTCR-engineered peripheral blood lymphocytes (PBL) plus high-dose aldesleukin following a non-myeloablative, lymphodepleting preparative regimen may result in objective tumor regression in patients with metastatic cancer including melanoma expressing the ESO antigen.

Eligibility:

- Age ≥ 15 years and ≤ 70 years. Patients aged 15-17 years must weigh at least 50 kg.
- HLA-A*0201 positive
- Metastatic cancer including melanoma whose tumors express the ESO antigen
- Previously received and have been a non-responder to or recurred after receiving standard care for metastatic disease
- No contraindications for high-dose aldesleukin administration

Design:

- Peripheral blood mononuclear cells (PBMC) obtained by leukapheresis will be cultured in the presence of anti-CD3 (OKT3) and aldesleukin to stimulate T-cell growth.
- Transduction is initiated by exposure of cells to retroviral vector supernatant containing the anti-ESO mTCR genes. This mTCR targets the exact same epitope as the hTCR.
- All patients will receive a non-myeloablative, lymphodepleting preparative regimen of cyclophosphamide and fludarabine.
- On Day 0, patients will receive anti-ESO mTCR gene-transduced PBMC and then begin high-dose aldesleukin.
- A complete evaluation of evaluable lesions will be conducted 6 weeks (± 2 weeks) following the administration of the cell product.
- The study will be conducted using a phase II optimal design. The objective will be to determine if the treatment regimen 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).
- A total of up to 43 patients may be enrolled (41, plus allowing for up to 2 non-evaluable patients).

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- To determine whether the administration of anti-ESO mTCR-engineered peripheral blood lymphocytes plus high-dose aldesleukin following a non-myeloablative, lymphodepleting preparative regimen may result in objective tumor regression in patients with metastatic cancer including melanoma expressing the ESO antigen.

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

1.2.1 Adoptive Cell Transfer Experience at the NCI Surgery Branch

The National Cancer Institute Surgery Branch (NCI-SB) has pioneered novel T-cell based cancer therapies for chemotherapy-refractory cancers and continues efforts to expand their application. This work has its foundation in the successful treatment of metastatic cutaneous melanoma with adoptive transfer of tumor infiltrating lymphocytes (TIL)[1]. We have reported the results of adoptive transfer therapy in 93 patients with metastatic melanoma who received TIL following a lymphodepleting regimen plus aldesleukin administration, with or without total body irradiation (**Figure 1**)[2]. Forty-three patients received a non-myeloablative chemotherapy consisting of 60 mg/kg cyclophosphamide daily x 2 and 25mg/m^2 fludarabine daily x 5 prior to cell transfer and aldesleukin administration. Twenty-five patients each also received the same chemotherapy agents in conjunction with either 200 or 1200 cGy total body irradiation (TBI) prior to cell infusion and aldesleukin administration. The overall objective response rate using RECIST criteria in these 93 patients was 56%. The clinical results in these three trials are shown in **Table 1**. There was one treatment related death in these 93 patients, which occurred in a patient who received 200cGy TBI who had an undetected diverticular abscess prior to beginning therapy. Of the 52 responding patients in this trial, 42 had disease that was refractory to aldesleukin therapy and 22 had disease that was refractory to prior aldesleukin plus chemotherapy. Thus, TIL therapy shows promise as an effective treatment for chemotherapy refractory metastatic cutaneous melanoma.

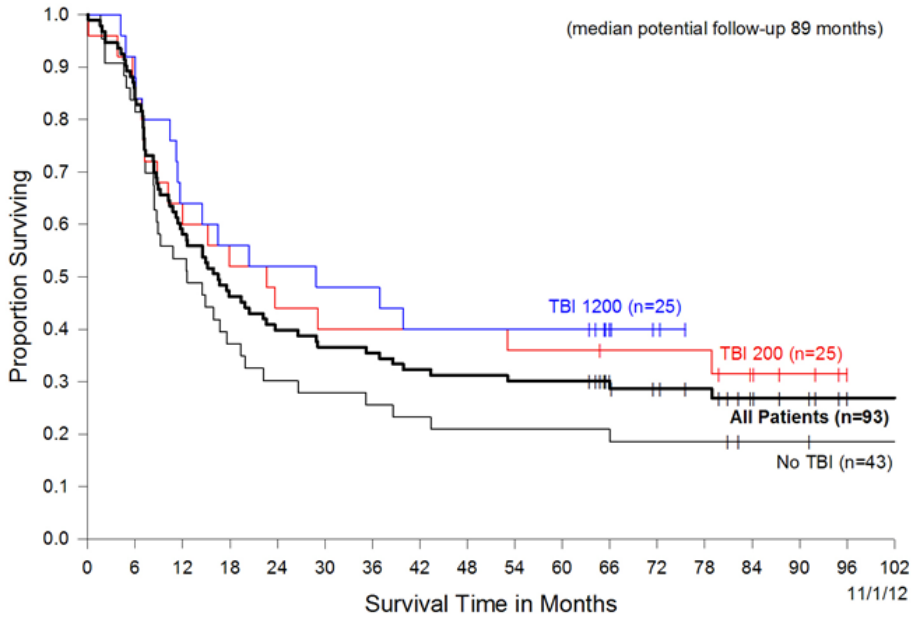

Figure 1. *Survival of patients with metastatic melanoma treated with autologous tumor-infiltrating lymphocytes and IL-2 following three different lymphoconditioning regimens.*

Table 1. Cell Transfer Therapy (11/1/2012). *Response rates and duration in patients with melanoma treated with tumor-infiltrating lymphocytes plus high-dose IL-2 following three different lymphoconditioning regimens.*

Treatment	Total	PR	CR	OR (%)
		number of patients (duration in months)		
No TBI	43	16 (37%) (84, 36, 29, 28, 14, 12, 11, 7, 7, 7, 7, 4, 4, 2, 2, 2)	5 (12%) (108+, 106+, 105+, 91+, 82+)	21 (49%)
200 TBI	25	8 (32%) (14, 9, 6, 6, 5, 4, 3, 3)	5 (20%) (95+, 92+, 87+, 84+, 64+)	13 (52%)
1200 TBI	25	8 (32%) (21, 13, 7, 6, 6, 5, 3, 2)	10 (40%) (75+, 72+, 71+, 66+, 66+, 65+, 65+, 64+, 63+, 19)	18(72%)

(20 complete responses: 19 ongoing at 63 to 108 months)

1.2.2 NY-ESO-1 T-Cell Receptor

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. 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 the surface of 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 isolated from mice immunized against the NY-ESO-1 cancer testis antigen 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 NCI-SB TCR gene transfer adoptive immunotherapy protocols using human and mouse TCRs.

1.2.3 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[3]. 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[4]. 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[5]. 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[6]. 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[7] and epitopes derived from the normal as well as alternative open reading frames of both gene products in the context of HLA-A31 have been described[8]. In addition, NY-ESO-1 epitopes are recognized in the context of multiple HLA class II restriction elements[9-11].

Utilizing modifications to a human anti-ESO TCR directed against the dominant NY-ESO-1:157-165 T-cell epitope, a Phase II clinical trial (08-C-0121) was opened for accrual in April 2008 and as of December 20, 2012, has accrued 34 evaluable patients treated for the first time with the anti-ESO TCR. Patients were entered into 4 cohorts. Cohorts 1 and 3 include patients with metastatic melanoma or renal cell cancer; cohorts 2 and 4 include patients with other types of metastatic cancer. In cohorts 3 and 4, patients also received the ALVAC-NY-ESO-1 vaccine. Thus, to date, we have enrolled 18 patients with metastatic melanoma, 16 patients with synovial sarcoma, and 1 patient with breast cancer. The vaccine was supplied by Sanofi Pasteur who then

withdrew the supply. There were no apparent effects of the vaccine and it is no longer available. The combined responses to therapy are shown in **Table 2**.

Table 2. *Response to therapy with NY-ESO-1 TCR (4/1/13)*

	Total	PR (%)	CR (%)	OR (%)
Melanoma	**21**	**5 (29%)** α (20+, 10**, 8, 5, 3+, 3)	**4 (19%)** (51+, 39+, 25, 23+**)	**10 (48%)**
Synovial Cell Sarcoma	**18**	**12 (67%)** (31+**, 12**, 10, 8, 7, 6, 5, 4+, 4, 3, 3**, 1*)	**0**	**12 (67%)**

α Durations of response in months are in parenthesis under the number of responders. "+" indicates on ongoing response.

* Meets criteria for response at first follow-up, but not official response

** Plus ALVAC vaccine

This regimen (08-C-0121) resulted in objective cancer regression in 50% (9/18) patients with melanoma, 4 of whom are ongoing, and in 63% (10/16) patients with synovial sarcoma 3 of which are ongoing. Non-hematologic and hematologic toxicities were those expected from IL-2 and the myelosuppressive chemotherapy and none were attributed to any off-tumor target toxicity due to recognition of NY-ESO-1. Grade 2, 3 and 4 toxicities attributable to the anti-ESO TCR are shown in **Table 3**. There were no mortalities related to the cell therapy; one patient died from sepsis secondary to myelosuppression from the chemotherapy, a second patient developed renal failure due to aldesleukin.

Table 3. *Adverse events possibly related to anti-NY-ESO-1.*

Adverse Event	Grade	number of events
Rash/desquamation	2	1
Bilirubin (hyperbilirubinemia)*	3	4
Dyspnea (shortness of breath)	2	1
Dyspnea (shortness of breath)	3	3
Renal failure*	4	1
* likely due to aldesleukin		

Robbins et al. reported preliminary results from this protocol in March of 2011[12]. The clinical responses observed in the 34 patients receiving anti-ESO TCR-engineered peripheral blood lymphocytes to date show a combined response rate by RECIST criteria for all arms of this study of 4 complete responses and 15 partial responses.

1.2.4 Murine TCR Directed Against NY-ESO-1

By introducing a TCR targeting the NY-ESO-1 antigen, large numbers of T-cells with a defined antigen specificity can be generated, resulting in the clinical responses seen in our past clinical trial targeting this antigen. However, given the short duration of some responses seen in the current clinical trial, methods to increase the potential efficacy of the TCR were investigated. One possible reason for decreased efficacy is that by introducing a TCR, mixed TCR dimers can be formed, which could harbor potentially harmful specificities and off-target effects, as well as decreasing the TCR expression of both the introduced and endogenous TCRs[13]. Studies have shown that the pairing of endogenous and introduced TCR chains in TCR gene-modified T-cells can lead to the formation of self-reactive TCRs, leading to cytokine-driven autoimmune pathology in mouse models[14]. These toxicities have not been seen in our prior studies targeting the same epitope as in the proposed trial. Murine-human hybrid TCRs have been investigated as a method to improve the expression of the transduced TCR. These studies demonstrated that by constructing TCRs with a murine constant region in place of the human constant region, a higher expression of the receptors were found on the surface of the human lymphocytes, caused by the preferential pairing of the murine constant regions[15]. The murine-human TCRs mediated higher levels of cytokine secretion and cell lysis, which was associated with improved CD3 stability[15].

To generate high avidity mTCRs against NY-ESO-1 antigen, we employed a transgenic mouse model that expresses the human HLA-A*0201 molecule. Transgenic mice expressing the full-length HLA-A*0201 molecule were immunized with a previously identified naturally processed and presented HLA-A*0201 restricted peptide from NY-ESO-1 (SLLMWITQC). Following two immunizations, murine T-cells were harvested from the spleen and stimulated *in vitro* with the respective peptide and IL-2. Bulk T-cell cultures were tested for specific reactivity using LPS-blast cells and T2 cells both pulsed with the relevant peptide after three *in vitro* stimulations. Reactive T-cells from positive wells from both the first and the third bulk stimulations were then cloned by limiting dilution and tested for antigen specific reactivity.

