Somatic Genome Editing
Academic Development Perspective

Bruce L. Levine, Ph.D.

Center for Cellular Immunotherapies, University of Pennsylvania

Co-Founder, Tmunity Therapeutics

President Elect, International Society for Cell and Gene Therapy

@BLLPHD

TMUNITY™

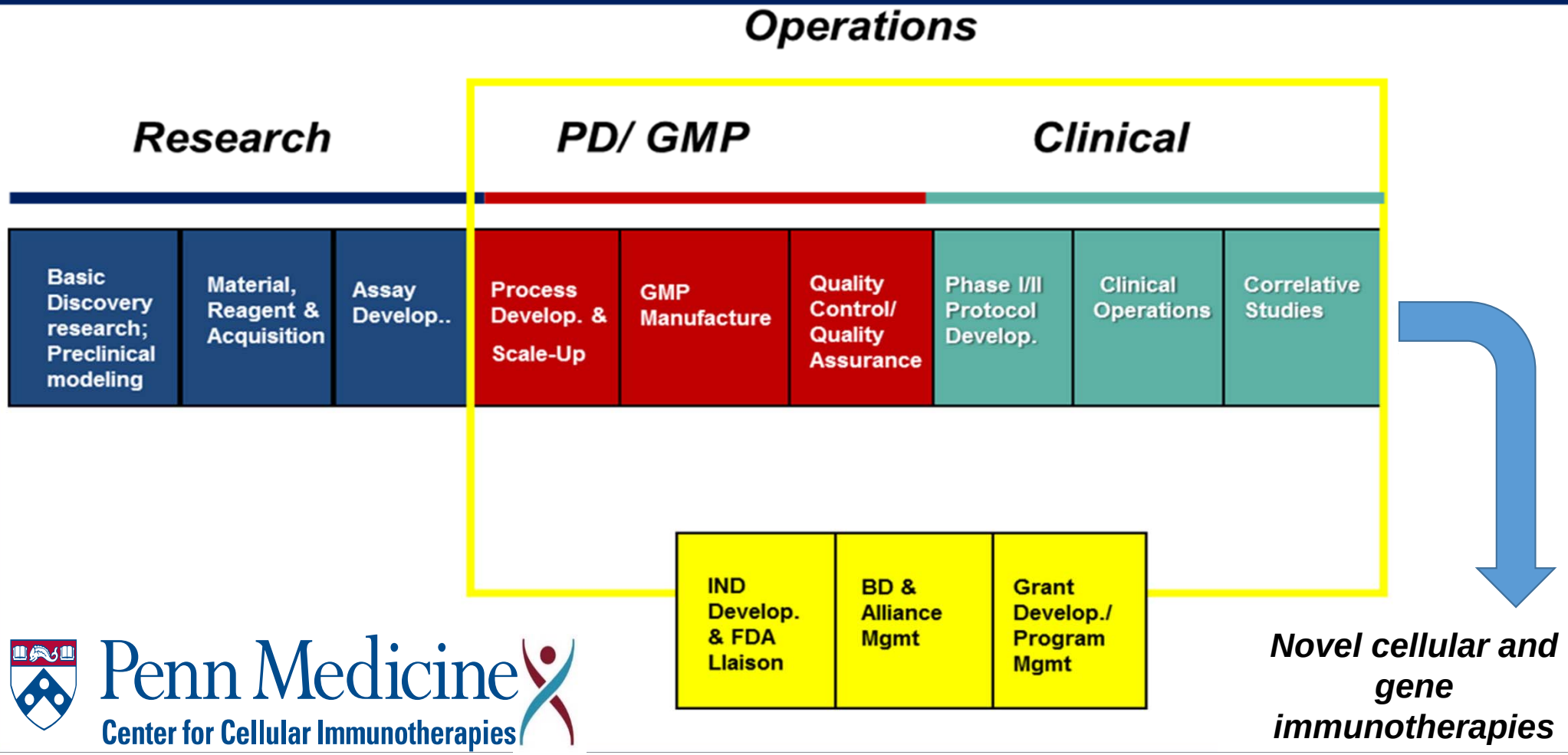
Penn
University of Pennsylvania

International Society
ISCT
Cell & Gene Therapy

Conflict of Interest Statement

- Declaration of financial interest due to intellectual property and patents in the field of cell and gene therapy.
- University of Pennsylvania Alliance with Novartis
- Consultant for CRC Oncology, Cure Genetics, Novartis
- Scientific Advisory Board for Avectas, Brammer Bio, Incysus, Vycellix
- Co-Founder and equity Tmunity Therapeutics
- Conflict of interest is managed in accordance with University of Pennsylvania policy and oversight

Center for Cellular Immunotherapies: Internal Translational Research Infrastructure



Penn Medicine
Center for Cellular Immunotherapies

CCI First In Human Trials

1st Trials of CAR T Cells (Cell Genesys)

Blood. 2000 Jul 15;96(2):467
Blood. 2000 Aug 1;96(3):785

1st Trial of Lentiviral Transduced Cells (VIRxSYS)

PNAS. 2006 Nov 14;103(46):17372

1st Trial of Genome Edited Cells (Sangamo)

NEJM 2014 Mar 6;370(10):901

1st Trial of Lentiviral Transduced TCR T Cells in Cancer (Adaptimmune)

Nat Med. 2015 Aug;21(8):914

1st Trial of RNA Electroporated CAR T Cells in Cancer (MaxCyte)

Cancer Res. 2010 Nov 15;70(22):9053

1st Trial of Lentiviral Transduced CAR T Cells in Cancer (licensed to Novartis)

NEJM 2011 Aug 25;365(8):725
Sci Transl Med. 2011 Aug 10;3(95):95ra73

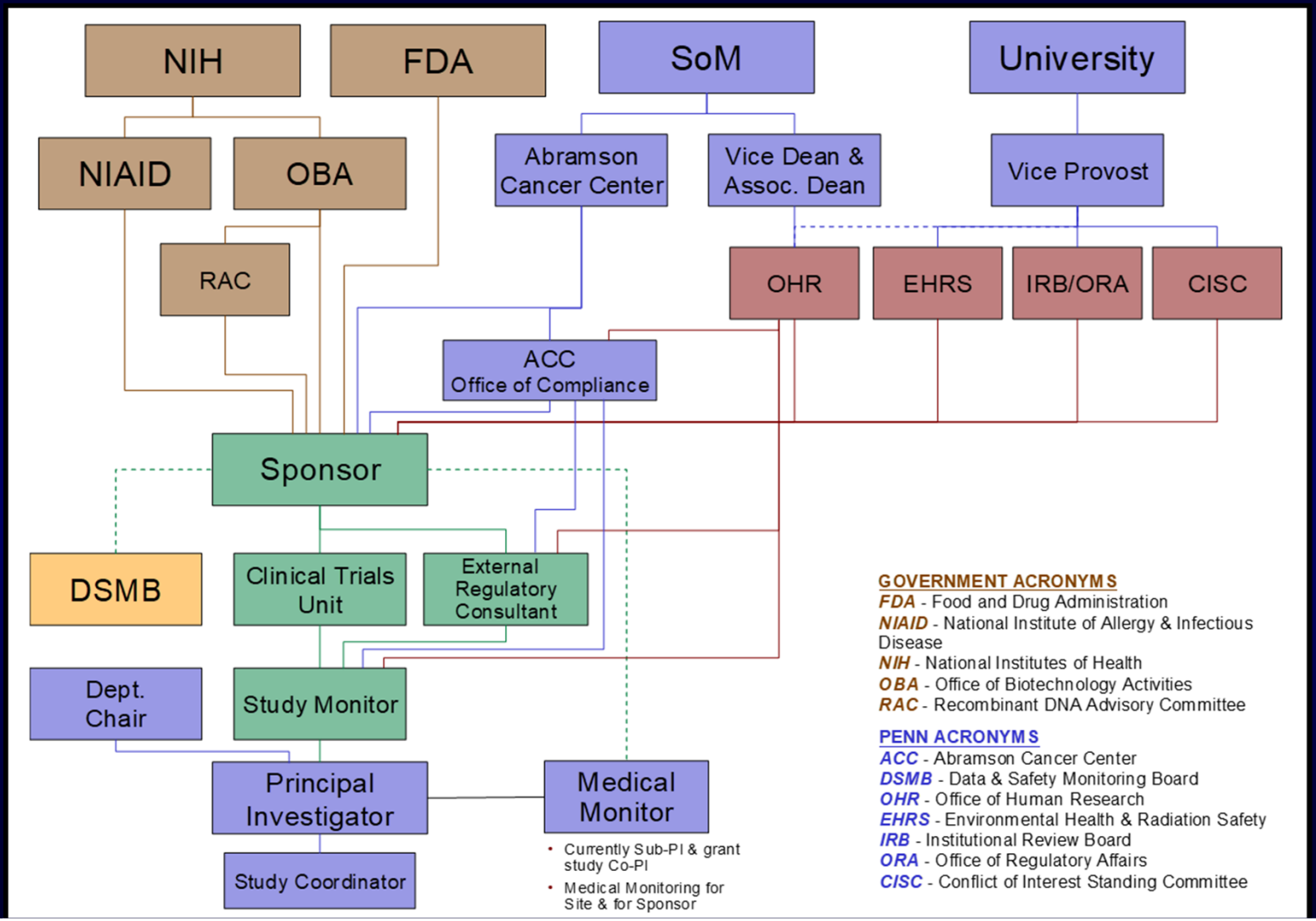
First Global Trial of CAR T Cells (Novartis)

