THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center

Genetically engineered receptors in adoptive cell therapies

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11/04/2011

3:15 PM to 3:45 PM

International Society for Biological Therapy of Cancer (iSBTc)

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Children's Cancer Hospital

The following relationships exist related to this presentation:

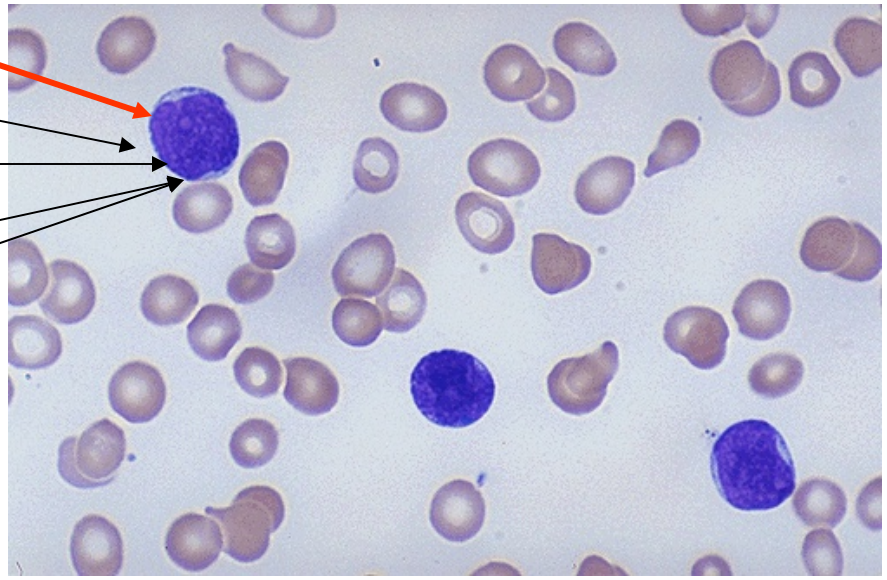
No Relationships to Disclose

Tumor-specific T cells

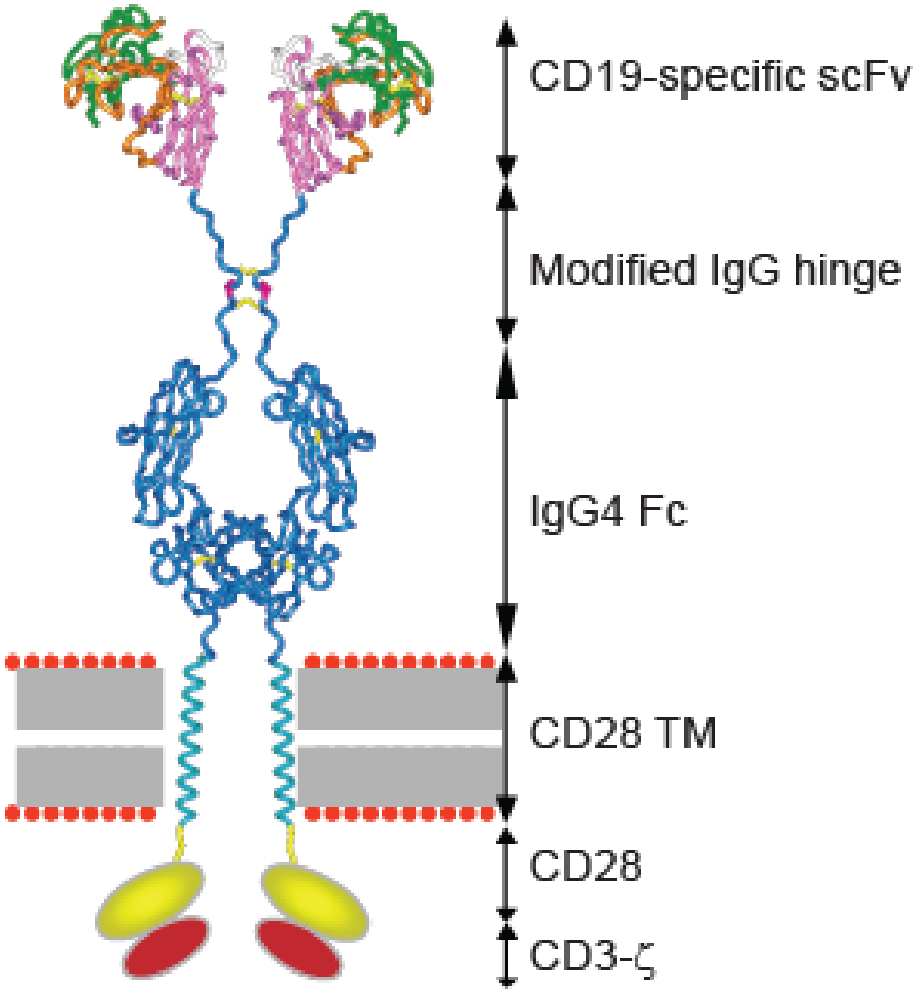
- T cells that recognize tumor-associated antigen (TAA) through introduced chimeric antigen receptor independent of MHC
- T cells that recognize TAA though endogenous $\alpha\beta$ T-cell receptor (TCR) in context of MHC

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

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

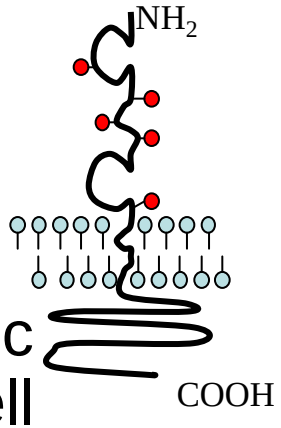


CD19-specific CAR

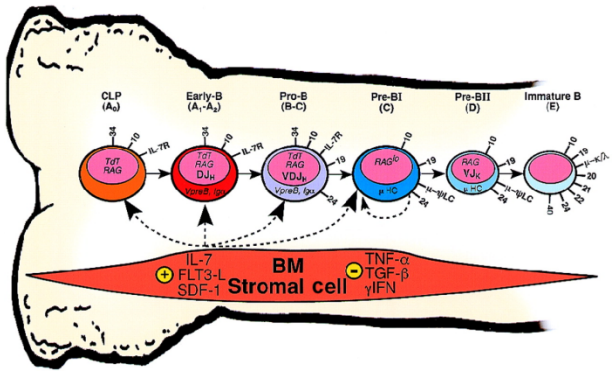


Rationale

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
  - Reprogramming culturing μ-environment
  - Cytokines
- CAR
  - 1st generation
  - 2nd generation
  - 3rd generation
- Type of T-cell
  - Memory
  - Naïve

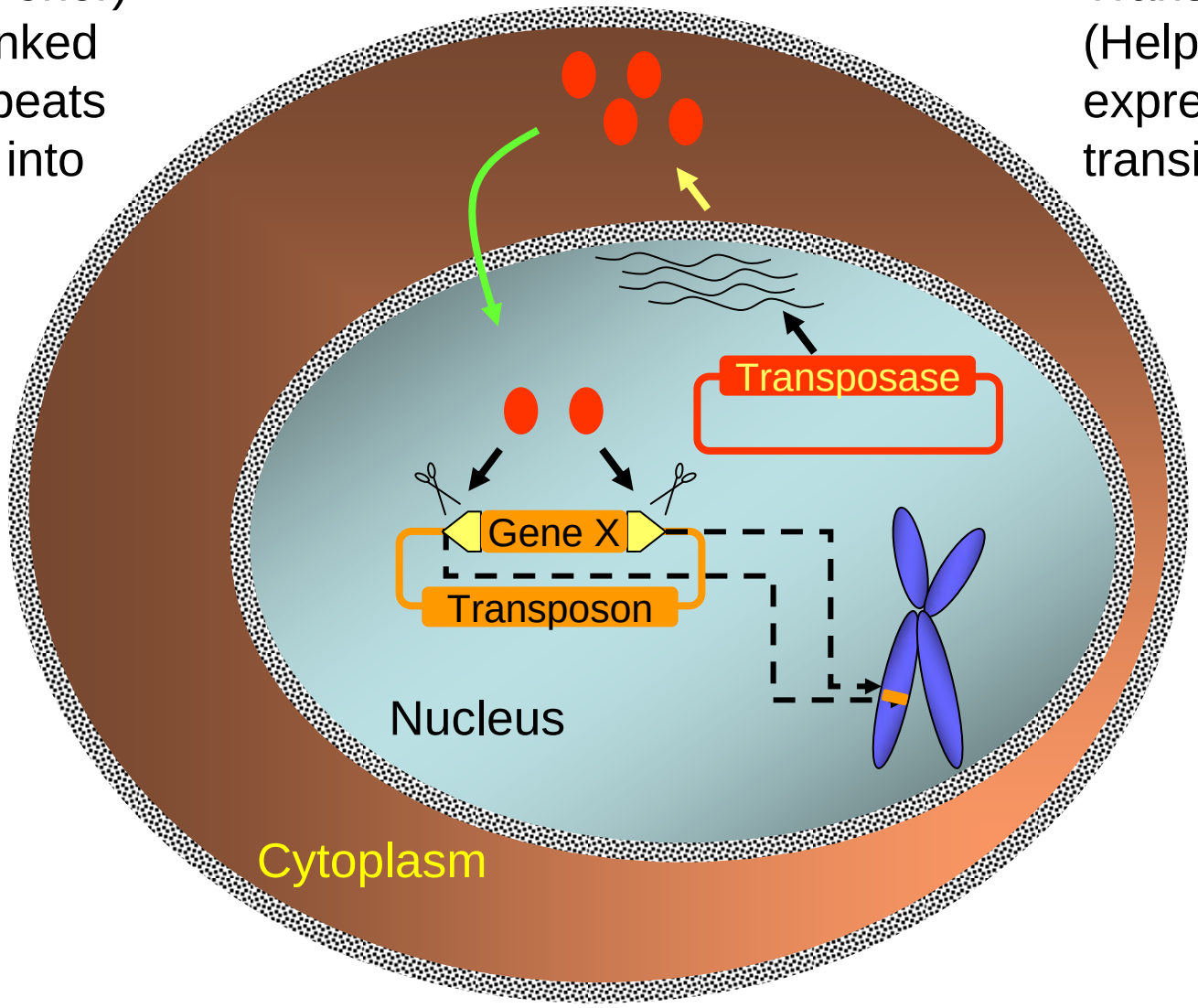
Most clinically-effective T-cell therapies includes *ex vivo* antigen-dependent proliferation