TCR α- and β-chains from each tumor reactive T-cell clone were cloned using SMART™ RACE cDNA amplification kit with gene specific primers in the constant region of mouse TCR α and β-chains. After the identification of the variable regions of α- and β-chains and the specific constant region of the β-chain, specific primers were used to amplify the full length TCR α and β-chains from cDNA. The TCR α- and β-chains that showed the highest level of specific reactivity were then cloned into an MSGV1-based retroviral vectors with expression cassettes consisting of MSGV1 ESO-157muTCRA2aB and MSGV1 ESO-157 muTCRB2aA (**Figure 2A**). The TCR expression in this vector is driven by the viral LTR, α and β chains are expressed as a single open reading frame using the 2A linker peptide[16, 17]. Human PBL were stimulated for 3 days and then transduced twice. FACS analysis of transduced PBL using the anti-mouse TCR-β chain revealed that both $CD8^+$ and $CD4^+$ cells had been transduced with these TCR vectors (**Figure 2B**). These transduced PBL were then expanded using our rapid expansion protocol (REP), along with the human NY-ESO-1 TCR used in our current clinical trial for comparison. The percentage of transduced cells was evaluated after one stimulation and after REP (**Figure 2C**), which showed an increase in the percentage of positively transduced mTCR cells after REP.

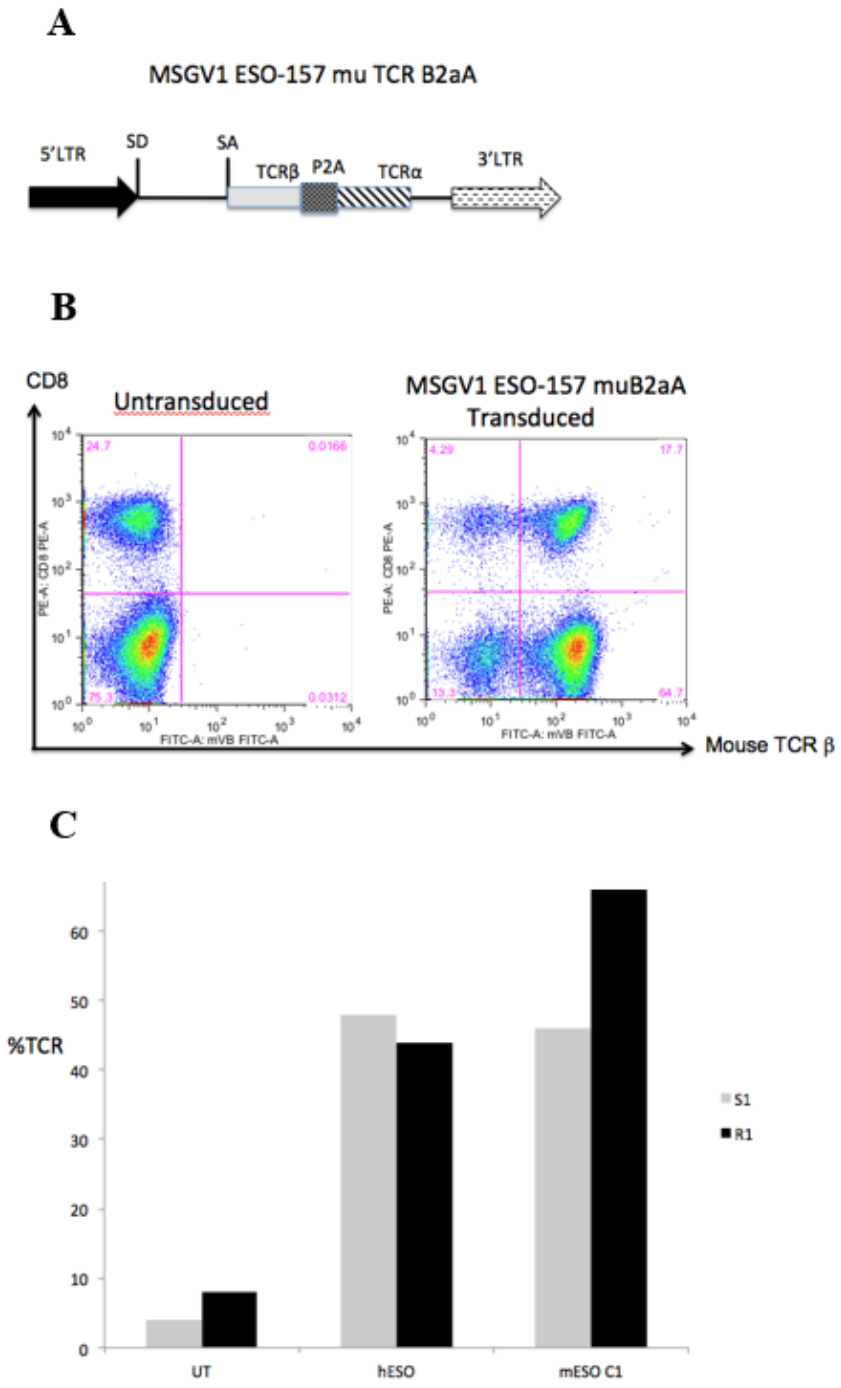

Figure 2. (A) *Schematic illustration of the MSGV1 based retroviral vector encoding anti ESO-157 mu T cell receptor expression cassette. TCR α and β chains are linked with furin- -spacer (SGSG)-P2A ribosomal skip peptide sequence.* ***(B)*** *Flow cytometric analysis of ESO mTCR transduced PBL, representative of eleven different donors.* ***(C)*** *Percentage of TCR positive cells (ESO TCR vs ESO mTCR) after stimulation with OKT3 (S1) and after a rapid expansion protocol (R1), representative of four different donors.*

To evaluate the recognition by the respective NY-ESO-1 TCRs, transduced PBL were subjected to co-culture assay with peptide pulsed T2 cells. mTCR transduced PBL specifically secreted IFN-γ upon encounter with the antigenic peptide in a dose dependent manner (**Figure 3**), and at higher levels after REP. PBL transduced with the ESO mTCR recognized T2 cells pulsed with as little as 0.1 ng/ml NY-ESO-1 peptide indicating that the TCR was a relatively high avidity receptor. To assess the specific recognition of tumor cells, TCR engineered PBL were co-cultured with a panel of HLA-A*0201^{+} and HLA-A*0201^{-} melanoma and lung tumor derived cell lines. Specific release of IFN-γ was observed when the TCR engineered PBL were co-cultured with HLA- A*0201^{+}/NY-ESO-1^{+} cell lines but not HLA-A*0201^{-}/NY-ESO-1^{+} or HLA-A*0201^{+}/NY-ESO-1^{-} cell lines (**Figure 4**). Higher levels of IFN-γ were secreted by the mTCR when compared with the current human TCR in use. These cells were also capable of specific cell lysis of HLA-A*0201^{+}/NY-ESO-1^{+} tumor cell lines (**Figure 5**).

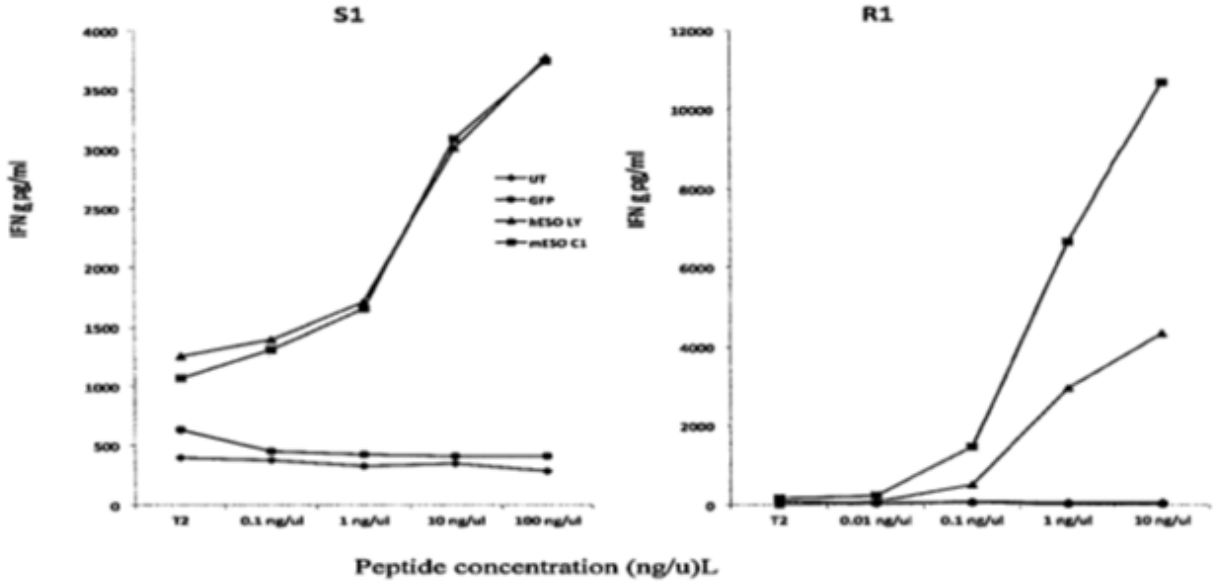

Figure 3. *Recognition of peptide pulsed T2 cells by the ESO TCR and ESO mTCR transduced PBL. Human PBL expressing TCR and mTCR against NY-ESO-1, GFP (negative control) and untransduced PBL (negative control) were co-cultured for 16 hours with T2 cells that were previously pulsed with different concentrations of peptide, representative of four different donors.*

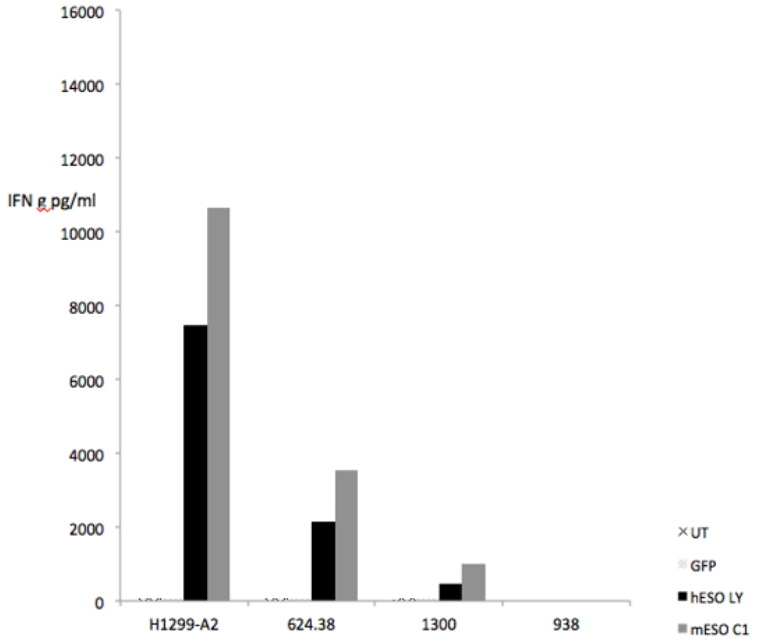

Figure 4. *Recognition of NY-ESO-1+, A2+ tumor cell lines (H1299-A2, 624.38 and 1300) and NY-ESO-1+, A2- tumor cell lines (938). ESO TCR and ESO mTCR transduced PBL were co-cultured for 16 hours with tumor target cell lines (H1299-A2=non-small cell lung cancer, ESO+, A2+, 624.38= melanoma, ESO+, A2+, 1300= melanoma, ESO+, A2+, 938= melanoma, ESO+, A2-) and IFN gamma levels were then measured, representative of 8 different donors.*

