TITLE: A Phase I/IIa Study of TGFβ Blockade in TCR-Engineered T-Cell Cancer Immunotherapy in Patients with Advanced Malignancies.

Roswell Park Cancer Institute

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List of Abbreviations

ACT, Adoptive cell transfer

AE, Adverse event

CBC, Complete blood count

CFR, Code of federal regulations

CMV, Cytomegalovirus

CR, Complete response

CRF, Case report form

CRP, C reactive protein

CT, Computer tomography

CTL, Cytotoxic T lymphocytes

DC, Dendritic cells

DLT, Dose limiting toxicity

DSMB, Data Safety and Monitoring Board

EBV, Epstein-Barr virus

ELISPOT, Enzyme linked immunospot assay

FDA, Food and Drug Administration

[^{18}F]FDG, [^{18}F]fluorodeoxy-glucose

GaLV, Gibbon ape leukemia virus

GCP, Good Clinical Practices

GCV, Ganciclovir

GM-CSF, Granulocyte-macrophage colony stimulating factor

GMP, Good Manufacturing Practices

GVHD, Graft-versus-host disease

GVL, Graft-versus-leukemia

HBV, Hepatitis B virus

HCV, Hepatitis C virus

HCT, Hematopoietic cell transplantation

H&E, Hematoxylin eosin staining

HEPA, High-efficiency particulate air

HIV, Human immunodeficiency virus

HLA, Human leukocyte antigen

ICF, Informed consent form

IBC, Institutional Biosafety Committee

i.d., Intradermal

IHC, Immunohistochemistry

irRECIST, Immune-Related Response Criteria

IU VPF, Indiana University Vector Production Facility

i.v, Intravenous

IQAC, Internal Quality Assurance Committee

IRB, Institutional Review Board

ISPRC, Internal Scientific Peer Review Committee

IL, Interleukin

LTR, Long terminal repeat

MHC, Major histocompatibility complex

MLV, Moloney Leukemia Virus

MRI, Magnetic resonance imaging

N.C.I., National Cancer Institute

NGVL, National Gene Vector Laboratory

N.I.H., National Institutes of Health

PBMC, Peripheral blood mononuclear cells

PCR, Polymerase chain reaction

PD, Progressive disease

PET, Positron emitting tomography

PFT, Pulmonary Function Test

PR, Partial response

PTT, Partial Thromboplastin Time

RAC, Recombinant Advisory Committee

RCR, Replication competent retrovirus

RECIST 1.1, Response Evaluation Criteria in Solid Tumors

RPCI, Roswell Park Cancer Institute

SAE, Serious adverse event

SD, Stable disease

SUV, Standardized uptake value

TSH, Thyroid stimulating hormone

TCR, T cell receptor

TGF**β**, Transforming Growth Factor-beta

SYNOPSIS

Title / Phase	**A Phase I/IIa Study of TGFβ Blockade in TCR-Engineered T-Cell Cancer Immunotherapy in Patients with Advanced Malignancies.**
Roswell Park Cancer Institute Study Number	I 258514
Roswell Park Cancer Institute Investigator	Grant/Scientific PI: Richard Koya MD, PhD Clinical PI: Philip McCarthy, MD
Sponsor	National Institutes of Health/ National Cancer Institute
Study Drug(S)	NY-ESO-1 TCR/ dnTGFβRII transgenic T cells Decitabine (Cohort 4 only)
Objectives	**Primary:** • To evaluate the safety and feasibility of adoptive transfer of autologous NY-ESO-1 TCR/dn TGFβ-RII transgenic T-cells in combination with Decitabine **Secondary:** • To measure the persistence of these genetically modified T cells • To study the T cell populations generated that correlates with higher anti-tumor responses • Assess clinical response and progression free survival **Exploratory:** • Gut microbiota pre and post treatment to evaluate the role of microbiota on the therapeutic efficacy of the proposed therapy.
Study Design	This is an open label Phase I/IIa clinical trial. Patients with NY-ESO-1-positive advanced malignancies who are HLA-A*0201-positive, and HIV, hepatitis B and C seronegative will be enrolled in the study providing they meet the remaining eligibility criteria. Upon enrollment, patients will undergo leukapheresis on day -6 for T cell collection and their cells will be genetically engineered and expanded ex-vivo. 5 days prior to ACT, patients will receive a conditioning regimen consisting of 45 mg/kg IV cyclophosphamide (adjusted body weight if > 120% of ideal body weight) for two days as an outpatient or inpatient, according to the investigator's discretion to create space for the transgenic cells. The cell product will be infused fresh after lot release on day 0 as an inpatient. This is a 3 + (3) design dose escalation study with the first cohort receiving 10e7, second cohort 10e8 and the third cohort 10e9 NY-ESO-1 TCR/dnTGFb-RII transgenic cells (0.5e9 or higher, up to 1e9 acceptable) as a single dose, calculated based on the transduction efficiency by tetramer assay. Cohort 4 will receive the MTD of NY-ESO-1 TCR/dnTGFb-RII transgenic cells defined in the first 3 cohorts in combination with Decitabine and will follow the 3 + (3) rule.

	There will be at least a 3 weeks staggering of infusions between the first two patients of each cohort in order to allow time to assess toxicities. Patients will be followed daily while in the hospital and discharged when the condition is stable as per clinical judgement of the treating physicians with an Absolute neutrophil count (ANC) of >500/uL and platelet count of > 20,000/uL. Thereafter, weekly follow ups for 4 weeks, then at 8 and 12 weeks, and at months 6, and 9 or until progression, whichever comes first. A biopsy for NY-ESO-1 testing and research will be performed at baseline if archival tissue is not available for testing. For cohort 4, with the addition of decitabine, NY-ESO-1 positivity will be recorded but is not required for eligibility. Optional tumor biopsies at 6 weeks post ACT (± 1 week) and upon progression, for research purpose may be done. Patients will undergo disease monitoring by CT scan at baseline, week 6, week 12, and at 6 months and 9 months, or at progression. At 9 months, the interventional portion of the protocol ends and long term follow up begins. Subjects who responded initially after the T cell infusion may be eligible (Section 6.3) for a second infusion at any time after disease progression is confirmed.
Target Accrual and Study Duration	A maximum of 27 patients at RPCI will be enrolled. The number of patients required is a function of the unknown dose-toxicity relationship. Accrual is expected to take up to 4 years.
Study Procedures	Patients who have solid tumors refractory to treatment that are either Stage IV or locally advanced will be considered for screening in this trial. Patients must be HLA-A*0201 (HLA-A2.1) and have NY-ESO-1 positive malignancy by IHC and/or by RNA expression (NY-ESO-1 positivity not needed for Cohort 4), age should be greater than or equal to 12 years old with a life expectancy of greater than 3 months as assessed by a study physician, ECOG performance status (PS) 0 or 1, have a minimum of one measurable lesion meeting the criteria for measurable disease according to Immune-Related Response Criteria (irRECIST), adequate bone marrow, hepatic, cardiac and pulmonary function determined within 30-60 days prior to enrollment and able to undergo leukapheresis procedure. Patient or legal representative must understand the investigational nature of this study and sign an Independent Ethics Committee/Institutional Review Board approved written informed consent form prior to receiving any study related procedure. Patients will be excluded from this study if there is previously known hypersensitivity to any of the agents used in this study, received systemic treatment for cancer, including immunotherapy, within one month prior to initiation of dosing within this protocol, history of, or significant evidence of risk for chronic inflammatory or autoimmune disease, have a known active infection (including HIV, HCV, HBV, CMV), have active brain metastasis or are pregnant or breast-feeding.

Statistical Analysis	This is a phase I/IIa trial, principally designed to assess the safety (defined by dose limiting toxicities, DLT) and feasibility (defined by sufficient numbers of circulating, lot-release confirmed T cells) of treating patients with advanced malignancies with escalating doses of genetically engineered T cells expressing NY-ESO-1 TCR together with a dnTGFb-RII transgene administered after lympho-depleting conditioning chemotherapy and in cohort 4, after decitabine and lympho-depleting conditioning chemotherapy. The assessment of safety and feasibility is based on studying cohorts of three subjects, with the addition of 3 subjects in case of toxicities meeting the definition of DLT in one of the three subjects in a cohort. If two or more subjects have DLT in a cohort, that cohort will be considered beyond the MTD and an intermediate dosing may be explored to better define the MTD of this combination. A minimum of three evaluable subjects will be treated at each of the three cohorts. In cohort 4, a total of 9 patients will be enrolled to receive the MTD of NY-ESO-1 TCR/dnTGFb-RII transgenic cells defined in the first 3 cohorts, in combination with decitabine. There is limited power to establish efficacy and therefore efficacy analysis will be primarily descriptive.

ROSWELL PARK CANCER INSTITUTE

INVESTIGATOR STUDY ELIGIBILITY VERIFICATION FORM

Patient Name: _____

Medical Record No.: _____

Title: A Phase I/IIa Study of TGFβ Blockade in TCR-Engineered T-Cell Cancer Immunotherapy in Patients with Advanced Malignancies

INCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "Yes" or "N/A" for patient enrollment.**	**Date**
☐	☐	☐	1. Patients with solid tumors as described below: a) Inoperable or metastatic (advanced) melanoma: - Has received, is intolerant, or refused a CTLA-4 inhibitor (Ipilimumab) or a PD-1 inhibitor (Nivolumab or Pembrolizumab) as monotherapy and/or a combination of Ipilumumab and Nivolumab. - Has received or is intolerant of a BRAF inhibitor or the combination of BRAF and MEK inhibitors for BRAFv600 mutant melanoma and a PD-1 inhibitor as monotherapy or in combination. b) Inoperable or metastatic (advanced) ovarian, primary peritoneal or fallopian tube carcinoma: - Has received platinum containing chemotherapy and has platinum refractory or resistant disease that has progressed on second line therapy - If platinum sensitive disease, should have received ≥2 lines of chemotherapy. - May have received PARP inhibitors, bevacizumab or other targeted VEGF inhibitor therapy c) Inoperable or metastatic (advanced) synovial sarcoma: - Should have received and progressed on ≥ two lines of systemic therapy d) Subjects with other histologies: Must have previously received two lines of systemic standard care (or effective salvage chemotherapy regimens) for metastatic disease, if known to be effective for that disease, and have been deemed either non-responders (progressive disease) or have recurred.	
☐	☐	☐	2. For Cohorts 1, 2 and 3 only: Patient's tumor must be positive by histological or molecular assay for NY-ES0-1, according to the	

INCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "Yes" or "N/A" for patient enrollment.**	**Date**
			screening algorithm as described in Section 9.1 under "tumor biopsy". Historical results may be used. For Cohort 4, NY-ESO-1 results will be noted but NY-ESO-1 positivity not required for eligibility.	
☐	☐	☐	3. HLA-A*0201 (HLA-A2.1) positivity by molecular subtyping (blood test or buccal swab, historical documentation acceptable)	
☐	☐	☐	4. Age ≥ 18 years old (Cohort 4: Age ≥ 12 years old)	
☐	☐	☐	5. Life expectancy greater than 3 months assessed by a study physician	
☐	☐	☐	6. Have been informed of other treatment options.	
☐	☐	☐	7. A minimum of one measurable lesion defined as: • Meeting the criteria for measurable disease according to Immune-related Response Criteria (irRECIST) • For patients with skin metastases, lesions selected as non-completely biopsied target lesion(s) that can be accurately measured and recorded by color photography with a ruler to document the size of the target lesion(s)	
☐	☐	☐	8. No restriction based on prior treatments, but at least 4 weeks from prior immunotherapy or prior investigational agents. **Note:** Patients who have suffered >grade 2 irAEs during previous checkpoint inhibitor therapy should be excluded.	
☐	☐	☐	9. ECOG Performance Status (PS) of 0 or 1 (**Appendix A**).	
☐	☐	☐	10. Must have adequate venous access for apheresis.	
☐	☐	☐	**11.** Women of childbearing potential and men must agree to use effective methods of birth control for the duration of the study and 6 months after. Methods for effective birth control include: condoms, diaphragm or cervical cap with spermicide, intrauterine device, and hormonal contraception. It is recommended that a combination of two methods be used.	
☐	☐	☐	12. Patients must have normal organ and marrow function as defined below: • Leukocytes: ≥ 3,000/mcl • Absolute Neutrophil Count: ≥ 1,000/mcl • Platelets: ≥ 100,000/mcl • Total bilirubin: ≤ 1.5 ULN • AST (SGOT)/ALT (SGPT): ≤ 2.5 x institutional upper limit of normal • Creatinine: ≤ 2 X ULN; if creatinine > 2 X ULN, Creatinine clearance must be > 60 ml/min	
☐	☐	☐	13. Must be willing and able to accept the leukapheresis procedure.	

INCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "Yes" or "N/A" for patient enrollment.**	**Date**
☐	☐	☐	14. At screening, must have tissue available for NY-ESO-1 testing (if not previously performed) or be willing and able to undergo a fresh tissue biopsy.	
☐	☐	☐	15. Patient or legal representative must understand the investigational nature of this study and sign an Independent Ethics Committee/Institutional Review Board approved written informed consent form prior to receiving any study related procedure.	
☐	☐	☐	16. Participant must agree to and arrange for a caregiver (age ≥ 18 years old) available 24 hours a day/7 days a week and arrange for lodging within a 45 minute drive to Roswell Park and transportation for a period of time after discharge from the hospital. The exact amount of time will depend on the individual status as determined by the treating physician,	

Investigator Signature: _____ Date: _____

INVESTIGATOR STUDY ELIGIBILITY VERIFICATION FORM

Patient Name: _____

Medical Record No.: _____

Title: A Phase I/IIa Study of TGFβ Blockade in TCR-Engineered T-Cell Cancer Immunotherapy in Patients with Advanced Malignancies

EXCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "No" or "N/A" for patient enrollment.**	**Date**
☐	☐	☐	1. Previously known hypersensitivity to any of the agents used in this study	
☐	☐	☐	2. Currently receiving any other investigational agents. **Note:** Patients who have suffered >grade 2 irAEs during previous checkpoint inhibitor therapy should be excluded.	
☐	☐	☐	3. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements. EKG will be done at screening. Cardiac stress test will be done as clinically indicated, the specific test to be chosen at the discretion of the treating physician.	
☐	☐	☐	4. History of severe autoimmune disease requiring steroids or other immunosuppressive treatments.	
☐	☐	☐	5. History of inflammatory bowel disease, celiac disease, or other chronic gastrointestinal conditions associated with diarrhea or bleeding, which in the investigator's opinion would place the patient at an increased risk for adverse effect or current acute colitis of any origin. Treated cases with no active disease are eligible.	
☐	☐	☐	6. Potential requirement for systemic corticosteroids or concurrent immunosuppressive drugs based on prior history or received systemic steroids within the last 4 weeks prior to enrollment (inhaled or topical steroids at standard doses or isolated use of steroids as premedication for medical procedures to minimize allergic reaction [e.g. CT scan dye] are allowed)	
☐	☐	☐	7. Known active infection with HIV, Hepatitis B, Hepatitis C, or CMV.	
☐	☐	☐	8. Known cases of clinically active brain metastases (brain MRI as clinically indicated). Prior evidence of brain metastasis successfully treated with surgery or radiation therapy will not be exclusion for participation as long as they are deemed under control at the time of study enrollment.	

EXCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "No" or "N/A" for patient enrollment.**	**Date**
☐	☐	☐	9. Dementia or significantly altered mental status that would prohibit the understanding or rendering of compliance with the requirements of this protocol even with caregiver support.	
☐	☐	☐	10. Pregnancy or breast-feeding. Female patients must be surgically sterile or be postmenopausal for two years, or must agree to use effective contraception during the period of treatment and 6 months after. All female patients with reproductive potential must have a negative pregnancy test (serum/urine) within 48 hours from starting the conditioning chemotherapy.	
☐	☐	☐	11. Lack of availability of a patient for immunological and clinical follow-up assessment.	

Patient meets all entry criteria: ☐ **Yes** ☐ **No**
If "NO", do not enroll patient in study.

Investigator Signature: _____ **Date:** _____

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IN-TEXT TABLES

APPENDICES

1 BACKGROUND

1.1 NY-ESO-1 Antigen as a Target for Immunotherapy

NY-ESO-1 belongs to a family of genes referred to as Cancer-Testis Antigens (1). CTAs are generally only expressed embryonically and are not expressed in adult somatic tissues, but can be re-expressed in malignancies(2). This is precisely the case for NY-ESO-1. Studies have indicated that the NY-ESO-1 protein is only expressed in male spermatogonia, but evidence for its expression in all other adult somatic tissues has not been found (3-6). On the other hand, the NY-ESO-1 protein has been observed to range in expression from 10-40% across a wide range of malignancies, and >80% in synovial sarcoma (5, 7-9). At Roswell Park Cancer Institute, Dr. Kunle Odunsi (co-investigator for this grant), detected expression of NY-ESO-1 in epithelial ovarian cancer by RT-PCR and/or IHC in 82 of 190 (43%) specimens (8). Together, these observations indicate that NY-ESO-1 is an ideal target for cellular based immunotherapies. In addition, a lack of MHC-I in the testes greatly reduces the potential for TCR based immunotherapeutics to induce orchitis (10).

1.2 Rationale for Immune-based Therapies for Advanced Malignancies

Progress in our understanding of how the immune system recognizes and kills cancer cells has led to the notion that high levels of circulating, tumor-antigen-specific T cells, may be required for effective antitumor responses. Active immunotherapy with several forms of cancer vaccines has shown that antigen-specific T cells can be activated and occasionally lead to antitumor responses (11, 12). However, the circulating levels of antigen-specific T cells and tumor response rates are much lower than desirable (13).

Adoptive transfer of clonally-expanded, tumor antigen-specific lymphocytes to lymphopenic hosts after nonmyeloablative conditioning chemotherapy has resulted in cell proliferation and persistent clonal repopulation (14). This approach has resulted in an objective response rate of over 50% in patients with metastatic melanoma, that holds up in larger groups of patients (15). The major limitation for the broad clinical applicability of this approach is the requirement for large-scale ex vivo lymphocyte culture expansion (up to 1011 cells) (14), which restricts this approach to a highly select group of patients.

1.3 Lessons Learned from Targeting Tumor Associated Antigens with Immunotherapy

Multiple lines of evidence suggest that most tumor responses to immunotherapy involve the activation of tumor antigen-specific CD8+ cytotoxic T lymphocytes (CTL) (16). These immune cells have a high affinity receptor, the TCR that specifically recognizes an 8-10 amino acid peptide of its ligand in a complex within an MHC molecule. Recently a trial conducted at the NCI utilizing ACT directed against the NY-ESO-1 peptide demonstrated a clinical response in 5/11 melanoma patients (with 2 patients experiencing a complete response with 20+ and 22+ months of follow-up) and a clinical response in 4/6 patients with synovial sarcoma (17). This provides an impetus for extending this type of therapy to other solid tumors without viable treatment options.

1.4 Why Gene Therapy

The adoptive transfer of large numbers of antigen-specific T cells leads to the highest percentage of tumor regressions reported in patients with melanoma, but this approach is only feasible in a minority of patients where TIL can be cloned and expanded from melanoma metastases (14, 15). Genetic engineering of T cells with optimal TCRs would make this approach more broadly applicable. Currently, there is no other approach that would allow the generation of large numbers of uniformly specific T cells directed against tumor associated antigens in a short (less than one week) ex vivo cell manipulation. Therefore, gene transfer techniques are the only approach that would allow testing this concept in human subjects, as supported by preliminary data from a published clinical trial at the Surgery Branch/NCI (18).

1.5 TCR Gene Therapy

The transfer of TCR genes is necessary and sufficient to endow recipient T cells with the specificity of donor T cells (19). Genetically modified T cells carrying foreign TCRs respond to target antigen recognition through the transgenic TCR both *in vitro* and *in vivo*, leading to effective immune responses to viral and tumor challenges in murine adoptive transfer models (20). T cells redirected by TCR gene transfer are fully functional after transfer into mice, and have been shown to expand dramatically (over three logs) after encounter with their cognate antigen *in vivo* (21). Preliminary data in humans provides evidence that the same is true in patients with metastatic melanoma and synovial sarcoma (17, 18, 22).

1.6 Retroviral Vector-based Human Gene Medicine

Retroviral-mediated gene transfer is an efficient means for the expression of transgene into actively dividing primary cells. Up to 99% of T lymphocytes can be transduced if cells are non-specifically activated with anti-CD3 and IL-2 (18, 22).

1.7 NY-ESO-1 TCR-engineered Adoptive Cell Transfer (ACT) Clinical Experience

1.7.1 Use of Other TCRs in Humans

The Surgery Branch/NCI has published the first report of genetically engineered NY-ESO-1 TCR therapy for melanoma and synovial sarcoma. In addition, there are 4 currently open trials employing this mode of therapy.

- NCI trial 08-C-0121 treated 17 patients using the MSGV-1G4-α95:LY retroviral vector, a similar vector, and the same packaging cell line proposed in the current study. This study enrolled patients with synovial cell sarcoma and metastatic melanoma. 5/11 melanoma patients experienced a clinical response, with 2 patients experiencing a long term complete regression with 20+ and 22+ months of follow up. In addition, 4/6 synovial cell sarcoma patients experienced a clinical response; one patient had a partial response that lasted 18 months (17).
- Currently, there are 4 accruing clinical trials which employ genetically engineered TCRs against NY-ESO-1 (NCI trials: 01343043, 01350401, 00670748, 01352286 and 01697527). These trials are being conducted in patients with metastatic melanoma and

synovial sarcoma and are occurring at the NCI, the University of Pennsylvania, Washington University in St. Louis and, the University of California, Los Angeles.

1.7.2 Use of the NY-ESO-1 TCRs in Humans

NCI trial 08-C-0121 (OBA# 0712-886) uses the same NY-ESO-1 TCR and retroviral vector construct proposed in the current protocol (22, 23). Below we list the major similarities and differences between both clinical trials.

Major similarities:

- TCR: Both clinical trials use the same MSGV-1G4-α95:LY generated by mutagenesis of particular TCR residues.
- Retrovirus: Both clinical trials use the same retrovirus vector backbone.
- Target cells: Both experiences include the transduction of activated PBMC. Since a TCR can only be expressed and functional on the cell surface in cells that are CD3 positive, this allows the TCR engineering of both CD8+ CTL and CD4+ T helper cells. Therefore, CD4+ T cells acquire a TCR that redirects them to recognize antigen presented by MHC class I molecules.
- Clinical trial design: Primary endpoints of both are safety and feasibility with a secondary end point of antitumor activity.

Major differences:

- Incorporation of dnTGFβRII.
- Rapid Expansion Protocol (REP): The NY ESO TCR clinical trial at the Surgery Branch/NCI included a 2-week ex vivo expansion after TCR engineering of lymphocytes following the REP protocol described by Riddell and colleagues (24). The clinical trial proposed will not include this forced ex vivo T cell expansion. The main reason is that several preclinical models suggest that extended ex vivo expansion of lymphocytes before adoptive transfer results in terminally differentiated cells with limited in vivo proliferation ability (25, 26). Therefore, TCR transduced PBMC will only be kept for up to 96 hours in culture after the first transduction and before re-infusing into patients.
- Conditioning regimen: Both use a non-myelodepleting but lympho-depleting chemotherapy conditioning regimen but NCI uses cyclophosphamide and fludarabine and we propose to use only cyclophosphamide at a lower dose to allow homeostatic proliferation of the adoptively transferred TCR transgenic cells.
- Cell dose for adoptive transfer: a dose escalation 3 cohort design ranging from 10^7 to 10^9 cells, the highest dose being two logs lower than in the clinical trial conducted at the Surgery Branch/NCI (17), where up to 10^{11} TCR transgenic cells were infused to patients.
- IL-2 dosing: The initial NCI trial used high dose IL-2 post-adoptive transfer as means to provide helper cytokines to support the proliferation of adoptively transferred TCR transgenic cells. Our trial will not include IL-2. This modification is based on preliminary data from subsequent trials (as presented and discussed at National Institutes of Health

(NIH), Office of Biotechnology Activities - T Cell Immunotherapy – Optimizing Trial Design, Bethesda, MD, September 10-11, 2013), which indicate that patients can achieve clinical responses without the need of IL-2 administration in TCR-engineered T cell ACT. Furthermore, administration of IL-2 has been associated with increased toxicity and there is evidence that high dose IL-2 may impair the generation of effective memory cells (27).

