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InCellerate, Inc. Founder

Adaptive Immunotherapy with T Cells

June 1, 2012

8:00 am – 1:00 pm

Hyatt Regency McCormick Place in Chicago, IL

Laurence J.N. Cooper

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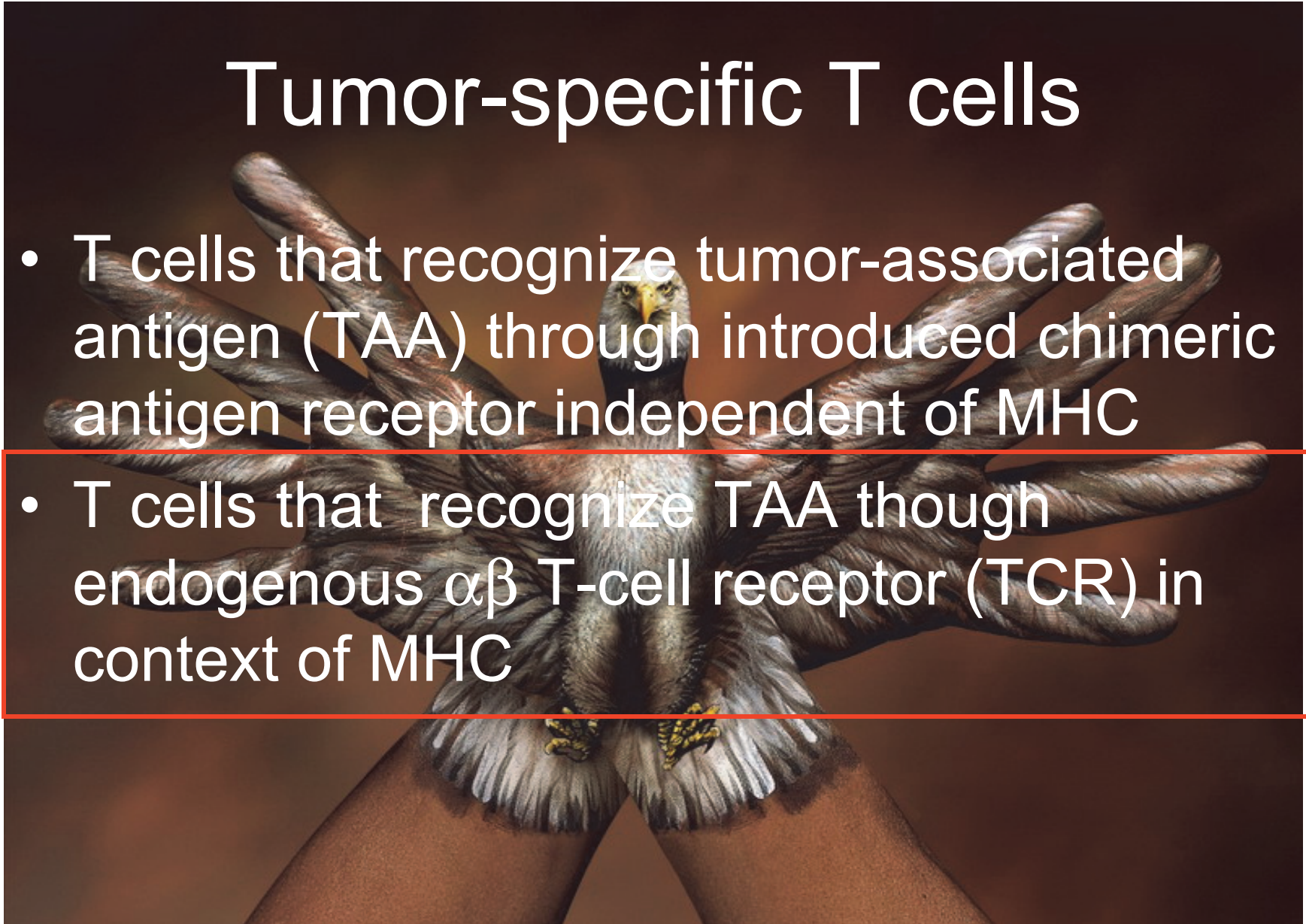
Society for Immunotherapy of Cancer

Bias of this presentation

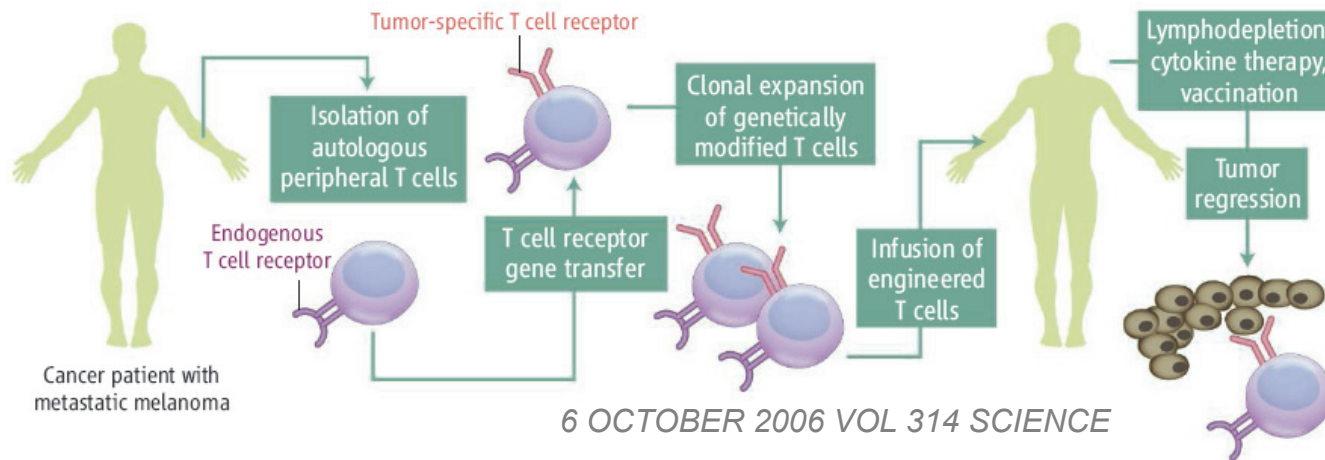
- Combine gene therapy with T-cell therapy to overcome issues of immune tolerance
- Use of mouse and humans to harvest desired immune receptors
- Common platforms for the development and release of T cells with redirected specificity

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 through endogenous $\alpha\beta$ T-cell receptor (TCR) in context of MHC



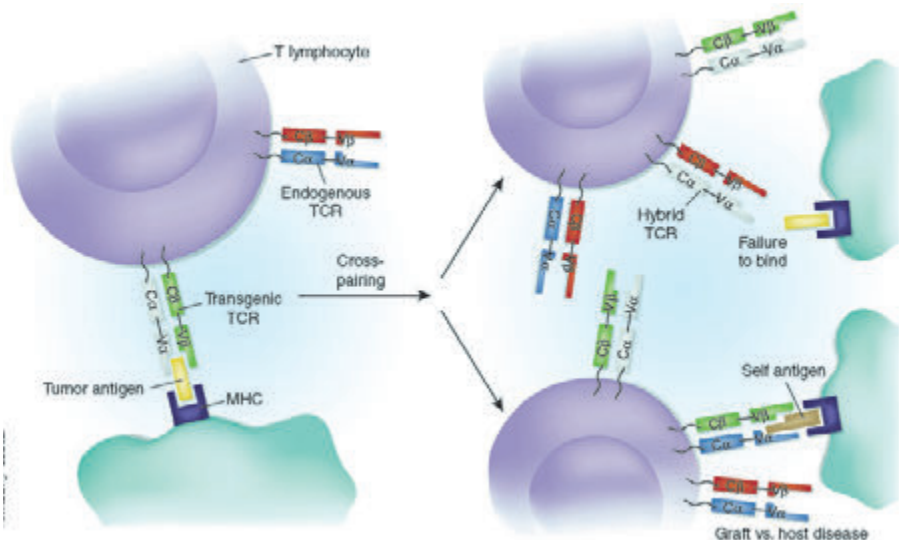
Harvesting and re-expressing melanoma-specific $\alpha\beta$ TCR