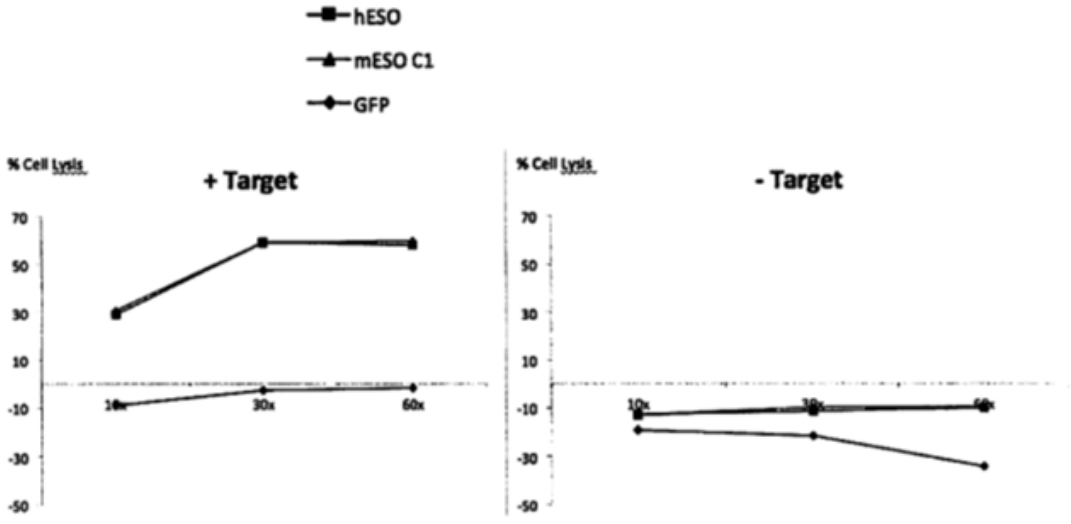

Figure 5. *Cell lysis assay comparing cell-specific lysis activity of the ESO TCR transduced PBL with ESO mTCR transduced PBL. Effector cells were co-cultured with an ESO+, A2+ tumor target (624.38, + control) and an ESO+, A2- tumor target (938, - control), representative of four different donors.*

1.2.5 Safety Considerations

The eligibility, design, treatment, and evaluation of patients in this protocol is the same as that already used to treat 40 patients using the human TCR. We saw no off-tumor toxicities when targeting this NY-ESO-1 epitope. 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, lymphodepleting preparative regimen and the administration of high-dose aldesleukin have expected toxicities, which are discussed in Section **7.3.1** of this study. The non-myeloablative, lymphodepleting preparative regimen used in this protocol has been administered to over 500 patients and all have reconstituted their hematopoietic systems. In October 2017, one patient enrolled to this protocol diagnosed with synovial cell sarcoma and extensive lung disease experienced a constellation of toxicities after receiving $69.1x10^9$ anti-ESO-1 murine TCR-gene engineered lymphocytes and one dose aldesleukin. Toxicities included mental status changes (somnolence, depressed consciousness), kidney and liver abnormalities, respiratory failure, and altered heart functioning. The events were determined to be possibly related to anti-ESO-1 cell infusion, aldesleukin infusion, and pulmonary compromise from tumor and prior surgeries.

In other protocols, we have administered up to $1.5x10^{11}$ 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. 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, WAS, and X-CGD using retroviral vector-mediated gene transfer into CD34+ hematopoietic stem 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. Murine TCRs have been used in four previous TCR gene therapy trials at the Surgery Branch (04-C-0241, 07-C-0174, 09-C-0047,11-C-0062). The introduction of murine or murinized TCRs and the possibility of immune responses against murine antigens has been studied in two of our clinical trials in which cancer patients were treated with murine TCRs specific for the antigens p53 and gp100; this study found that 23% of patients treated with the mTCRs developed antibodies directed towards the murine variable regions and not to the constant region common to all mTCR[18]. These antibodies were not detected for 3-4 months after cell transfer and the production of these antibodies was not associated with the level of transduced cell persistence or response to therapy.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria for Patients with Solid Tumor Cancers and Melanoma

2.1.1.1 Measurable (per RECIST v1.0 criteria) metastatic cancer or locally advanced refractory/recurrent malignancy including melanoma that expresses ESO as assessed by

one of the following methods: RT-PCR on tumor tissue, immunohistochemistry of resected tissue, or serum antibody reactive with ESO.

2.1.1.2 Confirmation of diagnosis of metastatic cancer including melanoma by the NCI Laboratory of Pathology.

2.1.1.3 Patients must have previously received first-line standard therapy (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.

2.1.1.4 Patients with 3 or fewer brain metastases that are less than 1 cm in diameter and asymptomatic are eligible. Lesions that have been treated with stereotactic radiosurgery must be clinically stable for 1 month after treatment for the patient to be eligible. Patients with surgically resected brain metastases are eligible.

2.1.1.5 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).

Note: Patients may have undergone minor surgical procedures within the past three weeks, as long as all toxicities have recovered to grade 1 or less.

Note: Patients who have previously received ipilimumab and have documented GI toxicity must have a normal colonoscopy with normal colonic biopsies.

2.1.2 Inclusion Criteria for Patients with Malignant Meningioma

2.1.2.1 Histologically proven recurrent meningioma or aggressive meningioma.

Note: Confirmation of ESO expression and pathology is not required in patients with definitive radiologic evidence of meningioma who are unresectable, and in whom radiation therapy without biopsy is the standard treatment.

2.1.2.2 Recurrent disease/progression after receiving all standard treatments, which must include the following:

- Surgical resection, if possible.
- Definitive radiation therapy for unresectable meningioma, or for recurrent meningioma after resection.

2.1.2.3 At least 4 weeks post-surgery, and must be at least 3 months post-radiation therapy, with resolution of related toxicities.

2.1.2.4 Measurable disease on MRI scan.

2.1.2.5 No history of intracranial hemorrhage.

2.1.2.6 Patients with a history of neurofibromatosis (NF) may have other stable CNS tumors, such as schwannoma, acoustic neuroma, or ependymoma only if those lesions have been stable for the past 6 months.

2.1.2.7 Patients must be on stable dose of steroids for at least 5 days prior to baseline imaging.

2.1.3 Inclusion Criteria for all Patients

2.1.3.1 Age $\geq$ 15 years and $\leq$ 70 years.

2.1.3.2 Patient, or their parent(s)/legal guardian(s) (if the patient is < 18 years of age), is able to understand and willing to sign a written informed consent. Written assent will be obtained for participants under the age of 18 as appropriate, as stated in Section **10.6**.

2.1.3.3 All participants ≥ 18 years of age must be willing to sign a durable power of attorney.

2.1.3.4 Clinical performance status of ECOG 0 or 1.

2.1.3.5 Patients aged 15-17 years weigh ≥ 50 kg.

2.1.3.6 HLA-A*0201 positive.

2.1.3.7 Patients of both genders must be willing to practice birth control from the time of enrollment on this study and for four months after treatment.

2.1.3.8 Women of child-bearing potential must have a negative pregnancy test because of the potentially dangerous effects of the treatment on the fetus.

2.1.3.9 Serology

- Seronegative for HIV antibody. (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who are HIV seropositive may have decreased immune-competence and thus may be less responsive to the experimental treatment and more susceptible to its toxicities.)
- Seronegative for hepatitis B antigen, and seronegative for hepatitis C antibody. If hepatitis C antibody test is positive, then patient must be tested for the presence of antigen by RT-PCR and be HCV RNA negative.

2.1.3.10 Hematology

- ANC > 1000/mm^3 without the support of filgrastim
- WBC ≥ 3000/mm^3
- Platelet count ≥ 100,000/mm^3
- Hemoglobin > 8.0 g/dL. Subjects may be transfused to reach this cut-off.

2.1.3.11 Chemistry

- Serum ALT/AST ≤ 2.5 x ULN
- Serum creatinine ≤ 1.6 mg/dL
- Total bilirubin ≤ 1.5 mg/dL, except in patients with Gilbert's Syndrome, who must have a total bilirubin < 3.0 mg/dL.

2.1.3.12 Subject must be co-enrolled on protocol 03-C-0277.

2.1.4 Exclusion Criteria

2.1.4.1 Women of child-bearing potential who are pregnant or breastfeeding because of the potentially dangerous effects of the treatment on the fetus or infant.

2.1.4.2 Any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease).

2.1.4.3 Active systemic infections requiring anti-infective treatment, coagulation disorders, or any other active or uncompensated major medical illnesses.

2.1.4.4 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).

2.1.4.5 Concurrent systemic steroid therapy.

2.1.4.6 History of severe immediate hypersensitivity reaction to cyclophosphamide, fludarabine, or aldesleukin.

2.1.4.7 History of coronary revascularization or ischemic symptoms.

2.1.4.8 Documented LVEF ≤ 45% tested in patients:

- Age ≥ 65 years
- With clinically significant atrial and/or ventricular arrhythmias, including but not limited to: atrial fibrillation, ventricular tachycardia, second- or third-degree heart block or have a history of ischemic heart disease and/or chest pain.

2.1.4.9 Documented FEV1 ≤ 60% predicted tested in patients with:

- A prolonged history of cigarette smoking (≥ 20 pack-year smoking history, with cessation within the past two years).
- Symptoms of respiratory dysfunction.

2.1.4.10 Patients who are receiving any other investigational agents.

2.2 SCREENING EVALUATION

Note: Testing for screening evaluation is conducted under the NCI-SB companion protocol, 99-C-0128 (Evaluation for NCI Surgery Branch Clinical Research Protocols).

2.2.1 Within 3 Months Prior to Starting the Preparative Regimen

- HIV antibody titer, HBsAg determination, and anti-HCV
- Anti-CMV antibody titer, HSV and VZV serology, and EBV panel (Note: Patients who are known to be positive do not need to be retested.)
- One of the following methods will be used to assess ESO expression: RT-PCR analysis of tumor biopsy, immunohistochemistry of resected tissue, or analysis of serum to evaluate for expression of ESO-1 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 ESO-1 antibody will be conducted in Dr. Rosenberg's laboratory using CLIA-approved methods. (Note: Testing is permitted to be conducted at any time prior to starting the preparative regimen.)
- Confirmation of diagnosis of metastatic cancer including melanoma by the NCI Laboratory of Pathology. (Note: Testing is permitted to be conducted at any time prior to starting the preparative regimen.)

2.2.2 Within 8 Weeks Prior to Starting the Preparative Regimen

- Pulmonary function testing for patients with a prolonged history of cigarette smoking (≥ 20 pack-year smoking history, with cessation within the past two years), symptoms of respiratory dysfunction, or other clinical indications which may include thoracic surgeries.
- Cardiac evaluation commensurate to patients' history and clinical presentation (e.g., stress thallium, echocardiogram, MUGA) for patients who are ≥ 65 years of age, or who 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 ≤ 45% will not be eligible. Patients < 65 years of age with cardiac risk factors (e.g., diabetes, hypertension, obesity) may undergo cardiac evaluations as noted above.

2.2.3 Within 4 Weeks Prior to Starting the Preparative Regimen

- Complete history and physical examination, including weight and vital signs, noting organ system involvement and any allergies/sensitivities to antibiotics. (Note: Patient history may be obtained within 8 weeks prior to starting the preparative regimen.)
- Patients with meningioma must undergo a neurological examination (Section **6.3.4.6**).
- Baseline evaluation to determine disease status. This may include CT, MRI, PET, and/or photography. Note: CT scans are not required for patients with meningioma.

2.2.4 Within 14 Days Prior to Starting the Preparative Regimen

- Screening blood tests:
  - CBC w/differential
  - Chemistries: Creatinine, ALT/GPT, AST/GOT, Total bilirubin
- Urinalysis, with culture if indicated

2.2.5 Within 7 Days Prior to Starting the Preparative Regimen

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

2.3 REGISTRATION AND TREATMENT ASSIGNMENT PROCEDURES

2.3.1 Prior to Registration for this Protocol

Patients will sign the consent for and enroll on the NCI-SB companion protocol 03-C-0277 (Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols), prior to leukapheresis for generation of the cell product. 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.

