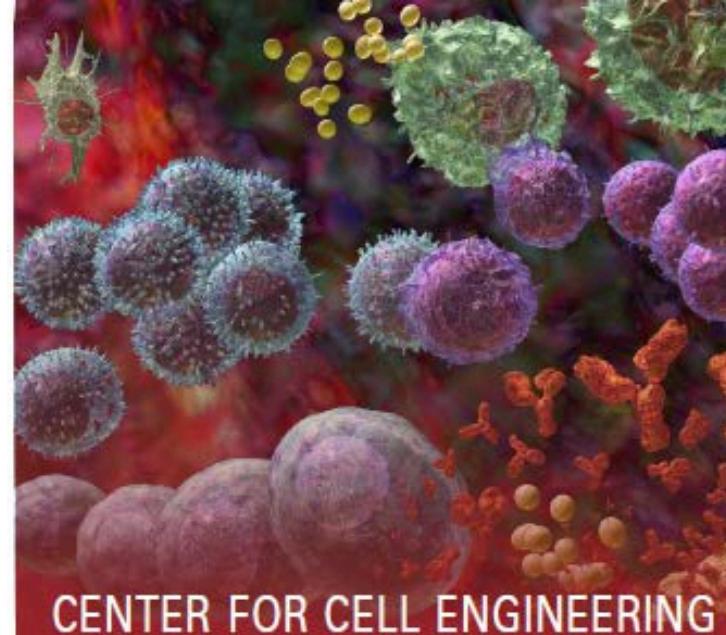


**The promise of T cell engineering  
CD19 CAR therapy  
A prologue to immune regenerative medicine  
Vast potential, patience, public education**

**Academies' Cell Therapy Workshop  
Washington, October 13, 2016**

Michel Sadelain, MD, PhD  
Director, Center for Cell Engineering  
Immunology Program, Sloan Kettering Institute  
Departments of Medicine and Pediatrics  
Memorial Sloan Kettering Cancer Center  
New York, NY



**CENTER FOR CELL ENGINEERING**

*Cell Engineering is part of the future to finding effective therapies to cure cancer and allied diseases*



**Memorial Sloan-Kettering  
Cancer Center**

# The rise of engineered T cells as cancer drugs

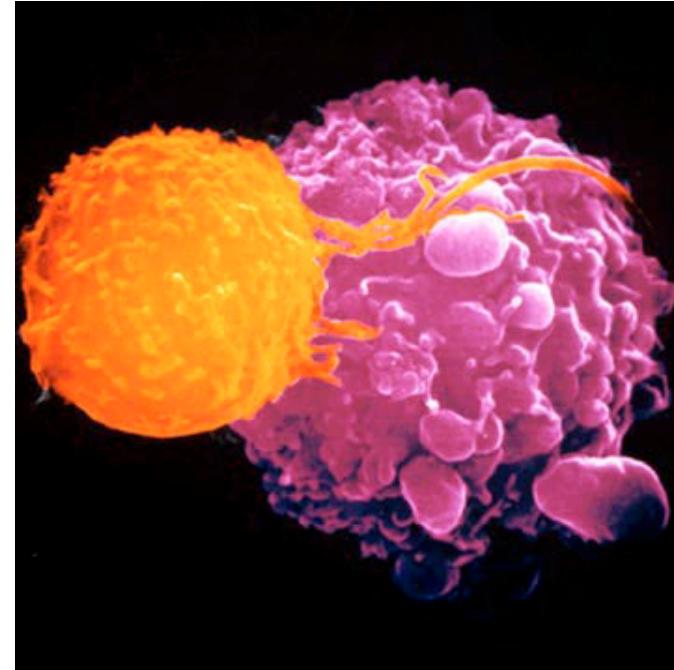
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- A major limitation of most existing cancer therapeutics is the lack of specificity or curative potential.
- Engineered T cells offer the prospect of combining specificity, potency and persistence from an optimized “living drug”.

- Safety
- Efficacy

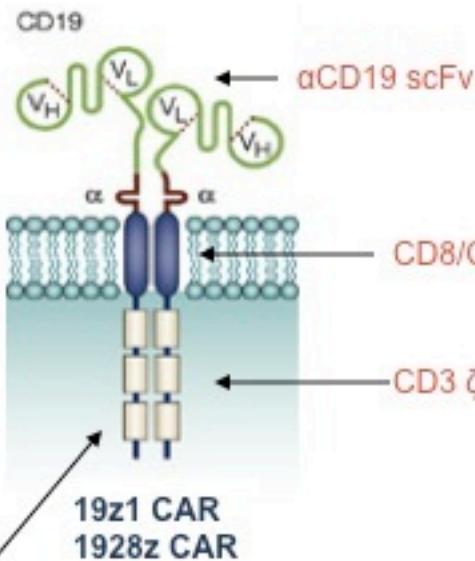
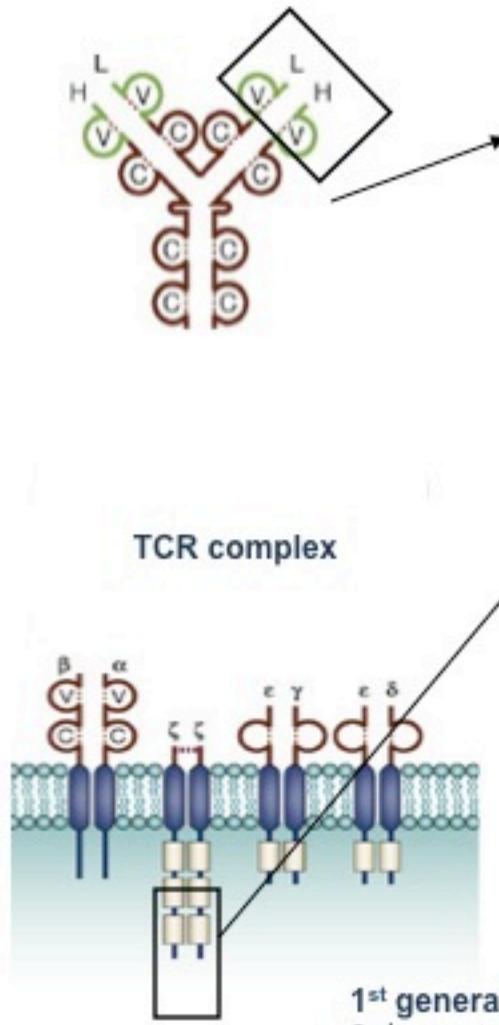
↔

