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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

Penn Medicine
Center for Cellular Immunotherapies

Penn
University of Pennsylvania
Parker Institute for Cancer Immunotherapy

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for CANCER IMMUNOTHERAPY
of MD ANDERSON, MEMORIAL SLOAN-KETTERING, PENN MEDICINE, STANFORD, UCLA, AND UCSF

NYCE Protocol 1604-1524: Investigative Team

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Background, Rationale and Cell Manufacturing

- 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

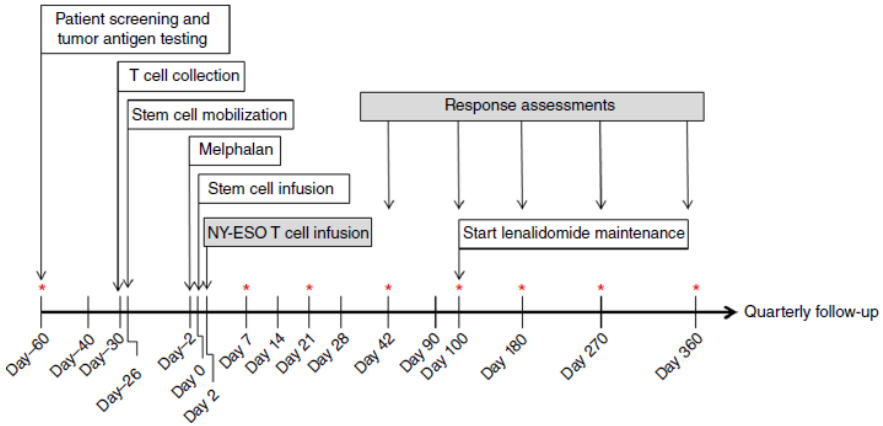
Melanoma	28 - 45%
Synovial and Myxoid Sarcoma	>70%
Advanced Myeloma	~50%

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

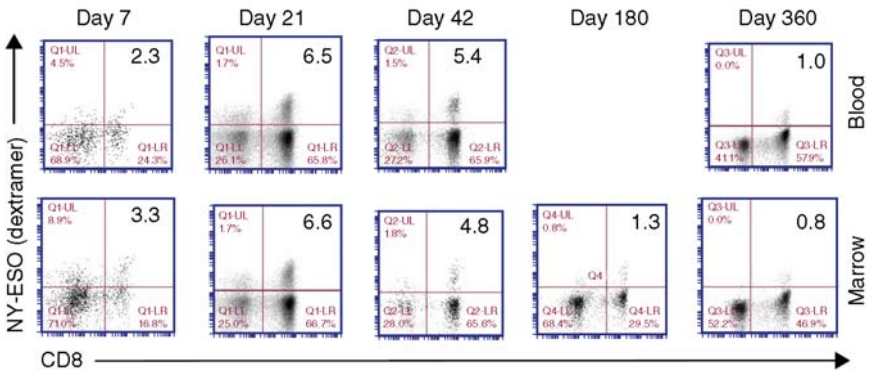
NY-ESO-1: Previous Experience with Vaccines and Adoptive T Cell Transfer

1. Baumgaertner P, Rufer N, Devevre E, Derre L, Rimoldi D, Geldhof C, Voelter V, Lienard D, Romero P, and Speiser DE. Ex vivo detectable human CD8 T-cell responses to cancer-testis antigens. *Cancer Research.* 2006;66(4):1912-6.
2. Odunsi K, Qian F, Matsuzaki J, Mhawech-Fauceglia P, Andrews C, Hoffman EW, Pan L, Ritter G, Villella J, Thomas B, et al. Vaccination with an NY-ESO-1 peptide of HLA class I/II specificities induces integrated humoral and T cell responses in ovarian cancer. *Proc Natl Acad Sci U S A.* 2007;104(31):12837-42.
3. Yuan J, Gnjatic S, Li H, Powel S, Gallardo HF, Ritter E, Ku GY, Jungbluth AA, Segal NH, Rasalan TS, et al. CTLA-4 blockade enhances polyfunctional NY-ESO-1 specific T cell responses in metastatic melanoma patients with clinical benefit. *Proc Natl Acad Sci U S A.* 2008;105(51):20410-5.
4. Hunder N, Wallen H, Cao J, Hendricks D, Reilly J, Rodmyre R, Jungbluth A, Gnjatic S, Thompson J, and Yee C. Treatment of metastatic melanoma with autologous CD4+ T cells against NY-ESO-1. *The New England journal of medicine.* 2008;358(25):2698.
5. Robbins PF, Morgan RA, Feldman SA, Yang JC, Sherry RM, Dudley ME, Wunderlich JR, Nahvi AV, Helman LJ, and Mackall CL. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. *Journal of Clinical Oncology.* 2011;29(7):917-24.
6. Rapoport AP, Stadtmauer EA, Binder-Scholl GK, Goloubeva O, Vogl DT, Lacey SF, Badros AZ, Garfall A, Weiss B, Finklestein J, et al. NY-ESO-1-specific TCR-engineered T cells mediate sustained antigen-specific antitumor effects in myeloma. *Nature Medicine.* 2015;21(8):914-21.

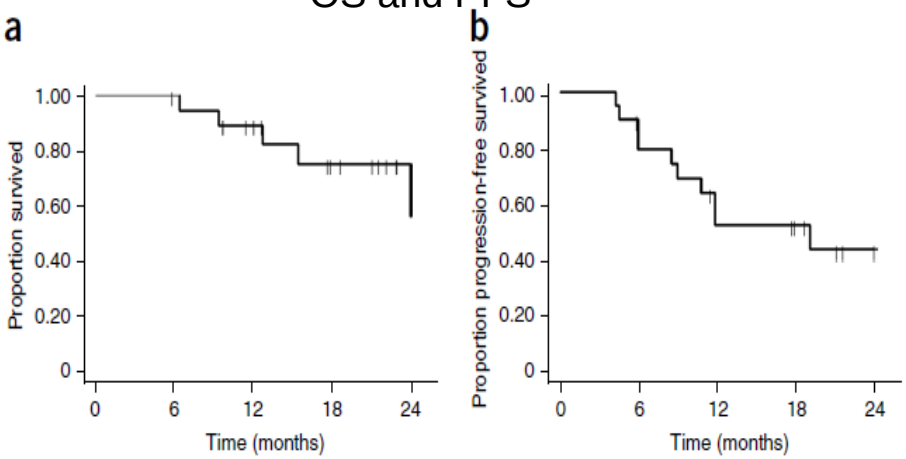
Experience with "Wild Type" NY-ESO-1 TCR T cells



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



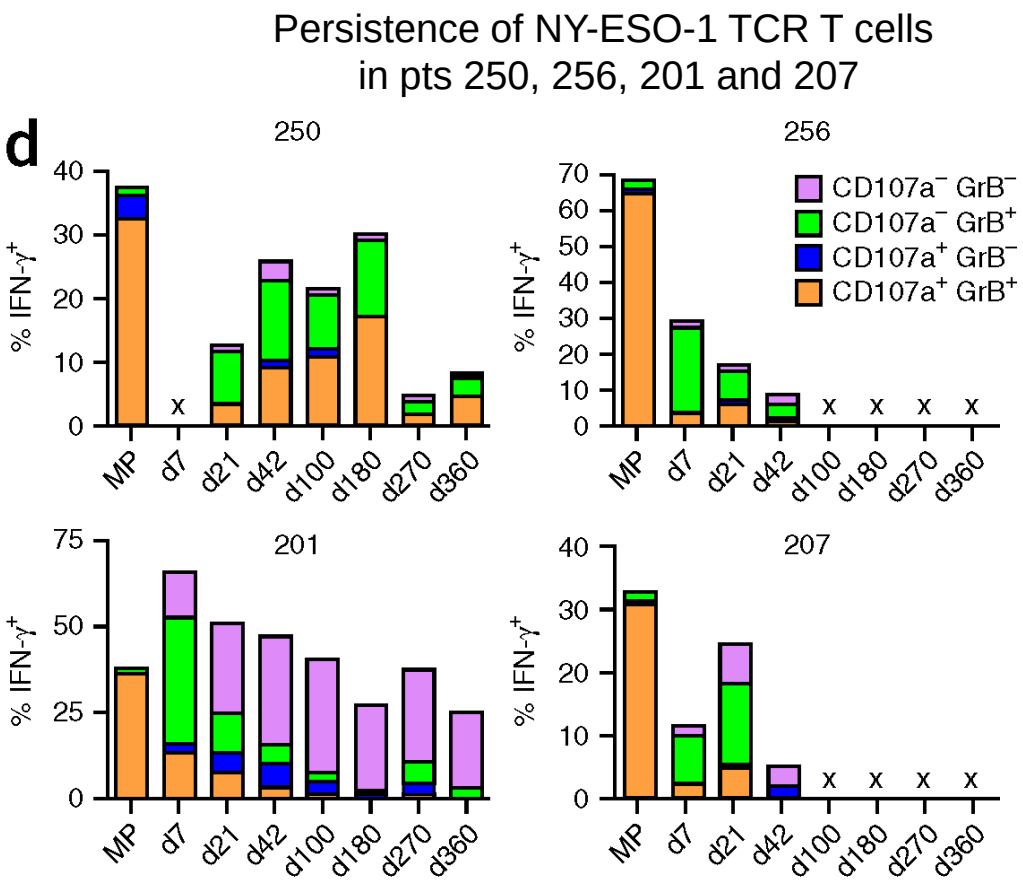
OS and PFS



- 20 pts with antigen-positive MM received an average 2.4 × 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 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]

1. Schietinger A & Greenberg PD. Tolerance and exhaustion: defining mechanisms of T cell dysfunction. Trends in immunology. 2014;35(2):51-60.
2. Postow, et al. Immune checkpoint blockade in cancer therapy. Journal of Clinical Oncology 33.17 (2015): 1974-1982.
3. van Loenen et al. Mixed T cell receptor dimers harbor potentially harmful neoreactivity. PNAS. 2010;107(24):10972-7.
4. Ochi et al. Novel adoptive T-cell immunotherapy using a WT1-specific TCR vector encoding silencers for endogenous TCRs shows marked antileukemia reactivity and safety. Blood. 2011;118(6):1495-503.

