This material contains content that is not fully supported by assistive technology. For accessibility assistance with the file, please contact:

Robert Jambou
[jambour@od.nih.gov -- 301.435.8267].
Phase I trial of NY-ESO-1 redirected CRISPR edited T cells (NYCE cells) engineered to express NY-ESO-1 TCR and gene edited to eliminate endogenous TCR and PD-1

Protocol # 1604-1524

NIH OBA RAC Public Review
June 21, 2016
# NYCE Protocol 1604-1524: Investigative Team

<table>
<thead>
<tr>
<th>Position</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol Chair (UPENN)</td>
<td>Edward Stadtmauer, M.D.</td>
</tr>
<tr>
<td>Protocol Co-chair (UCSF)</td>
<td>Thomas Martin, MD</td>
</tr>
<tr>
<td>Protocol Co-Chair (MDACC)</td>
<td>Cassian Yee, MD</td>
</tr>
<tr>
<td>Sub-Investigators</td>
<td></td>
</tr>
<tr>
<td>Kristy Weber, MD</td>
<td></td>
</tr>
<tr>
<td>Adam Cohen, MD</td>
<td></td>
</tr>
<tr>
<td>Regulatory Sponsor</td>
<td></td>
</tr>
<tr>
<td>University of Pennsylvania</td>
<td></td>
</tr>
<tr>
<td>Scientific Advisor</td>
<td>Carl H. June, MD (UPENN)</td>
</tr>
<tr>
<td>T Cell manufacturing</td>
<td></td>
</tr>
<tr>
<td>Bruce Levine, PhD</td>
<td></td>
</tr>
<tr>
<td>Andrew Fesnak, MD</td>
<td></td>
</tr>
<tr>
<td>Yangbing Zhao, MD PhD</td>
<td></td>
</tr>
<tr>
<td>Laboratory Team -Biomarkers</td>
<td></td>
</tr>
<tr>
<td>J. Joseph Melenhorst, PhD</td>
<td></td>
</tr>
<tr>
<td>Simon F. Lacey, PhD</td>
<td></td>
</tr>
<tr>
<td>Statistical Support</td>
<td></td>
</tr>
<tr>
<td>Wei-Ting Hwang, PhD</td>
<td></td>
</tr>
<tr>
<td>Administrative Director for PICI@Penn</td>
<td></td>
</tr>
<tr>
<td>Barbara Vance, PhD</td>
<td></td>
</tr>
<tr>
<td>CCI Translational Science Operations</td>
<td></td>
</tr>
<tr>
<td>Gabriela Plesa, MD PhD</td>
<td></td>
</tr>
</tbody>
</table>
• Many advanced tumors that are currently not curable express NY-ESO-1

• Our published data indicates that targeting of NY-ESO-1 with transgenic T cells is safe and has antitumor activity (Rapoport AP, Stadtmauer EA et al. Nature Medicine. 2015;21(8):914-21)

• Our preliminary data suggests that efficacy would be improved by increasing expression of the transgenic TCR and preventing exhaustion of the infused T cells
Tumor Expression of NY-ESO-1

• Adult expression of NY-ESO-1 is restricted to tumors and gametogenic tissues

• NY-ESO-1 expression on significant % of tumors

  Melanoma 28 - 45%
  Synovial and Myxoid Sarcoma >70%
  Advanced Myeloma ~50%

• Studies of NY-ESO-1 gene modified T cells show safety and evidence of antitumor activity in melanoma, sarcoma and myeloma


Experience with “Wild Type” NY-ESO-1 TCR T cells

Persistence of NY-ESO-1 TCR T cells in blood and marrow

- 20 pts with antigen-positive MM received an average $2.4 \times 10^9$ engineered T cells.
- Encouraging clinical responses were observed in 16 of 20 patients (80%) with advanced disease, with a median progression-free survival of 19.1 months.
- NY-ESO-1 TCR–engineered T cells were safe, trafficked to marrow and showed extended persistence that correlated with clinical activity against antigen-positive myeloma.