TCR gene therapy in patients with metastatic melanoma

Target Antigen	TCR $\alpha\beta$	Patient	Response	CR	PR	Reference
MART-1/A2	human	31	4		4	<i>Science</i> 2006; 314:126-129 <i>Blood</i> . 2009;114:535-546
MART-1/A2	human high avidity	20	6		6 (3, 4, 9, 16+, 17+, 17+)	<i>Blood</i> . 2009;114:535-546
gp100/A2	mouse	16	3	1 (14+)	2 (4, 3)	<i>Blood</i> . 2009;114:535-546
NYESO1/A2	human	11	5	2 (22+, 20+)	3 (3, 8, 9+)	<i>J Clin Oncol</i> 2011;29:917-924

Toxicities from TCR transfer



Nature Medicine 16,520–521(2010)

- GVHD due to the pairing of introduced and endogenous TCR chains in TCR gene-modified T cells in mouse model. *Nature Medicine* 16,565–570(2010)
- On the contrary, there has been no report of GVHD in human clinical trial so far. *Molecular Therapy* (2010) 18,1744–1745
- Further improvement of adoptive T cells therapy (e.g. long-lived T cells) may increase the potential risk of GVHD.

Potential Solutions

- Endogenous TCR can be disrupted by designed ZFN pairs to eliminate potential risk of GVHD *Nature Medicine* (2012) Published online 01 April 2012
- Other approaches to avoid mis-pairing include
 - i) use mouse derived TCR $\alpha\beta$ constant region *Cancer Res* 2006 66(17):8878-86.
 - ii) Cys modification *Blood*.2007;109:2331-2338., *Cancer Res.* 2007 67:3898-3903.
 - iii) siRNA to suppress endogenous TCR $\alpha\beta$ *Cancer Res.* 2009;69:9003-11.
 - iv) TCR $\alpha\beta$ gene transfer to $\gamma\delta$ T cells. *Cancer Res* 2006;66(6):3331-7.

Fratricide

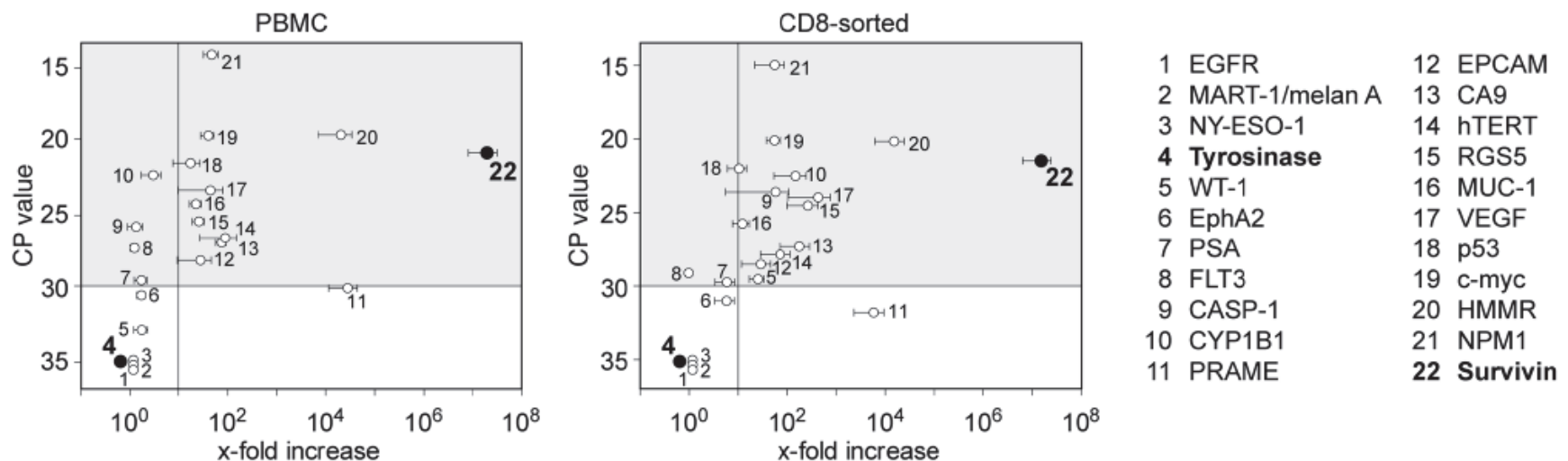
J Clin Invest. 2010;120(11):3869–3877.

MHC-restricted fratricide of human lymphocytes expressing survivin-specific transgenic T cell receptors

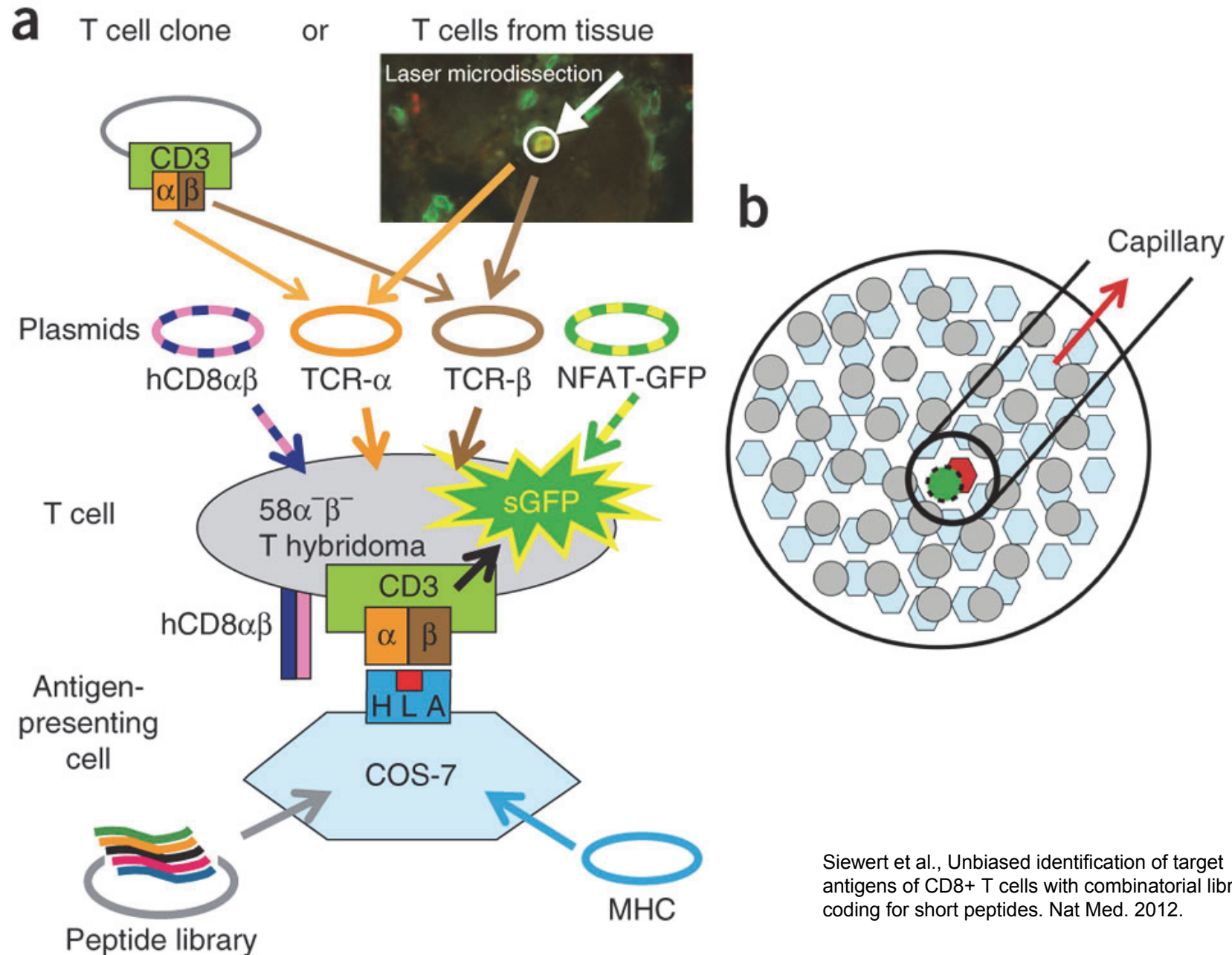
Matthias Leisegang,¹ Susanne Wilde,² Stefani Spranger,² Slavoljub Milosevic,² Bernhard Frankenberger,² Wolfgang Uckert,^{1,3} and Dolores J. Schendel^{2,4}