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

1. Schumacher TN. T-cell-receptor gene therapy. *Nat Rev Immunol.* 2002;2(7):512-9.
2. Bendle GM, Linnemann C, Hooijkaas AI, Bies L, de Witte MA, Jorritsma A, Kaiser ADM, Pouw N, Debets R, Kieback E, et al. Lethal graft-versus-host disease in mouse models of T cell receptor gene therapy. *Nature Medicine.* 2010;16(5):565-70.
3. Ochi T, Fujiwara H, Okamoto S, An J, Nagai K, Shirakata T, Mineno J, Kuzushima K, Shiku H, and Yasukawa M. Novel adoptive T-cell immunotherapy using a WT1-specific TCR vector encoding silencers for endogenous TCRs shows marked antileukemia reactivity and safety. *Blood.* 2011;118(6):1495-503.
4. Casey NP, Fujiwara H, Tanimoto K, Okamoto S, Mineno J, Kuzushima K, Shiku H, and Yasukawa M. A Functionally Superior Second-Generation Vector Expressing an Aurora Kinase-A-Specific T-Cell Receptor for Anti-Leukaemia Adoptive Immunotherapy. *PLoS ONE.* 2016;11(6):e0156896.
5. Schumann K, Lin S, Boyer E, Simeonov DR, Subramaniam M, Gate RE, Haliburton GE, Chun JY, Bluestone JA, and Doudna JA. Generation of knock-in primary human T cells using Cas9 ribonucleoproteins. *Proceedings of the National Academy of Sciences.* 2015;112(33):10437-42.

Previous Experience with Human Gene Editing

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 MARCH 6, 2014 VOL. 370 NO. 10

Gene Editing of *CCR5* in Autologous CD4 T Cells of Persons Infected with HIV

Pablo Tebas, M.D., David Stein, M.D., Winson W. Tang, M.D., Ian Frank, M.D., Shelley Q. Wang, M.D., Gary Lee, Ph.D., S. Kaye Spratt, Ph.D., Richard T. Surosky, Ph.D., Martin A. Giedlin, Ph.D., Geoff Nichol, M.D., Michael C. Holmes, Ph.D., Philip D. Gregory, Ph.D., Dale G. Ando, M.D., Michael Kalos, Ph.D., Ronald G. Collman, M.D., Gwendolyn Binder-Scholl, Ph.D., Gabriela Plesa, M.D., Ph.D., Wei-Ting Hwang, Ph.D., Bruce L. Levine, Ph.D., and Carl H. June, M.D.

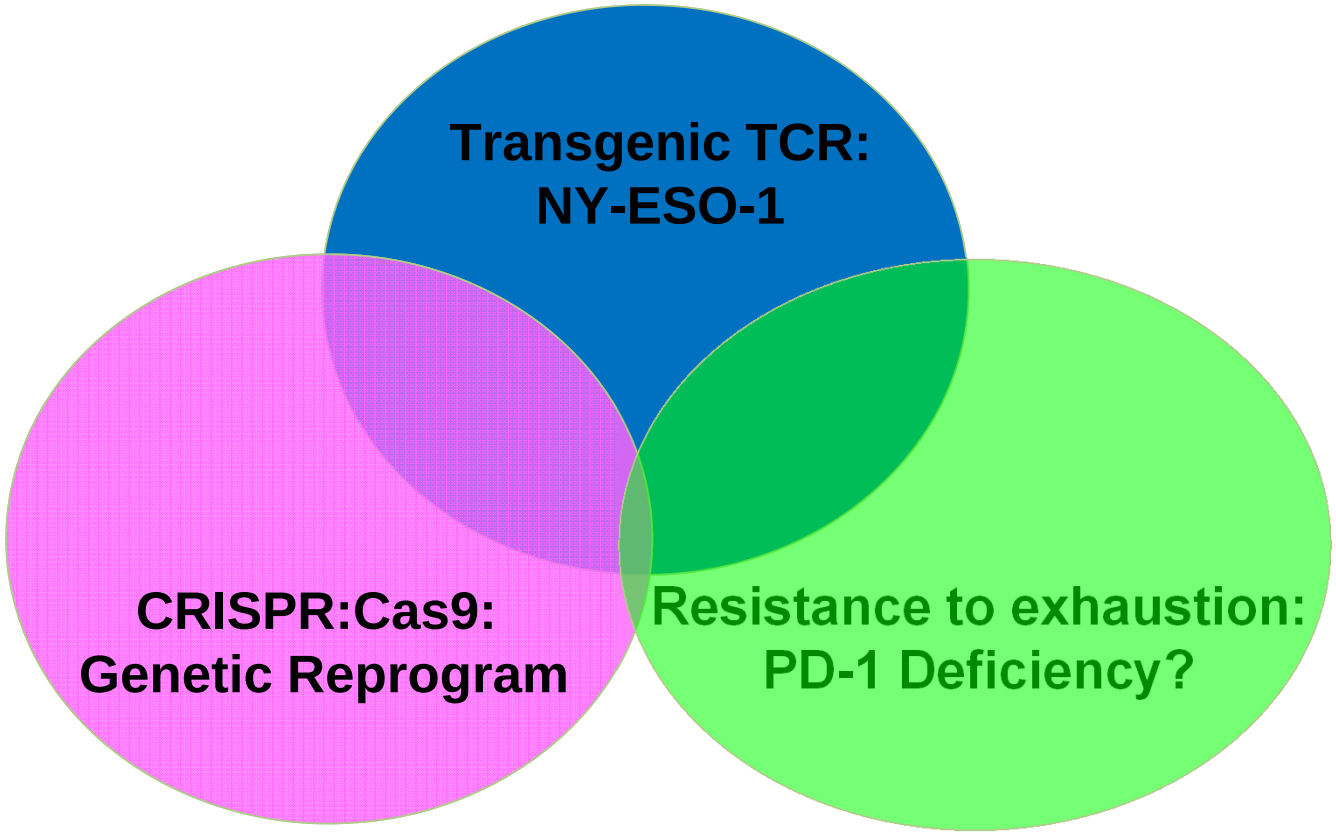
- **Open label, single dose study (NCT00842634)**
- **Study population: 3 cohorts**
- **Optional structured treatment interruption (STI) beginning 1 month post infusion (cohort 2)**
- **Single infusion of SB-728-T (5 - 10 x 10^9 cells)**
- **Translational time frame: Idea (2003) => Preclinical (2008) => Clinical (2014)**

Pilot Test of Adoptive Transfer of CCR5 Deleted CD4 T Cells using ZFNs to Introduce HIV resistance

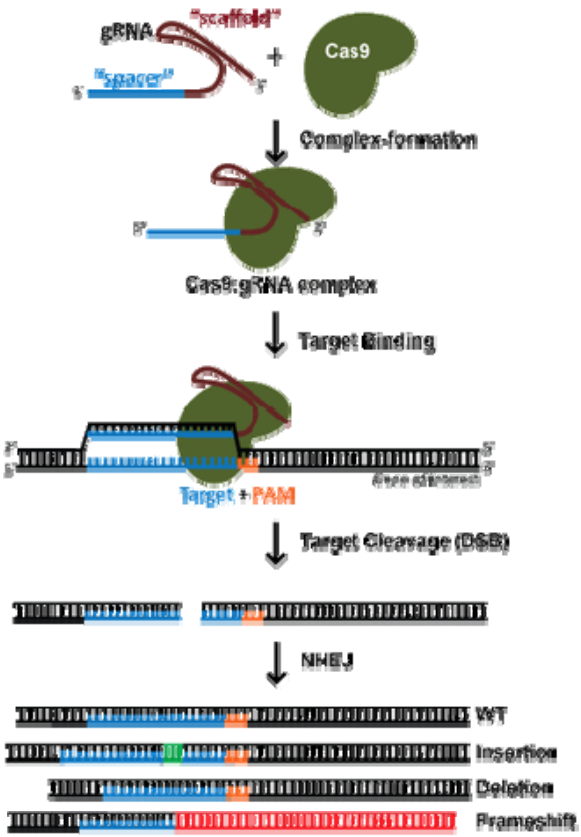
- **First patient infused July, 2009 with a single infusion of ZFN edited CD4 cells.**
- **As of 2016, more than 5% of CD4 cells are CCR5 deficient**
  => cells are permanently HIV resistant
- **No SAEs in the 12 patients treated**

Pablo Tebas, MD, Clinical PI, Protocol #806383

Cell manufacturing rationale for this protocol



CRISPR/Cas9 has potential to enhance T cell therapies



Patrick D. Hsu, et.al, Cell, 2014,157:1262-1274
Cong. Hsu, et.al, Science, 2013,339:819-822

addgene.org/crispr/guide

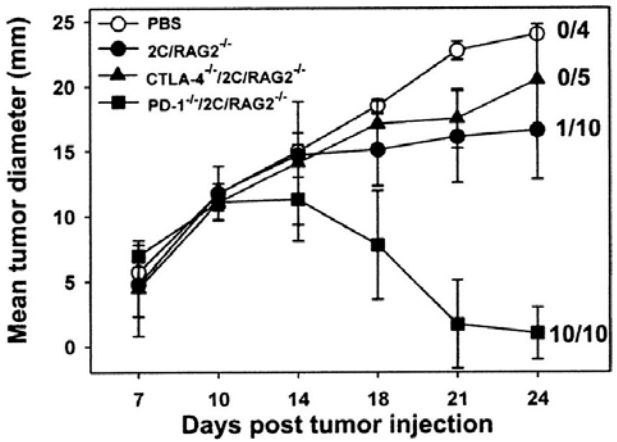
A rapidly advancing field

1. Torikai H, Reik A, Liu P-Q, Zhou Y, Zhang L, Maiti S, Huls H, Miller JC, Kebriaei P, Rabinovitch B, et al. A foundation for universal T-cell based immunotherapy: T cells engineered to express a CD19-specific chimeric-antigen-receptor and eliminate expression of endogenous TCR. *Blood.* 2012;119(24):5697-705.
2. Schumann K, Lin S, Boyer E, Simeonov DR, Subramaniam M, Gate RE, Haliburton GE, Chun JY, Bluestone JA, and Doudna JA. Generation of knock-in primary human T cells using Cas9 ribonucleoproteins. *Proceedings of the National Academy of Sciences.* 2015;112(33):10437-42.
3. Wang J, DeClercq JJ, Hayward SB, Li PW-L, Shivak DA, Gregory PD, Lee G, and Holmes MC. Highly efficient homology-driven genome editing in human T cells by combining zinc-finger nuclease mRNA and AAV6 donor delivery. *Nucleic acids research.* 2015:gkv1121.
4. Poirot L, Philip B, Schiffer-Mannioui C, Le Clerre D, Chion-Sotinel I, Derniame S, Potrel P, Bas C, Lemaire L, Galetto R, et al. Multiplex Genome-Edited T-cell Manufacturing Platform for "Off-the-Shelf" Adoptive T-cell Immunotherapies. *Cancer Res.* 2015;75(18):3853-64.

