Abbreviated Title: Anti-NY ESO-1 mTCR PBL

PROTOCOL TITLE

Pilot Study of Adoptive Cell Transfer for the Treatment 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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PRECIS

Background:

• The National Cancer Institute Surgery Branch (NCI-SB) has 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 (cancer/test antigen) double positive tumors, anti-ESO mTCR transduced T cells secreted significant amounts of IFN-γ with high specificity.

Objectives:

Primary objectives:

• To determine the safety, tolerability and feasibility of lymphodepleting conditioning followed by infusion of anti-NY ESO-1 murine TCR-gene engineered lymphocytes and aldesleukin for the treatment of metastatic cancer expressing the NY ESO-1 antigen in patients that are HLA-A2 positive.

Secondary objectives:

• Determine the in vivo survival of mTCR gene-engineered cells.

• As an exploratory endpoint, determine the objective response rate to this regimen using RECIST criteria as a pilot to inform on a follow-up Phase II trial if primary endpoints are met. This endpoint is exploratory and will not drive statistics.

Eligibility:

Patients who are HLA-A*0201 positive and 15 years of age or older, and must have:

• Metastatic cancer other than melanoma whose tumors express the ESO antigen;

• Patients must have previously received and have been a non-responder to or recurred after receiving standard care for metastatic disease;

• Patients may not have:

• 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 in order 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 lymphocyte depleting 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.

• A total of up to 10 patients may be enrolled (8, plus allowing for up to 2 non-evaluable patients).

• As this is a safety and feasibility study using genetically engineered lymphocytes, the trial will be monitored by the Data Safety and Monitoring Committee of the Albert Einstein Cancer Center.

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1. INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1. Primary Objective:

• To determine the safety and tolerability of the administration of anti-ESO (cancer/test antigen) mTCR (T cell receptor)-engineered peripheral blood lymphocytes plus high-dose aldesleukin following a nonmyeloablative lymphoid depleting preparative regimen in HLA-A2 positive patients with metastatic cancer expressing the ESO antigen.

1.1.2. Secondary Objective(s)

• Determine the in vivo survival of TCR gene-engineered cells.

• Determine the objective response rate by RECIST criteria.

1.2 BACKGROUND AND RATIONALE

1.2.1 Adoptive Cell Transfer experience at the Surgery Branch, NCI:

The 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]. The NCI-SB has 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 q daily x 2 and 25mg/m^2 fludarabine q 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.

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 Surgery Branch 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. Cohort 1 and 3 include patients with metastatic melanoma or renal cell cancer; cohort 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, up until the end of 2012, NCI-SB has 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.

This regimen (08-C-0121) resulted in objective cancer regression in 50% (9/18) of 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.

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 defined antigen specificity can be generated, resulting in the clinical responses seen in previous clinical trial at NCI-SB 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 at NCI-SB 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 regions15. 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, NCI-SB 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™ (Switching Mechanism At the 5′-end of RNA Transcript) RACE (Rapid Amplification of cDNA Ends) 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[17,18]. 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.

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).

Safety Considerations

The non-myeloablative chemotherapy and the administration of high dose aldesleukin have expected toxicities, which are discussed in Section 6.4 of this study. The non-myeloabalative chemotherapy used in this protocol has been administered to over 500 patients and all have reconstituted their hematopoietic systems.

In other protocols at NCI-SB, they have administered up to 1.5 X 10^{11} TIL with widely heterogeneous reactivity including CD4+, CD8+, and NK cells without difficulty. As discussed above, the expansion of tumor reactive cells is a desirable outcome following the infusion of antigen reactive T cells. NCI-SB does not believe the transfer of these gene-modified cells has a significant risk for malignant transformation in this patient population. While the risk of insertional mutagenesis is a known possibility using retroviral vectors, this has only been observed in the setting of infants treated for XSCID using retroviral vector-mediated gene transfer into CD34+ bone marrow cells. In the case of retroviral vector-mediated gene transfer into mature T cells, there has been no evidence of long-term toxicities associated with these procedures since the first NCI sponsored gene transfer study in 1989. Although continued follow-up of all gene therapy patients will be required, data suggest that the introduction of retroviral vectors transduced into mature T cells is a safe procedure. While NCI-SB 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, and 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 their 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[16]. 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 FOR ENROLLMENT AND COLLECTION / HARVEST

a. Cancer that expresses NY-ESO-1 as assessed by immunohistochemistry of resected tissue.

Note: As noted in the Screening Protocol (IRB Protocol #2016-6553), Immunohistochemistry verification of ESO expression will be carried out in the Montefiore Medical Center, CLIA-approved pathology laboratory. Specifically, Monoclonal anti-NY-ESO-1 antibody (mouse IgG1 subtype), clone E978 (Sigma Aldrich; St. Louis, MO, USA), will be validated on sections of formalin fixed, paraffin embedded normal testis, which will then serve as a control tissue. Clinical samples for testing will be obtained from recent, archival paraffin-embedded tissue of tumor resections. This tissue will be microtome sectioned at 5 microns thickness, mounted on glass slides, deparaffinized and rehydrated. Slides will be quenched for 10 minutes at room temperature in 3% aqueous hydrogen peroxide to block endogenous tissue peroxidase activity, given antigen retrieval by steaming for 30 minutes, incubated for 120 min with NY-ESO-1 primary antibody, diluted 1:100 with Invitrogen antibody diluent, incubated with secondary reagent Invision plus, Dako DAB+ chromogen for 4 minutes, counterstained with hematoxylin, dehydrated through graded ethanol solutions, and cover-slipped. These slides will be used to measure NY-ESO-1 expression by semi-quantitative scoring as the percentage of tumor cells staining positively, staining intensity (0, negative; 1+, weak to moderate; 2+, strong), and subcellular location (cytoplasmic and/or nuclear).

b. Patients must be HLA-A*0201 positive

c. Demonstration of metastatic disease and confirmation of diagnosis by the Laboratory of Pathology at the Montefiore Medical Center.

d. Measurable disease by RECIST criteria

e. Patients must have previously received systemic standard care (or effective salvage chemotherapy regimens) for metastatic disease, if known to be effective for that disease, and have been either non-responders (progressive disease) or have recurred.

f. 3 or fewer brain metastases.

Note: If lesions are symptomatic or greater than or equal to 1 cm each, these lesions must have been treated and stable for 3 months for the patient to be eligible.

g. Greater than or equal to 15 years of age and less than or equal to 66 years of age.

h. Willing to sign a health care proxy (for patients 18 years of age or older).

Because confusion is a possible side effect of aldesleukin administration, a healthcare proxy should be signed by the patient to identify a surrogate to make decisions if a patient becomes unable to make decisions.

i. Able to understand and sign the Informed Consent Document

j. Clinical performance status of ECOG 0 or 1

k. Life expectancy of greater than three months

l. Patients of both genders must be willing to practice birth control from the time of enrollment on this study and for up to four months after cells are no longer detected in the blood

m. Serology:

- Seronegative for HIV antibody. (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who are HIV seropositive can have decreased immune-competence and thus be less responsive to the experimental treatment and more susceptible to its toxicities.)
- 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.

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

o. Hematology

- Absolute neutrophil count greater than $1000/mm^3$ without the support of filgrastim
- WBC $\geq 3000/mm^3$
- Platelet count $\geq 100{,}000/mm^3$
- Hemoglobin > 8.0 g/dl

p. Chemistry:

- Serum ALT/AST $\leq$ to 2.5 times the upper limit of normal
- Serum creatinine $\leq$ to 1.6 mg/dl
- Total bilirubin $\leq$ to 1.5 mg/dl, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 3.0 mg/dl.

2.2 ELIGIBILITY FOR TREATMENT

2.2.1 INCLUSION CRITERIA

a. At least 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 3 weeks, as long as all toxicities have recovered to grade 1 or less or as specified in the eligibility criteria in Section 2.1.

b. Four to six weeks must have elapsed from the time of any antibody therapy that could affect an anti-cancer immune response, including anti-CTLA 4 therapy, at the time the patient receives the preparative regimen to allow antibody levels to decline.

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

c. Four weeks from prior radiation therapy

d. Clinical performance status of ECOG 0 or 1

e. Life expectancy of greater than three months

f. Hematology

- Absolute neutrophil count greater than $1000/mm^3$ without the support of filgrastim
- Platelet count $\geq 100,000/mm^3$
- Hemoglobin > 8.0 g/dl

g. Chemistry:

- Serum ALT/AST $\leq$ to 2.5 times the upper limit of normal
- Serum creatinine $\leq$ to 1.6 mg/dl
- Total bilirubin $\leq$ to 1.5 mg/dl, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 3.0 mg/dl.

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

i. Patients of both genders must be willing to practice birth control from the time of enrollment on this study and for up to four months after cells are no longer detected in the blood

2.2.2 EXCLUSION CRITERIA

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

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

c. Active systemic infections, coagulation disorders or other major medical illnesses of the cardiovascular, respiratory or immune system,

d. History of myocardial infarction, cardiac arrhythmias, obstructive or restrictive pulmonary disease.

e. History of coronary revascularization

f. 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).

g. Concurrent systemic steroid therapy.

h. History of severe immediate hypersensitivity reaction to any of the agents used in this study.

i. History of or active CNS disease (such as multiple sclerosis)

j. Documented LVEF of less than or equal to 45%.

k. Significant pulmonary dysfunction based on pulmonary function testing DLCO less than 60%, FEV1 less than 60% expected, FVC less than 60 %

l. Patients with Grade 2 and 3 penicillin allergy will be excluded from the protocol, which involves respiratory and/or cardiovascular systems and presents like an anaphylactic reaction; there is anaphylaxis when, in addition to mucocutaneous symptoms there are airway symptoms or hypotension or associated symptoms like hypotonia, syncope; the respiratory signs and symptoms may be laryngeal (tightness in the throat, dysphagia, dysphonia, hoarseness, stridor) or pulmonary (dyspnea, cough, wheezing/bronchospasm, hypoxemia).