Rapoport, Stadtmauer et al; Nat Med, 2015
Evidence suggesting that NY-ESO-1 T cells become exhausted

Persistence of NY-ESO-1 TCR T cells in pts 250, 256, 201 and 207

- Persistence and function of gene-modified cells in NY-ESO-1 TCR-positive CD8+ T cells were evaluated for functionality in the cell product (MP) and from d21 to d360 after infusion.
- Production of IFN-γ, granzyme B and CD107a in response to antigen-loaded T2 target cells was measured.
- Subsets of IFN-γ+ cells with or without expression of granzyme B and CD107a are represented by the various colors in each histogram.
- Pt 201 has persistent NY-ESO-1 T cells that increasingly fail to secrete IFN-γ+ and are granzyme B neg.

Rapoport, Stadtmauer et al; Nat Med, 2015
Limitations of Current NY-ESO-1 Transduced T Cells That Retain Endogenous TCR Expression

• Studies show many gene-modified T cells become exhausted, cease to proliferate, may lose function or fail to persist\(^1\)
• Signaling between PD-1 and PD-L1/PD-L2 leading to checkpoint blockade\(^2\)
• Mispairing of endogenous TCR with NY-ESO-1 TCR transduced T cells may generate new specificities that could be toxic\(^3\)
• Retention of the endogenous TCR can limit expression and functionality of the transgenic TCR\(^4\)

Genetic Editing May Increase Function of Transgenic TCRs and Increase Resistance to Exhaustion


Previous Experience with Human Gene Editing

- Open label, single dose study (NCT00842634)
- Study population: 3 cohorts
- Optional structured treatment interruption (STI) beginning 1 month post infusion (cohort 2)
- Single infusion of SB-728-T (5 - 10 x 10^9 cells)
Pilot Test of Adoptive Transfer of CCR5 Deleted CD4 T Cells using ZFNs to Introduce HIV resistance

• First patient infused July, 2009 with a single infusion of ZFN edited CD4 cells.
• As of 2016, more than 5% of CD4 cells are CCR5 deficient
  => cells are permanently HIV resistant
• No SAEs in the 12 patients treated

Pablo Tebas, MD, Clinical PI, Protocol #806383
Cell manufacturing rationale for this protocol

- Transgenic TCR: NY-ESO-1
- CRISPR:Cas9: Genetic Reprogram
- Resistance to exhaustion: PD-1 Deficiency?
CRISPR/Cas9 has potential to enhance T cell therapies

A rapidly advancing field


addgene.org/crispr/guide
Adoptive transfer of PD-1 deficient T cells

- Adoptive transfer of PD-1 deficient T cells has not been extensively studied, unlike the administration of PD-1 antagonistic antibodies

- PD-1–deficient CD8+ TCR transgenic T cells caused potent tumor rejection in an adoptive transfer model under conditions in which wild-type T cells as well as CTLA-4–deficient T cells failed (Blank et al. PD-L1/B7H-1 inhibits the effector phase of tumor rejection by T cell receptor (TCR) transgenic CD8+ T cells. Cancer Res. 2004;64(3):1140-5.

In studies of chronic viral infection:

- Early enhancement of proliferation and function of PD-1 KO P14 cells
- Reduced survival of PD-1 KO P14 cells during T cell contraction
- Diminished long term proliferation and stability of CD8+ T cells in absence of PD-1

NY-ESO-1 CRISPR (TCR-PD1) Edited (NYCE cells)
Manufacturing

Expected T Cell Populations \(2^4 = 16\)

<table>
<thead>
<tr>
<th></th>
<th>NYES01-TCR WT</th>
<th>NYES01+ TCR WT</th>
<th>NYES01+ TCR A-B+</th>
<th>NYES01+ TCR B-A+</th>
<th>NYES01+ TCR B-A+</th>
<th>NYES01+ TCR A-B+</th>
<th>NYES01+ TCR A-B-</th>
<th>NYES01+ TCR A-B-</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD1 WT</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>PD1 -</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>15</td>
<td>16</td>
</tr>
</tbody>
</table>
NY-ESO-1 CRISPR (TCR-PD1) Edited (NYCE cells)
Manufacturing

