Adoptive transfer of T cells genetically modified using the *Sleeping Beauty* system

Laurence J.N. Cooper

Adoptive Transfer session

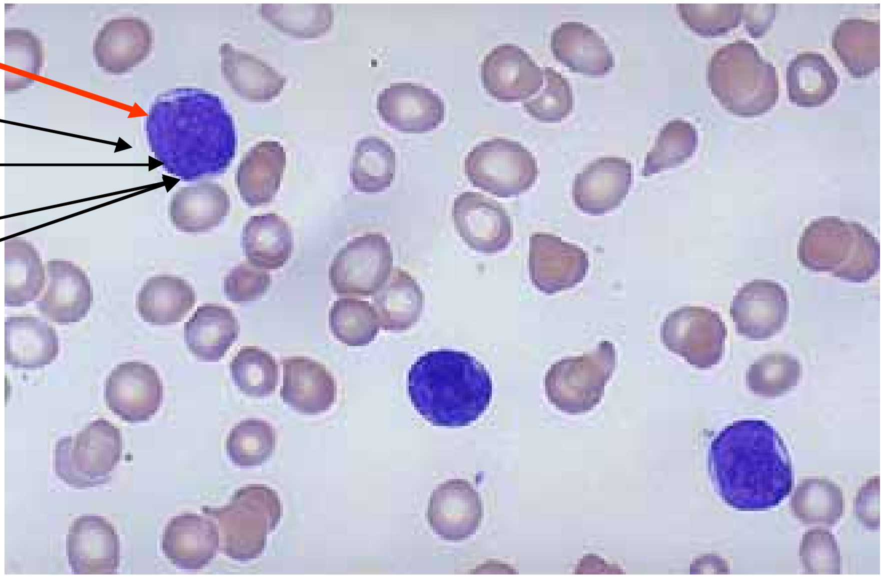
Saturday, October 31st

9:45 AM to 10:15 AM

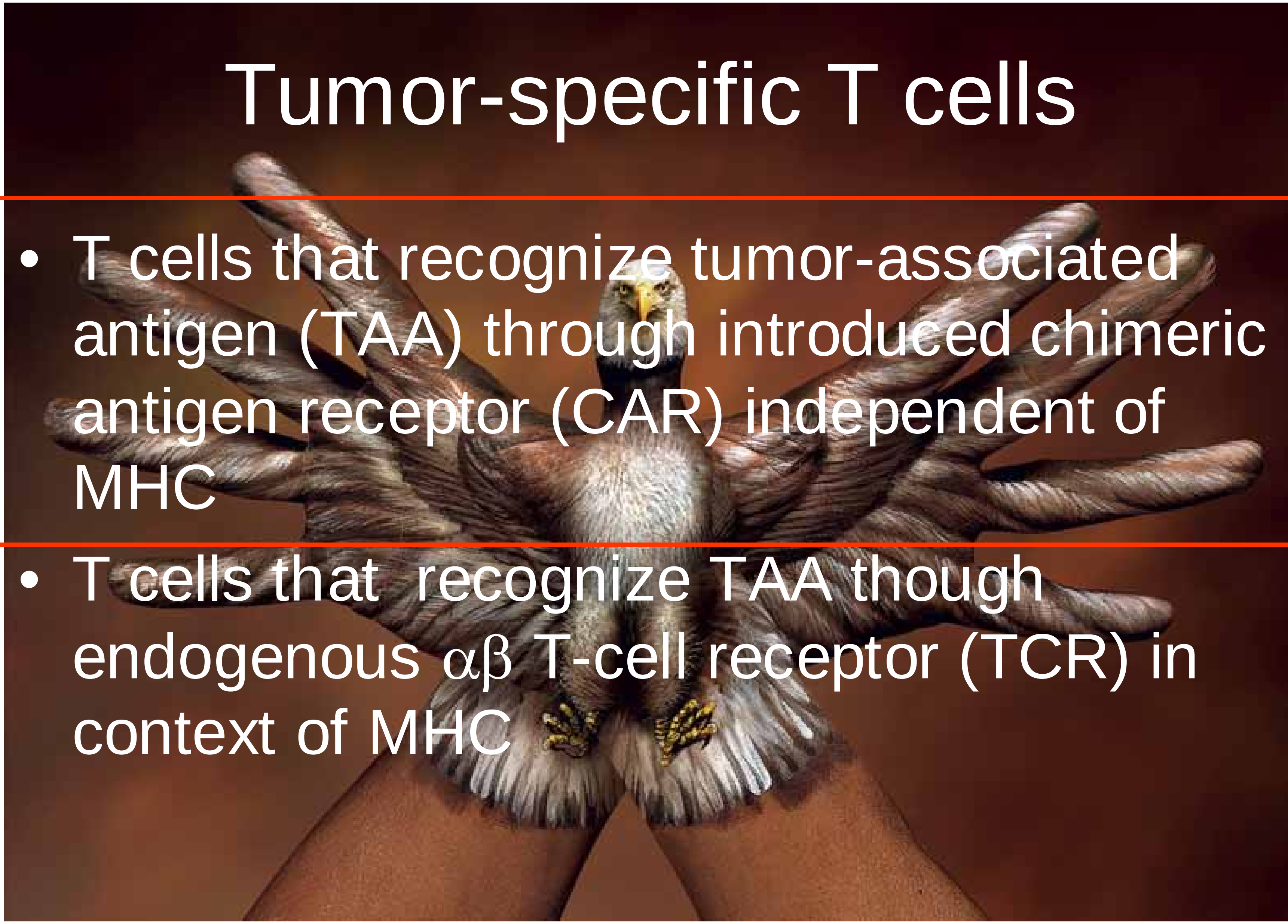
24th iSBTc

Immunotherapy options for B-lineage (CD19$^+$) ALL and lymphoma

- T-cell therapy
- NK-cell therapy
- Antibody therapy
- Immunocytokines
- Vaccination



Tumor-specific T cells



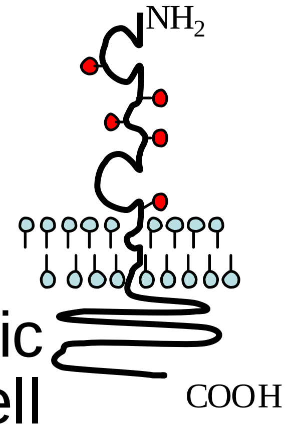
- T cells that recognize tumor-associated antigen (TAA) through introduced chimeric antigen receptor (CAR) independent of MHC
- T cells that recognize TAA though endogenous $\alpha\beta$ T-cell receptor (TCR) in context of MHC

Clinical studies using CAR^{+} T cells

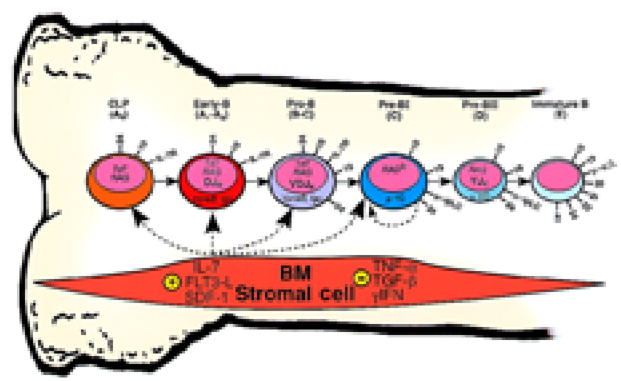
Target	CAR specificity	Major toxicity	References
HIV	CD4	None	Walker RE, Bechtel CM, Natarajan V, et al. Long-term in vivo survival of receptor-modified syngeneic T cells in patients with human immunodeficiency virus infection. Blood. 2000;96:467-474.
HIV	CD4	None	Mitsuyasu RT, Anton PA, Deeks SG, et al. Prolonged survival and tissue trafficking following adoptive transfer of CD4zeta gene-modified autologous CD4(+) and CD8(+) T cells in human immunodeficiency virus-infected subjects. Blood. 2000;96:785-793.
Renal cell cancer	Carbonic anhydrase IX	Liver	Lamers CH, Sleijfer S, Vulto AG, et al. Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. J Clin Oncol. 2006;24:220-222.
Ovarian cancer	α-folate receptor	None	Kershaw MH, Westwood JA, Parker LL, et al. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. Clin Cancer Res. 2006;12:6106-6115.
Neuroblastoma	CE7R	None	Park JR, DiGiusto DL, Slovak M, et al. Adoptive transfer of chimeric antigen receptor re-directed cytolytic T lymphocyte clones in patients with neuroblastoma. Mol Ther. 2007;15:825-833.
Lymphoma	CD19	None	Jensen MC, Popplewell L, DiGiusto DL, et al. A First-in-Human Clinical Trial of Adoptive Therapy Using CD19-Specific Chimeric Antigen Receptor Re-Directed T Cells for Recurrent/Refractory Follicular Lymphoma ASGT Seattle 2007. [abstract]
CLL	CD19	None	Brentjens RJ, Hollyman D, Weiss M, et al. A phase I trial for the treatment of purine analog-refractory chronic lymphocytic leukemia using autologous T cells genetically targeted to the B cell specific antigen CD19. 2008 ASCO Annual Meeting Proceedings. [abstract]
Lymphoma	CD20	None	Till BG, Jensen MC, Wang J, et al. Adoptive immunotherapy for indolent non-Hodgkin lymphoma and mantle cell lymphoma using genetically modified autologous CD20-specific T cells. Blood. 2008;112:2261-22671.
Neuroblastoma	GD_2	None	Pule MA, Savoldo B, Myers GD, et al. Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. Nat Med. 2008;14:1264-1270.
Glioblastoma	IL-13 receptor	None	Yaghoubi SS, Jensen MC, Satyamurthy N, et al. Noninvasive detection of therapeutic cytolytic T cells with (18)F-FHBG PET in a patient with glioma. Nat Clin Pract Oncol. 2008 Nov 18. [Epub ahead of print]