- Potency
- Specificity
- Long-acting



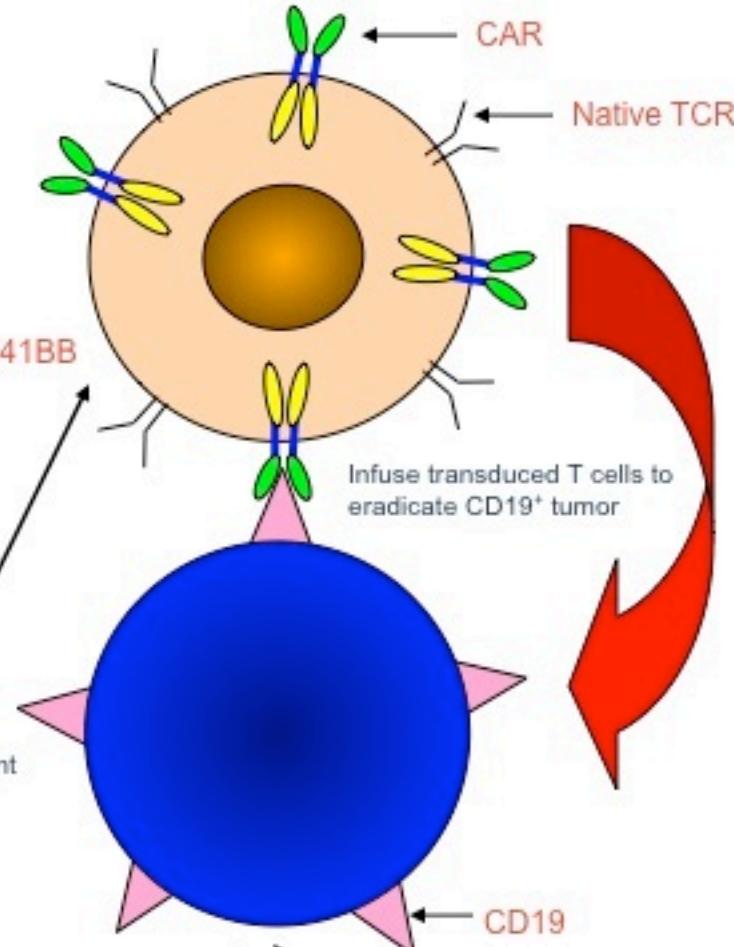
# CAR targeted T cells to increase anti-tumor functions

murine /human mAb



19z1 CAR  
1928z CAR

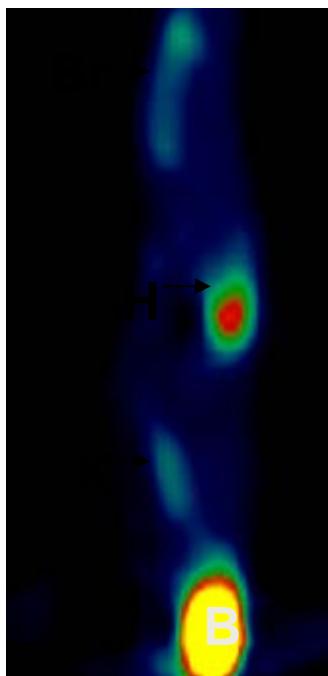
Transduce patient  
T cells *ex vivo*



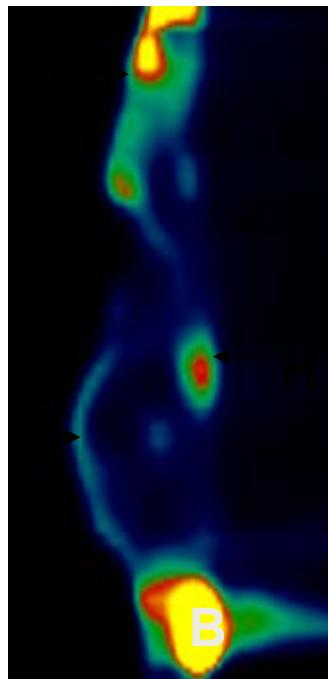
1<sup>st</sup> generation CAR: 19z1 (CD8)  
2<sup>nd</sup> generation CAR: 1928z (CD28) or 41BB, OX40  
3<sup>rd</sup> generation CAR: 2 costim. domains/CD3z

Maher, *Nat Biotech* 2002  
Brentjens, *Nat Med*, 2003  
Gade, *Cancer Res*, 2005  
Brentjens, *Clin Can Res* 2007  
Stephen, *Nat Med*, 2007  
Brentjens & Rivière, *Blood* 2011

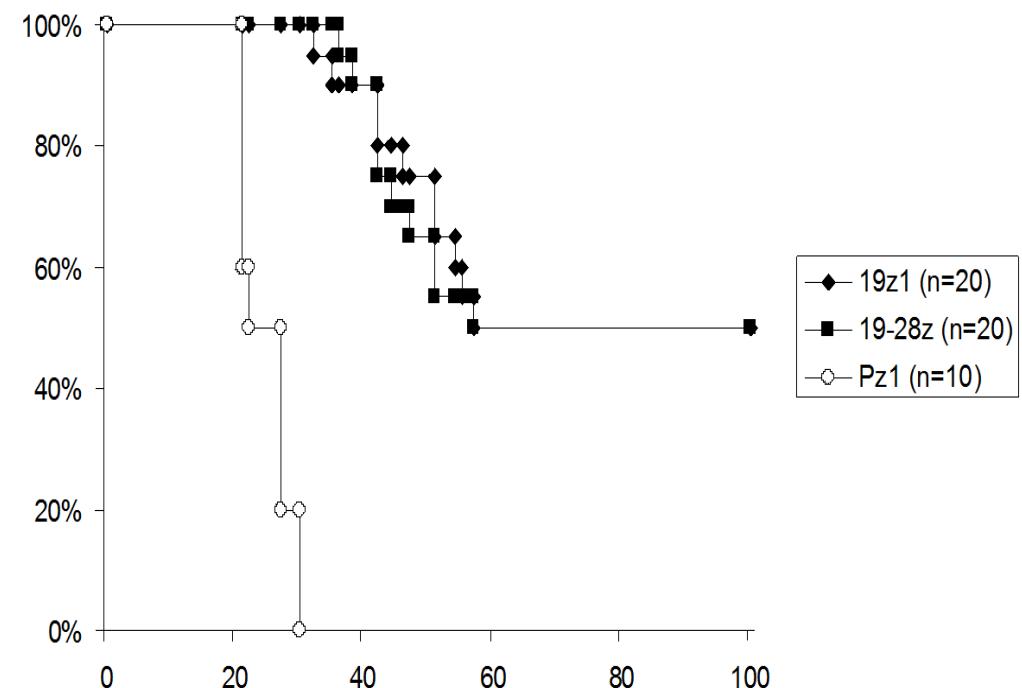
# Human CD19 CAR-targeted peripheral blood T cells eradicate systemic B lymphoma and ALL in mice