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

Sleeping Beauty Transposition

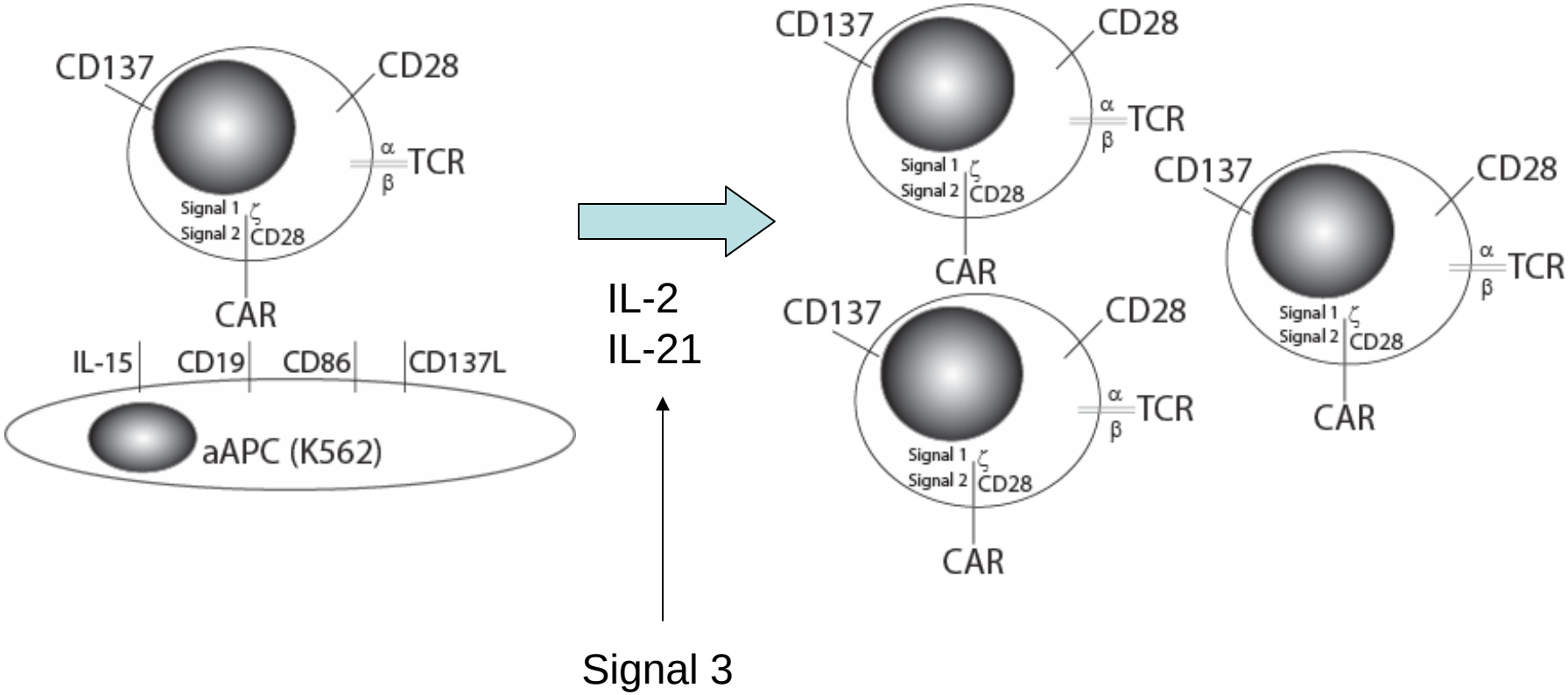
Transposon (Donor) sequences flanked by inverted repeats are integrated into genome