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

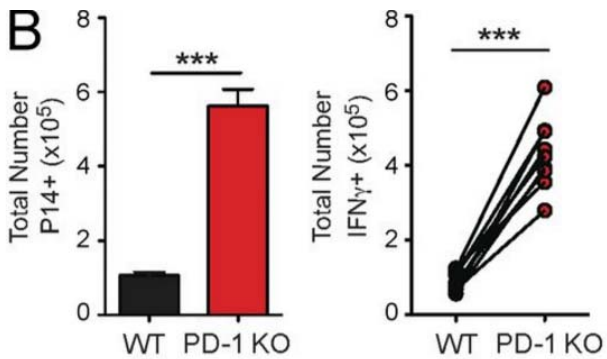
- PD-1–deficient CD8+ TCR transgenic T cells caused potent tumor rejection in an adoptive transfer model under conditions in which wild-type T cells as well as CTLA-4–deficient T cells failed (Blank et al. PD-L1/B7H-1 inhibits the effector phase of tumor rejection by T cell receptor (TCR) transgenic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



Blank, et al. Cancer Res. 2004;64(3):1140-5.

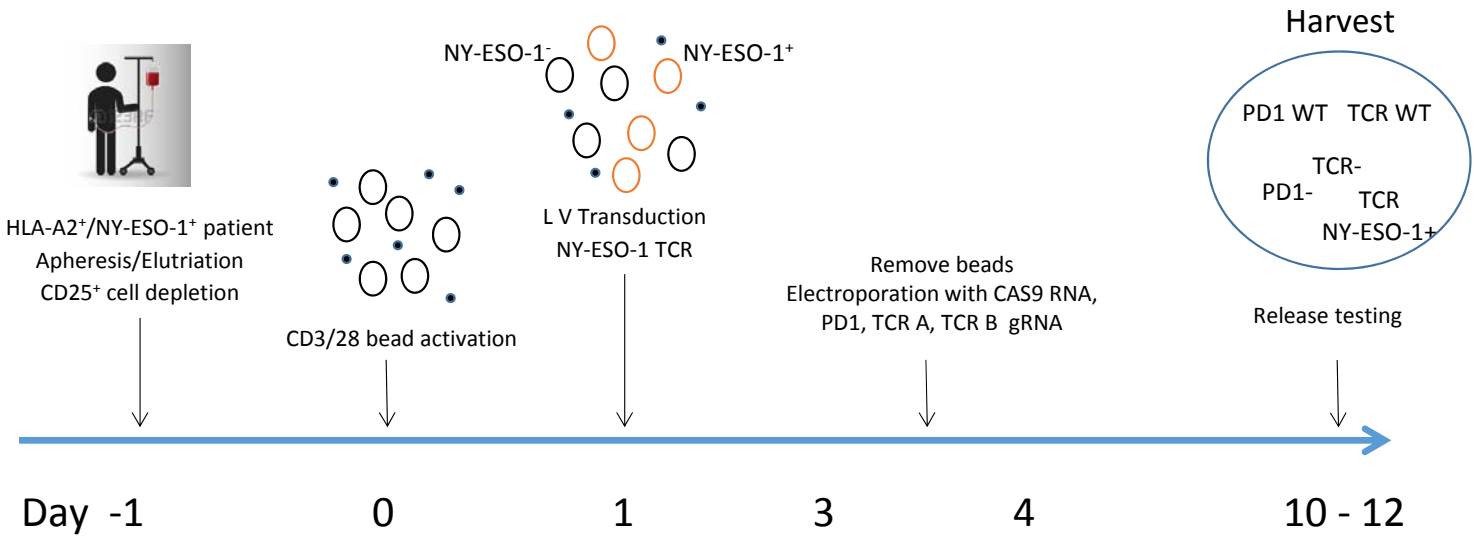
In studies of chronic viral infection:

- 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



Odorizzi PM et al. J Exp Med. 2015;212(7):1125-37.

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

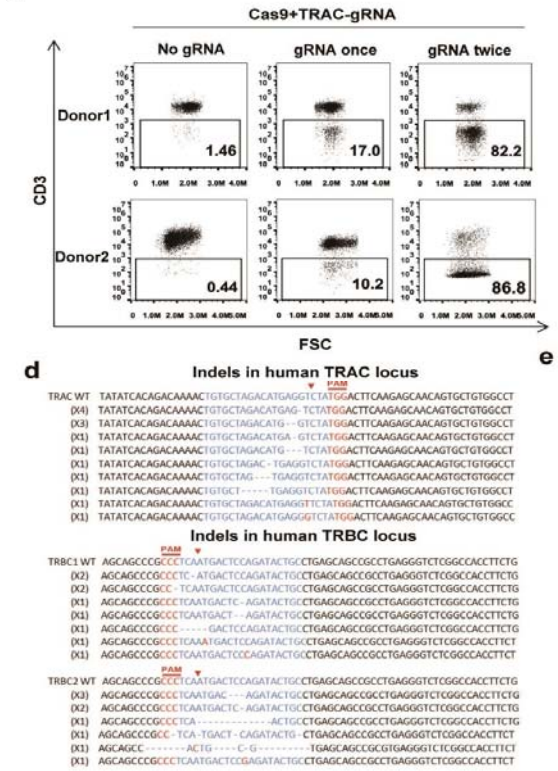


Expected T Cell Populations ($2^4 = 16$)

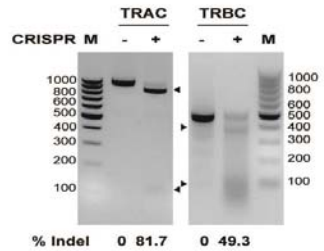
	NYES01- TCR WT	NYES01+ TCR WT	NYES01- TCR A-B+	NYES01+ TCR A-B+	NYES01- TCR B-A+	NYES01+ TCR B-A+	NYES01- TCR A-B-	NYES01+ TCR A-B-
PD1 WT	1	2	3	4	5	6	7	8
PD1 -	9	10	11	12	13	14	15	16