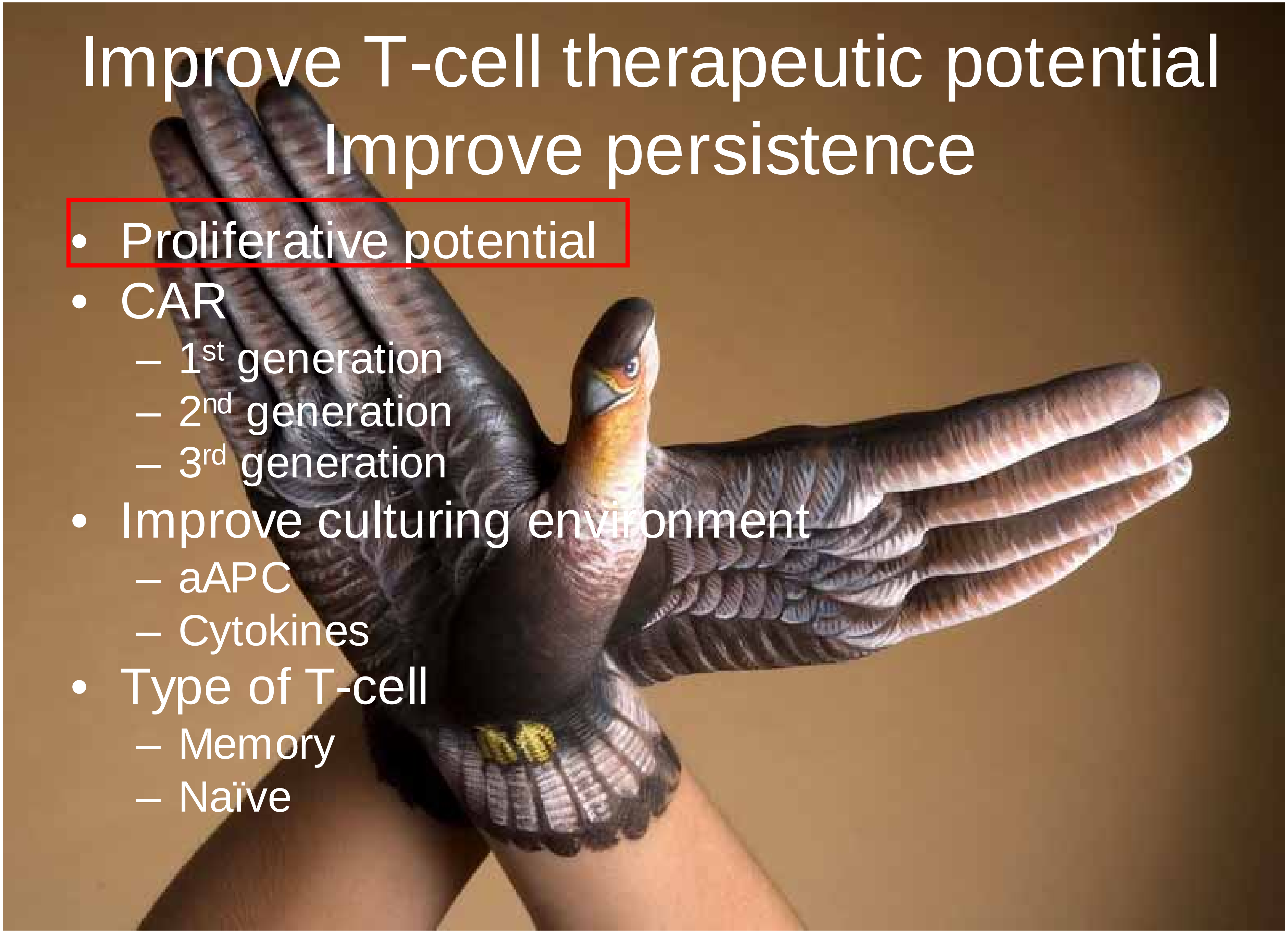
Rationale

Targeting CD19 determinant on B cells



- CD19 antigen is a 95 kDa B lineage-specific membrane glycoprotein, found on >95% of B-cell lymphomas and B-ALL cells;
- CD19 is rarely lost during the process of neoplastic transformation, but disappears upon differentiation to mature plasma cells;
- CD19 is not expressed on hematopoietic stem cells, nor on normal tissues outside the B lineage;
- CD19 is not shed into the circulation.





Improve T-cell therapeutic potential
Improve persistence

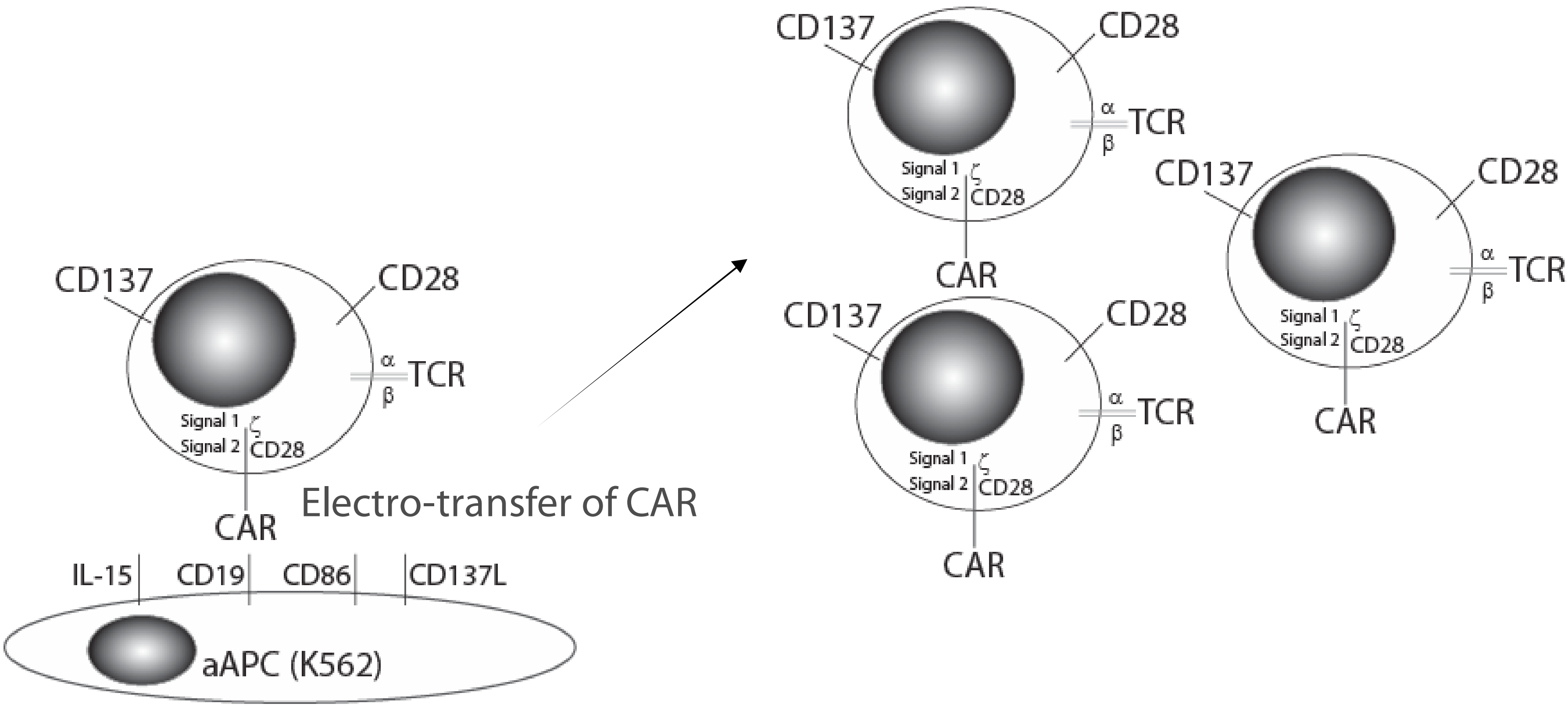
- Proliferative potential
- CAR
  - 1st generation
  - 2nd generation
  - 3rd generation
- Improve culturing environment
  - aAPC
  - Cytokines
- Type of T-cell
  - Memory
  - Naïve