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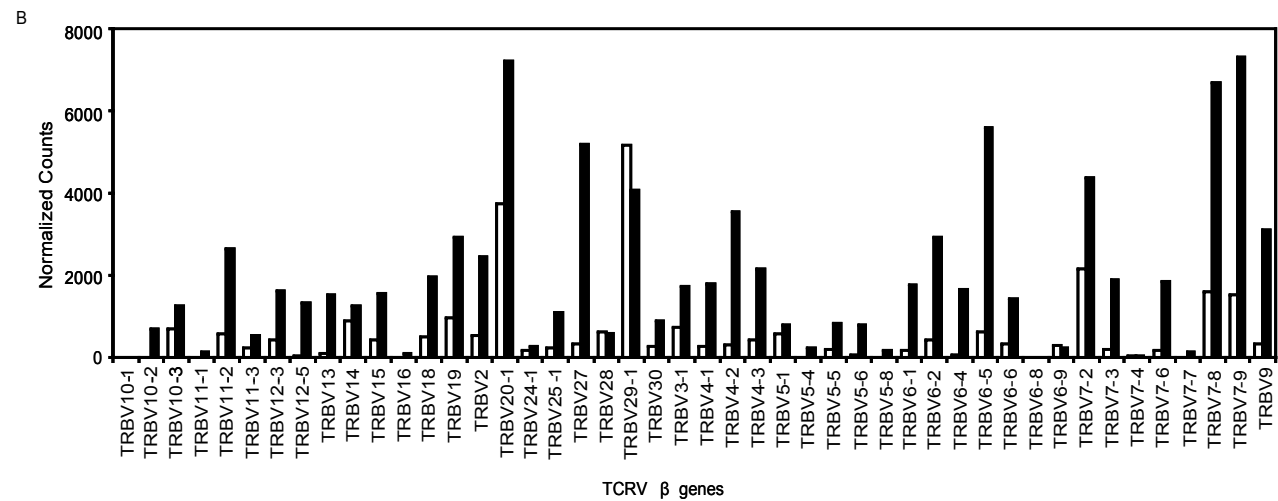
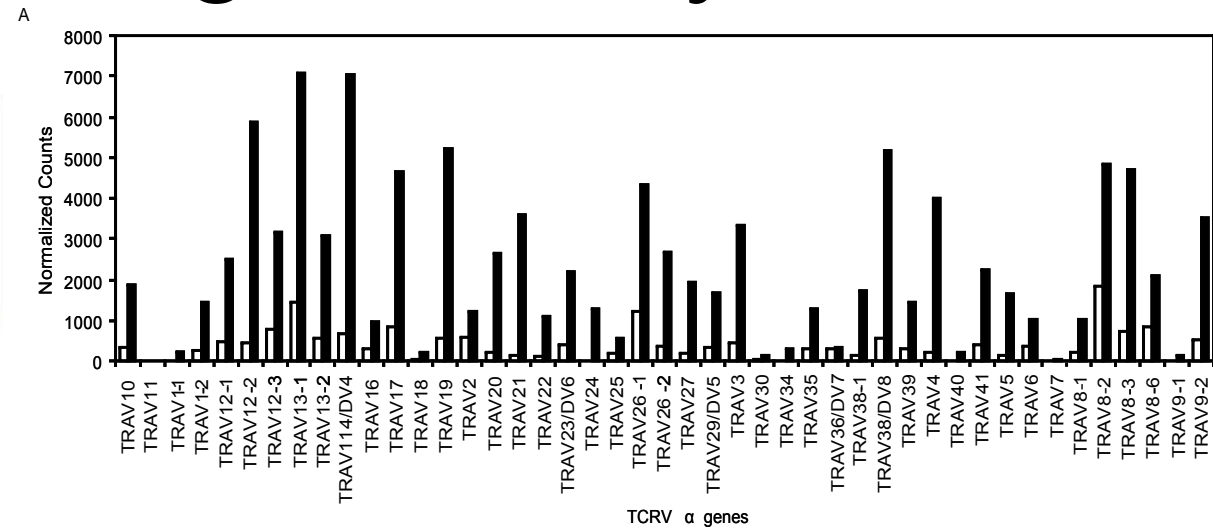
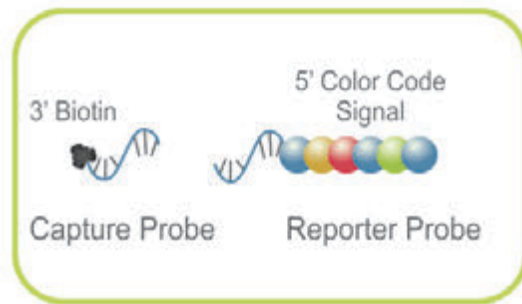


Identification of target antigens for CD8⁺ T cells



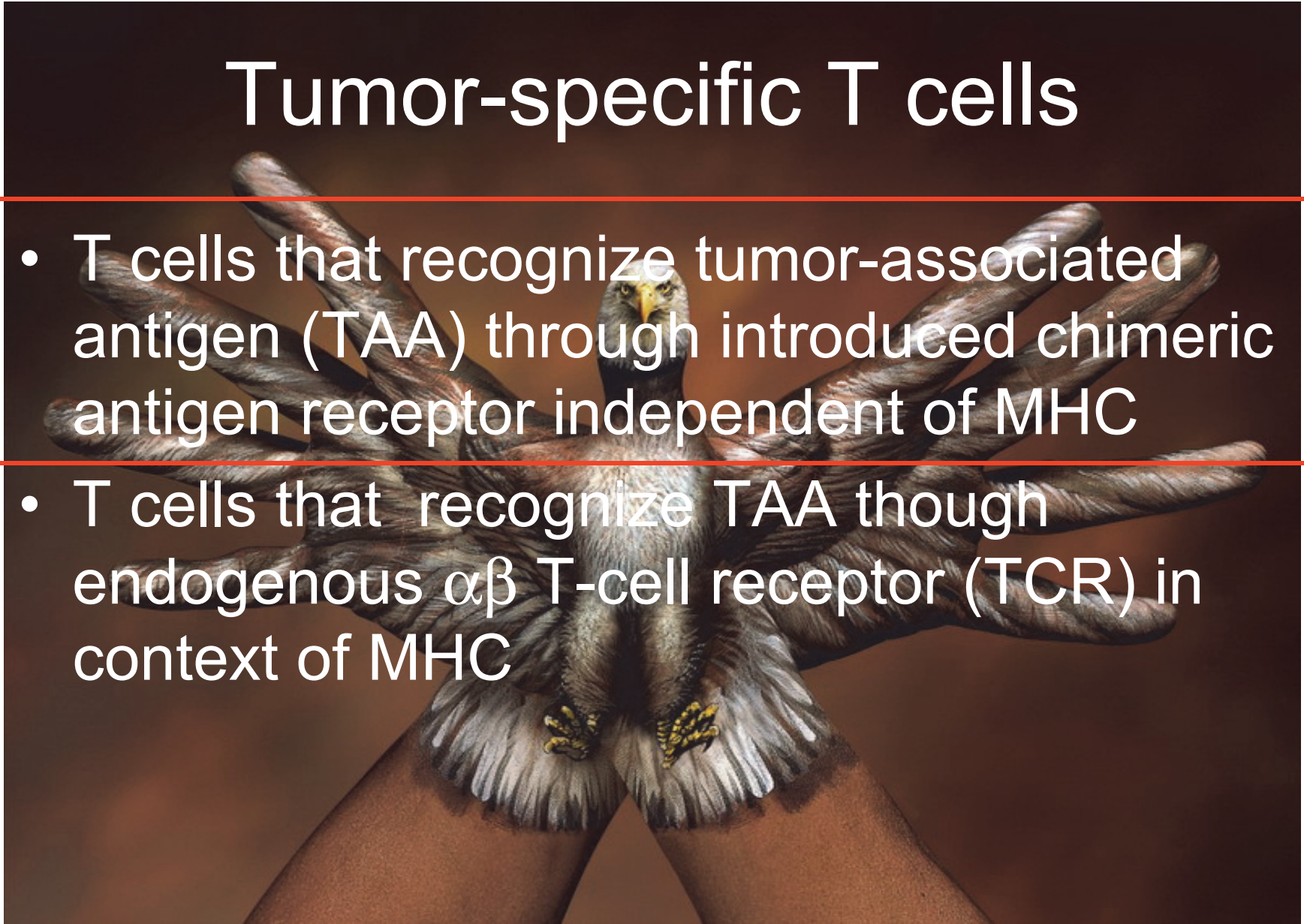
Siewert et al., Unbiased identification of target antigens of CD8⁺ T cells with combinatorial libraries coding for short peptides. Nat Med. 2012.