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

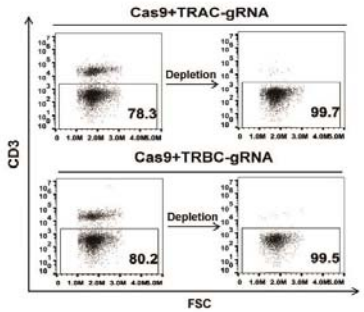
TCR Disruption



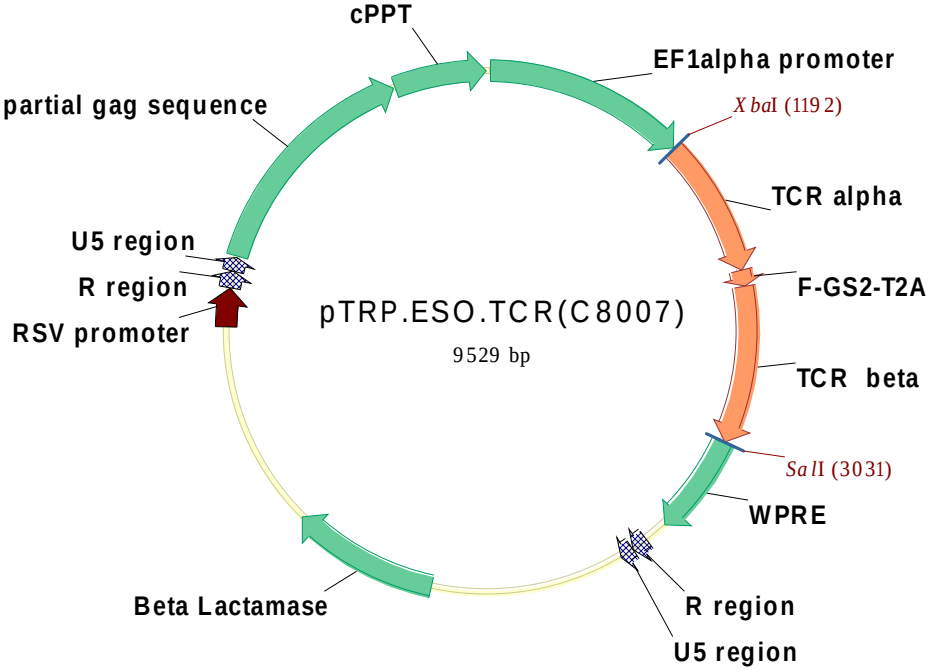
Surveyor Nuclease



CD3 Expression



Lentiviral Vector Map

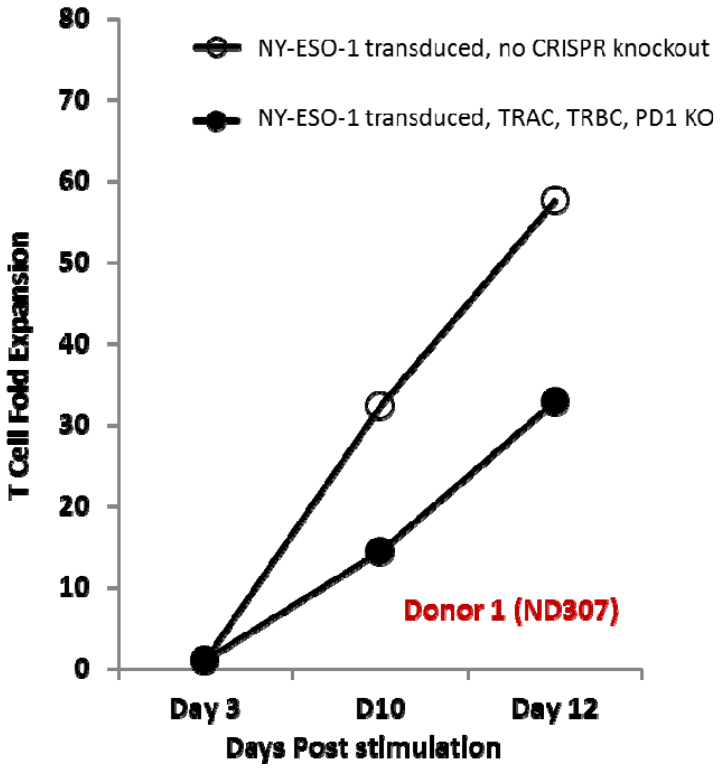


Gene Disruption in Edited T Cells Manufacturing

Frequencies in Healthy Donors

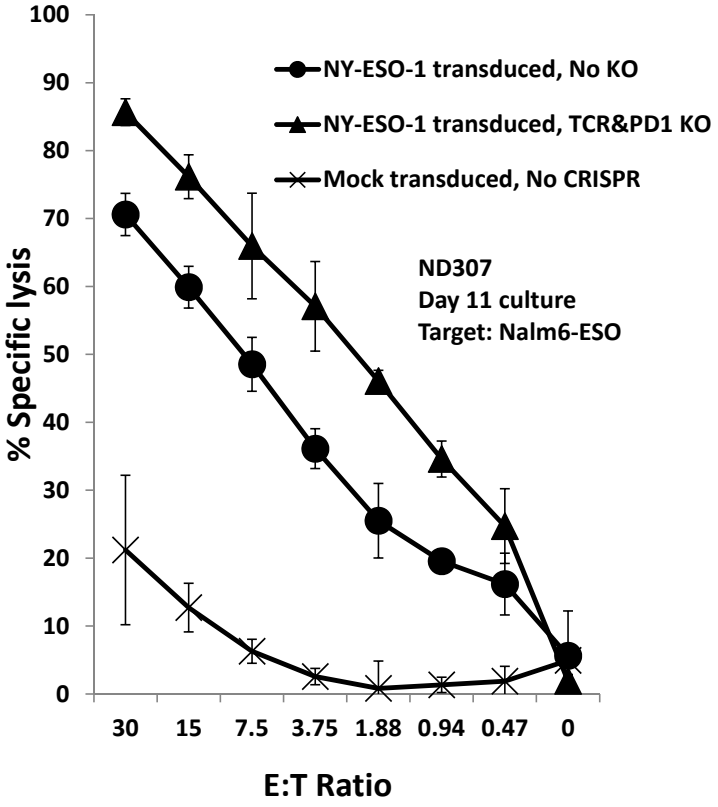
Gene	Number of Donors Tested	Average % Disruption (Range)
TRAC	5	79.6 (62.0 – 92.3)
TRBC	10	76.6 (69.5 – 83.1)
TRAC + TRBC	16	73.2 (57.1 – 92.1)
PDCD1 (PD1)	6	53.1 (47.5 – 56.1)

Expansion in vitro

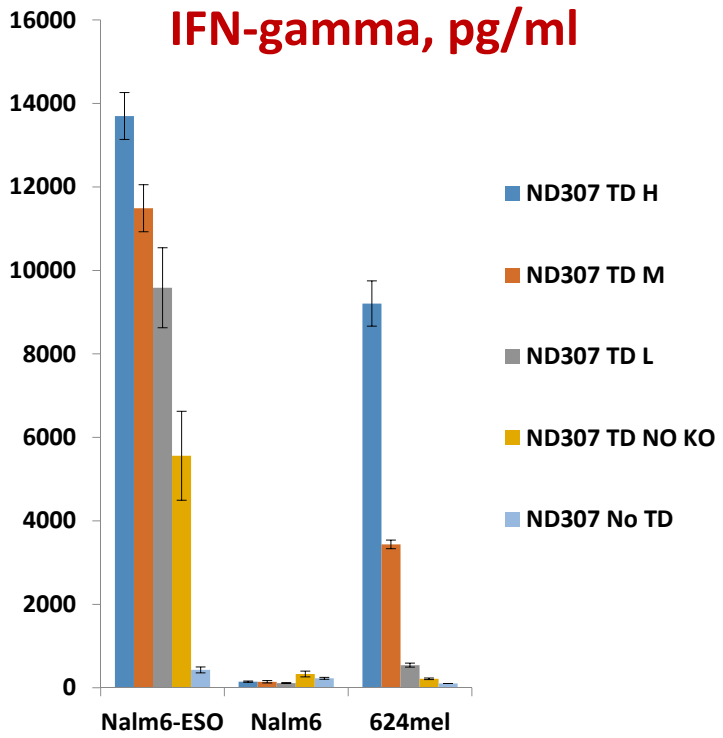


NY-ESO-1 CRISPR (TCR-PD1) Edited (NYCE cells) Function *In Vitro*

Increased lysis

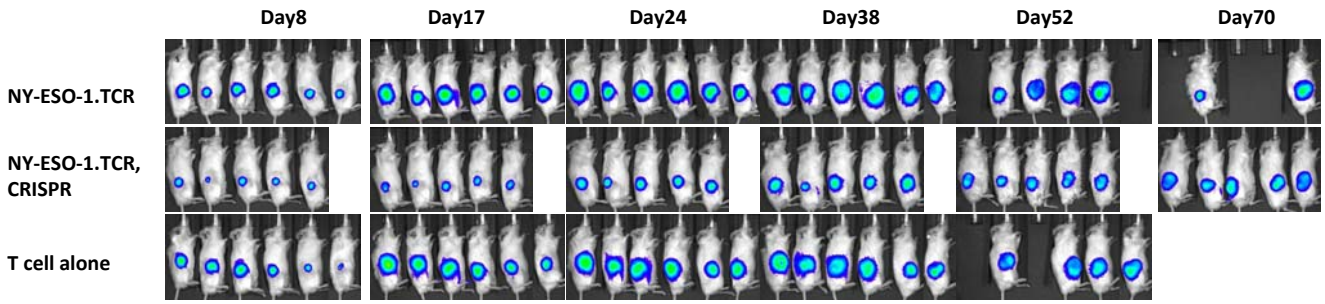


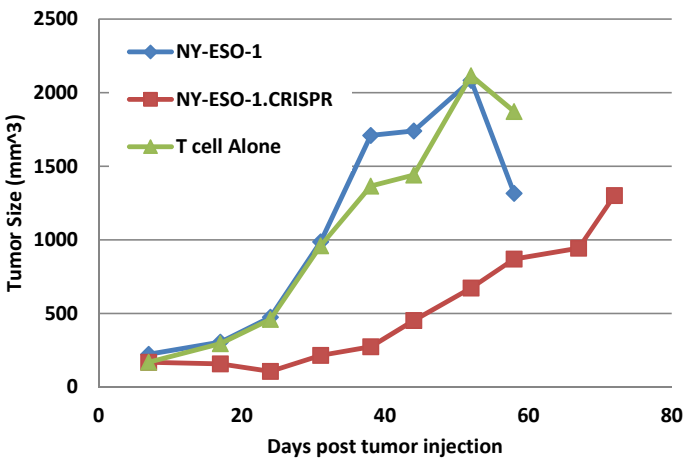
Cytokine secretion: effect of gRNA dose

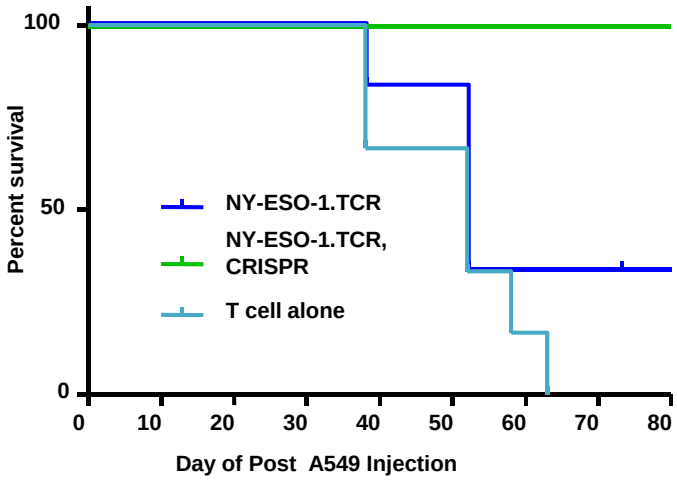


	Nalm6-ESO	Nalm6	624mel
HLA A2	+	+	+
NY-ESO-1	+	-	+

Pre-clinical studies: Triple-Edited NYCE T cells *In Vivo*







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:
  - Harrison DE. Competitive repopulation: a new assay for long-term stem cell functional capacity. Blood. 1980;55(1):77-81.
  - Zon LI. Intrinsic and extrinsic control of haematopoietic stem-cell self-renewal. Nature. 2008;453(7193):306-13.
  - Milone MC et al. Chimeric Receptors Containing CD137 Signal Transduction Domains Mediate Enhanced Survival of T Cells and Increased Antileukemic Efficacy In Vivo. Mol Ther. 2009;17(8):1453-64.
  - Savoldo B et al. CD28 costimulation improves expansion and persistence of chimeric antigen receptor–modified T cells in lymphoma patients. J Clin Invest. 2011;121(5):1822.

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

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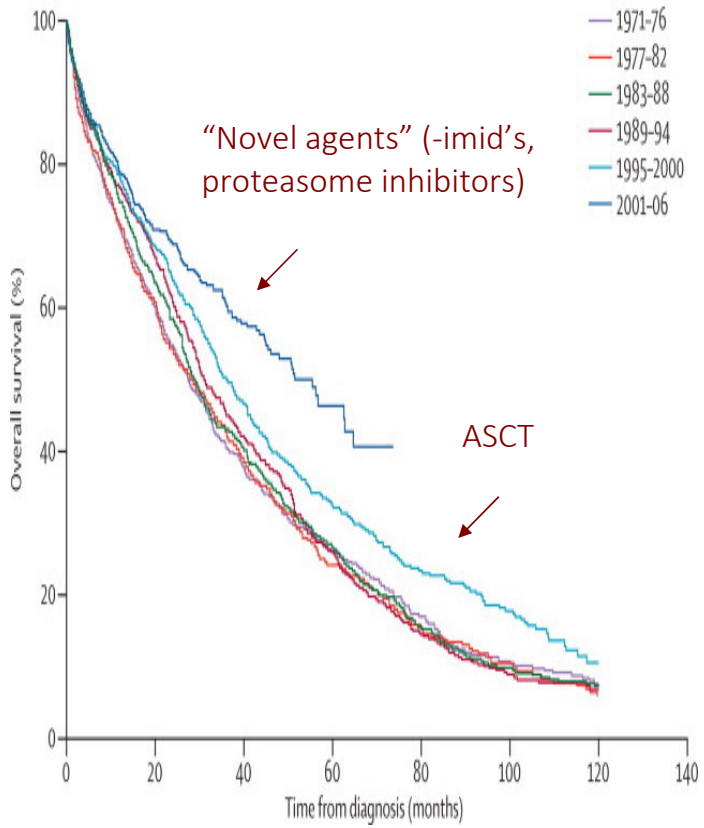
Study Background

- **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:

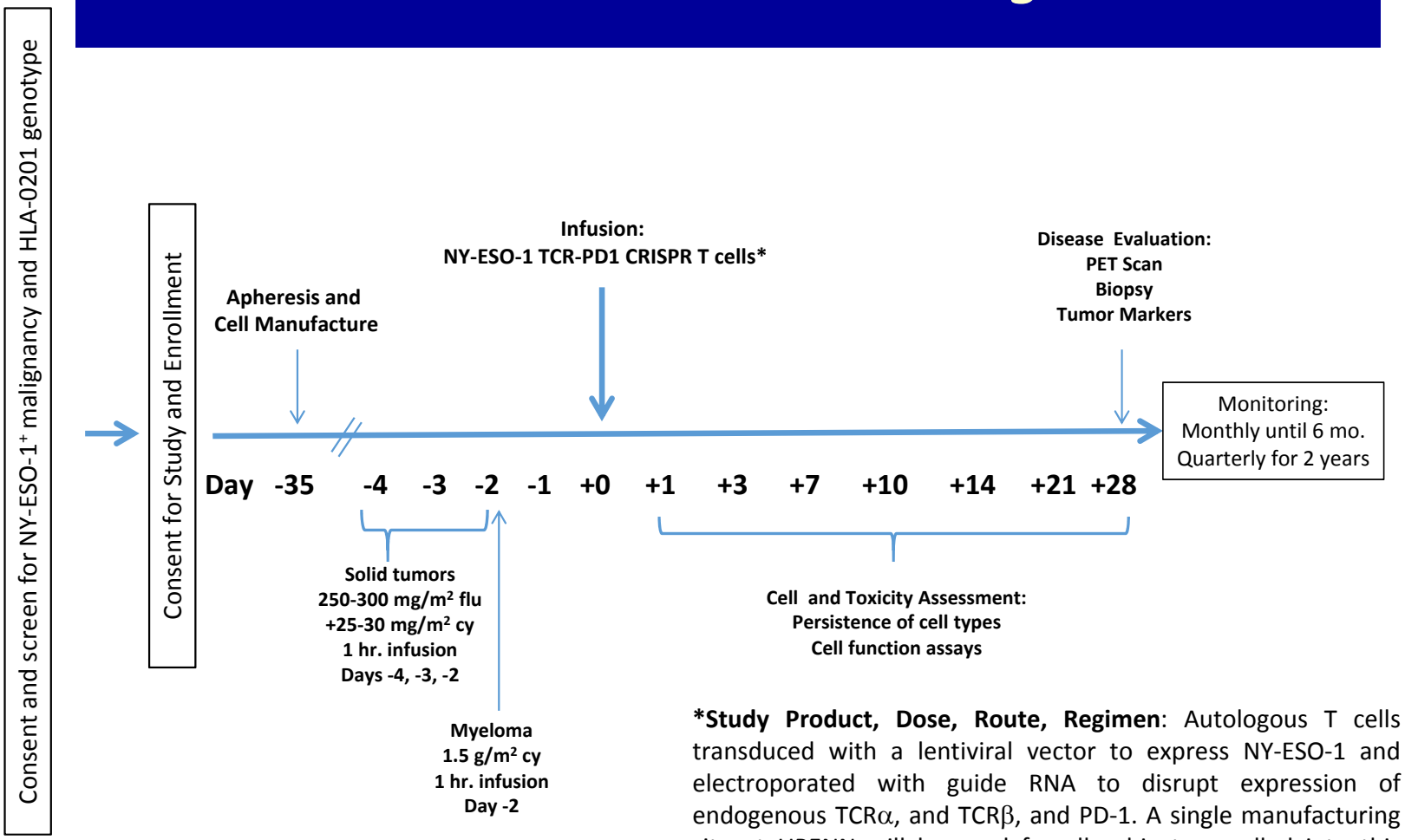
-Clinical assessment: anti-tumor responses and survival

-NYCE cells engraftment, persistence, and trafficking

-NYCE cells bioactivity

-NYCE cells immunogenicity

NYCE Clinical Trial Design



***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 $1x10^8$ cells/kg will be given i.v.

Study Aspect Rationale

- **Rationale for TCR^{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 my 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 patents 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

Protocol Management - II

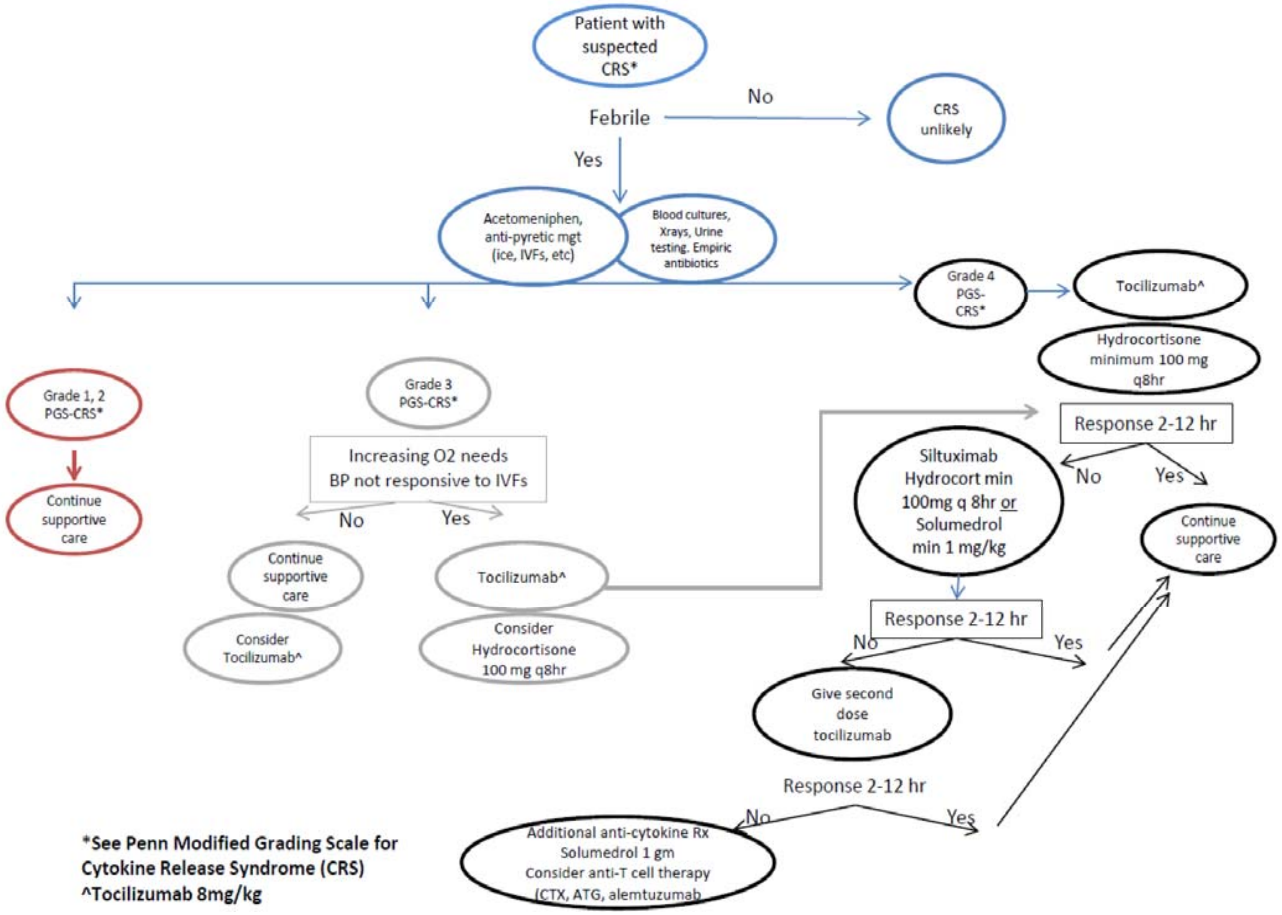
- **Disease Control**
  - Patients are allowed to receive standard therapies to maintain disease control after apheresis and up to 2 weeks prior to infusion. Patients with disease progression (or cell manufacturing failure) will be replaced. The patients with cell manufacturing failure will be included 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

Management of Cytokine Release Syndrome



Lee DW et al. How I treat: current concepts in the diagnosis and management of cytokine release syndrome. Blood. 2014;124(2):188-95.

Plan for Sample Analysis

Simon F. Lacey, Ph.D. Translational and Correlative Studies Laboratory

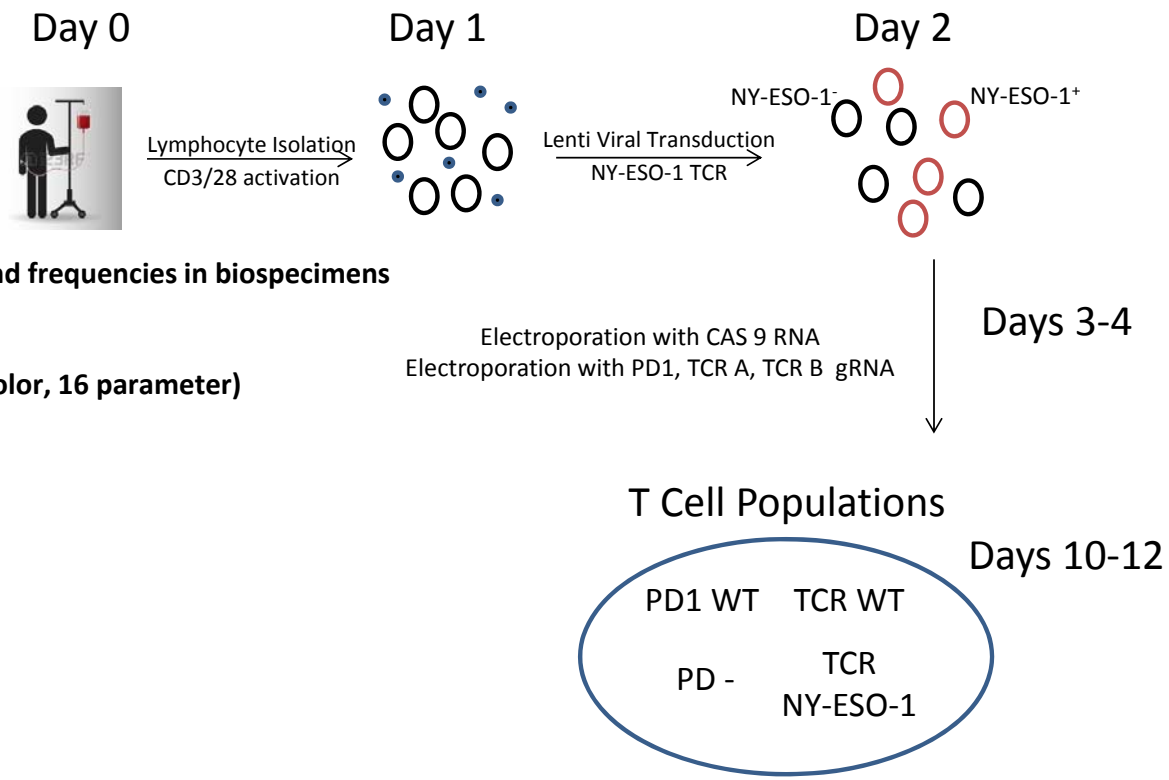
Jos Melenhorst, Ph.D. Product Development & Correlative Sciences laboratory