Most clinically-effective T-cell therapies includes *ex vivo* antigen-dependent proliferation

- Therefore we developed culture systems *ex vivo* that select for T cells that can sustain CAR-dependent proliferation *in vivo*

Experimental design
Programming the μenvironment

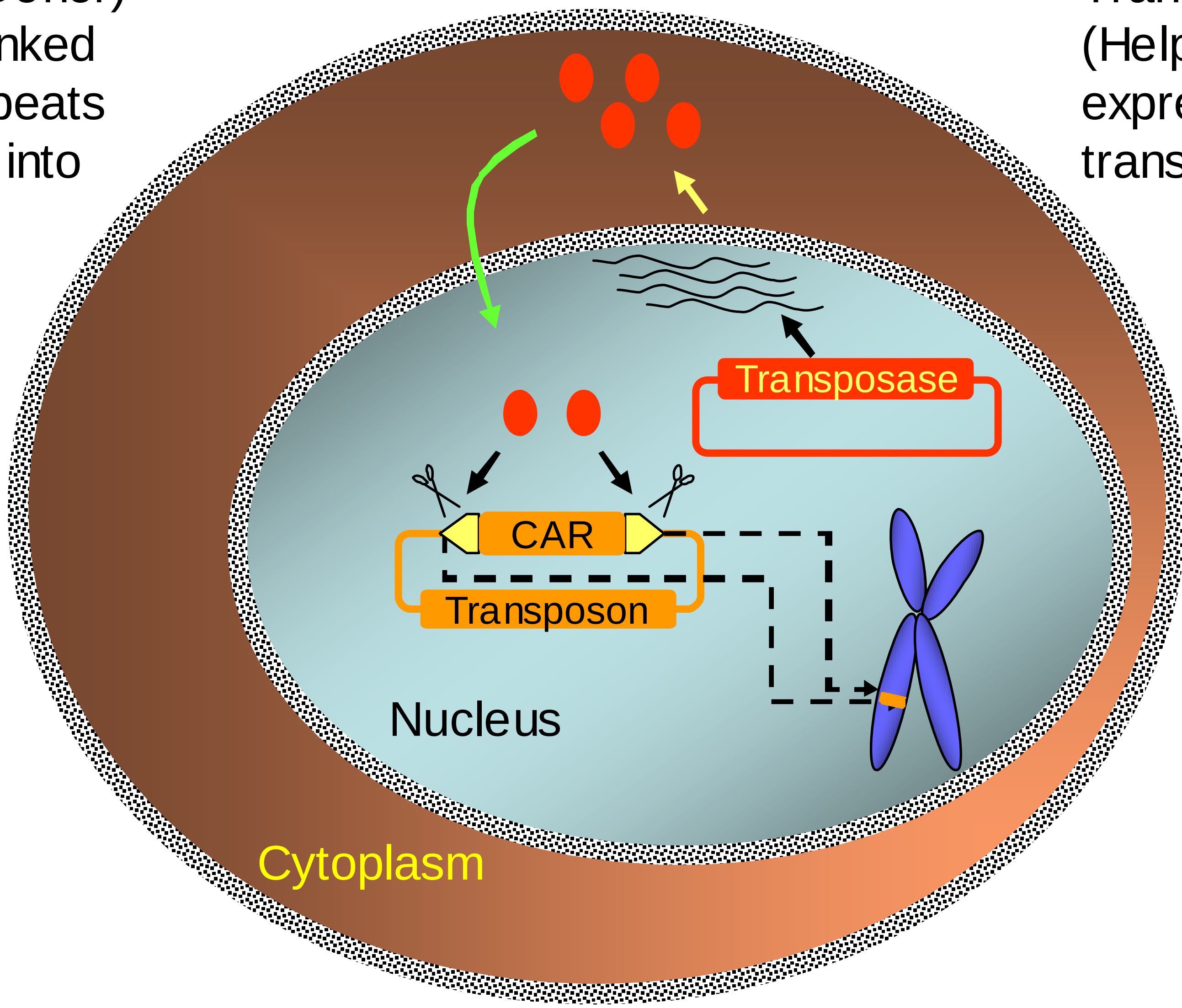
Numerically expand T cells under polarizing conditions



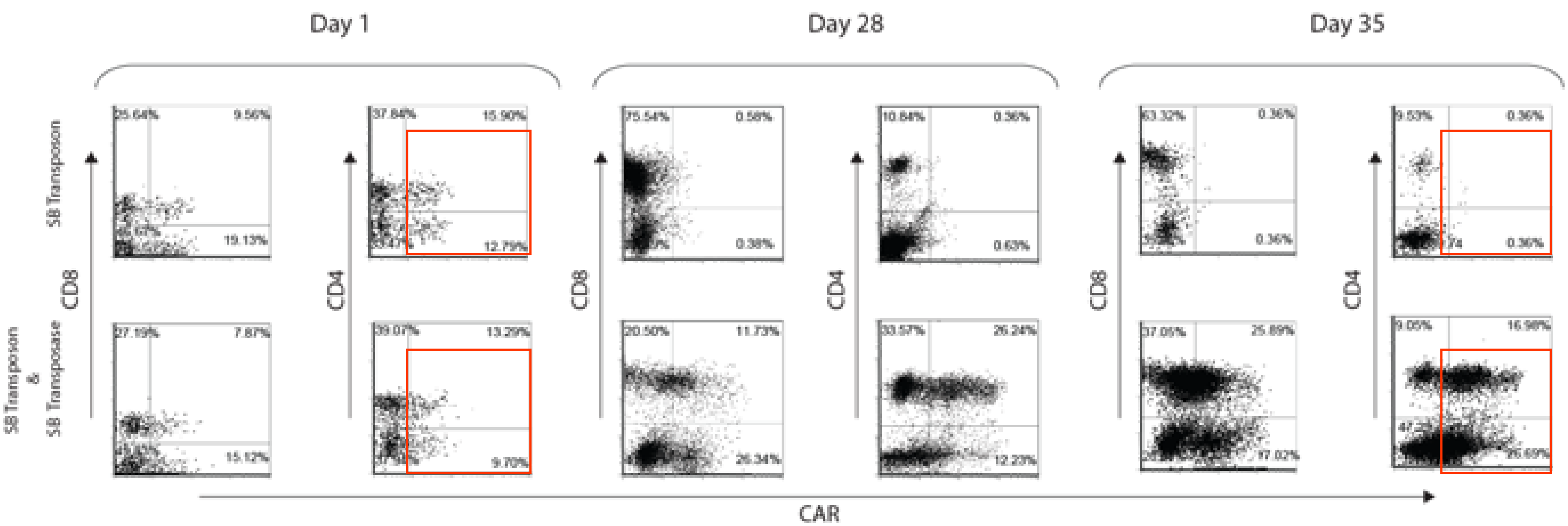
Sleeping Beauty Transposition

Transposon (Donor) sequences flanked by inverted repeats are integrated into genome