2.3.2 Registration Procedure

Authorized staff must register an eligible candidate with the NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the website

(http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and sent via encrypted email to the NCI-CRO at ncicentralregistration-l@mail.nih.gov. After confirmation of eligibility at the NCI-CRO, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol. Verification of registration will be forwarded electronically via email to the research team. A recorder is available during non-working hours.

2.3.3 Treatment Assignment Procedures

2.3.3.1 Cohorts

Number	Name	Description
1	*ESO-Expressing Cancers*	Patients with metastatic, solid tumor cancers (including melanoma and meningioma) that expresses the ESO antigen

2.3.3.2 Arms

Number	Name	Description
1	*Experimental Therapy*	Non-myeloablative, lymphodepleting preparative regimen of cyclophosphamide and fludarabine + anti-ESO murine TCR transduced PBL + high-dose aldesleukin

2.3.3.3 Randomization and Arm Assignment

This is a non-randomized study. All patients will be directly assigned based on cohort as follows:

- Patients in Cohorts 1-2 will be directly assigned to Arm 1.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

3.1.1 Pre-Treatment Phase: Cell Preparation Performed on 03-C-0277

PBMC will be obtained by leukapheresis (approximately $1x10^{10}$ cells). Whole PBMC will be cultured in the presence of anti-CD3 (OKT3) and aldesleukin to stimulate T-cell growth. Transduction is initiated by exposure of approximately $1x10^{7}$ to $5x10^{8}$ cells to supernatant containing the anti-ESO 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. Cells will be administered at a dose of between $1x10^{9}$ to $2x10^{11}$ lymphocytes and will be given over 20-30 minutes.

3.1.2 Treatment Phase

The study will be conducted using a Phase II design. Patients will receive up to $2x10^{11}$ anti-ESO mTCR engineered PBL. A minimum of approximately $1x10^{9}$ cells will be given. The cells administered vary depending on their growth characteristic. The percent of TCR+ cells will be recorded for each patient and the number of TCR+ cells administered per kg will be calculated for each patient at the time of the IRB continuing review, the annual report to the FDA, and in the event of any serious adverse events related to the cell product.

Patients will receive no other experimental agents while on this protocol.

Patients will receive the standard NCI-SB non-myeloablative, lymphodepleting preparative regimen consisting of cyclophosphamide and fludarabine followed by IV infusion of anti-ESO mTCR engineered PBL and aldesleukin. All 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.

3.2 PROTOCOL STOPPING RULES

New subject enrollment to the protocol will be temporarily halted if any of the following conditions are met, and discussions will be had with the FDA or IRB regarding safety and the need for protocol revisions if applicable:

- Two or more patients develop a grade 3 or greater toxicity at any point in the study attributable to the cell infusion that does not resolve to grade 2 within 10 days.
- If one of the first three 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.
- If one or more treatment-related deaths occur due to the cell infusion, we will promptly discuss this with the IRB and the FDA.

3.3 DRUG ADMINISTRATION

3.3.1 Preparative Regimen with Cyclophosphamide and Fludarabine

Treatment will be according to the schedule described below and in Section **3.3.4**. Starting on Day -6, study medication start times for drugs given once daily should be given within 2 hours of the scheduled time. Chemotherapy infusions may be slowed or delayed as medically indicated. Administration of diuretics, electrolyte replacement, and hydration and monitoring of electrolytes should all be performed as clinically indicated.

Days -7 and -6

Approximately 6 Hours Prior to Cyclophosphamide

Hydrate: Begin hydration with 0.9% Sodium Chloride Injection containing 20 mEq/L of potassium chloride at 1.5-2.0 mL/kg/hour (starting approximately 6 hours pre-cyclophosphamide and continuing until 24 hours after last cyclophosphamide infusion). Rate and composition of fluid may be altered based on clinical indications. The hydration rate will be capped at 250 mL/hour. At any time during the preparative regimen, if urine output is < 1.0 mL/kg/hour or if body weight > 2 kg over pre-cyclophosphamide value, furosemide 10-20 mg IV may be administered.

Approximately 1 Hour Prior to Cyclophosphamide

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

Cyclophosphamide 60 mg/kg/day x 2 days IV in 250 mL D5W infused simultaneously with mesna 15 mg/kg/day over 1 hour x 2 days. If patient is obese (BMI > 35), drug dosage will be calculated using practical weight as described in **Appendix A**.

A decreased dose of cyclophosphamide at 30 mg/kg/day (x 2 days) will be considered for patients who have a history of prolonged hematologic recovery from prior chemotherapy treatments.

Immediately Following the End of Cyclophosphamide

Begin mesna infusion at 3 mg/kg/hour intravenously diluted in a suitable diluent (see Section **11.2.1**) over 23 hours after each cyclophosphamide dose. If patient is obese (BMI > 35), drug dosage will be calculated using practical weight as described in **Appendix A**.

Days -7 to -3

Fludarabine 25 mg/m^2/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 **Appendix A**. (Fludarabine will be started approximately 1-2 hours after the cyclophosphamide and mesna on Days -7 and -6.)

3.3.2 Cell Infusion

Day 0 (2-4 Days After the Last Dose of Fludarabine)

The patient's PBMC transduced cells are delivered to the Patient Care Unit by a staff member from the Surgery Branch Cell Production Facility (SB-CPF). Cells will be administered at a dose of between 1x10^9 to 2x10^{11} lymphocytes. Prior to infusion, the cell product identity label is double-checked by two authorized staff (MD or RN), and an identification of the product and documentation of administration are entered in the patient's chart. The cells are to be infused intravenously on the Patient Care Unit over 20-30 minutes or as clinically determined by an investigator for patient safety via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping.

3.3.3 Aldesleukin Administration

Days 0-5 (Day 0 = Day of Cell Infusion)

- Beginning on Day 1 or 2, filgrastim will be administered subcutaneously at a dose of 300 mcg/day (dose may be adjusted as clinically indicated). Filgrastim administration will continue daily until neutrophil count > 1x10^9/L x 3 days or > 5x10^9/L.
- Aldesleukin will be given as described below.

Aldesleukin will be administered at a dose of 720,000 IU/kg (based on total body weight) as an intravenous bolus over a 15-minute period approximately every 8 hours beginning within 24 hours of cell infusion and continuing for up to 5 days (maximum 15 doses). Doses will be preferentially administered every eight hours; however, up to 24 hours may elapse between doses depending on patient tolerance. Aldesleukin dosing will be stopped if toxicities are not sufficiently recovered with supportive measures within 24 hours of the last dose of aldesleukin. Doses will be delayed or stopped 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 **Appendix B**. Toxicities will be managed as outlined in **Appendix C**. Dosing may be held or stopped at the discretion of the treating investigator. (**Appendix D** 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 about their medical care if the patient becomes incapacitated or cognitively impaired.

3.3.4 Treatment Schedule

Day	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5
Therapy													
Cyclophosphamide (60 mg/kg)	X	X											
Fludarabine (25 mg/m^2)	X	X	X	X	X								
Anti-ESO mTCR transduced PBL								X[1]					
Aldesleukin (720,000 IU/kg)								X[2]	X	X	X	X	X
Filgrastim[3] (5 mcg/kg)									X	X	X	X	X
TMP/SMX[4] 160 mg/800 mg (example)								X		X		X	
Fluconazole[5] (400 mg PO)								X	X	X	X	X	X
Valacyclovir PO or Acyclovir IV[6]								X	X	X	X	X	X

[1]Two to four days after the last dose of fludarabine.

[2]Initiate within 24 hours after cell infusion.

[3]Continue until neutrophil 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 x2, starting Day 0 or within one week of anticipated lymphopenia.

[5]Continue until ANC > 1000/mm^3

[6]In patients positive for HSV or VZV, continue for at least 6 months and until CD4 > 200 x2.

3.4 BASELINE EVALUATION

Note: Refer to Section **5** for research evaluations.

3.4.1 Within 14 Days Prior to Starting the Preparative Regimen

- Apheresis, as indicated
- Baseline blood tests:
  - CBC w/differential
  - Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric acid, Creatinine kinase, Lactate dehydrogenase, Total protein
  - PT-INR/PTT
  - TBNK

- Thyroid Panel
- Anti-CMV antibody titer, HSV and VZV serology, and EBV panel (may be performed within 3 months prior to starting the preparative regimen)

- Urinalysis, with culture if indicated
- Chest x-ray
- EKG

3.5 ON-STUDY EVALUATION

3.5.1 During the Preparative Regimen (Daily)

- CBC w/differential
- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric acid, Creatinine kinase, Lactate dehydrogenase, Total protein
- PT-INR/PTT (every 3 days)
- Urinalysis, as needed
- Weight, as indicated

3.5.2 Post-Cell Infusion

- Vital signs (with neuro checks for patients with meningioma) will be monitored hourly (± 15 minutes) for four hours and then routinely (every 4-6 hours) unless otherwise clinically indicated.

  Note: Neuro checks do not need to be continued after the first 4 hours if they are normal.

3.5.3 During Hospitalization (Every 1-2 Days)

- Physical examination, including weight and vital signs, as clinically indicated
- Toxicity assessment, including a review of systems, as clinically indicated
- CBC w/differential
- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric acid, Creatinine kinase, Lactate dehydrogenase, Total protein
- Once total lymphocyte count is > 200/mm^3, TBNK for peripheral blood CD4 count will be drawn weekly (while the patient is hospitalized). See Section **5.5** for post-cell infusion evaluations.
- Other tests will be performed as clinically indicated.

3.6 POST-TREATMENT (FOLLOW-UP) EVALUATION

- All patients will return to the NIH Clinical Center for their first follow-up evaluation for response 6 weeks (± 2 weeks) following the administration of the cell product.
- Patients who have received multiple transfusions during the treatment phase or have been discharged with grade 3 or greater significant adverse events should be evaluated by the referring physician within two weeks of discharge and repeat labs as appropriate to be faxed to the research team. Patients will receive appropriate treatment as determined by their treating physician.
- Patients who are unable or unwilling to return for follow-up evaluations may be followed via phone or email contact. A request will be made to send laboratory, imaging, and physician exam reports performed by their treating physician. Any outstanding toxicities will be reviewed with the patient.

3.6.1 Time-Period of Evaluations

Patients who experience stable disease, a partial response, or a complete response, or have unresolved toxicities after their first follow-up evaluation, will return to the NIH Clinical Center as noted below:

- Week 12 (± 2 weeks)
- Every 3 months (± 1 month) x3
- Every 6 months (± 1 month) x 2 years
- As per PI discretion for subsequent years

Note: Patients may be seen more frequently as clinically indicated.

3.6.2 Scheduled Evaluations

At each scheduled evaluation for response, patients will undergo:

- Physical examination, including ECOG
- Weight and vital signs
- Toxicity assessment, including a review of systems
- CBC w/differential
- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric acid, Creatinine kinase, Lactate dehydrogenase, Total protein
- PT-INR/PTT
- Urinalysis, as needed
- Thyroid Panel, as clinically indicated
- TBNK

- Imaging studies as per baseline assessment. If clinically indicated, other scans or x-rays may be performed, e.g. CT, MRI, and/or PET.
- A 5-liter apheresis may be performed at the first follow-up visit. If the patient is unable to undergo apheresis, approximately 96 mL of blood may be obtained. Subsequently, approximately 60 mL of blood will be obtained at follow-up visits for at least 3 months. PBMC will be cryopreserved so that immunologic testing may be performed. This will be performed on protocol 03-C-0277.
- Detection of RCR and persistence of TCR gene transduced cells (see Section **5.8**). This will be performed on the NCI-SB long-term follow-up protocol 09-C-0161 (Follow up Protocol for Subjects Previously Enrolled in NCI Surgery Branch Studies).
- 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. This will be performed on protocol 09-C-0161.