Tumor Free



Untreated  
4 weeks



Brentjens et al, *Nat Med*, 2003

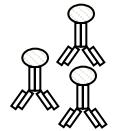
# CAR T cell Manufacturing Flow

Przybylowski et al, Gene Ther, 2006



Day 0  
Apheresis  
Thaw/Wash  
(Cell Saver5)

Incubation  
Dynabeads CD3/28



Apheresis  
Patient T cells



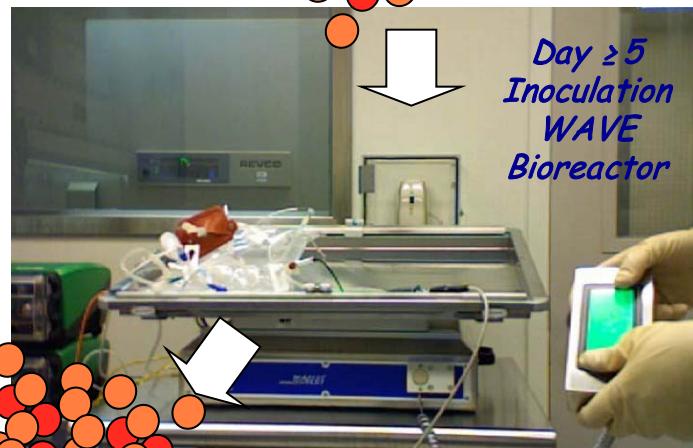
Selection  
ClinExVivo MPC

Day 3-4  
Transduction  
SFG-CAR  
Vector

CD3+ enriched  
activated  
T cells



CAR  
transduced  
T cells



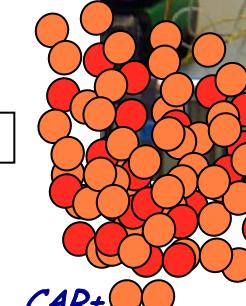
Day ≥ 5  
Inoculation  
WAVE  
Bioreactor



Wash  
(Cell Saver5)