Transposase (Helper) expression is transient



Numeric expansion of CAR$^+$ T cells on aAPC



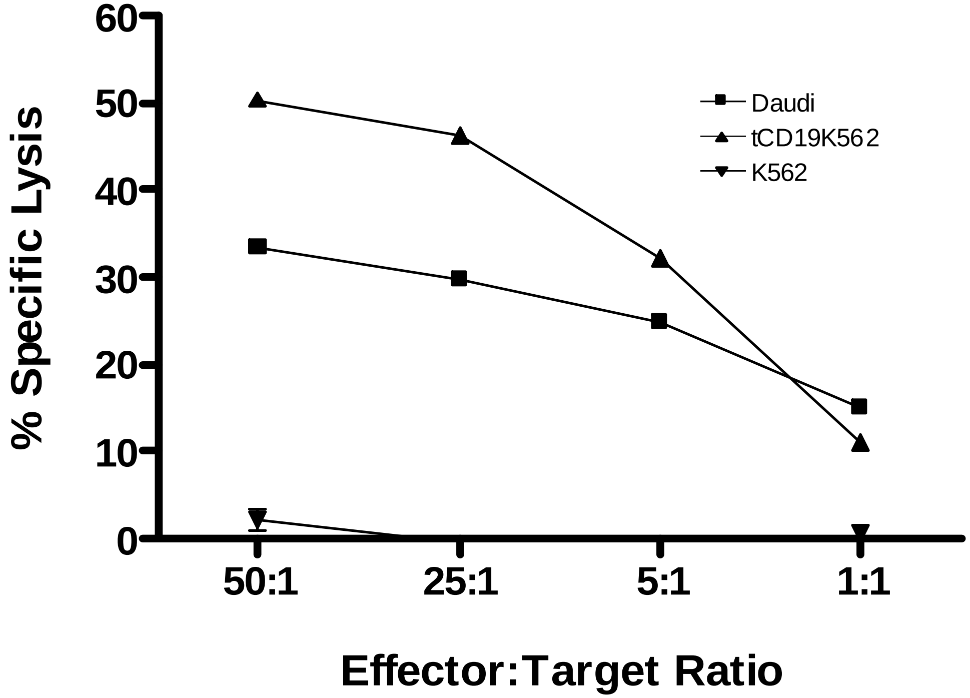
Relative efficiency of stable gene expression by *SB* system

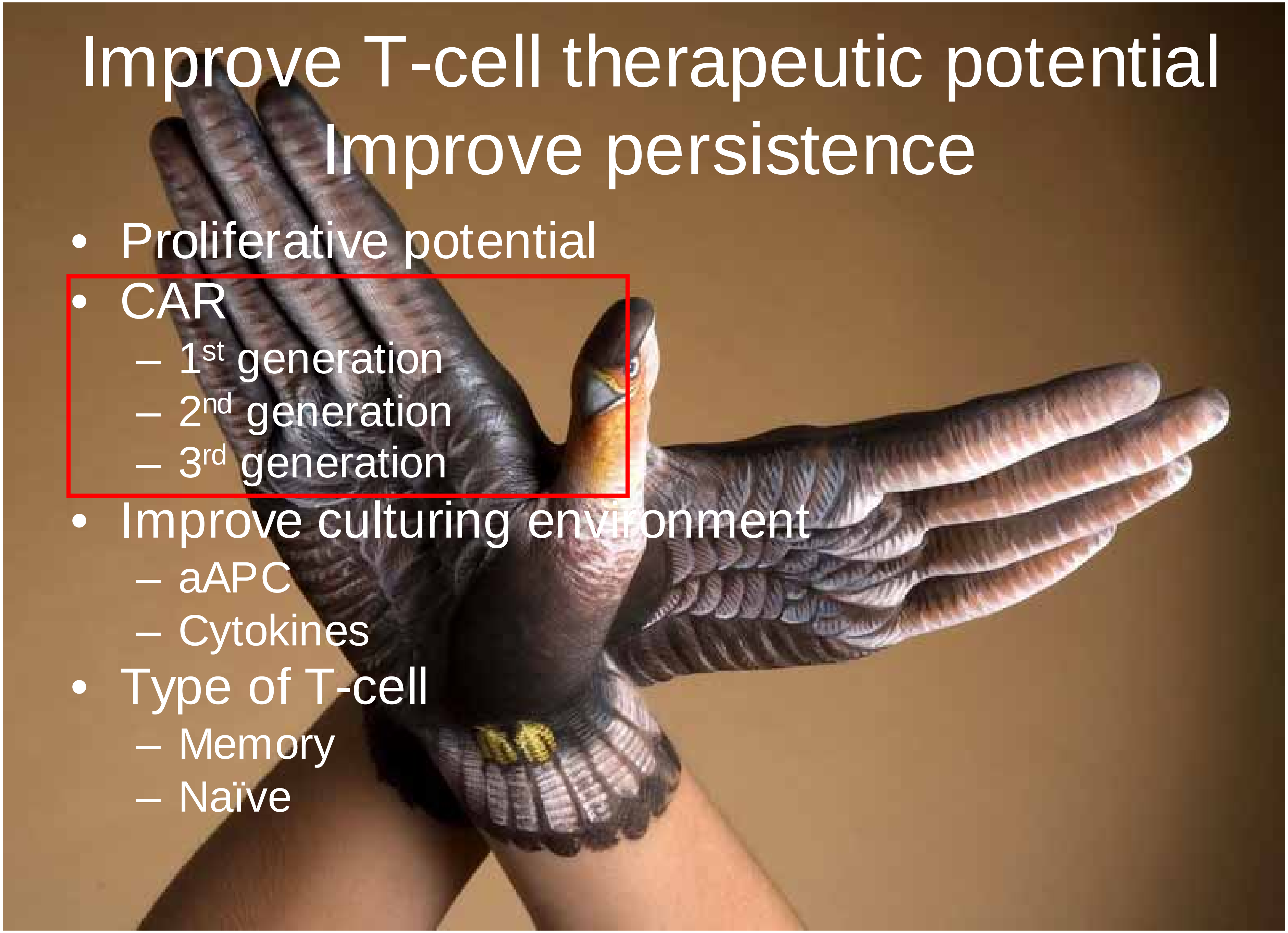
DNA plasmid	Day 0			Day 28		
	$CD4^+CAR^+$	**$CD8^+CAR^+$**	**Total CAR^+**	**$CD4^+CAR^+$**	**$CD8^+CAR^+$**	**Total CAR^+**
No DNA	0.63	0.18	1.61			
SB_{ase}	0.96	0.75	1.16			
SB_{on}	15.9	9.5	27.0	0.36	0.36	0.6
SB_{on} & SB_{ase}	13.29	7.87	22.0	16.98	25.89	43.0