NEJM 2018 Feb 1;378(5):439

First FDA Approved Gene Therapy (Novartis, Kymriah™)

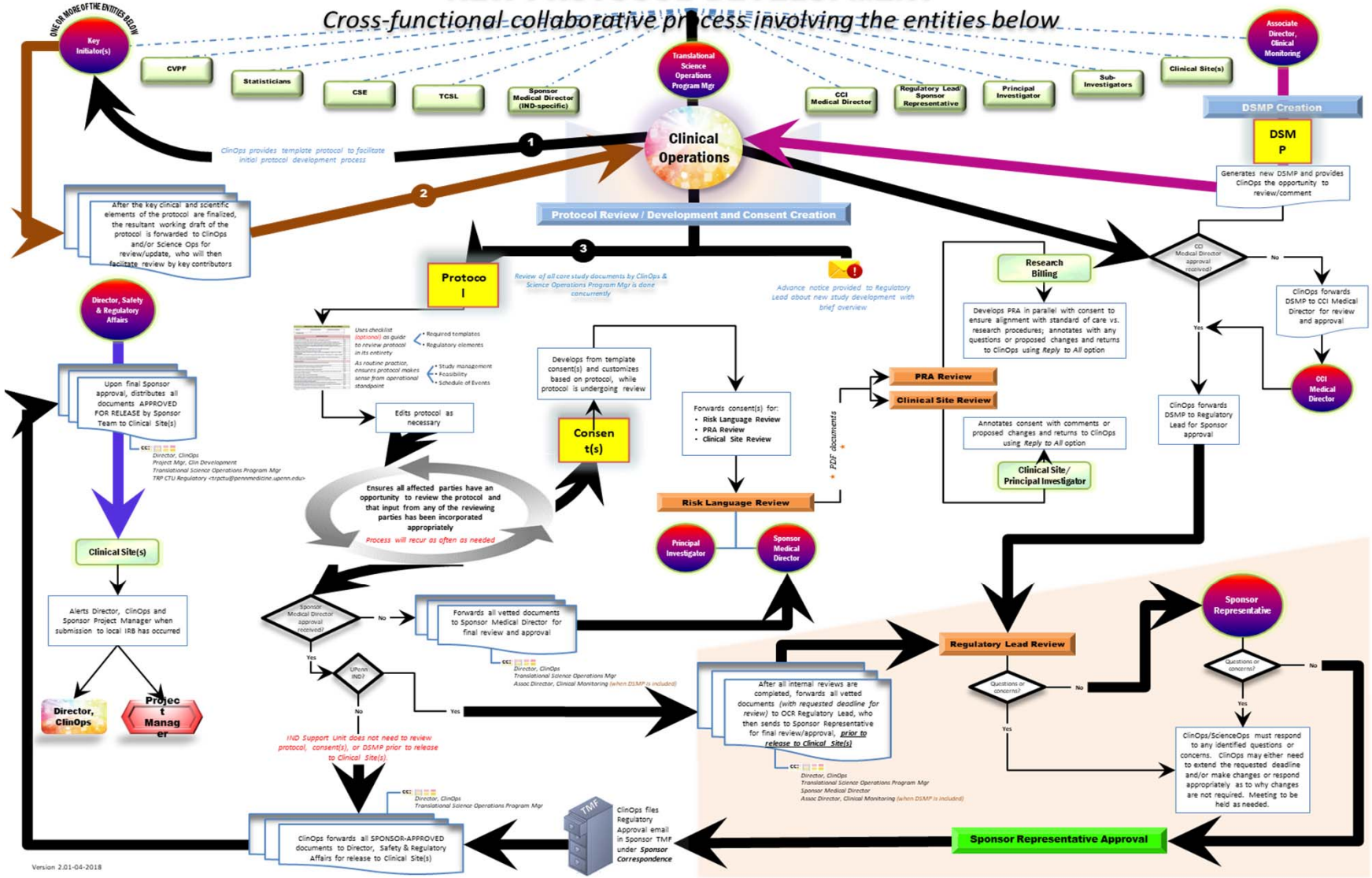
www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm574058.htm

Regulatory Oversight for Physician Sponsored FDA IND



NEW PROTOCOL DEVELOPMENT

Cross-functional collaborative process involving the entities below



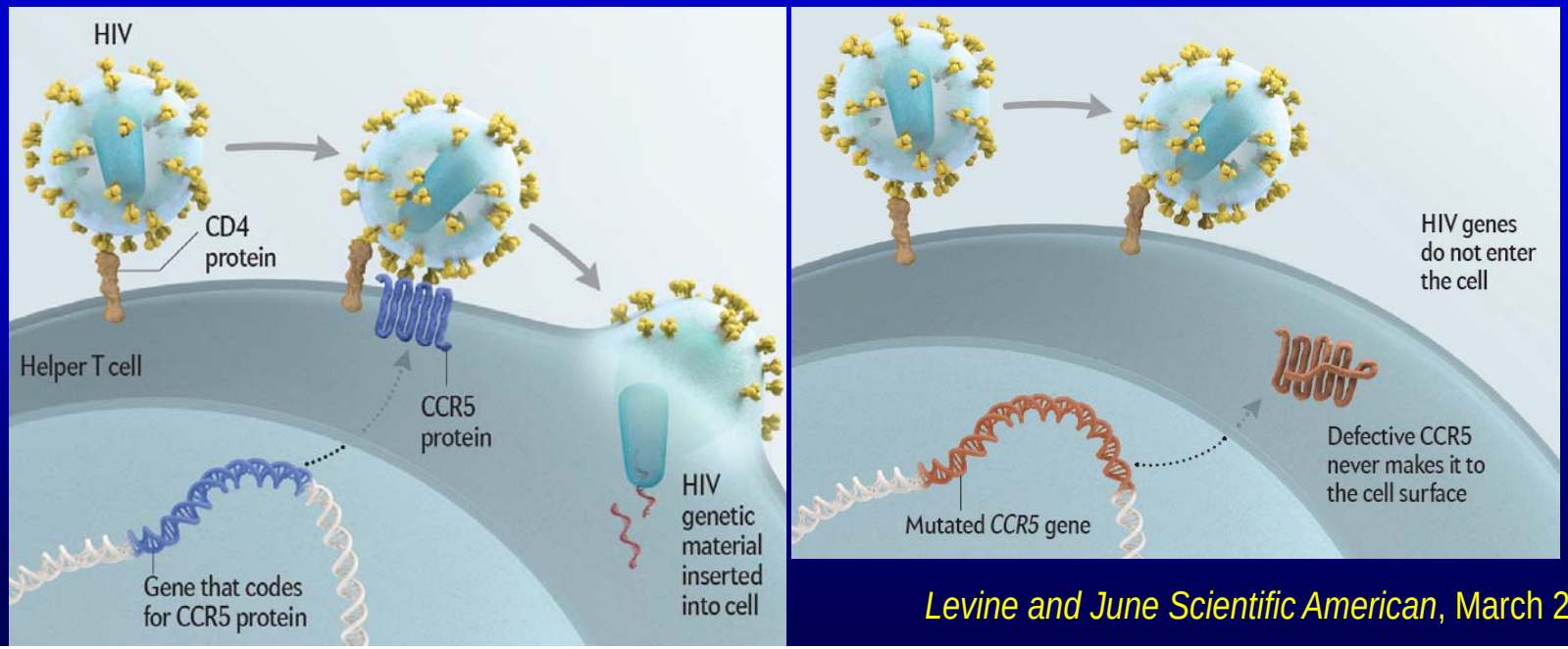
- The systems in place for federal and proper local regulatory oversight in the US are very complex
- Scientific, technical, funding hurdles create a high bar for entry
- If complied with

Clinical Development of Gene Editing

Part 1: ZFN KO of CCR5

Why Target CCR5 in HIV?

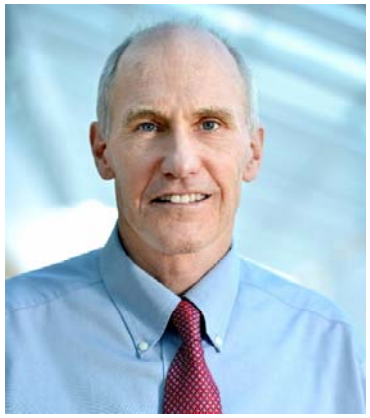
- HIV (R5 virus) targets CD4 T-cells by binding to CCR5, one of the major co-receptors for HIV entry
- CCR5 delta-32 mutation produces a nonfunctional protein
  - Homozygotes are resistant to HIV infection
  - Heterozygotes have slower disease progression



Levine and June Scientific American, March 2012

ZFN Mediated Disruption of CCR5 to Create CCR5 deficient CD4 Cells for HIV Therapy

Recombinant Advisory Committee Meeting
June 20, 2007



UPENN IPCP CFAR Center for AIDS Research

SANGAMO

Sangamo BioSciences, Inc.