Transposase (Helper) expression is transient

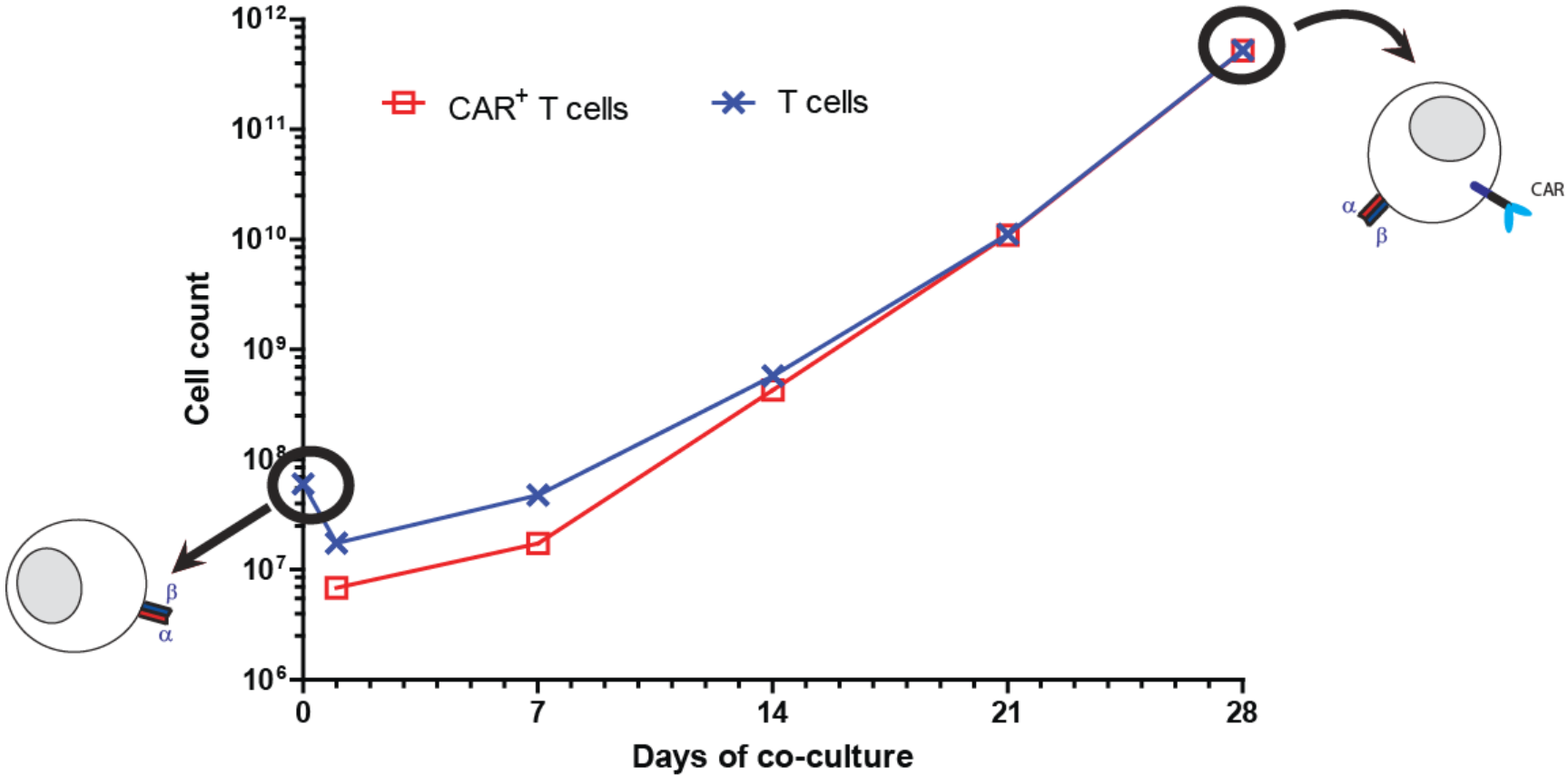


Experimental design

Re-programming T cells in culture

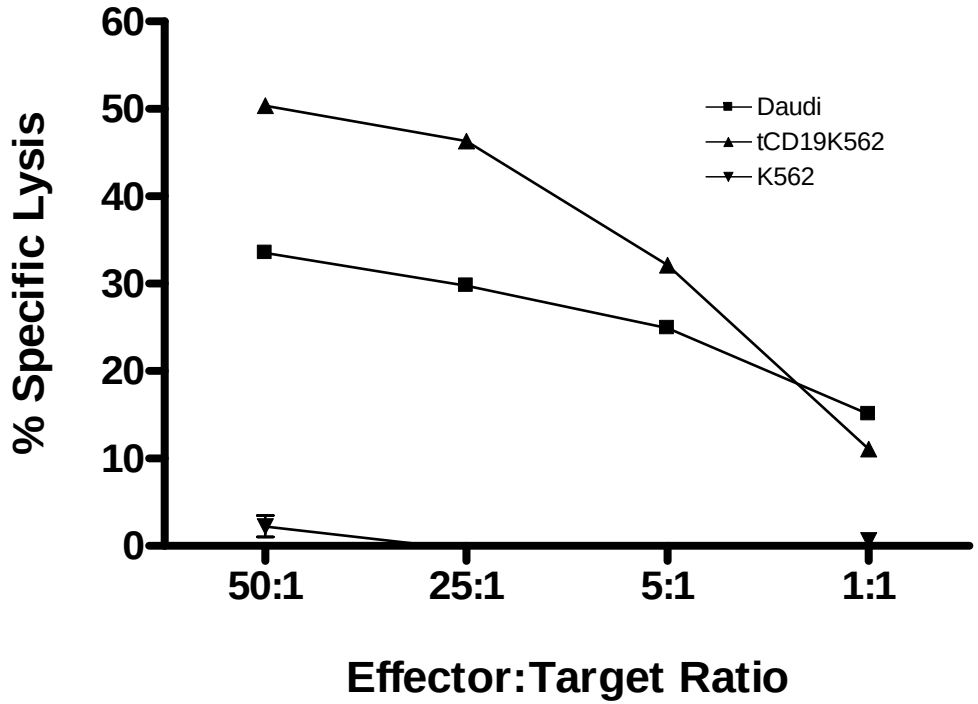


Production of T cells expressing CAR



SB T-cell data

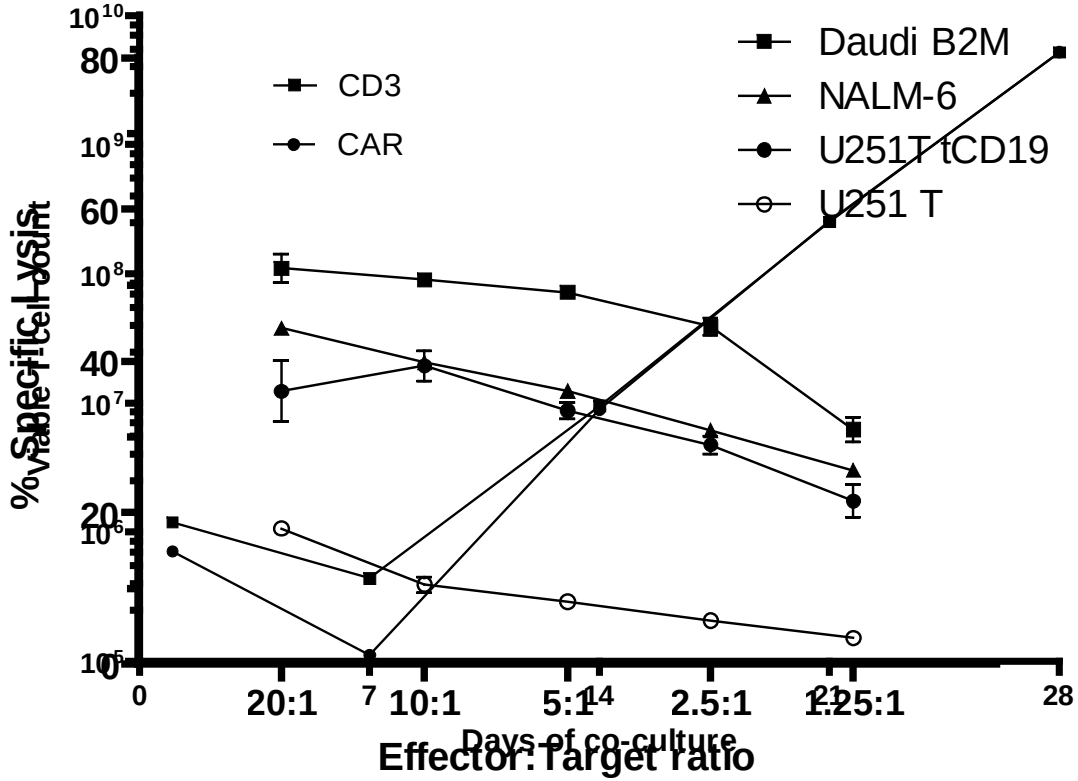
CD19-dependent cytotoxicity



T cells killing tumor cells expressing CD19

Time lapse

CD19-specific T cells from umbilical cord blood



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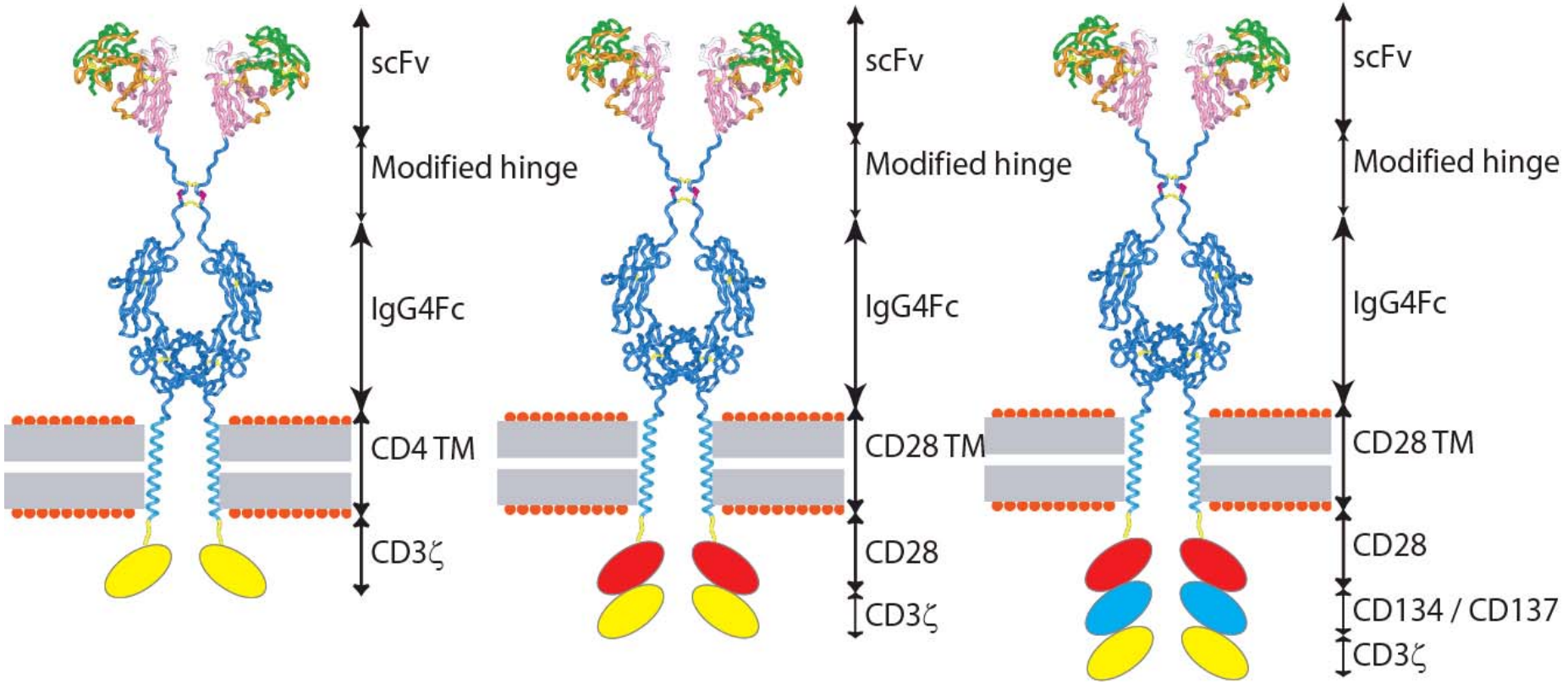
CD19-specific CARs

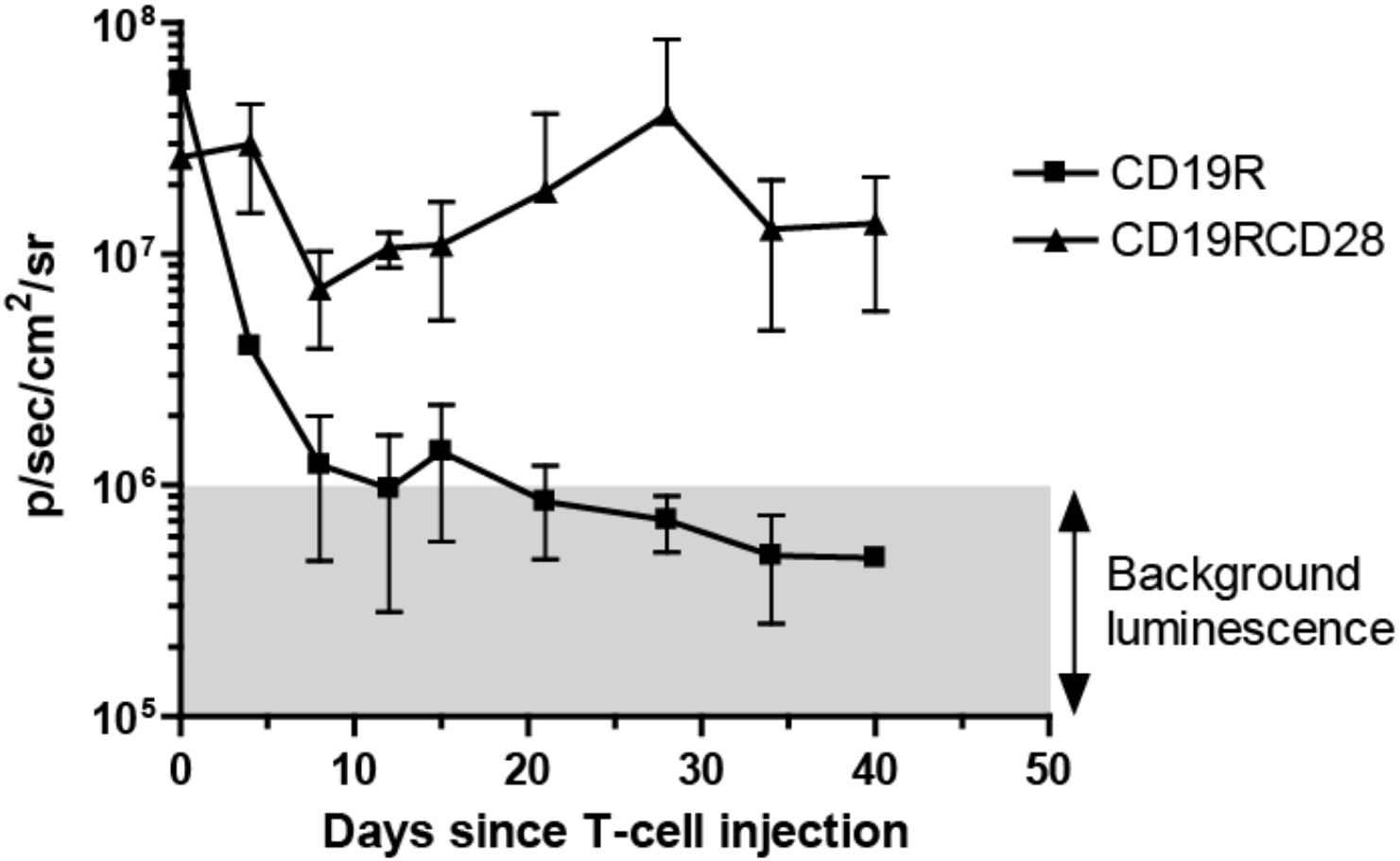
1st generation (CD19R)

2nd generation (CD19RCD28)