DAY ≥ 10  
Debeading  
ClinExVivo MPC

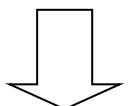


CAR+  
expanded T cells  
Isabelle Riviere  
Xiuyan Wang



Cryopreservation

Biosafety/  
QC release tests



Patient Infusion

Validation  
In vivo antitumor activity  
in SCID mice, CTL



Hollyman et al, J. Immunother, 2009

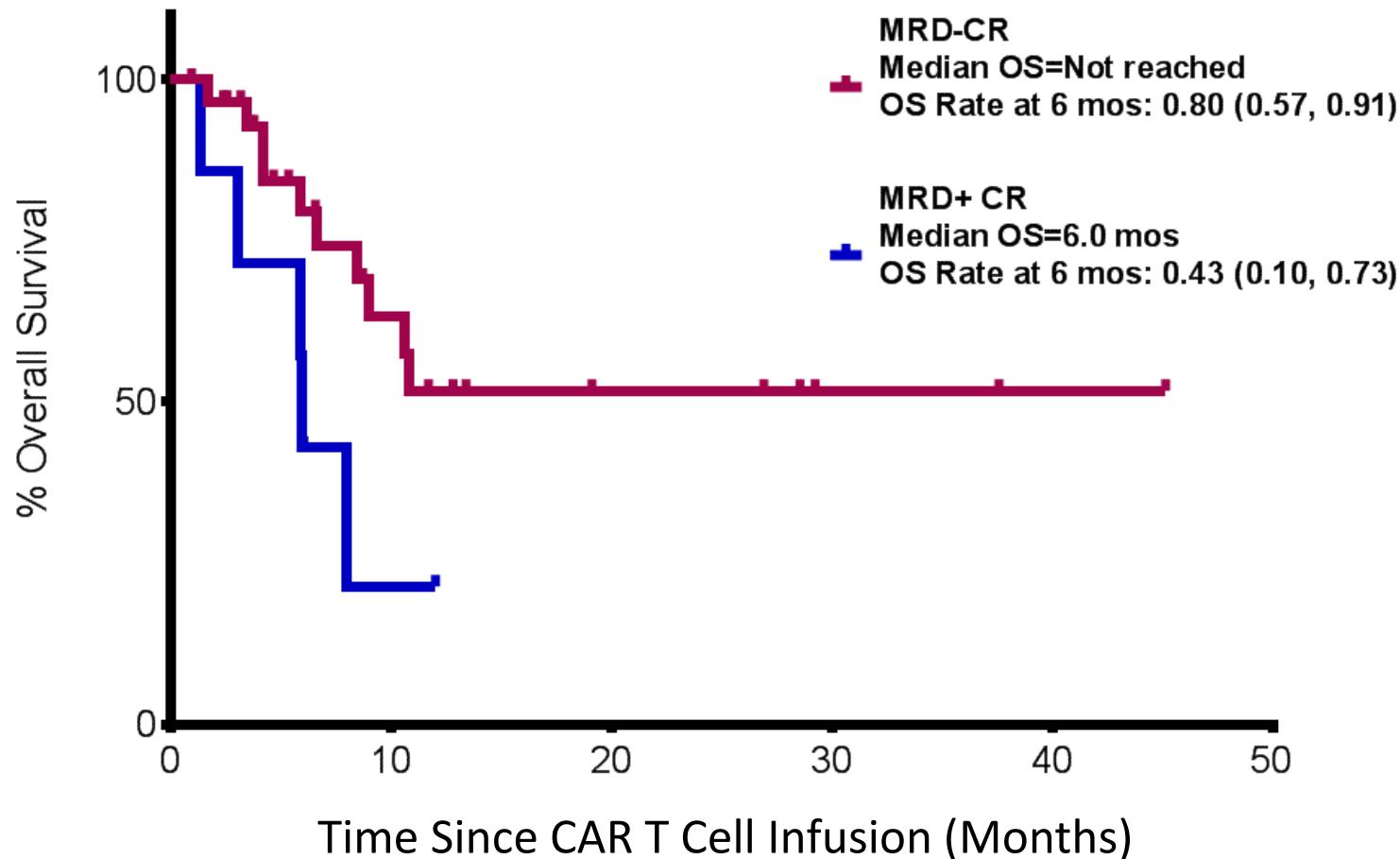
# Relapsed, chemo-refractory adult ALL

## Summary of Clinical Outcomes (MSKCC, ASH 2015)

	<b>Number of Patients N=45 (%) [95% CI]</b>
Overall CR Rate	37/45 (82%) [68 – 92]
Morphologic disease ( $\geq 5\%$ blasts)	18/24 (75%) [53 – 90]
Minimal disease ( $< 5\%$ blasts)	19/21 (91%) [70 – 99]
Overall MRD Negative CR Rate*	30/36 (83%)
Mean Time to CR (SD)	22 days (9.4)

\*Assessed among those patients who achieved CR and evaluable for MRD analysis (n=36)

# Overall Survival By MRD Status After CAR T Cell Treatment



20 December 2013 | \$10

# Science

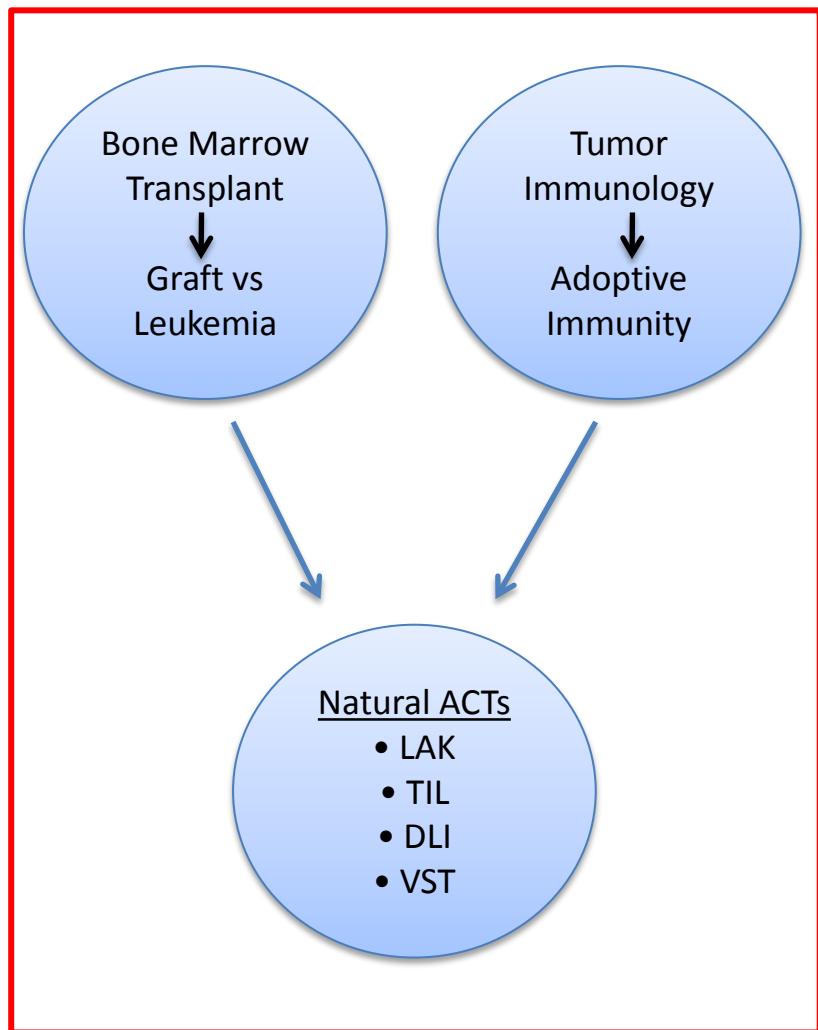
Breakthrough of the Year  
**Cancer  
Immunotherapy**