2.3 SCREENING EVALUATION

2.3.1 Within 4 Weeks Prior To Starting The Chemotherapy Regimen:

a. Complete history and physical examination, including weight, ECOG, and vital signs noting in detail the exact size and location of any lesions that exist. (**Note**: patient history may be obtained within 8 weeks.)

b. Chest x-ray

c. EKG

d. Baseline CT of the chest, abdomen and pelvis, and brain MRI to evaluate the status of disease. Additional scans and x-rays may be performed if clinically indicated based on patients' signs and symptoms.

e. Pulmonary Function Testing

f. Cardiac evaluation (either stress thallium, echocardiogram, MUGA)

g. Infectious Disease markers (performed within 30 days of conditioning start)

Send Routine Infectious Screening Panel to NYBC

Perform HSV I/II IgG and EBV IgG in house

h. Verification that HLA typing is completed (testing is permitted to be conducted at any time prior to this point)

i. Tumor tissue would be tested, as per the screening protocol, which is a separate study, (IRB Protocol #2016-6553), for NY-ESO-1 expression.

2.4 REGISTRATION PROCEDURES

All patients will be registered through the Clinical Trials Office at Montefiore Medical Center (Telephone: 718-379-6861) Monday through Friday 9:00am – 5:00pm Eastern Standard Time. Eligibility Checklist with supporting documentation, On Study Form and the signed Patient Consent Form must be emailed to the clinical trials office at Montefiore Medical Center, cpdmu-registration@montefiore.org, at the time of registration and prior to patient treatment.

At the time of registration, all eligibility criteria must be checked. Patients must meet all of the eligibility requirements listed Section 2.0. Patients must not start protocol treatment prior to registration.

It is the treating physician's responsibility to review all data submitted to the Clinical Trials Office for accuracy and completeness and he/she must sign the off study form.

3. STUDY IMPLEMENTATION

3.1 STUDY DESIGN

The study will be conducted as a pilot study of 10 patients meeting eligibility criteria. Patients will receive up to 2.0 X 10^{11} anti-ESO mTCR engineered PBL. A minimum of approximately 1.0 X 10^{9} cells will be given. The cells administered vary depending on their growth characteristics. 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 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 non-myeloablative, lymphodepleting preparative regimen consisting of cyclophosphamide and fludarabine followed by an 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.

3.1.1 Cell Preparation:

Indiana University Vector Production Facility (IUVPF) will receive Anti-NY-ESO-1 Murine TCR-Gene master cell bank from the NCI-SB. A cell line will be cloned, tested, and selected to be a suitable producer and then a cell bank will be established and characterized for this cell line. The cells required for expansion will be manipulated in a dedicated tissue culture environment using dedicated materials. All open manipulations will be in Class II Type A2 Biological Safety Cabinets. Certification assays will be performed and results will meet below stated criteria (Table 3.1). Production of the supernatant will be conducted in compliance with GMP for Phase I/II submissions to the FDA. Upon completion of the certification assays, Dr. Ira Braunschweig will receive a final study report that will include results for the certification assays, and Certificate of Analysis. Quality Assurance review of all work will be performed, documented and approval will be included in the report.

Table 3.1

CONTAMINANTS	AEROBIC AND ANAEROBIC CULTURE FOR BACTERIA AND FUNGUS	NO GROWTH WITHIN 14 DAYS
	MYCOPLASMA CULTURE AND VERO INDICATOR CELLS	NEGATIVE FOR THE PRESENCE OF MYCOPLASMA
	IN-VITRO VIRAL ASSAY UTILIZING MRC-5, VERO AND 3T3 CELLS	NO CPE OR HEMADSORPTION
ENDOTOXIN	LIMULUS AMEBOCYTE LYSATE	LESS THAN 0.33 EU/mL
REPLICATION COMPETENT RETROVIRUS:	S+L- (PG-4) (293 INFECTION) 5% OF VECTOR	NO EVIDENCE OF RCR
GAL-V	S+L- (PG-4) (293 CO- CULTURE) 10^8 CELLS FROM PRODUCTION RUN	NO EVIDENCE OF RCR
VECTOR INSERT STABILITY	TRANSDUCTION OF HeLa CELLS AND ANALYSIS BY	BAND CONSISTENT WITH PREDICTED FRAGMENT SIZE
POTENCY	PER SPONSOR	PER SPONSOR
VECTOR FUNCTION	PER SPONSOR	PER SPONSOR

PBMC will be obtained by leukapheresis (approximately 1 X 10^{10} cells) at the Montefiore Cellular Therapy Unit and subsequently sent to New York Blood Center (NYBC) by courier. Whole PBMC will be cultured in the presence of anti-CD3+ (OKT3) and aldesleukin in order to stimulate T cell growth. Transduction is initiated by exposure of approximately 1 X 10^7 to 5 X 10^8 cells to a 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 biological activity defined as gamma-interferon secretion must be at least 200 pg/ml. Successfully transduced cells will then be sent back to Montefiore Cellular Therapy Unit by courier, where the cells will be administered at a dose range of 1 X 10^9 to 2 X 10^{11} lymphocytes and will be infused over 20-30 minutes.

3.1.2 Protocol Stopping Rules:

The study will be temporarily halted pending discussions with the FDA and Institutional Review Board (IRB) regarding safety and the need for protocol revisions if any of the following conditions are met:

• Two or more patients develop a grade 3 or greater toxicity at any point in the study not attributable to the chemotherapy preparative regimen or aldesleukin (or circumstances unrelated to the study).

• 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

3.2 TREATMENT PLAN

DAYS -21 to -8

Pre-conditioning labs sent and reviewed (see section 3.3.1 for details), including pregnancy testing for females of childbearing age.

Confirm product expansion with Processing Facility and shipment plan.

DAYS -7 and -6

Chemotherapy infusions should be planned for morning administration.

Hydration: Begin hydration with 0.9% Sodium Chloride Injection containing 10 meq/L of potassium chloride at 2.6 ml/kg/hr (starting 12 hours pre-cyclophosphamide and continuing until at least 24 hours after last cyclophosphamide infusion). At any time during the preparative regimen, if urine output <1.5 ml/kg/hr or if body weight >2 kg over pre-cyclophosphamide value, furosemide IV may be administered.

Cyclophosphamide 60 mg/kg/day IV in 500 ml D5W X 2 days over 1 hr. If patient is obese (BMI > 35) medication dosage will be calculated using practical weight as described in Table 4.

Begin Mesna infusion with each cyclophosphamide dose at 60 mg/kg/day intravenously diluted in a suitable diluent (see pharmaceutical section) over 24 hours , or per institutional standard.. If patient is obese (BMI > 35) medication dosage will be calculated using practical weight as described in Table 4.

DAYS -5 to -1

Fludarabine 25 mg/m^2/day IVPB will be given

daily over 30 minutes. If patient is obese (BMI > 35) medication dosage will be calculated using practical weight as described in Table 4.

DAY – 1

Pre-meds for IL2 , fluids, Tylenol,

DAY 0

Cell Infusion (one to four days after the last dose of fludarabine): Twenty four hours must elapse between cellular infusion and the completion of fludarabine.• Cells will be infused intravenously (IV) on the Patient Care Unit over 20 to 30 minutes via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping.

*Cell dose: refer to section 3.1

Aldesleukin

• Aldesleukin 600,000 IU/kg IV (based on total body weight) over 15 minutes approximately every eight hours (+/- 1 hour) beginning as early as 3 hours and within 24 hours after cell infusion and continuing for up to 5 days (maximum of 15 doses).

DAY 1-4:

Start filgrastim on Day 1.Administer subcutaneously at a dose of 5 mcg/kg/day (not to exceed 300 mcg/day). Filgrastim administration will continue daily until neutrophil count > 1.0 $x10^9$/L X 3 days or > 5.0 $x10^9$/L.

Table 3.2

Day	-7	-6	-5	-4	-3	-2	-1	0[1]	1	2	3	4
Therapy												
Cyclophosphamide (60 mg/kg)	X	X										
Fludarabine (25 mg/m²)			X	X	X	X	X					
Anti-ESO mTCR								X				
Aldesleukin								X	X	X	X	X
Filgrastim[3] (5 mcg/kg/day)									X	X	X	X

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

[2]Initiate within 24 hours after cell infusion

[3]Continue until neutrophils count > 1X10^9/L for 3 consecutive days or > 5x10^9/L.

Aldesleukin will be administered at a dose of 600,000 IU/kg (based on total body weight) as an intravenous bolus over a 15 minute period approximately every eight hours *(+/- 1hr)* beginning within 24 hours of cell infusion and continuing for up to 5 days (maximum 15 doses). Total number of doses will be determined by the clinical judgment of the investigating physician.

Doses will be skipped if patients reach Grade 3 or 4 toxicity due to aldesleukin except for the reversible Grade 3 toxicities common to aldesleukin such as diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes as detailed in Appendix 1. Toxicities will be managed as outlined in Appendix 2. If these toxicities can be easily reversed within 24 hours by supportive measures then additional doses may be given. If greater than 2 doses of aldesleukin are skipped, aldesleukin administration will be stopped. (Appendix 3 lists the toxicities seen in patients treated with aldesleukin at the NIH Clinical Center).