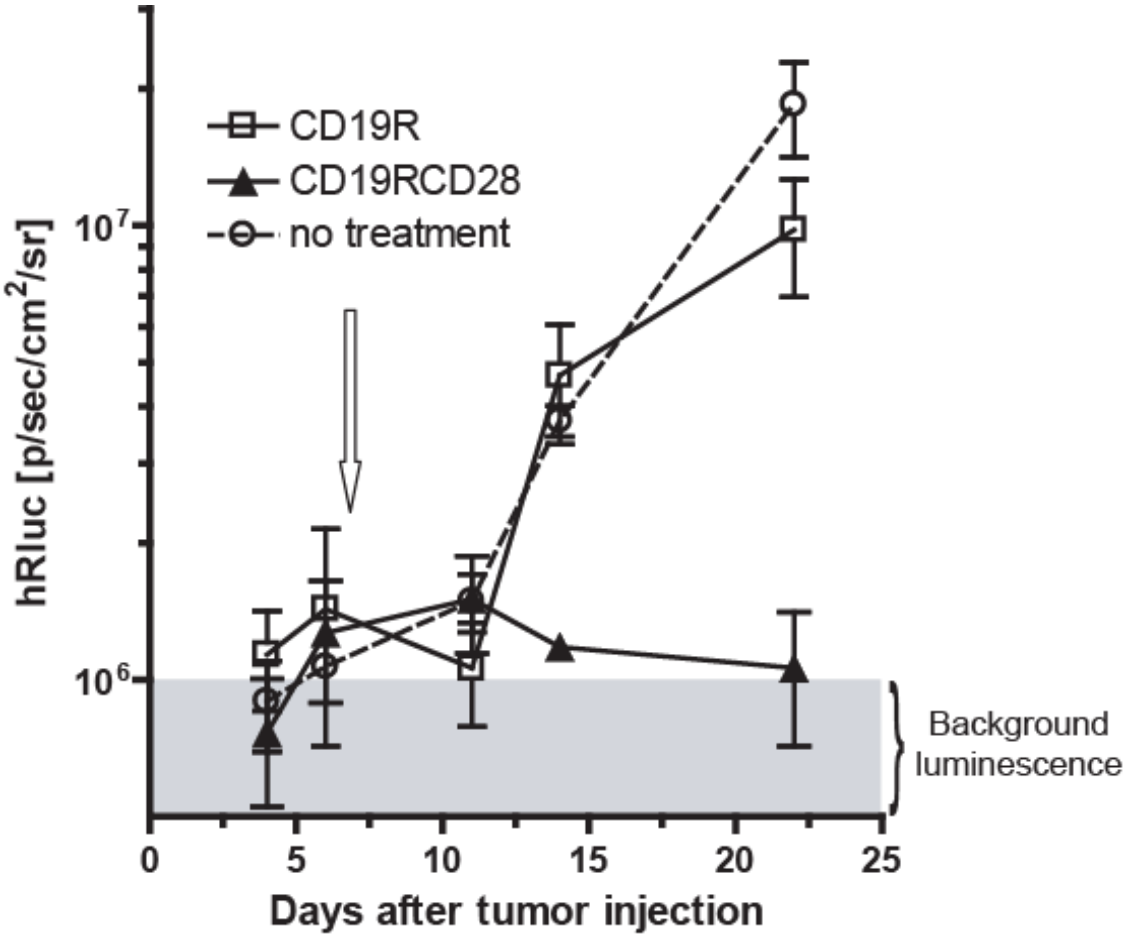
3rd generation (CD19RCD28CD134 or, CD19RCD28CD137)



Relative *in vivo* T-cell persistence

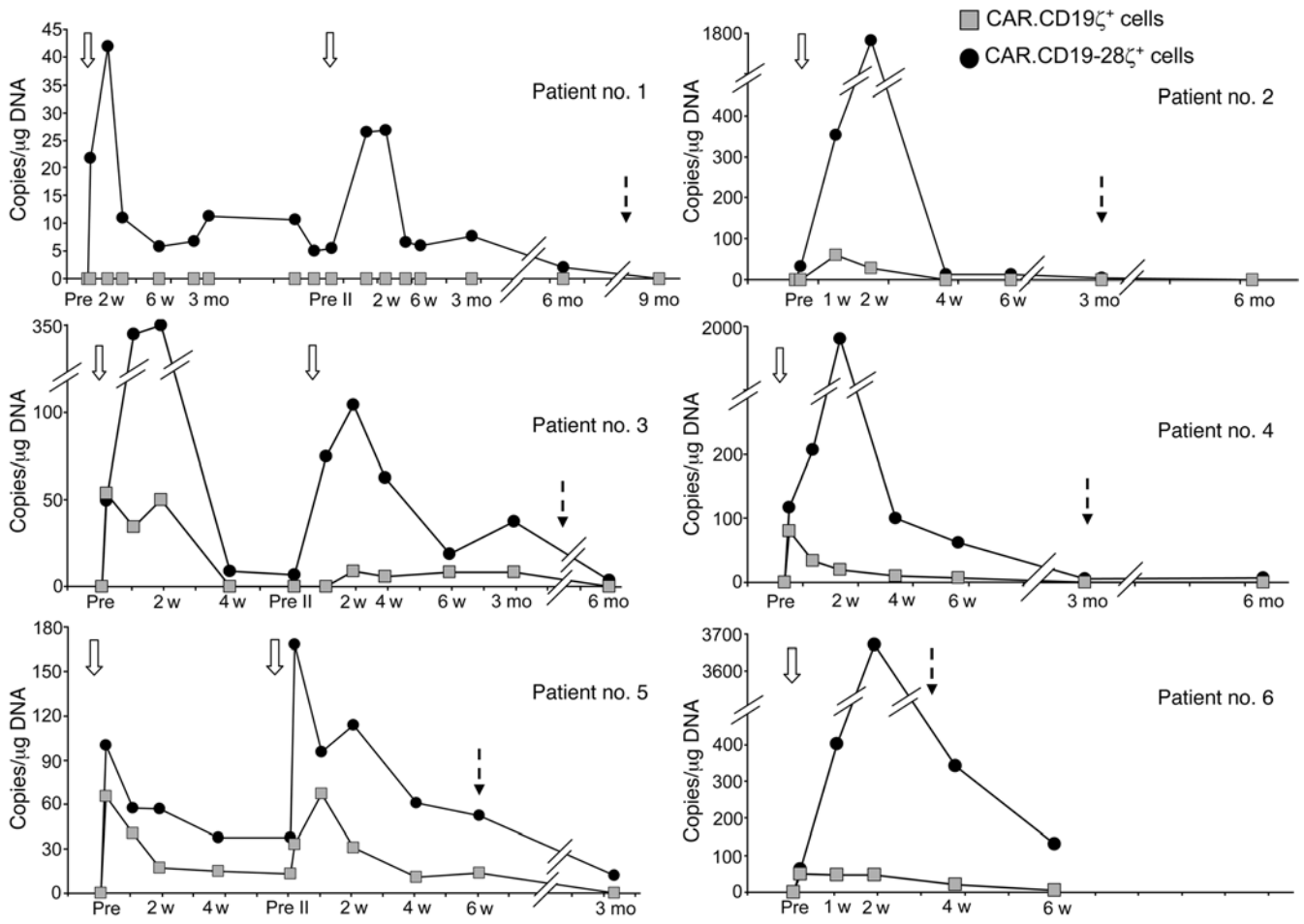


Relative anti-tumor effect



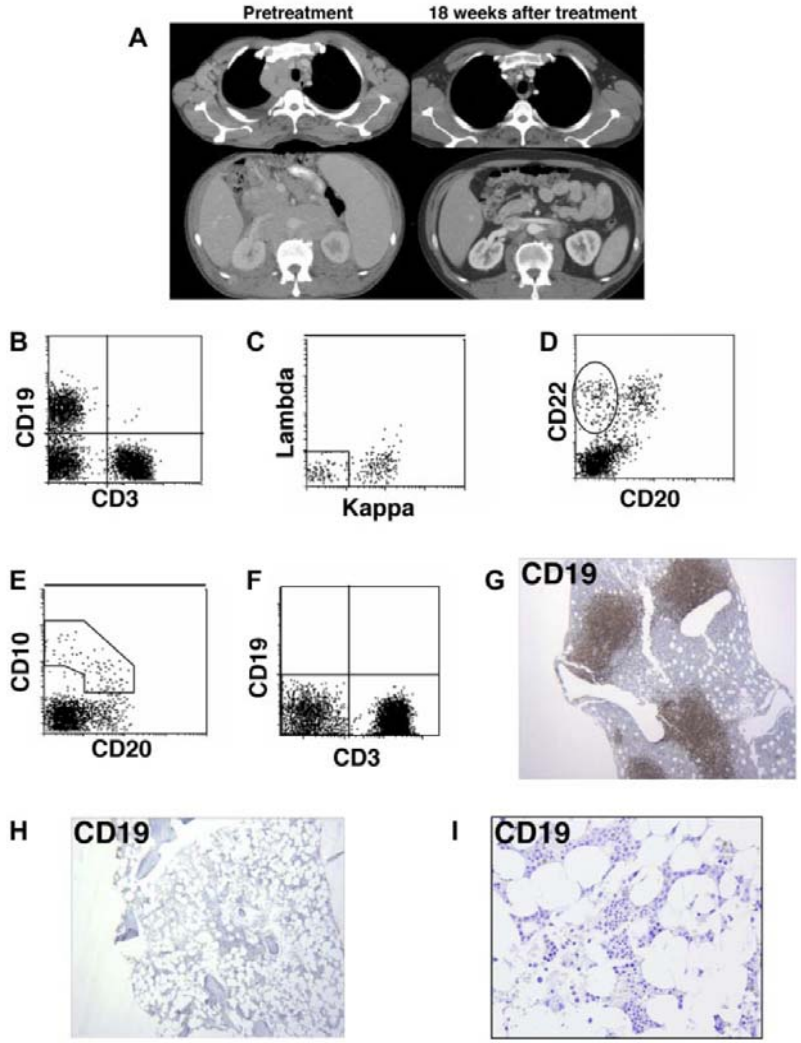
CD28 costimulation improves expansion and persistence of CAR+ T cells in lymphoma patients