Oligoclonality



Tumor-specific T cells

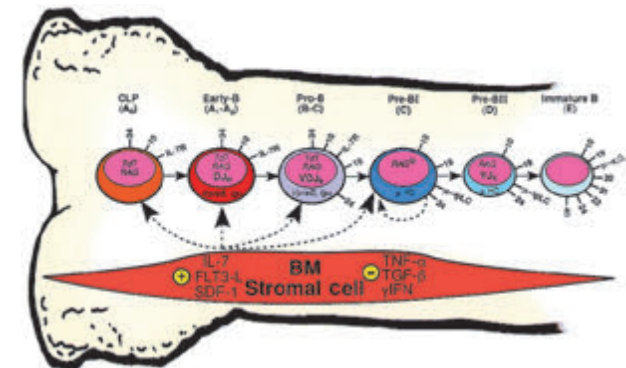
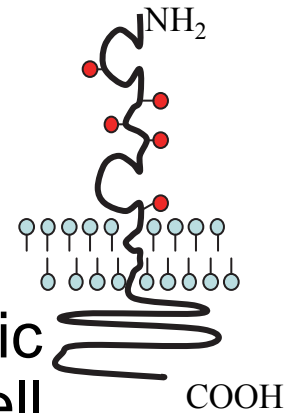
- T cells that recognize tumor-associated antigen (TAA) through introduced chimeric antigen receptor independent of MHC
- T cells that recognize TAA through endogenous $\alpha\beta$ T-cell receptor (TCR) in context of MHC



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.