T cells on the attack

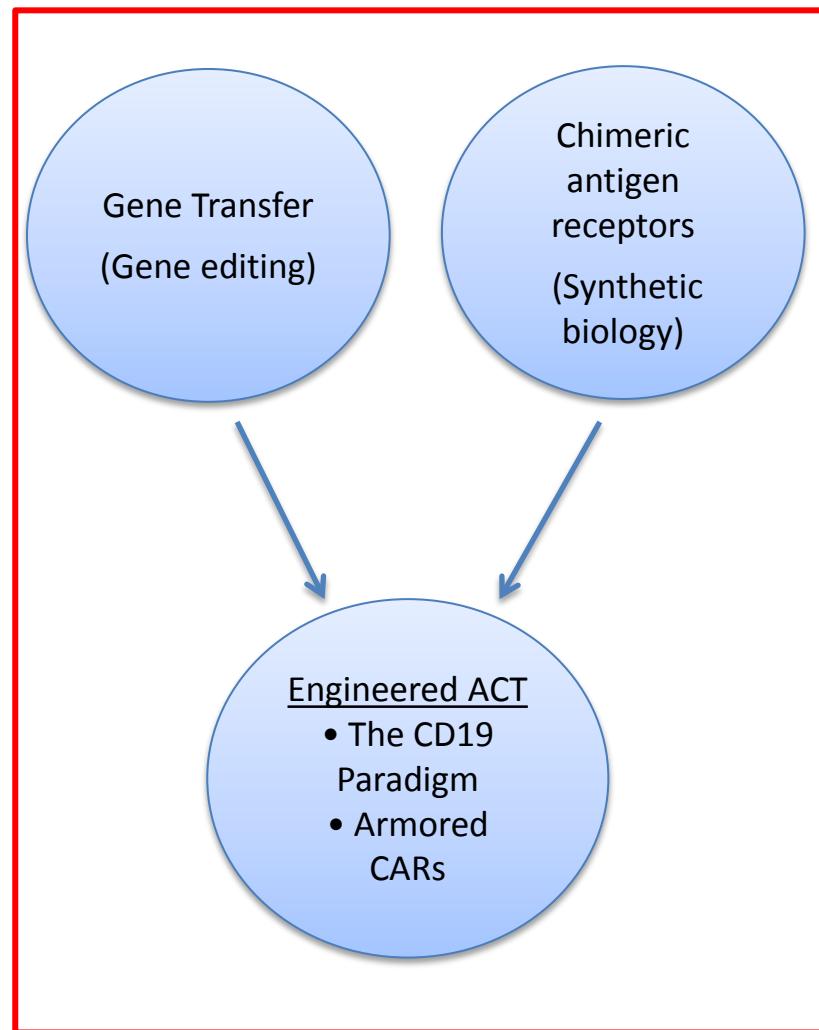


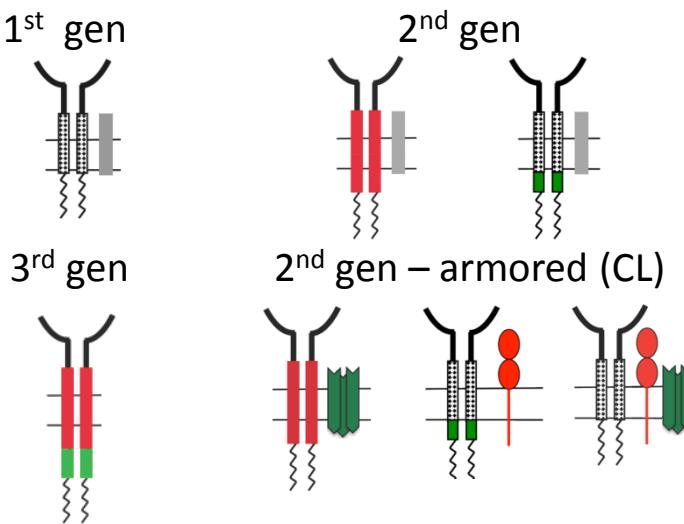
AAAS

## Define and Isolate Natural T Cells



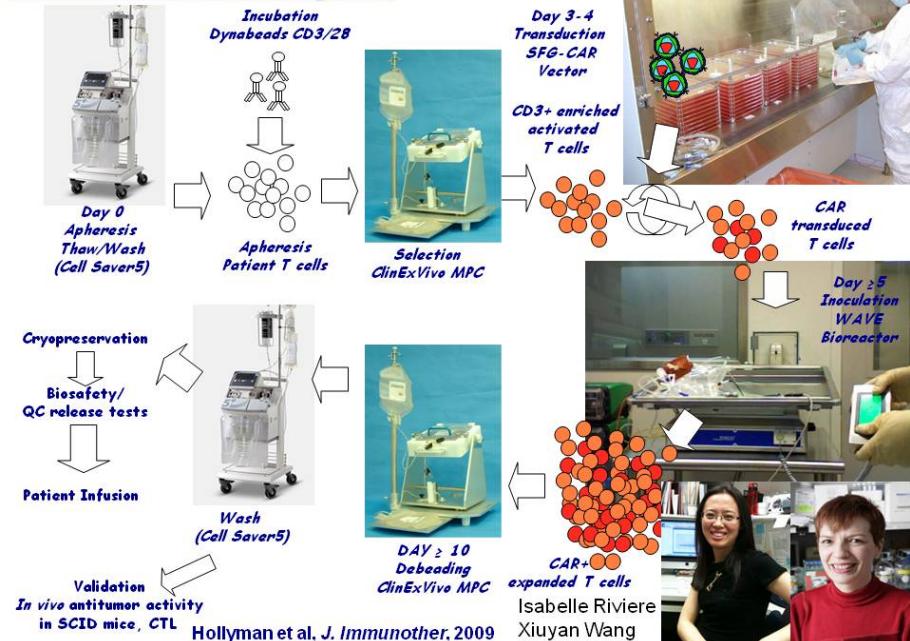
## Design and Manufacture T Cells





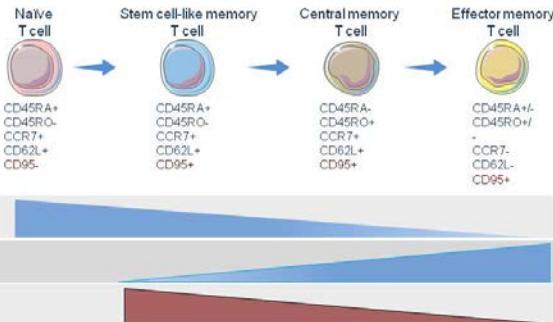
- 2<sup>nd</sup> gen CARs
- CAR + X (armored CARs)

### CAR T cell Manufacturing Flow



### Adoptive transfer of T cells for cancer immunotherapy

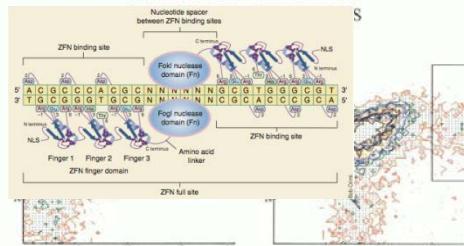
#### - Differentiation of memory T cells:



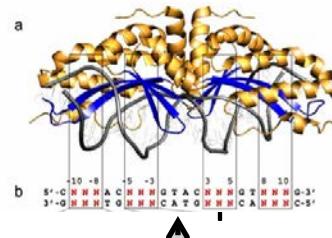
(Adapted from Restifo NP et al., *Nat Rev Immunol*, 2012)

- CD4/CD8 ratio (defined composition)
- T cell subset (eg TCM)

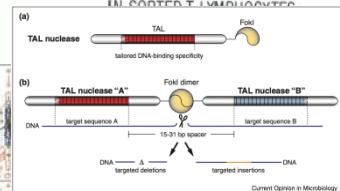
### Zinc-finger nuclease (ZFN)