71 fold

SB T-cell data

CD19-dependent cytotoxicity

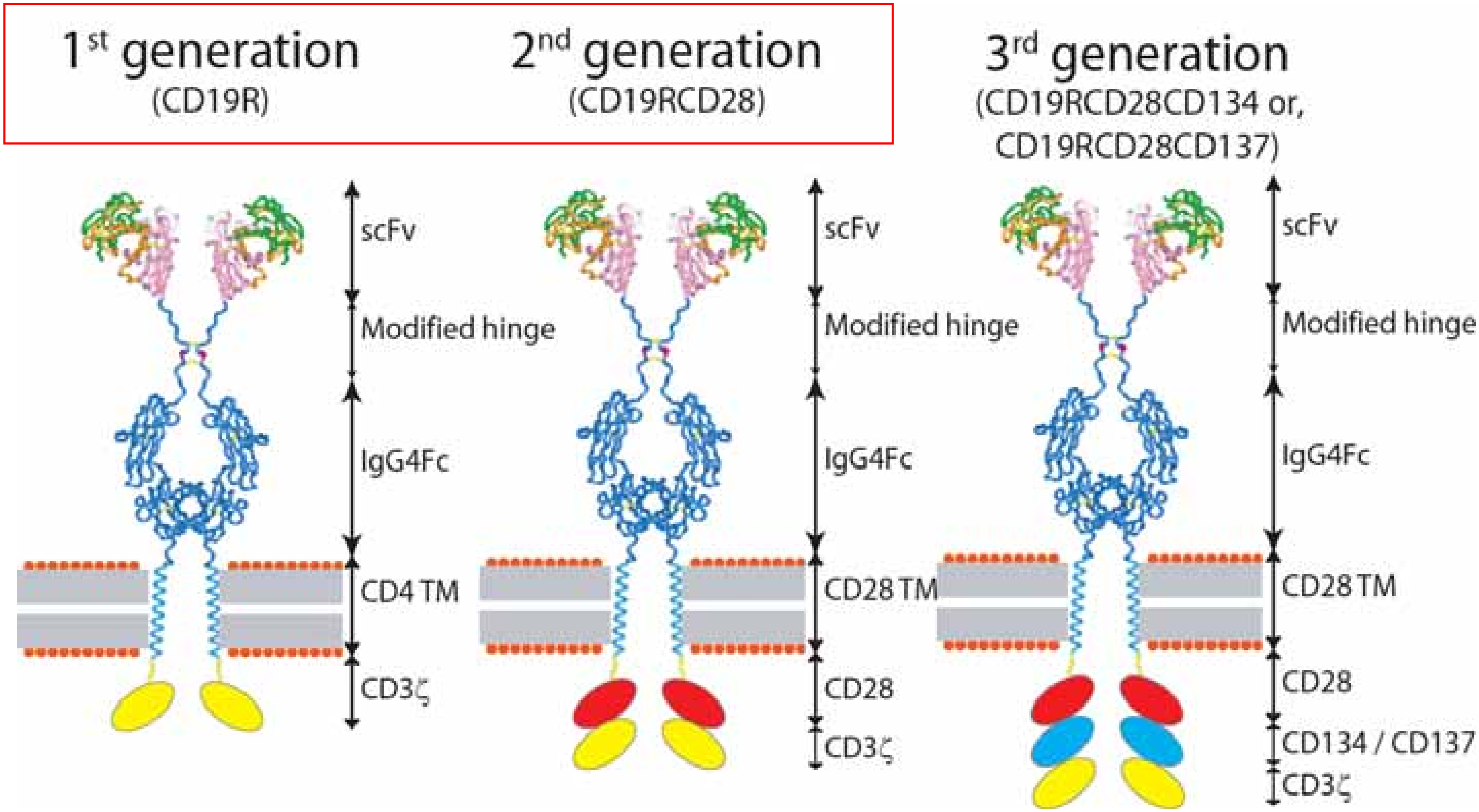




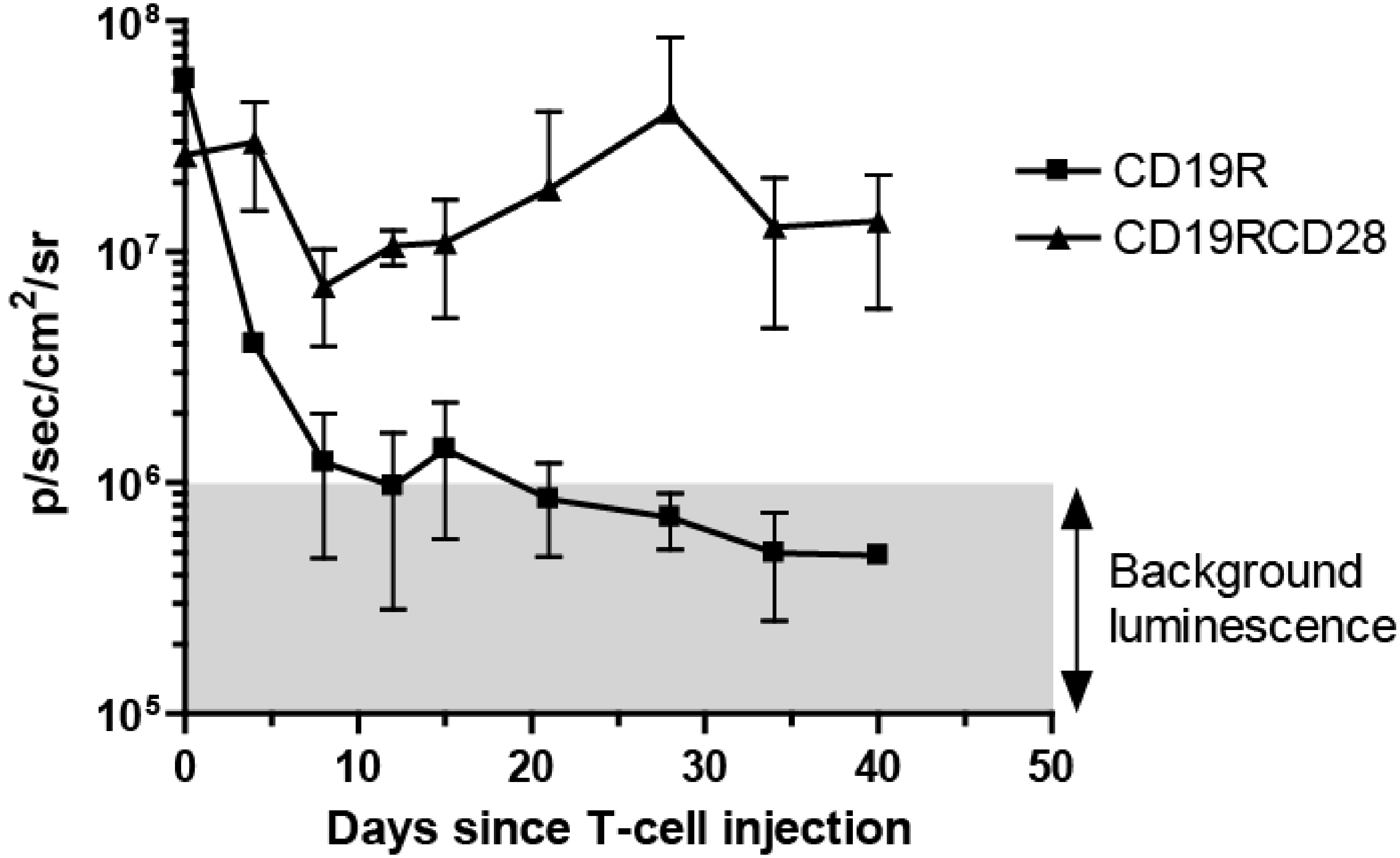
Improve T-cell therapeutic potential Improve persistence

- Proliferative potential
- CAR
  - 1st generation
  - 2nd generation
  - 3rd generation
- Improve culturing environment
  - aAPC
  - Cytokines
- Type of T-cell
  - Memory
  - Naïve