3.7 STUDY ASSESSMENT CALENDAR

Assessments	Prior to Starting Preparative Regimen					Prior to Preparative Regimen	During Preparative Regimen *(Daily)*	Prior to Cell Infusion	Post-Cell Infusion	During Hospitalization *(Every 1-2 Days)*	Post-Treatment Follow-up[1]
	Within 3 Months	Within 8 Weeks	Within 4 Weeks	Within 14 Days	Within 7 Days						
Confirmation of diagnosis by NCI Lab of Pathology[2]	X										
Assessment of ESO expression[3]	X										
Medical history[4]			X								
Physical exam			X							X[5]	X[6]
Neurological exam[7]			X						X[8]		

1 All patients will return to the NIH Clinical Center for their first follow-up evaluation for response 6 weeks (± 2 weeks) following the administration of the cell product. Patients who experience stable disease, a partial response, or a complete response, or have unresolved toxicities after their first follow-up evaluation, will return to the NIH Clinical Center at week 12 (± 2 weeks), every 3 months (± 1 month) x 3, every 6 months (± 1 month) x 2 years, and then per PI discretion for subsequent years. Patients may be seen more frequently as clinically indicated. See Section **3.6**.

2 Confirmation of diagnosis of metastatic cancer including melanoma. Testing is permitted to be conducted at any time prior to starting the preparative regimen.

3 One of the following methods will be used to assess ESO expression: RT-PCR analysis of tumor biopsy, immunohistochemistry of resected tissue, or analysis of serum to evaluate for expression of ESO-1 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 ESO-1 antibody will be conducted in Dr. Rosenberg's laboratory using CLIA-approved methods. Testing is permitted to be conducted at any time prior to starting the preparative regimen.

4 Note organ system involvement and any allergies/sensitivities to antibiotics. Patient history may be obtained within 8 weeks prior to starting the preparative regimen.

5 As needed or clinically indicated.

6 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. This will be performed on protocol 09-C-0161.

7 For patients with meningioma.

8 Neuro checks for patients with meningioma will be done hourly (± 15 minutes) for four hours and then routinely (every 4-6 hours) unless otherwise clinically indicated. Neuro checks do not need to be continued after the first 4 hours if they are normal.

Assessments	Prior to Starting Preparative Regimen					Prior to Preparative Regimen	During Preparative Regimen *(Daily)*	Prior to Cell Infusion	Post-Cell Infusion	During Hospitalization *(Every 1-2 Days)*	Post-Treatment Follow-up[1]
	Within 3 Months	Within 8 Weeks	Within 4 Weeks	Within 14 Days	Within 7 Days						
Performance score (ECOG)[9]					X						X
Weight			X				X[5]			X	X
Vital signs			X						X[10]	X	X
β-HCG pregnancy test[11]					X						
Urinalysis[12]				X			X				X
Pulmonary function test[13]		X									
Cardiac evaluation[14]		X									
EKG				X							
Toxicity assessment[15]										X[5]	X
Serology											
HIV antibody, HBsAg, anti-HCV	X										
Anti-CMV, HSV and VZV serology, EBV panel[16]	X			X							
Laboratory Procedures											
CBC w/differential				X			X			X	X

9 ECOG of 0 or 1.
10 Vital signs will be monitored hourly (± 15 minutes) for four hours and then routinely (every 4-6 hours) unless otherwise clinically indicated.
11 Serum or urine; on all women of child-bearing potential.
12 With culture if indicated.
13 For patients with a prolonged history of cigarette smoking (≥ 20 pack-year smoking history, with cessation within the past two years), symptoms of respiratory dysfunction, or other clinical indications which may include thoracic surgeries.
14 Commensurate to patients' history and clinical presentation (e.g., stress thallium, echocardiogram, MUGA) for patients who are ≥ 65 years of age, or who 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 ≤ 45% will not be eligible. Patients < 65 years of age with cardiac risk factors (e.g., diabetes, hypertension, obesity) may undergo cardiac evaluation as noted above.
15 Including a review of systems.
16 Patients who are known to be positive do not need to be retested. May be performed within 3 months prior to starting the preparative regimen.

Assessments	Prior to Starting Preparative Regimen					Prior to Preparative Regimen	During Preparative Regimen *(Daily)*	Prior to Cell Infusion	Post-Cell Infusion	During Hospitalization *(Every 1-2 Days)*	Post-Treatment Follow-up[1]
	Within 3 Months	Within 8 Weeks	Within 4 Weeks	Within 14 Days	Within 7 Days						
Blood chemistries[17]				X			X			X	X
PT-INR/PTT				X			X[18]				X
TBNK				X						X[19]	X
Thyroid Panel				X							X[5]
Additional apheresis[20]				X[5]							X
Persistence and RCR[21]											X
Correlatives[22]											
CPT tubes (SB-CPF)						X			X		
SST tubes (Figg Lab)						X		X	X		
Imaging											
CT, MRI, PET, and/or photography			X[23]								X[24]
Chest x-ray				X							

17 **Screening:** Creatinine, ALT/GPT, AST/GOT, Total bilirubin. **All other times:** Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric acid, Creatinine kinase, Lactate dehydrogenase, Total protein.
18 Every 3 days.
19 Once total lymphocyte count is > 200/mm^3, TBNK for peripheral blood CD4 count will be drawn weekly (while the patient is hospitalized).
20 Apheresis may be performed prior to and 6 weeks (± 2 weeks) following the administration of the cell product. If the patient is unable to undergo apheresis, approximately 96 mL of blood may be obtained. Subsequently, approximately 60 mL of blood will be obtained at follow-up visits for at least 3 months. PBMC will be cryopreserved so that immunologic testing may be performed. This will be performed on protocol 03-C-0277.
21 Detection of RCR and persistence of TCR gene transduced cells (see Section **5.8**). This will be performed on protocol 09-C-0161.
22 Research samples, as described in Section **5**.
23 Baseline evaluation to determine disease status. CT scans are not required for patients with meningioma.
24 Imaging studies as per baseline assessment. If clinically indicated, other scans or x-rays may be performed.

3.8 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF-STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects compete an evaluation safety visit approximately 6 weeks (± 2 weeks) following administration of the cell product (at the first follow-up evaluation).

3.8.1 Criteria for Removal from Protocol Therapy

Patients will be taken off treatment for the following:

- Completion of first follow-up evaluation
- Progression of disease
- Patient requests to be withdrawn from protocol therapy
- Investigator discretion
- Positive pregnancy test

3.8.2 Off-Study Criteria

Patients will be taken off-study for the following:

- Completion of study follow-up period
- Progression of disease
- Patient begins a new therapy for their cancer
- Patient requests to be withdrawn from the study
- Significant noncompliance
- Investigator discretion
- Patient lost to follow-up
- Death

All patients will be co-enrolled on protocol 09-C-0161. Patients who are taken off-study for progressive disease or study closure on this treatment protocol may be followed on protocol 09-C-0161.

Once a subject is taken off study, no further data can be collected on this treatment protocol.

3.8.3 Off Protocol Therapy and Off-Study Procedure

Authorized staff must notify the NCI-CRO when a subject is taken off protocol therapy and when a subject is taken off-study. A Participant Status Update Form from the website (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to the NCI-CRO at ncicentralregistration-l@mail.nih.gov.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 INFECTION PROPHYLAXIS

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

4.1.1 Pneumocystis Jirovecii Pneumonia

All patients will receive the fixed combination of trimethoprim and sulfamethoxazole (TMP/SMX) as double strength (DS) tab (DS tabs = TMP 160 mg/tab, and SMX 800 mg/tab) PO daily three times a week on non-consecutive days, beginning Day 0 or within one week of anticipated lymphopenia.

Dapsone (in G6PD sufficient patient), atovaquone, or pentamidine may be substituted for TMP/SMX-DS in patients with sulfa allergies.

4.1.2 Herpes Simplex or Varicella Zoster Virus Prophylaxis

Patients with positive HSV or VZV serology will be given valacyclovir orally at a dose of 500 mg daily starting on the day of cell infusion, or acyclovir, 250 mg/m^2 IV every 12 hours 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, HSV, and VZV will continue for 6 months post-chemotherapy. If the CD4 count is < 200 at 6 months post-chemotherapy, prophylaxis will continue for at least 6 months and until the CD4 count is > 200 for two consecutive measures.

Note: A missed prophylactic dose will not be considered a protocol deviation, and thus a deviation will not be reported to the IRB (see sections **7.1.8** and **7.2**), if the patient is compliant with taking at least 75 percent of their required dose.

4.1.3 Fungal Prophylaxis (Fluconazole)

Patients will start fluconazole 400 mg PO starting on the day of cell infusion and continue until the absolute neutrophil count is > 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.

4.1.4 Empiric Antibiotics

Patients will start on broad-spectrum antibiotics in accordance with current institutional guidelines for fever of 38.3°C once or two temperatures ≥ 38.0°C at least one hour apart, AND an ANC < 500/mm^3. Infectious disease consultation will be obtained for all patients with unexplained fever or any infectious complications.

4.2 BLOOD PRODUCT SUPPORT

Using daily CBCs as a guide, the patient will receive platelets and packed red blood cells (PRBCs) as needed. As a general guideline, patients may be transfused for:

- Hemoglobin < 8 gm/dL
- Platelets < 10,000/mm^3

All blood products will be irradiated. Leukocyte filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused WBCs and decrease the risk of CMV infection.

4.3 OTHER CONCOMITANT MEDICATIONS TO CONTROL SIDE EFFECTS

Concomitant medications to control side effects of therapy may 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 every 4 hours), indomethacin (50-75 mg every 8 hours), and ranitidine (150 mg every 12 hours). 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 BIOSPECIMEN COLLECTION

Blood and tissue are tracked at the patient level and can be linked to all protocols on which the patient has been enrolled. Samples will be used to support the specific objectives listed in the treatment protocol(s), e.g., immunologic monitoring, cytokine levels, persistence, as well as to support long-term research efforts within the NCI-SB and with collaborators as specified in protocol 03-C-0277.

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.

5.1 SAMPLES SENT TO FIGG LAB

- Venous blood samples will be collected in either a 4-mL or an 8-mL SST tube to be processed for serum and stored for future research. Record the date and exact time of draw on the tube. Blood tubes may be kept in the refrigerator until pick-up.
- For sample pick-up, page 102-11964.
- For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).
- For questions regarding sample processing, contact Julie Barnes by email or at 240-760-6044.
- The samples will be processed, barcoded, and stored in the Figg Lab until requested by the investigator.

5.2 SAMPLES SENT TO THE SURGERY BRANCH CELL PRODUCTION FACILITY

- Venous blood samples will be collected in 8-mL CPT tubes to be processed and stored for future research. Record the date and exact time of draw on the tube. Blood tubes are kept at room temperature until pick-up.
- Samples will be picked up by the research nurse or designee and transported to the SB-CPF within 24 hours of blood draw.
- The samples will be processed, barcoded, and stored in SB-CPF.

5.3 PRIOR TO CHEMOTHERAPY ADMINISTRATION

- 5 CPT tubes (8 mL each): SB-CPF

- 1 SST tube (8 mL): Figg Lab
- 1 SST tube (4 mL): Daily, starting first day of chemotherapy through day of discharge. Send to Figg Lab.