a) TCR Disruption

b) Surveyor Nuclease

CD3 Expression

Lentiviral Vector Map

- cPPT
- EF1alpha promoter
- TCR alpha
- F-GS2-T2A
- TCR beta
- SaII (3031)
- WPRES
- Beta Lactamase

pTRP.ESO.TCR(C8007) 9529 bp
### Frequencies in Healthy Donors

<table>
<thead>
<tr>
<th>Gene</th>
<th>Number of Donors Tested</th>
<th>Average % Disruption (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRAC</td>
<td>5</td>
<td>79.6 (62.0 – 92.3)</td>
</tr>
<tr>
<td>TRBC</td>
<td>10</td>
<td>76.6 (69.5 – 83.1)</td>
</tr>
<tr>
<td>TRAC + TRBC</td>
<td>16</td>
<td>73.2 (57.1 – 92.1)</td>
</tr>
<tr>
<td>PDCD1 (PD1)</td>
<td>6</td>
<td>53.1 (47.5 – 56.1)</td>
</tr>
</tbody>
</table>

**Gene Disruption in Edited T Cells Manufacturing**

**Expansion in vitro**

- NY-ESO-1 transduced, no CRISPR knockout
- NY-ESO-1 transduced, TRAC, TRBC, PD1 KO
NY-ESO-1 CRISPR (TCR-PD1) Edited (NYCE cells) Function In Vitro

Increased lysis

Cytokine secretion: effect of gRNA dose

IFN-gamma, pg/ml

% Specific lysis

E:T Ratio

NY-ESO-1 transduced, No KO
NY-ESO-1 transduced, TCR&PD1 KO
Mock transduced, No CRISPR

ND307
Day 11 culture
Target: Nalm6-ESO

HLA A2 + + +
NY-ESO-1 + - +
Pre-clinical studies:
Triple-Edited NYCE T cells In Vivo

NY-ESO-1.TCR, CRISPR
T cell alone

Five million lung cancer cells (A549-ESO-CBG) that are HLA-A2+ and NY-ESO-1+ were inoculated subcutaneously at the right flank of NSG mice. Nine days post tumor injection, 10 million transduced T cells, including only NY-ESO-1 TCR transduced T cells (NY-ESO-1.TCR), NY-ESO-1 TCR transduced and PD1/TCR alpha/TCR beta triple knockout T cells (NY-ESO-1.TCR, CRISPR), were injected to the mice intravenously. Tumor growth was monitored by bioluminescence imaging, tumor size and survival.
Clinical Protocol: Competitive repopulation strategy

• The approach is to employ a competitive repopulation strategy to identify T cells with a favored phenotype for persistence and function.

• Previous examples of competitive repopulation strategies in adoptive transfer studies:
Clinical Protocol:
Phase I trial of NY-ESO-1 redirected CRISPR Edited T cells (NYCE cells) engineered to express NY-ESO-1 TCR and gene edited to eliminate endogenous TCR and PD-1

Edward Stadtmauer, MD
NYCE Protocol 1604-1524: Investigative Team

Protocol Chair (UPENN)
Edward Stadtmauer, M.D.

Protocol Co-chair (UCSF)
Thomas Martin, MD

Protocol Co-Chair (MDACC)
Cassian Yee, MD

Sub-Investigators
Kristy Weber, MD
Adam Cohen, MD

Regulatory Sponsor
University of Pennsylvania

Scientific Advisor
Carl H. June, MD (UPENN)