Study medication start times for drugs given once daily should be given within 2 hours of the scheduled time.

3.3 ON-STUDY EVALUATIONS

3.3.1Within 14 days prior to starting the chemotherapy regimen:

a. Baseline blood tests

- Chem 20 equivalent: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid)
- Thyroid panel : TSH, FT4, Total T4, Total T3, FT3
- CBC with differential and platelet count
- PT/PTT

b. Urinalysis; urine culture, if indicated

3.3.2 Within 7 days prior to starting the chemotherapy regimen:

a. β-HCG pregnancy test (serum or urine) on all women of child-bearing potential

b. ECOG performance status of 0 or 1

3.3.3 Prior to starting the preparative regimen:

If any results are beyond the criteria established for eligibility, the patient will not proceed until the abnormalities can be resolved.

3.3.4 Day of admission and during the preparative regimen::

• daily Complete Blood Count

• daily Chem 20 equivalent: Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid

• daily urinalysis : If any results from labs sent on day of admission are beyond the criteria established for eligibility, the patient will not proceed until the abnormalities are resolved within the parameters for eligibility.

3.3.5 Prior to cell infusion:

Blood samples for analysis for detection of Replication Competent Retrovirus (RCR) by PCR as described in Section 5.1.3.

3.3.6 After cell infusion:

• Vital signs (including neuro checks) will be monitored hourly(+/- 15 minutes) for four hours and then routinely (every 4-6 hours) unless otherwise clinically indicated

• Daily

- o A review of systems and physical exam as clinically indicated
- o CBC
- o Chem 20 equivalent: Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid

• Other tests will be performed as clinically indicated. Infectious evaluation per institutional guidelines.

3.4 POST STUDY EVALUATION (FOLLOW-UP)

3.4.1 All patients will return to the Montefiore-Einstein Center for Cancer Care for evaluation 6 weeks (+/- 2 weeks) following the administration of the cell product.

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

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

Note: Patients may be seen more frequently as clinically indicated

3.4.3 At each scheduled evaluation patients will undergo:

- Physical examination
- Toxicity assessment, including a review of systems.
- Chem 20 equivalent: Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid
- Complete blood count
- Thyroid panel as clinically indicated
- CT of the chest, abdomen and pelvis. This end of course evaluation will be used to determine tumor response. If clinically indicated, other scans or x-rays may be performed, e.g. brain MRI, bone scan.
- Visual symptoms will be evaluated and if changes have occurred from baseline, i.e. changes in visual acuity, an ophthalmologic consult will be performed.

3.4.4 Detection of RCR and persistence of TCR gene transduced cells: (see section 5.1.3)

3.4.5 Long-term follow up of patients receiving gene transfer:

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

3.4.6 Patients who are unable or unwilling to return for follow up:

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

3.5 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

3.5.1 Off treatment criteria

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

- Grade 3 autoimmunity that involves vital organs (heart, kidneys, brain, eye, liver, colon, adrenal gland, lungs).
- If a patient experiences grade 3 or 4 toxicity due to cell infusion (reaction to cellular product or infusion reaction) the patient will receive no further treatment.
- Completion of treatment

3.5.2 Off study criteria

Patients will be taken off study for the following:

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

Note: Patients must be followed until all adverse events have resolved to grade 2 or less with the exception of lymphopenia and alopecia. If an adverse even is not expected to resolve to grade 2 or less this will be noted in the patient medical record and the patient may be taken off study.

4. CONCOMITANT MEDICATIONS/MEASURES

Administration of diuretics, electrolyte replacement, and hydration and monitoring of electrolytes should all be performed as clinically indicated – the doses and times noted below are offered only as examples.)

4.1 INFECTION PROPHYLAXIS

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

4.1.1 Pneumocystis 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) P.O. daily three times a week on non-consecutive days, beginning between days -5 and -8. Pentamidine will be substituted for TMP/SMX-DS in patients with sulfa allergies. It will be administered aerosolized at 300 mg per nebulizer within one week of chemotherapy start date.

4.1.2 Herpes Virus Prophylaxis:

Patients with positive HSV serology will be given valacyclovir orally at a dose of 500 mg daily the day after chemotherapy ends, or acyclovir, 250 mg/m^2 IV every 12 hrs if the patient is not able to take medication by mouth. Reversible renal insufficiency has been reported with IV but not oral acyclovir. Neurologic toxicity

including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs has been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made or the drug will be discontinued. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling. Prophylaxis for Pneumocystis and Herpes will continue for 6 months post chemotherapy. If the CD4+ count is less than 200 at 6 months post chemotherapy, prophylaxis will continue until the CD4+ count is greater than 200 for 2 consecutive measures.

4.1.3 Fungal Prophylaxis:

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

4.1.4 Empiric Antibiotics:

Patients will start on broad-spectrum antibiotics for fever of 38.3°C once or two temperatures of 38.0°C or above at least one hour apart, AND an ANC <500/mm3. Aminoglycosides should be avoided unless clear evidence of sepsis. Infectious disease consultation will be obtained for all patients with unexplained fever or any infectious complications. Treatment of fever will be per institutional guidelines. Cefazoline dosing will be determined according to neutrophil parameters for treatment (Neutrophils 200/mm3).

4.2 BLOOD PRODUCT SUPPORT

Using daily CBC's as a guide, the patient will receive platelets and packed red blood cells (PRBC's) as needed. Transfusion support parameters will be per institutional standards. All blood products will be irradiated. Leukocyte filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused WBC's and decrease the risk of CMV infection.

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 q4h), indomethacin (50-75 mg q6h) and ranitidine (150 mg g12h). If patients require steroid therapy they will be taken off treatment. Patients who require transfusions will receive irradiated blood products. Ondansetron 0.15 mg/kg/dose IV every 8 hours will be administered for nausea and vomiting. Additional antiemetics will be administered as needed for nausea and vomiting uncontrolled by ondansetron.

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

5. BIOSPECIMEN COLLECTION

Correlative Studies for Research: 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 POST CELL INFUSION EVALUATIONS

5.1.1 Once total lymphocyte count is greater than 200/mm^3, the following samples will be drawn and sent to New York Blood Center lab :

o 5 CPT tubes (8 ml each) NYBC needs to be contacted by email the day before the visit and again the day of the patient's visit to confirm samples are being drawn. The tubes are to be labeled with the following information : NY-ESO-1, patient first and last name, subject id number, date of samples being collected. Once tubes placed in bioharzard bag, they need to be placed in the Stem Cell Lab manager custody until the courier sent by NYBC comes to the HCT lab to collect them. The personnel responsible for taking the samples to the HCT lab , the lab manager and the NYBC courier must complete the requisition form (see protocol appendix 7).

o 1 SST tube (8 ml)

o 1 SST tube (4 ml) : one sample pre-infusion of experimental treatment and one sample post-infusion of experimental treatment

5.1.2 Immunological Testing:

Lymphocytes will be tested directly and following in vitro culture using some or all of 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.

5.1.3 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.

• Due to nature of these studies, it is possible that expansion of specific T-cell clones will be observed as tumor reactive T-cells proliferate in response to tumor antigens. Therefore, care will be taken to track T-cell persistence both immunologically and molecularly. Blood samples (5-10 mL) for persistence of TCR transduced cells will be obtained at 6 weeks (at time of post evaluation scan) after cell infusion, then at 3, 6, 12 months, and then annually thereafter. If any patient shows a high level of persistence of TCR gene transduced cells at month 6 (by semi quantitative DNA-PCR using primers specific for vector sequences) the previously archived samples will be subjected to techniques that would allow the identification of clonality of persisting TCR gene transduced cells. Such techniques may include T cell cloning or LAM-PCR. If a predominant or monoclonal T cell clone derived from TCR gene transduced cells is identified during the follow-up, the integration site and sequence will be identified and subsequently analyzed against human genome database to determine whether the sequences are associated with any known human cancers. If a predominant integration site is observed, the T cell cloning or LAM-PCR test will be used at an interval of no more than three months after the first observation to see if the clone persists or is transient. In all instances where monoclonality is persistent and particularly in instances where there is expansion of the clone, regardless of whether or not the sequence is known to be associated with a known human cancer, the subject should be monitored closely for signs of malignancy, so that treatment, if available, may be initiated early.

6. DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

Complete research records or medical records must be maintained on each patient treated on the protocol for both scheduled and unscheduled evaluations. These records should include primary documentation (e.g. laboratory report slips, X-ray reports, scan reports, pathology reports, physician's notes, etc.) which confirm that:

- The patient will meet all eligibility criteria
- Signed informed consent will be obtained prior to treatment
- Treatment will be given according to protocol (dated notes about doses given, complications, and clinical outcomes).
- Toxicity will be assessed according to protocol.
- Response will be assessed according to protocol (X-ray, CT-scan, MRI, lab reports, date noted on clinical assessment, as appropriate).
- MMC Drug Accountability Records will be kept for each patient.

6.2 DATA REPORTING

All patients must have signed an informed consent form and an on-study (confirmation of eligibility) form filled out and signed by a participating Investigator before entering on the study.

Toxicity will be scored using CTCAE Version 4.0 for toxicity and adverse event reporting at each post treatment visit. All adverse events, whether observed by the investigator or reported by the patient, must be recorded in the Adverse Event log with details about the duration and intensity of each episode, the action taken with respect to the study treatment, and the patient's outcome. The investigator must evaluate each adverse event for its relationship to the study treatment and for its seriousness.