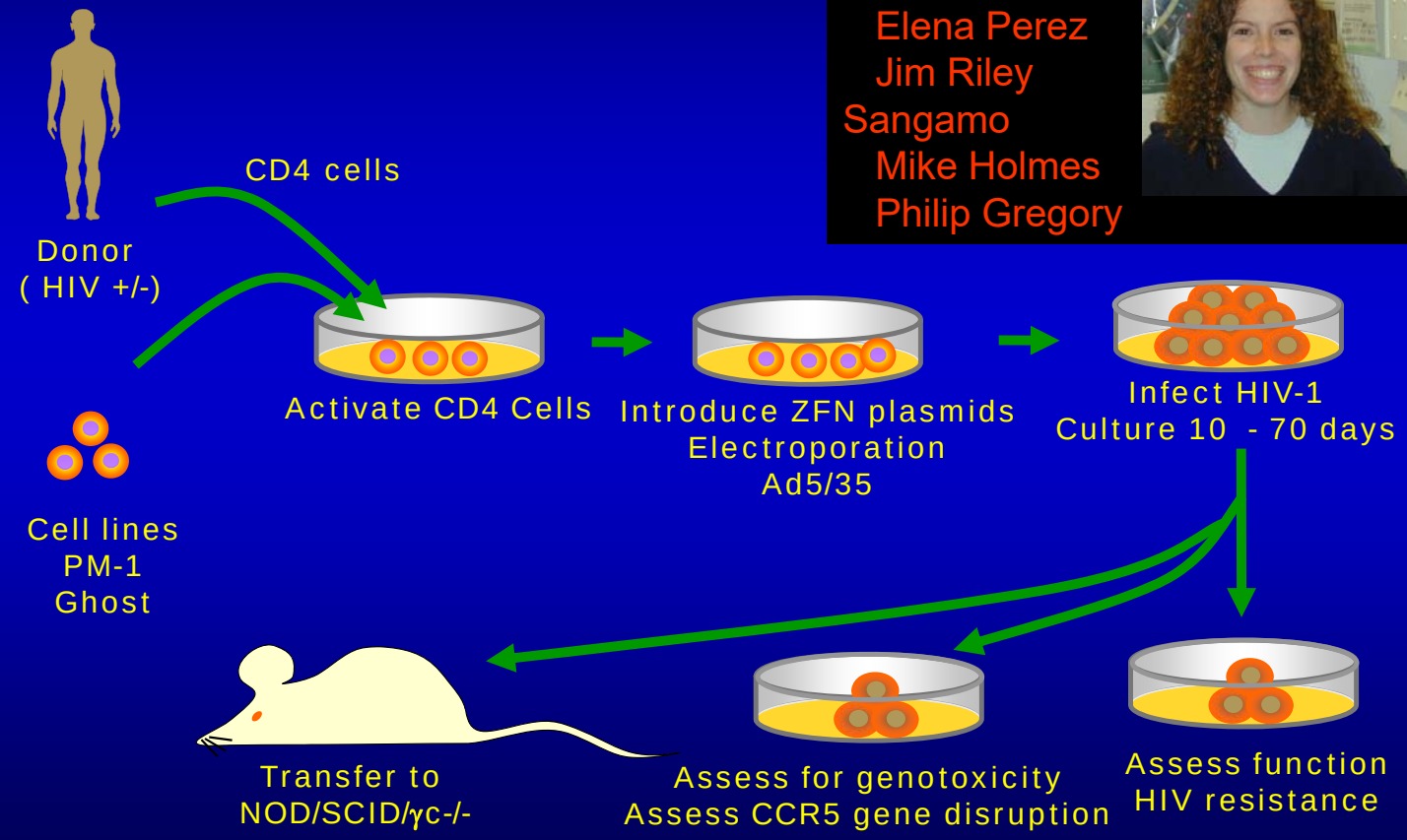
nature biotechnology

Establishment of HIV-1 resistance in CD4+ T cells by genome editing using zinc-finger nucleases

Elena E Perez[1,2], Jianbin Wang[3], Jeffrey C Miller[3], Yann Jouvenot[3,4], Kenneth A Kim[3], Olga Liu[1], Nathaniel Wang[3], Gary Lee[3], Victor V Bartsevich[3], Ya-Li Lee[3], Dmitry Y Guschin[3], Igor Rupniewski[3], Adam J Waite[3], Carmine Carpenito[1], Richard G Carroll[1], Jordan S Orange[2], Fyodor D Urnov[3], Edward J Rebar[3], Dale Ando[3], Philip D Gregory[3], James L Riley[1], Michael C Holmes[3] & Carl H June[1]

VOLUME 26 NUMBER 7 JULY 2008 NATURE BIOTECHNOLOGY

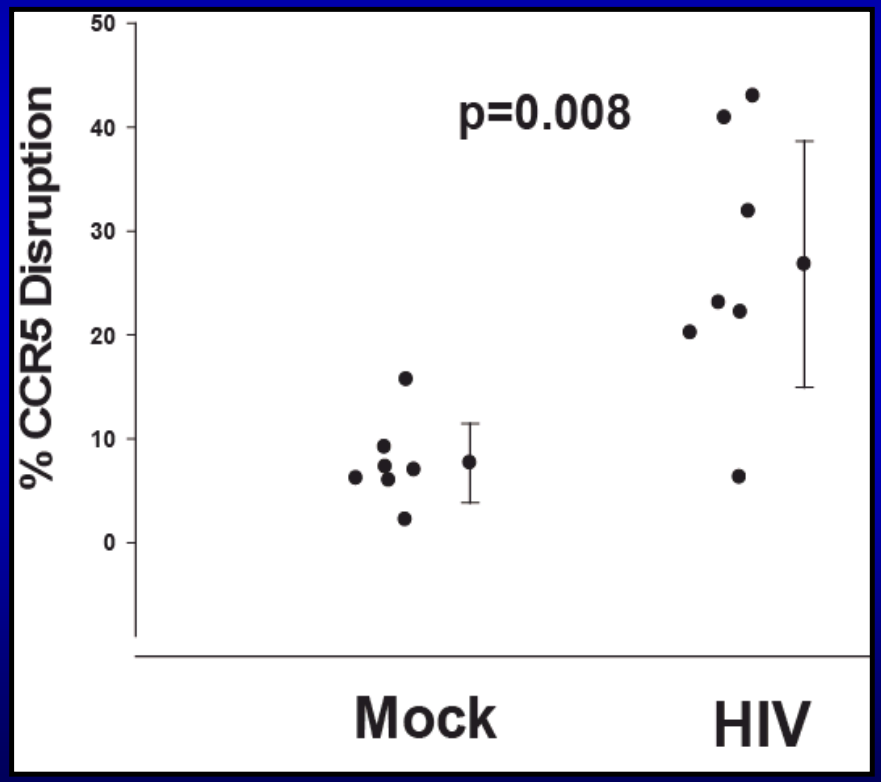
Approach to Pre-Clinical Testing of CCR5-ZFN



VOLUME 26 NUMBER 7 JULY 2008 NATURE BIOTECHNOLOGY

In Vivo Selection of CCR5-ZFN Modified Cells in NOD/SCID IL-2Rγ^{null} Mice

Primary CD4+ T Cells Isolated from Spleen Day 40 after HIV Challenge



HUMAN GENE THERAPY 24:245–258 (March 2013)