T Cell manufacturing
Bruce Levine, PhD
Andrew Fesnak, MD
Yangbing Zhao, MD PhD

Laboratory Team -Biomarkers
J. Joseph Melenhorst, PhD
Simon F. Lacey, PhD

Statistical Support
Wei-Ting Hwang, PhD

Administrative Director for PICI@Penn
Barbara Vance, PhD

CCI Translational Science Operations
Gabriela Plesa, MD PhD
Study Background

- Despite significant incremental improvements in the therapy of relapsed and refractory multiple myeloma, and unresectable synovial cell sarcoma, myxoid/round cell liposarcoma and melanoma, these diseases remain almost uniformly fatal.
- NY-ESO-1 is highly expressed in these poor-prognosis diseases:
  - SCS/MRCL >70%
  - Multiple Myeloma (50%)
  - Melanoma (28 - 45%)
- NY-ESO-1 directed T cells have been used to treat myeloma, sarcoma and melanoma with reports of response and safety:
  - Myeloma (UPENN, UMARYLAND)
    - Blood derived T cells gene modified with lentiviral vector encoding NY-ESO-1 TCR
  - Melanoma (Fred Hutch, MSKCC)
    - Blood derived T cells targeting NY-ESO-1 were isolated cultured and expanded
  - Sarcoma (NCI)
    - Blood derived T cells gene modified with retrovirus encoding NY-ESO-1 TCR
- Lack of response and progression have been associated with T cell exhaustion and lack of T cell persistence which may be mediated by PD-1:
  - Disrupting expression of endogenous TCR and PD-1 may increase the persistence of the therapeutic T cells
  - Result: increased safety and effectiveness
- Myeloma, sarcoma and melanoma have tumor tissue amenable to biopsy, permitting analysis of tumor, T cells and tumor microenvironment.

Kumar et al., Blood, 2008; Rollig et al., Lancet, 2014
NYCE Clinical Trial Objectives

Primary:
- Determine safety and feasibility of an infusion of autologous NY-ESO-1 transgenic TCR expressing and endogenous TCR and PD-1 gene edited (CRISPER) T Cells
- Evaluate Manufacturing feasibility

Secondary:
- Clinical assessment: anti-tumor responses and survival
- NYCE cells engraftment, persistence, and trafficking
- NYCE cells bioactivity
- NYCE cells immunogenicity
Consent and screen for NY-ESO-1+ malignancy and HLA-0201 genotype

Consent for Study and Enrollment

Day -35 -4 -3 -2 -1 +0 +1 +3 +7 +10 +14 +21 +28

Apheresis and Cell Manufacture

Infusion: NY-ESO-1 TCR-PD1 CRISPR T cells*

Monitoring: Monthly until 6 mo.
Quarterly for 2 years

Disease Evaluation:
PET Scan
Biopsy
Tumor Markers

Cell and Toxicity Assessment:
Persistence of cell types
Cell function assays

Solid tumors
250-300 mg/m² flu
+25-30 mg/m² cy
1 hr. infusion
Days -4, -3, -2

Myeloma
1.5 g/m² cy
1 hr. infusion
Day -2

*Study Product, Dose, Route, Regimen: Autologous T cells transduced with a lentiviral vector to express NY-ESO-1 and electroporated with guide RNA to disrupt expression of endogenous TCRα, and TCRβ, and PD-1. A single manufacturing site at UPENN will be used for all subjects enrolled into this study. A single dose of 1x10⁸ cells/kg will be given i.v.
• **Rationale for TCR\textsuperscript{endo} gene disruption**
  Safety: Minimize mispairing with recombinant NY-ESO-1 TCR
  - reduce the risk for autoreactivity (autoimmunity)
  Potency: enhance recombinant NY-ESO-1 TCR expression on the cell surface
  - reduces recombinant and endogenous TCR expression competition

• **Rationale for PD-1 gene disruption**
  Potency: create “checkpoint” resistant T cells
  - PD-1 is a T cell exhaustion marker and may lead to reduced or defective memory T cells

• **Dose Rationale**
  Safety established in previous clinical trials with NY-ESO-1 TCR modified cells at higher doses: 10^{10}-10^{11} cells

• **Patient Population Rationale**
  Tumors with NY-ESO-1 expression
  Poor prognosis with available therapies
  HLA-A0201 allele
  - The NY-ESO-1 TCR expressing T cell must complex with HLA-A0201 for successful activation
Inclusion Criteria