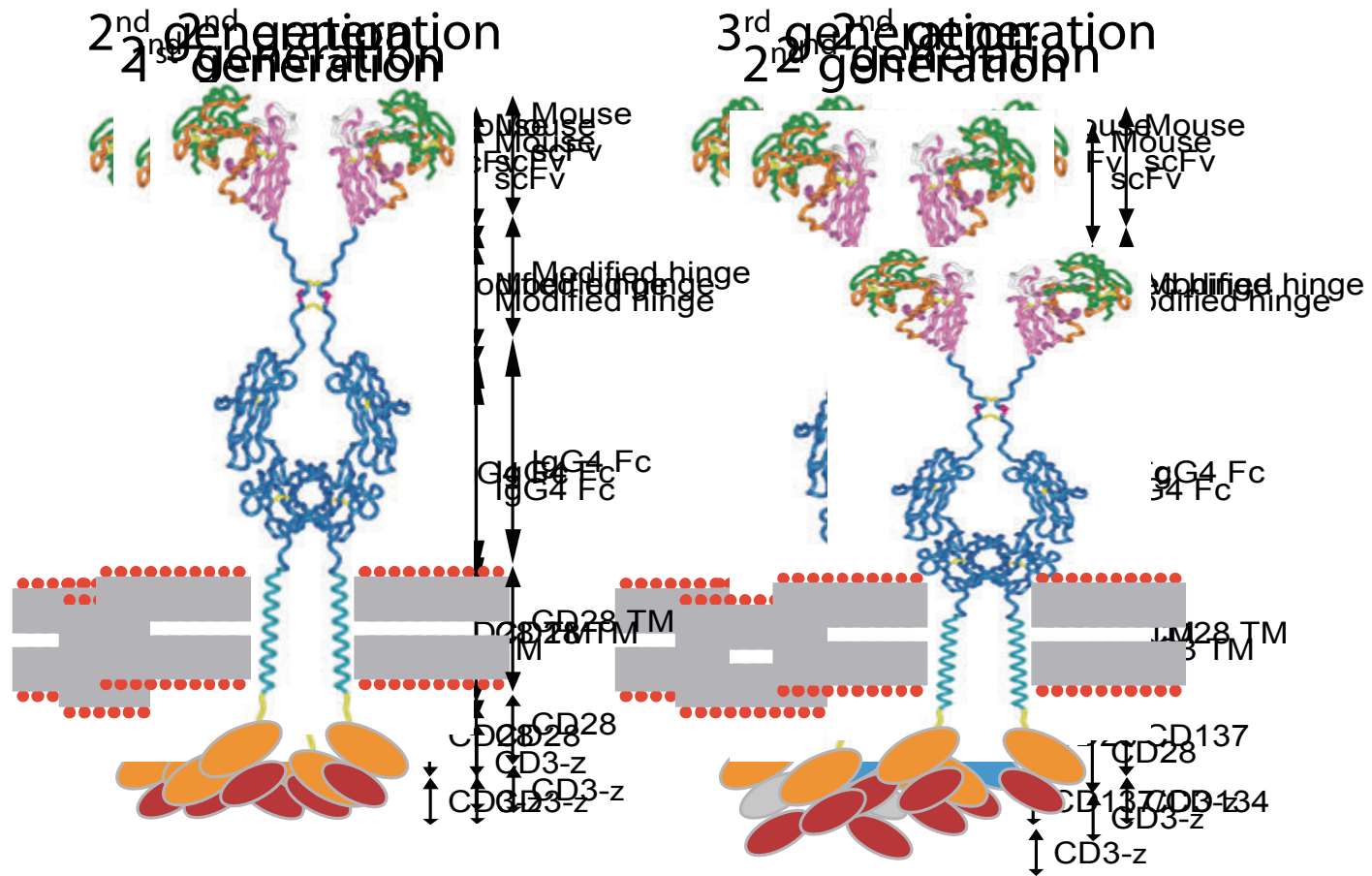


Clinical trials in USA infusing CAR⁺ T cells

	Antigen	Tumor target	Viral-specific T cell	Lympho-depletion	CAR generation	ClinicalTrial.gov identifier	Enrolling	SAE	Gene transfer
1	Kappa light chain	B-NHL and B-CLL	No	Yes	First and second	NCT00881920	Yes	TBM	Virus
2	CD19	Lymphoma/leukemia (B-NHL) and CLL	No	No	First and second	NCT00586391	Yes	TBM	Virus
3	CD19	Advanced B-NHL/CLL	Yes	No	First and second	NCT00709033	Yes	TBM	Virus
4	CD19	Lymphoma and leukemia	No	Yes	Second	NCT00924326*	Yes	TBM	Virus
5	CD19	ALL (post-HSCT)	Yes	No	Second	NCT00840853	Yes	TBM	Virus
6	CD19	Follicular NHL	No	Yes	First	NCT00182650	No	No	Electroporation
7	CD19	CLL	No	Yes/no	Second	NCT00466531*	Yes	Yes (1 Death)	Virus
8	CD19	B-NHL/leukemia	No	Yes	First and second	NCT00891215	Yes	TBM	Virus
9	CD19	B-cell leukemia, CLL and B-NHL	No	No	Second	NCT01087294	Yes	TBM	Virus
10	CD19	B-ALL	No	Yes	Second	NCT01044069	Yes	TBM	Virus
11	CD19	B-lymphoid malignancies	No	Yes	Second	NCT00968760	No	TBM	Electroporation (SB system)
12	CD20	Relapsed/refractory B-NHL	No	Yes	First	NCT00012207*	No	No	Electroporation
13	CD20	Mantle cell lymphoma or indolent B-NHL	No	Yes	Third	NCT00621452	Yes	TBM	Electroporation
14	GD ₂	Neuroblastoma	Yes	Yes	First	NCT00085930*	Yes	No	Virus
15	CEA	Adenocarcinoma	No	No	First	NCT00004178	No	No	Virus
16	PSMA	Prostate cancer	No	Yes	First	NCT00664196	Yes	TBM	Virus
17	CD171/L1-CAM	Neuroblastoma	No	No	First	NCT00006480	No	No	Electroporation
18	FR	Ovarian epithelial cancer	No	No	First	NCT00019136	No	No	Virus
19	CEA	Stomach carcinoma	No	No	Second	NCT00429078	Yes	TBM	Virus
20	CEA	Breast cancer	No	No	Second	NCT00673829	Yes	TBM	Virus
21	CEA	Colorectal carcinoma	No	No	Second	NCT00673322	Yes	TBM	Virus
22	IL-13R α 2	Glioblastoma	No	NA	Second	NCT00730613	No	No	Electroporation
23	ERBB2 (HER2/neu)	Metastatic cancer	No	Yes	Third	NCT00924287	No	Yes (1 Death)	Virus
24	HER2/neu	Lung malignancy	Yes	No	Second	NCT00889954	Yes	TBM	Virus
25	HER2/neu	Advanced osteosarcoma	No	No	Second	NCT00902044	Yes	TBM	Virus

Blood. 2010 Aug 19;116(7):1035-44.

CAR design



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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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 *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 vitro* (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 recipients can avoid graft-versus-host disease, which occurs with the infusion of unselected polyclonal donor T cells (15, 16). 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_{EM}), 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 prolif-

erate rapidly if responded 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 CD45^{RO} cytotoxic 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_H 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_H 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_{CM} subset of T_H 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_H . By contrast, T_E clones derived from T_{EM} persist long term after adoptive transfer, migrate to T_H niches, reacquire phenotypic properties of T_H , and respond to antigen challenge.

Results

Characterization of CMV-specific CD8⁺ T cell clones from CD45^{RO} T_{CM} and CD45^{RO} 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)

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

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Edited by Philippe March, Howard Hughes Medical Institute/National Jewish, Denver, CO, and approved August 28, 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 naive T cells. We sought to investigate the efficacy of tumor-specific effector cells derived from naive 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 naive, rather than central memory T cells, gave rise to an effector population that mediated superior antitumor immunity upon adoptive transfer. Effector cells developed from naive T cells lost the expression of CD45^{RO} 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, naive-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 naive 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 HIV-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 (TCRs) into reproductive mature T cells. These genetically modified T cells can specifically recognize tumor cells *in vivo*, and can induce objective tumor regression following infusion into patients (9).

The ability to engineer tumor recognition, persists 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 naive (T_N), central memory (T_{CM}), and effector memory (T_{EM}) populations, each with distinct phenotypic and functional characteristics (11). *In vivo* stimulation of these subsets induces their proliferation and differentiation into the cytotoxic effector cells (T_E) used for patient treatment. While the nature of CD8⁺ T-cell subsets is well defined (12), the heritable influence of these populations on the traits of their effector cell progeny is not well studied (13, 14). Understanding this relationship may 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 naive 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 (22) 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 status have not focused on the relative efficacy of naive T cells (23–25). With the emergence of TCR gene therapy, naive 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 naive or central memory origin, and we report the superior efficacy of effector cells derived directly from naive T cells for adoptive immunotherapy of cancer.

Results

We used the push I TCR transgenic model of adoptive immunotherapy to study the development, function, and efficacy of tumor-specific effector cells differentiated from naive 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 push I splenocytes (Fig. S1). These cells displayed not only the phenotype but also the functional qualities needed to raise and control memory cells as, in response to antigenic stimulation, IFN- γ production and proliferation were more efficient in the T_{CM} subset (Fig. 1A and B) (12). Evaluating clinical protocols, effector CD8⁺ T cells were generated from each population by two stimulations (Fig. 1C) (9). For simplicity, effector cells of naive or central memory origin were termed T_{E1} and T_{E2} , respectively.

Effector Cells Generated from Naive or Central Memory Cells Acquire Cytotoxic Effector Cell Phenotype and Function. Both T_{E1} and T_{E2} demonstrated high levels of specific target killing consistent with effector CD8⁺ T-cell function (Fig. 1D). They also expressed the cytotoxic granule proteins that typify effector cells, perforin 1,

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

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Which T-cell sub-population to genetically modify?

ARTICLES

nature
medicine



immunity
Article

A human memory T cell subset with stem cell-like properties

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Immunological memory is thought to depend on a stem cell-like, self-renewing population of lymphocytes capable of differentiating into effector cells in response to antigen re-exposure. Here we describe a long-lived human memory T cell population that has an enhanced capacity for self-renewal and a multipotent ability to derive central memory, effector memory and effector T cells. These cells, specific to multiple viral and self-tumor antigens, were found within a CD45RO⁺, CCR7⁺, CD45RA⁺, CD62L⁺, CD27⁺, CD28⁺ and IL-7R α ⁺ T cell compartment characteristic of naive T cells. However, they expressed large amounts of CD95, IL-2R β , CD133, and LFA-1, and showed numerous functional attributes distinctive of memory cells. Compared with known memory populations, these lymphocytes had increased proliferative capacity and more efficiently reconstituted immunodeficient hosts, and they mediated superior antitumor responses in a humanized mouse model. The identification of a human stem cell-like memory T cell population is of direct relevance to the design of vaccines and T cell therapies.

Long-lived self-renewing memory lymphocytes are a hallmark feature of the adaptive immune system in response to pathogens and tumors^{1–3}. In organ systems, nonreplicating, terminally differentiated cells are continually replenished by the progeny of less differentiated stem cells. Similarly, it has been suggested that memory cells might contain stem cell-like cells capable of regenerating fully differentiated effector lymphocytes in response to antigenic stimuli^{4,5}. Indeed, several characteristics of stem cells can be found to certain degrees in memory B and T cells, including selective transcriptional profiles⁶, the capacity for self-renewal and the multipotency to differentiate into progeny with diverse fates^{7,8}.

The memory T cell compartment is heterogeneous and has been conventionally divided into two subsets on the basis of the expression of the lymph node homing molecules CD62L and CCR7 (ref. 7). Central memory T cells (T_{CM} cells) highly express CD62L and CCR7 and were thought to be the stem cell-like memory subset, whereas CD62L⁺CCR7⁺ effector memory T cells (T_{EM} cells) are considered to be committed progenitor cells that undergo terminal differentiation after a limited number of divisions^{9,10}. The recent identification in mice of a population of memory T cells with enhanced stem cell-like qualities compared with conventional T_{CM} cells adds complexity to this dichotomous view¹¹. These memory T cells, which were designated memory stem T cells (T_{MS} cells), have a CD4^{hi}CD25^{hi} phenotype

phenotype similar to that of naive T cells (T_N cells), but they coexpress stem cell antigen-1 (Sca-1) and high levels of the antiapoptotic molecule Bcl-2. The T_{MS} cells also express the β -chain of the IL-2 and IL-15 receptor (IL-2R β), and the chemokine (CXCR3) receptor (CXCR3). Whether a similar memory T cell population exists in humans is currently under intensive investigation¹².