J Clin Invest. 2011 May 2;121(5):1822-6



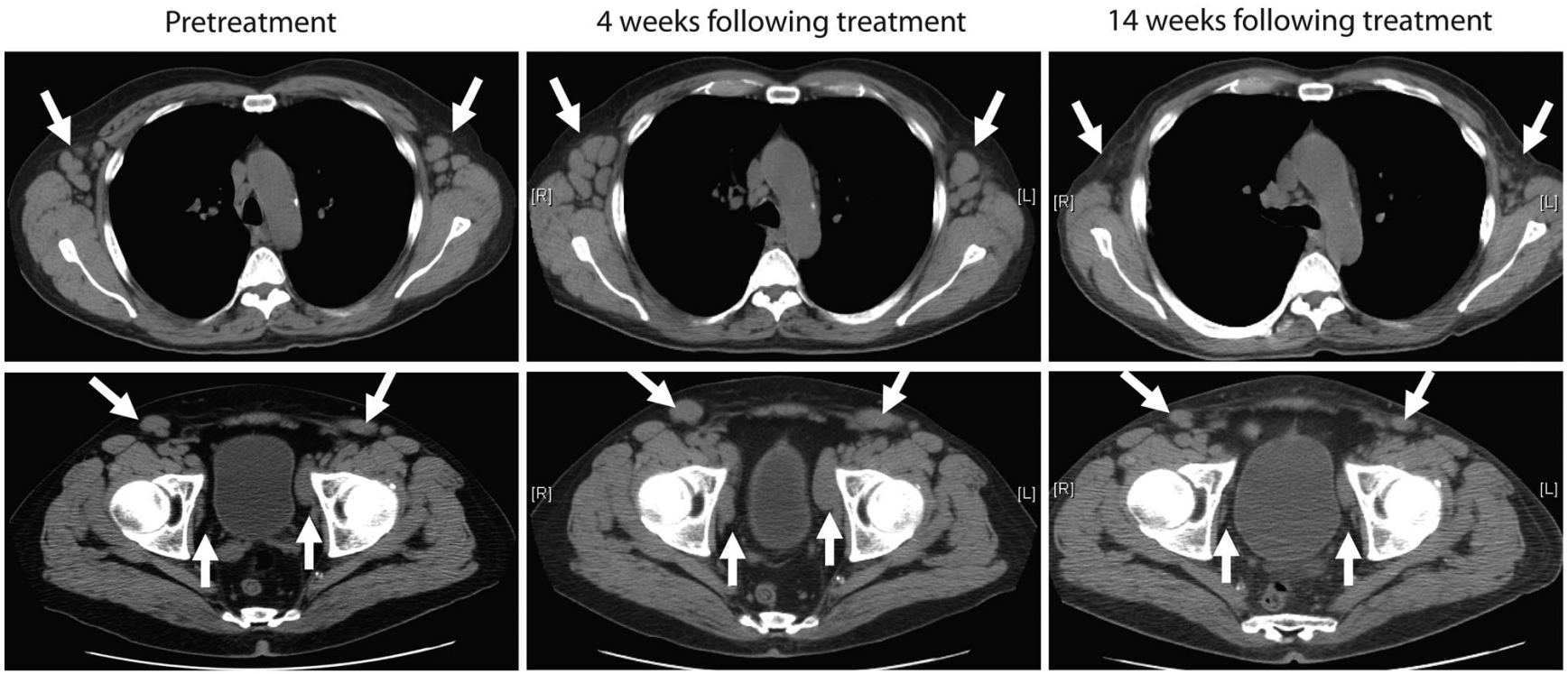
Signaling through chimeric CD28 results in anti-tumor responses

Blood. 2010 Nov 18;116(20):4099-102.

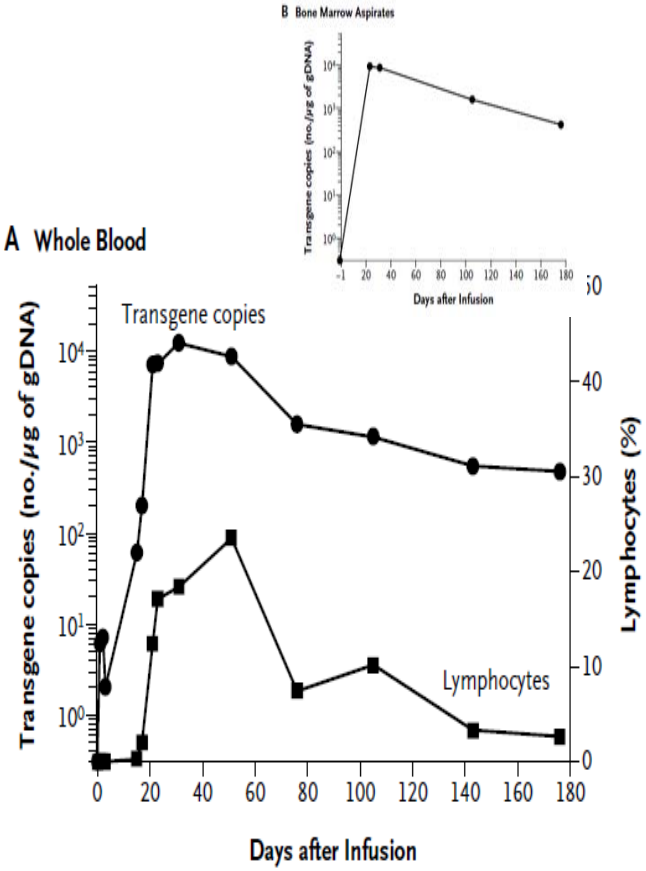


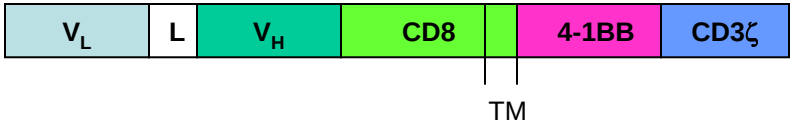
Signaling through chimeric CD28 results in anti-tumor responses

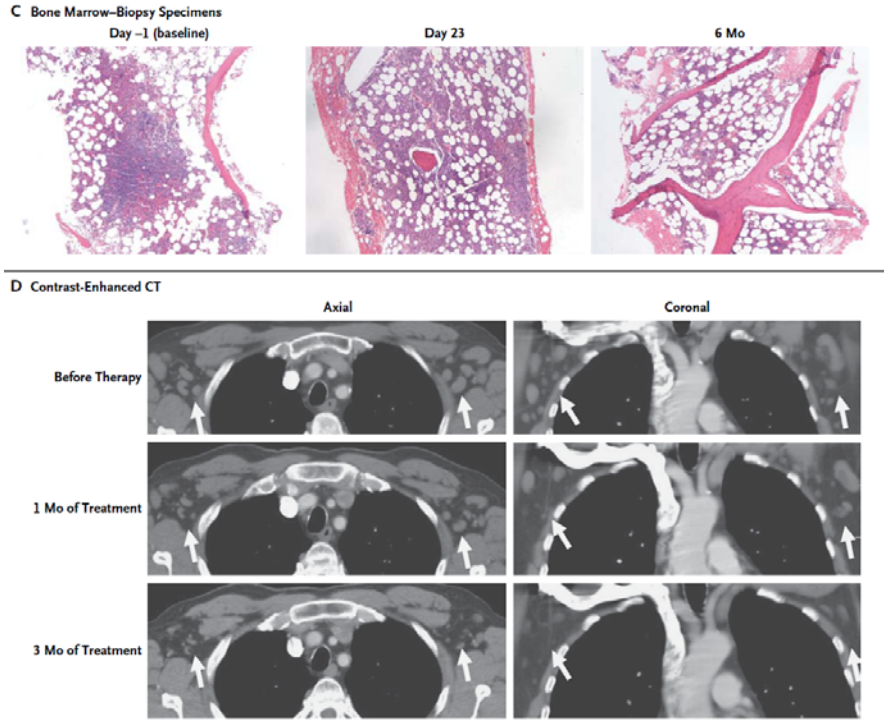
Blood. 2011 Aug 17. [Epub ahead of print]



Signaling through chimeric CD137 results in anti-tumor responses







Sci Transl Med. 2011 Aug 10;3(95):95ra73

N Engl J Med. 2011 Aug 25;365(8):725-33.

Comparing clinical effects of CD19-specific CAR$^+$ T cells

Institute	CD19$^+$ Disease	Clinical Trial.gov	Gene Transfer Method	Extracellular Scaffold	scFv clone	CAR Signaling	Loss of normal B cells?
U Penn	CLL	NCT01029366	Lentivirus	CD8alpha	FMC63	CD137 and CD3-zeta	YES
NCI	Follicular Lymphoma	NCT00924326	Retrovirus		FMC63	CD28 and CD3-zeta	YES
MSKCC	CLL and B-ALL	NCT00466531 and NCT01044069	Retrovirus	CD8alpha	SJ25C1	CD28 and CD3-zeta	YES
BCM	B-NHL or CLL	NCT00586391	Retrovirus	IgG1 Fc	FMC63	CD3 zeta vs. CD28 and CD3-zeta	YES
COH	Follicular Lymphoma	NCT00182650	Electroporation	IgG4 Fc	FMC63	CD3 zeta	NO

NIH STRAP grant will compare

- Common pool of patients with CLL (one protocol)
- Admixture of 1:1
  - T cells manufactured at MSKCC
    - Signaling through CD28
  - T cells manufactured at U PN
    - Signaling through CD137