6.2.1 Routine Data Reporting:

Data will be captured in the Montefiore Medical Center (MMC) C3D web based reporting system. A minimum of 25% of the data will be source data verified. Grade 1 and 2 lab toxicities and medications used to treat adverse events will be maintained in the source documents but will not be captured in C3D. Only the following outside labs will be captured in C3D:

Hemoglobin, WBC, ANC, Platelets, ALT/AST, total bilirubin, Creatinine {other labs associated with a serious adverse event may be captured as appropriate}

6.3 RESPONSE CRITERIA

Clinical Response will be determined using the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1).

6.3.1 Evaluation of Target Lesions[1]:

• Complete Response (CR): Disappearance of all target lesions

• Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions taking as reference the baseline sum LD.

• Progression (PD): At least a 20% increase in the sum of LD of target lesions taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.

• Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD.

6.3.2 Evaluation of Non-target Lesions[2]:

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

• Non-Complete Response: Persistence of one or more non-target lesions

• Progression (PD): Appearance of one or more new lesions. Unequivocal progression of existing non-target lesions

[1] All measurable lesions up to a maximum of 10 lesions representative of all involved organs should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.
[2] All other lesions (or sites of disease) should be identified as **non-target lesions** and should also be recorded at baseline. Measurements are not required, and these lesions should be followed as "present" or "absent."

6.3.3 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.

Targer 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 Confirmatory Measurement/Duration of Response:

Confirmation

Response assessments 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.

6.4 TOXICITY CRITERIA

This study will utilize the CTCAE version 4.0 for toxicity and adverse event reporting. A copy of the CTCAE v4.0 can be downloaded from the CTEP home page (http://ctep.cancer.gov). All appropriate treatment areas should have access to a copy of the CTCAE 4.0.

Over 200 patients have been treated in the Surgery Branch, NCI with TCR-transduced PBL targeting different antigens. Of these patients, 40 have received TCR-transduced PBL targeting NY-ESO-1. Early toxicities related specifically to the infusion of the cells (those which are seen immediately following cell infusion and prior to aldesleukin administration) are generally mild and include fevers, chills, headache, and malaise. Toxicities which occur following administration of cells can include dyspnea and hypoxia. Toxicities which occur following administration of aldesleukin but are thought to be related to the cells include immune mediated events such as vitiligo, transient uveitis, hearing loss and vestibular dysfunction. To ensure safety using this treatment, the IRB will review safety data on all protocols annually at the time of continuing review. Data will be presented for both the recent 6 month period and for the entire length of time the protocol has been open. The toxicity data for review will include all toxicities captured on the protocol and will be presented in individual tables as follows:

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

Toxicities seen on protocols using this non-myeloablative regimen that occur during the follow up period are rare but have included EBV lymphoma following prolonged lymphopenia, herpes zoster infection, and sensory neuropathy likely related to fludarabine. Side effects of drugs used in this nonmyeloablative regimen include:

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

Toxicities: Hematologic toxicity occurring with cyclophosphamide usually includes leukopenia and thrombocytopenia. Anorexia, nausea and vomiting, rash and alopecia occur, especially after highdose 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.

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

syndrome has been rarely observed in fludarabine treatment of CLL. Treatment on previous adoptive cell therapy protocols in the Surgery Branch have caused persistently low (below 200) CD4+ counts, and one patient developed polyneuropathy manifested by vision blindness, and motor and sensory defects.

High-dose aldesleukin: a variety of side effects have been associated with high-dose aldesleukin administration. A listing of these side effects in 525 patients treated with high dose aldesleukin at the NCI is listed in Appendix 1.
Toxicities: Expected toxicities of aldesleukin are listed in the product label and in Appendix 1 and 2. Grade 3 toxicities common to aldesleukin include diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes as detailed in Appendix 1. Additional grade 3 and 4 toxicities seen with aldesleukin are detailed in Appendix 2.

Valacyclovir: Common side effects include headache, upset stomach, nausea, vomiting, diarrhea or constipation. Rare serious side effects include hemolytic uremic syndrome and thrombotic thrombocytopenic purpura.

Acyclovir: 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.

Fluconazole: 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.

Ondansetron*:* It can cause headache, dizziness, myalgias, drowsiness, malaise, and weakness. Less common side effects include chest pain, hypotension, pruritis, constipation and urinary retention.

Furosemide**:** 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.

7. SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

An adverse event can occur on any clinical trial; it is the responsibility of the Principal Investigator and his/her research team to identify, review and report all necessary adverse events to the institutional IRB, the sponsor and governmental agencies (i.e., NCI and/or FDA) as appropriate. Adverse events should be identified through standard, routine protocol review and clinical assessment of each subject participating in the clinical trial. This review should be timely in order to meet the requirements for adverse event reporting defined below.

Adverse Event (AE)

Any untoward or unfavorable 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 the research, whether or not considered related to the subject's participation in the research. Adverse Events encompass both physical and/or psychological harms.

In the context of multicenter clinical trials, Adverse Events can be characterized as either internal adverse events or external adverse events. From the perspective of one particular institution engaged in a multicenter clinical trial, internal adverse events are those adverse events experienced by subjects enrolled by the investigator(s) at that institution, whereas external adverse events are those adverse events experienced by subjects enrolled by investigators at other institutions engaged in the clinical trial. In the context of a single-center clinical trial, all Adverse Events would be considered internal adverse events. Adverse events will be reported to FDA as per 21 CFR 312.32.

Unanticipated Problem

Any event, deviation, or problem that meets ALL of the following criteria:

- unexpected; AND
- possibly, probably or definitely related to study participation; AND
- fatal, life-threatening, or serious OR suggests greater risk of harm to study participant(s)or others than was previously known or recognized.

Unexpected

An event can be categorized as unexpected if it occurs in one or more subjects participating in a research protocol; and the nature, severity, or frequency of which is not consistent with either:
The known or foreseeable risk of adverse events associated with the procedures involved in the research that are described in protocol-related documents such as: the IRB-approved research protocol; any applicable investigator brochure: the current IRB-approved informed consent document; or other relevant sources of

information, such as product labeling and package inserts; or the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject's predisposing risk factor profile for the adverse event.

Serious Adverse Event (SAE)

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

- death,
- a life-threatening adverse reaction,
- inpatient hospitalization or prolongation of existing hospitalization,
- persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious 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 above.

7.2 DETERMINATION OF REPORTING REQUIREMENTS

Reportable Events

Any event that meets the definition of an Unanticipated Problem must be reported to the IRB within 5 business days. For multicenter studies, this would include such events that occur at external sites.

Other events that must be reported to the IRB include:

- The death of a participant in a "greater-than-minimal-risk" protocol being conducted at a site under the jurisdiction of the Einstein IRB, even if "anticipated", if it occurs within 30 days of a study-related procedure or the administration of a study drug.
- A Protocol Deviation that may place the participant or others at greater medical, physiological, social risk or economic risk than was previously known or recognized.
- Any deviation from IRB or Institutional Policy or Procedure which has the potential to adversely impact one or more subject or the overall integrity of data collected.
- Any reporting the PI is required to report directly to the FDA (e.g. the PI is the sponsor- Investigator, a protocol involving the use of an HUD).
- Any incident, experience, or outcome that indicates that the participant or others were placed at greater medical, physiological, social risk or economic risk than was previously known or recognized.
- Any reporting that the IRB cites as a condition of approval of the protocol.

- Complaint from a participant or other individual when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Deviation from the IRB Informed Consent Policy.
- Systematic data collection errors.
- Breach of confidentiality.
- Any action taken to eliminate an apparent immediate hazard to a research subject.
- Incarceration of a research subject.
- Sponsor or regulatory audit that requires corrective action.
- Suspension or restriction of an Investigator's clinical professional license.
- Disqualification or suspension of the Investigator by the FDA, NIH or other agency.

A record of non-reportable events and deviations must be maintained by the PI in a log and for greater than minimal risk studies must be submitted to the IRB as part of the annual review of the protocol. Adverse events (AEs) will be recorded in the case report form for the duration of the trial, regardless of whether or not the event(s) are considered related to trial medication. All AEs considered related to trial medication will be followed until resolution even if this occurs post-trial.

Reporting requirements may include the following considerations:

1) whether the patient has received an investigational or commercial agent;

2) the characteristics of the adverse event including the grade (severity), the relationship to the study therapy (attribution), and the prior experience (expectedness) of the adverse event

3) the phase (1, 2, or 3) of the trial

4) whether or not hospitalization or prolongation of hospitalization was associated with the event.

An investigational agent is a protocol drug administered under an Investigational New Drug Application (IND). In some instances, the investigational agent may be available commercially, but is actually being tested for indications not included in the approved package label.

Steps to determine if an adverse event is to be reported in an expedited manner:

Step 1: Identify the type of event using the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE can be downloaded from the CTEP home page (http://ctep.cancer.gov). Additionally, if assistance is needed, the NCI has an Index to the CTCAE that provides help for classifying and locating terms. All appropriate treatment locations should have access to a copy of the CTCAE.

Step 2: Grade the event using the NCI CTCAE.

Step 3: Determine whether the adverse event is related to the protocol therapy (investigational or commercial). Attribution categories are as follows: Unrelated, Unlikely, Possible, Probable, and Definite.

Step 4: Determine the prior experience of the adverse event. Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is NOT listed in section 6.5. the current NCI Agent-Specific Adverse Event List for the investigational agent.
Step 5: Review the Additional instructions, requirements, and exceptions for this protocol specific requirements for expedited reporting of specific adverse events that require special monitoring.
Step 6: Determine if the protocol treatment given prior to the adverse event included investigational agent(s), a commercial agent(s), or a combination of investigational and commercial agents.