A human CD8⁺ memory T cell population has been described that shares phenotypic and functional characteristics with hematopoietic stem cells including the expression of the stem cell marker α -KIT and the ability to repel cellular toxins through the ATP-binding cassette (ABC)-superfamily multidrug efflux protein ABCB1 (ref. 13). However, recent data revealed that these cells are predominantly V α 2.2⁺ myeloid-associated invariant T cells (MAITs)¹⁴. More recently, others have¹⁵ speculated that CD4^{hi}CD25^{hi} T cells that highly express β -catenin, a molecule associated with the generation of mouse T_{SCM} cells¹¹, represent human T_{SCM} cells, but the definitive identification of human T_{SCM} cells remains to be accomplished.

RESULTS

Identification of human T memory stem cells

We previously found that mouse T_{SCM} cells can be generated efficiently *in vitro* by triggering Wnt signaling during T cell priming using Wnt3A or inhibitors of glycogen synthase kinase-3 β (GSK-3 β)^{16,17}.

A Distinct Subset of Self-Renewing Human Memory CD8⁺ T Cells Survives Cytotoxic Chemotherapy

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DOI: 10.1038/nm.2008.015

SUMMARY

The mechanisms that maintain human T cell memory during normal and perturbed homeostasis are not fully understood. The repeated induction of profound lymphocytopenia in patients undergoing multiple cycles of cytotoxic chemotherapy infrequently results in severe infections with viruses controlled by memory T cells, suggesting that some memory T cells survive chemotherapy and restore immunity. Here, we identified a distinct subpopulation of memory CD8⁺ T cells with the ability to rapidly efflux and survive exposure to chemotherapy drugs *in vitro* and *in vivo*. T cells with high efflux capacity shared expression of molecules with hematopoietic stem cells, were quiescent in nonlymphocytopenic individuals, and were induced to proliferate in patients rendered lymphocytopenic after chemotherapy. Effluxing T cells differentiated into noneffluxing subsets in response to antigen stimulation and inflammatory signals, thereby contributing to repopulation of memory cells after chemotherapy.

INTRODUCTION

A hallmark of adaptive immunity to pathogens is the establishment of long-lived memory T cells that are able to rapidly respond to reinfection and control reactivation of persistent pathogens. After clearance of primary viral infection in mice, CD8⁺ memory T cells remain for the life of the animal (Murak-Krishna et al., 1998). In humans, memory T cells elicited in response to smallpox vaccination persist for 75 years in the absence of re-exposure to the virus (Hammarlund et al., 2003). The durability of T cell memory under normal homeostasis is due in part to slow cell division mediated by cytokines such as IL-15 (Ludger et al., 2002; Zhang et al., 1998), but the mechanisms by which T cell memory is maintained when homeostasis is perturbed by toxic environmental or iatrogenic

clinical infection with CMV or EBV either during the lymphocyte nadir or after recovery of lymphocyte numbers (Sung et al., 2008). The absence of infection in AM, patients undergoing chemotherapy suggests that sufficient virus-specific memory T cells survive chemotherapy and reconstitute functional, long-lived immunity thereafter.

Two broad subsets of memory T cells, termed central memory (T_{CM}) and effector memory (T_{EM}) cells, have been identified that differ in phenotype and function (Sallusto et al., 1999). In humans, these subsets have considerable heterogeneity, which could potentially include subpopulations that serve a distinct role in reconstituting memory T cells after chemotherapy, analogous to the reconstitution of hematopoiesis by hematopoietic stem cells (HSCs). The mechanisms by which HSCs are resistant to chemotherapy are related both to cell quiescence and the overexpression of ATP-binding cassette (ABC) superfamily multidrug efflux proteins that protect cells from toxic xenobiotics and endogenous metabolites (Chaudhary and Rimmann, 1991; Goffman et al., 2002; Maderi et al., 2008). We used ABCB1-mediated efflux of the fluorescent marker rhodamine-123 (Rh123) to determine whether CD8⁺ memory T cells might employ a similar mechanism. We identified a quiescent subpopulation of polyclonal memory CD8⁺ T cells in both T_{CM} and T_{EM} cell fractions that have high multidrug cotransporter activity and a distinct phenotype. The effluxing CD8⁺ T cells contain virus-specific cells, are induced to proliferate during lymphocytopenia, and can self-renew and differentiate into the more prevalent non-effluxing memory subsets. Thus, distinct CD8⁺ memory T cells employ conserved resistance mechanisms utilized by stem cells of diverse origin and exhibit a stem cell-like capacity for self-renewal and differentiation.

RESULTS

CD8⁺ Virus-Specific T Lymphocytes Persist after Cytotoxic Chemotherapy

Patients with AM, treated with chemotherapy that includes ABCB1 substrates such as doxorubicin and etoposide develop profound transient bone marrow hypoplasia and peripheral lymphocyte depletion (Figure 1A) (Bernan et al., 1991). These patients rarely succumb to infection from acute or persistent viruses, suggesting that some CD8⁺ memory T cells resist chemotherapy and replenish the memory T cell pool during lymphocyte recovery. We examined whether CD8⁺ T cells specific for CMV, EBV, and influenza were present in blood obtained from adults after recovery from chemotherapy that included an ABCB1 substrate drug and induced a lymphocyte

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Which T-cell sub-population to genetically modify?



Immunology Article

Th17 Cells Are Long Lived and Retain a Stem Cell-like Molecular Signature

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SUMMARY

Th17 cells have been described as short lived, but this view is at odds with their capacity to trigger protracted damage to normal and transformed tissues. We report that Th17 cells, despite displaying low expression of CD27 and other phenotypic markers of terminal differentiation, efficiently eradicated tumors and caused autoimmunity, were long lived, and maintained a core molecular signature resembling early memory CD8⁺ cells with stem cell-like properties. In addition, we found that Th17 cells had high expression of *Tcf7*, a direct target of the Wnt and β -catenin signaling axis, and accumulated β -catenin, a feature observed in stem cells. In vivo, Th17 cells gave rise to Th1-like effector cell progeny and also self-renewed and persisted as IL-17A-secreting cells. Multipotency was required for Th17 cell-mediated tumor eradication because effector cells deficient in IFN- γ or IL-17A had impaired activity. Thus, Th17 cells are not always short lived and are a less-differentiated subset capable of superior persistence and functionality.

INTRODUCTION

A key feature of adaptive immunity is the ability to generate long-lived populations of self-remembering memory cells; however, the evolutionary benefits of having robust anamnestic responses are balanced against the burden and hazard of maintaining large numbers of antigen-specific lymphocytes. Upon antigen stimulation, both CD8⁺ and CD4⁺ T cells experience a stereotypical clonal expansion followed by a contraction phase and the formation of memory (Kaech et al., 2002). Although CD8⁺ memory can be retained almost indefinitely, the ability of CD4⁺ cells to persist

is less understood and appears to be dependent upon the conditions of initial antigenic exposure (Romano et al., 2001; McKinstry et al., 2010; Taylor and Jenkins, 2011; Williams et al., 2008). The relative efficiency with which different CD4⁺ T cell subsets enter into the memory pool is the matter of discussion (MacLeod et al., 2009), and the analysis of memory formation is complicated because some polarized T helper (Th) cell subsets are meta-stable and experience plasticity (Zygmunt and Weinreb, 2010).