• **All Subjects:**
  Subjects must be ≥ 18 years of age.
  Subjects must have an ECOG performance status of 0-2.
  Subjects must be HLA-A2 positive and have NY-ESO-1 expression on tumor biopsy.
  Subjects of reproductive potential must agree to use acceptable birth control methods.
  Subjects must have adequate vital organ function:
    - Serum creatinine ≤ 2.5 or estimated creatinine clearance ≥30 ml/min and not dialysis-dependent
    - ANC ≥1000/µl and platelet count ≥50,000/µl (≥30,000/µl if BM plasma cells are ≥50% for MM patients)
    - SGOT ≤ 3x the upper limit of normal and total bilirubin ≤ 2.0 mg/dl
    - Left ventricular ejection fraction (LVEF) ≥ 45%
• **MM Subjects**
  Confirmed prior diagnosis of active MM as defined by the IMWG criteria.
  Subjects must have measurable disease
  Relapsed or refractory disease after either one of the following:
    - At least 3 prior regimens, which must have contained an alkylating agent, proteasome inhibitor, and IMiD.
    - At least 2 prior regimens if “double-refractory” to a proteasome inhibitor and IMiD.
  Be at least 90 days since autologous stem cell transplant, if performed.
  Recovered from toxicities from prior therapies.
• **Melanoma Subjects**
  Subjects must have a confirmed prior diagnosis of melanoma.
  Progressed after at least 4 therapy lines.
  Patients must have measurable disease.
• **Sarcoma Subjects**
  Subjects must have a confirmed prior diagnosis of synovial sarcoma or MRCL.
  Patients with proven metastatic disease or surgically inoperable local recurrence that have failed first line treatment.
  Patients must have measurable disease.
Exclusion Criteria

- Be pregnant or lactating.
- Inadequate venous access for or contraindications to leukapheresis.
- Active and uncontrolled infection.
- Active hepatitis B, hepatitis C, or HIV infection.
- Uncontrolled medical or psychiatric disorder that would preclude participation as outlined.
- Have NYHA Class III or IV heart failure, unstable angina, or a history of recent (within 6 months) myocardial infarction or sustained (>30 seconds) ventricular tachyarrhythmias.
- Received prior gene therapy or gene-modified cellular immunotherapy. Subject may have received non-gene-modified autologous T-cells in association with an anti-myeloma vaccine (e.g., hTERT or MAGEA3) or vaccination against infectious agents (e.g., influenza or pneumococcus).
- Active auto-immune diseases, or have a history of severe (as judged by the principal investigator) autoimmune disease requiring prolonged immunosuppressive therapy.
- Prior allogeneic stem cell transplant.
- Prior or active CNS involvement with myeloma. Subjects with calvarial disease that extends intracranially and involves the dura will be excluded, even if CSF is negative for myeloma.
Protocol Management - I

- **Enrollment (18 total evaluable, 6 myeloma, 6 sarcoma, 6 melanoma)**
  A ‘basket’ design will be used
  - Myeloma (UPENN 3 and UCSF 3)
  - Sarcoma (MD Anderson 3 and UCSF 3)
  - Melanoma (MD Anderson 6)

The first 3 patients will be treated in 4 week intervals with the next patient treated after the day 28 evaluation. If no untoward safety signal, the remainder of enrollment will proceed as subjects are accrued.

The UPENN site will open first, followed by opening the other sites. The first patients will be those with multiple myeloma as this patient population has been previously tested for safety of NY-ESO-1 TCR redirected T cells at UPENN. Patients enrollment will be coordinated by a dedicated project manager within the Regulatory Sponsor team at UPENN, ensuring balanced allocation at the 3 sites and coordination with the UPENN CVPF manufacturing slots availability.

- **Cell collection and manufacturing**
  A single manufacturing site at UPENN will be used for all subjects enrolled. Collection will be at the individual sites.

- **Lymphodepleting Chemotherapy**
  Fludarabine and cyclophosphamide will be given prior to T cell infusion for solid tumors
  - standard doses for these agents for other diseases and prior cellular therapy protocols will be used
  Cyclophosphamide alone will be given prior to T cell infusion for myeloma
  - standard dose given prior to previous NY-ESO-1 TCR cellular therapy protocol
• Disease Control
  ➢ Patients are allowed to receive standard therapies to maintain disease control after apheresis and up to 2 weeks prior to infusion. Patients with disease progression (or cell manufacturing failure) will be replaced. The patients with cell manufacturing failure will be included in the analysis of feasibility endpoint.