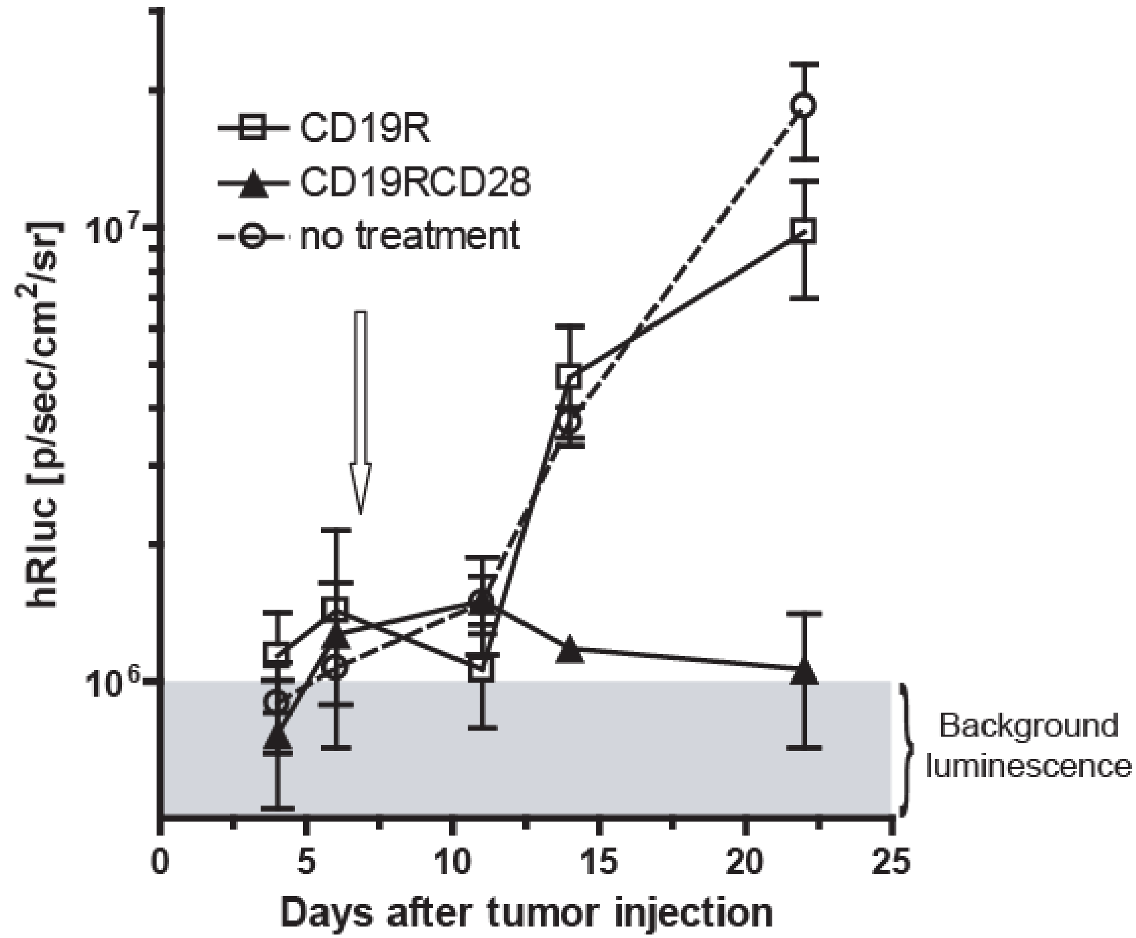
CD19-specific CARs

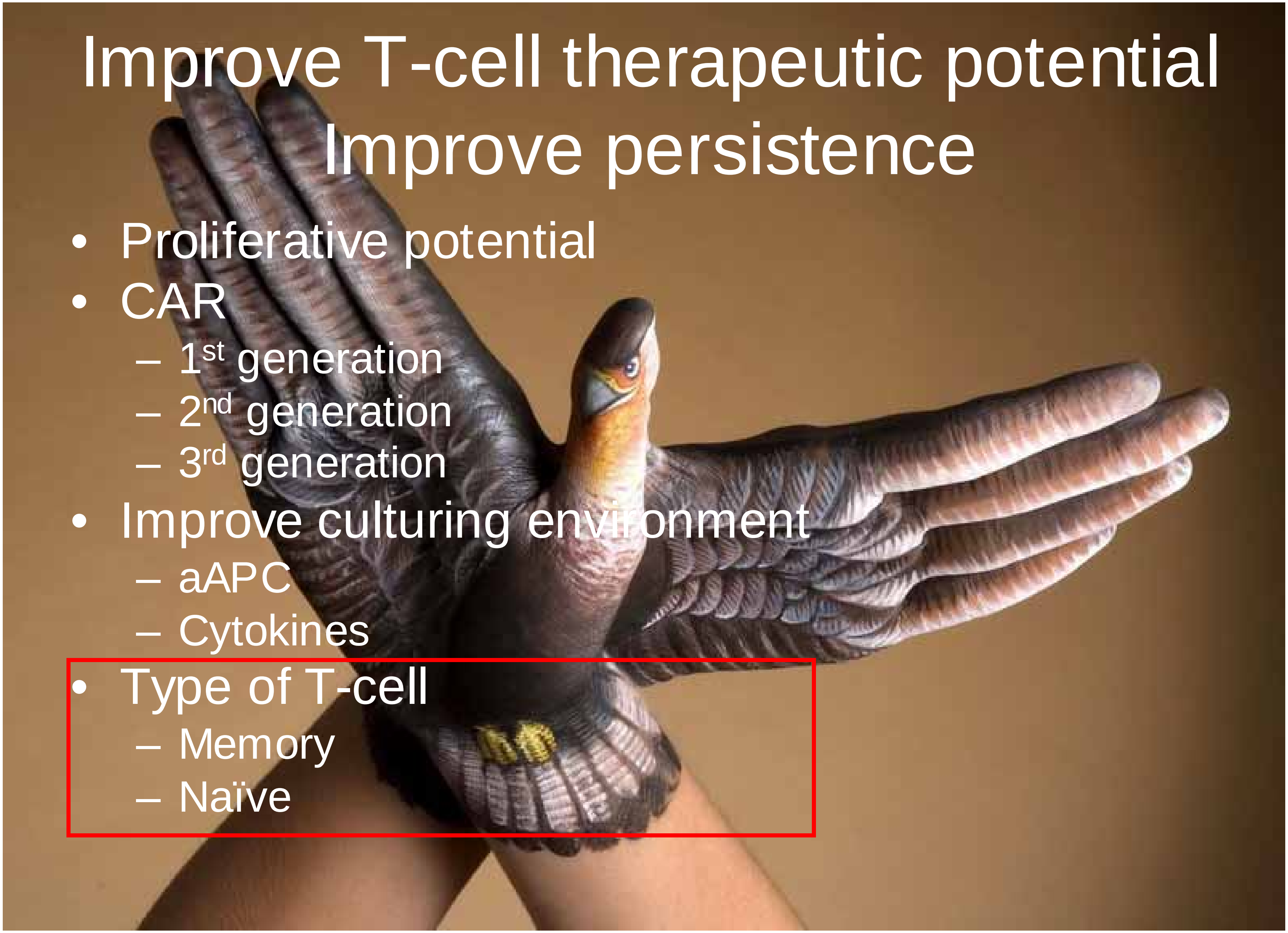


Relative *in vivo* T-cell persistence



Relative anti-tumor effect

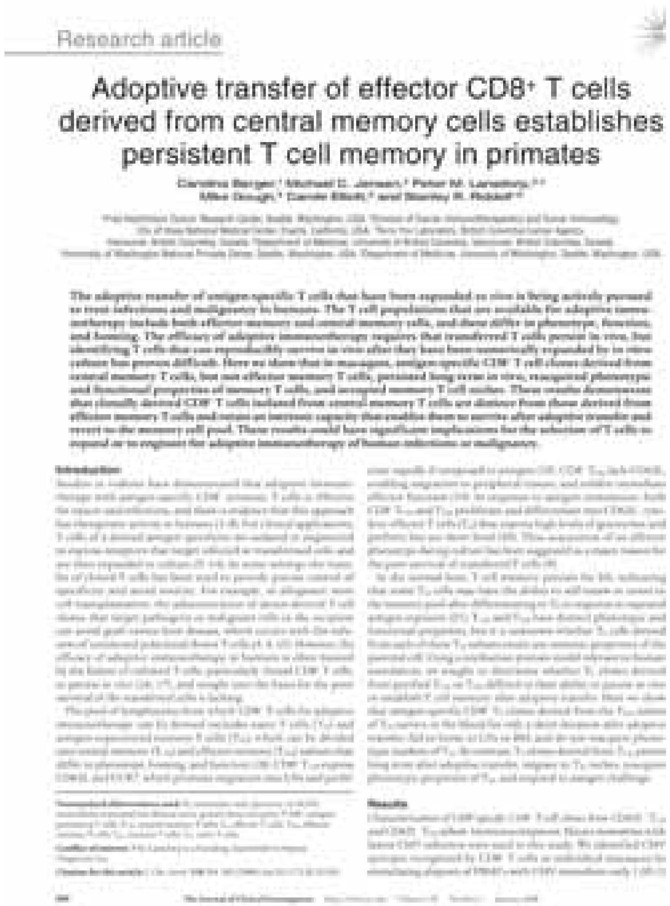




Improve T-cell therapeutic potential
Improve persistence

- Proliferative potential
- CAR
  - 1st generation
  - 2nd generation
  - 3rd generation
- Improve culturing environment
  - aAPC
  - Cytokines
- Type of T-cell
  - Memory
  - Naïve

Which T-cell sub-population to genetically modify?



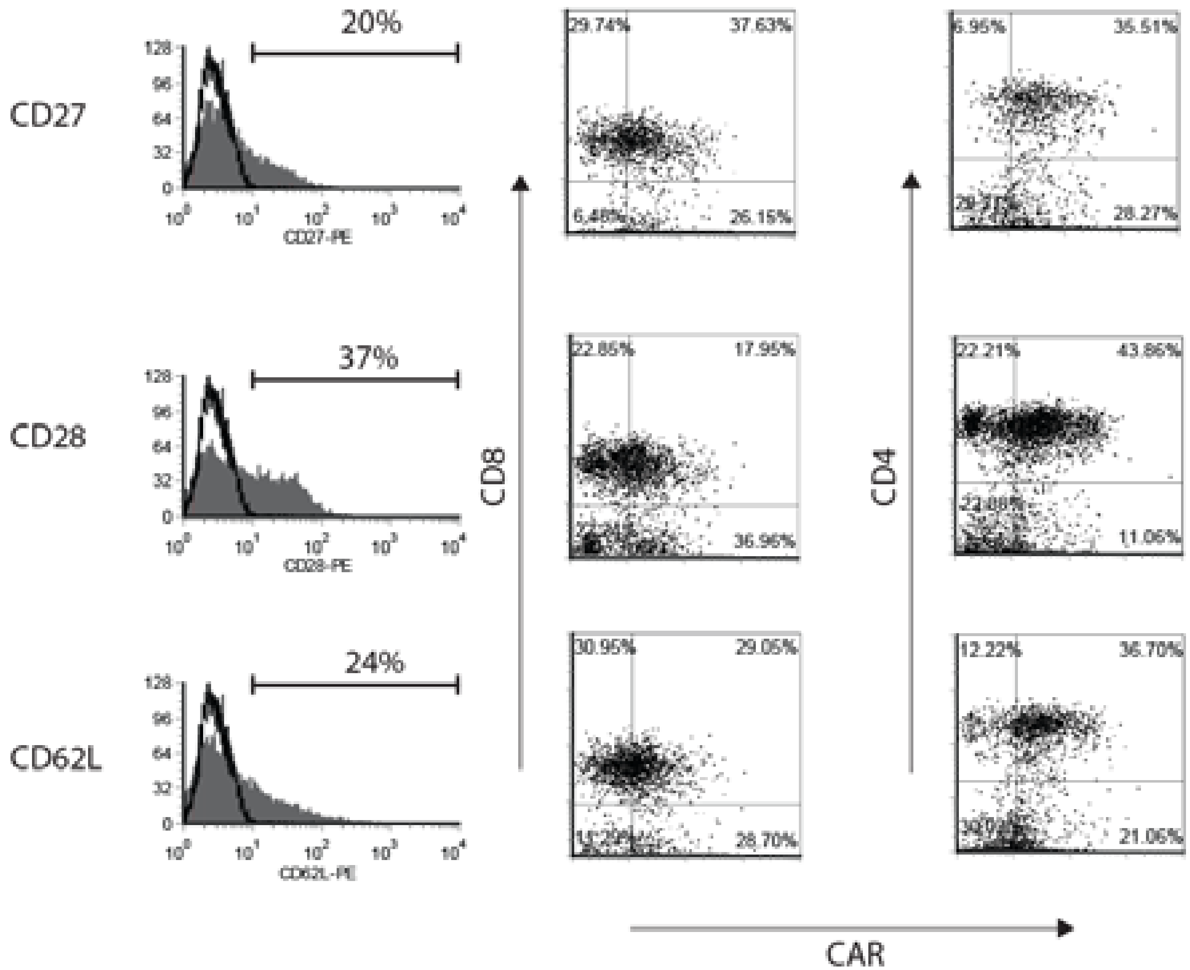
Research article

Adoptive transfer of effector CD8+ T cells derived from central memory cells establishes persistent T cell memory in primates