DOI: 10.1089/hum.2012.172

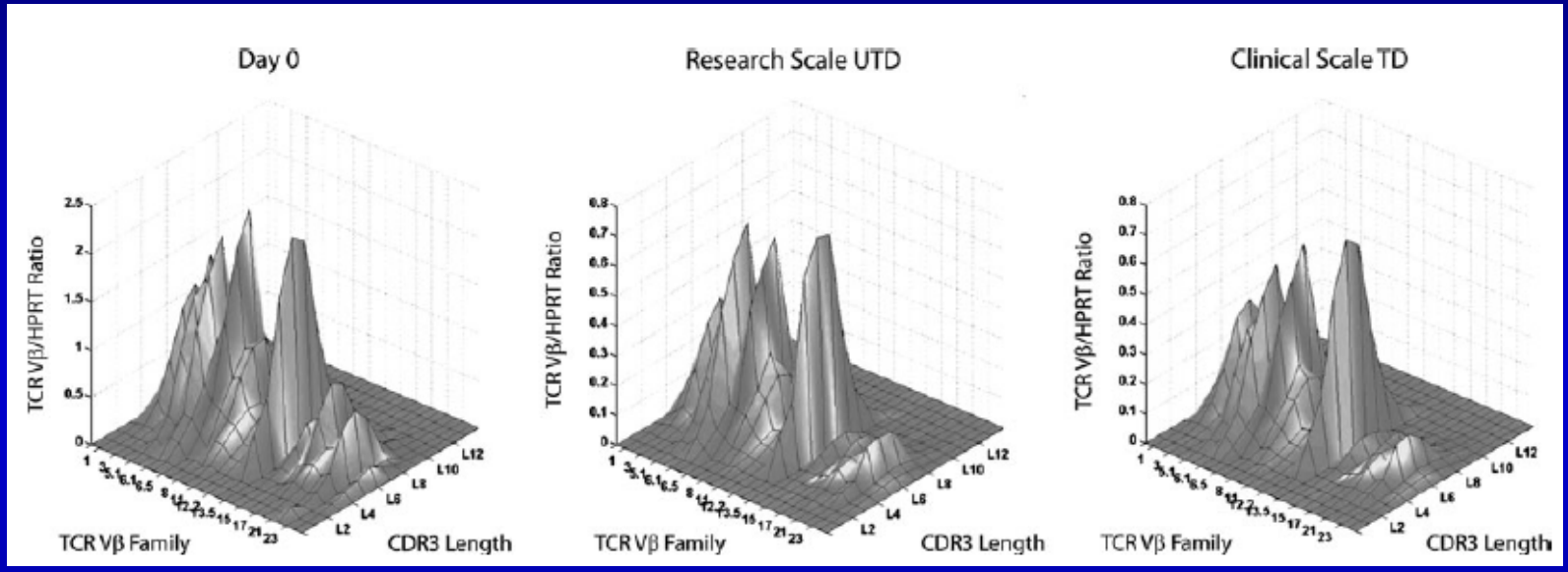
Research Articles

Efficient Clinical Scale Gene Modification via Zinc Finger Nuclease–Targeted Disruption of the HIV Co-receptor CCR5

Dawn A. Maier,[1,*] Andrea L. Brennan,[1,*] Shuguang Jiang,[1,*] Gwendolyn K. Binder-Scholl,[1,†] Gary Lee,[2] Gabriela Plesa,[1] Zhaohui Zheng,[1] Julio Cotte,[1] Carmine Carpenito,[1,‡] Travis Wood,[2] S. Kaye Spratt,[2] Dale Ando,[2] Philip Gregory,[2] Michael C. Holmes,[2] Elena. E. Perez,[1,§] James L. Riley,[3] Richard G. Carroll,[1,¶] Carl H. June,[1] and Bruce L. Levine[1]

Research scale to clinical scale
Gene delivery efficiency
Disruption
Off target
T cell function
In vivo pharm/tox

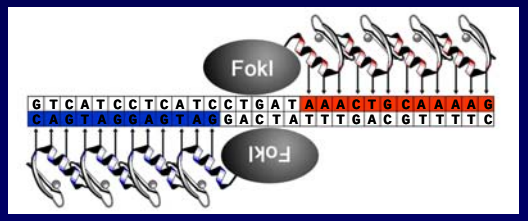
High Definition T Cell receptor Repertoire Following CCR5 ZFN gene disruption



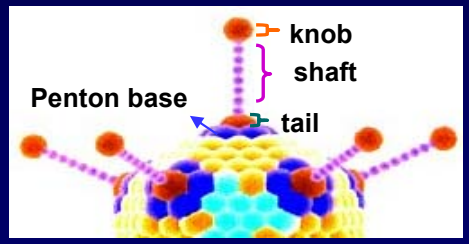
- No change compared to unmodified cultures
- Karyotyping also showed no differences
- Extensive animal studies show no increase in adverse events/deaths

Human Gene Therapy 24:245–258 (March 2013)

Ex Vivo CCR5 Genetic Modification of CD4+ T-cells Via Zinc Finger Nucleases in HIV+ Pts

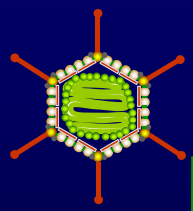


Ad5/F35 Chimeric Vector



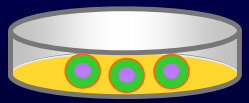
- Evaluated potential off target sites
- Cel-1 surveyor nuclease assay
- 53PB1 foci assay,
- karyotype analysis
- long term culture to assess for T cell transformation

SB-728



Pheresis

Enrich CD4+



Expand, Cryopreserve, Test cell product

Infuse

Follow Safety, CD4/HIV, CCR5 selection

Clinicaltrials.gov NCT00842634

First Use of Gene Edited Cells in Humans

July 27, 2009

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

Experience in Human Genome Editing

Pablo Tebas

The NEW ENGLAND JOURNAL of MEDICINE

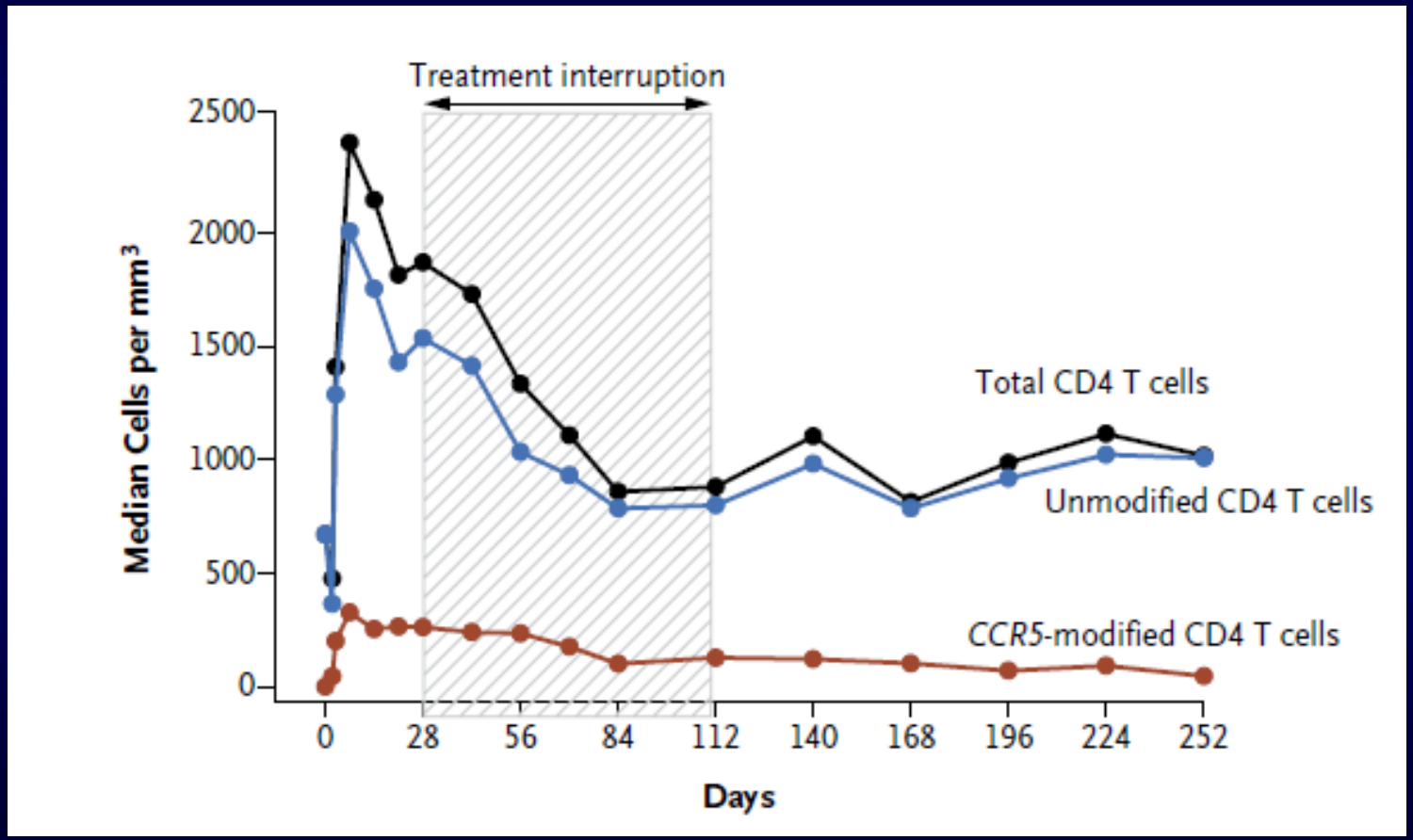
ESTABLISHED IN 1812 MARCH 6, 2014 VOL. 370 NO. 10