2 PRELIMINARY DATA

2.1 Origin and Functionality of the NY-ESO-1 TCR

The original retroviral vector, MSGV1-A2aB-1G4A-LY3H10, expressing the NY-ESO-1 TCR was developed by Drs. Steven Rosenberg and Paul Robbins from the NCI. The parental 1G4 clone recognizing NY-ESO-$1_{157-165}$ was originally derived from phage display libraries (28). Modifications of the CDR regions in the alpha and beta chains of the TCR indicated that higher affinity NY-ESO-$1_{157-165}$ recognizing TCRs could be generated. However, this development came with a loss of antigen specificity (29).

However, some substitutions, e.g. the (alpha95:LY) in the CD3alpha region did not engender a loss of antigen specificity while increasing sensitivity after being transferred into CD4+ or CD8+ cells. Moreover, functionally, these substitutions led to an increased responsiveness in *in vitro* assays (29). As such, the modified 1G4 clone with the alpha chain mutation was selected to be used in an ACT trial at the NCI in tumors expressing NY-ESO-1.

2.2 Use of the NY-ESO-1 TCR Retroviral Vector (MSGV1-A2aB-1G4A-LY3H10) in Human Subjects at the Surgery Branch/NCI

Investigators at the Surgery Branch of the NCI recently reported on 17 patients with NY-ESO-1+ metastatic melanoma and synovial sarcoma treated with the ACT of lymphocytes expressing the NY-ESO-1 TCR. The Surgery Branch protocol differs with the UCLA protocol in that up to 1.3 x 10^{11} TCR transgenic cells were administered, and that no DC vaccine was included. Patients in the NCI trial were conditioned with cyclophosphamide and fludarabine (25 mg/m^2/day x 5 days). The protocol was feasible and safe, with no reported grade 3 or 4 toxicities. Objective clinical responses were seen in 4/6 patients with synovial sarcoma and 5/11 patients with melanoma (17). In this protocol, we will use lower cell dose and 2 days low dose cyclophosphamide regimen with no IL2.

3 RATIONALE

3.1 Rationale for TCR Transgenic Adoptive Cell Transfer Therapy

The adoptive transfer of large numbers of clonally-expanded antigen-specific T cells into patients with melanoma that have received a conditioning regimen to deplete endogenous lymphocytes (non-myeloablative but lympho-depleting), together with high doses of interleukin-2 (IL-2), results in the highest rate of melanoma responses (up to 70%) reported to date (14, 15 Dudley, 2008 #4580, 30, 31). This approach provides a proof-of-principle for adoptive transfer immunotherapy for metastatic melanoma, but it is difficult to implement outside of pilot studies given its requirement for extensive ex vivo manipulations. This roadblock may be overcome and may also be extended to other malignancies by the ex vivo transduction of specific TCR genes that

recognize tumor associated antigens. In this project we will test the hypothesis that human PBMC engineered to express a high affinity TCR specific for the human cancer testes antigen NY-ESO-1 coupled with dnTGFβRII is safe, feasible and can induce objective tumor responses in human subjects with advanced malignancies.

3.2 Rationale for Incorporating dnTGFβRII

Tumor secreted TGFβ is a major contributor to the immunosuppressive properties of the tumor microenvironment(32, 33). TGFβ1 inhibits T cell function (proliferation and activation), antigen-presentation by professional antigen-presenting cells, and promotes maintenance of T regulatory (Treg) cells. Most human cancers produce large amounts of active TGFβ, which also support tumor growth, invasion and metastasis. Based on a preclinical animal model (pmel-1/B16 melanoma), and an analysis of public and our data so far for patients treated with TCR-engineered ACT, we hypothesize that incorporating a dominant-negative TGFβ receptor II (dnTGFβRII) into the NY-ESO-1 TCR retroviral vector used to transduce patient T cells will render these effector cells insensitive to TGFβ, resulting in an enhancement of their effector function in the tumor microenvironment.

3.3 Rationale for adding decitabine

The ability of the immune system to recognize and reject tumors is dependent on several factors that include the expression of immunogenic target antigens on tumor cells, generation of high frequencies of tumor antigen (TA) specific T cells with potent effector function, and capacity to overcome several mechanisms by which tumors escape immune attack. Although several *in vitro* and *in vivo* studies have demonstrated that the amount and duration of antigen stimulation can influence antigen-specific T cell responses (34, 35), the majority of cancer immunotherapy studies have focused on the generation of TA-specific T cells without concomitant manipulation of antigen expression. This is a critical consideration because (i) TAs are generally not expressed at 100% frequency in cancers; (ii) even when expressed, the antigen density may be low; and (iii) expression is often heterogeneous with areas of tumor that are distinctly positive or negative for the TA. Moreover, in the latter scenario, the generation of tumor specific immunity may result in tumor "immunosculpting" that could influence the ability to evade immune eradication. In this regard, a number of human immunotherapy clinical trials, have demonstrated evidence of immunoediting with either loss of antigen expression (e.g. MART-1) or MHC class I expression (15, 36, 37)

NY-ESO-1 expression is regulated by DNA methylation in EOC cells and tumor tissues, and decitabine treatment induces NY-ESO-1 expression in EOC cell lines (38). Additionally, heterogeneous intratumor expression of NY-ESO-1, which frequently occurs in EOC and other tumor types, is associated with the DNA methylation status of the *NY-ESO-1* promoter and the global methylation status of the tumor cells (38). These observations and other work suggest that antigen expression is an important limitation for CT antigen immunotherapy, and epigenetic therapy is a means to overcome this limitation.

We therefore hypothesize that the combination of epigenetic modification (Decitabine) and adoptive T cell therapy (ACT) will be safe and improve progression free survival (PFS) and/or

overall survival (OS) in patients with advanced malignancies. Epigenetic therapy using a demethylating agent 5-aza-2' –deoxycytydine (Decitabine) will increase cancer-testis antigen (CTA) expression, minimize emergence of antigen loss variants, and induce an immune signature that would further sensitize patients for ACT. Furthermore, this combinatorial strategy would allow inclusion of patients without NY-ESO-1 expression.

3.4 Rationale for lowering the eligible age to ≥ 12 years old

The dose escalation portion of this study has been completed with a total of 9 adult subjects infused with the study drug, NY-ESO-1 TCR/dn TGFβ-RII transgenic T-cells, without DLT (dose limiting toxicity). The last 3 patients in Cohort 3 received the maximum target dose for this study. We also treated a pediatric patient on a compassionate basis with the same combination therapy of NY-ESO-1 TCR/dn TGFβ-RII transgenic T-cells and decitabine as per Cohort 4 of this study. Therefore, the eligible age for this next Cohort 4 is being lowered to ≥ 12 years old to include adolescent patients with cancers that are relapsed after or refractory to standard therapy with no curative options.

Fixed cell dose justification

The same fixed dose (MTD) of NY-ESO-1 TCR/ dnTGFβRII cells at 1 x 10^9 cells iv (0.5e9 or higher, up to 1e9 acceptable) will be utilized for the pediatric patients as well. In the previously reported trial of NY-ESO-1 TCR T-cells for patients with advanced synovial sarcoma (NCT01353043)(39), eligible patients were ≥ 4 years old and patients ≥40 kg received the minimum cell dose of at least 1x10^9 transduced NY-ESO-1^{c}.^{59}T cells with a maximum of 6x10^9 transduced cells. The target dose for this protocol was 5x10^9 transduced NY-ESO-1^{c}.^{59}T cells, which is 5 times more than the MTD in our study. Patients <40 kg were dosed per body weight with a minimum of 0.025x10^9 transduced cells/kg, with a target dose of 0.125 x10^9 transduced cells/kg. With this, for a 20 kg child, the dose would be 2.5 x10^9 cells, which is still 2.5 times higher than the MTD in our study.

Relapse of solid tumors in pediatrics

Solid tumors make up about 30% of all pediatric cancers. Children with therapy-refractory or relapsed malignant solid tumors such as sarcomas, wilm's tumor, and neuroblastoma have minimal treatment options (40). The use of multiagent chemotherapy or radiotherapy results in only temporary control of disease. Efforts have been made in recent years to find novel approaches for treatment. NY-ESO-1 has been found to be variably expressed in a variety of pediatric solid tumors including osteosarcoma, synovial sarcoma, rhabdomyosarcoma, melanoma and neuroblastoma with expression identified in 25-90% of tumors (40-42). Ongoing and published trials on targeted NY-ESO-1 therapies and immunotherapy mainly concern adult patients, but the somatic biology of pediatric sarcomas have many similarities to adult disease. A phase I trial in 2015 showed that a peptide vaccine targeting cancer tesis antigens including NY-ESO-1 was well tolerated in children with relapsed neuroblastoma and sarcomas with 1/10 patients having a complete response to therapy (41). Of the other nine patients, one had an initial tumor response with subsequent progression and a second who had multiply relapsed neuroblastoma had prior tumor resection, but remained disease free for >2 years from receipt of the vaccine. Additionally, in a previously reported trial of NY-ESO-1 TCR T-cells for patients with advanced synovial sarcoma

(NCT01353043), at least one pediatric aged patient experienced a partial response lasting 32 weeks (39)(Ramachandran 2019). This highlights the potential therapeutic efficacy of targeting cancer testis antigens including NY-ESO-1 in pediatric solid tumors.

Pediatric experience with NY-ESO-1 TCR/dn TGFβ-RII transgenic T-cells in combination with decitabine

On a compassionate basis, we have treated one pediatric patient with NY-ESO-1 TCR/ dnTGFβRII transgenic T cells and decitabine as proposed in the Cohort 4 of this current protocol. The patient is a 15 years old, with multiply relapsed osteosarcoma who developed progressive disease while undergoing prior salvage chemotherapy. The patient was HLA-A*0201 positive, but tumor was NY-ESO-1 negative, thus he was treated with inclusion of decitabine to induce NY-ESO-1 expression. Following apheresis for collection of T-cells, he underwent conditioning chemotherapy with decitabine and cyclophosphamide. He was then infused with the successfully engineered transgenic T-cells. The patient tolerated the conditioning well with only mild-moderate chemotherapy induced nausea controlled with antiemetic therapies. He also tolerated the T-cell infusion without complication. He was admitted for observation following the T-cell infusion. Markers of inflammation and cytokine release syndrome were monitored throughout his admission. The patient remained clinically stable though did develop myelosuppression believed to be secondary to the decitabine and cyclophosphamide regimen. He also developed a coagulopathy with Grade 1 prolongation of the PTT, elevated D-dimer (though with no clinical evidence of DIC) and an elevated CRP, but otherwise his lab evaluations remained normal. The only grade 3 or greater toxicity was hematologic with grade 3 febrile neutropenia that developed day +16 following T-cell infusion, grade 3 anemia requiring PRBC transfusion and grade 4 thrombocytopenia requiring a single platelet transfusion. Blood counts had recovered within 1 month of the therapy. Though he tolerated the therapy well, this patient developed progressive disease 1 month following the T-cell therapy. Considering the potential, there is a need to explore this type of therapy in adults as well as pediatric patients.

4 OBJECTIVES

4.1 Primary Objectives

To evaluate the safety and feasibility of adoptive transfer of autologous NY-ESO-1 TCR/dn TGFβRII transgenic T cells.

4.1.1 Safety

The NY-ESO-1 TCR and the retroviral vector MSGV1-A2aB-1G4A-LY3H10 has already been tested in a completed and reported phase 2 clinical trial by investigators at the Surgery Branch/NCI (17). In this trial, no grade 3 or 4 side effects were reported in 17 melanoma and synovial cell sarcoma patients. In addition, CD4 cells trained to recognize NY-ESO-1 were used successfully in one patient with metastatic melanoma without any acute or long term toxicities (26 months) (43). At Roswell Park Cancer Institute, Dr. Kunle Odunsi (co-investigator for this grant) has been running a clinical trial with ACT utilizing NY-ESO-1 TCR transgenic cells after a lymphodepletion regimen and collection of experimental/clinical data is ongoing. At my previous institution, UCLA, we ran a clinical trial targeting another melanoma antigen with ACT utilizing

MART1 F5 TCR transgenic cells after a lymphodepletion regimen, followed with DC vaccination and high dose IL-2. Three out of 14 patients had a DLT; one patient experienced marrow aplasia that has been ascribed to the conditioning regimen of the protocol and extensive bone marrow infiltration by melanoma, and two other patients required intubation for bilateral pneumonia worsened by high-dose IL-2-induced capillary leak syndrome. Because of these DLTs, future patients have received a less intensive conditioning regimen with 4 instead of 5 days of fludarabine conditioning, the regimen of high dose IL-2 has been changed to low-dose IL-2 (37, 44), and the number of infused transgenic CTLs has decreased to 10^9. As such, patients in this trial will receive a less intensive low dose cyclophosphamide conditioning regimen, IL-2 will not be administered, and dose escalation will start with very low numbers of cells (10^7). Altogether this setting will substantially minimize the potential toxicity, improving the safety level for our patients.

After establishing the MTD for the NY-ESO-1 TCR/dnTGFβRII transgenic cells, combination with decitabine will be tested in cohort 4 using the 3+3 rule.

4.1.2 Feasibility

This protocol attempts to administer a cell-based therapy that requires laboratory manipulation within the setting of Good Manufacturing Practices (GMP). This will require strict lot release criteria of the final product before administration. Feasibility will be determined at every cohort, and if 2 out of 6 patients cannot receive the intended cellular therapies, or there is suboptimal TCR transgenic cell persistence *in vivo*, further accrual will not be warranted. The feasibility assessment will be based on:

i) Potential problems in the manufacturing of NY-ESO-1 TCR/ dnTGFβRII engineered PBMC.

ii) Potential problems in the delivery of the proposed NY-ESO-1 TCR/ dnTGFβRII cell therapy.

4.2 Secondary Objectives

4.2.1 NY-ESO-1 TCR/ dnTGFβRII Transgenic T Cell Persistence

The persistence of NY-ESO-1 TCR/ dnTGFβRII-engineered cells will be determined by analyzing serial peripheral blood samples (described in **Section 8.4.3.2**) for the presence of T cells with the NY-ESO-1 TCR by tetramer analysis. In addition, and when technically feasible, samples from tumor sites will be biopsied and assessed for the presence of the NY-ESO-1 transgenic cells. V5 tagged 2A sequence linked to the dnTGFβRII and quantitative PCR will allow the assessment of NY-ESO-1 TCR/dnTGFβRII transgenic cells. This consecutive analysis from various time-points after infusion will allow us to test if TGFβ signaling blockage in T cells will allow survival, expansion and persistent NY-ESO-1 TCR engineered cells.

4.2.2 Study of transgenic T cell differentiation.

In order to study T cell differentiation that correlates with higher anti-tumor responses, we will study the different T cell subpopulations by immunophenotypic assays of NY-ESO-1 TCR/dnTGFβRII transgenic cells, using multicolor flow cytometry and immunohistochemistry.

PBMC and tumor infiltrating lymphocytes will be harvested at determined time points. We will be able to test the hypothesis that NY-ESO-1 TCR/ dnTGFβRII T cells will generate T memory cells, since blockage of immuno-suppressive signals by TGFβ would potentially allow better cell survival and hence facilitate further development of T memory phenotypes.

4.2.3 Functional studies of transgenic T cells

In order to test the hypothesis that NY-ESO-1 TCR/dnTGFβRII will constitute in cells more efficient in inducing tumor regression, we will perform specific T cell functional assays. PBMC and tumor infiltrating lymphocytes will be harvested at determined time points. We will use our methodology based on combination of NY-ESO-1 (157-165) tetramer and peptide stimulation to analyze TIL and PBMC samples with intracellular effector cytokine staining of TNFα and IFNγ, and Granzyme B/CD107a,b for cytotoxic T cell function. With our expertise in polychromatic flow cytometry, we will also be able to perform simultaneous multifunctional assessment of the transgenic cells. We will include bar-coded carrier cells, if necessary, to minimize cell loss.

4.2.4 Clinical Response

Objective tumor responses will be determined by irRECIST Immune-Related Response Criteria. The rate of irCR plus irPR will be used to explore antitumor activity. Response assessment will be performed by comparing standard CT imaging scans and photographs of target lesions from baseline with repeated imaging tests obtained at day +90 after the TCR transgenic PBMC adoptive transfer.

4.3 Exploratory Objectives

- Gut microbiota pre and post treatment to evaluate the role of microbiota on the therapeutic efficacy of the proposed therapy.

4.3.1 Gut Microbiota

Microbiome sequencing and bacterial 16s RNA expression will be studied to explore the therapeutic efficacy on changes in gut microbiota due to the proposed therapy. After initial eligibility has been determined, stool samples will be collected within 1 week of apheresis (day -6) and 12 weeks post T cell infusion. Samples will be stored at the DBBR laboratory at Roswell Park Cancer Institute and microbiome sequencing will be done at the Genomics Shared Resource (GSR), Roswell Park Cancer Institute.

The human microbiome plays an essential role in digestion and nutrient absorption, epithelial homeostasis, angiogenesis, and in the proper function of nervous and immune systems (45, 46). Disruption of the microbiome could lead to chronic inflammation, altered immune responses and carcinogenesis (47, 48). Most recent research shows that there is a complex reciprocal modulation between gut microbiota and the human brain, which involves interaction of the central nervous system, sympathetic and parasympathetic branches of the autonomic nervous system, the enteric nervous system, and elements of the neuroendocrine and neuroimmune systems. For example, some microbiota synthesize neurotransmitters directly (e.g., gamma-amino butyric acid) while many modulate the synthesis of neurotransmitters, such as serotonin, dopamine and

norepinephrine, and brain-derived neurotropic factor (49). Microbiotic abnormalities have been linked to reductions in neurotransmitter levels, cognitive deficits, anxiety, depression, chronic pain and fatigue (50, 51).

The composition of the microbiota also regulates the levels of tryptophan in the systemic circulation as well levels and nature of tryptophan catabolites (TRYCATs). These catabolites influence epithelial barrier integrity and the presence of an inflammatory or tolerogenic environment in the intestine and beyond (52). The composition of the microbiota also determines the levels and ratios of short chain fatty acids, such as butyrate and propionate, which are key energy source for colonocytes (53). Dysbiosis (altered microbiome) can lead to reduced levels of butyrate, and therefore may have adverse effects on epithelial barrier integrity, increase bacterial translocation into the systemic circulation, effects energy homeostasis, and the T helper 17/regulatory/T cell balance. (49, 53).

Increasing evidence suggests that different gut, skin, and vaginal microbiota profiles may be related to the etiology of certain gynecological cancers, such as cervical cancer, uterine cancer, and ovarian cancer (54). Patients' severity of side effects and response to certain cancer treatments can also be impacted by gut microbiome composition (47). In a recent study by Vetizou *et al.* it was shown that anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota and the authors showed the key role for *Bacteroidales* in the immunostimulatory effects of CTLA-4 blockade both in mice and patients (55). In another recent landmark paper by Sivan *et al.* (56) was shown that *Bifidobacterium* as associated with the antitumor effects and oral administration of Bifidobacterium alone improved tumor control to the same degree as PD-L1-specific antibody therapy, and combination treatment nearly abolished tumor outgrowth. Thus, the authors suggested that manipulating the microbiota may modulate cancer immunotherapy (56). Since data is more mature for gut microbiome and much less is known on pelvic/peritoneal or vaginal microbiome, only samples for gut microbiota will be collected in his study.

We will also ask our patients to complete basic surveys (refer to Appendix G and Appendix H) on their nutrition and lifestyle in order to gather a more comprehensive data on the interaction of microbiome and the immune system.

5 METHODOLOGY

5.1 Study Design

This is a single center, open label, Phase I/IIa study to assess the safety, feasibility, and objective tumor response of NY-ESO-1 coupled with dnTGFβRII in human subjects with advanced malignancies. Using the MTD of NY-ESO-1 TCR/dnTGFβRII transgenic cells determined in the first 3 cohorts, a fourth cohort (Cohort 4) will test the combination with decitabine.

Patients will undergo an initial leukapheresis to collect PBMC. The manufacture of transgenic T cells will be started on the day of leukapheresis. Immediately after processing the PBMC, $1x10^8$ to $3x10^9$ PBMC will be put in activation media containing OKT3 (anti-CD3 antibody) and IL-2 for two days, and the rest will be cryopreserved. Following activation, cells are transduced twice on two consecutive days with NY-ESO-1 TCR/ dnTGFβRII retrovirus in RetroNectin®-coated plates. Transduced cells will be expanded ex vivo for 4 days after the first transduction and then

put into infusion bags to be infused intravenously, fresh, on the day of harvest as soon as the lot release is cleared.

For Cohorts 1, 2 and 3, the next day after leukapheresis, patient will start receiving the non-myeloablative conditioning chemotherapy on Day -5 and -4 (45 mg/kg/day IV cyclophosphamide) to create space for the transgenic T cells. On day 0, patient will receive the cell infusion.

For Cohort 4, after leukapheresis, on the same day, Day -6, the patient will start receiving decitabine (20 mg/m^2 /day IV) for a total of 3 days (Day -6 to -4) followed by non-myeloablative conditioning chemotherapy on Day -3 and -2 (45 mg/kg/day IV cyclophosphamide). On day 0, similar to cohorts 1, 2 and 3, patient will receive the cell infusion.

In the event the attending physician decides that the patient will not be clinically fit to receive the adoptive cell transfer within 24 hours of the scheduled day of cell harvest, the cells will be cryopreserved on the harvest day for later use. Patients will undergo repeated peripheral blood sampling, CT scanning and biopsies of tumor deposits (see **Figure 1**).

Figure 1 Study Schematic (Cohorts 1, 2, and 3)

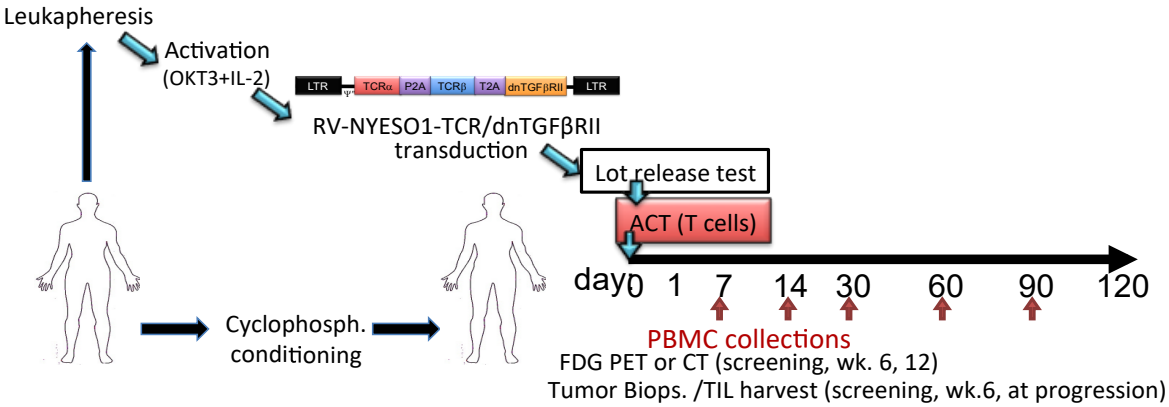

Figure 1: Schematic of clinical trial procedures. Patients will undergo leukapheresis to collect PBMC, which will be activated *in vitro* followed by transduction with retrovirus NY-ESO-1 TCR//dnTGFβRII. After passing lot release tests, patients pre-conditioned with lymphodepleting chemotherapy will receive the transgenic T cells. We will collect peripheral blood, perform PET/CT scanning and biopsies of tumors.