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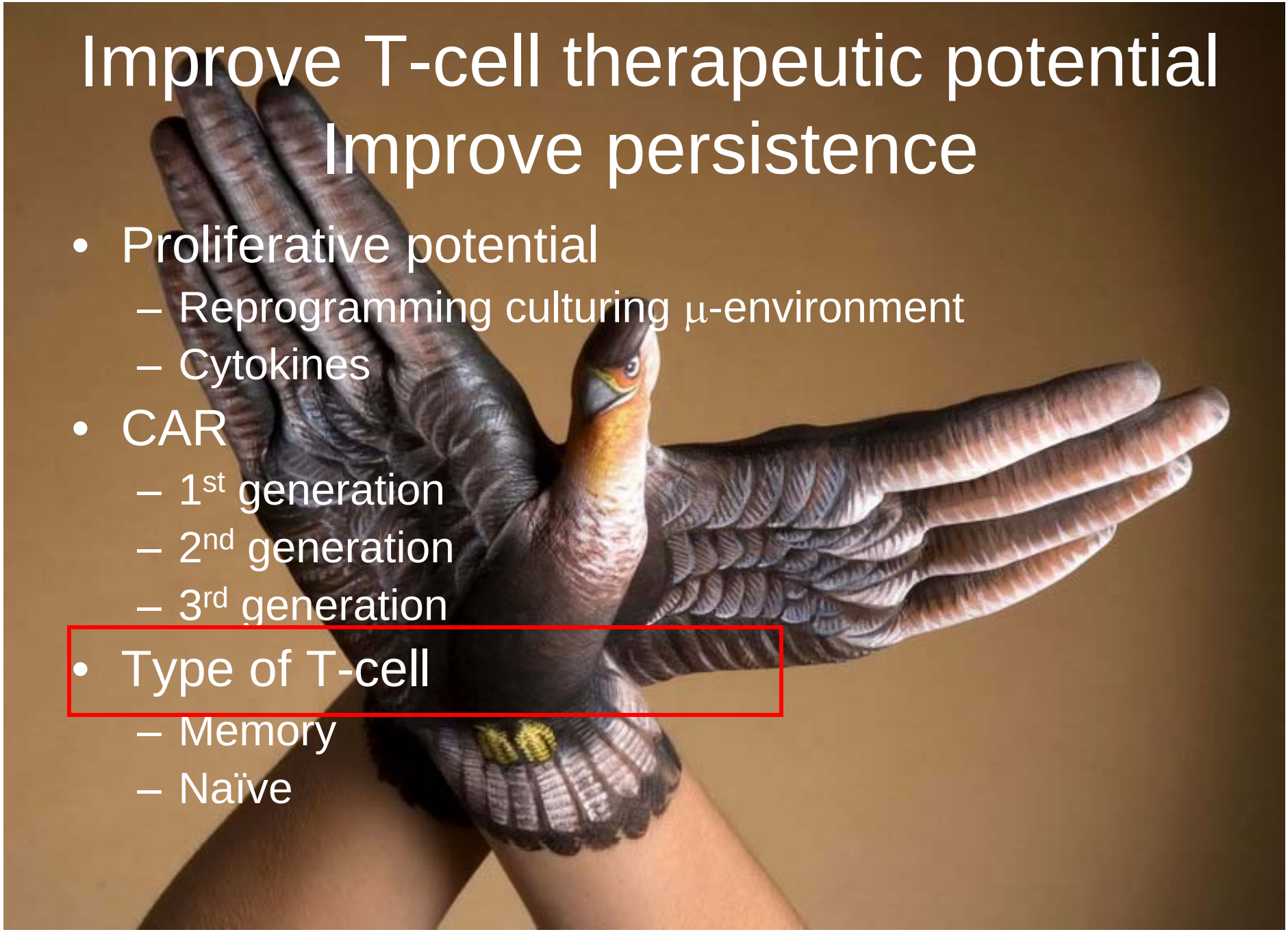

Which T-cell sub-population to genetically modify?

Research article

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

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[1]Fred Hutchinson Cancer Research Center, Seattle, Washington, USA. [2]Division of Cancer Immunotherapeutics and Tumor Immunology, City of Hope National Medical Center, Duarte, California, USA. [3]Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, British Columbia, Canada. [4]Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada. [5]University of Washington National Primate Center, Seattle, Washington, USA. [6]Department of Medicine, University of Washington, Seattle, Washington, USA.

The adoptive transfer of antigen-specific T cells that have been expanded ex vivo is being actively pursued to treat infections and malignancy in humans. The T cell populations that are available for adoptive immunotherapy include both effector memory and central memory cells, and these differ in phenotype, function, and homing. The efficacy of adoptive immunotherapy requires that transferred T cells persist in vivo, but identifying T cells that can reproducibly survive in vivo after they have been numerically expanded by in vitro culture has proven difficult. Here we show that in macaques, antigen-specific CD8+ T cell clones derived from central memory T cells, but not effector memory T cells, persisted long-term in vivo, reacquired phenotypic and functional properties of memory T cells, and occupied memory T cell niches. These results demonstrate that clonally derived CD8+ T cells isolated from central memory T cells are distinct from those derived from effector memory T cells and retain an intrinsic capacity that enables them to survive after adoptive transfer and revert to the memory cell pool. These results could have significant implications for the selection of T cells to expand or to engineer for adoptive immunotherapy of human infections or malignancy.

Introduction

Studies in rodents have demonstrated that adoptive immunotherapy with antigen-specific CD8+ cytotoxic T cells is effective for cancer and infections, and there is evidence that this approach has therapeutic activity in humans (1–8). For clinical applications, T cells of a desired antigen specificity are isolated or engineered to express receptors that target infected or transformed cells and are then expanded in culture (9–14). In some settings the transfer of cloned T cells has been used to provide precise control of specificity and avoid toxicity. For example, in allogeneic stem cell transplantation, the administration of donor-derived T cell clones that target pathogens or malignant cells in the recipient can avoid graft-versus-host disease, which occurs with the infusion of unselected polyclonal donor T cells (3, 4, 15). However, the efficacy of adoptive immunotherapy in humans is often limited by the failure of cultured T cells, particularly cloned CD8+ T cells, to persist in vivo (16, 17), and insight into the basis for the poor survival of the transferred cells is lacking.

The pool of lymphocytes from which CD8+ T cells for adoptive immunotherapy can be derived includes naive T cells (T_N) and antigen-experienced memory T cells (T_M), which can be divided into central memory (T_{CM}) and effector memory (T_{EM}) subsets that differ in phenotype, homing, and function (18). CD8+ T_{CM} express CD62L and CCR7, which promote migration into LNs and proliferate rapidly if reexposed to antigen (19). CD8+ T_{EM} lack CD62L, enabling migration to peripheral tissues, and exhibit immediate effector function (19). In response to antigen stimulation, both CD8+ T_{CM} and T_{EM} proliferate and differentiate into CD62L− cytolytic effector T cells (T_E) that express high levels of granzymes and perforin but are short lived (20). Thus acquisition of an effector phenotype during culture has been suggested as a major reason for the poor survival of transferred T cells (9).

In the normal host, T cell memory persists for life, indicating that some T_M cells may have the ability to self-renew or revert to the memory pool after differentiating to T_E in response to repeated antigen exposure (21). T_{CM} and T_{EM} have distinct phenotypic and functional properties, but it is unknown whether T_E cells derived from each of these T_M subsets retain any intrinsic properties of the parental cell. Using a nonhuman primate model relevant to human translation, we sought to determine whether T_E clones derived from purified T_{CM} or T_{EM} differed in their ability to persist in vivo or establish T cell memory after adoptive transfer. Here we show that antigen-specific CD8+ T_E clones derived from the T_{EM} subset of T_M survive in the blood for only a short duration after adoptive transfer, fail to home to LNs or BM, and do not reacquire phenotypic markers of T_M. By contrast, T_E clones derived from T_{CM} persist long term after adoptive transfer, migrate to T_M niches, reacquire phenotypic properties of T_M, and respond to antigen challenge.

Results

Characterization of CMV-specific CD8+ T cell clones from CD62L+ T_{CM} and CD62L− T_{EM} subsets. Immunocompetent *Macaca nemestrina* with latent CMV infection were used in this study. We identified CMV epitopes recognized by CD8+ T cells in individual macaques by stimulating aliquots of PBMCs with CMV immediate early 1 (IE-1)

Nonstandard abbreviations used: IE, immediate early (protein); ΔLNGFR, intracellular truncated low-affinity nerve growth factor receptor; TAPC, antigen-presenting T cells; T_{CM}, central memory T cells; T_E, effector T cells; T_{EM}, effector memory T cells; T_M, memory T cells; T_N, naive T cells.

Conflict of interest: P.M. Lansdorp is a founding shareholder in Repeat Diagnostic Inc.

Citation for this article: *J. Clin. Invest.* **118**:294–305 (2008). doi:10.1172/JCI32103.

PNAS PNAS PNAS

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

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[a]National Cancer Institute, Bethesda, MD 20892; [b]National Heart, Lung, and Blood Institute, Bethesda, MD 20824; and [c]Functional Genomics and Proteomics Facility, Critical Care Medicine Department, National Institutes of Health, Bethesda, MD 20892

Edited by Philippa Marrack, Howard Hughes Medical Institute/National Jewish, Denver, CO, and approved August 24, 2009 (received for review July 9, 2009)

Effector cells derived from central memory CD8+ T cells were reported to engraft and survive better than those derived from effector memory populations, suggesting that they are superior for use in adoptive immunotherapy studies. However, previous studies did not evaluate the relative efficacy of effector cells derived from naïve T cells. We sought to investigate the efficacy of tumor-specific effector cells derived from naïve or central memory T-cell subsets using transgenic or retrovirally transduced T cells engineered to express a tumor-specific T-cell receptor. We found that naïve, rather than central memory T cells, gave rise to an effector population that mediated superior antitumor immunity upon adoptive transfer. Effector cells developed from naïve T cells lost the expression of CD62L more rapidly than those derived from central memory T cells, but did not acquire the expression of KLRG-1, a marker for terminal differentiation and replicative senescence. Consistent with this KLRG-1− phenotype, naïve-derived cells were capable of a greater proliferative burst and had enhanced cytokine production after adoptive transfer. These results indicate that insertion of genes that confer antitumor specificity into naïve rather than central memory CD8+ T cells may allow superior efficacy upon adoptive transfer.

Infusion of tumor-reactive T cells to treat cancer is transitioning from a promising possibility to a successful reality. Adoptive immunotherapy with T cells can effectively treat patients with EBV-associated malignancies and metastatic melanoma, and application of this treatment is broadening as our ability to generate T cells targeting diverse tumor antigens improves (1–10). Our expanding capacity to target novel antigens is driven, in part, by advances in genetic engineering that permit high efficiency transfer of genes encoding tumor specific T-cell receptors (TCR) into open repertoire mature T cells. These genetically modified T cells can specifically recognize tumor cells in vitro, and can induce objective tumor regression following infusion into patients (9).

The ability to engineer tumor recognition permits not only targeting of any antigen for which a specific TCR can be identified, but also selection of the CD8+ T-cell subset from which the cells for therapy will be generated. Resting CD8+ T cells exist as naïve (T_N), central memory (T_{CM}), and effector memory (T_{EM}) populations, each with distinct phenotypic and functional characteristics (11). In vitro stimulation of these subsets induces their proliferation and differentiation into the cytolytic effector cells (T_{EFF}) used for patient treatment. While the nature of CD8+ T-cell subsets is well defined (12), the heritable influence of those populations on the traits of their effector cell progeny is not well studied (13, 14). Understanding this relationship might be important for generating optimal effector cells for patient treatment.