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

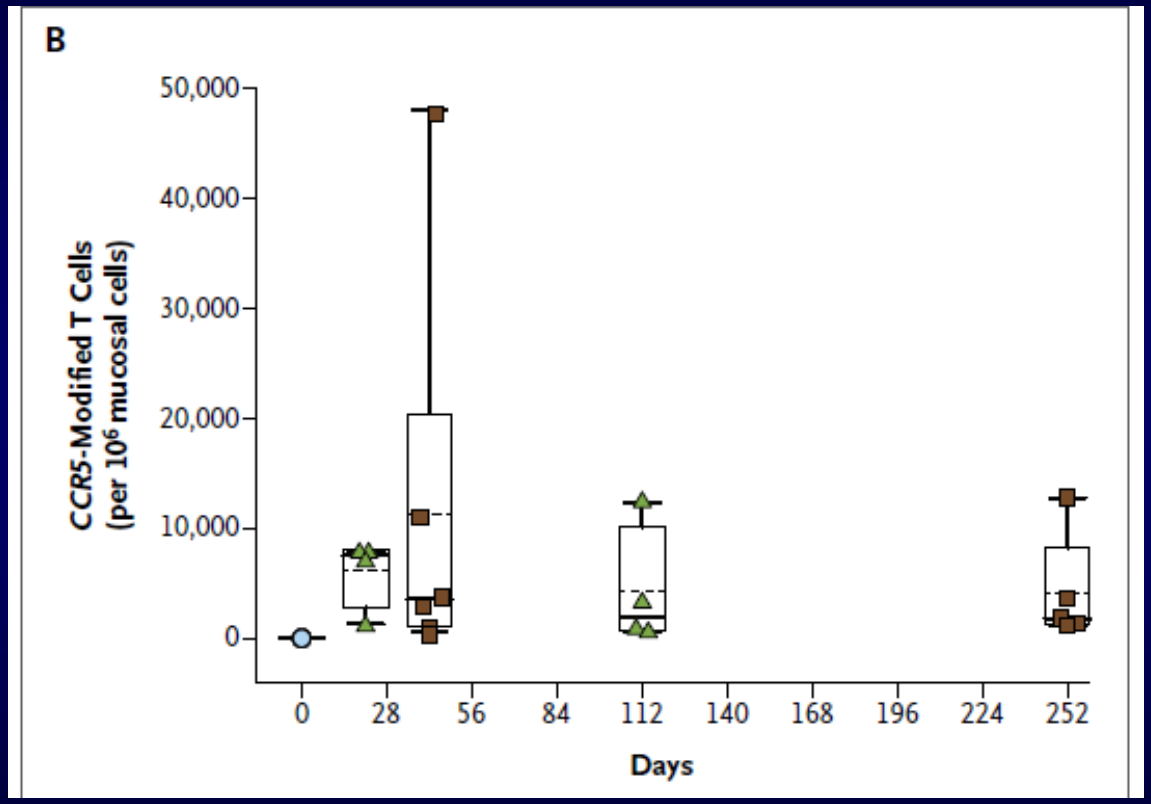
Pablo Tebas, M.D., David Stein, M.D., Winson W. Tang, M.D., Ian Frank, M.D., Shelley Q. Wang, M.D., Gary Lee, Ph.D., S. Kaye Spratt, Ph.D., Richard T. Surosky, Ph.D., Martin A. Giedlin, Ph.D., Geoff Nichol, M.D., Michael C. Holmes, Ph.D., Philip D. Gregory, Ph.D., Dale G. Ando, M.D., Michael Kalos, Ph.D., 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.

- **Phase I Study: NCT00842634**
- **Single infusion of CCR5 edited CD4 cells (5 - 10 x 10^9 cells)**
- **Translational time frame: Idea (2003) => Preclinical (2008) => Clinical (2014)**

CD4 Count Decay:
Unmodified vs Gene-Edited T Cells



CCR5-Modified T Cells Traffic to Rectal Mucosa



- **R5 gene disruption levels in rectal mucosal CD4s qualitatively tracks with the disruption levels in peripheral blood CD4s**

Genome Editing with ZFNs: Key Points

- Treatment with gene edited CD4 T-cells is well tolerated HIV subjects.
- Data supports proposed mechanism that CCR5 modification prevents HIV infection in CD4 T-cells.
- Durable increases in total CD4 T cells, normalization of CD4:CD8 ratio.
- CCR5-modified CD4 T cells detected in gut mucosa, demonstrating homing and persistence
- Delay/decrease in viral setpoint during drug treatment interruption in a subset of study subjects
- Survival advantage of CCR5-modified cells during ARV treatment interruption
- ZFN edited cells persist for >5 yrs. No gene editing related SAE's.

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Tebas, et al.. 2014. Gene Editing of CCR5 in Autologous CD4 T-cells of Persons Infected with HIV. *N Engl J Med 370:901-910.*

Clinical Development of Gene Editing

Part 2:
CRISPR 3X KO + Lentivector Transduction

NY-ESO-1–specific TCR–engineered T cells mediate sustained antigen-specific antitumor effects in myeloma

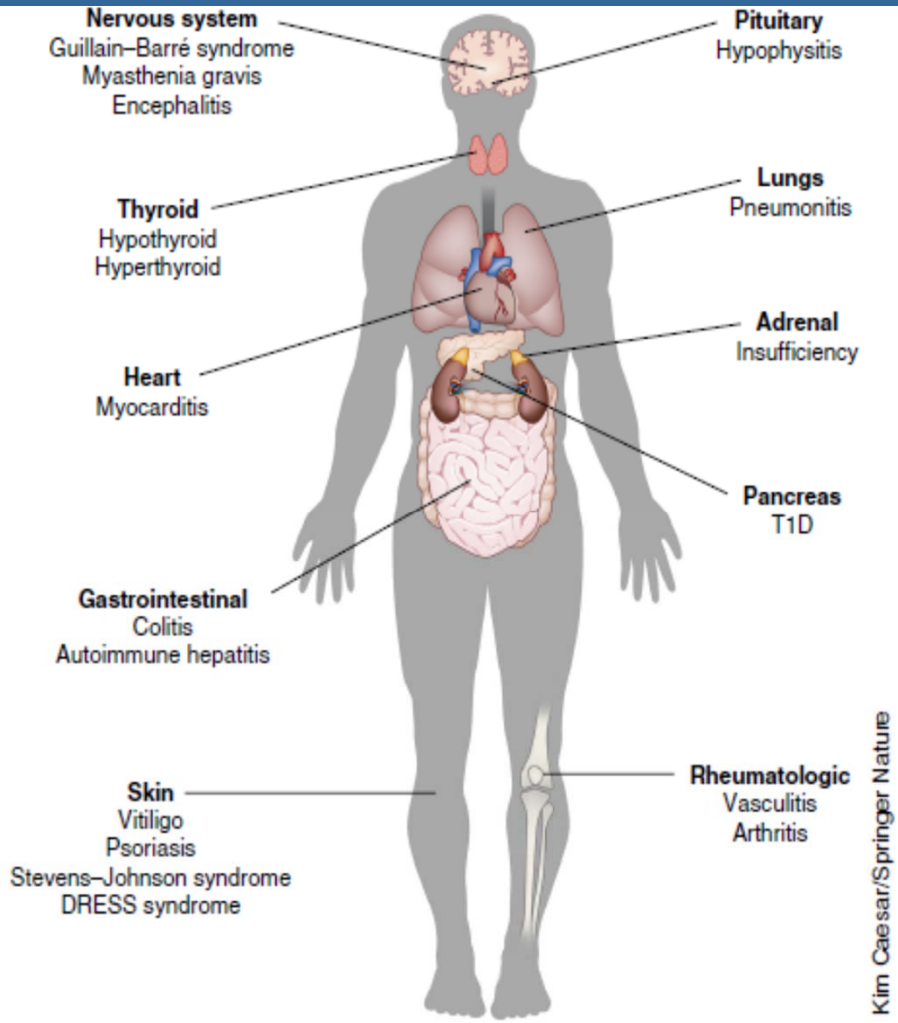
Encouraging clinical responses were observed in 16 of 20 patients (80%) with advanced disease, with a median progression-free survival of 19.1 months. NY-ESO-1–LAGE-1 TCR–engineered T cells were safe, trafficked to marrow and showed extended persistence that correlated with clinical activity against antigen-positive myeloma.

VOLUME 21 | NUMBER 8 | AUGUST 2015 NATURE MEDICINE

Evidence suggests that NY-ESO-1 T cells become exhausted

Autoimmunity – The Achilles Heel of Undirected Checkpoint Immunotherapy

Checkpoint blockade toxicity:
Spares no organ system



June, Warshauer, Bluestone

VOLUME 23 | NUMBER 5 | MAY 2017 NATURE MEDICINE

PHASE I TRIAL OF AUTOLOGOUS T CELLS ENGINEERED TO EXPRESS NY-ESO-1 TCR AND GENE EDITED TO ELIMINATE ENDOGENOUS TCR AND PD-1

Principal Investigator Overall and UPENN	*Edward Stadtmauer, M.D.* *Department of Medicine* *University of Pennsylvania School of Medicine* *Philadelphia, Pennsylvania 19104*
Principal Investigator UCSF	*Thomas Martin, MD* *Department of Medicine* *University of California, San Francisco*
Principal Investigator MDACC	*Cassian Yee, MD* *Department of Immunology* *MD Anderson Cancer Center*

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- **Rationale #1: long term goal is to generate checkpoint resistant T cells**
- **Rationale #2: deletion of endogenous TCR decrease risk of autoimmunity?**
- **First-ex-China evaluation of safety and feasibility of CRISPR/Cas9 technology**