The schema for cohort 4 is the same as above except Decitabine will be given after leukapheresis on Days -6 to -4 (total 3 days) and Cyclophosphamide will be given on Days -3 and -2.

All patients will sign an informed consent prior to study related tests. All patients will meet the inclusion and exclusion criteria summarized in **Section 6.1**and **Section 6.2**. Conditioning chemo could be as an outpatient or inpatient, according to the investigator's discretion but ACT will be done as an inpatient.

Subjects in whom disease control was observed after the T cell infusion may be eligible for a second transgenic T cell infusion if the subject still meets the eligibility criteria in Section 6 below.

5.2 Target Accrual and Study Duration

A maximum of 27 patients at RPCI will be enrolled. Accrual is expected to take up to 4 years.

6 PATIENT SELECTION

6.1 Inclusion Criteria

To be included in this study, patients must meet the following criteria:

1. Patients with solid tumors as described below:
   a) Inoperable or metastatic (advanced) melanoma:

- Has received, is intolerant, or refused a CTLA-4 inhibitor (Ipilimumab) or a PD-1 inhibitor (Nivolumab or Pembrolizumab) as monotherapy and/or a combination of Ipilimumab and Nivolumab.
- Has received or is intolerant of a BRAF inhibitor or the combination of BRAF and MEK inhibitors for BRAFv600 mutant melanoma and a PD-1 inhibitor as monotherapy or in combination.

b) Inoperable or metastatic (advanced) ovarian, primary peritoneal or fallopian tube carcinoma:
- Has received platinum containing chemotherapy and has platinum refractory or resistant disease that has progressed on second line therapy
- If platinum sensitive disease, should have received ≥2 lines of chemotherapy.
- May have received PARP inhibitors, bevacizumab or other targeted VEGF inhibitor therapy

c) Inoperable or metastatic (advanced) synovial sarcoma:
- Should have received and progressed on ≥ two lines of systemic therapy

d) Subjects with other histologies:
- Must have previously received two lines of systemic standard care (or effective salvage chemotherapy regimens) for metastatic disease, if known to be effective for that disease, and have been deemed either non-responders (progressive disease) or have recurred.

2. For cohorts 1, 2 and 3 only: Patient's tumor must be positive by histological or molecular assay for NY-ESO-1, according to the screening algorithm as described in Section 9.1 under "tumor biopsy". Historical results may be used.

   For Cohort 4, NY-ESO-1 results will be noted but NY-ESO-1 positivity is not required for eligibility.
3. HLA-A*0201 (HLA-A2.1) positivity by molecular subtyping (blood test or buccal swab, historical documentation acceptable).
4. Age ≥ 18 years old, (Cohort 4: Age ≥ 12 years old).
5. Life expectancy greater than 3 months assessed by a study physician.
6. Have been informed of other treatment options.
7. A minimum of one measurable lesion defined as:
   - Meeting the criteria for measurable disease according irRECIST criteria.
   - For patients with skin metastases, lesions selected as non-completely biopsied target lesion(s) that can be accurately measured and recorded by color photography with a ruler to document the size of the target lesion(s).
8. No restriction based on prior treatments but at least 4 weeks from prior immunotherapy, or prior investigational agents. **Note:** Patients who have suffered >grade 2 irAEs during previous checkpoint inhibitor therapy should be excluded.
9. ECOG performance status (PS) 0 or 1 (**Appendix A**).

10. Must have adequate venous access for apheresis.
11. Women of childbearing potential and men must agree to use effective methods of birth control for the duration of the study and 6 months after. Methods for effective birth control include: condoms, diaphragm or cervical cap with spermicide, intrauterine device, and hormonal contraception. It is recommended that a combination of two methods be used.
12. Patients must have normal organ and marrow function as defined below:
    - Leukocytes: ≥ 3,000/mcl
    - Absolute Neutrophil Count: ≥ 1,000/mcl
    - Platelets: ≥ 100,000/mcl
    - Total bilirubin: ≤ 1.5 ULN
    - AST (SGOT)/ALT (SGPT): ≤ 2.5 x institutional upper limit of normal
    - Creatinine: ≤ 2X ULN; if creatinine > 2X ULN, creatinine clearance should be > 60 ml/min.
13. Must be willing and able to accept the leukapheresis procedure.
14. At screening, must have tissue available for NY-ESO-1 testing (if not previously performed) or be willing and able to undergo a fresh tissue biopsy.
15. Patient must understand the investigational nature of this study and sign an Independent Ethics Committee/Institutional Review Board approved written informed consent form prior to receiving any study related procedure.
16. Participant must agree to and arrange for a caregiver (age ≥ 18 years old) available 24 hours a day/ 7 days a week and arrange for lodging within a45 minute drive to Roswell Park and transportation for a period of time after discharge from the hospital. The exact amount of time will depend on the individual status as determined by the treating physician.

6.2 Exclusion Criteria

Patients will be excluded from this study for the following:

1. Previously known hypersensitivity to any of the agents used in this study.
2. Currently receiving any other investigational agents. **Note:** Patients who have suffered >grade 2 irAEs during previous checkpoint inhibitor therapy should be excluded.
3. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements. EKG will be done at screening. Cardiac stress test will be done as clinically indicated, the specific test to be chosen at the discretion of the treating physician.
4. History of severe autoimmune disease requiring steroids or other immunosuppressive treatments.
5. History of inflammatory bowel disease, celiac disease, or other chronic gastrointestinal conditions associated with diarrhea or bleeding, which in the investigator's opinion would

place the patient at an increased risk for adverse effect or current acute colitis of any origin. Treated cases with no active disease are eligible.

6. Potential requirement for systemic corticosteroids or concurrent immunosuppressive drugs based on prior history or received systemic steroids within the last 4 weeks prior to enrollment (inhaled or topical steroids at standard doses or isolated use of steroids as premedication for medical procedures to minimize allergic reaction [e.g. CT scan dye] are allowed).
7. Known active infection with HIV, Hepatitis B, Hepatitis C or CMV.
8. Known cases of clinically active brain metastases (brain MRI as clinically indicated). Prior evidence of brain metastasis successfully treated with surgery or radiation therapy will not be exclusion for participation as long as they are deemed under control at the time of study enrollment.
9. Dementia or significantly altered mental status that would prohibit the understanding or rendering of compliance with the requirements of this protocol even with caregiver support.
10. Pregnancy or breast-feeding. Female patients must be surgically sterile or be postmenopausal for two years, or must agree to use effective contraception during the period of treatment and 6 months after. All female patients with reproductive potential must have a negative pregnancy test (serum/urine) within 48 hours from starting the conditioning chemotherapy.
11. Lack of availability of a patient for immunological and clinical follow-up assessment.

6.3 Eligibility Criteria for a Second Transgenic T cell infusion

Subjects in whom disease control was observed after the T cell infusion may be eligible for a second transgenic T cell infusion if the subject meets the following criteria:

- Patient attained confirmed disease control (either CR, PR or SD) after the first transgenic T cell infusion.
- Patient still meets the eligibility criteria in Section 6.1 and 6.2.
- A second T cell infusion is discussed and the patient agrees to receive it.
- Either cryopreserved extra cells from the first T cell product is available or cryopreserved autologous PBMC is available for the manufacture of a second transgenic T cell product.

6.4 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this study. Subjects of any gender and ethnicity with advanced solid tumor expressing NY-ESO-1 will be considered in the proposed clinical trials (NY-ESO-1 positivity not required for participants enrolling in cohort 4). There has been and will certainly be no discrimination due to gender or ethnicity, but the epidemiology of the disease and HLA haplotypes may result in uneven minority population distribution.

6.5 Subject Recruitment

Study subjects will be invited to participate by the study investigators at RPCI as part of the discussion of standard and experimental therapy for locally advanced or metastatic melanoma, epithelial ovarian cancer, synovial sarcoma or other advanced malignancies expressing NY-ESO-1 for which no suitable standard of care exists. Patients may access the study investigators through:

a) Subjects may be referred to RPCI from outside clinics. No patient will be approached without a referral or agreement from the patient's physician in order to uphold patient confidentiality

b) Subjects may be identified by the study investigators from their own patient pools while discussing standard and experimental options for advanced malignancies.

c) Study flyers will be posted in public places, including the oncology clinic waiting room, for subject self-referral.

d) Subjects may self-refer to this study when inquiring about clinical trial options at RPCI.

e) This study will be listed at the NIH web site listing active clinical trials, which also assures trial registration at a public site.

7 TREATMENT PLAN

7.1 Dosing and Administration

Cell therapy will be administered on an inpatient basis. All patients will receive the same combined therapy of: 1) a lympho-depleting preparative regimen (outpatient or inpatient), 2) infusion of autologous PBMC retrovirally-transduced with NY-ESO-1 TCR/ dnTGFβRII. Cohort 4 will receive decitabine prior to lympho-depleting preparative regimen and NY-ESO-1 TCR/ dnTGFβRII transgenic T cells.

All patients will undergo leukapheresis to collect PBMC [study Day (-6)]. The manufacture of transgenic T cells will be started on the day of leukapheresis (transduced cells will be expanded ex vivo for 4 days after the first transduction and then put into infusion bags to be infused intravenously, fresh, on the day of harvest as soon as the lot release is cleared).

Patients in cohorts 1, 2 and 3 will receive the non-myeloablative conditioning chemotherapy with cyclophosphamide [intravenous injection of 45 mg/kg/day (use adjusted body weight if > 120% of ideal body weight; Male: IBW= 50 + 0.91 (Ht in cm -152), Female: IBW= 45 + 0.91 (Ht in cm-152)) study Day (-5) and study Day (-4)]. On study Day 0, the patient will receive a single dose of the transgenic NY-ESO-1 TCR/dnTGFβRII PBMC cell infusion as an inpatient.

Patients in cohort 4 will receive Decitabine at a dose of 20 mg/m^2 /day IV daily on days -6 after leukapheresis to -4 of treatment, for a total of 3 days. On days -3 and -2, patients will receive the conditioning regimen consisting of 45 mg/kg/day IV cyclophosphamide for two days to create space for the transgenic T cells. On study Day 0, the patient will receive a single dose of the transgenic NY-ESO-1 TCR/dnTGFβRII PBMC cell infusion as an inpatient.

Reported adverse events (AEs) and potential risks are described in **Section 8.1.2.1.** Appropriate dose modifications are described in **Section 7.4.**

7.1.1 Leukapheresis for NY-ESO-1 TCR PBMC Product

On Day (-6), patients will undergo a 12 liter leukapheresis at the RPCI Blood Donor Facility, which is performed as an outpatient procedure. The leukapheresis product will be transferred to the GMP Suite for cell processing, at the Therapeutic Cell Production Facility, Center for Immunotherapy for the manufacture of the PBMC expressing NY-ESO-1 TCR/ dnTGFβRII cells for infusion on Day 0 (Appendix B).

7.1.2 Cyclophosphamide:

For Cohorts 1, 2 and 3, non-myeloablative chemotherapy with cyclophosphamide 45 mg/kg in 500 cc NS IV (CTX) is administered in accordance with Institutional standards on Day-5 and Day-4 (adjusted body weight will be used if > 120% of ideal body weight, Male: IBW= 50 + 0.91 (Ht in cm -152), Female: IBW= 45 + 0.91 (Ht in cm- 152)).

For Cohort 4, non-myeloablative chemotherapy with the same dose of cyclophosphamide 45 mg/kg in 500 cc NS IV (CTX) is administered over 2 hours or in accordance with Institutional standards on Day-3 and Day-2 (adjusted body weight will be used if > 120% of ideal body weight, Male: IBW= 50 + 0.91 (Ht in cm -152), Female: IBW= 45 + 0.91 (Ht in cm- 152)). This shift is because cohort 4 receives decitabine. **Note:** For pediatric patients, the following Traub equation for IBW will be utilized: for children shorter than 5 ft, the IBW in kilograms is calculated as ([height in inches]^2~ 1.65)/1000; for boys taller than 5 ft, the IBW in kilograms is calculated as 39 + (2.27 x [height in inches − 60]); for girls taller than 5 ft, the IBW in kilograms is calculated as 42 + (2.27 x [height in inches − 60]).

Treatment will be administered on an outpatient or inpatient basis, according to the investigator's discretion. Premedication is recommended and may be given in accordance with Institutional standards. Aspirin may be substituted for acetaminophen for patients allergic to acetaminophen. Hydration with normal saline (250 cc/ hour) is given for 2 hours, or a bolus infusion if suitable or in accordance with institutional standards. Mesna (15 mg/kg in 50 cc NS) can be administered IV over 30 minutes for 2 doses starting immediately prior to and at 4 hours after the completion of cyclophosphamide. Premedication with anti- emetics, chosen as determined by the investigator, is provided and as needed after the CTX.

At the investigators' discretion, patients may be hospitalized for the cytoreductive chemotherapy.

7.1.2.1 Toxicities Associated with Cyclophosphamide

Hematologic toxicity occurring with cyclophosphamide usually includes leukopenia and thrombocytopenia. Anorexia, nausea and vomiting, rash and alopecia occur, especially after high-dose cyclophosphamide; diarrhea, hemorrhagic colitis, infertility, and mucosal and oral ulceration have been reported. Sterile hemorrhagic cystitis occurs in about 20% of patients; severity can range from microscopic hematuria to extensive cystitis with bladder fibrosis. Although the incidence of hemorrhagic cystitis associated with cyclophosphamide appears to be lower than that associated with ifosfamide, mesna (sodium 2-mercaptoethanesulfonate) has been used prophylactically as an

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, alkalization 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 (SIADH) 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.

7.1.3 Decitabine

Only Cohort 4 receives Decitabine (20 mg/m^2 IV) after leukapheresis, administered daily on Days -6 to -4 by IV infusion over 1 hour. Decitabine treatment is planned to be administered on an outpatient basis, however, patient may be hospitalized if clinically indicated.

7.1.3.1 Toxicities Associated with Decitabine

Expected toxicities of decitabine are listed in the product label. Most commonly occurring adverse reactions are neutropenia, thrombocytopenia, anemia, febrile neutropenia, leukopenia, lymphadenopathy, fatigue, pyrexia, nausea, cough, petechiae, constipation, diarrhea and hyperglycemia.

7.1.4 Transgenic NY-ESO-1 TCR/dnTGFβRII PBMC Infusion: Day 0

The TCR genetically engineered PBMC product will be transported to the in-patient unit on ice in a rigid-walled container with a tight fitting lid (i.e. Tupperware; Nalgene container etc.) and labeled with a biohazard label. The T cell Infusion will be administered through a peripheral IV without lymphocyte filter, a Hohn catheter or central line by rapid infusion to gravity over approximately 15 minutes. Vital signs are recorded prior to, during, and at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, and 4 hours post-infusion. If any patient develops a temperature> 38.5ºC (101.3ºF), the Principal Investigator should be notified. Patients will be hospitalized for the cell infusion and discharged when patient condition is stable and approved by the attending physician.

In the event that any patient develops sepsis or bacteremia following the T cell infusion, appropriate cultures and medical management should be initiated. If a contaminated T cell product is suspected, the cell product can be re-tested for sterility using archived samples.

7.1.5 Second Transgenic NY-ESO-1 TCR/dnTGFβRII PBMC Infusion

Eligible patients who showed initial response/disease control may receive a second T cell infusion at any time after progression is confirmed. Bridging therapy is allowed before the second T cell dosing. For this second T cell infusion, all cohort patients will receive the same highest study dose (Cohort 3 dose of 0.5e9 transgenic T cells or higher, up to 1e9). The Chemo-conditioning, decitabine (for Cohort 4), and T cell infusion will follow a similar procedure and schedule as described in Sections 7.1.2, 7.1.3 and 7.1.4 respectively and Section 9 Study Procedures. The second T cell infusion may be administered without chemo-conditioning in the following conditions:

- Evidence of marrow involvement that may cause count recovery failure after chem-conditioning
- Patient had delayed count recovery after the chemo-conditioning for the first T cell infusion (prolonged neutropenia: ANC <500 for >15 days and prolonged thrombocytopenia: platelet <50,000/μL for >15 days, with growth factor support)
- Presence of any other risk for delayed count recovery as determined by the principal investigator or the treating physician
- Presence of transgenic T cells in the peripheral blood

Note: Biopsy tissue may be collected before and after the 2nd T cell infusion when a biopsy is clinically indicated. Blood/serum sample collections will follow the same schedule as for the first infusion. With the re-start of this collection schedule after the second infusion, the initial schedule after the first infusion will be stopped. The starting point of LTFU for up to 15 years will start from the second T cell infusion date. Apheresis and Microbiome/stool samples will not be collected for this second cycle.

7.2 Cohort Management

The Phase 1 part of the study will have three cohorts of three patients, with three patients added to any cohort with a dose limiting toxicity (DLT). If 2 out of 3 to 6 subjects have a DLT, dose escalation will be halted and the cohort will be declared beyond the maximum tolerable dose (MTD). Subsequently, an additional cohort # 4 with a total of 9 patients will receive NY-ESO-1 TCR/dnTGFb-RII transgenic cells at the highest tolerated dose in combination with decitabine. Overall, accrual is expected to be between 18-27 patients [18 if no DLT occurs and 27 if DLT occurs in all dose levels (Cohort 1 has been completed with 3 patients), it will be 9 subjects plus 3 extra per dose level, and the possibility of an expanded cohort of 9 patients at the highest tolerated dose level determined (MTD)].

Participants who withdraw early after receiving the T-cell infusion will be considered evaluable and will not be replaced in their position in the cohort. Participants who have not yet been infused will not be considered evaluable and will be replaced with another patient.

Table 1 provides a description of the doses of the proposed combined therapy with a dose escalation of NY-ESO-1 TCR / dnTGFβRII transgenic cells in 4 cohorts of patients.

Table 1 Dose Escalation for NY-ESO-1 TCR / dnTGFβRII Transgenic Cells

Cohort	Number of patients	Decitabine	Cyclophosphamide Conditioning	NY-ESO-1 TCR/ dnTGFβRII cell dose
1	3 (+3)	None	45 mg/kg x 2 days	1 x 10^7 iv x 1 infusion
2	3 (+3)	None	45 mg/kg x 2 days	1 x 10^8 iv x 1 infusion
3	3 (+3)	None	45 mg/kg x 2 days	1 x 10^9 iv x 1 infusion (0.5e9 or higher, up to 1e9 acceptable)
4	3 (+3) +6	20 mg/m^2 x 3 days	45 mg/kg x 2 days	MTD: 1 x 10^9 iv x 1 infusion (0.5e9 or higher, up to 1e9 acceptable) *

* After completion of the first 3 cohorts, the Cohort 3 dose of 1 x 10^9 iv (0.5e9 or higher, up to 1e9 acceptable) has been declared as the MTD.

7.3 Definition of Dose-Limiting Toxicity

A dose limiting toxicity (DLT) is defined as something which is both:

- Any untoward or adverse event which seems at least possibly related to the engineered T cell therapy, *plus*:
- Any Grade 3 or higher event that is sustained for more than 1 week, excepting autoimmune disorders and chemotherapy related adverse events. For cytokine release syndrome, the event must be a grade 4 adverse event and sustain at grade 4 for more than1week to be considered a DLT.

Any Grade 3 or higher autoimmune disorder that cannot be resolved to less than or equal to a Grade 2 autoimmune toxicity within 100 days should also be deemed a DLT.

Any chemotherapy-related event should not normally be considered a DLT unless the investigator is under the reasonable impression that the modified TCR therapy specifically has made the event worse in which case it will be considered a DLT.

The DLT time period is 30 days post T cell infusion.

7.4 Dose Modifications

Each patient receives only a single dose of transgenic T cells and hence dose modification within a patient not anticipated.

7.4.1 Treatment Delay

Each patient receives only a single dose of transgenic T cells and hence treatment delay not anticipated. In the event the patient is not clinically fit to receive the gene-modified NY-ESO-1 TCR cell infusion within 24 hours of the scheduled day of cell harvest, the cells will be cryopreserved on the harvest day for later use when the physician determines the patient's condition to be fit. If in case a patient already received chemo-conditioning, but not able to receive

the cells as scheduled for medical reasons, the cryopreserved cells may be infused within 2 weeks after the completion of chemo-conditioning. For more than 2 weeks delay, based on clinical judgement and patient's acceptance, patient may receive a second lymphodepletion (chemo-conditioning) at a time patient is determined by the investigator to be clinically fit to receive the cell infusion. The first T cell infusion should take place within 6 months of the cell manufacture date.

7.5 General Concomitant Medication and Supportive Care

7.5.1 Supportive Therapy on Day 0, Administration of T cells

About 30 minutes to 1 hour prior to Infusion, subjects are given acetaminophen 650 mg p.o. and diphenhydramine 50 mg p.o. The T cell Infusion will be administered through a peripheral IV without lymphocyte filter, a Hohn catheter or central line by rapid infusion to gravity over approximately15 minutes. Vital signs during infusion will be done before, after, and q15 minutes if longer than 15 minutes infusion.

Mild transient symptoms have been observed with LAK and TIL cell infusions and, with infusions of antigen-specific T cell clones. The management of these symptoms is outlined below or, can be done in accordance with institute standards:

- Fever, chills and temperature elevations > 101˚F will be managed with acetaminophen 650 mg p.o. q 4-6 hours. All subjects that develop fever or chills will have a blood culture drawn.
- Headaches may be managed with acetaminophen following a neurologic examination.
- Nausea, vomiting will be treated with a non-steroidal anti-emetic of choice.
- Hypotension will initially be managed by intravenous fluid administration and further measures as dictated by standard medical practice.
- Hypoxemia will initially be managed with supplemental oxygen and further measures as dictated by standard medical practice.

7.5.2 Potential Toxicities Warranting Ablation of Adoptively Transferred T Cells

The following will be considered toxicities warranting ablation of transferred T cell clones by corticosteroid administration:

- Grade 3 or greater toxicity occurring in any other organ system following administration of antigen-specific cytotoxic T cells not attributable to underlying metastatic cancer and excluding known toxicities of cyclophosphamide.
- Any grade 3 or greater toxicities warranting ablation of cells by corticosteroids will be submitted to the FDA as an expedited SAE.

7.5.2.1 **Treatment with Corticosteroids**

- Patients will receive corticosteroids if treatment-related toxicity warranting abrogation of T cells is observed.
- The in vivo frequency of infused CD8+ T cells will be assayed immediately prior to and 48 hours after the start of steroid therapy.
- All patients may be hospitalized for the first 48 hours for monitoring. The following dose schedule may be used or as per institutional practices:

Day 1	Intravenous methylprednisolone* at 2 mg/kg
Day 2	Intravenous methylprednisolone* at 2 mg/kg
Day 3 - 4	Prednisone at 30 mg po b.i.d.
Day 5 - 6	Prednisone at 15 mg po b.i.d.
Day 7 - 8	Prednisone at 10 mg po b.i.d.
Day 9 - 10	Prednisone at 10 mg po q.d
Day 11 - 12	Prednisone at 5 mg po q.d.