PNAS

Adoptively transferred effector cells derived from naïve rather than central memory CD8+ T cells mediate superior antitumor immunity

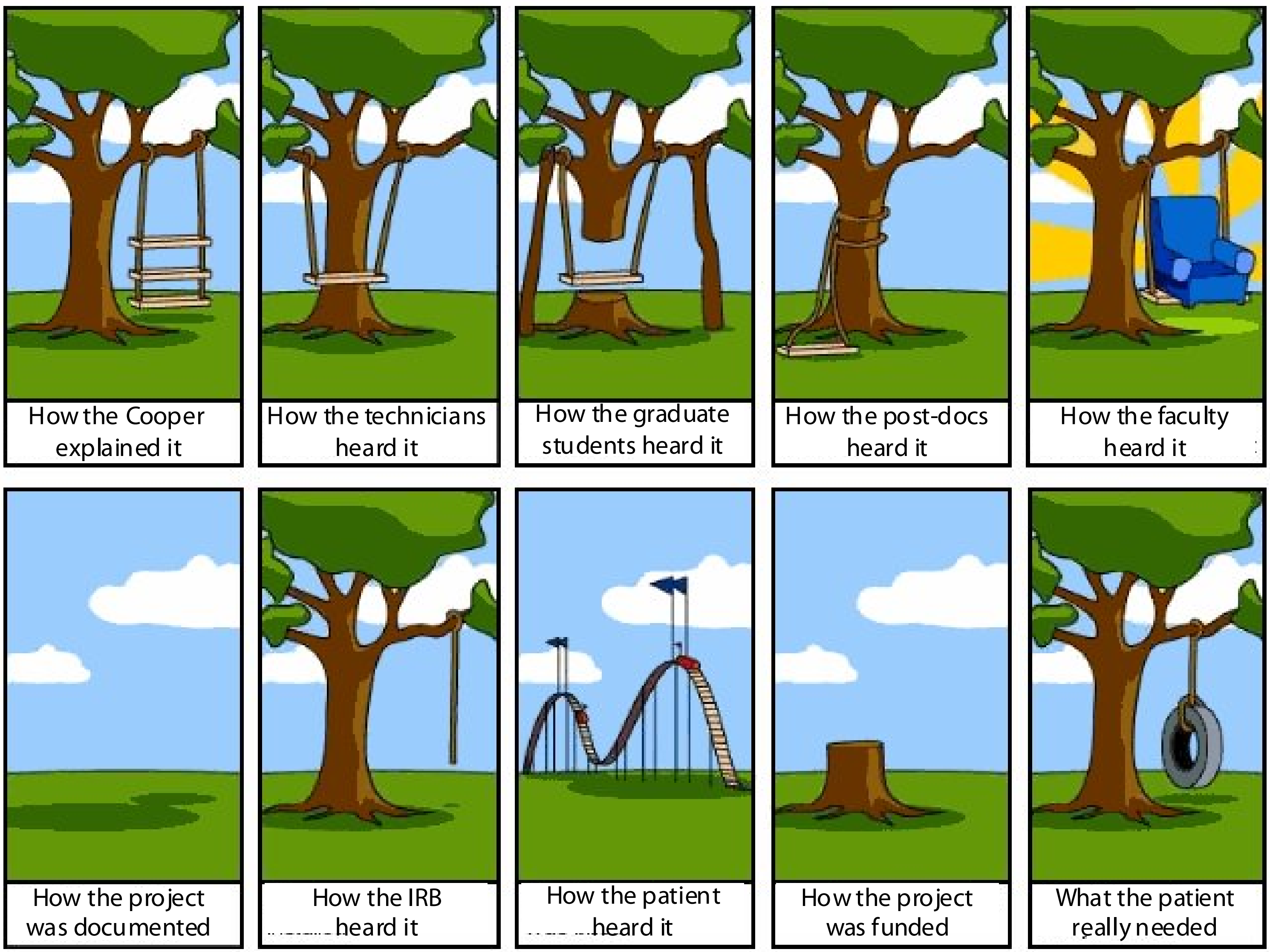
Outgrowth of CAR$^+$ T cells with memory cell phenotype