7.3 REPORTING METHODS

Any event that meets the definition of an Unanticipated Problem must be reported to the IRB within 5 business days. The Unanticipated Problem Report Form can be found on the Einstein IRB website: http://einstein.yu.edu/administration/institutional-review-board/forms.aspx. All Reportable Events must be submitted to the IRB within 5 business days of the identification of the event by the research staff. Only the Principal Investigator may sign off on reportable event submissions, although any member of the research team may initiate the report. A record of non-reportable events and deviations must be maintained by the PI in a log and for greater than minimal risk studies must be submitted to the IRB as part of the annual review of the protocol.

7.4 THE TRIAL MONITORING

This trial will be monitored by the Albert Einstein Cancer Center Data Safety Monitoring Committee (AECC DSMC). A copy of the monitoring plan is maintained at the CPDMU. The DSMC as part of its function performs quarterly reviews of Clinical Trials Compliance Audits, monthly reviews Adverse Events Reports, and monthly reviews of internally monitored Phase I/Phase II trials for accrual and response. Other monitoring activities are established as necessary in a protocol specific manner.
This trial will be part of the monthly Quality Assurance Audits. Each patient will be evaluated within 8 weeks of registration. This permits evaluation of consent, eligibility, and treatment/dose modification/Adverse Event (AE) reporting, and data quality for the first cycle (or month) of treatment.
This trial may also be eligible for quarterly Quality Enhancement Audit. Each audit consists of a review of regulatory documents, pharmacy drug accountability (if applicable) and patient case review, confirming eligibility, protocol compliance and source documentation
The results of the audit will be presented at the following month's DSMC meeting. The DSMC has the authority to close trial to patient accrual should the risk to patients be excessive or results require a corrective plan from the Principal Investigator. All study suspensions and closures will be forwarded to the IRB and study sponsor. All audit reports are forwarded to the DSMC and presented to the DSMC by the Audit Committee Coordinator.

8.0 REGULATORY CONSIDERATIONS

8.1 PROTECTION OF HUMAN SUBJECTS

The Investigator must ensure that patients or their legally acceptable representatives are clearly and fully informed about the purpose, potential risks and other critical issues regarding clinical trials in which they volunteer to participate. Preparation of the consent form is the responsibility of the Investigator and must include all elements required by CFR 21 Part 50.25 and the local IRB.

8.2 COMPLIANCE WITH THE PROTOCOL AND PROTOCOL REVISIONS

The study must be conducted as described in this approved protocol. All revisions to the protocol must be provided to Dr. Braunschweig and Montefiore Medical center. The Investigator should not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the IRB/IEC of an Amendment, except where necessary to eliminate an immediate hazard(s) to study patients.

If the revision is an Administrative Letter, Investigators must inform their IRB(s)/IEC(s).

The Investigator must ensure that patients or their legally acceptable representatives are clearly and fully informed about the purpose, potential risks and other critical issues regarding clinical trials in which they volunteer to participate. Preparation of the consent form is the responsibility of the Investigator and must include all elements required by CFR 21 Part 50.25 and the local IRB.

9.0 DATA MONITORING / QUALITY ASSURANCE/ RECORD RETENTION

Investigator responsibilities are set out in the ICH guideline for Good Clinical Practice (GCP) and in the US Code of Federal Regulations.

The Montefiore Medical Center, as coordinator of this study, is responsible for ensuring proper conduct of the study with regard to protocol adherence and the validity of the data recorded on the case report forms. The Principal Investigator will monitor this study. The case report forms will be monitored every 3 months against submitted support documents for accuracy, completeness, adherence to the protocol and regulatory compliance.

U.S. FDA regulations (21CFR312.62[c] require all records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs, consents forms, laboratory test results and medication inventory records, must be retained by the Principal Investigator for 2 years after marketing application approval. If no application is filed, these records must be kept 2 years after the investigation is discontinued and the FDA and the applicable local health authorities are notified.

10.0 DATA SAFETY AND MONITORING BOARDS

This trial will be monitored by the Albert Einstein Cancer Center Data Safety Monitoring Committee (AECC DSMC). A copy of the monitoring plan is maintained at the CPDMU**.** The DSMC as part of its function performs quarterly reviews of Clinical Trials Compliance Audits, monthly reviews Adverse Events Reports, and monthly reviews of internally monitored Phase I/Phase II trials for accrual and response. Other monitoring activities are established as necessary in a protocol specific manner**.**

The details of the monitoring are outlined in section 7.4.

11. STATISTICAL CONSIDERATIONS

11.1 SAMPLE SIZE

This is a pilot study whose primary objective is to determine the safety, feasibility and tolerability of the combination of, lymphocyte-depleting chemotherapy, and an infusion of anti-ESO mTCR-gene engineered lymphocytes and high dose aldesleukin. Ten patients meeting eligibility criteria will be enrolled on the trial and will be examined to assess the primary endpoint.

11.2 STATISTICAL DATA ANALYSIS

For the primary endpoint of the study, we will first enroll 5 patients and assess safety and toxicity. Interim analysis and stopping rules are listed below. Secondary endpoints will be purely exploratory and will make use of descriptive statistics. The response rate calculation and persistence of gene altered T-cells will be used to inform the design of a future phase II trial. 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.

11.3 INTERIM ANALYSIS DECISION RULES

Patient toxicity will be assessed throughout the trial. After 5 patients have been accrued we will hold accrual for 30 days past the completion of treatment for the 5th patient. This will allow us to assess safety before enrolling additional patients. If there is a death or an irreversible grade IV toxicity among the first 5 patients, attributable to the therapy, we will halt accrual.

12. HUMAN SUBJECTS PROTECTIONS

12.1. 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.

12.2. EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

The experimental treatment has a chance to provide clinical benefit though it is not known if it will do so. The success of this effort cannot be predicted at this time. Because all patients in this protocol have incurable metastatic cancers the potential benefit is thought to outweigh the risks. The risks are outlined in section 6.4.

12.3. CONSENT PROCESS AND DOCUMENTATION

If the patient has a tumor that is found to be NY-ESO-1 positive by immunohistochemistry, the patient or legal guardian, will be presented with a detailed description of the protocol treatment informed consent. The informed consent will be obtained according to institutional practice. For subjects under the age of 18 years old (minors) assent will be obtained on the main Informed Consent form. The consent form will be kept in the patient's permanent medical record and on file with the principal investigator.

In order to maintain patient confidentiality, all case report forms, study reports and communications relating to the study will identify subjects by initials and assigned subject numbers; subjects should not be identified by name. In accordance with local, national or federal regulations, the investigator will allow the personnel of data monitoring committee access to all pertinent medical records in order to verify the data gathered on the case report forms. Regulatory agencies such as the US Food and Drug Administration (FDA) may also request access to all study records, including source documentation for inspection.

13. PHARMACEUTICAL INFORMATION

13.1 INTERLEUKIN-2 (ALDESLEUKIN, PROLEUKIN, RECOMBINANT HUMAN INTERLEUKIN 2)

How Supplied: Interleukin-2 (aldesleukin) will be provided by the Montefiore 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 (20 - 80C) 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, 20 – 300C.

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.

13.2 FLUDARABINE

(Please refer to 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 Montefiore Medical CenterClinical Pharmacy Department from commercial sources. Fludarabine is supplied in a 50 mg vial as a fludarabine phosphate powder in the form of a white, lyophilized solid cake.

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

Storage: Intact vials should be stored refrigerated (2-8°C).

Administration: Fludarabine is administered as an IV infusion in 100 ml 0.9% sodium chloride, USP over 15 to 30 minutes. The doses will be based on body surface area (BSA). If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Table 4.

13.3 CYCLOPHOSPHAMIDE

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

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

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

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

Administration: It will be diluted in 500 ml D5W and infused 30 minutes to 1 hour before the Cytoxan. The dose will be based on the patient's body weight. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Table 4.

13.4 ANTI-ESO TCR TRANSDUCED PBL

The procedure for expanding the human PBL and the Certificate of Analysis (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 4. The PBL will be transduced with retroviral supernatant containing the alpha chain and beta chain genes of the anti-ESO mTCR.

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.

The NY-ESO-1 TCR is detected by a flow cytometric assay using a customized iTAg™ MHC Tetramer: iTAg Tetramer/PE - HLA-A*02:01 NY-ESO-1 (SLLMWITQC). The titer was measured as transducing units per milliliter.

Supernatent will be stored at the Indiana University Vector Production Facility. 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.

13.5 MESNA

(Sodium 2-mercaptoethanesulfonate, Mesnum, Mesnex, NSC-113891):

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

Description: Mesna will be obtained commercially by the Clinical Center Pharmacy Department and is supplied as a 100 mg/ml solution.

Storage: Intact ampoules are stored at room temperature.

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

Administration: Dilute to concentrations less than or equal to 20 mg mesna/ml fluid in D5W or 0.9% NaCl and to be administered intravenously as a continuous infusion. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Table 4. Toxicities include nausea, vomiting and diarrhea.

13.6 FILGRASTIM

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

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

13.7 TRIMETHOPRIM AND SULFAMETHOXAZOLE DOUBLE STRENGTH (TMP / SMX DS)

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

13.8 AEROSOLIZED PENTAMIDINE IN PLACE OF TMP/SMX DS

Patients with sulfa allergies will receive aerosolized Pentamidine 300 mg per nebulizer with one week prior to admission and continued monthly until the CD4+ count is above 200 on two consecutive follow up lab studies and for at least 6 months post chemotherapy. Pentamidine Isethionate will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prevent the occurrence of PCP

infections. It is supplied in 300 mg vials of lyophilized powder and will be administered via nebulizer. Toxicities reported with the use of Pentamidine include metallic taste, coughing, bronchospasm in heavy smokers and asthmatics; increased incidence of spontaneous pneumothorax in patients with previous PCP infection or pneumatoceles, or hypoglycemia.