Penn Medicine
Center for Cellular Immunotherapies

Penn
University of Pennsylvania
Parker Institute for Cancer Immunotherapy

PARKER INSTITUTE
for CANCER IMMUNOTHERAPY
of MD ANDERSON, MEMORIAL SLOAN-KETTERING, PENN MEDICINE, STANFORD, UCLA, AND UCSF

Plan for Sample Analysis



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

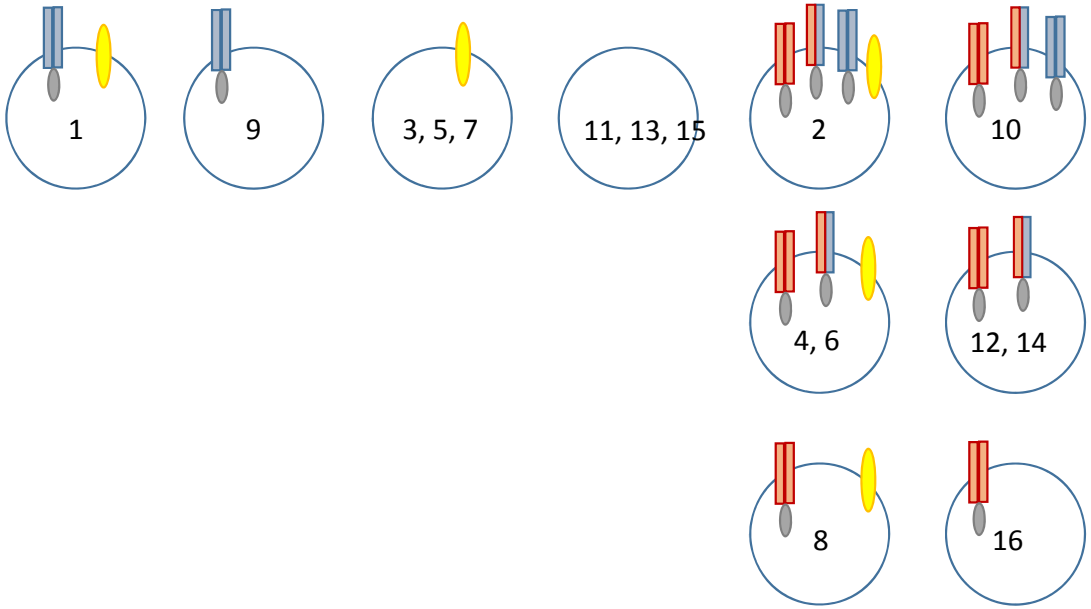
- **Flow Cytometry**
  - **NY-ESO-1 Dextramers**
  - **Stimulation and checkpoint inhibitor expression (14-color, 16 parameter)**
  - **TCR^{endo} staining with cocktail of Vβ mAbs**

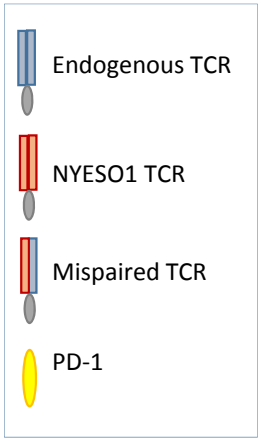
- **Detection of Indels due to CAS9 gene editing**
  - **Endonuclease digestion (Surveyor)**
  - **Digital PCR to assess gene disruption frequency**
  - **Targeting sequencing in TCR domains, and PD1**

- **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



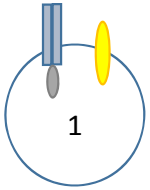


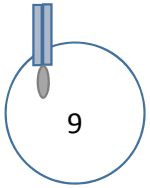
Genotype

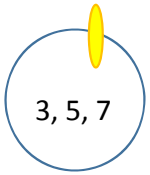
PD1+	PD1-	PD1+	PD1-	PD1+	PD1-
TCR A+B+	TCR A+B+	3:TCR A-B+	11:TCR A-B+	2:TCRA+B+	10:TCRA+B+
		5:TCR B-A+	13:TCR B-A+	4:TCR A-B+	12:TCR A-B+
		7:TCR A-B-	15:TCR A-B-	6:TCR B-A+	14:TCR B-A+
				8:TCR A-B-	16:TCR A-B-

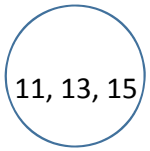
Expected T Cell Populations: fitness and function

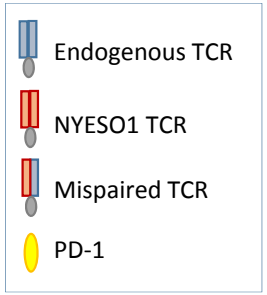
Phenotype











Genotype

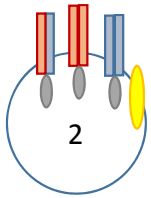
PD1+ TCR A+B+	PD1- TCR A+B+	PD1+ 3:TCR A-B+ 5:TCR B-A+ 7:TCR A-B-	PD1- 11:TCR A-B+ 13:TCR B-A+ 15:TCR A-B-
Wild-type T cells that were not modified during manufacturing. Safety is expected while efficacy is not.	**T cells with PD-1 disruption that retains endogenous TCR. These cells are anticipated to 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 ($2^4 = 16$)

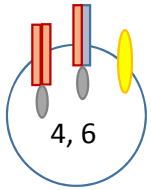
	NYESO1- TCR WT	NYESO1+ TCR WT	NYESO1- TCR A-B+	NYESO1+ TCR A-B+	NYESO1- TCR B-A+	NYESO1+ TCR B-A+	NYESO1- TCR A-B-	NYESO1+ TCR A-B-
PD1 WT	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
PD1 -	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**

Expected T Cell Populations (cont.)

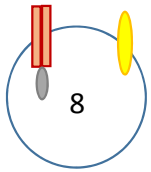
Phenotype



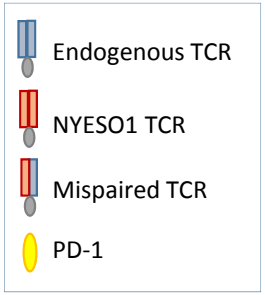
NY-ESO-1 specific T cells without gene disruption. Previously tested in patients with multiple myeloma with demonstrated safety.



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.



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

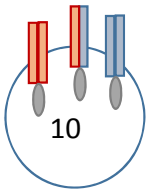
PD1+
2:TCRA+B+
4:TCR A-B+
6:TCR B-A+
8:TCR A-B-

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

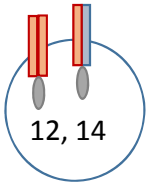
	NYESO1- TCR WT	NYESO1+ TCR WT	NYESO1- TCR A-B+	NYESO1+ TCR A-B+	NYESO1- TCR B-A+	NYESO1+ TCR B-A+	NYESO1- TCR A-B-	NYESO1+ TCR A-B-
PD1 WT	1	2	3	4	5	6	7	8
PD1 -	9	10	11	12	13	14	15	16

Expected T Cell Populations (cont.)

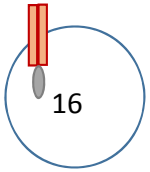
Phenotype

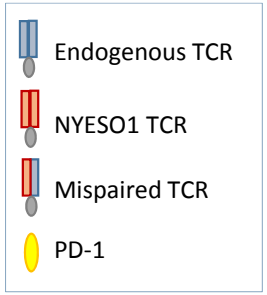


NY-ESO-1 specific T cells with PD-1 disruption. Expected anti-tumor efficacy and less susceptible to exhaustion by PD-1 expression



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.