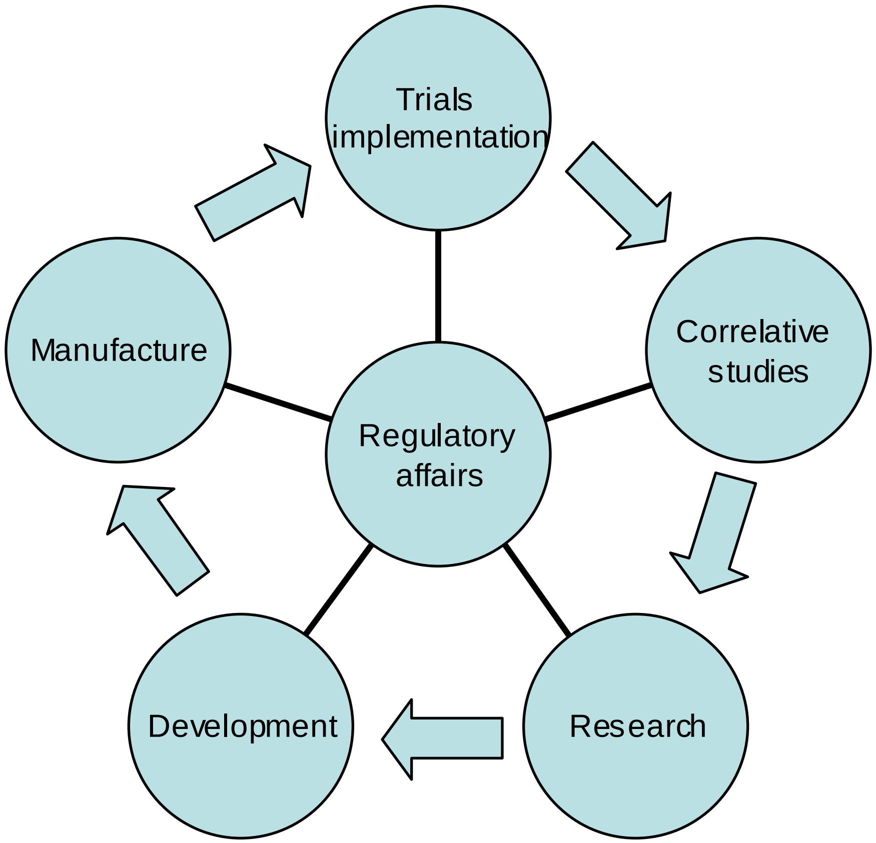

Safety of transposition

Translation to clinical trials

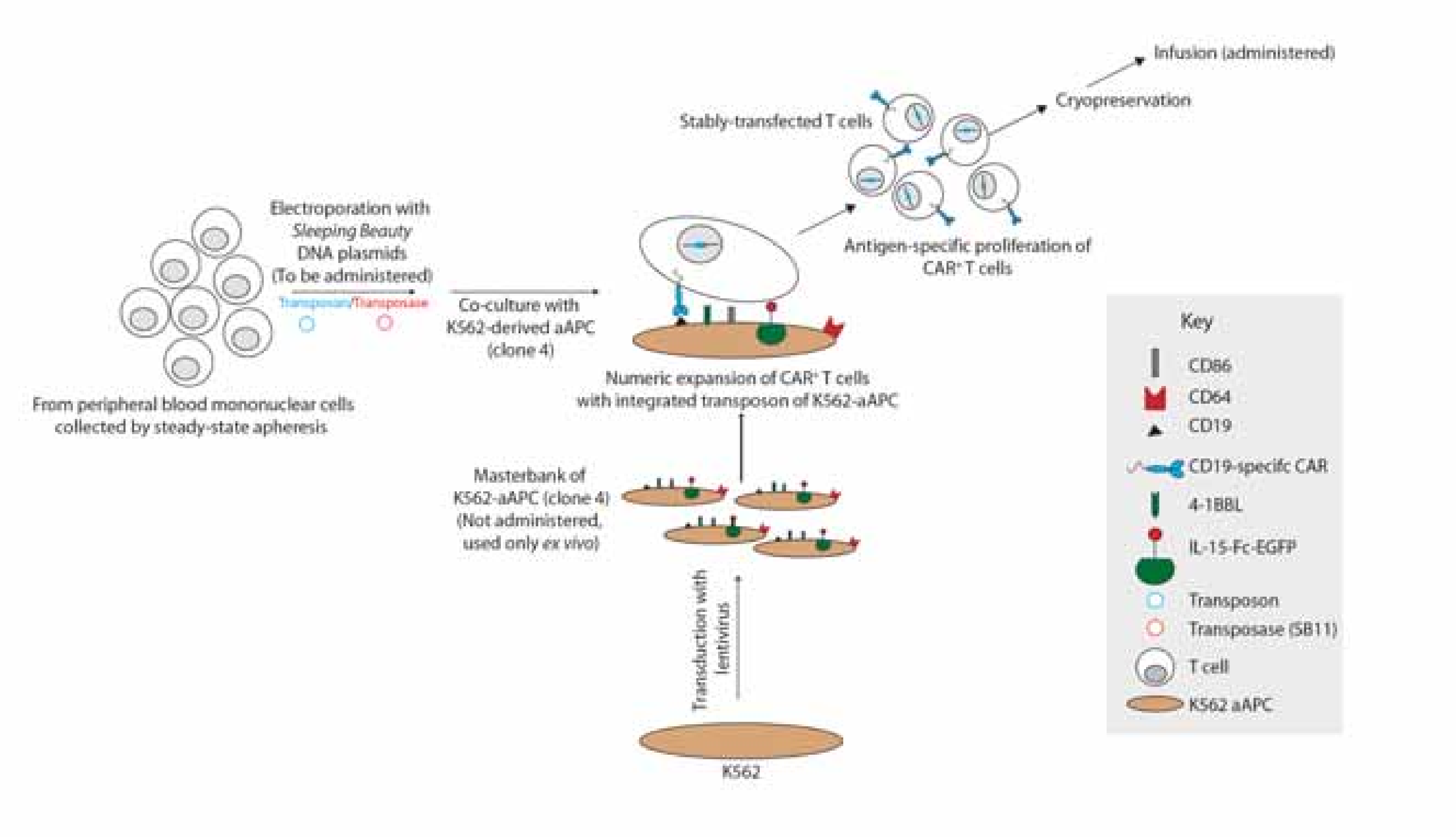
Applied Cellular Therapy (ACT)



Translational pipeline



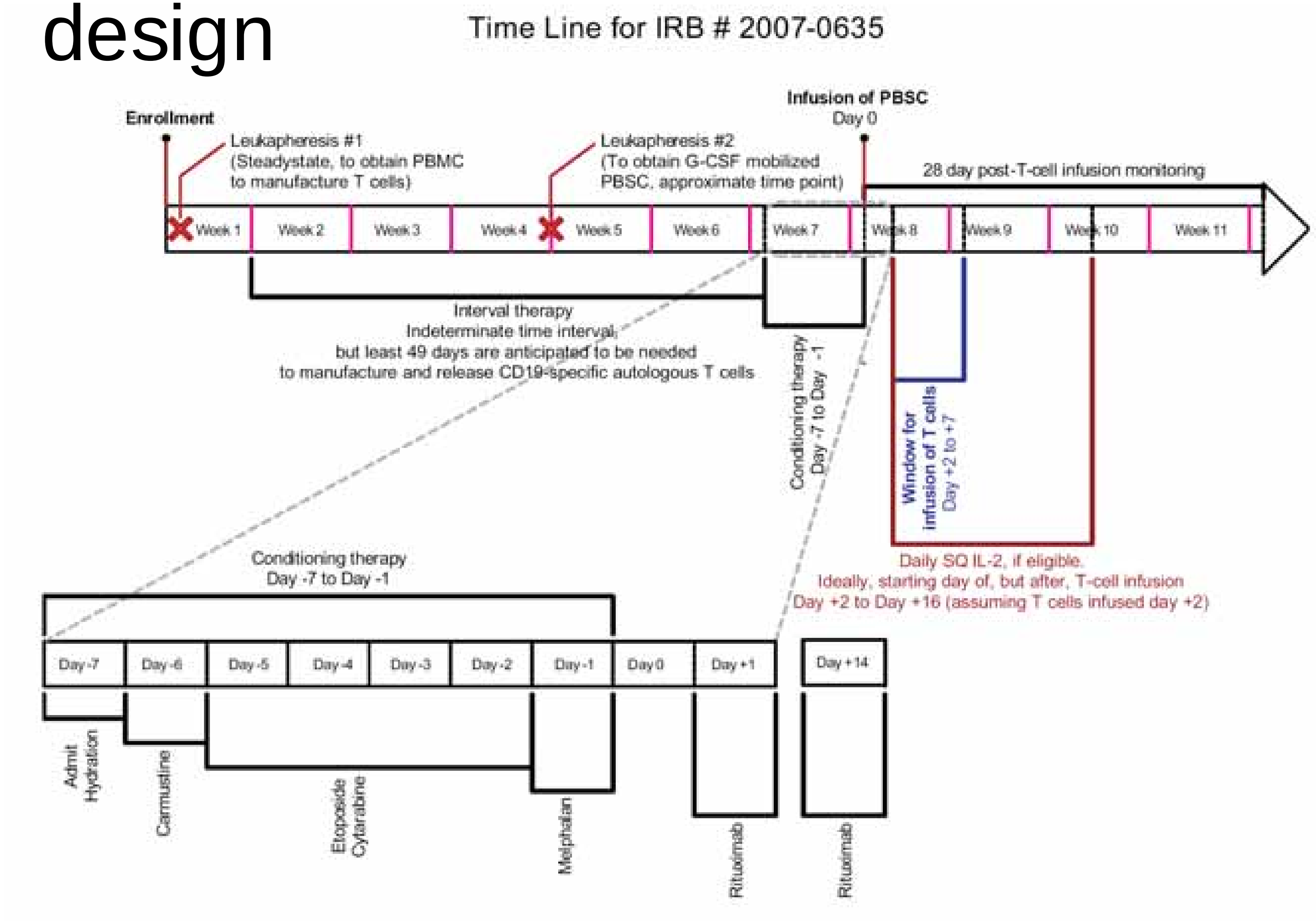
Schematic



Infusion (administered)
Cryopreservation
Stably-transfected T cells
Antigen-specific proliferation of CAR⁺ T cells
Electroporation with *Sleeping Beauty* DNA plasmids (To be administered)
Transposon/Transposase
Co-culture with K562-derived aAPC (clone 4)
From peripheral blood mononuclear cells collected by steady-state apheresis
Numeric expansion of CAR⁺ T cells with integrated transposon of K562-aAPC
Masterbank of K562-aAPC (clone 4) (Not administered, used only *ex vivo*)
Transduction with lentivirus
K562
Key
CD86
CD64
CD19
CD19-specifc CAR
4-1BBL
IL-15-Fc-EGFP
Transposon
Transposase (SB11)
T cell
K562 aAPC

Trial design

Principal Investigator = Dr. Partow Kebriaei



T-cell trials at MDACC

Trial	Agent	Preclinical	Phase I
Lymphoma	Autologous CD19-specific T cells	→	X
ALL and lymphoma	Allogeneic CD19-specific T cells	→	X
ALL and lymphoma	Allogeneic CD19-specific UCB-derived T cells	→	X

NK-cell trials at MDACC

Trial	Agent	Preclinical	Phase I	Phase II
Neuroblastoma	Haploidentical NK cells	→	X	
ALL	Haploidentical NK cells and Epratuzumab	→	X	
AML	Haploidentical NK cells	→	→	X

Future developments

- Altering the host environment to facilitate persistence and function of transferred T cells
- Altering intrinsic properties of T cells that are selected or engineered for therapy

Lessons and Take Home Messages

- Key points
  - *SB* system can be used to genetically modify human T cells
- Potential impact on the field
  - *SB* system can be adapted for human application
- Lessons learned
  - Genetically modified T cells can be generated with improved persistence and therapeutic potential

THANKS!


THE UNIVERSITY OF TEXAS
MD ANDERSON
CANCER CENTER
Making Cancer History®
children's
cancer
hospital™