The characteristics of CD8+ T-cell subsets have been elucidated primarily through study of viral infection (15–17). In this setting, memory cells are superior to naïve cells due to their increased precursor frequency (18), their rapid proliferation and their efficient acquisition of effector functions (12). However, these qualities might not be advantageous for adoptive immunotherapy where the precursor frequency is determined by the number of cells infused, and differentiation into effector cells occurs before cell infusion. Indeed, recent studies intimate this possibility; in nonhuman primates, induction of effector memory cells has been uniquely successful in protecting from simian immunodeficiency virus (19) yet, in another macaque study, adoptively transferred effector cells generated from effector memory cells rapidly perished (20).

Previous studies on the influence of CD8+ T cell differentiation states have not focused on the relative efficacy of naïve T cells (20–23). With the emergence of TCR gene therapy, naïve cells, which represent the most common CD8+ T-cell phenotype in many patients, have become an important potential source of effector cells. Herein we investigate the lineage relationship and therapeutic efficacy of effector cells of naïve or central memory origin, and we report the superior efficacy of effector cells derived directly from naïve T cells for adoptive immunotherapy of cancer.

Results

We used the pmel-1 TCR transgenic model of adoptive immunotherapy to study the development, function, and efficacy of tumor specific effector cells differentiated from naïve or central memory progenitors. This model reproduces the clinical challenge of breaking tolerance to a shared tumor/self antigen to induce regression of large, established tumors (24). Tumor-specific CD8+ T-cell populations enriched for T_N or T_{CM} phenotype cells were isolated from pmel-1 splenocytes (Fig. S1). These cells displayed not only the phenotypic but also the functional qualities ascribed to naïve and central memory cells as, in response to antigenic stimulation, IFN-γ production and proliferation were more efficient in the T_{CM} subset (Fig. 1 *A* and *B*) (12). Emulating clinical protocol, effector CD8+ T cells were generated from each population by two stimulations (Fig. 1*C*) (9). For simplicity, effector cells of naïve or central memory origin were termed T_{EFF}^{N} and T_{EFF}^{CM}, respectively.

Effector Cells Generated from Naïve or Central Memory Cells Acquire Cytolytic Effector Cell Phenotype and Function. Both T_{EFF}^{N} and T_{EFF}^{CM} demonstrated high levels of specific target killing consistent with effector CD8+ T-cell function (Fig. 1*D*). They also expressed the cytolytic granule proteins that typify effector cells, perforin 1,

Author contributions: C.S.H., Z.A.B., L.S.-P., R.L.D., S.A.R., and N.P.R. designed research; C.S.H., Z.A.B., L.C., L.G., R.S., Z.Y., L.S.-P., P.M., C.L., D.C.P., Y.J., R.N.R., W.J.L. performed research; C.S.H., Z.A.B., S.J.K., R.L.D., and N.P.R. analyzed data; and C.S.H., Z.A.B., and N.P.R. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/cgi/content/full/0907448106/DCSupplemental.

Improve T-cell therapeutic potential
Improve persistence

- Factors that influence persistence
  - Recipient
  - T cell

Manipulating…

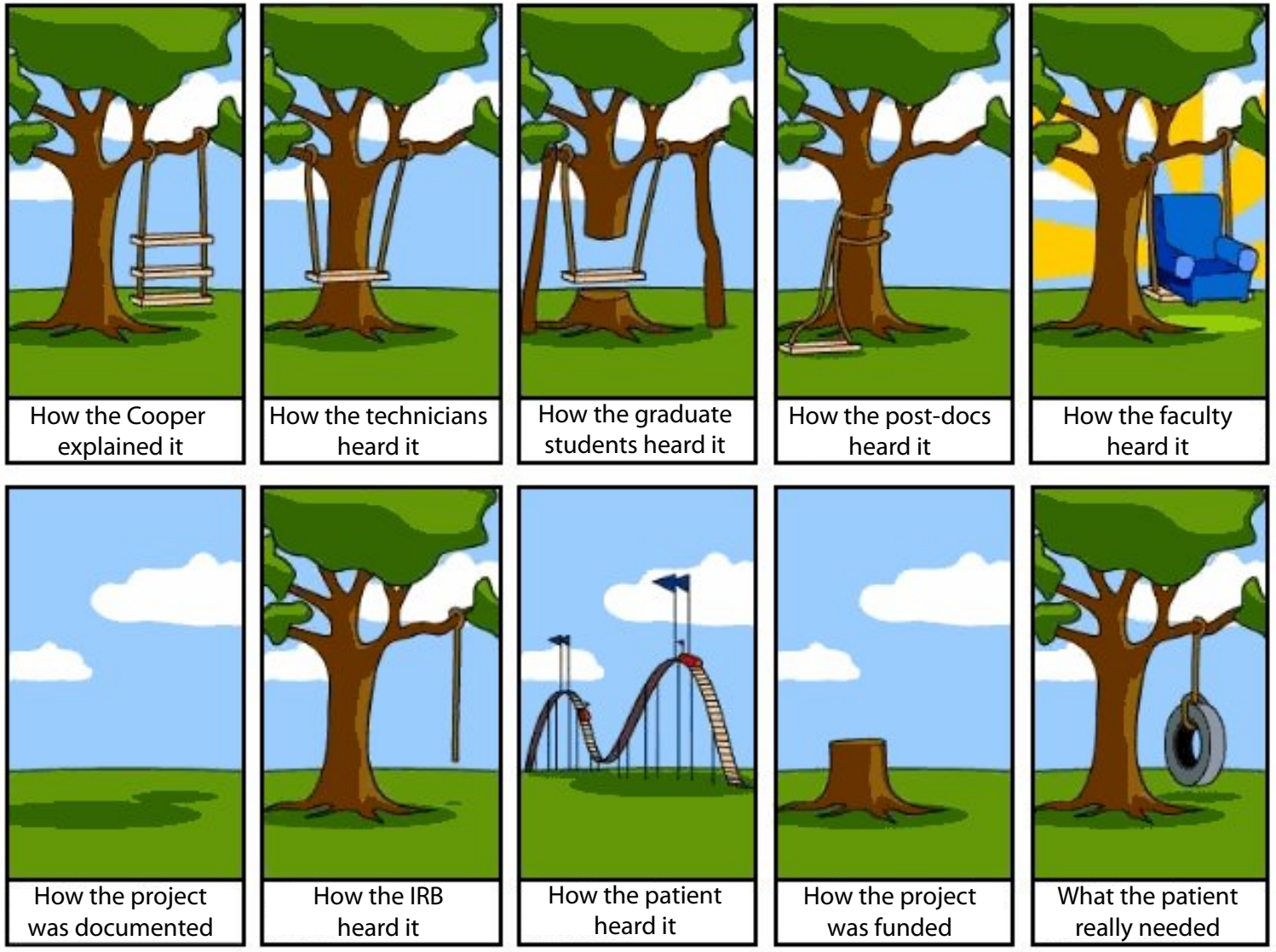
- Recipient
  - Lymphoablation
  - Cytokines (supraphysiologic dosing)
    - IL-2, IL-7, IL-15, IL-21
- T cells
  - CAR
    - Endodomains
  - Cellular substrate
    - Memory, “stem cell”, naïve
    - “Bi-specific” T cells
  - Co-stimulation for improved potency and homing
    - Cytokines and receptors, chemokine receptors

Clinical application of *SB* system

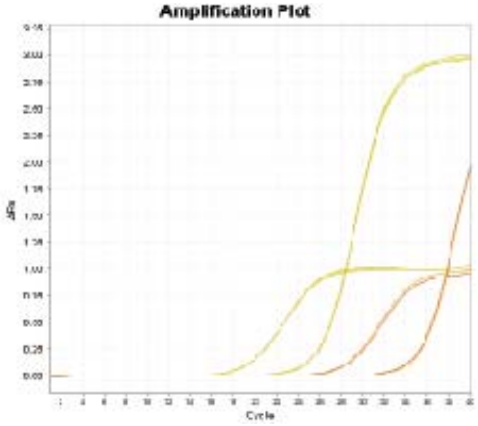
Non-viral gene transfer

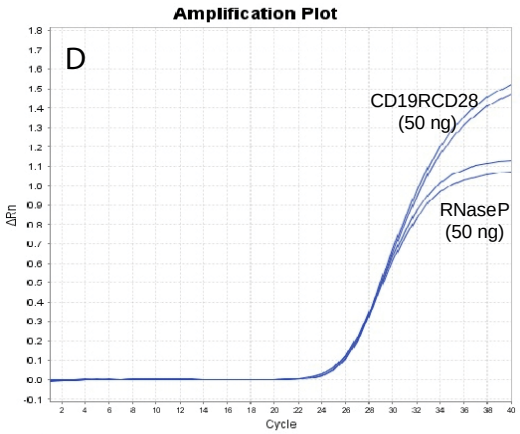
- DNA plasmids are less expensive to produce and require less sophisticated infrastructure compared with producing clinical grade recombinant retrovirus
- Facilitates design and redesign of CAR (other transgenes)
- Two transposons can be synchronously electrotransferred
  - Produce CAR^+ T cells and CAR^+TK^+ T cells for PET imaging (and conditional ablation)

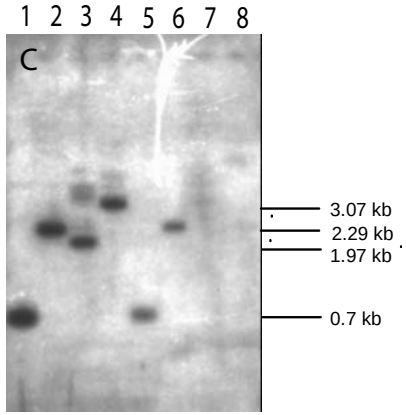
Applied Cellular Therapy (ACT)