5.4 PRIOR TO CELL INFUSION (BASELINE SAMPLE FOR CYTOKINE ANALYSIS)

- 1 SST tube (8 mL): Figg Lab

5.5 POST-CELL INFUSION EVALUATIONS

Once total lymphocyte count is > 200/mm^3, the following samples will be drawn and sent to the SB-CPF on Monday, Wednesday, and Friday x5, and then weekly (while the patient is hospitalized):

- 5 CPT tubes (8 mL each): SB-CPF
- 1 SST tube (8 mL): Figg Lab

5.6 SAMPLES COLLECTION SCHEDULE

Test	Volume Blood	Type of Tube	Collection Point	Disposition
Research blood	52 mL	CPT and SST	Prior to chemo	Deliver SST tubes to Figg Lab. Deliver CPT tubes to SB-CPF.
Research blood	8 mL	SST	Prior to cell infusion	Deliver to Figg Lab.
Research blood	48 mL	CPT and SST	Post-cell infusion and at follow-up visits[1]	Deliver SST tubes to Figg Lab. Deliver CPT tubes to SB-CPF

[1] Research blood will be obtained at follow-up visits for at least 3 months.

5.7 IMMUNOLOGICAL TESTING

- Apheresis may be performed prior to and 6 weeks (± 2 weeks) following the administration of the cell product. At other time points, patient PBL will be obtained from whole blood by purification using centrifugation on a Ficoll cushion. Aliquots of these PBMC will be cryopreserved for immunological monitoring of cell function and 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 using some or all the following tests. Direct immunological monitoring will consist of quantifying T-cells reactive with targets FACS analysis using mouse V-beta antibody. 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 transduced PBMC cryopreserved at the time of infusion. In general, differences of 2- to 3-fold in these assays are indicative of true biologic differences.

5.8 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. Note: Samples will be batched and assayed at the conclusion of the study.
- Patients will be co-enrolled on protocol 09-C-0161 and will adhere to the follow-up schedule described in that protocol. 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 NCI-SB research team.

5.9 SAMPLE STORAGE, TRACKING AND DISPOSITION FOR SURGERY BRANCH CELL PRODUCTION FACILITY

Blood and tissue collected during the course of this study will follow the Cell Tracking and Labeling System established by the SB-CPF. 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 NCI-SB laboratories at specified temperatures with alarm systems in place. Serum samples will be sent to the Blood Processing Core (BPC) for storage. Samples will be barcoded and stored on site or offsite at NCI Frederick Central Repository Services in Frederick, MD. All samples collected (blood or tissue) are entered into a central computer database with identification and storage location, and this database is backed up every night.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed and reported as such to the IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e. broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB.

Blood and tissue collected during the course of this study will be stored, tracked, and disposed of as specified in protocol 03-C-0277.

5.10 SAMPLE STORAGE, TRACKING AND DISPOSITION FOR FIGG LAB

5.10.1 Sample Data Collection

All samples sent to the BPC will be barcoded, with data entered and stored in the LABrador (aka LabSamples) utilized by the BPC, and data will be updated to the NCI-SB central computer database weekly. This is a secure program, with access to LABrador limited to defined Figg Lab personnel, who are issued individual user accounts. Installation of LABrador is limited to computers specified by Dr. Figg. These computers all have a password-restricted login screen. All Figg Lab personnel with access to patient information annually complete the NIH online Protection of Human Subjects course.

LABrador creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without LABrador access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.10.2 Sample Storage and Destruction

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in LABrador. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB-approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator (PI) to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed and reported as such to the IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e. broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the LABrador. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password-protected electronic system (C3D) and ensuring data accuracy, consistency and timeliness. The PI, associate investigators (AI), research nurses, and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant. Data will be entered into the NCI CCR C3D database.

All adverse events (AEs), including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Patients will be followed for AEs until their first follow-up evaluation (6 weeks (± 2 weeks) following administration of the cell product) or until off-study, whichever comes first.

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

All AEs must be recorded on the AE case report form unless otherwise noted below in Section **6.1.1**.

End of study procedures: Data will be stored according to HHS and FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

6.1.1 Exclusions to Routine Adverse Event Reporting

Patients will be receiving multiple agents, which include commercially available agents (fludarabine, cyclophosphamide, aldesleukin, and supportive medications) in combination with the investigational agents; therefore, grade 1 events not related to the cell product will not be reported/recorded.

6.2 DATA SHARING PLAN

6.2.1 Human Data Sharing Plan

De-identified human data generated for use in future and ongoing research will be shared through a NIH-funded or approved repository (ClinicalTrials.gov) and BTRIS. At the completion

data analysis, data will be submitted to ClinicalTrials.gov either before publication or at the time of publication or shortly thereafter. Data may also be used to support long-term research efforts within the NCI-SB, and de-identified data may also be shared with collaborators as specified in protocol 03-C-0277.

6.2.2 Genomic Data Sharing Plan

The NIH Genomic Data Sharing Policy does not apply to this study.

6.3 RESPONSE CRITERIA

For the purposes of this study, patients should be re-evaluated for response at 6 and 12 weeks (± 2 weeks), then every 3 months (± 1 month) x3, then every 6 months (± 1 month) x 2 years following administration of the cell product, then as per PI discretion. In addition to a baseline scan, confirmatory scans should also be obtained at least 4 weeks (but not less than 4 weeks) following initial documentation of objective response.

Response and progression will be evaluated in this study using the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.0).

6.3.1 Definitions

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

Evaluable for objective response: Only those patients who have measurable disease present at baseline, have received at least one course of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of course 1 will also be considered evaluable.)

Evaluable non-target disease response: Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one course of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

6.3.2 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as:

- By chest x-ray: ≥ 20 mm
- By CT scan:
  - Scan slice thickness 5 mm or under: ≥ 10 mm
  - Scan slice thickness > 5 mm: double the slice thickness
- With calipers on clinical exam: ≥ 10 mm

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be >15 mm in short axis when assessed by CT scan (CT scan slice thickness

recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease: All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

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

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

6.3.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

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

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

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans). Ideally, the same type of scanner should be used, and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

FDG-PET: While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions based on FDG-PET imaging can be identified according to the following algorithm:

- Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
- No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing based on the anatomic images, this is not PD.
- FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

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

6.3.4 Response Criteria

6.3.4.1 Evaluation of Target Lesions

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 based on 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.

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.

6.3.4.2 Evaluation of Non-Target Lesions

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."

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.

6.3.4.3 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

6.3.4.4 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.

6.3.4.5 Central Radiology Review

We will centrally (NCI) review all MRI scans that have been designated as a partial or complete response. Dr. John Butman of NIH Neuro-Radiology or his designee will assist in the review of all MRI scans.

6.3.4.6 Neurological Examination

Since it is not used for determining response, an objective assessment of the neurological exam will not be required data. Nevertheless, the neurological exam could be useful as a corollary piece of data to support or refute the validity of subtle MRI changes. Thus, we will request (but not require) that the following information will be recorded at each follow-up visit:

- Normal versus abnormal neurologic exam
- Status of neurologic exam compared to last exam:
  - Definitely better
  - Possibly better
  - Stable
  - Possibly worse
  - Definitely worse

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

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's Brochure or is not listed at the specificity or severity that has been observed; or, if an Investigator's 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's 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 Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event

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 disability/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.

7.1.6 Disability

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

7.1.7 Life-Threatening Adverse Drug Experience

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.

7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved research protocol.

7.1.9 Non-Compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

1. 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**
2. Is related or possibly related to participation in the research; **AND**
3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 IRB AND CLINICAL DIRECTOR (CD) REPORTING

7.2.1 IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report in the NIH Problem Form to the IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease
- All protocol deviations
- All unanticipated problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance.
3. A tabular summary of the following adverse events:
   - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research
   - All Grade 3 and 4 events that are possibly, probably or definitely related to the research
   - All Grade 5 events regardless of attribution
   - All serious events regardless of attribution.

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

7.2.3 IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the IRB.

7.2.4 Request for Waiver from IRB Reporting: Anticipated Protocol Deviations and Expected Non-UP Adverse Events

Patients may receive prophylaxis for pneumocystis, HSV, and VZV for at least 6 months post-chemotherapy (see Section **4.1.2**). The investigators are requesting a waiver from IRB reporting for a missed prophylactic dose. A missed prophylactic dose will not be considered a protocol deviation, and thus a deviation will not be reported to the IRB, if the patient is compliant with taking at least 75 percent of their required dose.

If the rate of these events exceeds the rate anticipated in the protocol (i.e., the patient takes less than 75 percent of their required dose), the events will be classified and reported as though they are unanticipated problems.

7.3 IND SPONSOR REPORTING CRITERIA

From the time the subject receives the investigational agent/intervention to the time of the first follow-up evaluation (6 weeks (± 2 weeks) following the administration of the cell product), the investigator must immediately report to the sponsor, using the mandatory MedWatch Form FDA 3500A or equivalent, any serious adverse event, whether or not considered drug-related, including those listed in the protocol or Investigator's Brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event. For serious adverse events that occur after the first follow-up evaluation, only those events that have an attribution of at least possibly related to the agent/intervention will be reported.

Required timing for reporting per the above guidelines:

- Death (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- Other serious adverse events as well as deaths due to progressive disease must be reported within one business day.

Events will be submitted to the Center for Cancer Research (CCR) at: CCRsafety@mail.nih.gov and to the CCR PI and study coordinator.

7.3.1 Waiver of Expedited Reporting to CCR

The investigators are requesting a waiver from reporting specific events in an expedited manner to the CCR. Patients will be receiving commercially available agents, such as fludarabine, cyclophosphamide, and aldesleukin. The majority of toxicities observed on NCI-SB adoptive cell therapy (ACT) protocols are expected toxicities of the non-myeloablative, lymphodepleting preparative regimen or IL-2 and occur in approximately 95% of the patients enrolled, therefore, we are requesting a waiver from reporting the following events in an expedited manner to the CCR:

- Grade 3 or greater myelosuppression, defined as lymphopenia, neutropenia, decreased hemoglobin, and thrombocytopenia.
- Grade 3 or greater nausea, vomiting, mucositis - oral, anorexia, diarrhea, fever, chills, fatigue, and rash maculo-papular.
- Grade 3 hypoxia, dyspnea, hematuria, hypotension, sinus tachycardia, urine output decreased, confusion, infections, and febrile neutropenia.

The PI will submit a summary table of all grade 3-5 events, whether or not considered related to the product, every 6 months. The report shall include the number of patients treated in the timeframe, the number of events per AE term per grade which occurred in the 6-month timeframe and in total since the start of the study, attribution, and type/category of serious.

Reports will be submitted to the Center for Cancer Research (CCR) at: CCRsafety@mail.nih.gov

7.3.2 Reporting Pregnancy

7.3.2.1 Maternal Exposure

If a patient becomes pregnant during treatment and for the first four months following treatment, the study treatment should be discontinued immediately and the pregnancy should be reported to the Sponsor. The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agents (s) should be documented in box B5 of the MedWatch form "Describe Event or Problem".

Pregnancy itself is not regarded as an SAE. However, as patients who become pregnant on study risk intrauterine exposure of the fetus to agents which may be teratogenic, the CCR is requesting that pregnancy should be reported in an expedited manner as grade 3 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy)" under the "Pregnancy, puerperium and perinatal conditions" SOC.

Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

If any pregnancy occurs during the course of the study, then the investigator should inform the Sponsor within one day, i.e., immediately, but **no later than 24 hours** of when he or she becomes aware of it.

The designated Sponsor representative will work with the investigator to ensure that all relevant information is provided to the Sponsor within 1 to 5 calendar days for SAEs and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

7.3.2.2 Paternal Exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 120 days after the last dose of aldesleukin.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 120 days after the last dose should, if possible, be followed up and documented.

7.4 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.4.1 Serious Adverse Event Reports to IBC

The PI (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of the cell product 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 cell product, but are not fatal or life-threatening, much be reported to the NIH IBC as soon as possible, but not later than 15 calendar days after the investigator's initial receipt of the information. Adverse events may be reported by using the MedWatch Form FDA 3500A or equivalent.