• Patient monitoring
  ➢ Our plan for this protocol is that patients will be followed closely following the infusion of the NYCE cells using deep sequencing, quantitative PCR and digital droplet PCR. Cases with unusual kinetics of the infused cells, i.e. any patients with secondary expansions of gene-modified cells, will examined in detail for clonal T cell abnormalities.
  ➢ Our protocol is designed to assess for off-target editing of the CRISPR/Cas9 as a primary safety endpoint. Patients will be followed per FDA guidelines for 15 years to gather clinical and laboratory information on the potential consequences of off target editing
Management of Potential Toxicity

1. Uncontrolled T cell proliferation
   • corticosteroids and chemotherapy
2. Immunogenicity of product
   • Cas9 immunogenicity tested as secondary endpoint
3. Transformation
   • Not seen in hundreds of subjects treated with LV modified T cells;
     • Risk due to CRISPR off target disruption is not known
4. CRS
   • Supportive therapy, anti-cytokines (next slide)
5. TLS (usually associated with burden of disease)
   • Fluids, rasburicase, prophylactic allopurinol, close monitoring
6. Off target disruption
   • Secondary clonal T cell expansions to be investigated in detail
7. Autoimmunity
   • Possible; safety evaluated as primary endpoint
8. Chemotherapy adverse effects
   • Standard care
9. RCL (replication competent lentivirus) (will be assessed up to 2 years)
   • Consultation with FDA
Plan for Sample Analysis

Simon F. Lacey, Ph.D. Translational and Correlative Studies Laboratory
Jos Melenhorst, Ph.D. Product Development & Correlative Sciences laboratory
Plan for Sample Analysis

Assays to detect integrity of gene editing and cell populations and frequencies in biospecimens

- **Flow Cytometry**
  - NY-ESO-1 Dextramers
  - Stimulation and checkpoint inhibitor expression (14-color, 16 parameter)
  - TCR<sub>endo</sub> staining with cocktail of Vβ mAbs

- **Detection of Indels due to CAS9 gene editing**
  - Endonuclease digestion (Surveyor)
  - Digital PCR to assess gene disruption frequency
  - Targeting sequencing in TCR domains, and PD1

- **Detection of off target effects**
  - Targeted deep sequencing
  - Sequencing of gene panels
  - Whole genome vs whole exome sequencing
  - Long term cell culture to detect clonal advantage or transformation
Expected T Cell Populations: Genotype and Phenotype

Phenotype

1
9
3, 5, 7
11, 13, 15
2
10
4, 6
12, 14
8
16

Genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>PD1+ TCR A+B+</th>
<th>PD1- TCR A+B+</th>
<th>PD1+ TCR A-B+</th>
<th>PD1- TCR A-B+</th>
<th>PD1+ TCR A-B+</th>
<th>PD1- TCR A-B+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>5:TCR B-A+</td>
<td>4:TCR A-B+</td>
<td>4:TCR A-B+</td>
<td>12:TCR A-B+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7:TCR A-B-</td>
<td>6:TCR B-A+</td>
<td>6:TCR B-A+</td>
<td>14:TCR B-A+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11:TCR A-B-</td>
<td>11:TCR A-B-</td>
<td>16:TCR A-B-</td>
</tr>
</tbody>
</table>
**Expected T Cell Populations: fitness and function**

**Phenotype**

- 1
- 9
- 3, 5, 7
- 11, 13, 15

**Genotype**

**PD1+**
- TCR A+B+

**PD1-**
- TCR A+B+

**PD1+**
- 3:TCR A-B+
- 5:TCR B-A+
- 7:TCR A-B-

**PD1-**
- 11:TCR A-B+
- 13:TCR B-A+
- 15:TCR A-B-

- **Wild-type T cells that were not modified during manufacturing. Safety is expected while efficacy is not.**

- **T cells with PD-1 disruption that retains endogenous TCR. These cells are anticipated to have a low toxicity risk based on data from systemic therapy with PD-1 antagonists. Increased neo-Ag responses??**

- **T cells with endogenous TCR disruption. These cells are expected to be rare, have low potential for toxicity and should not persist after adoptive transfer.**