13.9 HERPES VIRUS PROPHYLAXIS

13.9.1 Valacyclovir (Valtrex):

Valacyclovir will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used orally to prevent the occurrence of herpes virus infections in patients with positive HSV serology. It is supplied in 500 mg tablets. Valcyclovir will be started the day after the last dose of fludarabine at a dose of 500 mg orally daily if the patient is able to tolerate oral intake. See package insert for dosing adjustments in patients with renal impairment. 13.9.2 Acyclovir:

Acyclovir will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prevent the occurrence of herpes virus infections in patients who cannot take oral medications. It is supplied as powder for injection in 500 mg/vials. Reconstitute in 10 mL of sterile water for injection to a concentration of 50 mg/mL. Reconstituted solutions should be used within 12 hours. IV solutions should be diluted to a concentration of 7mg/mL or less and infused over 1 hour to avoid renal damage.

13.10 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. For IV administration in patients who cannot tolerate the oral preparation, Fluconazole comes in 2 MG/ML solution for injection, and prepared according to Clinical Center Pharmacy standard procedures. It should be administered at a maximum IV rate of 200 mg/hr.

13.11 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. Consult the package insert for specific dosing instructions.

13.12 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.

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FIGURES, TABLES & APPENDICES **Table 1**. Response rates and duration in patients with melanoma treated with tumor infiltrating lymphocytes plus high-dose aldesleukin following three different lymphoconditioning regimens.

Cell Transfer Therapy (11/1/12)

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)

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

	Total	PR	CR	OR
		Number of patients (duration in months)		
Melanoma	21	5 (29%) 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%)

+continuing response

*meets criteria for response at first follow up, but not official response yet

**plus ALVAC vaccine

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		

Table 4:

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.

Figure 1

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

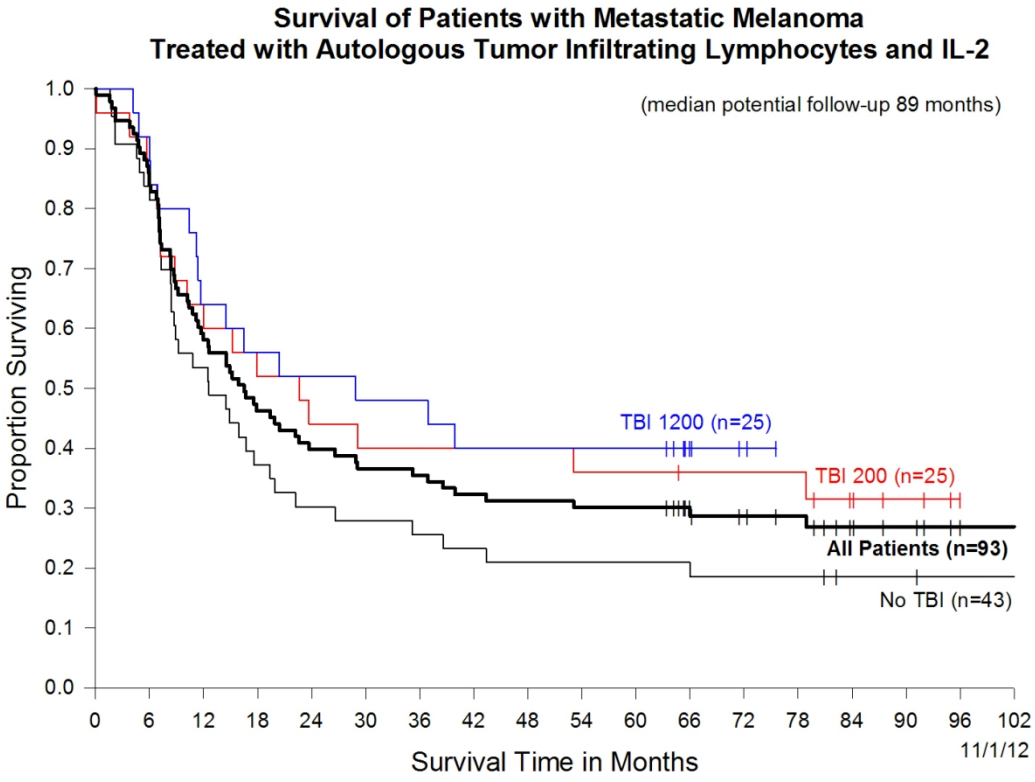


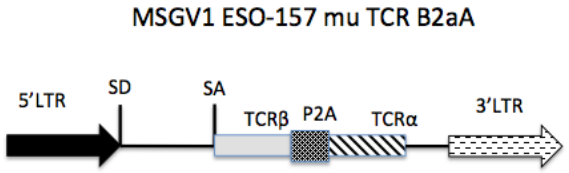
Figure 2.

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.

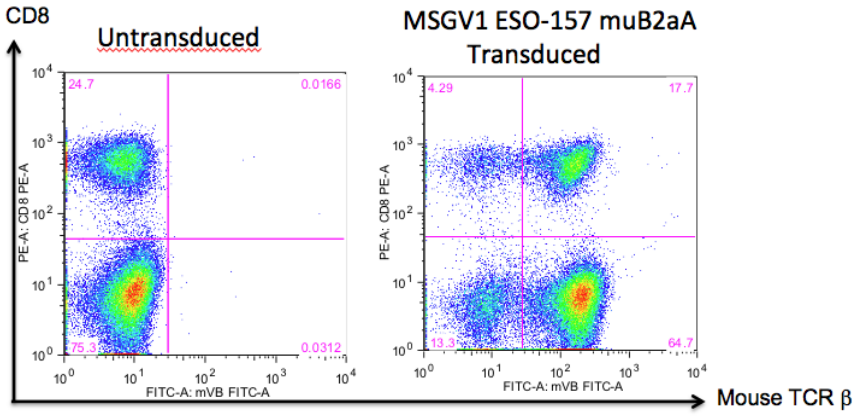
B. Flow cytometric analysis of ESO mTCR transduced PBL, representative of eleven different donors.

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.

A.



B.



C.

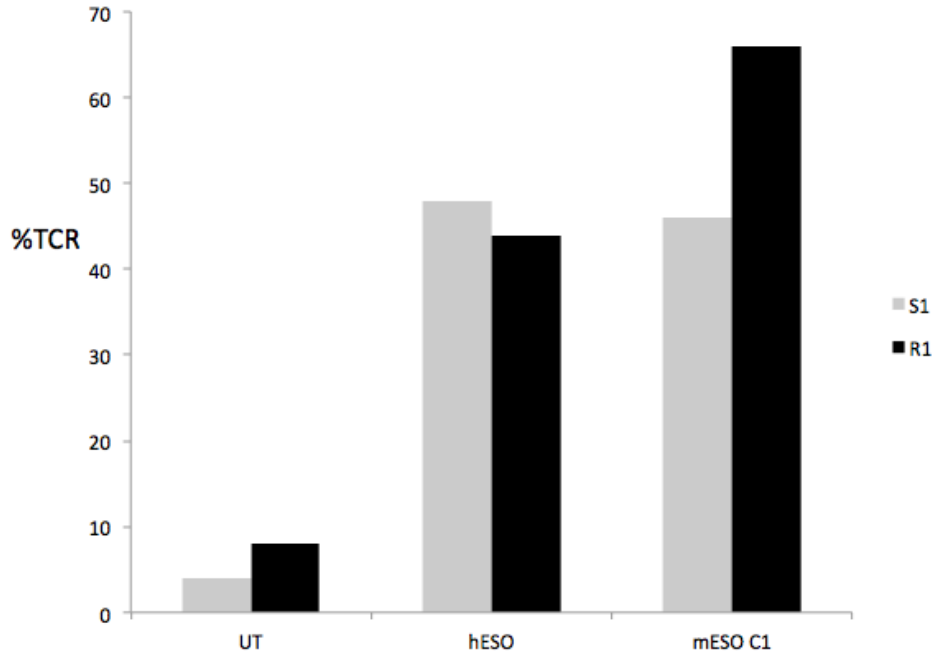


Figure 3
Recognition of peptide pulsed T2 cells by the ESO TCR and ESO mTCR transduced PBL. Human PBL expressing TCR and mTCR against NY-ESO-1, GFP (negative control) and untransduced PBL (negative control) were co-cultured for 16h with T2 cells that were previously pulsed with different concentrations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