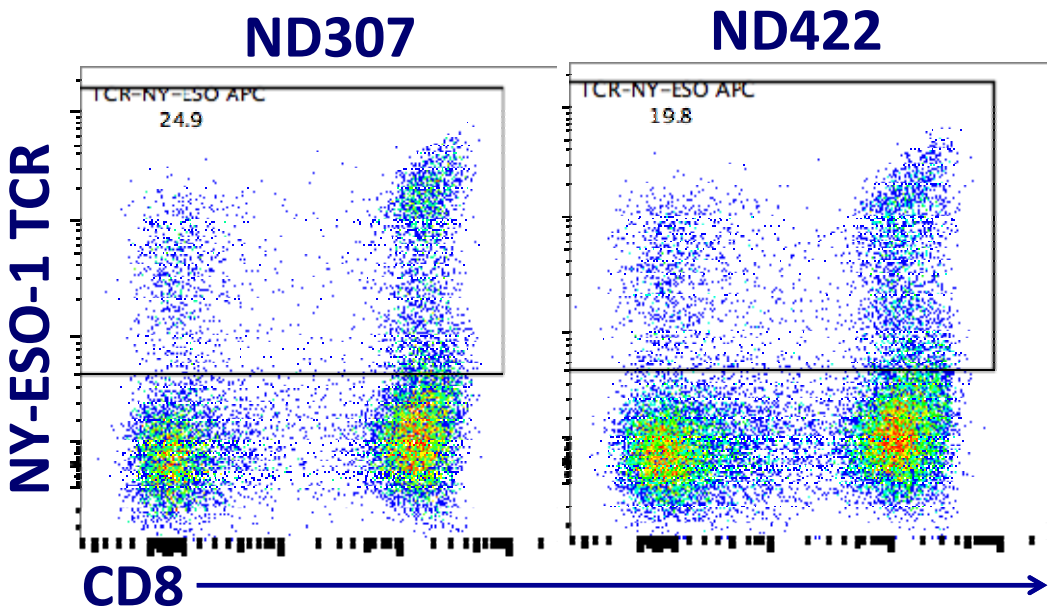
Genotype

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

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

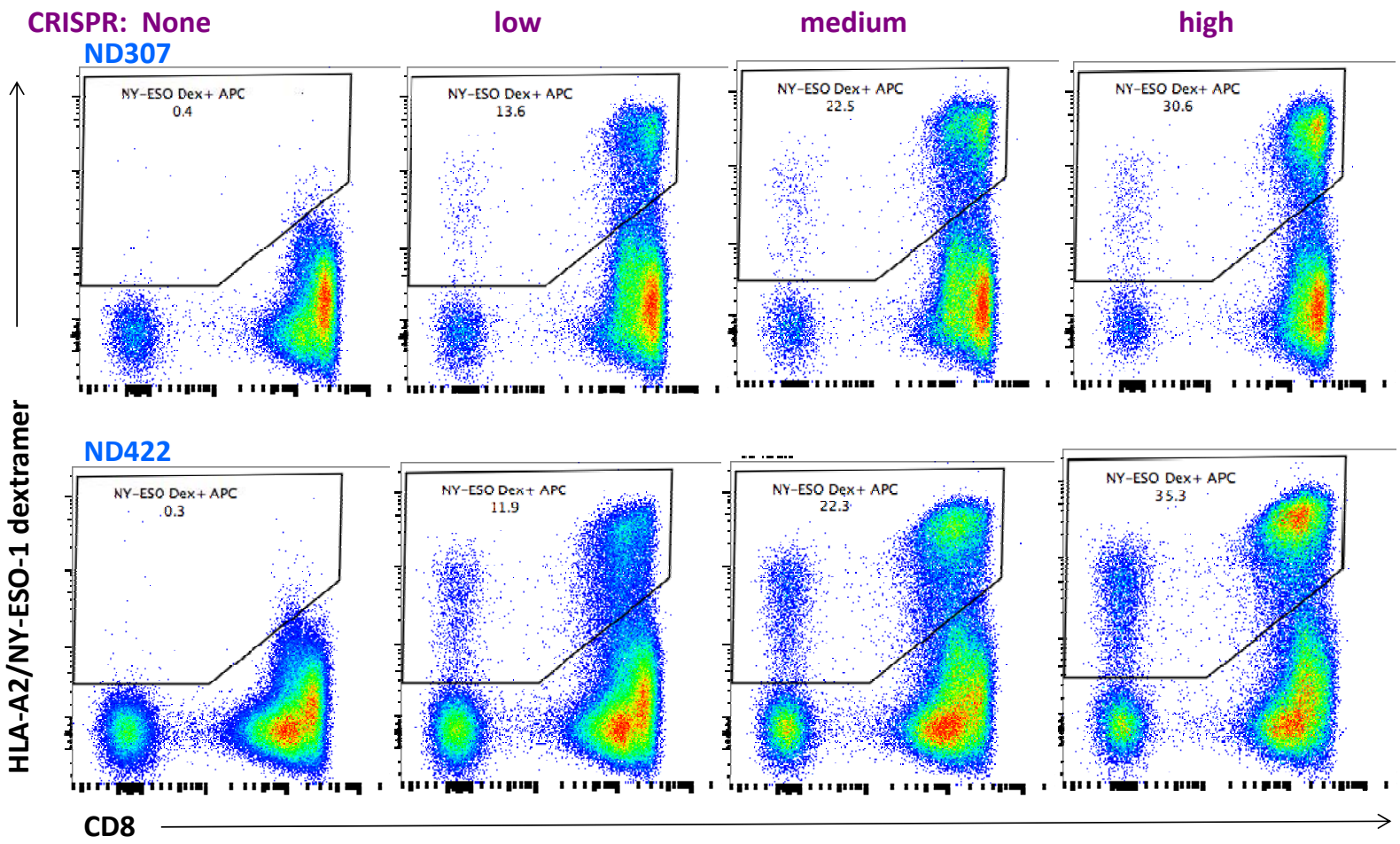
	NYES01- TCR WT	NYES01+ TCR WT	NYES01- TCR A-B+	NYES01+ TCR A-B+	NYES01- TCR B-A+	NYES01+ TCR B-A+	NYES01- TCR A-B-	NYES01+ TCR A-B-
PD1 WT	1	2	3	4	5	6	7	8
PD1 -	9	10	11	12	13	14	15	16

Detection of NY-ESO-1 specific TCR using HLA-A2/SLLMWITQC dextramer identifies transgenic TCR expression on both CD8+ and CD4+ NYCE T cells



NY-ESO-1 expression analysis in low CRISPR-treated donor T cells; % TCR+ cells		
T cell subset	**Donor ND307**	**Donor ND422**
CD4+	16.7	13.5
CD8+	29.9	24.9

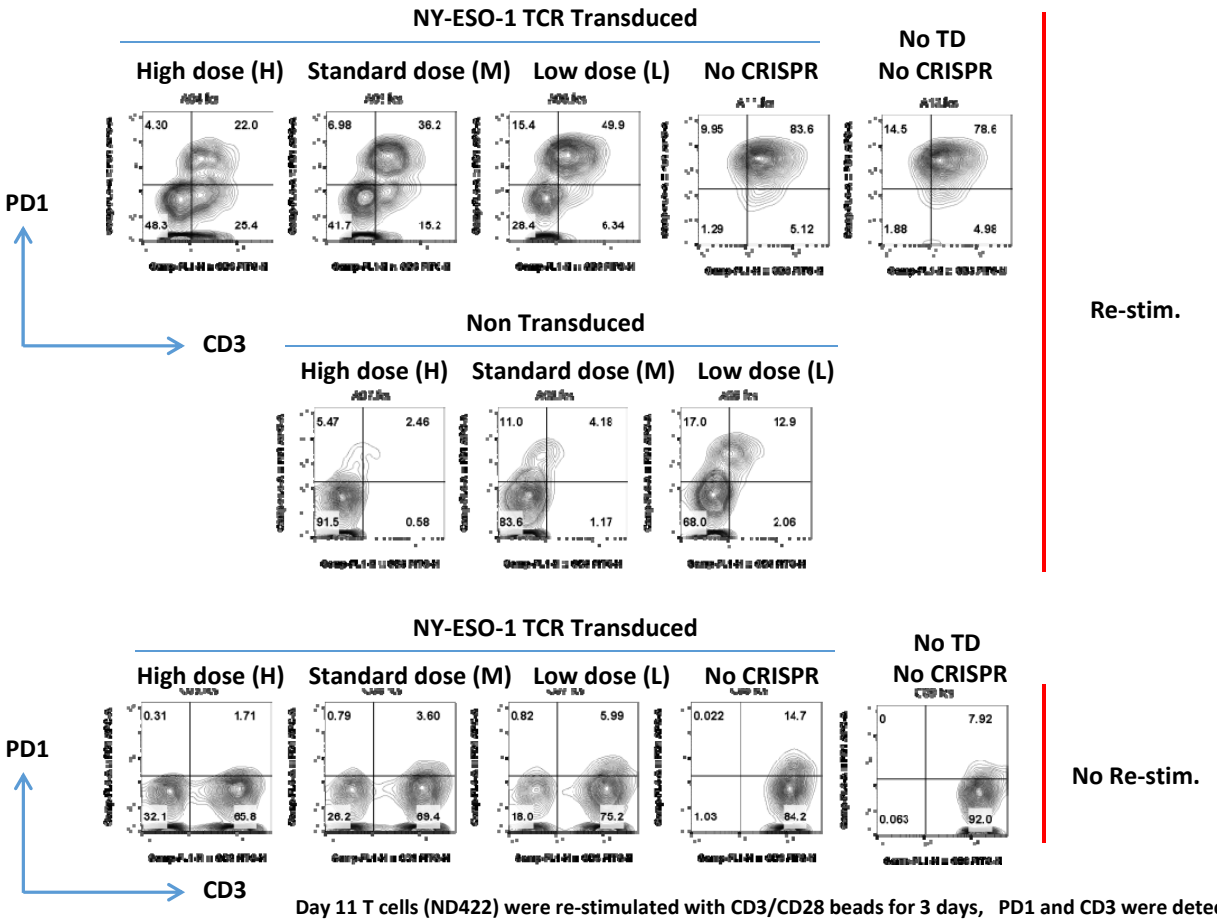
Increased frequency of NY-ESO-1 dextramer+ T cells with increased CRISPR gRNA dose



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

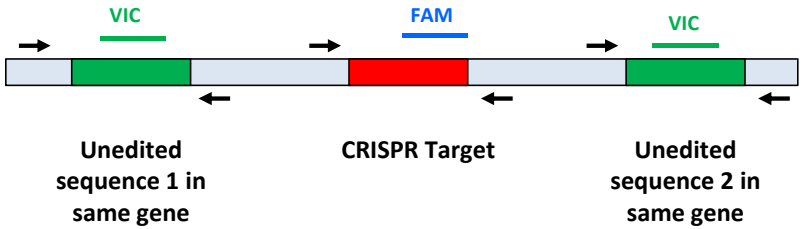
PD-1 Expression on TCR Transduced, Triple CRISPR-Edited T cells



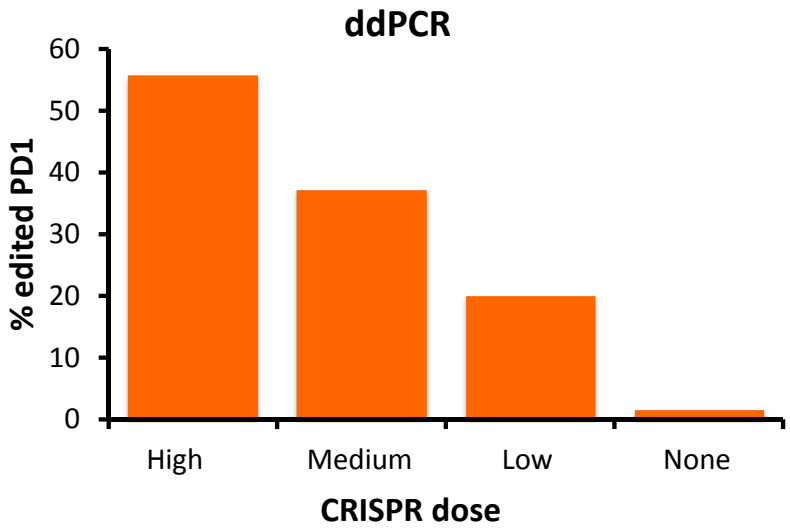
Day 11 T cells (ND422) were re-stimulated with CD3/CD28 beads for 3 days, PD1 and CD3 were detected by flow cytometry (upper and middle panel), non re-stimulated T cells were used as controls (bottom).

Digital-PCR Assay of PD-1 Gene-Editing in NY-ESO-1 TCR Transduced, Triple Edited T cells

A.