*Consider bismuth, ranitidine and misoprostol ulcer prophylaxis, Dexamethasone may be used instead at 0.5 mg/kg.

7.5.2.2 **Treatment with Tocilizumab or Infliximab/Etanercept**

Engineered T cell therapy is rapidly advancing, and potent clinical responses have been associated with acute cytokine release syndrome which often requires intensive care to manage(57, 58). Recent studies in adoptive cell therapy and immunotherapy with blinatumomab have revealed that the use of the anti-IL 6 humanized antibody, Tocilizumab, and the anti-TNF-α antibody, etanercept or infliximab, can resolve CRS within hours and with a single dose (57). There have also been well characterized pre-clinical and clinical reports of Tocilizumab making a marked difference to patients with intractable/steroid resistant, grade IV (grading is from 1-mild to IV four-severe) graft versus host disease after transplantation.

In one case of CRS, the use of steroids did not abrogate CRS, though this was subsequently controlled by the anti-cytokine antibodies (57) implying that the antibodies not only provide an adequate alternative to steroids but that they are in fact likely a superior alternative. Furthermore, with the avoidance of steroids, these patients have been able to go on and have good responses to therapy. This is preferable as multiple pre-clinical and clinical reports (59) indicate that high dose steroids largely destroy the effects of the T cell therapy on the target. Although these anti-cytokine agents are associated with immune suppression when dosed repeatedly in the context of disease like arthritis or psoriasis there is little evidence to suggest risk in this on-off dosing context where the immediate benefits may well be life-saving (57, 58, 60).

Due to the 1-2 day turn-around time reasonably expected for cytokine results, it is reasonable to consider administration of Tocilizumab based on clinical evidence of CRS, and before having the cytokine data in hand. Tocilizumab has been used at a dose of 8 mg/kg stat and etanercept at a single dose of 50mg.

To abrogate acute cytokine release treatment with Tocilizumab or Etanercept, the treatment doses below are recommended; however, treatment is to be given per investigator's discretion and in accordance with institutional practices:

- Tocilizumab 8 mg/kg immediately, IV (max 800 mg)
- Etanercept 50 mg one single dose, SC
- Infliximab 10 mg/kg, IV

Other additional agents that could be potentially used are Basiliximab (Simulect), an IL-2 receptor target, Siltuximab (Sylvant), an anti-IL-6 monoclonal antibody, Anakinra (Kineret), an IL-1R antagonist.

7.5.2.3 **Tocilizumab: Drug Information**

Initial U.S. Approval: 2013

U.S. BRAND NAMES: ACTEMRA8 (tocilizumab) ADMINISTRATION: Injection, for intravenous infusion.

ADVERSE REACTIONS: Most common adverse reactions (incidence ~ 5%): upper respiratory tract infections, nasopharyngitis, headache, hypertension, increased ALT.

WARNINGS AND PRECAUTIONS

- Serious Infections
    - Serious and sometimes fatal infections due to bacterial, mycobacterial, invasive fungal, viral, protozoa, or other opportunistic pathogens have been reported in patients receiving immunosuppressive agents including ACTEMRA for rheumatoid arthritis. The most common serious infections included pneumonia, urinary tract Infection, cellulitis, herpes zoster, gastroenteritis, diverticulitis, sepsis and bacterial arthritis. Among opportunistic infections, tuberculosis, cryptococcus, aspergillosis, candidiasis, and pneumocystosis were reported with ACTEMRA. Other serious infections, not reported in clinical studies, may also occur (e.g., histoplasmosis, coccidioidomycosis, and listeriosis). Patients have presented with disseminated rather than localized disease, and were often taking concomitant immuno-suppressants such as methotrexate or corticosteroids which in addition to rheumatoid arthritis may predispose them to infections.
    - ACTEMRA should not be administered in patients with an active infection, including localized infections. The risks and benefits of treatment should be considered prior to initiating ACTEMRA in patients:
        - with chronic or recurrent infection;
        - who have been exposed to tuberculosis;
        - with a history of serious or an opportunistic infection who,
        - have resided or travelled in areas of endemic tuberculosis or endemic mycoses; or, with underlying conditions that may predispose them to infection.

- Patients should be closely monitored for the development of signs and symptoms of infection during and after treatment with ACTEMRA, as signs and symptoms of acute inflammation may be lessened due to suppression of the acute phase reactants.
- ACTEMRA should be interrupted if a patient develops a serious infection, an opportunistic infection, or sepsis. A patient who develops a new infection during treatment with ACTEMRA should undergo a prompt and complete diagnostic workup appropriate for an immunocompromised patient, appropriate antimicrobial therapy should be initiated, and the patient should be closely monitored.

- Tuberculosis
  - Patients should be evaluated for tuberculosis risk factors and tested for latent Infection prior to initiating ACTEMRA. Anti-tuberculosis therapy should also be considered prior to initiation of ACTEMRA in patients with a past history of latent or active tuberculosis in whom an adequate course of treatment cannot be confirmed, and for patients with a negative test for latent tuberculosis but having risk factors for tuberculosis infection. Consultation with a physician with expertise in the treatment of tuberculosis is recommended to aid in the decision whether initiating antituberculosis therapy is appropriate for an individual patient.
  - Patients should be closely monitored for the development of signs and symptoms of tuberculosis including patients who tested negative for latent tuberculosis infection prior to initiating therapy.
  - It is recommended that patients be screened for latent tuberculosis infection prior to starting ACTEMRA.
  - The incidence of tuberculosis in worldwide clinical development programs is 0.1%. Patients with latent tuberculosis should be treated with standard antimycobacterial therapy before initiating ACTEMRA.
- Viral Reactivation
  - Viral reactivation has been reported with immunosuppressive biologic therapies and cases of herpes zoster exacerbation were observed in clinical studies with ACTEMRA. No cases of Hepatitis B reactivation were observed in the trials; however patients who screened positive for hepatitis were excluded.
- Gastrointestinal Perforations
  - Events of gastrointestinal perforation have been reported in clinical trials, primarily as complications of diverticulitis. ACTEMRA should be used with caution in patients who may be at increased risk for gastrointestinal perforation. Patients presenting with new onset abdominal symptoms should be evaluated promptly for early identification of gastrointestinal perforation.
- Laboratory Parameters

- Neutrophils: Treatment with ACTEMRA was associated with a higher incidence of neutropenia. Infections have been uncommonly reported in association with treatment-related neutropenia in long-term extension studies and postmarketing clinical experience. It is not recommended to initiate ACTEMRA treatment in patients with a low neutrophil count L [absolute neutrophil count (ANC) <2000/mm3]. In patients who develop an absolute neutrophil count <500/mm3 treatment is not recommended. Neutrophils should be monitored every 4 to 8 weeks.
- Platelets: Treatment with ACTEMRA was associated with a reduction in platelet counts. Treatment-related reduction in platelets was not associated with serious bleeding events in clinical trials.- It is not recommended to initiate ACTEMRA treatment in patients with a platelet count below 100,000/mm3. In patients who develop a platelet count <50,000/mm3 treatment is not recommended. Platelets should be monitored every 4 to 8 weeks.

- Liver Function Tests: Treatment with ACTEMRA was associated with a higher incidence of transaminase elevations. These elevations did not result in apparent permanent or clinically evident hepatic injury in clinical trials. Increased frequency and magnitude of these elevations was observed when potentially hepatotoxic drugs (e.g., MTX) were used in combination with ACTEMRA. In one case, a patient who had received ACTEMRA 8 mg/kg monotherapy without elevations In transaminases experienced elevation in AST to above 10x ULN and elevation in ALT to above 16x ULN when MTX was initiated in combination with ACTEMRA. Transaminases normalized when both treatments were held, but elevations recurred when MTX and ACTEMRA were restarted at lower doses. Elevations resolved when MTX and ACTEMRA were discontinued. It is not recommended to initiate ACTEMRA treatment in patients with elevated transaminases ALT or AST> 1.5x ULN. In patients who develop elevated ALT or AST> 5x ULN treatment is not recommended. ALT and AST levels should be monitored every 4 to 8 weeks. When clinically indicated, other liver function tests such as bilirubin should be considered.
- Lipids: Treatment with ACTEMRA was associated with increases in lipid parameters such as total cholesterol, triglycerides, LDL cholesterol, and/or HDL cholesterol. Assessment of lipid parameters should be performed approximately 4 to 8 weeks following initiation of ACTEMRA therapy, then at approximately 6 month intervals. Patients should be managed according to clinical guidelines [e.g., National Cholesterol Educational Program (NCEP)] for the management of hyperlipidemia.
- Immunosuppression: The impact of treatment with ACTEMRA on the development of malignancies is not known but malignancies were observed in clinical studies. ACTEMRA is an immunosuppressant, and treatment with immunosuppressants may result in an increased risk of malignancies.
- Hypersensitivity Reactions: Serious hypersensitivity reactions, including anaphylaxis, have been reported in association with infusion of ACTEMRA. Appropriate medical treatment should be available for immediate use in the event of an anaphylactic reaction during administration of ACTEMRA.

- Demyelinating Disorders: The impact of treatment with ACTEMRA on demyelinating disorders is not known, but multiple sclerosis and chronic inflammatory demyelinating polyneuropathy were reported rarely in clinical studies. Patients should be closely monitored for signs and symptoms potentially Indicative of demyelinating disorders. Prescribers should exercise caution in considering the use of ACTEMRA in patients with preexisting or recent onset demyelinating disorders.
- Active Hepatic Disease and Hepatic Impairment: Treatment with ACTEMRA is not recommended in patients with active hepatic disease or hepatic impairment.
- Vaccinations: Live vaccines should not be given concurrently with ACTEMRA as clinical safety has not been established. No data are available on the secondary transmission of infection from persons receiving live vaccines to patients receiving ACTEMRA. No data are available on the effectiveness of vaccination in patients receiving ACTEMRA. Because IL-6 inhibition may interfere with the normal immune response to new antigens, patients should be brought up to date on all recommended vaccinations, except for live vaccines, prior to initiation of therapy with ACTEMRA.

MECHANISM of ACTION: Tocilizumab binds specifically to both soluble and membrane-bound IL-6 receptors (sIL-6R and mIL-6R), and has been shown to inhibit IL-6-mediated signaling through these receptors. IL-6 is a pleiotropic pro-inflammatory cytokine produced by a variety of cell types including T- and B-cells, lymphocytes, monocytes and fibroblasts. IL-6 has been shown to be involved in diverse physiological processes such as T-cell activation, induction of immunoglobulin secretion, initiation of hepatic acute phase protein synthesis, and stimulation of hematopoietic precursor cell proliferation and differentiation. IL-6 is also produced by synovial and endothelial cells leading to local production of IL-6 in joints affected by inflammatory processes such as rheumatoid arthritis.

7.5.2.4 **Infliximab: Drug Information**

Initial U.S. Approval: 1998

U.S. BRAND NAMES - Remicade® (Infliximab)

ADMINISTRATION: Lyophilized Concentrate for Injection, for Intravenous Use

ADVERSE REACTIONS

- SIGNIFICANT: Most common adverse reactions (>10%) – infections (e.g. upper respiratory, sinusitis, and pharyngitis), infusion-related reactions, headache, and abdominal pain.
- WARNINGS AND PRECAUTIONS
  - Serious infections – do not give REMICADE during an active infection. If an infection develops, monitor carefully and stop REMICADE if infection becomes serious.
  - Invasive fungal infections – for patients who develop a systemic illness on REMICADE, consider empiric antifungal therapy for those who reside or travel to regions where mycoses are endemic

- Malignancies – the incidence of malignancies including lymphoma was greater in REMICADE treated patients than in controls. Due to the risk of HSTCL carefully assess the risk/benefit especially if the patient has Crohn's disease or ulcerative colitis, is male, and is receiving azathioprine or 6-mercaptopurine treatment.
- Hepatotoxicity – rare severe hepatic reactions, some fatal or necessitating liver transplantation. Stop REMICADE in cases of jaundice and/or marked liver enzyme elevations.
- Heart failure –new onset or worsening symptoms may occur.
- Cytopenias – advise patients to seek immediate medical attention if signs and symptoms develop, and consider stopping REMICADE.
- Hypersensitivity – serious infusion reactions including anaphylaxis or serum sickness-like reactions may occur.
- Demyelinating disease –exacerbation or new onset may occur.
- Lupus-like syndrome – stop REMICADE if syndrome develops.
- Live vaccines or therapeutic infectious agents – should not be given with REMICADE.

MECHANISM OF ACTION: Infliximab neutralizes the biological activity of TNFαby binding with high affinity to the soluble and transmembrane forms of TNFαand inhibits binding of TNF

α with its receptors. Infliximab does not neutralize TNFβ (lymphotoxin-α), a related cytokine that utilizes the same receptors as TNFα. Biological activities attributed to TNFα include: induction of pro-inflammatory cytokines such as interleukins (IL) 1 and 6, enhancement of leukocyte migration by increasing endothelial layer permeability and expression of adhesion molecules by endothelial cells and leukocytes, activation of neutrophil and eosinophil functional activity, induction of acute phase reactants and other liver proteins, as well as tissue degrading enzymes produced by synoviocytes and/or chondrocytes. Cells expressing transmembrane TNF

α bound by infliximab can be lysed in vitro or in vivo. Infliximab inhibits the functional activity of TNFαin a wide variety of in vitro bioassays utilizing human fibroblasts, endothelial cells, neutrophils, B and T- lymphocytes and epithelial cells. The relationship of these biological response markers to the mechanism(s) by which REMICADE exerts its clinical effects is unknown. Anti-TNFα antibodies reduce disease activity in the cotton-top tamarin colitis model, and decrease synovitis and joint erosions in a murine model of collagen-induced arthritis. Infliximab prevents disease in transgenic mice that develop polyarthritis as a result of constitutive expression of human TNFα, and when administered after disease onset, allows eroded joints to heal.

7.5.2.5 **Etanercept: Drug Information**

Initial U.S. Approval: 1998

U.S. BRAND NAMES - Enbrel® (etanercept)

ADMINISTRATION: Solution for Subcutaneous Use

ADVERSE REACTIONS

- SIGNIFICANT: Most common adverse reactions (incidence > 5%): infections and injection site reactions.
- WARNINGS AND PRECAUTIONS
  - Do not start Enbrel during an active infection. If an infection develops, monitor carefully and stop Enbrel if infection becomes serious.
  - Consider empiric anti-fungal therapy for patients at risk for invasive fungal infections who develop a severe systemic illness on Enbrel (those who reside or travel to regions where mycoses are endemic).
  - Demyelinating disease, exacerbation or new onset, may occur.
  - Cases of lymphoma have been observed in patients receiving TNF blocking agents. Congestive heart failure, worsening or new onset, may occur.
  - Advise patients to seek immediate medical attention if symptoms of pancytopenia or aplastic anemia develop, and consider stopping Enbrel.
  - Monitor patients previously infected with hepatitis B virus for reactivation during and several months after therapy. If reactivation occurs, consider stopping Enbrel and beginning anti-viral therapy.
  - Anaphylaxis or serious allergic reactions may occur.
  - Stop Enbrel if lupus-like syndrome or autoimmune hepatitis develops.

MECHANISM OF ACTION: TNF is a naturally occurring cytokine that is involved in normal inflammatory and immune responses. It plays an important role in the inflammatory processes of rheumatoid arthritis (RA), polyarticular juvenile idiopathic arthritis (JIA), psoriatic arthritis (PsA), and ankylosing spondylitis (AS) and the resulting joint pathology. In addition, TNF plays a role in the inflammatory process of plaque psoriasis (PsO). Elevated levels of TNF are found in involved tissues and fluids of patients with RA, JIA, PsA, AS, and PsO. Two distinct receptors for TNF (TNFRs), a 55 kilodalton protein (p55) and a 75 kilodalton protein (p75), exist naturally as monomeric molecules on cell surfaces and in soluble forms. Biological activity of TNF is dependent upon binding to either cell surface TNFR. Etanercept is a dimeric soluble form of the p75 TNF receptor that can bind TNF molecules. Etanercept inhibits binding of TNF-α and TNF-α (lymphotoxin alpha (LT-α]) to cell surface TNFRs, rendering TNF biologically inactive. In in vitro studies, large complexes of etanercept with TNF-α were not detected and cells expressing transmembrane TNF (that binds Enbrel) are not lysed in the presence or absence of complement.

7.6 Permissible Systemic Therapies During the Protocol On-Study Period

The on-study period is defined as 9 months from the date of the TCR engineered ACT infusion unless there is overt tumor progression or dose limiting toxicities leading to patient discontinuation.

Systemic immune suppressive therapies will be avoided during this time, but they may be permissible in the following situations:

- Systemic corticosteroids: Used in case there is suspicious or evidence of an autoimmune reaction resulting in serious adverse effects.

7.7 Study Duration

Patients may remain on study and continue to receive treatment in the absence of disease progression, unacceptable toxicity or withdrawal from study, intercurrent illness that prevents further administration of treatment, participant demonstrates an inability/refusal to comply with oral medication regime, and participant withdraws from study.

At week 12 post-adoptive cell transfer, patients will be evaluated for antitumor activity after undergoing re-staging exams and laboratory tests. Patient with SD, PR or CR will be offered to continue on protocol after discussion of the study results. Patients with PD will be offered to terminate participation. However, given the phenomenon of delayed responses with tumor immunotherapy approaches, the final decision to continue or not on study will be at the discretion of the treating physician and the patient, regardless of the tumor response assessment.

7.8 Study Discontinuation

Upon treatment discontinuation all end of treatment evaluations and tests will be conducted. All patients who discontinue due to an AE must be followed until the event resolves or stabilizes. Appropriate medical care should be provided until signs and symptoms have abated, stabilized, or until abnormal laboratory findings have returned to acceptable or pre-study limits. The final status of the AE will be reported in the patient's medical records and the appropriate eCRF.

Reasons for treatment discontinuation should be classified as follows:

- Death
- Progressive disease
- Toxicity; related or unrelated to treatment
- Investigator judgment
    - The Investigator may discontinue a participant if, in his/her judgment, it is in the best interest of the participant to do so.
- Noncompliance
- Patient voluntary withdrawal
    - A patient may withdraw from the study at any time, for any reason. If a patient discontinues treatment, an attempt should be made to obtain information regarding the reason for withdrawal.
- Sponsor decision.

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

8 INVESTIGATIONAL AGENTS

8.1 PBMC Expressing NY-ESO-1 TCR/ dnTGFβRII

Overview: A leukapheresis product obtained from study patients will be transferred to the GMP Suite, at the Therapeutic Cell Production Facility, Center for Immunotherapy. PBMC will be isolated by gradient centrifugation, and an aliquot of these cells will be cultured for 48 hours in the presence of anti-CD3 (OKT3) and IL-2 in order to stimulate T-cell growth to prepare for viral vector transduction. Stimulated PBMC will be transduced with a clinical grade retrovirus vector expressing NY-ESO-1 TCR/ dnTGFβRII using RetroNectin®-coated plates on two consecutive days and kept in culture for 96 hours from the time of the first retroviral transduction. Transduced cells will be harvested and infused fresh as soon as the lot release criteria are cleared. On the infusion day, Cohorts 1, 2 and 3 patients will have completed the non-myeloablative but lymphocyte depleting preparative regimen consisting of cyclophosphamide and Cohort 4 patients will have completed their decitabine and cyclophosphamide regimen.

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

8.1.1 Retroviral Vector NY-ESO-1 TCR/ dnTGFβRII

Product Description: This vector contains the 5' and 3' LTR of the murine stem cell virus as well as a packaging signal containing splicing donor and acceptor sites. In this TCR vector, the alpha and beta chains of the NY-ESO-1 TCR are separated by a picornavirus P2A "self-cleaving" sequence. The particular vector that is being used in the this trial contains a modified version of the non-codon optimized parental 1G4 vector in which the amino acids 95 and 96 of the CDR3 of the alpha chain have been modified to leucine (L) and tyrosine(Y) (29). We generated the NY-ESO-1 TCR/ dnTGFβRII by incorporating a dnTGFβRII into the NY-ESO-1 retrovirus. This new clinical grade vector is being manufactured at the Indiana University Viral Production Facility (IU VPF, Kenneth Cornetta, Director).

Classification: Immunotherapeutic.

Active Ingredient: This retroviral vector have two active transgenes from a TCR, the alpha and beta chains of a high affinity TCR that jointly recognizes the NY-ESO-$1_{157-165}$ epitope in the context of HLA-A*0201, and a dnTGFβRII, which blocks the effect of TGFβ.

- **NY-ESO-1 TCR:** The 1G4 high affinity TCR pair specific for NY-ESO-$1_{157-165}$ presented by HLA-A*0201 was originally cloned from a patient with metastatic melanoma (61). The TCR was engineered for higher specificity for NY-ESO-1 by modifying the CDR of the

alpha chain of the TCR, leading to the current version of the 1G4 TCR used in this study. (29)

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

- **NY-ESO-1 TCR:** The NY-ESO-1 TCR is expected to redirect the specificity of the TCR transgenic T cells to the NY-ESO-$1_{157-165}$ peptide presented by HLA-A2*0201 on the surface of cancer cells.
- Incorporating a dominant-negative TGFβ receptor II (dnTGFβRII) into the NY-ESO-1 TCR retroviral vector used to transduce patient T cells will render these effector cells insensitive to TGFβ, resulting in an enhancement of their effector function in the tumor microenvironment.

Manufacturing Information for the Clinical Grade Vector: The clinical grade vector to express NY-ESO-1 TCR/ dnTGFβRII is being generated at the Indiana University Vector Production Facility (IU VPF). The retroviral vector supernatant will be prepared and preserved following cGMP conditions at IU VPF. The physical titer will be determined by RNA dot blot according to the manufacturer's certificate. The supernatant will be stored upon the completion of production at least at –70° C at IU VPF. Upon shipment on dry ice, the supernatant will be stored at –80° C at the GMP suite. This facility is equipped with around-the-clock temperature monitoring. Supernatant will be thawed and used for *in vitro* transduction of activated PBMC. There will be no re-use of the same unit of supernatant for different patients. The retroviral titer has been shown to be stable after immediate thawing and immediate use (tissue culture wells previously coated with RetroNectin®). Handling of the vector should follow the guidelines of Biosafety Level-2 (BSL-2).

How Supplied: The clinical grade vector is supplied by IU VPF following their Standard Operating Procedures (SOP) for retroviral vector suspension, labeling and cryopreservation.

Storage: Single use aliquots are stored in a viral bank established in a dedicated –80°C freezer with central, computerized monitoring system, with alarm and recording of all GMP storage systems located at the restricted access GMP Suite, at RPCI.

Stability: Under the conditions of continuous storage at –80°C without freeze-thawing, it is expected that the retroviral vector is stable for at least 5 years. Aliquots of this vector will be recertified annually throughout the study period with selected assays for purity, identity and potency.

Dose and Schedule: For *ex vivo* use only.

Route of Administration. All use of this retrovirus vector is *ex vivo*, and no direct injection will be done in human subjects.

Prior Human Experience: This retrovirus vector expressing the NY-ESO-1 TCR similarly used to transduce human PBMC has already been administered to humans within a phase 2 clinical trial at the Surgery Branch/NCI (22). The prior human experience is as follows:

- **Retrovirus Vectors:** Retroviral vectors have undergone extensive testing in humans. Their main safety caveat is the development of insertional mutagenesis, which seems to be a rare

event and may be related to the expressed transgene and the immune competency of the host. Over 250 patients in over 40 clinical trials have received stem cells genetically modified with retroviral vectors (62), and the only cases of malignant transformation are the two infants in a clinical trial inserting the gamma C gene (a surface cytokine receptor) to HSC of infants with X-linked severe combined immune deficiency (SCID) (63, 64).