- **T cells with endogenous TCR and PD-1 disruption; expected to be rare, have low potential for toxicity and should not persist after adoptive transfer.**

**Expected T Cell Populations** \(2^4 = 16\)

<table>
<thead>
<tr>
<th>NYES01- TCR WT</th>
<th>NYES01+ TCR WT</th>
<th>NYES01- TCR A-B+</th>
<th>NYES01+ TCR A-B+</th>
<th>NYES01- TCR B-A+</th>
<th>NYES01+ TCR B-A+</th>
<th>NYES01- TCR A-B-</th>
<th>NYES01+ TCR A-B-</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD1 WT</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>PD1 -</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>15</td>
</tr>
</tbody>
</table>
**Expected T Cell Populations (cont.)**

**Phenotype**

2

NY-ESO-1 specific T cells without gene disruption. Previously tested in patients with multiple myeloma with demonstrated safety.

4, 6

NY-ESO-1 specific T cells with endogenous TCR disruption. Expected enhanced anti-tumor activity compared to cell population 2, have reduced, but not absent, potential for mispairing and susceptible to exhaustion by PD-1 expression.

8

NY-ESO-1 specific T cells with endogenous TCR disruption. Expected enhanced anti-tumor activity compared to population 2. Absent potential for mispairing, but susceptible to exhaustion by PD-1 expression.

**Genotype**

<table>
<thead>
<tr>
<th>PD1+</th>
<th>2:TCRA+B+</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:TCR A-B+</td>
<td></td>
</tr>
<tr>
<td>6:TCR B-A+</td>
<td></td>
</tr>
<tr>
<td>8:TCR A-B-</td>
<td></td>
</tr>
</tbody>
</table>

**Expected T Cell Populations**

$2^4 = 16$

<table>
<thead>
<tr>
<th>Expected Position (Number)</th>
<th>NYES01+</th>
<th>NYES01-</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCR WT</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>TCR A+B+</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>TCR B-A+</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>TCR B-A-</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>TCR A-B+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCR A-B-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PD1 WT</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD1 -</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>15</td>
<td>16</td>
</tr>
</tbody>
</table>
**Expected T Cell Populations (cont.)**

**Phenotype**

- **10**
  - NY-ESO-1 specific T cells with PD-1 disruption. Expected anti-tumor efficacy and less susceptible to exhaustion by PD-1 expression.

- **12, 14**
  - NY-ESO-1 specific T cells with endogenous TCR and PD-1 disruption. Expected reduced potential for mispairing. May have enhanced effector function. Expected to be less susceptible to exhaustion by PD-1 expression.

- **16**
  - PD1- 10:TCRA+B+ 12:TCR A-B+ 14:TCR B-A+ 16:TCR A-B-

**Genotype**

<table>
<thead>
<tr>
<th>Expected T Cell Populations ($2^4 = 16$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NYESO1- TCR WT</td>
</tr>
<tr>
<td>PD1 WT</td>
</tr>
<tr>
<td>PD1-</td>
</tr>
</tbody>
</table>
Detection of NY-ESO-1 specific TCR using HLA-A2/SLLMWITQC dextramer identifies transgenic TCR expression on both CD8+ and CD4+ NYCE T cells.

<table>
<thead>
<tr>
<th>T cell subset</th>
<th>Donor ND307</th>
<th>Donor ND422</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+</td>
<td>16.7</td>
<td>13.5</td>
</tr>
<tr>
<td>CD8+</td>
<td>29.9</td>
<td>24.9</td>
</tr>
</tbody>
</table>
Increased frequency of NY-ESO-1 dextramer+ T cells with increased CRISPR gRNA dose

These 8 samples were also analyzed for:
1. Long term culture (CVPF);
2. Molecular and flow assay (TCSL);
3. Karyotyping;
4. NGS for off-target testing
Day 11 T cells (ND422) were re-stimulated with CD3/CD28 beads for 3 days, PD1 and CD3 were detected by flow cytometry (upper and middle panel), non-re-stimulated T cells were used as controls (bottom).
Digital-PCR Assay of PD-1 Gene-Editing in NY-ESO-1 TCR Transduced, Triple Edited T cells

A. Primers/probes amplify two sites in PD-1 gene which are not affected by CRISPR/Cas9 editing, (VIC probes), and one probe (FAM Probe) only amplifies the target gene if unedited.