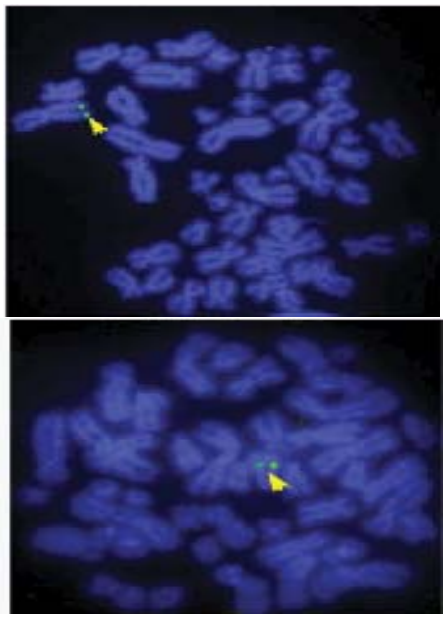


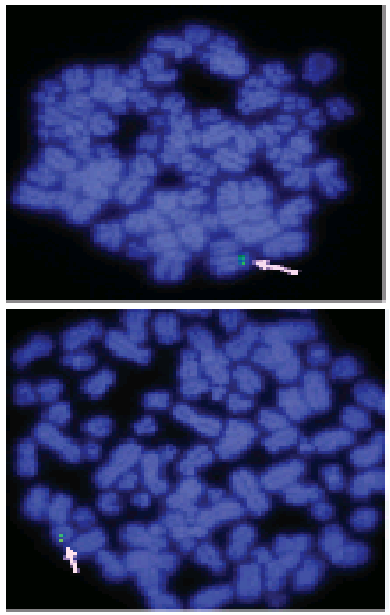
Single integration of *SB* transposon

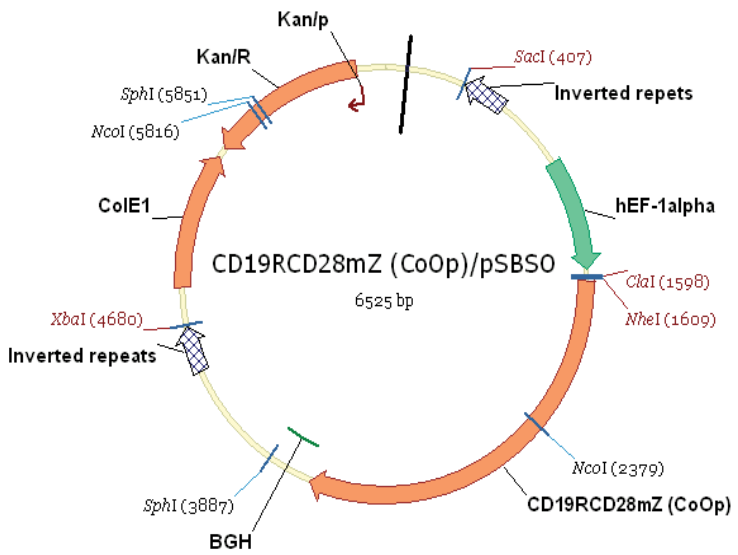










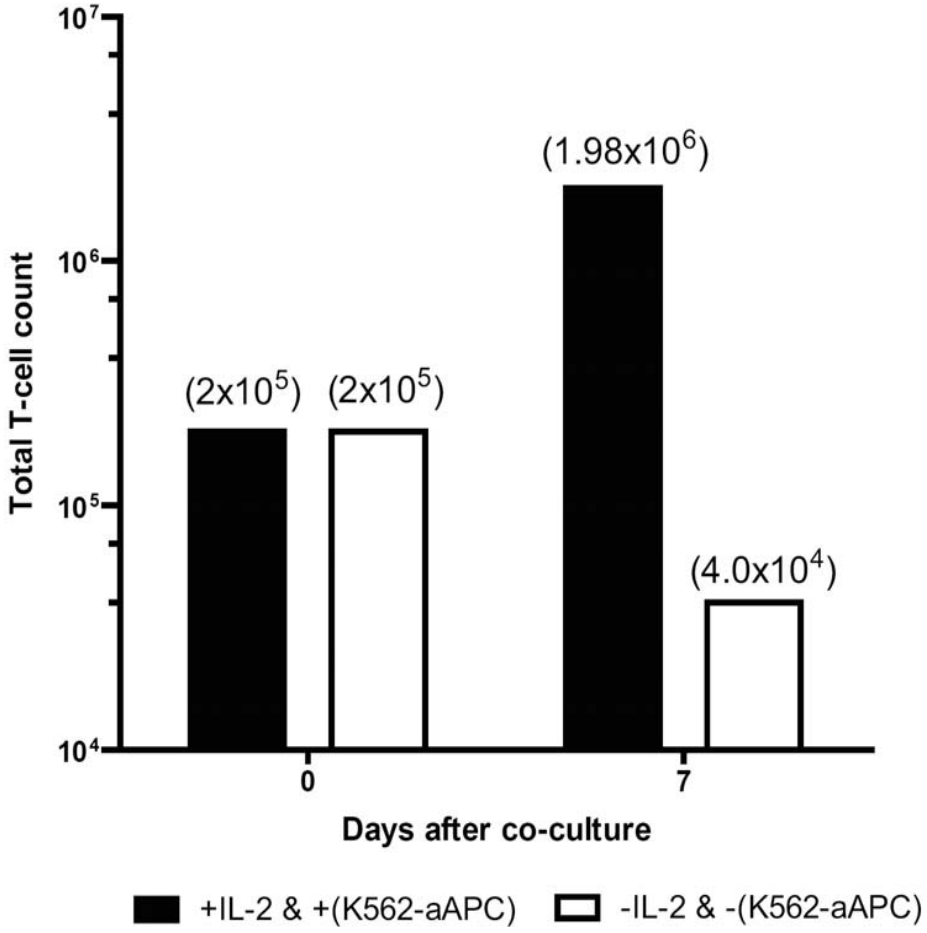


Preclinical Integration Statistics

- 33 individual samples
  - Raw sequence overlap among samples **< 5%**

- Total = 7,436,108 raw reads
  - Unique reads **687,176 (9.24 %)**
    - **IR/DR present** **99.998 %**
    - **TA present** **99. 9%**
  - Human Genomic Sequence Matches **87 %**
  - Mapped insertion Sites **>11,000**
  - Insertions in Intergenic Regions **56 %**
  - Insertions within a Gene **44 %**
    - **Intronic** **96.5 %**
    - **Exonic** **3.5 % (mostly non-coding)**
  - Insertion in Repeat Regions **>25,000**
- No obvious hot spots
- SB insertions into TSS correlates with quiescent T cell expression profile (vs activated T cell profile for retroviral transductions)
- Preference for AT rich regions (gene poor regions)
- Preference for repeat/replicated regions (≥50% of genome)

Absence of T-cell autonomous growth

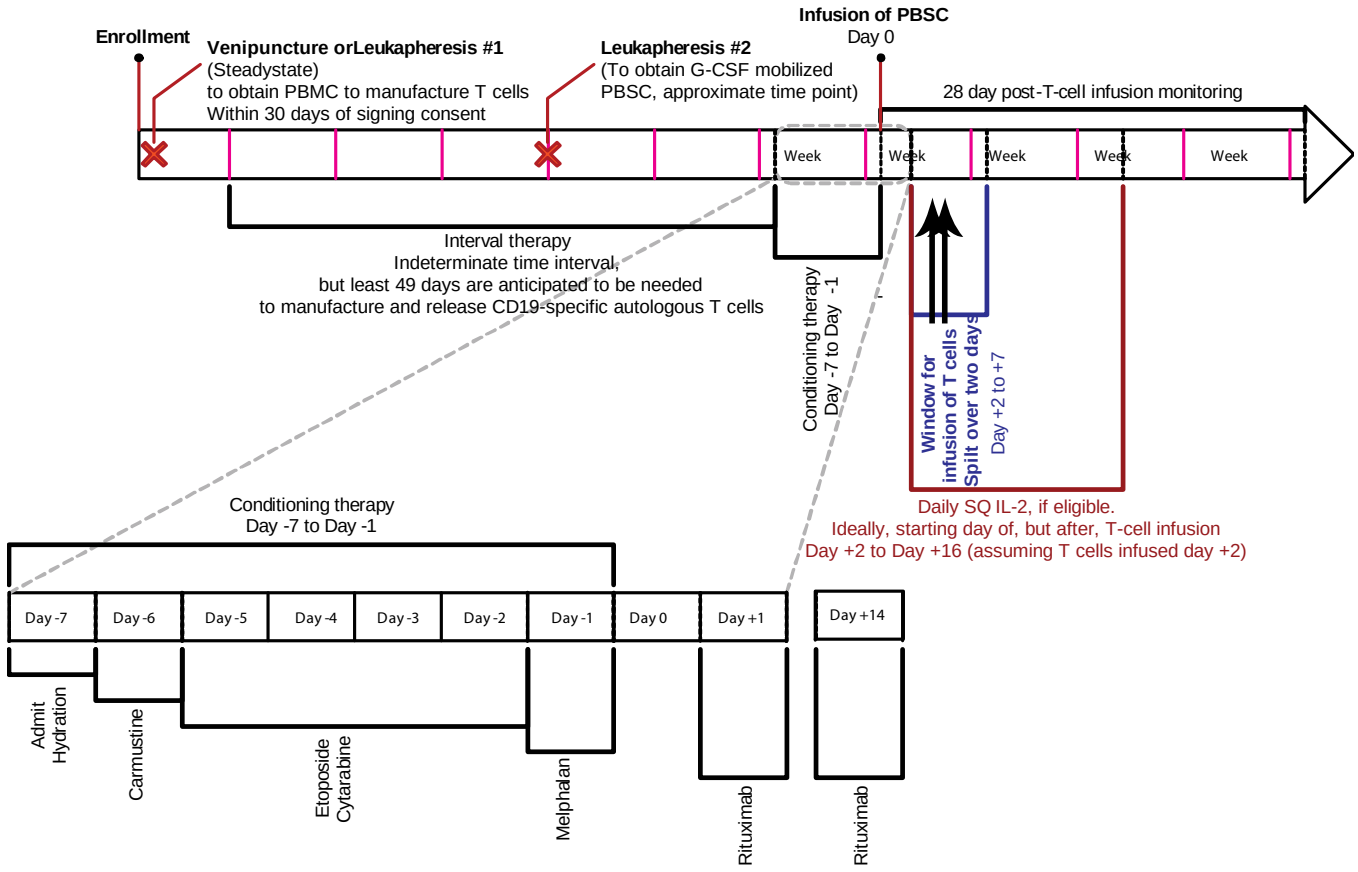


Manufacturing T cells

Trial design

Principal Investigator = Dr. Partow Kebriaei

Time Line for IRB #2007-0635



CAR^{+} T-cell trials at MDACC

Trial	Agent	Preclinical	NIH-OBA	IND
ALL and lymphoma	Autologous CD19-specific T cells	→	X	Enrolling
ALL and lymphoma	Allogeneic CD19-specific T cells	→	X	Approved
ALL and lymphoma	Allogeneic CD19-specific UCB-derived T cells	→	X	Approved

Thanks

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Children's Cancer Hospital

University of Minnesota