NY-ESO-1 TCR CRISPR Triple Edited (TCRα TCRβ PD1) T Cell Study Objectives

Objectives	Primary: Determine safety profile of a single infusion of autologous t cells modified to express NY-ESO-1 transgenic TCR and gene edited at the endogenous TCR and PD-1 (CRISPR edited T cells) Secondary: 1. Describe anti-tumor responses and survival after infusion 2. Evaluate manufacturing feasibility 3. Determine engraftment, persistence, and trafficking of NY-ESO-1 redirected CRISPR cells 4. Evaluate bioactivity of NY-ESO-1 redirected CRISPR cells 5. Describe the incidence of immunogenicity

Adult patients HLA-A2*0201 positive who have relapsed/refractory tumors expressing NY-ESO-1 antigen. Patients with myeloma, synovial sarcoma, melanoma

What a Gene Editing Investigator May Be Asked

- Worst case scenario: induced off-target effects that induce transformation, and a form of hematologic malignancy
- FDA: Off target sites identification needs to be based on not only in silico analysis but also on unbiased assays.
- # gRNAs evaluated, detailed description of manufacture, # non-complementary bases tolerated, sequence of Cas9, purity, ratio of free vs complexed protein, stability, residual
- Laboratory test, validated laboratory test, CLIA test
- Long term culture assay
- Cell product potency

First-in-human Feasibility And Safety Of Multiplexed Genetic Engineering: Lentivector NY-ESO-1 TCR And CRISPR/Cas9 Gene Editing To Eliminate Endogenous TCR And PD-1

- 3 subjects treated
- Post-infusion products showed in vivo expansion, stable persistence
- Safety to date demonstrated
- Feasibility of multiplex gene editing and lentivector gene delivery

What Happens When Things Go
-Right
-Wrong
-Strange

What is the Appropriate Level of . . .

- Scientific expertise of developing center?
- Manufacturing and analytics expertise?
- Regulatory expertise of developing/manufacturing center?
- Clinical and monitoring expertise of medical center?
- Funding for patient lifetime(s) follow-up?
- Scientific expertise of regulatory center?
- Local/regional/national governmental monitoring & enforcement?

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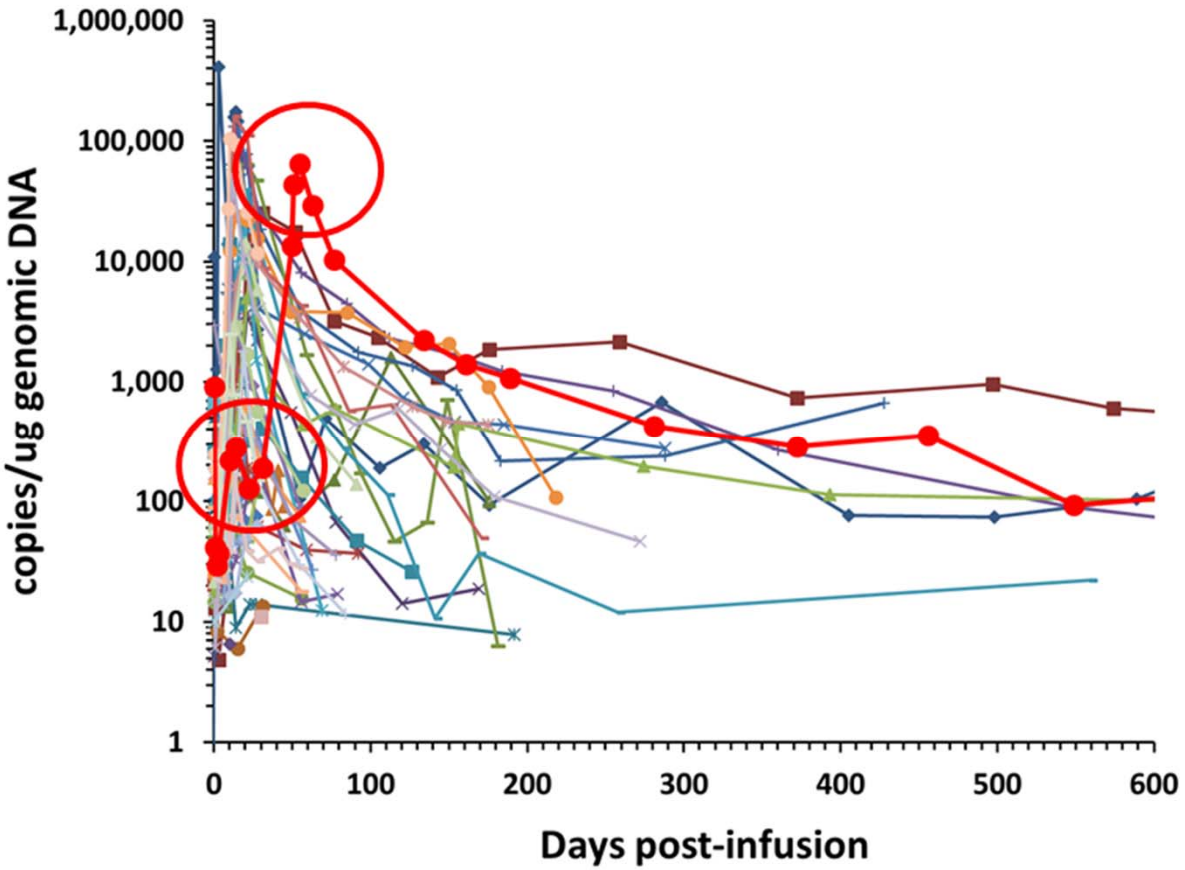
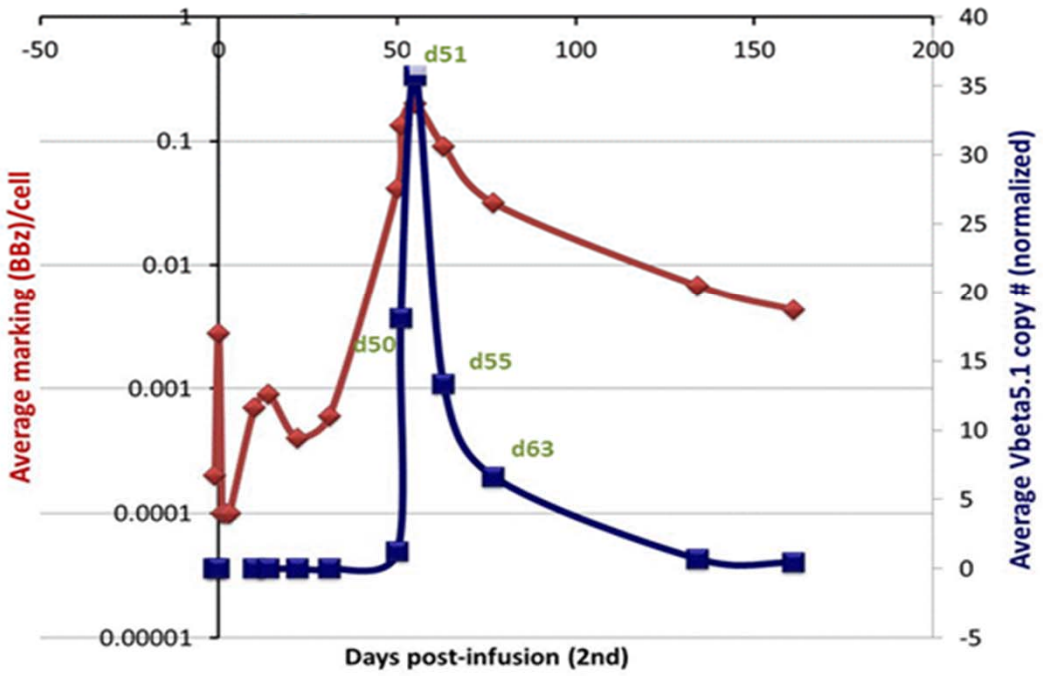
Lessons From Outliers