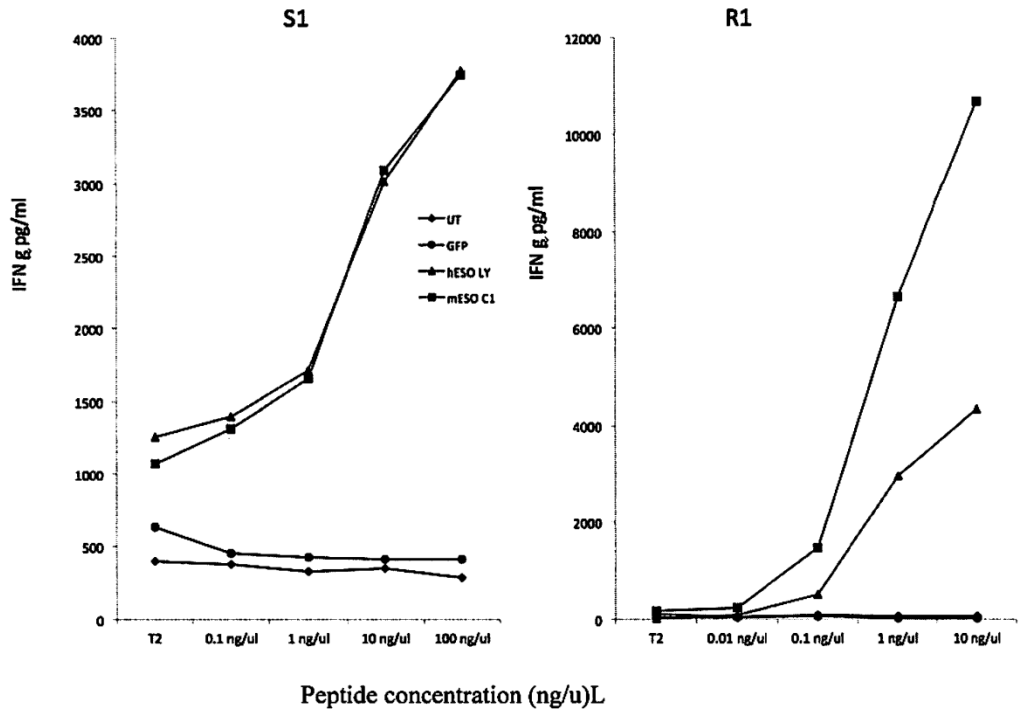


Figure 4

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.

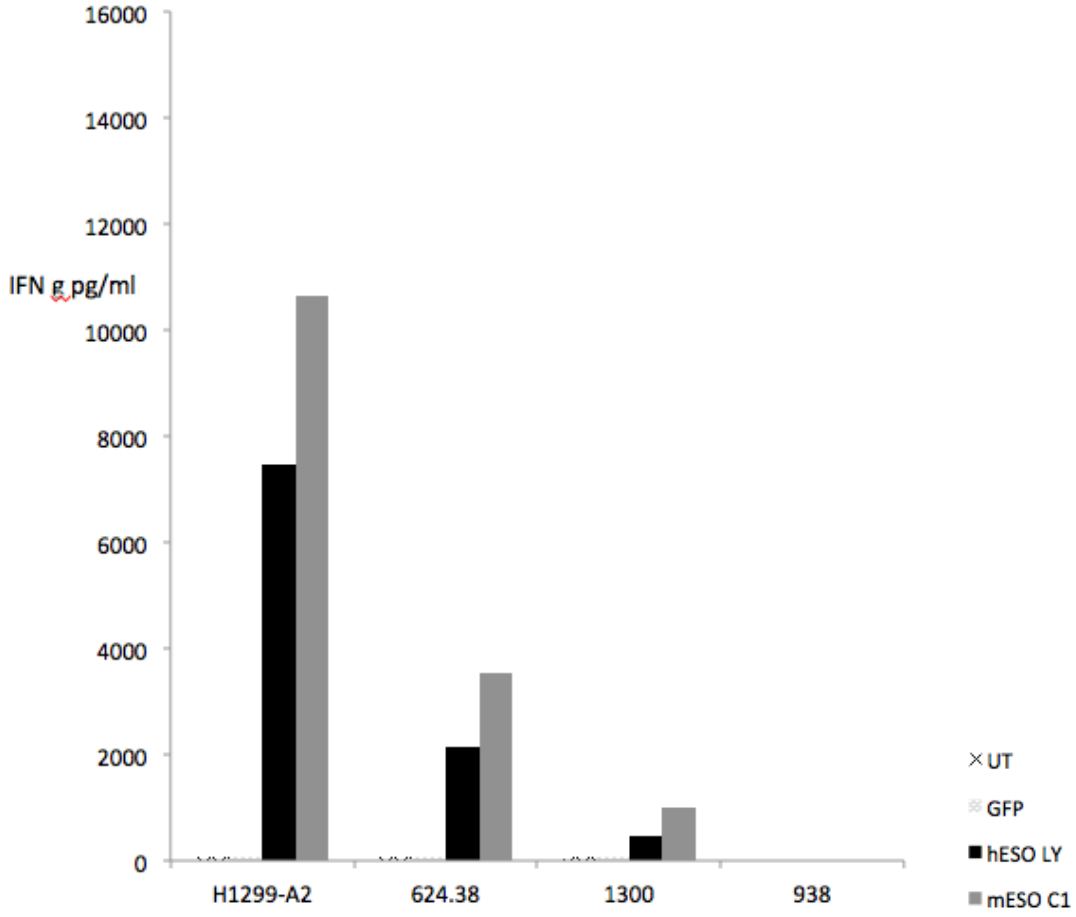
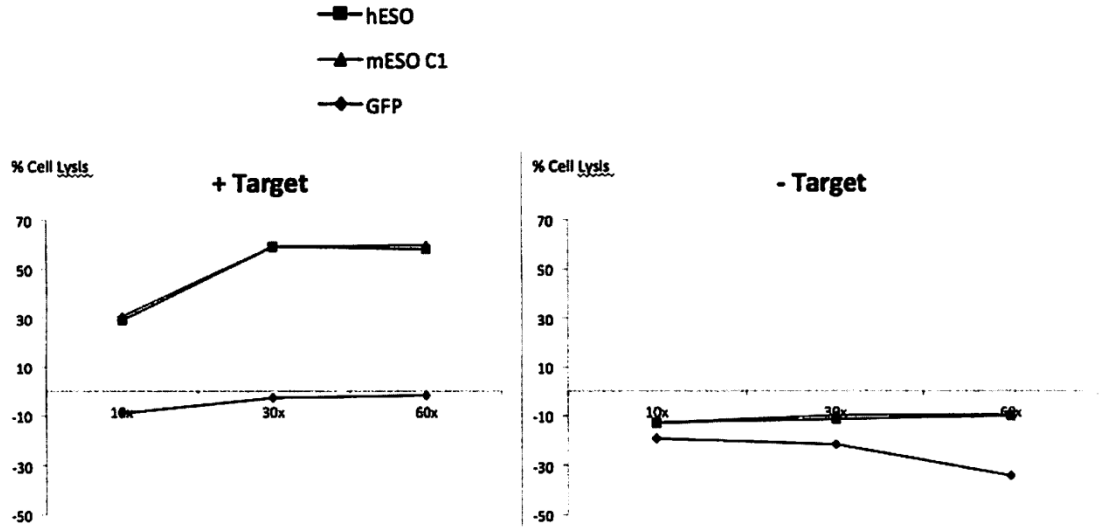


Figure 5
Cell lysis assay comparing cell specific lysis activity of the ESO TCR transduced PBL with ESO mTCR transduced PBL. Effector cells were co-cultured with an ESO+, A2+ tumor target (624.38, + control) and an ESO+, A2- tumor target (938, - control), representative of four different donors.



Appendix 1:

Adverse Events Occurring In ≥10% Of Patients Treated With Aldesleukin (N=525)[1]

Body System	% Patients	Body System	% Patients
Body as a Whole		*Metabolic and Nutritional Disorders*	
Chills	52	Bilirubinemia	40
Fever	29	Creatinine increase	33
Malaise	27	Peripheral edema	28
Asthenia	23	SGOT increase	23
Infection	13	Weight gain	16
Pain	12	Edema	15
Abdominal pain	11	Acidosis	12
Abdomen enlarged	10	Hypomagnesemia	12
Cardiovascular		Hypocalcemia	11
Hypotension	71	Alkaline phosphatase incr	10
Tachycardia	23	*Nervous*	
Vasodilation	13	Confusion	34
Supraventricular tachycardia	12	Somnolence	22
Cardiovascular disorder[a]	11	Anxiety	12
Arrhythmia	10	Dizziness	11
Digestive		*Respiratory*	
Diarrhea	67	Dyspnea	43
Vomiting	50	Lung disorder[b]	24
Nausea	35	Respiratory disorder[c]	11
Stomatitis	22	Cough increase	11
Anorexia	20	Rhinitis	10
Nausea and vomiting	19	*Skin and Appendages*	
Hemic and Lymphatic		Rash	42
Thrombocytopenia	37	Pruritus	24
Anemia	29	Exfoliative dermatitis	18
Leukopenia	16	*Urogenital*	
Oliguria	63		

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

Appendix 2

Expected IL-2 Toxicities and their Management

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

			occur simultaneously	
Hyperbilirubinemia	3 or 4	Observation	If other toxicities occur simultaneously	No
Anemia	3 or 4	Transfusion with PRBCs	If uncontrolled despite all supportive measures	No
Thrombocytopenia	3 or 4	Transfusion with platelets	If uncontrolled despite all supportive measures	No
Edema/Weight gain	3	Diuretics prn	No	No
Hypotension	3	Fluid resuscitation Vasopressor support	If uncontrolled despite all supportive measures	No
Dyspnea	3 or 4	Oxygen or ventilatory support	If requires ventilatory support	No
Oliguria	3 or 4	Fluid boluses or dopamine at renal doses	If uncontrolled despite all supportive measures	No
Increased creatinine	3 or 4	Observation	Yes (grade 4)	No
Renal failure	3 or 4	Dialysis	Yes	Yes
Pleural effusion	3	Thoracentesis	If uncontrolled despite all supportive measures	No
Bowel perforation	3	Surgical intervention	Yes	Yes
Confusion	3	Observation	Yes	No
Somnolence	3 or 4	Intubation for airway protection	Yes	Yes
Arrhythmia	3	Correction of fluid and electrolyte imbalances; chemical conversion or electrical conversion therapy	If uncontrolled despite all supportive measures	No
Elevated Troponin	3 or 4	Observation	Yes	If changes in LV function

levels				have not improved to baseline by next dose
Myocardial Infarction	4	Supportive care	Yes	Yes
Elevated transaminases	3 or 4	Observation	For grade 4 without liver metastases	If changes have not improved to baseline by next dose
Hyperbilirubinemia	3 or 4	Observation	For grade 4 without liver metastases	If changes have not improved to baseline by next dose
Electrolyte imbalances	3 or 4	Electrolyte replacement	If uncontrolled despite all supportive measures	No
Neutropenia	4	Observation	No	No

*Unless the toxicity is not reversed within 12 hours

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

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

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

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

* Eleven patients are in two protocols.