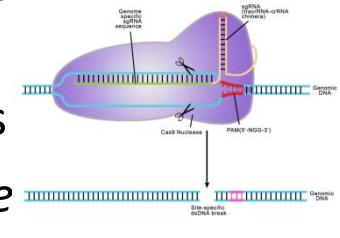
### Meganuclease



### TALE nuclease (TALEN)

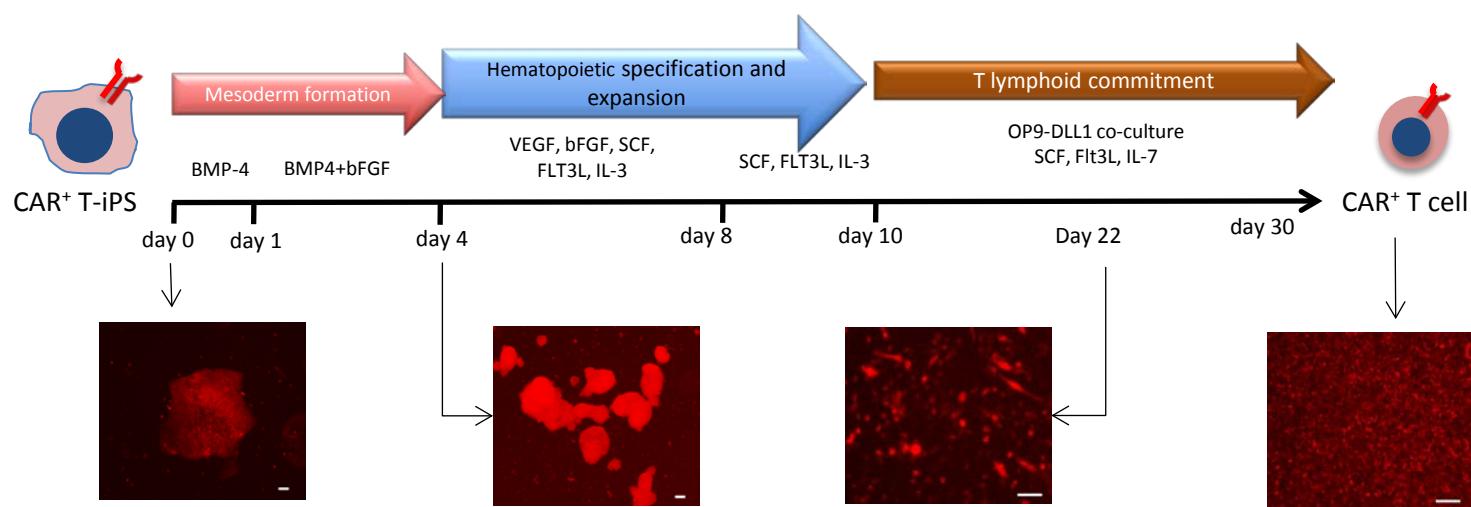
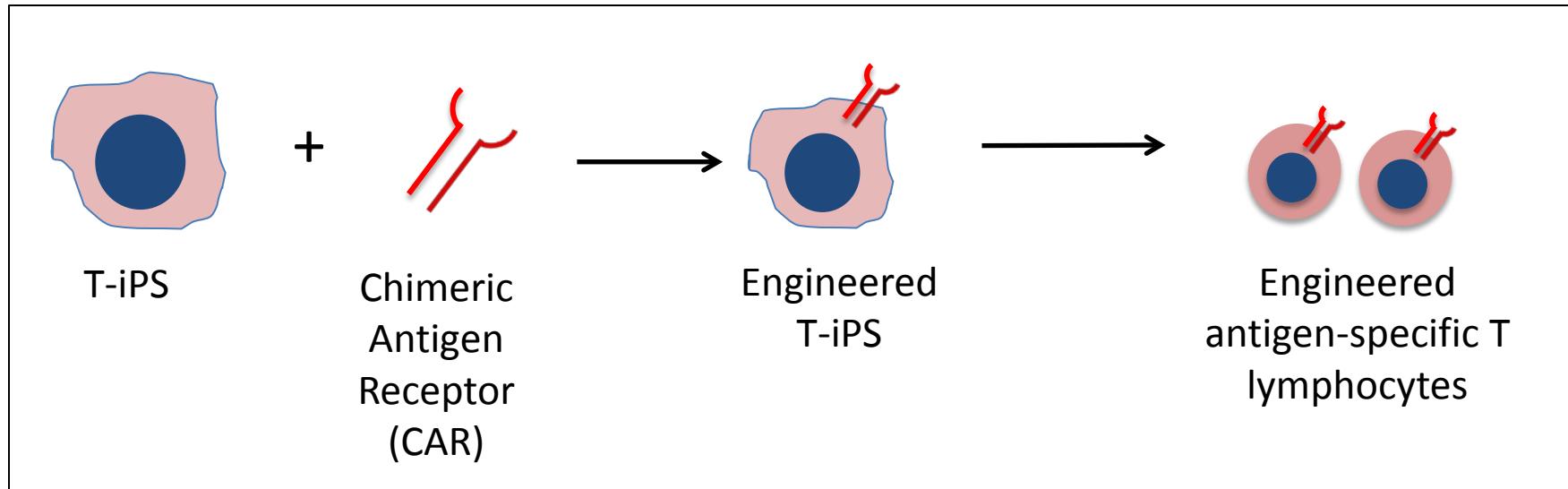