- CLL Patient #10: Delayed eradication of CLL

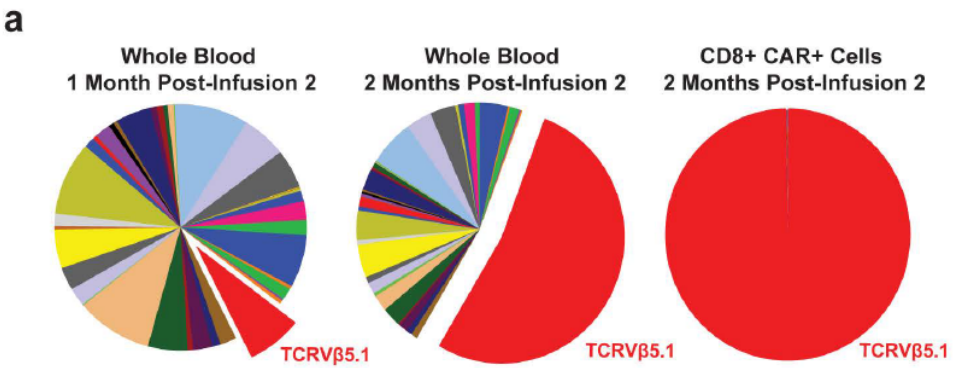


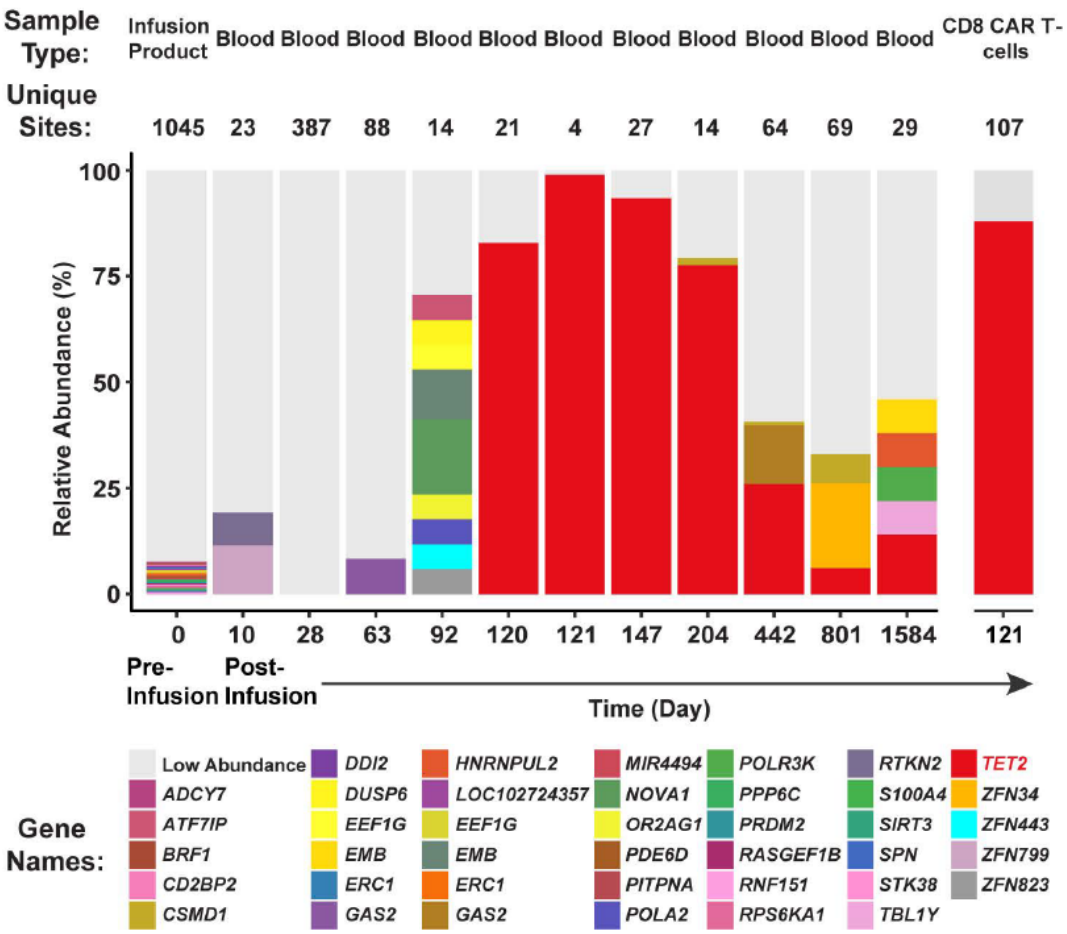
Rapid Expansion and Contraction of Clonal CTL019 Population



Peak expansion of TCR Vb5.1 correlates with CTL019 peak expansion

In Vivo Expansion of Clonal CTL019 Cell Population





LETTER

https://doi.org/10.1038/s41586-018-0178-z

Disruption of *TET2* promotes the therapeutic efficacy of CD19-targeted T cells

Joseph A. Fraietta[1,2,3,4], Christopher L. Nobles[5], Morgan A. Sammons[6,10], Stefan Lundh[1,2], Shannon A. Carty[2,11], Tyler J. Reich[1,2], Alexandria P. Cogdill[1,2], Jennifer J. D. Morrissette[3], Jamie E. DeNizio[7,8], Shantan Reddy[5], Young Hwang[5], Mercy Gohil[1,2], Irina Kulikovskaya[1,2], Farzana Nazimuddin[1,2], Minnal Gupta[1,2], Fang Chen[1,2], John K. Everett[5], Katherine A. Alexander[6], Enrique Lin-Shiao[6], Marvin H. Gee[9], Xiaojun Liu[1,2], Regina M. Young[1,2], David Ambrose[1,2], Yan Wang[1,2], Jun Xu[1,2], Martha S. Jordan[2,3], Katherine T. Marcucci[1,2], Bruce L. Levine[1,2,3], K. Christopher Garcia[9], Yangbing Zhao[1,2], Michael Kalos[1,2,3], David L. Porter[1,2,7], Rahul M. Kohli[5,7,8], Simon F. Lacey[1,2,3], Shelley L. Berger[6], Frederic D. Bushman[5], Carl H. June[1,2,3,4]* & J. Joseph Melenhorst[1,2,3,4]*

Accrual of Patient Safety Data

Example from Retroviral/Lentiviral Gene Transfer

Patient Safety Years of Genetically Modified T cells

University of Pennsylvania

March, 2018

Trial	Engineered T Cell	# Patients Infused	Safety (Patient-Years)	# Patients Alive (as of last date enrolled in study/LTFU)
Sangamo ZFN (HIV)	Ad5/35 zinc finger nuclease	12	74.4	12
CD4z CAR (HIV) includes CG trials	Retroviral CAR	44	783.6	44
SB-728mR CCR5 ZFN	CCR5 ZFN	11	10.9	11
MAZ-Takara (HIV)	Retroviral MazF	10	22.7	10
VirxSys VRX496 (HIV)	Lentiviral antisense HIVenv	20	204.1	20
Adaptimmune (HIV)	Lentiviral gag TCR	2	10.8	2
Adaptimmune Myeloma and Sarcoma	Lentiviral NY-ESO1 TCR	21	100.8	21
Penn/Novartis CART19/CTL019	Lentiviral 19:BBz CAR	311	467.3	214
EGFR	Lentiviral CART-EGFR	10	8.5	3
UPCC19214 CART-MESO-19	CART-MESO-19	3	3.2	2
UPCC31213	CART-MESO	15	10.2	2
UPCC31415	CART22	3	1.5	1
UPCC14415	CART-BCMA	14	10.9	10
Total		**476**	**1709**	**352**

Absence of Genotoxicity in T Cells with Retroviral and Lentiviral Vectors

Absence of Replication-Competent Retrovirus in Vectors, T Cell Products, and Patient Follow-Up Samples.
Lyon D, Lapteva N, Gee AP. Mol Ther. 2018 Jan 3;26(1):6-7.

Seek and You Will Not Find: Ending the Hunt for Replication-Competent Retroviruses during Human Gene Therapy.
Heslop HE, Brenner MK. Mol Ther. 2018 Jan 3;26(1):1-2.

Retroviral and Lentiviral Safety Analysis of Gene-Modified T Cell Products and Infused HIV and Oncology Patients.
Marcucci KT, Jadlowsky JK, Hwang WT, Suhoski-Davis M, Gonzalez VE, Kulikovskaya I, Gupta M, Lacey SF, Plesa G, Chew A, Melenhorst JJ, Levine BL, June CH. Mol Ther. 2018 Jan 3;26(1):269-279.

Absence of Replication-Competent Lentivirus in the Clinic: Analysis of Infused T Cell Products.
Cornetta K, Duffy L, Turtle CJ, Jensen M, Forman S, Binder-Scholl G, Fry T, Chew A, Maloney DG, June CH. Mol Ther. 2018 Jan 3;26(1):280-288.

☞ Revisit Test Requirements for Retro/Lenti-Transduced T Cell Products

FDA Efforts to Advance Development of Gene Therapies

Long Term Follow-up After Administration of Human Gene Therapy Products; Draft Guidance for Industry

Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up; Draft Guidance for Industry

July 11, 2018

Separating Hope from Hype

Tomorrow's treatments today—that's the promise of a growing number of companies offering cell therapies untested in rigorous clinical trials. Some experts say the claims must be challenged

Selling the Stem Cell Dream

IF YOU SUFFER FROM AN INCURABLE neurological disease such as multiple sclerosis (MS), Parkinson's, amyotrophic lateral sclerosis (ALS), or Huntington's disease, a clinic in the Netherlands says it may be able to help you. inject them. Almost all have Web sites to advertise the promise of the new therapies, often with hopeful case reports. The sites help recruit patients with what regular medicine cannot provide: a hope of recovery.

trying to find out more, too, although they say it can be impossible to get even basic facts about the treatments.