7.4.2 Annual Reports to IBC

Within 60 days after the one-year anniversary of the date on which the IBC approved the initial protocol, and after each subsequent anniversary until the trial is completed, the PI (or delegate) shall submit the information described below. Alternatively, the IRB continuing review report can be sent to the IBC in lieu of a separate report. Please include the IBC protocol number on the report.

7.4.2.1 Clinical Trial Information

A brief summary of the status of the trial in progress or completed during the previous year. The summary is required to include the following information:

- Title and purpose of the trial
- Clinical site
- Principal Investigator
- Clinical protocol identifiers
- Participant population (such as disease indication and general age group, e.g., adult or pediatric)
- Total number of participants planned for inclusion in the trial; the number entered into the trial to date whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons
- Status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed
- If the trial has been completed, a brief description of any study results.

7.4.2.2 Progress Report and Data Analysis

Information obtained during the previous year's clinical and non-clinical investigations, including:

- Narrative or tabular summary showing the most frequent and most serious adverse experiences by body system
- Summary of all serious adverse events submitted during the past year
- Summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications
- If any deaths have occurred, the number of participants who died during participation in the investigation and causes of death

- Brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

7.5 DATA AND SAFETY MONITORING PLAN

7.5.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis when patients are being actively treated on the trial to discuss each patient. Decisions about enrollment will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the PI or AI. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be reported to the IRB using iRIS.

The PI will review adverse event and response data on each patient to ensure safety and data accuracy. The PI will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.5.2 Sponsor Monitoring Plan

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary study endpoints; adherence to the protocol, regulations, and SOPs; and human subject's protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by a CCR contractor. 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.

7.5.3 Safety Monitoring Committee (SMC)

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the PI and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 STATISTICAL CONSIDERATIONS

The primary objective of this trial is to determine whether the combination of high-dose aldesleukin, lymphodepleting chemotherapy, and an infusion of anti-ESO mTCR-gene engineered lymphocytes is associated with a modest fraction of patients who express ESO that can experience a clinical response (PR + CR) to therapy.

The study will be conducted using a phase II optimal design[19]. The objective will be to determine if the combination of high-dose aldesleukin, lymphodepleting 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).

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%.

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.

A total of up to 43 patients may be required (41 plus up to 2 inevaluable patients). Provided that about 2-4 patients per month will be able to be enrolled onto this trial, approximately 2 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 21 patients.

9 COLLABORATIVE AGREEMENTS

We have established a Cooperative Research and Development Agreement (CRADAs #02716 and #03168) with Kite Pharma, Inc., and will be sharing data with them.

10 HUMAN SUBJECTS PROTECTIONS

10.1 RATIONALE FOR SUBJECT 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.

10.2 PARTICIPATION OF CHILDREN

The use of the non-myeloablative, lymphodepleting preparative 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 under the age of 15 to this risk without further evidence of benefit. We will only treat adolescents who are of adult stature (must be ≥ 50 kg) and thus can safely receive the protocol treatment regimen; we do not anticipate the need for consultation with the pediatric oncology service although this will always remain an option.

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 younger pediatric population to evaluate potential benefit in that patient population.

10.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (Section **10.5**), all subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have a pre-determined substitute decision maker, the procedures described in MAS Policy 87-4 and OHSRP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

10.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

The experimental treatment has a chance to provide clinical benefit though this is unknown. The NCI-SB has extensive experience with ACT following treatment with high-dose aldesleukin, however, this experimental treatment is only available at a very few centers throughout the country. Although we have seen responses to this treatment, we do not know if this change in our process will improve patient outcome. The risks associated with ACT are substantial, including a delay in treatment due to the need to harvest and grow the cells; a surgical procedure (possibly major) to obtain tumor for the cell product; the possibility that a cell product cannot be generated; infection and sepsis due to the non-myeloablative, lymphodepleting preparative regimen; intubation; renal toxicities due to aldesleukin; and death. The risks in this treatment are detailed in Section **11**.

10.5 Risk/Benefit Analysis

Because all patients in this protocol have metastatic cancer and limited life expectancies, the potential benefit is thought to outweigh the potential risks. The risk/benefit analysis for adults with the capacity to consent, as well as for adults who may become unable to provide consent, is greater than minimal risk but presenting the prospect of direct benefit to individual subjects based on the risks and potential benefits described in Section **10.4**. The risk/benefit analysis for pediatric patients falls under Category 2, 45 CFR 46.405, which is greater than minimal risk but presenting the prospect of direct benefit to the individual child subjects involved in the research.

10.6 Consent Process and Documentation

Patients are initially consented on protocols 99-C-0128 and 03-C-0277. If the patient has a tumor that is found to be NY-ESO-1 positive by immunohistochemistry, and if the lymphocytes can be generated for infusion and the patient meets the thorough screening for eligibility, the patient and their parent(s) or legal guardian(s) if applicable, 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. If the patient is under 18 years of age, the consent process includes both the patient and the parent(s)/guardian(s), and a signed informed consent document will be obtained prior to entry onto the study. The informed consent document is given to the patient, and the parent(s)/guardian(s) if applicable, who is requested to review it and to ask questions prior to agreeing to participate in receiving treatment on 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 PI, AI, or clinical fellow is responsible for completing the consent process and a copy of the consent is offered to the patient, and the parent(s)/guardian(s), if applicable.

The investigators are requesting a waiver from the IRB to allow only one parent to sign the informed consent to enter a child on the protocol. Because many patients must travel to the NIH from long distances at substantial expense, requiring both parents to be present for the consent process could be a financial hardship for many families. When guardianship status of the child is uncertain, documentation of custody status must be obtained. In situations where there is joint custody of a child, both parents must sign consent. If only one parent can be present at NIH, the other parent's consent can be obtained by telephone via the procedure described in Section **10.6.2**.

Where deemed appropriate by the clinician and the child's parent(s) or legal guardian(s), the child will also be included in all discussions about the trial and age-appropriate language will be used to describe the procedures and tests involved in this study, along with the risks, discomforts and benefits of participation. The parent(s)/guardian(s) will sign and date the informed consent to provide permission for the child to take part in the study. Written assent will be obtained from children as the study holds out the prospect of direct benefit that is important to the health and well-being of the child and is available only in the context of the research. Written assent will be obtained as appropriate for children ages 15-17, and the parent(s)/guardian(s) will sign on the designated line on the informed consent attesting to the fact that the child has given assent. The consent/assent process will be documented in the child's medical record, including the assessment of the child's ability to provide written assent, as applicable. All children will be

contacted after they have reached the age of 18 (see Section **10.6.1**) to determine whether they wish to continue on the trial and informed consent will be obtained from them at that time.

10.6.1 Consent for Minors when they Reach the Age of Majority

When a pediatric subject reaches age 18, continued participation (including ongoing interactions with the subject or continued analysis of identifiable data) will require consenting of the now adult with the standard protocol consent document to ensure legally effective informed consent has been obtained. We request waiver of informed consent for those individuals who become lost to follow-up during their participation in the research study.

Requirements for Waiver of Consent consistent with 45 CFR 46.116 (d):

1. The research involves no more than minimal risk to the subjects.
    a. Analysis of samples and data from this study involves no additional risks to subjects.
2. The waiver or alteration will not adversely affect the rights and welfare of the subjects.
    a. Retention of these samples or data does not affect the welfare of subjects.
3. The research could not practicably be carried out without the waiver or alteration.
    a. Considering the length of time between the minor's last contact with the research team and their age of majority, it will likely be very difficult to locate them again. A significant reduction in the number of samples analyzed is likely to impact the quality of the research.
4. Whenever appropriate, the subjects will be provided with additional pertinent information after participation.
    a. We request a waiver of consent for those subjects who have been lost to follow-up or who have been taken off-study before reaching the age of majority.

10.6.2 Telephone Consent

In situations where the second parent is unable to be physically present to give consent, the informed consent document will be sent to that parent or legally authorized representative. An explanation of the study will be provided over the telephone after the second parent or legally authorized representative has had the opportunity to read the consent form. The second parent or legally authorized representative will sign and date the informed consent. A witness to the second parent or legally authorized representative's signature will sign and date the consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone.

A fully executed copy will be returned via mail for the subject's records.

The informed consent process will be documented on a progress note by the consenting investigator and a copy of the informed consent document and note will be kept in the subject's research record.

10.6.3 Informed Consent of Non-English Speaking Subjects

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB-approved consent document, the PI and/or those authorized to obtain informed

consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, OHSRP SOP 12, 45 CFR 46.117 (b) (2), and 21 CFR 50.27 (b) (2). The summary that will be used is the English version of the extant IRB-approved consent document. Signed copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation (using either the long-translated form or the short form). Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24-48 hours if possible).

We request prospective IRB approval of the use of the short form process for non-English speaking subjects and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form.

11 PHARMACEUTICAL INFORMATION

Cyclophosphamide, fludarabine, and aldesleukin, the commercial drugs used in this study, will not alter labeling of the FDA-approved drugs. The investigation is not intended to support a new indication for use or any other significant changes to labeling or advertising in cyclophosphamide, fludarabine, or aldesleukin. The investigation does not involve a route of administration or dosage level in use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug products.

11.1 INVESTIGATIONAL REGIMEN

11.1.1 Anti-ESO TCR Transduced PBL

The procedure for expanding the human PBL and the CoA are similar to those approved by the Food and Drug Administration, and used at the NCI in ongoing protocols evaluating cell therapy in the NCI-SB. The CoA is included in **Appendix E**. The PBL will be transduced with retroviral supernatant containing the α-chain and β-chain genes of the anti-ESO mTCR.

11.1.1.1 Retroviral Vector Containing the anti-ESO TCR Gene

The retroviral vector supernatant (PG13-ESO-157m TCR (C1) 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). Transgenic mice expressing full-length human HLA-A*0201 gene were obtained from the Jackson Laboratory (Bar Harbor, ME) and immunized with human NY-ESO-1 peptide (SLLMWITQC). Murine T-cells reactive to this peptide were used as a source to isolate the TCR alpha and beta chain genes (TCR alpha chain TRAV6D and beta chain TRBV26). Based on the DNA sequence of the TCR alpha and beta chains, a DNA sequence was chemically synthesized to link the beta and alpha TCR genes using a peptide linker. This synthetic DNA was then inserted into the gamma-retroviral vector MSGV1 to produce the murine anti-NY-ESO-1 TCR vector, (MSGV1 ESO-157 muTCR B2aA). Plasmid DNA for this vector was used to make the PG13 virus producer cell clone. The alpha and beta chains are linked by a T2A peptide.

The physical titer will be determined by transduction of PBL with serial dilutions of the vector. TCR expression on the cell surface will be measured using FACS following staining with an anti-mouse constant region antibody. The titer will be measured as transducing units per milliliter. Portions of the supernatant will be stored at -80°C at NCI-SB, as well as at Fisher Bioservices in 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 *ex vivo* transduction of patient PBL. There will be no re-use of the same unit of supernatant 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.

Note: Penicillin, streptomycin, and gentamycin will not be used in the manufacture of products for patients with documented allergies to these drugs.

11.1.2 Interleukin-2 (Aldesleukin, Proleukin, Recombinant Human Interleukin 2)

How Supplied: Interleukin-2 (aldesleukin) will be provided by the NIH Clinical Pharmacy Department from commercial sources.