In a recent report, Th17 cells were characterized as short-lived effector cells with a limited capacity to persist that was attributed to extinction of IL-17A secretion and low expression of CD27, when compared with Th1 cells (Pepper et al., 2010). In this elegant study, the authors analyzed endogenous Th1 and Th17 cells induced upon infection, thus allowing for *in situ* glimpses at the “real” T cell response in a more naturalistic setting than reports based on cells generated *ex vivo* (Bjork and Spertus, 2010). However, the assertion that Th17 cells have a limited survival potential seems at odds with their protective role in antimicrobial immunity and the protracted tissue damage associated with Th17 cell responses in autoimmune diseases such as arthritis, multiple sclerosis, Crohn’s disease, uveitis, psoriasis, and graft-versus-host disease (Carlson et al., 2009; Maynard and Weaver, 2009; Sallusto and Lanzavecchia, 2008; Shi et al., 2008). The view that Th17 cells are short lived also seems contrary to the superior antitumor activity of adoptively transferred Th17 cells (Martin-Baron et al., 2009b; Muranski et al., 2008; Muranski and Restifo, 2009), in which persistence is critical to achieving complete tumor eradication (Shen et al., 2007; Zhou et al., 2009).

We therefore sought to study the phenotype, functional maturation, and survival of Th17 cells *in vivo* by using a T cell receptor (TCR) transgenic model in which CD4⁺ cells are specific for the TRP-1 tissue differentiation antigen expressed by normal and transformed melanocytes and are capable of eradicating large established tumors (Muranski et al., 2008). Although Th17 cells can become “Th1-like” (Bending et al., 2008; Lee et al., 2009; Palmer and Weaver, 2010; Wu et al., 2008), it remains unclear

RESEARCH ARTICLE

IMMUNOLOGY

The Inducible Costimulator (ICOS) Is Critical for the Development of Human T_H17 Cells

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¹Published 27 October 2010; Volume 15 Issue 10

Human T helper 17 (T_H17) cells regulate host defense, autoimmunity, and tumor immunity. Although cytokines that control human T_H17 cell development have been identified, the costimulatory molecules important for T_H17 cell generation are unknown. Here, we found that the inducible costimulator (ICOS) was critical for the differentiation and expansion of human T_H17 cells. Human cord blood contained a subset of CD161⁺CD4⁺ T cells that were recent emigrants from the thymus, expressed ICOS constitutively, and were imprinted as T_H17 cells through ICOS signaling. ICOS stimulation induced c-MAF, RORC2, and T-bet expression in these cells, leading to increased secretion of interleukin-21 (IL-21), IL-17, and interferon- γ (IFN- γ) compared with cells stimulated with CD28. Conversely, CD28 ligation abrogated ICOS costimulation, dampening RORC2 expression while promoting the expression of the aryl hydrocarbon receptor, which led to reduced secretion of IL-17 and enhanced production of IL-22 compared with cells stimulated with ICOS. Moreover, ICOS promoted the robust expansion of IL-17⁺IFN- γ ⁺ human T cells, and the antitumor activity of these cells after adoptive transfer into mice bearing large human tumors was superior to that of cells expanded with CD28. The therapeutic effectiveness of ICOS-expanded cells was associated with enhanced functionality and engraftment *in vivo*. These findings reveal a vital role for ICOS signaling in the generation and maintenance of human T_H17 cells and suggest that components of this pathway could be therapeutically targeted to treat cancer or chronic infection and, conversely, that interruption of this pathway may have utility in multiple sclerosis and other autoimmune syndromes. These findings have provided the rationale for designing new clinical trials for tumor immunotherapy.

INTRODUCTION

CD4⁺ T cells are important in regulating immunity to pathogens, allergic responses, asthma, and immunity to self or tumor tissues (1–3). Depending on the microenvironmental cues present, naive CD4⁺ T cells may differentiate into one of several T helper (T_H) cell lineages, including T_H1, T_H2, T_H17, T_H22, and regulatory T (T_{reg}) cells (4, 5). T_H1 and T_H2 cells are effector cells that express T-bet and GATA-3, respectively (6). In contrast, T_H17 cells suppress effector T cell functions and are essential for regulating autoimmune responses (8), and the recently described T_H22 cells secrete interleukin-22 (IL-22) and might be a subset of skin homing cells responsible for inflammation (7, 8). T_H17 cells augment host defense, have a major role in mucosal immunity, enhance a number of autoimmune diseases, and release cytokines, including IL-17A and IL-17F (9). The contribution of T_H17 cells to tissue immunity varies, showing the potential for both antitumor and proinflammatory activity (10). Therefore, identification of the mechanisms that control T_H17 responses is essential to understand tumor immunity.

The functions of cytokines [for example, transforming growth factor- β (TGF- β), IL-6, IL-18, IL-21, and IL-23] and transcription factors (such as RORC2 and RORC1) in human T_H17 cell development are distinct from T_H1 and T_H2 effector cells (11–16). Further, natural agonists for the aryl hydrocarbon receptor (AHR) augment murine

T_H17 cell differentiation (15). However, the specific costimulatory pathways that may influence T_H17 generation and stability remain to be elucidated.

Antigen-specific and antigen-nonspecific costimulatory signals from antigen-presenting cells (APCs) are necessary for the activation, differentiation, and function of T lymphocytes (16). CD28 is considered to be the primary *in vivo* signaling molecule on CD4⁺ T cells because of its early expression, and it is often used to generate IL-17-producing lymphocytes (12–14, 17–19). However, in addition to CD28, signaling via the inducible costimulator (ICOS, also called CD278) is required for optimal cytokine secretion, because both molecules are essential for optimal IL-17A secretion by murine T_H17 cells (20). Recent findings in murine models have revealed that ICOS amplifies T_H17 responses by inducing the expression of the transcription factor c-MAF and therefore transactivating IL-21 production (21). Although both CD28 and ICOS are important for the generation of murine T_H17 cells, their particular roles in regulating key genes in human T_H17 cells remain to be identified.

Here, we show that the nature of costimulation during CD4⁺ T cell activation critically regulates human T_H17 cell differentiation. ICOS, but not CD28, is necessary for optimal expansion and function of human T_H17 cells. Surprisingly, CD28 ligation abrogated the effects of ICOS costimulation. These data are surprising given that CD28 is often used to expand human T_H17 cells, and they raise the possibility that the full inflammatory potential of human T_H17 cells *in vivo* has not been fully reflected by previous *in vitro* studies. Of clinical relevance, genetically reprogrammed human T_H17 cells expanded with ICOS-mediated superior regression of human tumors compared to cells expanded with CD28. These findings reveal a key role for ICOS signaling in human T_H17 cell development and suggest new therapeutic approaches.

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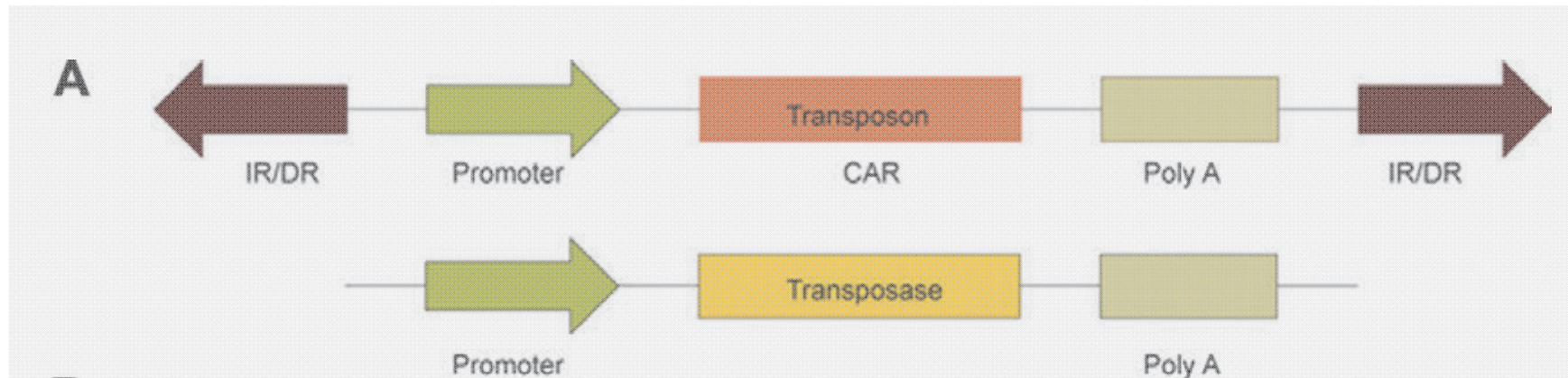
(Continued)