- **NY-ESO-1 TCR:** The same NY-ESO-1 TCR alpha and beta gene pair is being tested at the Surgery Branch/N.C.I. (Steven A. Rosenberg, P.I.) for its ability to redirect antigen specificity of human PBMC adoptively transferred to lymphopenic hosts in a protocol design similar to the one proposed herein (22). If we gain knowledge of the development of serious adverse events (SAE) related to the NY-ESO-1 TCR transgenic T cell administration, our protocol procedures and informed consent form will be modified accordingly.

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

8.1.2 PBMC Transgenic for NY-ESO-1 TCR / dnTGFβRII

Product Description: PBMC obtained from leukapheresis and activated during 48 hours with OKT3 (anti-CD3 antibody) and IL-2, infected by the clinical grade NY-ESO-1 TCR / dnTGFβRII retroviral vector supernatant on two consecutive days, maintained in culture for 4 days from the start of transduction in IL-2 media. Aliquots of these cells will be used to fulfill the lot release criteria.

Classification: Immunotherapeutic.

Active Ingredient: As described for the retrovirus vectors NY-ESO-1 TCR / dnTGFβRII

Mode of Action: The genetically modified PBMC expressing a transgenic surface NY-ESO-1 TCR will be expected to be redirected to the NY-ESO-1 antigen expressed by HLA-A2*0201 and NY-ESO-1 positive cancer cells. Recognition of the cognate antigen will result in the release of effector molecules from T cells, like the cytotoxic granules perforin and granzyme B, and activating cytokines like interferon-gamma, IL-2 and TNF-alpha.

Manufacturing Information: Genetically modified PBMC expressing NY-ESO-1 TCR / dnTGFβRII will be manufactured by transduction of previously activated PBMC (see **Appendix E and Appendix F**) using the clinical grade retroviral vectors.

Storage: PBMC transgenic for NY-ESO-1 TCR / dnTGFβRII will be harvested within 96 hours from the first transduction and infused fresh. If cryopreserved, cells will be stored in a centrally-monitored liquid nitrogen freezer in the GMP suite.

Stability: These genetically modified PBMC will be infused fresh. When stored in liquid nitrogen, PBMC are viable for over 2 years with minimal viability loss if not subject to freeze-thawing. Cells should be used within 6 months of manufacture. Extra cells cryopreserved from the first T cell product may be used within 2 years of manufacture for a second T cell infusion. If preparing a second T cell product utilizing cryopreserved PBMC from the first apheresis collection, the PBMC may be older than 2 years as long as the viability is >90%.

Dose and Schedule: Administered once on Day 0 of this protocol. Dose escalation of NY-ESO-1 TCR / dnTGFβRII transgenic cells will be in 3 cohorts of patients [10^7,10^8, and10^9 cells (0.5e9 or higher, up to 1e9 acceptable)].

Administration: Transgenic cells will be transported from the GMP suite to the hospital bed in infusion bags with over-wrap, on ice in a rigid-walled container with a tight fitting lid (i.e. Tupperware; Nalgene container etc.) labeled with a biohazard label.

Unused Transgenic Cells: Transgenic cells generated but not administered to patients will be banked cryopreserved for up to 5 years. Extra cells cryopreserved from the first T cell product may be used within 2 years of manufacture for a second T cell infusion. Cells may be used for additional *in vitro* testing beyond this period of time. Disposal of transgenic cells remaining in the bag and i. v. tubing after administration to patients will be done in a biohazard container that will be autoclaved before disposal.

Prior Human Experience: As described above, PBMC transduced with the same retrovirus vector is currently in clinical testing (22).

See **Appendix D** for the **Retrovirus Transduction Method.**

8.1.2.1 Reported Adverse Events and Potential Risks

Potential risks are derived from the viral vector, the transgenes it will express and the infusion of genetically modified cells:

- **Retroviral Vectors:** There are two potential serious anticipated risks associated with retroviral vectors. One is related to its ability to randomly and permanently integrate in the genome of target cells, which may lead to altered expression of genes in these cells potentially inducing oncogenic changes. The second one is related to the possibility of its recombination and generation of replication competent virus that could potentially lead to a HIV-like disease.
- **Risk of Insertional Mutagenesis with Retroviral Vectors.** The risks of insertional oncogenesis with integrating gene delivery vectors is likely a complex function of several factors, related to the type of vector used, the promoter and other transcriptional control elements of the vector, the transgene *per se*, as well as properties of the target cells. Moloney Leukemia Virus (MLV)-based gamma-retroviral vectors have been shown to have a high predilection for insertion into the 5' region of actively transcribed genes, which may increase the risks for trans-activation of transcription from the cellular gene promoter (65). Genes that modulate cell proliferation, such as the gamma C gene used to treat X-linked SCID may provide one component of a proliferative signal that plays a cooperative role in cellular transformation with cellular genes involved in cellular proliferation, such as LMO-2, when activated by insertional trans-activation (63). In contrast, effector proteins such as TCR and a truncated version of a receptor (TGFβRII) would not, a priori, be expected to confer an unregulated proliferative stimulus, although they may play a role in clonal expansion upon encounter with the cognate antigen. Furthermore, T cells transduced with dnTGFβRII did not lead to spontaneous lymphoproliferation in vivo (66). Recently, a decade-long safety/function of retroviral-modified chimeric antigen receptor T cells

study(67) showed no evidence of cell immortalization, and neither persistent clonal expansion as measured in >500 patient-years of follow-up. Hence given the natural history and poor prognosis of advanced cancer patients and large evidence from the retroviral-based ACT literature, we assess that the risks of leukemogenesis are very small and justifiable. Even so, we will monitor insertional mutagenesis events by repeated sampling of cells and study if dominant clones of long-term persisting (6 months and over) transgenic cells are clonal or polyclonal by analyzing the retroviral insertion sites and ruling out common integration sites (CIS)(68). Findings that would suggest proto-malignant behavior would lead us to discuss them with the FDA, and consider introducing suicide transgenes into the vector, such as sr39Thymidine Kinase (our group recently received a clinical GMP-grade virus batch for another future trial) or possible less immunogenic alternatives as they become available (69).

- **Risk of Replication Competent Retrovirus (RCR) with Retroviral Vectors.** Another risk is that genetic recombination within components of the vector system could lead to the generation of RCR. This event would have the potential of generating infective retrovirus that may lead to oncogenesis or immune deficiency. Our patients will be screened at baseline to be HIV seronegative. Since there will be no wild type HIV virus present, the possibility of providing the missing genes for the generation of infective retrovirus *in vivo* would be lower than when retroviral vectors are administered to HIV positive subjects. In a recent publication, testing of Master Cell Banks and patient follow-up from 29 clinical studies using gene-modified T cells manufactured with gamma-retroviral vectors from five different centers showed no RCR in all tests thus far (51), in line with our experience from our TCR trial. Nevertheless we will monitor for RCR risk by strictly following FDA guidelines.

8.1.2.2 NY-ESO-1 TCR:

The main potential toxicities from the transgenic alpha and beta NY-ESO-1 TCR genes expressed in PBMC are derived from the NY-ESO-1 specificity of the TCR, or the mispairing with endogenous TCR genes leading to cells with potential autoimmune specificity.

- Autoimmune Toxicity against NY-ESO-1 Positive Cells. Toxic events could be potentially derived from cytotoxic activity of the transgenic PBMC against NY-ESO-1 positive cells. However, studies to date have not detected expression of NY-ESO-1 in adult somatic tissues (3, 4, 6). While spermatogonia express the NY-ESO-1 protein, the testes do not express MHC-1, thereby markedly decreasing the potential of a cellular immune response against the testes. Furthermore, a recent trial conducted at the NCI in which 17 patients underwent ACT with a TCR directed against NY-ESO-1157-165 did not detect any grade 3 or 4 toxicities.
- Autoimmune Toxicity from Mispairing of TCR Chains. The alpha and beta chains of the NY-ESO-1 TCR could undergo heterologous pairing with endogenous alpha and beta chains of TCR expressed by the transduced T cells, leading to misdirected T cells with newly acquired TCR specificities. These would be unpredictable and not subjected to the thymic selection process, potentially resulting in T cells with auto-reactivities. If evidence

of autoimmunity developed, patients would receive immune suppressive therapy as clinically indicated based on the severity of symptoms, using medications like corticosteroids, cyclosporin-A, mycophenolate mofetil, anti-TNF-alpha antibodies or anti-thymocyte globulin .

8.1.2.3 NY-ESO-1/ dnTGFβRII

In addition to the potential toxicities listed above for NY-ESO-1 TCR, autoimmune toxicity from dnTGFβRII could occur**.**

Autoimmune Toxicity from dnTGFβRII: The TGFβ signaling pathway is necessary to maintain T cell homeostasis, and abrogation early in T cell development produces T cell hyperproliferation and autoimmunity(70). Due to developmental defects, transgenic mice that express dnTGFβRII in T cells present with CD8+ lymphoproliferative disorder and autoimmunity. The lymphoproliferating T cells were naive and IL-2 receptor negative, and exhibited distinct patterns of recirculation and homeostasis. By contrast, our transgenic cells have been activated and expanded *in vitro* and are post-thymic mature T cells that are dependent on antigen and growth factors for survival. In communication with Malcolm Brenner at Baylor, there was no evidence *in vitro* and *in vivo* that long-term expression of the mutant TGF receptor had any deleterious effects on the transduced cells, which remained antigen and cytokine dependent. In the event evidence of autoimmunity is observed, the patient will receive immune suppressive therapy as clinically indicated based on the severity of symptoms (corticosteroids, cyclosporin-A, mycophenolate mofetil, anti-TNF-alpha antibodies).

8.1.2.4 NY-ESO-1/ dnTGFβRII Transgenic T Cells

The adoptive transfer of an *ex vivo* modified autologous cellular product may lead to potential toxicities related to the cell manipulation.

Allergic Reaction and Cytokine Release Syndrome: The culture of cells *ex vivo* in media products to maintain lymphocyte viability, the use of reagents to activate cells, the retroviral transduction process, and the expression of foreign genes may lead to alterations in the cell product resulting in immediate untoward effects. The most serious would be infusion reactions leading to severe allergic reactions, or the triggering of a cytokine release syndrome. Therefore, the genetically modified cells will be re-infused in an inpatient setting, with continuous monitoring of vital signs and by experienced personnel within the bone marrow transplant (BMT) unit.

Contaminated Cell Product: *Ex vivo* culture and manipulation of cells may result in a cell product contaminated by bacteria, fungus, mycoplasma or virus. With the goal of detecting potential contaminants, the genetically modified cells will undergo intensive lot release testing prior to infusion.

8.2 Decitabine: Drug Information

Decitabine is commercially available and will be supplied by the study.

The date of receipt and the amount of drug received will be documented. Drug shipment records will be retained by the investigational pharmacist or designee.

The package insert will be followed when preparing, handling and disposing Decitabine.

8.3 Cyclophosphamide: Drug Information

U.S. BRAND NAMES — Cytoxan®

PHARMACOLOGIC CATEGORY: Antineoplastic Agent, Alkylating Agent

ADMINISTRATION:

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

ADVERSE REACTIONS

- **Significant** (Greater than 10%):
  - Dermatologic: Alopecia (40% to 60%) but hair will usually regrow although it may be a different color and/or texture. Hair loss usually begins 3-6 weeks after the start of therapy.
  - Endocrine & metabolic: Fertility: May cause sterility; interferes with oogenesis and spermatogenesis; may be irreversible in some patients; gonadal suppression (amenorrhea).
  - Gastrointestinal: Nausea and vomiting occur more frequently with larger doses, usually beginning 6-10 hours after administration; anorexia, diarrhea, mucositis, and stomatitis are also seen.
  - Genitourinary: Severe, potentially fatal acute hemorrhagic cystitis, believed to be a result of chemical irritation of the bladder by acrolein, a cyclophosphamide metabolite, occurs in 7% to 12% of patients and has been reported in up to 40% of patients in some series. Patients should be encouraged to drink plenty of fluids during therapy (most adults will require at least 2 L/day), void frequently, and avoid taking the drug at night. With large I.V. doses, I.V. hydration is usually recommended. The use of mesna and/or continuous bladder irrigation is rarely needed for doses <2 g/m2.
  - Hematologic: Thrombocytopenia and anemia are less common than leukopenia. Onset: 7 days, Nadir: 10-14 days, Recovery: 21 days.
- **From 1% to 10%:**
  - Cardiovascular: Facial flushing
  - Central nervous system: Headache

- Dermatologic: Skin rash
- Renal: SIADH may occur, usually with doses >50 mg/kg (or 1 g/m2); renal tubular necrosis, which usually resolves with discontinuation of the drug, is also reported.
- Respiratory: Nasal congestion occurs when I.V. doses are administered too rapidly (large doses via 30-60 minute infusion); patients experience runny eyes, rhinorrhea, sinus congestion, and sneezing during or immediately after the infusion. If needed, a decongestant or decongestant/antihistamine (e.g., pseudoephedrine or pseudoephedrine/triprolidine) can be used to prevent or relieve these symptoms.

- **Less than 1%** (Limited to important or life-threatening):
  - High-dose therapy may cause cardiac dysfunction manifested as CHF; cardiac necrosis or hemorrhagic myocarditis has occurred rarely, but may be fatal. Cyclophosphamide may also potentiate the cardiac toxicity of anthracyclines.
  - Other adverse reactions include anaphylactic reactions, darkening of skin/fingernails, dizziness, hemorrhagic colitis, hemorrhagic ureteritis, hepatotoxicity, hyperuricemia, hypokalemia, jaundice, neutrophilic eccrine hidradenitis, radiation recall, renal tubular necrosis, secondary malignancy (e.g., bladder carcinoma), Stevens-Johnson syndrome, toxic epidermal necrolysis; interstitial pneumonitis and pulmonary fibrosis are occasionally seen with high doses.
- **BMT:**
  - Cardiovascular: Heart failure, cardiac necrosis, pericardial tamponade
  - Endocrine & metabolic: Hyponatremia
  - Hematologic: Methemoglobinemia
  - Gastrointestinal: Severe nausea and vomiting
  - Miscellaneous: Hemorrhagic cystitis, secondary malignancy

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

8.3.1 Mesna: Drug Information

U.S. BRAND NAMES — Mesnex®

PHARMACOLOGIC CATEGORY: Antidote; Uroprotectant

ADMINISTRATION: For IV administration, the required dose of mesna should be withdrawn from the multidose vial labeled as containing 100 mg/mL and diluted with an appropriate volume of a compatible IV solution (i.e., 5% dextrose; 5% dextrose and 0.2, 0.33, or 0.45% sodium chloride; 0.9% sodium chloride; lactated Ringer's) to obtain a solution containing 20 mg/mL The

diluted solution may then be given by direct IV injection or infused IV over a period of 15–30 minutes.

ADVERSE REACTIONS: SIGNIFICANT

Mesna Monotherapy: Headache, injection site reactions, flushing, dizziness, nausea, vomiting, somnolence, diarrhea, anorexia, fever, pharyngitis, hyperesthesia, influenza-like symptoms, coughing, constipation, flatulence, rhinitis, rigors, back pain, rash, conjunctivitis, arthralgia.

Mesna Alone (frequency not defined):

- Cardiovascular: Flushing
- Central nervous system: Dizziness, fever, headache, hyperesthesia, somnolence
- Dermatologic: Rash
- Gastrointestinal: Anorexia, constipation, diarrhea, flatulence, nausea, taste alteration/bad taste (with oral administration), vomiting
- Local: Injection site reactions
- Neuromuscular: Arthralgia, back pain, rigors
- Ocular: Conjunctivitis
- Respiratory: Cough, pharyngitis, rhinitis
- Miscellaneous: Flu-like syndrome

Mesna Alone or in Combination: (Post-marketing and/or case reports) Allergic reaction, anaphylactic reaction, hypersensitivity, hyper-/hypotension, injection site erythema, injection site pain, limb pain, malaise, myalgia, platelets decreased, ST-segment increased, tachycardia, tachypnea, transaminases increased.

MECHANISM OF ACTION: In blood, mesna is oxidized to dimesna which in turn is reduced in the kidney back to mesna, supplying a free thiol group which binds to and inactivates acrolein, the urotoxic metabolite of ifosfamide and cyclophosphamide.

Cyclophosphamide and mesna will be provided to the study participant, at no cost, by the study sponsor.

8.4 Laboratory Reagents

8.4.1 OKT3

OKT3 (eBioscience), is an anti-CD3 antibody used to activate T cells in vitro by engaging the CD3 complex. In the current clinical trial, OKT3 will be strictly used ex vivo for the activation of PBMC for retroviral transduction. A clinical grade OKT3 termed Orthoclone (muromonab-CD3, Ortho-Biotech) is a therapeutic monoclonal antibody approved by the FDA to treat rejection of transplanted organs, including the heart, kidneys and liver. However, this antibody is currently not being produced by the clinical grade manufacture. When used in vivo OKT3 antibodies eliminate CD3 positive lymphocytes which is beneficial to treat graft rejections. It can induce anaphylactic

or anaphylactoid reactions, cytokine release syndrome and a variety of constitutional symptoms when administered systemically. The source of OKT3 antibody for the current studies is obtained with a certificate of analysis providing its manufacturing and product characterization. In addition, the batches used within this study are further characterized by performing the following tests: Gram stain, fungal stain, bacterial culture, fungal culture, mycoplasma culture, endotoxin assay.

8.4.2 Interleukin-2

Please refer to package insert for complete product Information. Human recombinant interleukin-2 is a highly purified protein with a molecular weight of approximately 15,300 daltons. It is a lymphokine produced by recombinant DNA technology using a genetically engineered E. coli strain containing an analog of the human interleukin-2 gene.

Mode of Action: Immunotherapeutic agent which stimulates T and NK cell responses.

How Supplied: Interleukin-2 (IL-2) is manufactured by Prometheus.

Formulation/Reconstitution: IL-2 is provided as single-use vials containing 22 million IU (~1.3 mg) IL-2 as a sterile, white to off-white lyophilized cake. In this protocol, IL-2 will be used only for in-vitro stimulation and expansion of PBMC at a concentration of 300 IU/ml. Harvested transgenic cells will be washed three times with normal saline before packaging into the infusion bag.

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

Dilution/Stability: Reconstituted IL-2 will be further diluted to prepare a stock solution for in-vitro use and stored at -80ºC in single use aliquots. One aliquot from this will be subjected to sterility tests and endotoxin assay. These aliquots will be used as single use up to the expiry date on the original vial.

Dose and Schedule: In-vitro use only at 300 IU/ML in the stimulation media and expansion media during the manufacture of the transgenic T cells.

Administration: In-vitro use only. IL-2 will not be administered directly to the patient.

Toxicities: Expected toxicities of IL-2 are listed in the product label. Grade III toxicities common to high dose IL-2 include diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes; it is not expected that these side effects will be manifested by in-vitro use of IL-2 during cell manufacture.

8.4.3 RetroNectin®

RetroNectin® (CH-296, Takara) is a chimeric peptide of recombinant human fibronectin fragments produced in E. coli, consisting of three functional domains: a central cell-binding domain (type III repeat, 8-10), heparin-binding domain II (type III repeat, 12-14), and a CS1 site within the alternatively-spliced IIICS region. It is a 574 amino acid protein with a molecular weight of 63 kDa. When coated on the surface of containers such as culture dishes, petri dishes, flasks or bags, RetroNectin® significantly enhances retrovirus-mediated gene transduction into mammalian cells. This enhancement is hypothetically due to co-localization of retroviral particles and target

cells on the molecules of RetroNectin®. Virus particles bind RetroNectin® via interaction with heparin-binding domain II, and target cells bind mainly through the interaction of the cell surface integrin receptor VLA-4 with the fibronectin CS1 site. Additionally, cells may also bind through the interaction of another fibronectin ligand (RGDS in repeat 10) within the central cell-binding domain with a corresponding integrin receptor VLA-5 on the cell surface.

8.4.3.1 Clinical Grade Retrovirus Lot Release Testing

Prior to use, the clinical grade retroviral vector will require fulfilling the lot release criteria: (1) Certificate of Analysis of the retroviral vector Master Cell Bank (MCB) and, (2) Certificate of Analysis of the Clinical grade retrovirus (see **Appendix F: C1 & C2)**.

8.4.3.2 Samples for Transgenic TCR Cell Persistence and Immune Monitoring

TCR and vector presence will be quantitated in PBMC samples, obtained before adoptive transfer and at weeks 1, 2, 4, 8, 12, and months 6 and 9. At 9 months, the interventional portion of the protocol ends and long term follow up begins. From years 1 to 5, every 6 months and then yearly up to 15 years if transgenic T cell is present in the peripheral blood. Analysis will be performed both using immune monitoring and molecular techniques. Detection of surface expression of the NY-ESO-1 TCR transgenic protein will be analyzed both by MHC tetramer or dextramer analysis and staining for the specific region. Molecular analysis of the persistence of cells bearing the NY-ESO-1 TCR cDNA will be done by real time PCR techniques using primers specific for the transgenes and retroviral vector sequences. This testing will provide data to estimate the in vivo survival of lymphocytes derived from the infused cells.

9 STUDY PROCEDURES

Informed consent ***MUST*** be completed prior to receiving any study related procedures.

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

9.1 Screening Evaluations

The initial screening period will be 30 days, but patients will be allowed to enroll after the 30-day initial screening period, for up to approximately 60 days, using the same screening procedure test results to avoid unnecessary assay duplication. A pregnancy test must be completed within 48 hours prior to dosing, if applicable.

Screening will occur in 2 stages. In the first stage, a patient will provide their written informed consent, and will provide a small amount of blood or buccal swab for HLA typing (see section **9.14.8)**. In addition, patient will be screened for NY-ESO-1 positivity using historical results, archival tissue or fresh biopsy.

If the patient is eligible based on HLA type and tumor antigen expression, then the patient will undergo the remainder of the screening procedures. This will include the following (see **Section 9.12** for a more complete definition of assessments):

- Informed consent: Must be completed prior to receiving any study-related procedures as described above.
- Medical history (**9.14.1, 9.14.2)**
- Targeted physical exam (**9.14.5**)
- Determination of performance status (**9.14.4**)
- CBC and differential (**9.14.7**)
- Complete metabolic and hepatic panel (**9.14.6)**
- Baseline CA-125 reading (for ovarian cancer patients)
- Urinalysis
- Screening for suitability for apheresis
- Cardiac function tests (Troponin/EKG)
- Cardiac stress test (Cardiac stress test will be done as clinically indicated, the specific test to be chosen at the discretion of the treating physician).
- Tumor Biopsy or acquisition of archival material (refer to section 9.16.1)

  Patients' tumor will be screened for NY-ESO-1 antigen expression by immunohistochemistry (IHC) and/or molecular analysis by qRT-PCR. At Roswell Park, NY-ESO-1 testing will be done in accordance with Protocol I 226412 titled "NY-ESO-1 Expression in Patients with Solid Tumors" or Protocol I 215512 titled “Analysis of Tumor Antigens, Immunity, and Genetic Changes in Gynecological Cancer Patients”. Data from prior IHC and/or molecular tests may be used.
- Baseline CT Scan

  A baseline CT scan will be done for baseline tumor assessment (An existing scan within 60 days of enrollment is acceptable).
- Brain MRI as clinically indicated
- Pregnancy Test

  All females of childbearing potential must have a negative serum or urine Pregnancy Test within 48 hours from the initial dose of conditioning chemotherapy in order to be eligible for participation. Females who have undergone surgical sterilization, or who have been postmenopausal for at least 2 years, are not considered to be of childbearing potential.
- Concomitant medications

9.2 Day (-6): Leukapheresis for NY-ESO-1 TCR T cell Product.

- Within 1 week of leukapheresis, after initial eligibility has been determined, the following will be performed:
    - Stool Sample Collection Questionnaire (refer to Appendix G)
    - Microbiome Collection Questionnaire (refer to Appendix H)
    - Microbiome Stool Collection (refer to Appendix I)
- Collection of blood for research
- Apheresis

After enrollment, the leukapheresis date (Day -6) will be scheduled based on the T cell manufacture schedule and infusion day (Day 0). All patients will undergo a 12L mononuclear cell apheresis procedure to collect steady-state T-cells. Patients will be admitted to the Blood Donor facility for leukapheresis that will be performed on an outpatient basis. Antecubital veins will be used, or when necessary, a central catheter will be placed. A standard collection protocol will be used to obtain the peripheral white blood cells with settings modified according to the Cobe Spectra Apheresis System recommendations (or other comparable machine) to obtain the best mononuclear cell yield.