The result, Weissman fears, may be that stem cell research—already under criticism for its use

Threats

- Bad actors coopting new technology terms
- Insufficient enforcement, “ethics dumping”
- Public confusion on new technology
- Moore’s Law in Biology
- Regulations and Guidances written in large part for small molecules and biologics
- Drug Industry and many regulators educated in the era of small molecules and biologics

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THANK YOU

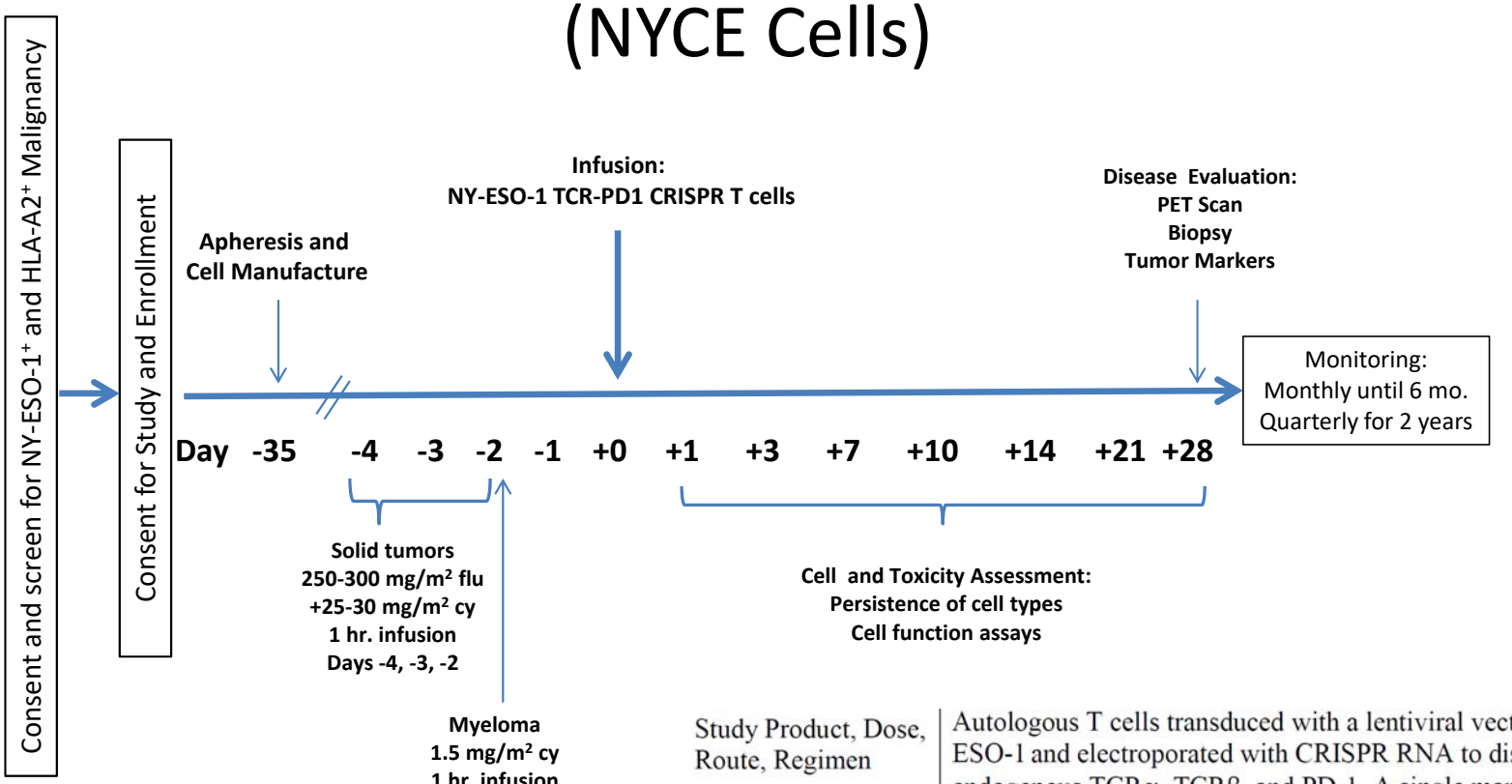
Penn Medicine
Center for Cellular Immunotherapies

UNIVERSITY OF PENNSYLVANIA
Abramson Cancer Center

Study Participants

FDA U.S. FOOD & DRUG ADMINISTRATION

NY-ESO-1 CRISPR (TCR-PD1) Triple Edited T Cell Study Schema (NYCE Cells)

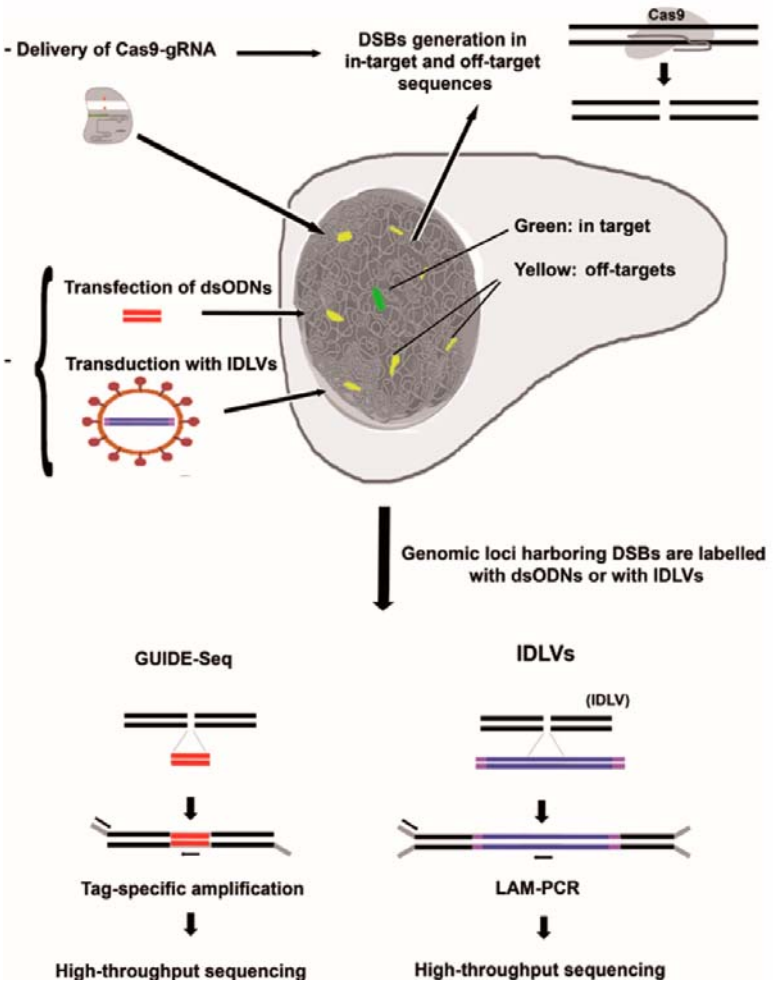


Study Product, Dose, Route, Regimen	Autologous T cells transduced with a lentiviral vector to express NY-ESO-1 and electroporated with CRISPR RNA to disrupt expression of endogenous TCRα, TCRβ, and PD-1. A single manufacturing site at UPENN will be used for all subjects enrolled into this study. A single dose of $1x10^8$cells/kg will be given i.v.

IND 17297 and Clinicaltrials.gov NCT03399448
Sponsor: Tmunity and Parker Institute for Cancer Immunotherapy

Assays to Detect Off-Target Editing

- Biased assays: shining the flashlight; "genome profiling"
  - ✓ In silico assays: ChipSeq and SELEX (Systemic Evolution of Ligands by EXponential Enrichment)
- Unbiased assays: genome wide; casts widest possible net
  - ✓ IDLV based assay: Gabriel et al. An unbiased genome-wide analysis of zinc-finger nuclease specificity. Nature biotechnology. 2011;29(9):816-23
  - ✓ Guide-seq, based on dsODN: Tsai et al. GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. *Nature biotechnology.* 2015;33(2):187-97.



Lessons Learned from Tet2 Disruption in CLL Patient #10

- Progeny derived from a **single** CTL019 TCRVβ5.1+ CD8+ T cell were responsible for the eradication of massive tumor burden in patient #10.
- Can the lowest effective dose of CAR T be a single cell?
- Unintentional knock out of Tet2 was responsible for enhanced CAR T function
- Since Tet2 can increase HSC stem cell renewal, would inhibition or intentional disruption of Tet2 increase CAR T cell proliferation and/or function?

Fraietta JA. Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells. *Nature.* 2018.

https://clinicaltrials.gov/ct2/show/NCT03164135

- Xu L, Yang H, Gao Y, et al. CRISPR/Cas9-Mediated CCR5 Ablation in Human Hematopoietic Stem/Progenitor Cells Confers HIV-1 Resistance In Vivo. Molecular Therapy 2017;25:1782-9.
- Safety of Transplantation of CRISPR CCR5 Modified CD34+ Cells in HIV-infected Subjects With Hematological Malignances
  - https://clinicaltrials.gov/ct2/show/NCT03164135

- NIH OBA RAC Meeting June 21, 2016
- Pre-clinical studies in vitro and in vivo
- Scale up
- Manufacturing, release criteria, for information assays
- Post-infusion sample analysis
- Clinical trial inclusion and exclusion criteria
- Patient monitoring and management
  - plan for potential toxicity
  - Plan for the unexpected observations: infrastructure must be in place