Formulation/Reconstitution: Aldesleukin, NSC #373364, is provided as single-use vials containing 22 million IU (~1.3 mg) IL-2 as a sterile, white to off-white lyophilized cake plus 50 mg mannitol and 0.18 mg sodium dodecyl sulfate, buffered with approximately 0.17 mg monobasic and 0.89 mg dibasic sodium phosphate to a pH of 7.5 (range 7.2 to 7.8). The vial is reconstituted with 1.2 mL of Sterile Water for Injection, USP, and the resultant concentration is 18 million IU/mL or 1.1 mg/mL. Diluent should be directed against the side of the vial to avoid excess foaming. Swirl contents gently until completely dissolved. Do not shake. Since vials contain no preservative, reconstituted solution should be used with 24 hours.

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

Dilution/Stability: Reconstituted 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 RIL-2. Dilutions of the reconstituted solution over a 1000-fold range (i.e., 1 mg/mL to 1 mcg/mL) are acceptable in either glass bottles or polyvinyl chloride bags. Aldesleukin is chemically stable for 48 hours at refrigerated and room temperatures, 2-30°C.

Administration: The dosage will be calculated based on total body weight. 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 B** and **Appendix C**. 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 B**. Additional grade 3 and 4 toxicities seen with aldesleukin are detailed in **Appendix C**.

11.1.3 Fludarabine

(Please refer to the FDA-approved package insert for complete product information)

Description: 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 15-30 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 **Appendix A**.

Toxicities: At doses of 25 mg/m^2/day for 5 days, the primary side effect is myelosuppression; however, thrombocytopenia is responsible for most cases of severe and life-threatening hematologic toxicity. Serious opportunistic infections have occurred in 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 ACT protocols in the NCI-SB have caused persistently low (below 200) CD4 counts, and one patient developed polyneuropathy manifested by vision blindness, and motor and sensory defects.

11.1.4 Cyclophosphamide

(Please refer to the 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 **Appendix A**.

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.

11.2 SUPPORT MEDICATIONS

11.2.1 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 (1-20 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 ≤ 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 **Appendix A**. Toxicities include nausea, vomiting and diarrhea.

11.2.2 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 µg/mL and 480 µg/1.6 mL vials. Filgrastim 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.

Filgrastim will be given as a daily subcutaneous injection. The side effects of filgrastim 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.

11.2.3 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 Day 0 or within one week of anticipated lymphopenia and continuing for at least 6 months and until the CD4 count is > 200 on two 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 8-14 days after beginning the standard dose. Neutropenia, a reduction in the number of neutrophils, can also occur.

Dapsone (in G6PD sufficient patient), atovaquone, or pentamidine will may be substituted for TMP/SMX-DS in patients with sulfa allergies.

11.2.3.1 Dapsone

Dapsone will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used for the prevention of Pneumocystis pneumonia. The dose is 100 mg by mouth daily, starting on Day 0 (± 7 days) and continuing at least 6 months and until the CD4+ count is > 200 on two consecutive lab studies. It is supplied as 25 mg and 100 mg tablets. Dapsone contains a sulfa group, although the cross reactivity in patients with sulfa allergies is quite low. Dapsone may be considered in patients with mild to moderate sulfa allergies. Dapsone should be avoided in patients with severe (i.e., a history of anaphylaxis or other equally serious reaction) reactions to sulfa drugs. Additionally, dapsone has been reported to cause hemolytic anemia is patients with G6PD deficiency. It is recommended that patients be tested for G6PD deficiency prior to the initiation of dapsone therapy. Dapsone is generally well tolerated, but may cause a number of hematologic adverse reactions, including increased reticulocyte counts, hemolysis, decreased hemoglobin, methemoglobinemia, agranulocytosis, anemia, and leukopenia. Other rare but serious adverse reactions include bullous exfoliative dermatitis, Stevens-Johnson syndrome, toxic epidermal necrolysis, pancreatitis, interstitial pneumonitis, and pulmonary eosinophilia. For more detailed information about adverse reactions, consult the package insert.

11.2.3.2 Atovaquone

Atovaquone will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used for the prevention of Pneumocystis pneumonia in patients who cannot tolerate or are allergic to sulfamethoxazole/trimethoprim, dapsone, or pentamidine. Atovaquone may be given as a single daily dose of 1500 mg orally or the dose may be split into 750 mg given orally twice daily. Atovaquone will be started on Day 0 (± 7 days) and will continue for at least 6 months and until the CD4+ count is > 200 on two consecutive lab studies. Atovaquone is supplied as an oral suspension containing 150 mg/mL. Common adverse reactions to atovaquone include: headache, rash, diarrhea, nausea, vomiting, abdominal pain, cough, and fever. Rare but serious adverse reactions include acute renal failure, hepatitis and hepatic failure, angioedema, pancreatitis, and Stevens-Johnson syndrome. For more detailed information about adverse reactions, consult the package insert.

11.2.3.3 Aerosolized Pentamidine

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.

11.2.4 Herpes and Varicella Zoster Virus Prophylaxis

11.2.4.1 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 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.

11.2.4.2 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 7 mg/mL or less and infused over one 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.

11.2.5 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 is prepared according to Clinical Center Pharmacy standard procedures. It should be administered at a maximum IV rate of 200 mg/hour.

11.2.6 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.

11.2.7 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.

12 REFERENCES

1. Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res. 2011;17:4550-7.

2. Dudley ME, Yang JC, Sherry R, Hughes MS, Royal R, Kammula U, et al. Adoptive cell therapy for patients with metastatic melanoma: Evaluation of intensive myeloablative chemoradiation preparative regimens. J Clin Oncol. 2008;26:5233-9.

3. Chen YT, Scanlan MJ, Sahin U, Tureci O, Gure AO, Tsang S, et al. A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. Proc Natl Acad Sci U S A. 1997;94(5):1914-8.

4. Scanlan MJ, Gure AO, Jungbluth AA, Old LJ, Chen YT. Cancer/testis antigens: an expanding family of targets for cancer immunotherapy. Immunol Rev. 2002;188:22-32.

5. Lethe B, Lucas S, Michaux L, De SC, Godelaine D, Serrano A, et al. LAGE-1, a new gene with tumor specificity. Int J Cancer. 1998;76(6):903-8.

6. Jager E, Chen YT, Drijfhout JW, Karbach J, Ringhoffer M, Jager D, et al. 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. 1998;187(2):265-70.

7. Aarnoudse CA, van den Doel PB, Heemskerk B, Schrier PI. Interleukin-2-induced, melanoma-specific T cells recognize CAMEL, an unexpected translation product of LAGE-1. Int J Cancer. 1999;82(3):442-8.

8. Wang RF, Johnston SL, Zeng G, Topalian SL, Schwartzentruber DJ, Rosenberg SA. A breast and melanoma-shared tumor antigen: T cell responses to antigenic peptides translated from different open reading frames. J Immunol. 1998;161(7):3598-606.

9. Chen Q, Jackson H, Parente P, Luke T, Rizkalla M, Tai TY, et al. 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. 2004;101(25):9363-8.

10. Jager E, Jager D, Karbach J, Chen YT, Ritter G, Nagata Y, et al. 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. 2000;191(4):625-30.

11. Zeng G, Touloukian CE, Wang X, Restifo NP, Rosenberg SA, Wang RF. Identification of CD4+ T cell epitopes from NY-ESO-1 presented by HLA-DR molecules. J Immunol. 2000;165(2):1153-9.

12. Robbins PF, Morgan RA, Feldman SA, Yang JC, Sherry RM, Dudley ME, et al. Tumor regression in patients with metastatic synovial sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. J Clin Oncol. 2011;29:917-24.

13. van Loenen MM, de Boer R, Amir AL, Hagedoorn RS, Volbeda GL, Willemze R, et al. Mixed T cell receptor dimers harbor potentially harmful neoreactivity. Proc Natl Acad Science,USA. 2010;107:10972-7.

14. Bendle GM, Linnemann C, Hooijkaas AI, Bies L, de Witte MA, Jorritsma A, et al. Lethal graft-versus-host disease in mouse models of T cell receptor gene therapy. Nat Med. 2010;16(5):565-70, 1p.

15. Cohen CJ, Zhao Y, Zheng Z, Rosenberg SA, Morgan RA. Enhanced antitumor activity of murine-human hybrid T-cell receptor (TCR) in human lymphocytes is associated with improved pairing and TCR/CD3 stability. Cancer Res. 2006;66:8878-86.

16. Wargo JA, Robbins PF, Li Y, Zhao Y, El-Gamil M, Caragacianu D, et al. Recognition of NY-ESO-1+ tumor cells by engineered lymphocytes is enhanced by improved vector design and epigenetic modulation of tumor antigen expression. Cancer Immunol Immunother. 2009;58(383):394.

17. Yang S, Cohen CJ, Peng PD, Zhao Y, Cassard L, Yu Z, et al. Development of optimal bicistronic lentiviral vectors facilitates high-level TCR gene expression and robust tumor cell recognition. Gene Ther. 2008;15:1411-23.

18. Davis JL, Theoret MR, Zheng Z, Lamers CH, Rosenberg SA, Morgan RA. Development of human anti-murine T-cell receptor antibodies in both responding and nonresponding patients enrolled in TCR gene therapy trials. Clin Cancer Res. 2011;16:5852-61.

19. Simon R. Optimal two-stage designs for phase II clinical trials. Control Clin Trials. 1989;10(1):1-10.

13 APPENDICES

13.1 APPENDIX A: MODIFICATION OF DOSE CALCULATIONS* IN PATIENTS WHOSE BMI IS GREATER THAN 35

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.

13.2 APPENDIX B: ADVERSE EVENTS OCCURRING IN ≥10% OF PATIENTS TREATED WITH ALDESLEUKIN (N=525)[1]

Body System	% Patients
Body as a Whole	
Chills	52
Fever	29
Malaise	27
Asthenia	23
Infection	13
Pain	12
Abdominal pain	11
Abdomen enlarged	10
Cardiovascular	
Hypotension	71
Tachycardia	23
Vasodilation	13
Supraventricular tachycardia	12
Cardiovascular disorder [a]	11
Arrhythmia	10
Digestive	
Diarrhea	67
Vomiting	50
Nausea	35
Stomatitis	22
Anorexia	20
Nausea and vomiting	19
Hemic and Lymphatic	
Thrombocytopenia	37
Anemia	29
Leukopenia	16

Body System	% Patients
Metabolic and Nutritional Disorders	
Bilirubinemia	40
Creatinine increase	33
Peripheral edema	28
SGOT increase	23
Weight gain	16
Edema	15
Acidosis	12
Hypomagnesemia	12
Hypocalcemia	11
Alkaline phosphatase increased	10
Nervous	
Confusion	34
Somnolence	22
Anxiety	12
Dizziness	11
Respiratory	
Dyspnea	43
Lung disorder [b]	24
Respiratory disorder [c]	11
Cough increase	11
Rhinitis	10
Skin and Appendages	
Rash	42
Pruritus	24
Exfoliative dermatitis	18
Urogenital	
Oliguria	63

Legend:

a. Cardiovascular disorder: fluctuations in blood pressure, asymptomatic ECG changes, CHF.
b. Lung disorder: physical findings associated with pulmonary congestion, rales, rhonchi.
c. Respiratory disorder: ARDS, CXR infiltrates, unspecified pulmonary changes.

[1]Source: Proleukin® Prescribing Information – June 2007

13.3 APPENDIX C: 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 q4h PO, 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 interspersed with activity	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	No

			supportive measures	
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.

13.4 APPENDIX D: 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
<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.

13.5 APPENDIX E: CERTIFICATE OF ANALYSIS – ANTI-NY-ESO-1 MTCR PBL

Date of preparation of final product:

Patient:

Tests performed on final product:

Test	*Method*	*Limits*	*Result*	*Tests performed by*	*Initials/ Date*
Cell viability[1]	trypan blue exclusion	>70%			
Total viable cell number[1]	visual microscopic count	>1 x10^8			
Tumor reactivity[2]	□□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: _____
Qualified Clinical or Laboratory Supervisor