### CRISPR-Cas9

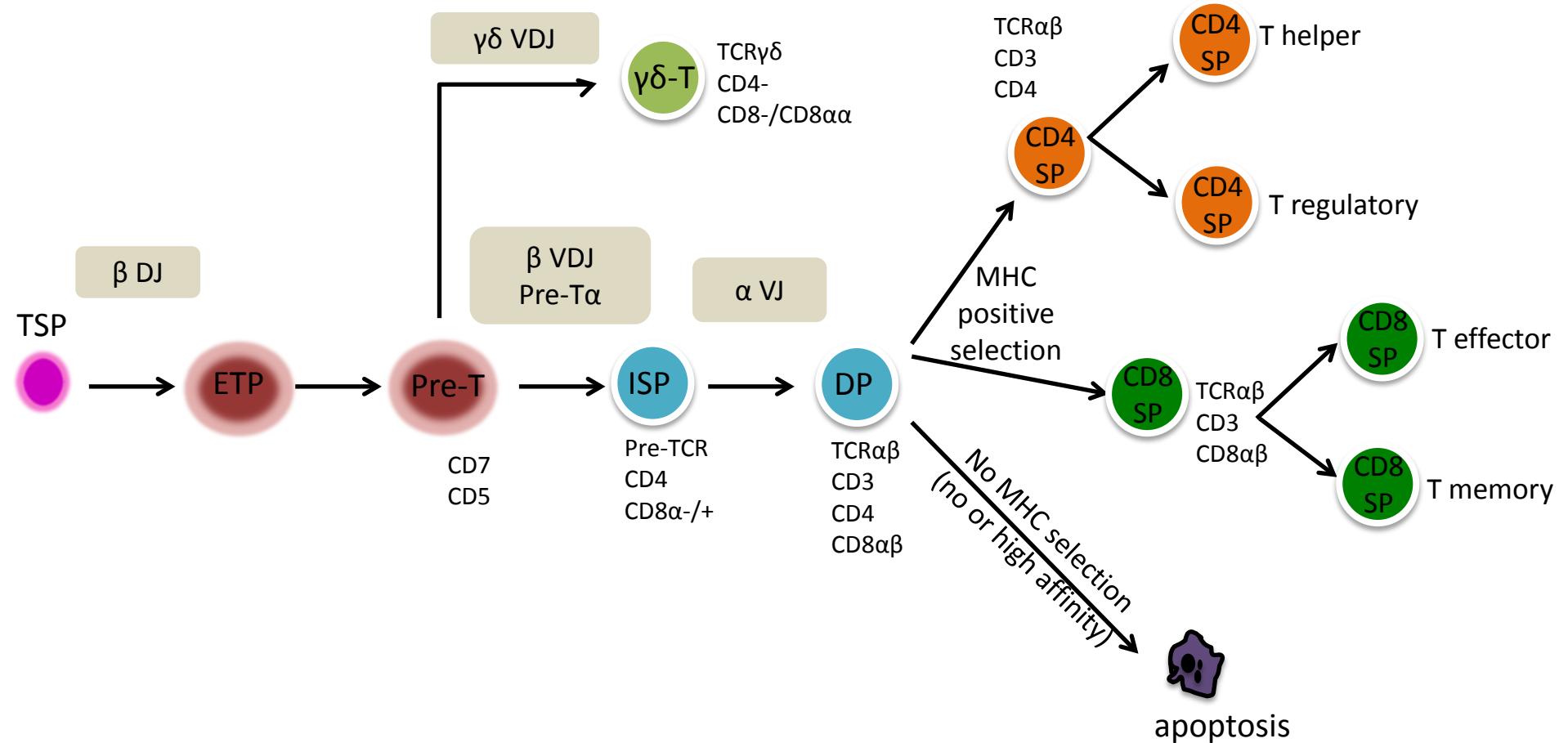


ctors  
(Slee

# T-iPS-derived CAR-targeted T cells

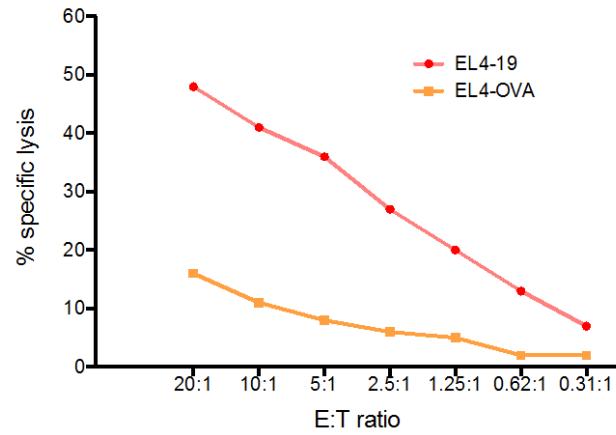


# How T cells are made (in the thymus)

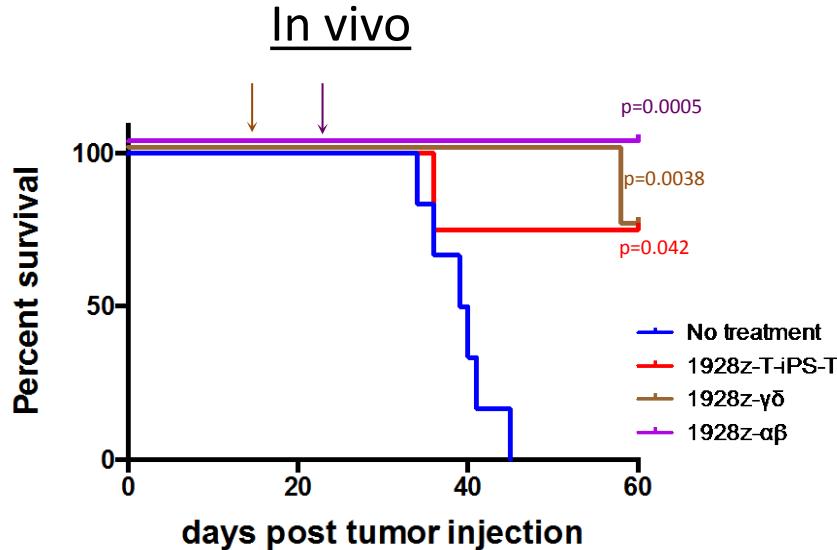


# 1928z-T-iPS-T cells lyse CD19+ tumor cells in vitro and in vivo

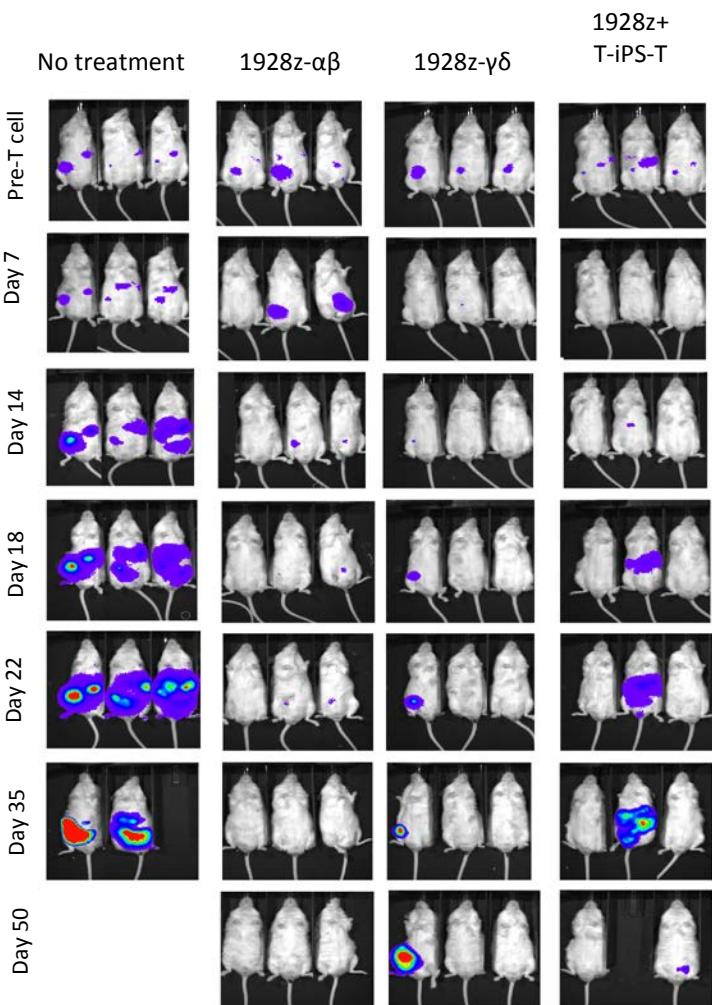
## In vitro



## In vivo

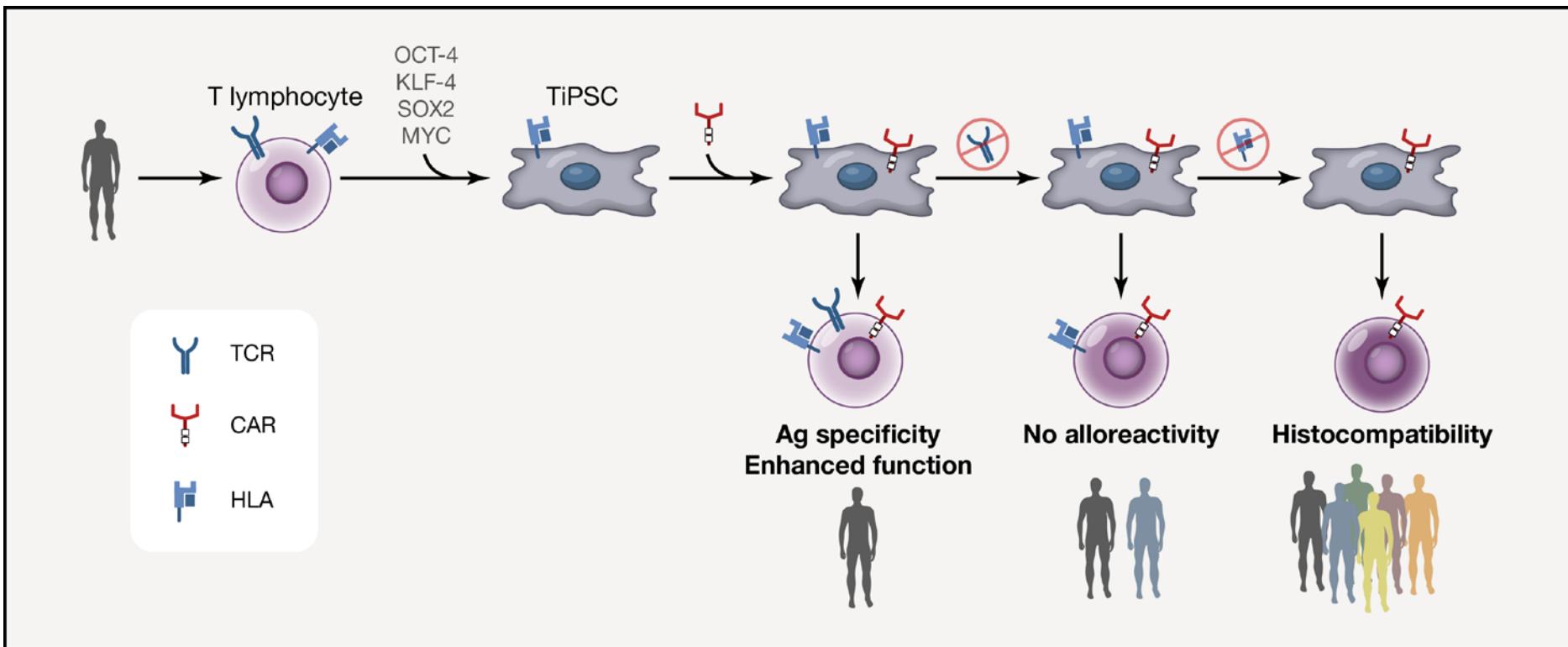


Themeli, *Nature Biotechnology*, 2013

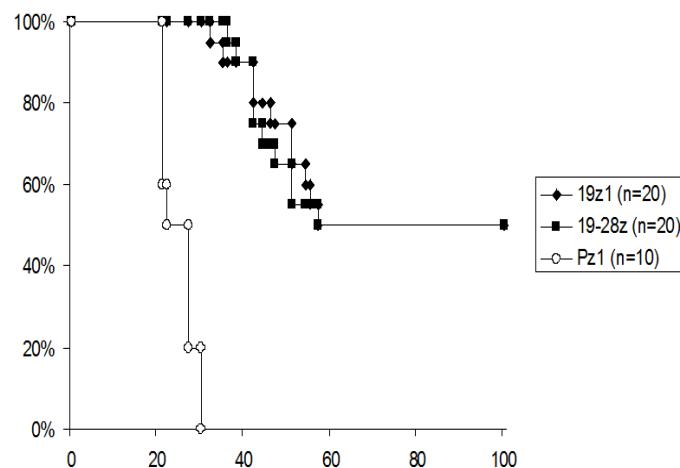


CD19+ Raji-FFLuc i.p.

# A perspective for large-scale generation of potent histocompatible therapeutic T cells



# From idea to proof-of-concept to clinical benefit CD19 CAR therapy 1992-2013



Mouse T cell Engineering  
Sadelain and Mulligan, 1992



CD19 CAR therapy POC  
Brentjens et al, 2003



Clinical trials  
FDA Breakthrough design., 2014

# Cell Therapy and Cell Engineering Facility

Isabelle Rivière, PhD, Director

- Non-profit; Grants, philanthropy, foundations
- Current GMP Facility: 6 cleanrooms
- New GMP facility opening 2014: 13 cleanrooms
- Cellular products (**CAR T cells, HSCs, DCs, NK cells, cord blood, hES/DA neurons, T-iPS**),
- RNA vectors, plasmid DNA, CRISPR/Cas9
- Phase I/II clinical trials



## CENTER FOR CELL ENGINEERING

*Cell Engineering is part of the future to finding effective therapies to cure cancer and allied diseases*



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