- Concomitant medications
- Adverse events

9.3 Cohort 4 only: Days -6 to -4 Decitabine

- Decitabine Administration: as described in **Section 7.1.3.**
- Concomitant medications
- Adverse events

9.4 Cohorts 1, 2 &3: Days –5 & –4; Cohort 4: Days -3 &-2 Chemotherapy Administration.

- Medical History
- Targeted Physical Exam
- ECOG Performance Status
- CBC, differential
- Complete Metabolic and Hepatic Panel
- Urinalysis
- Chemotherapy Administration: as described in **Section 8.1.2.**
- Concomitant medications

- Adverse events

9.5 Day 0: NY-ESO-1 TCR/dnTGFβRII T cell Infusion

- Subject will be admitted to the BMT ward at RPCI for the infusion.
- T cell infusion: The TCR genetically engineered PBMC product will be transported to the in-patient unit on ice in a rigid-walled container with a tight fitting lid (i.e. Tupperware; Nalgene container etc.) and labeled with a biohazard label. Infusion and monitoring procedures are described in **Section 8.1.2.**
- Research blood will be collected as follows:
  - Prior to infusion (SST: 2 cc)
  - At 1, 2 and 4 hours post infusion (SST: 2 cc)
- Medical history
- Targeted physical exam
- Determination of performance status (ECOG)
- CBC and differential
- Complete metabolic and hepatic panel
- CA-125 level (pre-infusion for ovarian cancer patients only)
- Urinalysis
- Concomitant medications
- Adverse events
- Troponin/EKG if clinically indicated.

9.6 Post Infusion Follow-Up (the week of Infusion)

Patients will be followed daily the week of infusion as inpatient/ outpatient. These visits will include the following procedures (urinalysis and EKG/troponin will be done if clinically indicated):

- Medical history
- Targeted physical exam
- Determination of performance status (ECOG)
- CBC and differential
- Complete metabolic and hepatic panel
- Ferritin as clinically indicated.
- Collection of blood for research (**Table 2**)

- Concomitant medications
- Adverse events

9.7 Hospital Discharge

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

- Absolute neutrophil count (ANC) > 500/μL
- Platelet count > 20,000/μL
- Hemodynamically stable
- Liver function tests stable
- Not requiring daily blood product infusion

9.8 Post Infusion Follow-Up (Weeks 1 to 4)

Patients will be followed weekly until week 4. There is a ±2 day window for these visits, which will include the following procedures:

- Medical history
- Targeted physical exam
- Determination of performance status (ECOG)
- CBC and differential
- Complete metabolic and hepatic panel
- Ferritin as clinically indicated
- CA-125 reading (Ovarian cancer patients, week 4 only)
- Concomitant medications
- Adverse events

Collection of blood for research at weeks 1, 2 and 4 (Collection of 100 ml of peripheral blood each time (less may be collected depending on patient condition). However, blood draws on these days should be performed if considered safe, and may be skipped or delayed because patients are likely to have chemotherapy-induced pancytopenia).

9.9 Post Infusion Follow-up (week 6)

- Tumor imaging (a restaging CT of the chest, abdomen and pelvis, will be performed to track changes in tumors)
- Optional biopsy (excisional or core biopsy whenever feasible.)

9.10 Post Infusion Follow-Up (Week 8 and Week 12)

Patients will be followed at Week 8 (± 2 days) and Week 12 (± 1 week). The reason for the smaller window at Week 8 is that this time point is important for staging and monitoring for adverse events. These visits will include the following procedures:

- Medical history
- Targeted physical exam
- Determination of performance status (ECOG)
- CBC and differential
- Complete metabolic and hepatic panel
- CA-125 reading (Ovarian cancer patient)
- Collection of blood for research
- Tumor Imaging (Patients will undergo restaging CT scans of the chest, abdomen and pelvis at week 12 ± 1 week)
- Concomitant medications
- Adverse events
- Microbiome Collection Questionnaire week 12 only (refer to Appendix H)
- Microbiome Stool Collection week 12 only (refer to Appendix I)

9.11 Post Infusion Follow-Up (6 months and 9 Months)

Patients will be followed at 6 months (± 2 weeks) and 9 months (± 2 weeks). These visits will include the following procedures:

- Medical history
- Targeted physical exam
- Determination of performance status (ECOG)
- CBC and differential
- Complete metabolic and hepatic panel
- CA-125 reading (ovarian cancer patients)
- Collection of blood for research
- Tumor Imaging
- Concomitant medications
- Adverse events

9.12 Re-Staging Evaluation: Week 12

At week 12 post-adoptive cell transfer (± 1 week), patients will be evaluated for antitumor activity after undergoing re-staging exams and laboratory tests. Patient with SD, PR or CR will be offered to continue on protocol after discussion of the study results. Patients with PD will be offered to terminate participation. However, given the phenomenon of delayed responses with tumor immunotherapy approaches, the final decision to continue or not on study will be at the discretion of the treating physician and the patient, regardless of the tumor response assessment.

9.13 Long-Term Follow-up Plan (Years 1 to 15)

Evaluations will be performed for up to 15 years on all subjects as specified by FDA, in accordance with recent guidelines for long-term follow-up (LTFU) set forth by the ASGT and the FDA. Please refer to **Table 2** for Long Term Follow-up.

The most recently released (November 2006) Guidance on Monitoring for Delayed Adverse Events states that for the first 5 years, all subjects should undergo monitoring of vector sequences every 6 months, and a full physical examination including a medical history, concomitant medications and examination of appropriate organ systems and a hemogram annually. For the final 10 years, if vector sequences are no longer detected in PBMCs, a one page questionnaire or post card may be sufficient for reporting of any adverse events and questions relating to the patient's status.

For the LTFU, subjects who have completed the 9 month visit, the first visit will occur at 1 year post T cell infusion and then every 6 months until the end of year 5 post T cell infusion. For subjects who do not complete the 3 month visit, the first visit will occur at 3 months post T cell infusion, the second visit will be 6 month post T cell infusion, the third visit will occur at 9 months post T cell infusion and the next visit will occur 1 year post T cell Infusion, and then every 6 months until the end of year 5 post T cell infusion.

Visits will include a medical history, physical exam, and blood tests for disease monitoring, CBC, chemistries, monitoring for persistence of vector modified cells and annually for RCR testing/archive. The physical exam and medical history (including concomitant medications and adverse events) will be conducted with careful attention to features possibly related to oncoretroviral diseases including: (1) New malignancies, (2) New incidence or exacerbation of a pre-existing neurologic disorder, (3) New incidence of exacerbation of a prior rheumatologic or other autoimmune disorder, and (4) New incidence of a hematologic disorder.

For the next 10 years, subjects will be asked to return for testing only if vector modified cells were detected in the previous year, and if so, then blood for persistence of vector modified cells and RCR testing {archive) will also be performed/collected. If no vector modified cells were detected in the previous year, the follow-up will be conducted by means of a clinical questionnaire completed via phone or through the mail by the study coordinator. The questionnaire will include the following questions:

- When did you last visit your personal physician?
- Has there been a change in your condition? Are there any adverse events to report?

- Did you have a blood test?
- Did your physician express any concerns with the test results?

Participants who come off the study prior to 12 months post cell infusion and are started on another treatment including standard of care due to disease progression will be asked to come for follow up visits at 3, 6 and 9 months when blood for Central Lab Immunemonitoring will be collected (70 cc: 7 Lavender-Top Tubes, used for PBMC for functional Assays/Immunophenotyping, cell pellets for Vector Copy number, RCR & RCL tests). **Patients receiving viral vector engineered cells are required by the FDA to be tested for RCR/RCL at these time points.** For 1 year and later time points, the same schedule as in Table 5 will be followed. Biopsy will be done at 3 months post infusion if clinically feasible.

The schedule of procedures and observations for this study is summarized in **Table 2** below.

Table 2 Schedule of Procedures and Observations

															LTFU Years 1-5*	
	Screening	Day -6	(Cohort 4 only) Day -6 to -4	Cohorts 1, 2 &3) Day -5 and -4	(Cohort 4 only) Day -3 and -2	Day 0	Day 1 to 3	Day 7	Week 2 to 4 (weekly ±2 days)	Week 6 (± 1 week)	Week 8 (±2 days)	Week 12 (±1 week)	6 months (±2 weeks)	9 months (±2 weeks)	Years 1,2,3,4,5 (±1 month)	Years 1.5,2.5,3.5,4.5 (±1 month)
Clinical Assessments[14]																
Informed Consent	X															
HLA Typing (Blood-10 ml Lavender Top tube or buccal swab)	X															
Medical History	X			X	X	X	X	X	X		X	X	X	X	X	X
Targeted Physical Exam	X			X	X	X[11]	X	X	X		X	X[12]	X	X	X	X
ECOG Performance Status	X			X	X	X	X	X	X		X	X	X	X	X	X
CBC, differential (2 ml lavender)	X			X	X	X	X	X	X		X	X	X	X	X	
Complete Metabolic & Hepatic Panel (2 ml SST)	X			X	X	X	X	X	X		X	X	X	X	X	
Ferritin Level							X[13]	X[13]	X[13]							
Urinalysis	X			X	X	X										
Troponin/EKG	X[2]					X[2]	X[2]									
Leukapheresis screen	X[15]															
CA-125 Monitoring (5 ml SST; ovarian cancer patients)	X					X[3]			X[5]		X	X	X	X		
Pregnancy Test[4]	X															
Concomitant medications (see 9.14.3)	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X
Adverse Event		X	X	X	X	X	X	X	X		X	X	X	X	X	X

															LTFU Years 1-5*	
	Screening	**Day -6**	**(Cohort 4 only) Day -6 to -4**	**Cohorts 1, 2 &3) Day -5 and -4**	**(Cohort 4 only) Day -3 and -2**	**Day 0**	**Day 1 to 3**	**Day 7**	**Week 2 to 4 (weekly ±2 days)**	**Week 6 (± 1 week)**	**Week 8 (±2 days)**	**Week 12 (±1 week)**	**6 months (±2 weeks)**	**9 months (±2 weeks)**	**Years 1,2,3,4,5 (±1 month)**	**Years 1.5,2.5,3.5,4.5 (±1 month)**
Stool Sample Collection Questionnaire[16]		X[16]														
Microbiome Collection Questionnaire[17]		X[17]										X				
Procedures[14]																
Cyclophosphamide & Mesna				X	X											
Decitabine[20]			X													
Apheresis 12L		X														
Inpatient Admission & T cell Infusion						X										
CT	X									X		X	X	X		
Brain MRI	X[1]															
Research Assessments[14]																
Biopsy and Tumor Antigen	X[6]									X[7,8]						
Blood for cell analyses (60 to 100 ml, Lavender-Top tube)								X	X[9]		X	X	X	X	X	X
PBMC (Immunophenotyping, Tetramer & functional analyses[19]		X						X	X[9]		X	X	X	X	X	X
Serum (2 ml SST)						X[10]	X	X	X[9]		X	X	X	X		
Microbiome Stool Collection[18]		X[18]										X				

* Follow-up until 15 years post infusion is required, but can be completed by phone call or postcard if gene modified cells are no longer detected in the patient.

1 Brain MRI only if clinically indicated.

2 2EKG will be done at screening. Cardiac stress test will be done as clinically indicated, the specific test to be chosen at the discretion of the treating physician. Troponin at screening; Troponin and EKG on Day 0, 1, 2, 3 if clinically indicated.

3 Day 0 pre-infusion.

4 Females only: within 48 hours of starting therapy.

5 Week 4 only.

6 Required only if archival is not available for NY-ESO testing (if not previously done) and for research.

7 Optional biopsies at Week 6 (± 1 week) and upon progression.

8 Antigen expression at Week 6 and upon progression if biopsy is performed.

9 Week 2 & 4 only.

10 2 ml pre-infusion and at 1, 2, and 4 hours post infusion.

11 T cells administered IV without lymphocyte filter, by rapid infusion over approximately15 minutes. Vital signs are recorded prior to, during, and at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, and 4 hours post-infusion. Refer to Section 7.5.1for supportive therapy on Day 0.

12 Response assessment for ending participation at Week 12.

13 Ferritin level as clinically indicated.

14 To facilitate scheduling, a (-1 day) window is allowed for assessments and procedures required on first day of cyclophosphamide treatment or Day 0 (day of T-cell infusion).

15 Venous access assessment and bone marrow transplant autotransplant serology order set (without CMV testing)

16 Stool Sample Collection questionnaire to be completed at time of stool microbiome collection [within 1 week of apheresis (day -6), after initial eligibility has been confirmed]. Refer to section 9.17.2 and Appendix G.

17 Microbiome Collection Questionnaire to be completed at time of stool microbiome collection [within 1 week of apheresis (day -6), after initial eligibility has been confirmed]. Refer to section 9.17.2 and Appendix H.

18 After initial eligibility has been confirmed, stool for microbiome will be collected within 1 week of apheresis (day-6) Refer to section 9.17.1 and Appendix I.

19 The PBMC will be processed from the 60 to 100 ml blood collected in lavender top tubes

20 To be started after leukapheresis

9.14 Definitions

9.14.1 Medical History and Physical Examination

The medical history must include all diagnoses and surgical procedures of major organ systems. Any allergies to any medications and their formulations must be documented.

9.14.2 Previous Medications

All prescription and nonprescription medication, vitamins, herbal and nutritional supplements, taken by the subject during the 30 days prior to screening will be recorded at the screening visit.

9.14.3 Concomitant Medications

Concomitant medications will be recorded in the medical record and on the appropriate eCRF. In addition, any additions, deletions, or changes of these medications will be documented.

9.14.4 ECOG Performance Status

Performance status will be assessed by ECOG. If necessary, for reporting purposes, the scale can be converted to Karnofsky using the standard scale shown in Appendix A.

9.14.5 Targeted Physical Examination and Vital Signs

A targeted physical exam will imply assessments in each of the following categories: General (weight, height, blood pressure, pulse, respiration rate, and temperature), head, eyes, ear, nose and throat examinations, lymph node examination, chest and cardiovascular system examination, breasts, respiratory system, abdominal examination, dermatological examination, and neurological examination including mental status assessment.

9.14.6 Complete Metabolic and Hepatic Panel

Complete metabolic panel will include measurements of sodium, potassium, chloride, bicarbonate, urea nitrogen, creatinine, total protein, albumin, calcium, and lactic dehydrogenase. Hepatic panel refers to liver function tests will include measurements of bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP). In the schedule of study procedures, chemistries and liver function tests are always performed together.

9.14.7 CBC with Differential

Hematology tests will include hemoglobin, hematocrit, platelet count, red cell count, and white cell count with differential count.

9.14.8 HLA A201 Screening

A high-resolution test for the HLA A2 allele is required. At the present time, only HLA A201 subjects are eligible, as the different subtypes (e.g., HLA A202) do not interact with the engineered TCRs. HLA testing will be performed by a CLIA certified laboratory (such as Quest Laboratories) as selected by each clinical site.

9.15 Research Blood Sample Collection and Processing

9.15.1 Day 6 in the Morning

On the morning of the day, a 12 liter leukapheresis will be performed at the Blood Donor Facility at RPCI, as an outpatient. Antecubital veins will be used, or when necessary, a central catheter will be placed. A standard collection protocol will be used to obtain the peripheral white blood cells with settings modified according to the Cobe Spectra Apheresis System recommendations (or other comparable machine) to obtain the best mononuclear cell yield. In order to obtain sufficient monocytes cells, leukapheresis time may vary from 90 minutes to 240 minutes. Cells will be transferred to the cGMP cell production facility at the Center for Immunotherapy (CCC building, 4th Floor) where the transgenic cells will be manufactured using the procedure described in **Appendix B** and **Appendix D**.

9.15.2 Immune Monitoring Analysis (blood for cell analyses)

Collection of 60 to 100 ml of peripheral blood (7 to 11 tubes of 10 ml lavender top EDTA tubes) is scheduled on study day +7 and study day +14. However, blood draws on these days should be performed if considered safe, and may be skipped or delayed because patients are likely to have chemotherapy-induced pancytopenia. These blood draws can be done +/- 6 days from the intended days.

100 ml of blood samples will also be collected on weeks 4, 8, and 12, and at months 6 and 9 post adoptive transfer as listed on **Table 2**. After 9 months, LTFU starts and during years 1 to 5, blood samples will be collected every 6 months until no transgenic cells are detectable in the peripheral blood.

Roswell Park Cancer Institute
Immune Analysis Shared Resource (IASR) CCC room 416
Attn: Junko Matsuzaki (I 285416 Samples)
Elm & Carlton Streets
Buffalo, NY 14263
(716) 845-8459
Junko.matsuzaki@RoswellPark.org

> **Note:** All investigator or analyzing research laboratories housing research samples need to maintain current Temperature Logs and study-specific Sample Tracking and Shipping Logs. The Principal Investigator/Laboratory Manager must ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens. This is required for both observational and interventional clinical studies collecting clinical samples.

9.15.3 Serum Analyses

Serum (2 ml SST) will be collected on infusion day (Day 0) pre-infusion, and at 1, 2 and 4 hours post-infusion, Days +1, +2, +3, +7, and weeks +2, +4, +8, +12, and at months +6 and +9.

The blood samples will be received by the Immune Analyses Facility at the Center for Immunotherapy (CCC building, 4th floor).

Roswell Park Cancer Institute
Immune Analysis Shared Resource (IASR) CCC room 416
Attn: Junko Matsuzaki (I 285416 Samples)
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Buffalo, NY 14263
(716) 845-8459
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9.16 Pathology

9.16.1 Screening Tumor Tissue Requirements

NY-ESO-1 Testing

Historical NY-ESO-1 results may be used. If not previously done, tumor tissue requirements for CT-antigens (e.g., NY-ESO-1) expression in tumor are as follows:

- Evaluation by Immunohistochemistry will occur at OmniSeq, LLC if required for eligibility (at PNSR if solely needed for research).
- A target minimum of 4 x 4 mm block of primary or metastatic tumor.
- If unable to release tumor block please submit: 5 unstained sections at 4-5 microns thickness on charged glass slides and 8 unstained sections at 10 microns thickness on uncharged glass slides, cut with molecular precautions.

A biopsy of a tumor lesion accessible to outpatient sampling either surgically or by image-guided biopsy will be collected if adequate archival tissue as described above is not available (collect 2 cores and process to FFPE, per site usual process, for NY-ESO-1 testing). For cohort 1, 2, and 3: This lesion will be required to be NY-ESO-1 positive by IHC or RNA analysis, compared to suitable positive and negative controls. For Cohort 4, NY-ESO-1 results will be noted but NY-ESO-1 positivity is not required for eligibility.

Tumor Tissue for Research

Confirmation of available archival tissue:

- Enough tissue for approximately 5 sections at 5 microns thickness and 15-30 sections at 8 microns as a block or as unstained charged slides; material to be requested for patients who

have a subsequent on-treatment biopsy or biopsy at progression. If archival material is extremely limited, a fresh biopsy will be done at the discretion of the investigator.

9.16.2 On Study Tumor Tissue Requirements

Optional biopsies, if clinically feasible, will be performed 6 weeks post ACT (± 1 week, or when the treating physicians deem that the patient is stable enough to undergo these procedures), and upon progression either as an inpatient or outpatient; optional research tumor tissue at progression will only be collected in conjunction with a standard of care procedure needed for patient care.. For each optional biopsy, 5 or more cores (or pieces of tumor tissue equivalent to 18 gauge or larger needle core) should be collected from a core or excisional biopsy procedure; if fewer than 5 cores are available, please process in the following order of priority:

- Cores 1 and 2: place in 10% NBF 12-24 hours (longer is acceptable), then process and embed into 1 block and deliver to IASR
- Core 3: place in cryovial and snap freeze in liquid nitrogen, then store at -80°C until delivery to IASR on dry ice
- Core 4: place in a cryovial of RNAlater, refrigerate overnight, then store at -80°C until delivery to IASR on dry ice
- Core 5+ : place fresh tissue in media provided by IASR (RPMI +10% FBS) and transport on ice pack as soon as possible to IASR for TIL processing.

Tumor tissue for research should be labeled as follows:

- Study # I 258514
- Patient Study ID #
- Time Point (Screening, 6W post-ACT, Progression)
- Collection Date
- Tumor site (e.g. abdominal mass, lymph node, etc.)

For patients having an optional biopsy, the pre-treatment archival “Tumor Tissue for Research” described in section 9.16.1 will also be requested at this time and delivered to IASR when ready.

In patients where follow up tumor biopsies are available, the following will be performed to attempt to detect the NY-ESO-1 TCR/ dnTGFβRII transduced PBMC. The additional tumor biopsies will be analyzed for tumor expression of the tumor antigen target (NY ESO 1, LAGE-1); HLA expression, and infiltration by lymphocytes. The qPCR will be performed by the Genomic Shared Resource (GSR), and the IHC by the Pathology Network Shared Resource (PNSR) at Roswell Park Cancer Institute. TIL analysis will be performed by the Immune Analysis Shared Resource (IASR) at the Center for Immunotherapy, Roswell Park Cancer Institute.

Roswell Park Cancer Institute
Correlative Science Pathology Office, GBSB S-636
Attn: I 285416 Samples

Elm & Carlton Streets
Buffalo, NY 14263
(716) 845-8917
CRSLabPathTeam@RoswellPark.org

Note: Specimens will be accepted Monday through Thursday only (not on a holiday or day before holiday). Please contact the lab for special arrangement if an exception is to be requested.

Note: All investigator or analyzing research laboratories housing research samples need to maintain current Temperature Logs and study-specific Sample Tracking and Shipping Logs. The Principal Investigator/Laboratory Manager must ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens. This is required for both observational and interventional clinical studies collecting clinical samples.

9.17 Gut Microbiota

9.17.1 Fecal microbiome collection

After initial eligibility has been determined, stool samples will be collected within 1 week of apheresis (day -6) and 12 weeks post T cell infusion. The stool specimen will be self-collected by participants. Patients will be provided with a sample collection kit. Patients will be provided with written instructions (Appendix I), and study staff will explain how to use the kits to collect fecal samples in the privacy of their own homes.