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

Appendix 5: Preparing Specimen for Mycoplasma Testing.

Purpose:

All cells intended for patient treatment and/or tissue culture lines, must be monitored for possible mycoplasma contamination. Samples for patient treatment will be pulled from the cultures on day 18 and will be labeled in the Regenerative Medicine laboratory before submission to a licensed clinical laboratory, LABS, Inc., CLIA ID 06D0717586. Mycoplasma is detected by an FDA-approved assay from Applied Biosystems. Turnover time from submission should be approximately one week.

Hazards:

All personnel should treat patients' tissues, blood and blood derived products as potentially infectious material. Universal precautions and infection control should be practiced at all times.

Mycoplasma testing for control tissue culture cell lines (TC).

1. Remove approximately 5e5 cells from TC to be tested.
2. Place in 50 ml centrifuge tube and top with antibiotic-free RPMI 1640, containing 10% FCS, in order to block trypsin-EDTA activity.
3. Spin at 600g, 8 min.
4. Resuspend the pellet in antibiotic free media or HBSS and centrifuge at 600g.
5. Aspirate the supernatant.
6. Resuspend the pellet in 10 ml antibiotic-free media.
7. Culture in a labeled T25 tissue culture flask and incubate horizontally at 37°C for minimum of 5 days.
8. Label a 15 ml tube with cell line # and date.
9. Mix the medium in the T25 flask by pipeting up and down.
10. Transfer approximately 5ml of the cell suspension into the labeled tube.
11. Follow directions included with shipping materials from LABS, Inc. for Kit Style #378188 Packing Instructions: Wet Ice Shipper to send sample to LABS.
12. Results are available on the LABS, Inc web portal approximately one week after sample submission.

Mycoplasma testing for Patient Treatment cells

1. Label a 15ml tube with sample #/UPN and the date.
2. Gently shake the flasks that need to be sampled.
   •For GRex flasks sample all flasks that are sampled for counting; otherwise, sample about 1/5 of the total number of the flasks. If there are 30 bags/flasks, choose 6.
3. Remove about one ml from each and pool in a 15 ml tube.
4. Count cells using standard cell counting technique.
5. Transfer 5 ml of the pooled samples into the labeled 15 ml tube.
6. Follow directions included with shipping materials from LABS, Inc. for Kit Style #378188 Packing Instructions: Wet Ice Shipper to send sample to LABS.
7. Results are available on the LABS, Inc web portal approximately one week after sample submission.

<u>Appendix 6:</u> Preparation of Cells for Patient Infusion

Purpose: To provide the standard operating procedure for the preparation of cells for infusion into patients after harvesting.

Hazards:
All personnel should treat patient's tissues, blood and blood derived products as potentially infectious material. Universal precautions and infection control should be practiced at all times.

All components in contact with cells or their media need to be sterile.

Procedures:
Set up supplies needed to prepare harvest product for infusion:

- Set up ring stand (wiped with alcohol moistened cloth) in the back of the hood.

- Label four 250 ml centrifuge tubes or four 300 ml bags with patient name (to receive the harvest product).

- Label a 250 ml tube as, "saline + 2.5% human albumin". Into this tube make a 1:10 dilution of 25% human albumin into saline (20 ml 25% albumin + 180 ml saline). Confirm addition of IL-2 in the final product and then add 50 CU/ml.

- Prepare a 300 ml bag to receive the final product. CLOSE ALL CLAMPS and insert a sampling site coupler.

-Attach a patient label directly to the bag. This should have notes added to the label identifying it as the infusion bag with the date.

-Prepare an INFUSION BAG LABEL. This label should include:

- Patient label stuck onto upper left corner
- Cell ID
- "IL-2" should be circled on the label
- Human Albumin (lot # and expiration date filled in)
- Number of cells (to be filled in later)
- Volume (to be filled in later)
- Expiration date/ time (to be filled in later)

- Label one aerobic micro bottle, one anaerobic micro bottle and one 1.5 ml tube to receive final product sample for microbiology testing. The label should include; patient initials, , "infusion" and date.

<u>Before cells are harvested:</u>

1. Endotoxin assay:

1. Remove approximately one ml from each flask and pool in a 15 ml tube.

2. Perform endotoxin assay by following directions in SOP 33.6009.1 The Endosafe Portable Test System for detection of endotoxin in cellular therapy products, current version.

2. Pull counts and stat Gram stain:

1. Remove approximately one ml from each flask and pool in a 15 ml tube.

2. Perform Gram stain by following directions in SOP 33.6010 Gram staining using the PREVI Color Gram (current version) and SOP 33.6011, Gram Stain slide preparation and interpretation (current version). A report of "No Microorganisms Seen" must be received prior to harvest.

3. Count the samples pulled and record on the REP 2 countdown sheet.

3. Check all results from testing:

- Confirm results from Co-culture, FACS and RCR.
- Receive results from Endotoxin assay.
- Enter all results on the patient treatment COA form.

<u>Processing harvested cells for patient infusion</u>

- After the cells have been harvested, they will arrive in one or two 1L transfer packs if harvested on the Fenwal or the donut if harvested on the Cobe.
- Insert spike/spike transfer tubing into transfer packs containing harvested cells and drain the contents into the labeled 250 ml centrifuge tubes or 300 ml transfer bags.
- Centrifuge @400g for 15 minutes.
- Aspirate the sup and re-suspend the pellets in the 2.5% Albumin/Saline to a final volume of 200 ml, making sure to rinse each tube as it is emptied.
- A 60ml syringe barrel should have been connected to the ring stand to serve as a funnel and the 300 ml patient Rx bag attached to the luer lok end of the syringe.
- Transfer the 200ml infusion product to the 300 ml bag by pouring through the 60ml syringe barrel (make sure the clamp to the tubing entering the bag is open).
- Close the clamp on the 300 ml bag, remove from the syringe barrel and heat seal.
- Using the 3 ml syringe, pull a count from the infusion bag.
- Count the cells (a 1:10 or 1:100 dilution will most likely be necessary).
- Record the count (#cells/ml, volume, total # cells, % viability).
- Calculate how many cells will be needed to freeze 30 vials @ 2e7/vial.
- Pull the cells for freeze vials plus a sample for micros. Put the cells for microbiology testing directly into a 250 ml tube for washing. Put the cells for freezing into a 50 ml centrifuge tube and keep on ice until ready to freeze in vials.
- Arrange for courier to pick up cells for delivery to Montefiore.

- Calculate the final number of cells after the freeze and micros volumes have been subtracted from the 200 ml volume.
- Enter the final volume, the final number of cells and the expiration date/time on the INFUSION BAG LABEL.
- Ensure that the patient treatment COA has been completed (including final number of cells and %Viability).
- Bring the infusion bag to the courier to be delivered for patient infusion.
- Record all counts and lot numbers.
- Freeze the infusion bag sample in vials.

Appendix 7: Standard Operating Procedure for the Safety Monitoring of Cells for Infusion into Patients after Cell Expansion and Harvest.

Safety testing of cell cultures for adoptive transfer from initial OKT3 stimulation to preparation for infusion will be performed. This cell culture, transduction, and expansion will occur over a 25 day period. Aliquots of prepared cells or suspension fluids will be tested for mycoplasma, fungal culture, bacterial culture (aerobic and anaerobic), gram stain, endotoxin contamination and free virus. Any positive testing will preclude cell transfer with the understanding that cultures may not test positive until sometime after infusion.

On day 18 of culture, cells will be sent for mycoplasma evaluation as described under SOP Preparing cells for mycoplasma testing. Mycoplasma will be detected by an FDA-approved assay from Applied Biosystems performed at a licensed clinical laboratory, LABS, Inc., CLIA ID 06D0717586 On day 23 DNA extracts will be tested by PCR for free virus.

On day 23 cells will be sent to licensed clinical laboratory, LABS, Inc., CLIA ID 06D0717586, for fungal culture, and anaerobic and aerobic bacterial cultures and Gram staining will be performed at New York Blood Center. On day 25, the day of cell infusion, an aliquot of cells will undergo a stat Gram stain and assay for endotoxin contamination using the Endosafe® - PTS™ Reader at New York Blood Center. Once cells are prepared for infusion, samples will again be sent for anaerobic and aerobic bacterial and fungal culture, as well as Gram staining.

Appendix 7 :

Protocol : Anti-NY ESO-1 mTCR PBL

Date sample collected :

Research Lab Requisition Form

Investigator's Name:
Site/Patient Number : _ _ _ _
Age : Gender :

☐5 CPT tubes / ambient

☐Bioharzard bag

Samples collected by :
Name :
Signature :
Date and time:

Samples received at the HCT lab by :
Name :
Signature :
Date and time :

Samples picked up by NYBC courier:
Name :
Signature
Date and time :