B. % PD-1 Gene Editing = FAM/VIC X 100
Quantification of gene disruption efficiency using digital PCR and Surveyor Assays

**digital PCR**

- **PD1**
- **TRAC**
- **TRBC**

**Surveyor**

- **PD1**
- **TRAC**
- **TRBC**
Plan for Assessment of Off-Target Effects

Assays conducted on infusion product and patient samples post infusion:

• Deep sequencing
• Digital droplet PCR (ddPCR)
• Karyotyping / FISH
• Long term culture of T cells
• Analysis to be driven by clinical outcome and/or evidence of clonal TCR expansion
Assessment of Off-Target Effects: Deep Sequencing

Number of small insertions and deletions at the off-target sites

<table>
<thead>
<tr>
<th>Target Gene</th>
<th>ND307</th>
<th>ND391</th>
<th>ND422</th>
<th>ND463</th>
<th>ND469</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High Dose</td>
<td>Med Dose</td>
<td>Low Dose</td>
<td>High Dose</td>
<td>Med Dose</td>
</tr>
<tr>
<td>PD1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TRAC</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TRBC</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Off-target indels

<table>
<thead>
<tr>
<th>Number of Samples having the indel at the off-target site</th>
<th>Chromosome Location (hg19)</th>
<th>Mutation description</th>
<th>HGVS mutation name</th>
<th>Mutation Frequency in the identified sample(s)</th>
<th>Number of supporting sequencing reads</th>
<th>Gene</th>
<th>Gene region</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 BS1 of 5</td>
<td>chr8:41631339</td>
<td>deletion of the C at chr8:4163339</td>
<td>NM_00037.3:c.28-15684del</td>
<td>0.45%</td>
<td>5</td>
<td>ANK1</td>
<td>intron 1</td>
</tr>
</tbody>
</table>
Long term culture to measure:
- total cell number
- population doubling
- viability
- cell volume

Serial transfer of patient T cells into NSG mice
Responses to Reviewers

Thank you and Questions?

1. Michael B. Atkins, MD, Professor of Oncology and Medicine (Hematology/Oncology), Georgetown University School of Medicine.

2. Paula Cannon, PhD, Professor in the Department of Molecular Microbiology & Immunology, University of Southern California Keck School of Medicine.

3. Mildred Cho, PhD, Professor Department of Pediatrics, Stanford University School of Medicine.
NATIONAL INSTITUTES OF HEALTH

CONSENT AND RELEASE

I hereby agree to allow the National Institutes of Health ("NIH") to use, with appropriate attribution, the following "works" on its websites and for other government purposes. Such government purposes may include distribution or display of copies of the work to the public. The following works may be used:

A PDF version of Slides Presented at the June 21-22, 2016 RAC meeting.

If the works include materials made by a third party, I have obtained permission to use them for the purposes recited above.

____________________________
Carl June, MD
Printed Name

____________________________
Signature

Date: __06-09-2016__
CONSSENT AND RELEASE

I hereby agree to allow the National Institutes of Health ("NIH") to use, with appropriate attribution, the following "works" on its websites and for other government purposes. Such government purposes may include distribution or display of copies of the work to the public. The following works may be used:

A PDF version of Slides Presented at the June 21-22, 2016 RAC meeting.

If the works include materials made by a third party, I have obtained permission to use them for the purposes recited above.

J. Joseph Melenhorst, PhD
Printed Name

Signature

Date: _06/09/2016_____
NATIONAL INSTITUTES OF HEALTH

CONSENT AND RELEASE

I hereby agree to allow the National Institutes of Health ("NIH") to use, with appropriate attribution, the following "works" on its websites and for other government purposes. Such government purposes may include distribution or display of copies of the work to the public. The following works may be used:

A PDF version of Slides Presented at the June 21-22, 2016 RAC meeting.

If the works include materials made by a third party, I have obtained permission to use them for the purposes recited above.

Edward Stromvall
Printed Name

Signature

Date: 6/4/18