We will instruct the participants to return the stool sample within 24 hours of collection. If patient is unable to provide the sample with home collection or enough sample for analysis, a digital rectal exam will be performed at the time of their study visit. All microbiome samples will be labeled with the participant's MR number, participant's initials, participant's study number, clinical study number, protocol time point, and protocol day. Collected stool samples will be sent to DBBR for de-identification, storage and future DNA extraction.

Roswell Park Cancer Institute
DBBR Laboratory
GBSB Bldg. 7th Floor, Rm. 726 via Tube station #86
Attn: Study Number-I 258514
Elm and Carlton Streets
Buffalo, NY 14263
Tel: 716-845-1036
Fax: 716-845-1350
warren.davis@roswellpark.org

Note: All investigator or analyzing research laboratories housing research samples need to maintain current Temperature Logs and study-specific Sample Tracking and Shipping Logs. The Principal Investigator/Laboratory Manager must ensure that the stated lab(s) have a process in

place to document the receipt/processing/storage/shipping of study-related samples/specimens. This is required for both observational and interventional clinical studies collecting clinical samples.

9.17.2 Questionnaires

The Stool Sample Collection Questionnaire (Appendix G) and the Microbiome Collection Questionnaire (Appendix H) were designed for patients enrolling in this study in order to broaden the information gathered on nutrition and basic lifestyle habits that have been shown to have an impact on human microbiome composition. The Stool Sample Questionnaire will be completed only once at screening. The Microbiome Collection Questionnaire will be completed at the same time points as stool collection (within 1 week of apheresis (day -6) and 12 weeks post T cell infusion). Paper questionnaires will be collected by the Clinical Research Coordinator on the day of the patient's visit and will be kept in a secured, locked up study folder in clinic. Data from collected questionnaires throughout the trial will be migrated and stored in a secure REDCap data base.

10 EFFICACY EVALUATIONS

10.1 Objective Tumor Response

All protocol-defined imaging studies must be performed at the investigative site or sponsor-approved facility using protocol-defined parameters. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Immune-Related Response Criteria (irRECIST) described by Nishino et al, 2013 (71) and Bohnsack et al, 2014 (72) will be used to assess objective tumor response.

10.2 Definition of Target and Non-Target Lesions

10.2.1 Target Lesions

- All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, will be identified as target lesions and recorded and measured at baseline
- Measurable lesions must be accurately measured in at least one dimension with a minimum size of:
    - 10 mm in the longest diameter by CT or MRI scan (or no less than double the slice thickness) for non- nodal lesions and ≥ 15 mm in short axis for nodal lesions.
    - 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).
    - 20 mm by chest X-ray
- A sum of the longest diameter (short axis for lymph nodes) of all target lesions will be calculated and reported as the baseline sum diameters. This will be used as reference to

further characterize the objective tumor response of the measurable dimension of the disease.

10.2.2 Non-Target Lesions

- Non-target lesions will include:
    - Measurable lesions not selected as target lesions
    - All sites of non-measurable disease, such as neoplastic masses that are too small to measure because their longest uninterrupted diameter is < 10 mm (or < two times the axial slice thickness), i.e., the longest perpendicular diameter is ≥ 10 and < 15 mm.
    - Other types of lesions that are confidently felt to represent neoplastic tissue, but are difficult to measure in a reproducible manner. These include bone metastases, leptomeningeal metastases, malignant ascites, pleural or pericardial effusions, ascites, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, ill-defined abdominal masses, skin lesions, etc.
- All lesions or sites of disease not recorded as target lesions (e.g., small lesions and non-measurable lesions) should be identified as non-target lesions and indicated as present in the source documents at baseline. There is no limit to the number of non-target lesions that can be recorded at baseline. The general location will also be documented on the images, drawing a regularly-shaped Region of Interest.
- Measurements of the non-target lesions will not be performed, but the presence or absence of each should be noted throughout follow-up and evaluation.

10.3 Lesions

Target and Non-Target Lymph Node Lesion Definitions

- To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Bone Lesions

- Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.
- Regardless of the imaging modality blastic bone lesions will not be selected as target lesions. Lytic or mixed lytic-blastic lesions with a measurable soft tissue component ≥ 10 mm can be selected as target lesions.

Brain Lesions

- Brain Lesions detected on brain scans can be considered as both target or non-target lesions.

Cystic and Necrotic Lesions as Target Lesions

- Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.
- Lesions that are partially cystic or necrotic can be selected as target lesions.
- The longest diameter of such a lesion will be added to the ***Total Measured Tumor Burden (TMTB)*** [1] of all target lesions at baseline.
- If other lesions with a non-liquid/non-necrotic component are present, those should be preferred.

Lesions with Prior Local Treatment

- During target lesion selection the radiologist will consider information on the anatomical sites of previous intervention (e.g. previous irradiation, RF-ablation, TACE, surgery, etc.).
- Lesions undergoing prior intervention will not be selected as target lesions unless there has been a demonstration of progress in the lesion.

No Disease at Baseline

- If a patient has no measurable and no non-measurable disease at baseline the radiologist will assign '***No Disease***' (irND) as the overall tumor assessment for any available follow-up time-points unless new measurable lesions are identified and contribute to the TMTB.

10.3.1 Follow-Up

Recording of Target and New Measurable Lesion Measurements

- The longest diameters of non-nodal target and new non-nodal measurable lesions, and short axes of nodal target and new nodal measurable lesions will be recorded. Together they determine the ***Total Measured Tumor Burden (TMTB)*** at follow-up.

$$\textbf{TMTB \% change} = \frac{\textbf{Baseline Tumor Burden} - \textbf{Current Tumor Burden}}{\textbf{Baseline Tumor Burden}} \times \mathbf{100}$$

Definition of New Measurable Lesions

- In order to be selected as new measurable lesions (≤ 2 lesions per organ, ≤ 5 lesions total, per time-point), new lesions must meet criteria as defined for baseline target lesion selection and meet the same minimum size requirements of 10 mm in long diameter and minimum 15 mm in short axis for new measurable lymph nodes. New measurable lesions shall be prioritized according to size, and the largest lesions shall be selected as new measured lesions.

Non-Target Lesion Assessment

- The RECIST 1.1 definitions for the assessment of non-target lesions apply (i.e., measurements of the non-target lesions will not be performed, but the presence or absence of each should be noted throughout follow-up and evaluation).
- The response of non-target lesions primarily contributes to the overall response assessments of irCR and irNon-CR/Non-PD (irNN).
- Non-target lesions do not affect irPR and irSD assessments.
- Only a massive and unequivocal worsening of non-target lesions alone, even without progress in the TMTB is indicative of irPD.

New Non-Measurable Lesions Definition and Assessment

- All new lesions not selected as new measurable lesions are considered new non-measurable lesions and are followed qualitatively.
- Only a massive and unequivocal progression of new non-measurable lesions leads to an overall assessment of irPD for the time-point.
- Persisting new non- measurable lesions prevent irCR.

10.4 irRECIST Overall Tumor Assessments

The irRECIST overall tumor assessment is based on the ***TMTB*** (total measured tumor burden) of measured target and new lesions, non-target lesion assessment and new non-measurable lesions.

Time point response assessments will be performed at 4 weeks, 12 weeks, 6 months and 9 months.

Conventional RECIST 1.1 will also be utilized during the trial however; RECIST 1.1 will not be used to determine disease progression. The irRECIST will be used for tumor response assessment at time of continuing review and will be used for IRB reporting and formal analysis.

- **irCR:** Complete disappearance of all measurable and non-measurable lesions. Lymph nodes must decrease to < 10 mm in short axis.
- **irPR:** Decrease of ≥ 30% in TMTB relative to baseline, non-target lesions are irNN, and no unequivocal progression of new non-measurable lesions.
- **irSD:** Failure to meet criteria for irCR or irPR in the absence of irPD.
- **irNN:** No target disease was identified at baseline and at follow-up the patient fails to meet criteria for irCR or irPD.
- **irPD:** Minimum 20% increase and minimum 5 mm absolute increase in TMTB compared to nadir, or irPD for non-target or new non-measurable lesions. Confirmation of progression is recommended minimum 4 weeks after the first irPD assessment.
- **irNE:** Used in exceptional cases where insufficient data exists.
- **irND:** In adjuvant setting when no disease is detected.

New Lesions: the presence of new lesion(s) does not define progression. The measurements of the new lesion(s) are included in the sum of the measurements (the sum of the measurements = the sum of the longest diameters of all target lesions and new lesions, if any).

10.5 Confirmation Measurement

A confirmatory assessment is required no less than 6 weeks after a PR or CR is deemed by irRECIST

10.6 Guidelines for Evaluation of Measurable Disease

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

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

- **Chest x-ray:** Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.
- **Conventional CT and MRI:** This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g., for body scans) however, conventional CT scan is the preferred modality to determine response to treatment.

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the irRECIST or RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

- **Ultrasound:** Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study,

confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

- **Tumor Markers:** Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a participant to be considered in complete clinical response.

11 SAFETY EVALUATION

11.1 Adverse Events

11.1.1 Definition

An adverse event or adverse experience (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Therefore, an AE can be ANY unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product (attribution of 'unrelated', 'unlikely', 'possible', 'probable', or 'definite').

An AE is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan in other study-related documents.

11.1.1.1 Diagnosis Versus Signs and Symptoms

If known, a diagnosis should be recorded on the CRF rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be clinically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded as an AE or SAE on the CRF. If a diagnosis is subsequently established, it should be reported as follow-up information.

11.1.1.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (e.g., cascade events or clinical sequelae) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the CRF.

However, clinically significant AEs occurring secondary to an initiating event that are separated in time should be recorded as independent events on the CRF. For example, if a severe gastrointestinal hemorrhage leads to renal failure, both events should be recorded separately on the CRF.

11.1.1.3 Abnormal Laboratory Values

Only clinically significant laboratory abnormalities that require active management will be recorded as AEs or SAEs on the CRF (e.g., abnormalities that require study drug dose

modification, discontinuation of study treatment, more frequent follow-up assessments, further diagnostic investigation, etc.).

If the clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5 x the upper limit of normal associated with cholecystitis), only the diagnosis (e.g., cholecystitis) needs to be recorded on the Adverse Event CRF.

If the clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded as an AE or SAE on the CRF. If the laboratory abnormality can be characterized by a precise clinical term, the clinical term should be recorded as the AE or SAE. For example, an elevated serum potassium level of 7 mEq/L should be recorded as "hyperkalemia".

Observations of the same clinically significant laboratory abnormality from visit to visit should not be repeatedly recorded as AEs or SAEs on the CRF, unless their severity, seriousness, or etiology changes.

11.1.1.4 Preexisting Medical Conditions (Baseline Signs and Symptoms)

A preexisting medical condition should be recorded as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When recording such events on an Adverse Event CRF, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

11.1.2 Grading and Relationship to Drug

The descriptions and grading scales found in the CTEP Version 4 of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. CTEP Version 4 of the CTCAE is identified and located at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. AEs not covered by specific terminology listed should be reported with common medical terminology, and documented according to the grading scales provided in the CTCAE Version 4.

The relationship of event to study drug will be documented by the Investigator as follows:

- **Unrelated:** The event is clearly related to other factors such as the patient's clinical state, other therapeutic interventions or concomitant drugs administered to the patient.
- **Unlikely:** The event is doubtfully related to investigational agent(s). The event was most likely related to other factors such as the patient's clinical state, other therapeutic interventions, or concomitant drugs.
- **Possible:** The event follows a reasonable temporal sequence from the time of drug administration, but could have been produced by other factors such as the patient's clinical state, other therapeutic interventions or concomitant drugs.
- **Probable:** The event follows a reasonable temporal sequence from the time of drug administration, and follows a known response pattern to the study drug. The event cannot be reasonably explained by other factors such as the patient's clinical state, therapeutic interventions or concomitant drugs.

- **Definite:** The event follows a reasonable temporal sequence from the time of drug administration, follows a known response pattern to the study drug, cannot be reasonably explained by other factors such as the patient's condition, therapeutic interventions or concomitant drugs; AND occurs immediately following study drug administration, improves upon stopping the drug, or reappears on re-exposure.

11.1.3 Reporting Adverse Events

Table 3 Guidelines for Routine Adverse Event Reporting for Phase 1 Studies (Regardless of Expectedness)

Attribution	Grade 1	Grade 2	Grade 3	Grade 4
Unrelated	X	X	X	X
Unlikely	X	X	X	X
Possible	X	X	X	X
Probable	X	X	X	X
Definite	X	X	X	X

Table 4 Guidelines for Routine Adverse Event Reporting Phase 2 Studies (Regardless of Expectedness)

Attribution	Grade 1	Grade 2	Grade 3	Grade 4
Unrelated			X	X
Unlikely			X	X
Possible	X	X	X	X
Probable	X	X	X	X
Definite	X	X	X	X

11.2 Routine AE's will be reported from the start date of intervention. Refer to section 9.13 for duration of follow-up AE reporting. Serious Adverse Events

11.2.1 Definition

A serious adverse event (SAE) is any adverse event (experience) that in the opinion of either the investigator or sponsor results in **ANY** of the following:

- Death.
- A life-threatening adverse event (experience). Any AE that places a patient or patient, in the view of the Investigator or sponsor, at immediate risk of death from the reaction as it occurred. It does NOT include an AE that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours).

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly or birth defect.
- Important Medical Event (IME) that, based upon medical judgment, may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

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

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

11.2.2 Reporting Serious Adverse Events

All new SAEs occurring from the date the patient signs the study consent until 30 days after the last intervention or a new treatment is started, whichever comes first, will be reported. The RPCI SAE Source Form is to be completed with all available information, including a brief narrative describing the SAE and any other relevant information.

SAEs occurring after the 30 day follow-up period that the investigator determines to be possibly, probably or definitely related to the study intervention should be reported.

SAEs identified as an Unanticipated Problem by the Investigator must be reported. Please refer to Section 11.5 for details on reporting Unanticipated Problems.

11.3 Investigator Reporting: Notifying the Study Sponsor

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

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

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

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

11.4 Follow-Up for Serious Adverse Events

All related SAEs should be followed to their resolution, until the study patient is lost to follow-up, the start of a new treatment, or until the study investigator assesses the event(s) as stable or irreversible. New information will be reported when it is received.

11.5 Unanticipated Problems

11.5.1 Definition

An Unanticipated Problem (UP) is any incident, experience, or outcome that meets all of the following criteria:

Unexpected (in terms of nature, severity, or frequency) given:

a) The research procedures that are described in the study-related documents, including study deviations, as well as issues related to compromise of patient privacy or confidentiality of data.

b) The characteristics of the patient population being studied.

Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research).

Suggests that the research places patients or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized and if in relation to an AE is also deemed Serious per Section 11.2.

11.5.2 Reporting Unanticipated Problems

The Reportable New Information (RNI) Form will be submitted to the CRS Quality Assurance (QA) Office within 1 business day of becoming aware of the Unanticipated Problem. After review, CRS QA Office will submit the RNI to the IRB.

When becoming aware of new information about an Unanticipated Problem, submit the updated information to CRS QA Office with an updated Reportable New Information Form. The site Investigator or designated research personnel will report all unanticipated problems to the IRB in accordance with their local institutional guidelines.

11.6 FDA Reporting

When RPCI is the IND holder the following describes the FDA reporting requirements by timeline for AEs and new safety findings that meet the criteria outlined below:

Within 7 Calendar Days

Any adverse event that meets ALL the following criteria:

- Related or possibly related to the use of the study drug;
- Unexpected; and
- Fatal or life-threatening.

Within 15 Calendar Days

Any adverse event that meets ALL the following criteria:

- Related or possibly related to the use of the study drug;
- Unexpected; and
- Serious but not fatal or life-threatening;

Or, meets ANY of the following criteria:

- A previous adverse event that is not initially deemed reportable but is later found to fit the criteria for reporting (report within 15 days from when event was deemed reportable).
- Any findings from other studies, including epidemiological studies, pooled analysis of multiple studies, or other clinical studies conducted with the study drug that suggest a significant risk in humans exposed to the drug.
- Any findings from animal or in vitro testing that suggest a significant risk for human participants including reports of mutagenicity, teratogenicity, or carcinogenicity or reports of significant organ toxicity at or near the expected human exposure.
- Any clinically important increase in the rate of occurrence of a serious, related or possibly related adverse event over that listed in the protocol or investigator brochure.

Sponsors are also required to identify in IND safety reports, all previous reports concerning similar adverse events and to analyze the significance of the current event in the light of the previous reports.

Reporting Process

The principal investigator or designee will complete and submit a FDA Form 3500A MedWatch for any event that meets the above criteria. Forms will be submitted to the CRS Compliance Office via email to CRSCompliance@RoswellPark.org.

11.7 Study Stopping and Non-stopping Rules

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

Absolute stopping rules for this clinical trial will be:

1. Any death that is possibly related to the NY-ESO-1 TCR (T cell receptor).
2. Two or more grade 4 events that are possibly related to the NY-ESO-1 TCR (T cell receptor).
3. Any event of hematological malignancy must be considered study stopping criteria until oncogenesis related to retroviral mutagenesis, EBV lymphoma and Post-Transplant Lymphoproliferative Disorder (PTLD) can be excluded.

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

11.8 Gene Therapy Study Reporting of Serious Adverse Events

Studies that involve Gene Therapy will report SAEs as described above and will be reported to the Office of Biotechnology Activities (OBA) / NIH.

Any SAE that is fatal or life-threatening, that is unexpected, and associated with the use of the gene transfer product must be reported to the FDA and NIH OBA as soon as possible, but not later than 7 calendar days after the Sponsor's initial receipt of the information.

SAEs that are unexpected and associated with the use of the gene transfer product, but are not fatal or life-threatening, must be reported to the FDA and NIH OBA as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information.

If, after further evaluation, an adverse event initially considered not to be associated with the use of the gene transfer product is subsequently determined to be associated, then the event must be reported to the NIH OBA within 15 days of the determination.

Any follow-up information relevant to a SAE must be reported within 15 calendar days of the Sponsor's receipt of the information. If a SAE occurs after the end of a clinical trial and is determined to be associated with the use of the gene transfer product, that event shall be reported to the NIH OBA within 15 calendar days of the determination.

The Principal Investigator or delegated research staff will submit a report to the CRS Compliance Office via email: CRS Compliance for all gene therapy SAEs. The Compliance office will forward the information to ORSP.

Completed reports may be sent via U.S. mail, courier service, e-mail, or facsimile to:

NIH Office of Biotechnology Activities
6705 Rockledge Drive, Suite 750
Bethesda, Maryland 20892-7985
(For all non-USPS deliveries use Zip Code 20817)
Telephone: 301-496-9838
Fax: 301-496-9839
E-mail address for Reporting Adverse Events: GeMCRIS@od.nih.gov
General E-mail: oba@od.nih.gov
Website: http://oba.od.nih.gov/oba/index.html

Additional information for reporting adverse events, including a report template, can be found at: http://oba.od.nih.gov/rdna/adverse_event_oba.html

12 DATA AND SAFETY MONITORING

Phase 1 studies will be reviewed at the scheduled Roswell Park Early Phase Clinical Trials (EPCT) meetings and the minutes are forwarded to the IRB for review.

The Research Monitor, Kelvin Lee, MD, PhD, is responsible to oversee the safety of the research and report observations/findings to the IRB or a designated institutional official. The Research Monitor will review all unanticipated problems involving risks to subjects or others associated with the protocol and provide an independent report of the event to the IRB. The Research Monitor may discuss the research protocol with the investigators; shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report; and shall have the responsibility to promptly report their observations and findings to the IRB or other designated official and the HRPO.

13 STATISTICAL METHODOLOGY

This is a Phase I/IIa clinical trial principally designed to assess the safety (defined by dose limiting toxicities, DLT) and feasibility (defined by sufficient numbers of circulating, lot-release confirmed T cells) of treating patients with advanced malignancies with escalating doses of infused genetically engineered PBMC expressing NY-ESO-1 TCR together with a dnTGFβ-RII transgene administered after lympho-depleting conditioning chemotherapy.

The phase I part of the study includes three cohorts of 3 subjects (+ 3 additional subjects in case of 1 DLT) will receive, 10^7, 10^8 and 10^9 NY-ESO-1/dnTGFβRII cells, respectively. The assessment of safety and feasibility is based on studying cohorts of 3 subjects, with the addition of 3 subjects in case of toxicities meeting the definition of DLT in one of the three first subjects in a cohort. If two or more subjects have DLT in a cohort, that cohort will be considered beyond the MTD and an intermediate dosing may be explored to better define the MTD of this combination. The Phase II part of the study will include an expanded cohort of 9 patients at the maximum tolerated dose.

13.1 Sample Size Determination

A maximum of 27 patients at RPCI will be enrolled. A minimum of 3 evaluable subjects will be treated at each of the 3 cohorts and 9 patients in cohort 4. Accrual is expected to take up to 4 years.

13.2 Definition of Evaluable Patients

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

13.3 Primary Study Endpoints

13.3.1 Safety

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

In this study, safety evaluations will continue and the same early stopping rules for DLTs will apply to this study. Safety remains the priority in all phases and stages of the investigational studies.

13.3.2 Feasibility

After entering 3 patients to each cohort, followed up for a minimum of 1 month after the last subject has received the infusion of the transgenic cells, an assessment of protocol feasibility will be done by the study investigators. The feasibility assessment will be based on potential problems in the manufacturing of NY-ESO-1/ dnTGFβRII engineered cells (Table 5**)**.

Table 5 Criteria to Declare the Study Unfeasible

Parameter	Criteria for Unfeasibility
Feasibility of generation transgenic cells that meet the lot release criteria.	3 or more preparations not meeting the lot release criteria in each cohort

13.4 Secondary Study Endpoints

13.4.1 Transgenic Cell Persistence

TCR and vector presence will be quantitated in PBMC samples, obtained before adoptive transfer and at weeks 1, 2, 4, 8, and 12 after transgenic cell adoptive transfer. Thereafter, sampling will be every 3 months during the first year, and then every 6 to 12 months as per Schedule of Procedures and Observations (refer to **Table 2**). Analysis will be performed both using immune monitoring and molecular techniques. Detection of surface expression of the NY-ESO-1 TCR transgenic protein will be analyzed both by MHC tetramer analysis and staining for the specific region. The V5 tagged 2A sequence linked to the dnTGFβRII and quantitative PCR will allow the assessment of the ratio of NY-ESO-1 TCR versus NY-ESO-1 TCR/dnTGFβRII transgenic cells.

13.4.1.1 Long Term Monitoring for Replication Competent Retrovirus (RCR)

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

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

13.4.1.2 Analysis of Retroviral Insertion Sites in Long Term Persisting NY-ESO-1 TCR Clones

It is possible that expansion of specific T-cell clones will be observed as tumor reactive T cells proliferate in response to tumor antigens. Therefore, care will be taken to track T cell persistence both immunologically and molecularly. Blood samples for persistence of TCR gene transduced cells will be obtained at 3, 6, and 12 months, and then annually thereafter. If any patient shows a high level of persistence of TCR gene transduced cells at month 6 (by semi quantitative DNA-PCR using primers specific for vector sequences) the previously archived samples will be subjected to techniques that would allow the identification of clonality of persisting TCR gene transduced cells. Such techniques may include T cell cloning or LAM-PCR. Clonality analysis is available through NGVL (73).

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.