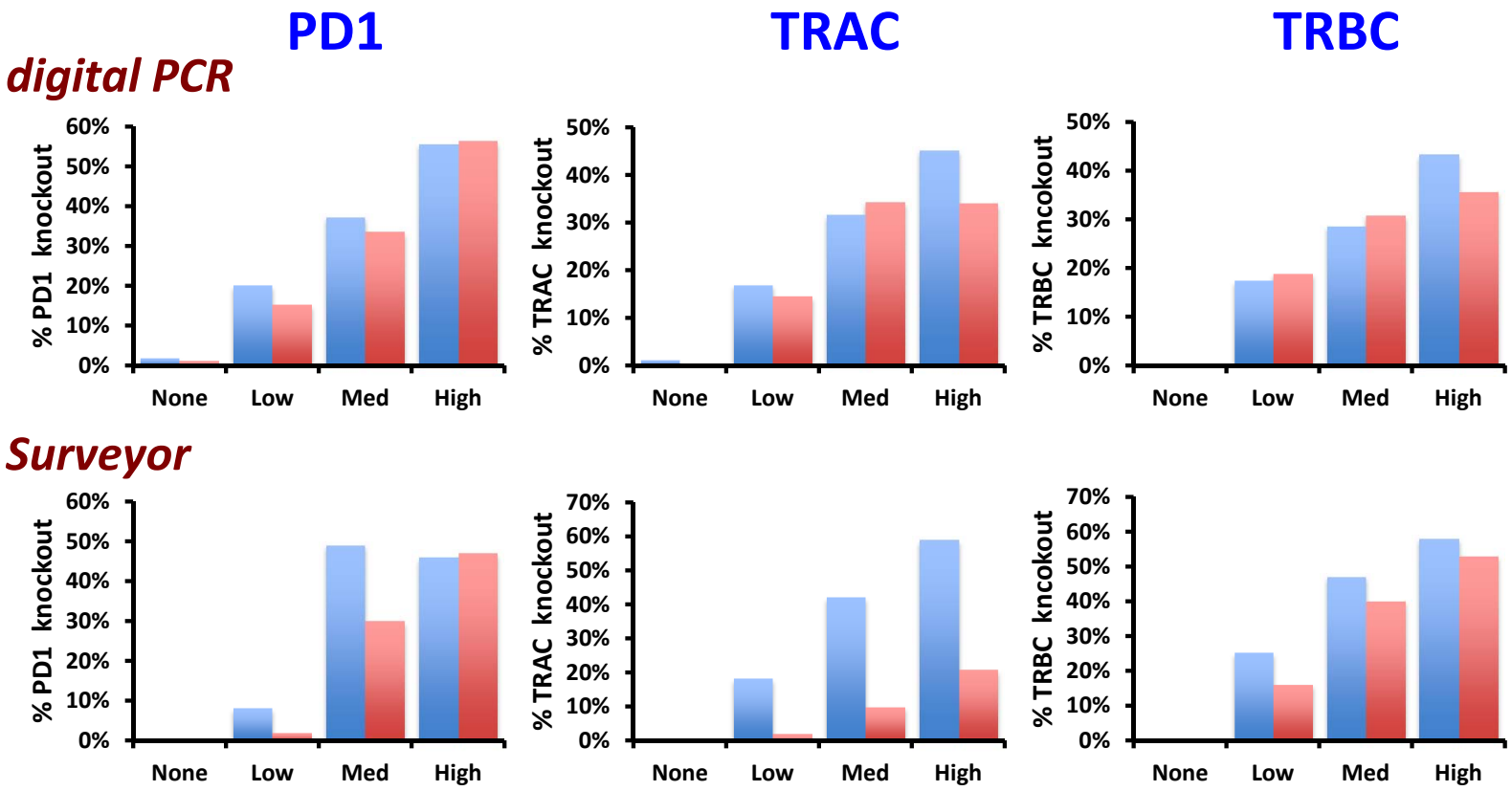
B.



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.

% PD-1 Gene Editing = FAM/VIC X 100

Quantification of gene disruption efficiency using digital PCR and Surveyor Assays



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

Target Gene	ND307			ND391	ND422			ND463	ND469
	High Dose	Med Dose	Low Dose	Med Dose	High Dose	Med Dose	Low Dose	Med Dose	Med Dose
PD1	0	0	0	0 Not targeted	0	0	0	0 Not targeted	0 Not targeted
TRAC	0	0	0	0 Not targeted	0	0	0	0 Not targeted	0 Not targeted
TRBC	0	1	0	0	0	0	0	0	0

Off-target indels

Number of Samples having the indels at the off-target site	Chromosome Location (hg19)	Mutation description	HGVS mutation name	Mutation Frequency in the identified sample(s)	Number of supporting sequencing reads	Gene	Gene region
1 out of 9	chr8:41631339	deletion of the C at chr8:4163339	NM_000037.3:c.28-15684del	0.45%	5	ANK1	intron 1

TRAC Sequencing sites

sequence	UCSC gene	locus (hg19)	Note
TGTGCTAGACATGAGGTCTATGG		chr14:+23016588	TRAC guide sequence
TGTGCTAGAGATTAGGTCTAGGG		chr5:+98609341	
AGTCCTAGACATGAGGTCTCAGG		chr22:+43998013	
TCTGCTAGTAATGAGGTCTACAG		chr13:-110662475	
TTTGCCAGAAATGAGGTCTACAG		chr2:-150299223	
TGGGTTAAAAATGAGGTCTATGG		chr18:-659011	
TGCGCTACACATAAGGTCTAGGG		chr5:+168703790	
TGTTATAGACATTAGGTCTACAG		chr10:-12763850	
TGTTCTTGACAGGAGGTCTAGGG		chr4:-90922429	
GGGTCTAGGCATGAGGTCTAAGG		chr22:-27933001	
TGTGACACAGATGAGGTCTACAG		chr6:-107598353	
TCTTCTAGATATGAGGTCTGAGG		chr16:+75379841	
TAACCTAGACATGAGGGCTAGGG		chr10:+116534477	
AATGATAGACATCAGGTCTACAG		chr3:-135045562	
CCTCCTAGACATTAGGTCTAAGG		chr12:-72404435	
TTTGCTAAAAATGAGGCCTACAG		chr10:+12677636	
TGTGGTATATATAAGGTCTATAG		chr7:+145619400	
AGTTCCAGACGTGAGGTCTATGG		chr19:+4811068	
TGTGCTGGACTTGAGGTCTTTAG		chr1:+85154310	
TCTGGTAGATATGAGGTCAATAG		chr2:-161344560	
AGTGCTGCACATGAGGCCTACAG	NM_178026	chr20:+33450540	
GGTTCTGGACATGAGGCCTAGAG		chr21:-46598307	
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TGTGTTACCCATGAGGTCTGTGG	NM_020402	chr11:-3686775	
TGGGCTGTACATGAGGCCTAGAG		chr2:-31180420	
TGTGCTGGGCATGAGGTCGAGGG		chr3:-174859848	
TAGGCTAGATATGACGTCTATGG		chr8:+81836556	
TGTGTTAGCTATGAGGTCTGCAG		chr10:-89800797	
TGTTTTAGAGATGAAGTCTAAAG		chr18:-33612451	
TGTGCTAGATATAAGGGCTAAGG		chr10:+119664019	
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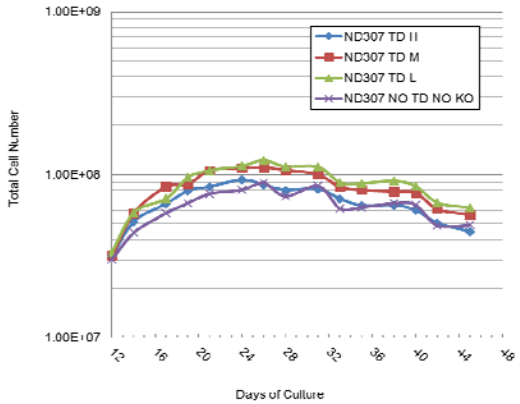
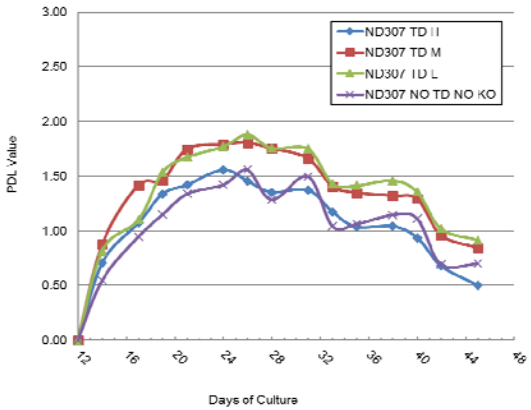
GGTCCTAGACATGAGGACTCTGG		chr19:-40402588	
GGTCCTAGACATGAGGACTCTGG		chr19:-40370648	
TGTGAAAGAAATGAGGTCAAAGG		chr4:-84930266	
TGTGGTAAACACCAGGTCTATAG		chr13:+107879766	
TGTGCCAGACATTAGGGCTAGAG		chr5:+4155089	
TGTTCTTGAAATGATGTCTAGGG		chr21:-30265732	
TGTTCTAGAAATCAGGTCTTAAG		chr2:-110407377	
TGTGTTAGAAATGAGGACTTGAG		chrX:-20417960	
CCTGCTAGACTTGATGTCTACAG		chr16:-85993980	
TGTGCTGGAACAGAGGTCTAAGG		chr1:+37001981	
CGTCCTAGACATGAGGTCAGGAG		chr2:-7488417	
TGTGCTAACCCTGAGGTCTTGGG		chr9:-72693940	

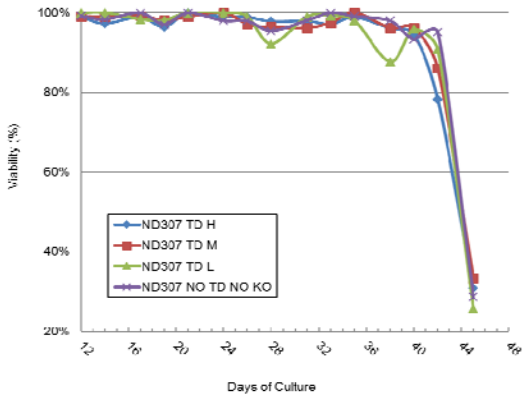
Assessment of Off-Target Effects: Long-term culture of manufactured T cells and patient samples

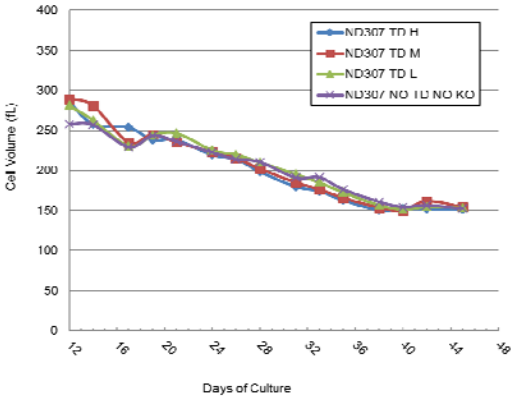
Long term culture to measure:
- total cell number
- population doubling
- viability
- cell volume

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.

1524.

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Carl June, MD
Printed Name

Carl H June
Signature

Date: 06-09-2016

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J. Joseph Melenhorst, PhD_____
Printed Name

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Date: _06/09/2016_____

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EDWARD STROTMAUER

Printed Name

Signature

Date: 6/9/16