13.4.1.3 Immunological Monitoring

Immunological monitoring will consist primarily of quantifying T cells bearing surface NY-ESO-1 TCR by NY-ESO-1(157-165) /MHC tetramer analysis. We will use our methodology based on combination of NY-ESO-1(157-165) tetramer and peptide stimulation to analyze PBMC samples with intracellular effector cytokine staining of TNFα and IFNγ, and Granzyme B / CD107a,b for cytotoxic T cell function. With our expertise in polychromatic flow cytometry (74-77) we will also be able to perform simultaneous multifunctional assessment (Almeida et al 2007) of the transgenic cells. Functional assays like ELISA and/or multi-cytokine array assays will complement the results. Immunological assays will be compared between 1) pre-infusion PBMC, 2) an aliquot of

the engineered PBL at the time of infusion, 3) cells recovered from patients' peripheral blood after adoptive transfer.

Additional testing may be performed with other assays, for example intracellular phosphoprotein staining for signaling networks, multiplexed microfluidic and nanotechnology-based immune monitoring assays (78).

13.4.2 Evaluation of NY-ESO-1 TCR Transgenic Cell Tumor Trafficking

13.4.2.1 Tumor Biopsies

For the numbers and functional phenotype of NY-ESO-1 TCR transgenic T cells accumulating at the tumor site, percutaneous or image-guided biopsies of tumor lesions between study days 20-40, with allowed variability from this range, will be performed.

The biopsies will be analyzed by H&E, IHC to quantify the numbers of T lymphocytes If the sufficient quantity of tissue is available, the study investigators will attempt to monitor the phenotype of the TIL obtained from tumor biopsy samples by MHC tetramer, multicolor flow cytometry (FACS), and other immune monitoring assays.

13.4.3 Antitumor Activity

13.4.3.1 Malignancy Status

Tumor response is an exploratory endpoint in this clinical trial. All patients who have received an infusion of NY-ESO-1 TCR/dnTGFβRII transgenic T cells and have tumor assessments at baseline and during the study follow up will be considered evaluable for tumor response.

Potential objective responses to this combinatorial immunotherapy will be recorded following the irRECIST (71, 72). Appropriate evaluations, including physical exam, pictures of visible lesions and imaging exams, will be evaluated at screening and then between study days 75-90, or at discontinuation of study. The duration of patient evaluation may be extended if additional outpatient visits are required to assess duration of tumor response or time to progression.

13.5 Efficacy Analysis

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

13.6 Safety Analysis

DLTs are the primary endpoint of this study and are used in the estimation of the MTD and the accompanying of the dose escalation decisions. However, no formal analyses of DLTs are planned.

Patients who do not have a DLT and who do not complete a full cycle of treatment will be considered non-evaluable for DLT.

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

In this study, safety evaluations will continue and the same early stopping rules for DLTs will apply to this study. Safety remains the priority in all phases and stages of the investigational studies.

13.7 Adverse Event

The frequency of toxicities will be tabulated by grade across all dose levels and cycles. The frequency of toxicities will also be tabulated for the dose estimated to be the MTD. All patients who receive any study treatment will be considered evaluable for toxicity.

13.8 Interim Analysis and Criteria for Early Termination of the Study

No explicit interim analyses are planned for this study.

However, this is a Phase 1 study and as such will be monitored and discussed by Roswell Park's EPCT Committee, which meets weekly. Drug safety will be monitored and evaluated continuously throughout the study including 30 day safety follow-up period by obtaining, reviewing and analyzing data on AEs, changes in laboratory values, vital signs, electrocardiograms (ECGs), and physical examination findings. Potential early termination decisions are an inherent part of the Phase 1 study monitoring.

14 ETHICAL AND REGULATORY STANDARDS

14.1 Ethical Principles

This study will not be initiated until the protocol and informed consent document(s) have been reviewed and approved by a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC). Each patient (or legal guardian) shall read, understand, and sign an instrument of informed consent prior to performance of any study-specific procedure. It is the responsibility of the investigator to ensure that the patient is made aware of the investigational nature of the treatment and that informed consent is given.

The Investigator is responsible for the retention of the patient log and patient records; although personal information may be reviewed by authorized persons, that information will be treated as strictly confidential and will not be made publicly available. The investigator is also responsible for obtaining patient authorization to access medical records and other applicable study specific information according to Health Insurance Portability and Accountability Act regulations (where applicable).

This study will be conducted in compliance with all applicable laws and regulations of the state and/or country and institution where the patient is treated, in accordance with the Declaration of

Helsinki, Good Clinical Practice, and according to the guidelines in this protocol, including attached appendices.

The IRB approved the consent form and protocol allowing for a legally authorized representative to consent on the patient's behalf if necessary, following IRB and institute policies. This is an early phase therapeutic clinical study in which patients have advanced disease that is resistant or intolerant to existing therapy, with no standard of care options and therefore has intended potential benefit to each individual patient in order to have a favorable benefit-risk relationship.

14.2 Informed Consent

The Investigator (or IRB approved designee) is responsible for obtaining written consent in advance, from each patient or the patient's legally authorized representative in accordance with ICH-GCP guidelines using the approved informed consent form, before any study specific procedures (including screening procedures) are performed. The informed consent form acknowledges all information that must be given to the patient according to ICH-GCP, including the purpose and nature of the study, the expected efficacy and possible side effects of the treatment(s), and specifying that refusal to participate will not influence further options for therapy. Any additional information that is applicable to the study must also be included. Additional national or institutionally mandated requirements for informed consent must also be adhered to. The patient should also be made aware that by signing the consent form, processing of sensitive clinical trial data and transfer to other countries for further processing is allowed.

The Investigator shall provide a copy of the signed consent form to the patient and the signed original shall be maintained in the Investigator File. A copy of the signed consent form must be filed in the patient file. At any stage, the patient may withdraw from the study and such a decision will not affect any further treatment options.

15 STUDY RESPONSIBILITIES

15.1 Data Collection

Data entry into the database is to be completed in a timely fashion (within 30 days) after the patient's clinic visit. If an AE is considered serious it is captured on both the Adverse Event page and the Serious Adverse Event Source Form, which is handled in an expedited fashion.

Data management activities will be performed using eClinical. eClinical is a suite of software tools that enables the collection, cleaning and viewing of clinical trial data. CRS data management will design the study-specific database and facilitate its development by the eClinical Information Technology team. Once the database design is approved by the Investigator, Statistician, and Clinical Research Coordinator, the database will be put into production and data entry can begin. Data can be entered and changed only by those with the rights to do so into the eCRFs (via the EXPeRT Module). eClinical is compliant with all relevant technical aspects of relevant GCP guidelines.

- The system can generate accurate copies of stored data and audit trail information in human readable form.

- System access is limited to authorized individuals through the controlled assignment of unique ID and password combinations.
- The system is designed to periodically force users to change their passwords and verifies that user ID and password combinations remain unique.
- The system automatically generates a permanent time-stamped audit trail of all user interactions.

When data entry is complete, data management will review the data and will query any missing, incomplete, or invalid data points for resolution by the Clinical Research Coordinator and Investigator. Once all queries have been resolved, the data can be released to the statistician for analysis.

15.2 Maintenance of Study Documents

Essential documents will be retained per RPCI's policy for 6 years from the study termination date. These documents could be retained for a longer period, however, if required by the applicable local regulatory requirements or by an agreement with RPCI.

16 ADMINISTRATIVE RULES

16.1 Revisions to the Protocol

RPCI may make such changes to the protocol as it deems necessary for safety reasons or as may be required by the U.S. FDA or other regulatory agencies. Revisions will be submitted to the IRB/ERC for written approval before implementation.

16.2 Termination of the Study

It is agreed that, for reasonable cause, either the RPCI Investigators or the Sponsor, may terminate this study, provided a written notice is submitted within the time period provided for in the Clinical Trial Agreement. In addition, RPCI may terminate the study at any time upon immediate notice if it believes termination is necessary for the safety of patients enrolled in the study.

16.3 Confidentiality

Any data, specimens, forms, reports, video recordings, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a limited access environment. All computer entry and networking programs will be done using PIDs only. Information will not be released without written authorization of the participant.

17 APPENDICES

Appendix A ECOG Performance Status Scores

Description	Status
Fully active, able to carry on all pre-disease performance without restriction.	0
Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.	1
Ambulatory and capable of all self-care but unable to carry out any work activities.	2
Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	3
Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	4
Dead	5

Appendix B Procedure for Generation of Experimental Cell Therapies

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

Generation of NY-ESO-1/ dnTGFβRII Transduced PBMC

Leukapheresis Procedure

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

PBMC Isolation

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

PBMC Activation

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

Clinical Grade Retrovirus Lot Release Testing

Prior to use, the clinical grade retroviral vector will require fulfilling the lot release criteria: (1) Certificate of Analysis of the retroviral vector Master Cell Bank (MCB) and, (2) Certificate of Analysis of the Clinical Grade Retrovirus (See **Appendix C**).

Appendix C Clinical Grade Retrovirus Lot Release Criteria

C1 Certificate of Analysis of the retroviral vector Master Cell Bank (MCB)

Test	Methods	Limits	Results	Date
Contaminants	Aerobic and anaerobic culture for bacteria and fungus	No growth within 14 days	No growth within 14 days	8/27/2014
	Mycoplasma culture and vero indicator cells	Negative for the presence of mycoplasma	Negative	7/15/2014
	In-vitro viral assay utilizing 3T3, MRC-5 and Vero cells	No CPE or hemadsorption	No CPE or hemadsorption	8/27/2014
	Bovine viral assay*	Negative for cytopathic effect, hemadsorption and the presence of 7 specific bovine viruses using IFA	Negative for cytopathic effect, hemadsorption and the presence of 7 specific bovine viruses using IFA	8/25/2014
	Porcine viral assay*	Negative for cytopathic effect, hemadsorption and the Presence of porcine Viruses, BVDV Reovirus and rabies	Negative for cytopathic effect, hemadsorption and the Presence of porcine Viruses, BVDV Reovirus and rabies	8/25/2014
	In-vivo viral assay*	No evidence of viral contamination	Negative for the presence of adventitious viral contaminants	8/13/2014
	MAP/LCM*	No evidence of viral contamination	No evidence of viral contamination	8/15/2014
	Transmission E.M.*	No Vital particles other than retro-virus like particles and no other identifiable microbial agents.	No Vital particles other than retro-virus like particles and no other identifiable microbial agents	8/8/2014
	SV40 large T antigen (PCR)*	Negative	<10 copies/0.2 ug DNA	8/28/2014
	E1a (PCR)*	Negative	<10 copies/0.2 ug DNA	8/28/2014
Human Vital Contaminants	HTLV (PCR)*	Negative	Negative	7/7/2014
	HAV (PCR)*	Negative	Negative	7/7/2014
	HCV (PCR)*	Negative	Negative	7/7/2014
	HBV (PCR)*	Negative	Negative	7/7/2014
	SV40 (PCR)*	Negative	Negative	7/7/2014
	HIV-I, HIV-II (PCR)*	Negative	Negative	7/7/2014
	HHV 6, HHV 7, HHV 8 (PCR)*	Negative	Negative	7/7/2014
	CMV (PCR)*	Negative	Negative	7/7/2014
	EBV (PCR)*	Negative	Negative	7/7/2014
	Human parvovirus B19	Negative	Negative	7/7/2014
	HSV 1/2	Negative	Negative	7/7/2014

Test	Methods	Limits	Results	Date
Replication Competent Retrovirus: GAL-V	S+L- (PG-4/293) amplification) 5% of MCB supernatant	No Evidence of RCR	No Evidence of RCR	8/27/2014
Replication Competent Retrovirus: GAL-V	S+L- (PG-4/293 co-culture) 1% of MBC	No Evidence of RCR	No Evidence of RCR	8/27/2014
Identity	ADA Isoenzyme	Cells confirmed to be Of murine origin	Cells confirmed to of murine origin	8/28/2014
Vector insert stability	Transduction of HeLa cells and analysis by Southern blotting	Band consistent with predicted fragment size	Band consistent with predicted fragment size	8/28/2014

C2 Certificate of Analysis of the Clinical grade retrovirus

Test	Methods	Limits	Results	Date
Contaminants	Aerobic and anaerobic culture for Bacteria and Fungus	No growth 14 days	No growth 14 days	7/17/2015
	Mycoplasma culture and Vero indicator cells	Negative for the presence of Mycoplasma	Negative for the presence of Mycoplasma	5/29/2015
	In-Vitro viral assay utilizing 3T3, MRC-5 And Vero cells	No CPE or hemadsorption	No CPE or hemadsorption	7/17/2015
Endotoxin	Limulus amebocyte lysate	Less than 0.33 EU/mL	Harvest 1-7: less than 0.06 EU/mL	7/17/2015
Replication competent retrovirus: GAL-V	S+L-(PG-4) (293 infection) 5% of vector supernatant	No Evidence of RCR	No Evidence of RCR	7/10/2015
	S+L-(PG-4) (293 co-culture) 10^8 cells from production run	No Evidence of RCR	No Evidence of RCR	7/17/2015
Transgene expression	NY-ESO-1(157-165) tetramer staining in activated PBMC	> 30% NY-ESO-1(157-165) tetramer positive cells among CD3+ T lymphocytes	Harvest 1-7: 49.6 to 60% NY-ESO-1(157-165) tetramer positive cells among CD3+ T lymphocytes	2/27/2015
Potency	NY-ESO-1(157-165) peptide and antigen-specific IFN-γ production by ELISA	> 30,000 pg/ml of IFN-γ production upon NY-ESO-1(157-165) peptide stimulation using NY-ESO-1(157-165) peptide-pulsed K562/A2.1 cells	Harvest 1-7: > 100,000 pg/ml of IFN-γ production upon NY-ESO-1(157-165) peptide stimulation using NY-ESO-1+ HLA2+ cell line	3/6/2015

Appendix D Retrovirus Transduction and Cell Expansion Method

The retroviral vectors will be stored in a monitored and locked < = -70ºC freezer in the GMP Suite, Transductions will be performed in RetroNectin®-coated 6-well plates.

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

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

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

Transgenic PBMC Product Washing and Bagging

After completion of the cell transduction procedure and short term ex vivo expansion, the NY-ESO-1/ dnTGFβRII transduced PBMC products are subjected to two washings, resuspended in saline containing 1% HSA, put into infusion bags and kept in a 4 degrees refrigerator until lot release clearance and i. v. infusion. Lot release tests are performed on aliquots of this final product. In the event patient is not able to receive the infusion within 24 hours of the scheduled harvest, cells will be re-suspended in cryopreservation medium and put into an infusion bag. Cryopreservation will be at < -130oC in a monitored liquid nitrogen freezer at the GMP suite.

Product Labeling

The final NY-ESO-1/ dnTGFβRII transgenic PBMC bags will be labeled using a preprinted study label with the following information:

- Patient ID. Number
- Patient's initials
- Subject laboratory clinical trial code
- Date of cell preparation, with an expiration date of 24 hours if fresh and of 6 months if cryopreserved
- Initials of person who prepared the vaccine
- Labeled with "For autologous use only"

- Labeled as “Not Tested for Biohazards” (due to no specific testing for HIV, HepC, HepB and other adventitious viruses in the final product)

Appendix E In-process Testing and Final Product Lot Release Testing

In Process Testing: We will conduct the following in-process testings:

Appendix E 1 Activated PBMC (Day 2) In-process Testing

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

Appendix E 2 Transduced PBMC (Day 5) In-process Testing

Test Item	Acceptable Criteria
MycoAlert test	Negative ;ratio<1 (reading B/reading A)
TCR transgene expression	>30% of T cells by NY-ESO-1(157-165) tetramer staining
TCR transgene functionality	> 5,000 pg/ml/million cells of NY-ESO-1 specific IFN-γ production by ELISA

Appendix F Final Product Testing and Lot Release Criteria

The results that will be required before administering the cells to the patients are described (**F1**), along with the results that need to be in process at that time but results may not be available at the time of administration to patients are described **(F2**

Appendix F 1 NY-ESO-1/ dnTGFβRII Transgenic PBMC Lot Release Testing with Results Available Before Administration to Patients

Test Item	Acceptable Criteria
Viability	≥ 70%
Gram stain[a]	Negative
Endotoxin Assay [b]	≤ 5 EU/ kg body weight per dose
Mycoplasma rapid test[c]	Negative ;ratio<1 (reading B/reading A)
TCR transgene expression	>30% of T cells by NY-ESO-1(157-165) tetramer staining

a. Performed on the final product. All sterility cultures will be followed up to 14 days. If the cultures become positive after the patient has received the cells, the patient will be started on empiric antibiotics.

b. Performed on the final product using the Limulus amebocyte lysate read using the Quantitative Chromogenic Procedure analyzed using a OCL-1000 (Bio Whittaker), or the Endosafe® - PTS™ (Portable Test System: Charles River Laboratories International, Inc.) assays.

c. If first line MycoAlert test becomes positive, it will be repeated with a second sample from the final product.

Appendix F 2 NY-ESO-1/ dnTGFβRII Transgenic PBMC Lot Release Testing with Results Available After Administration to Patients

Test Item	Acceptable Criteria
Bacterial and fungal culture	No growth x 14 days
GalV S^+ /L^- RCR assay	Sample archived for later use if required. Not tested prospectively in all samples.
TCR transgene functionality	> 5,000 pg/ml/million cells of NY-ESO-1 specific IFN-γ production by ELISA

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

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

PCR test, the stored sample of the final product will be tested. The patient will be contacted, two peripheral blood draws will be collected for confirmation, and the patient will be started on therapy with combination antiretrovirals used to treat HIV infection.

Notification of Regulatory Agencies for Positive Results of TCR Transgenic Cell Administration to Patients.

If a preparation with bacterial, fungal, mycoplasma or RCR contamination has been administered to a patient, this event should be reported to the following agencies and committees within 48 hours of first knowledge:

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

Additional Optional Testing of TCR Transgenic Cells. The release tests will be used to determine whether the final cell product can be released for infusion (see Tables above). In addition, the cell product may undergo further characterization apart from the release testing. The vector-specific proviral copy number per cell may be determined. The sites of integration may be defined as may be the structure of the integrated transgene. Detailed flow cytometry and TCR chain usage analysis may be performed. The product may be further characterized for measurable transgene products (**F3**)

Appendix F 3 NY-ESO-1/ dnTGFβRII Transgenic PBMC Optional Additional Testing

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

Appendix G Stool Sample Collection Questionnaire

Patient Name:
MRN:
Date:

Stool Sample Collection Questionnaire

1. **What is your race? (one or more categories may be selected)**
   - American Indian or Alaska Native
   - Black or African American
   - White
   - Asian, please specify:_____
   - Native Hawaiian/Other specific Islander:
   - Other Race, please specify:_____

2. **Are you Hispanic or Spanish origin?**
   - No
   - Yes

3. **Were you breastfed as an infant?**
   - No
   - Yes
   - I don't know

4. **Were you delivered via vaginal delivery or C-section?**
   - Vaginal delivery
   - C-section
   - I don't know

5. **Have you had an appendectomy?**
   - No
   - Yes
   - I don't know

6. **Have you ever had any bowel surgery/removal a portion of your bowel or stomach?**
   - No
   - Yes, please specify_____
   - I don't know

7. Were you ever diagnosed with an autoimmune disease?

- No _____
- Yes, please specify_____
- I don't know _____

Appendix H Microbiome Collection Questionnaire

Patient Name:
MRN:
Date:

MICROBIOME COLLECTION QUESTIONNAIRE

1. **Have you had any fevers or infection in the past 2 weeks?**
   - o No
   - o Yes, please specify:_____

2. **Have you taken antibiotics or antifungal agents in the past 4 weeks?**
   - o No
   - o Yes, please tell us which medication(s):_____
     Why were you given antibiotics? _____
     For how long did you take it? _____

3. **How many times a week do you eat red meat?**
   - o I don't eat red meat
   - o 1-3 times a week
   - o More than 3 times a week

4. **How many times a week you eat cruciferous vegetables such as cabbage, broccoli, kale, and cauliflower?**
   - o I don't eat cruciferous vegetables
   - o 1-3 times a week
   - o More than 3 times a week

5. **Do you eat yogurt?**
   - o I don't eat yogurt
   - o 1-3 times a week (please specify which kind)_____
   - o More than 3 times a week (please specify which kind)_____

6. **Do you take probiotics?**
   - o No
   - o Yes, please indicate how often and brand name if known:
     _____

7. **Are there foods you must avoid?**
   - o No
   - o Yes, please list the food(s):_____
   - o Foods you avoid for religious, personal, or cultural

reasons:_____
- Foods your Doctor told you to avoid:_____

8. If you are CURRENTLY on a special diet, please indicate below:
- Not on a special diet
- Weight loss
- Weight gain
- Vegetarian
- Diet for diabetes
- Diet for heart disease
- Diet for kidney disease
- Other:_____

9. Are you currently taking prescribed or over-the-counter medications to lose weight or maintain your current weight?
- No
- Yes, I am on these weight loss medications:_____

10. Are you taking laxatives to have a bowel movement on a regular base?
- No
- Yes, please describe which kind and how often:_____

11. How would you describe your bowel movements in the past 2 weeks:
- Regular bowel movements every 1-2 days with no change
- Frequent issues diarrhea, please describe_____
- Frequent issues with constipation, please describe_____

12. Do you participate in regular physical activity?
- No
- Yes, please describe what exercise do you like to do?_____
  How long? _____
  How many times a week? _____

13. Do you smoke?
- No
- Yes (please indicate how many cigarettes a day)_____

Appendix I Stool Collection Instructions

STOOL SAMPLE COLLECTION INSTRUCTIONS

On the morning of your visit (or the day prior, but within 24 hours of your visit), please collect a small stool sample and fill out the Stool Sample Collection Questionnaire.

Before starting:

Please check to make sure that you have received the following:

1. A white stool collection container and toilet ring
2. Two (2) plastic tubes with a scoop attached to each lid.
3. A plastic zip-lock bag with an orange biohazard sign
4. A pair of disposable gloves

If something is missing, or if any of the following instructions are unclear, please call the research nurse.

Getting ready to collect:

- Line the white collection container with toilet paper. It is important that you do NOT use the bag with the orange biohazard sign on it, because that bag cannot be flushed down the toilet.
- Follow the instructions on the container lid for placing the container under the toilet seat. The toilet seat and collection kit should be set up like this:

- Sit on the toilet, relax, and collect a stool sample into the container.

Getting sample into the vials we provide:

1. Put on the gloves.
2. Remove the lid from the first tube provided. Using the scoop that is attached to the lid, place 2 pea-sized scoops into the tube. Please do not collect more than 2 pea-sized scoops.
3. Screw the lid onto the tube, and **make sure it is on properly and tightly**.
4. Repeat steps 2-3 with the other tube provided.

5. Place both tubes into the plastic zip-lock bag with the red biohazard sign on it, and **seal it tightly**.

Disposing of the leftover stool:

Dispose of the contents of the collection container (toilet paper, leftover stool) in the toilet.

Dispose of the white plastic collection container in the regular trash.

Remove the gloves, and throw them in the trash. **Do NOT** flush any part of the white collection container or the gloves down your toilet.

Completing the form:

When you are finished and have washed your hands, complete the "Stool Sample Collection Questionnaire" form (on the following page).

Storing before delivery:

Store the collection bag in a cool place, but not the refrigerator. Bring the tubes in the biohazard bag and the Stool Sample Collection Form with you on the morning of your visit, and give them to the research nurse.

Clinic Use Only
Study ID:
Date:
Collection:

STOOL SAMPLE COLLECTION QUESTIONNAIRE

Did you have any problems or concerns with the stool collection? (Please describe):

_____

_____

_____

1) Date stool sample collected: _____-_____-_____ **(MM/DD/YYYY)**

2) Time of collection: _____: _____ **(Hr:Min)** ☐**AM** ☐**PM**

PLEASE BRING THIS QUESTIONNAIRE AND THE SAMPLE MATERIALS TO YOUR NEXT VISIT.

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