Published clinical data to date infusing CAR⁺ T cells targeting CD19

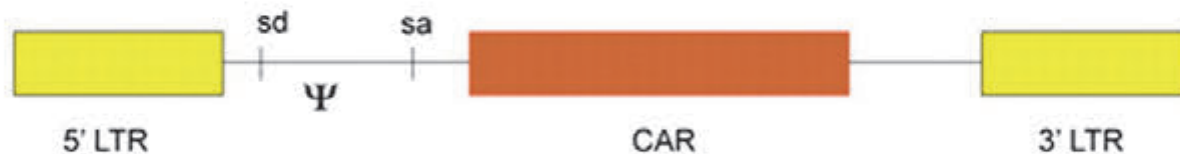
Institute	CD19 ⁺ Disease	Clinical Trial.gov Identifiers	Chemotherapy prior to T-cell infusion	IL-2 after T-cell infusion	Gene transfer approach	CAR scaffold to append scFv	Mouse mAb clone used to derive scFv	CAR signaling endodomain(s)	Loss of normal B cells?
U Penn	CLL	NCT01029366	Yes	No	Lentivirus	CD8alpha	FMC63	CD137 and CD3-zeta	Yes
NCI	Follicular Lymphoma and CLL	NCT00924326	Yes	Yes	Retrovirus	Truncated CD28	FMC63	CD28 and CD3-zeta	Yes
MSKCC	CLL and B-ALL	NCT00466531 and NCT01044069	Yes	No	Retrovirus	Truncated CD28	SJ25C1	CD28 and CD3-zeta	Yes
BCM	B-NHL or CLL	NCT00586391	No	No	Retrovirus	IgG1 CH ₂ CH ₃ domains	FMC63	CD3 zeta vs. CD28 and CD3-zeta	Yes
COH	Follicular Lymphoma	NCT00182650	Yes	Yes	Electroporation	IgG4 hinge and CH ₂ -CH ₃ domains	FMC63	CD3 zeta	No

Blood. 2012 Mar 22;119(12):2700-2.

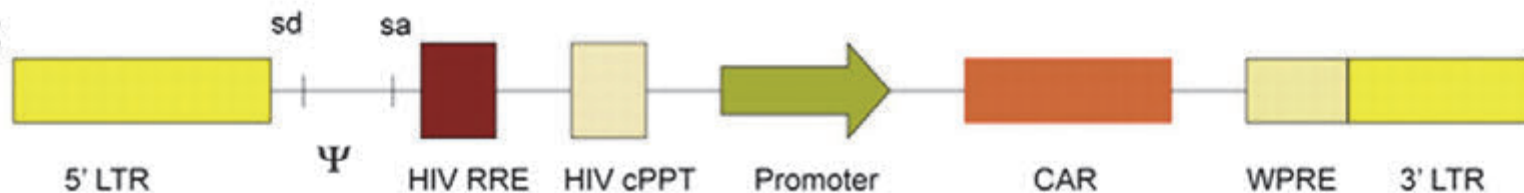
Vector systems to express CAR transgenes used in clinical trials



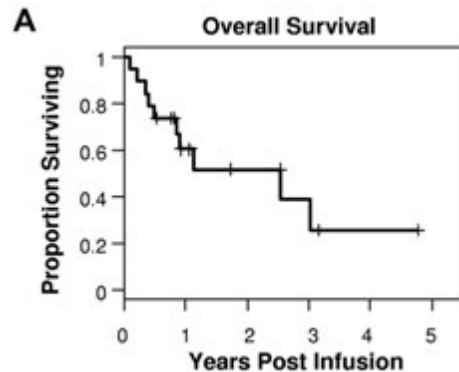
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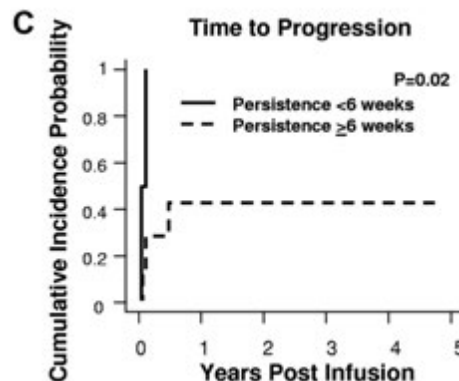
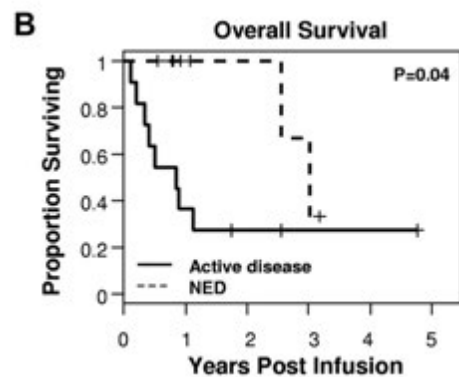
C



GD₂-targeted CAR⁺ T cells in patients with neuroblastoma

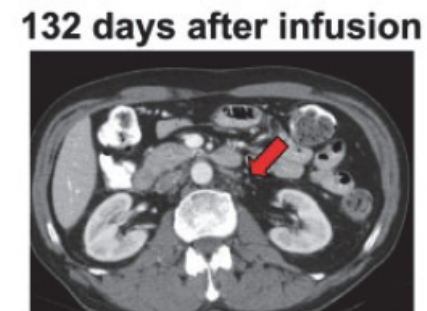
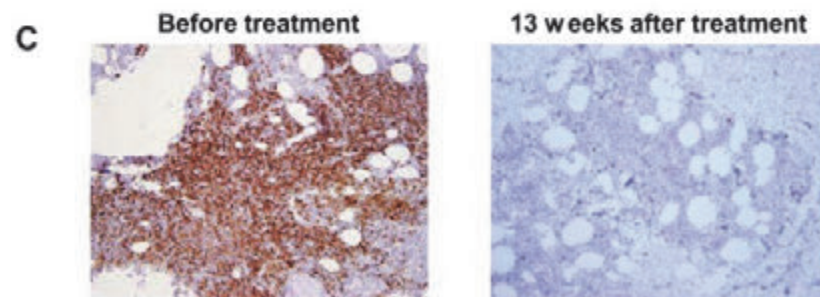
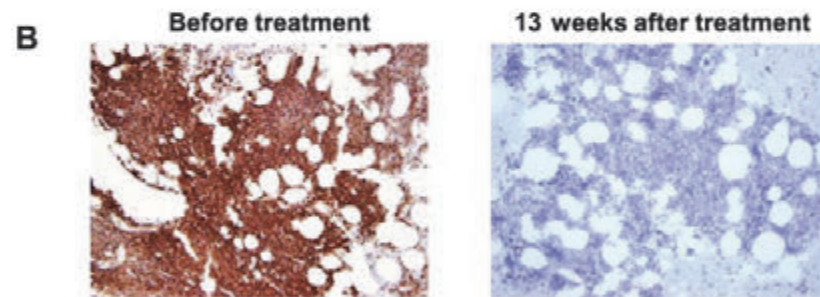
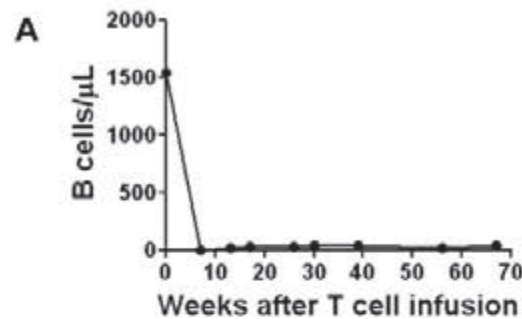


Antitumor activity and long-term fate of CAR⁺ T cells in patients with neuroblastoma

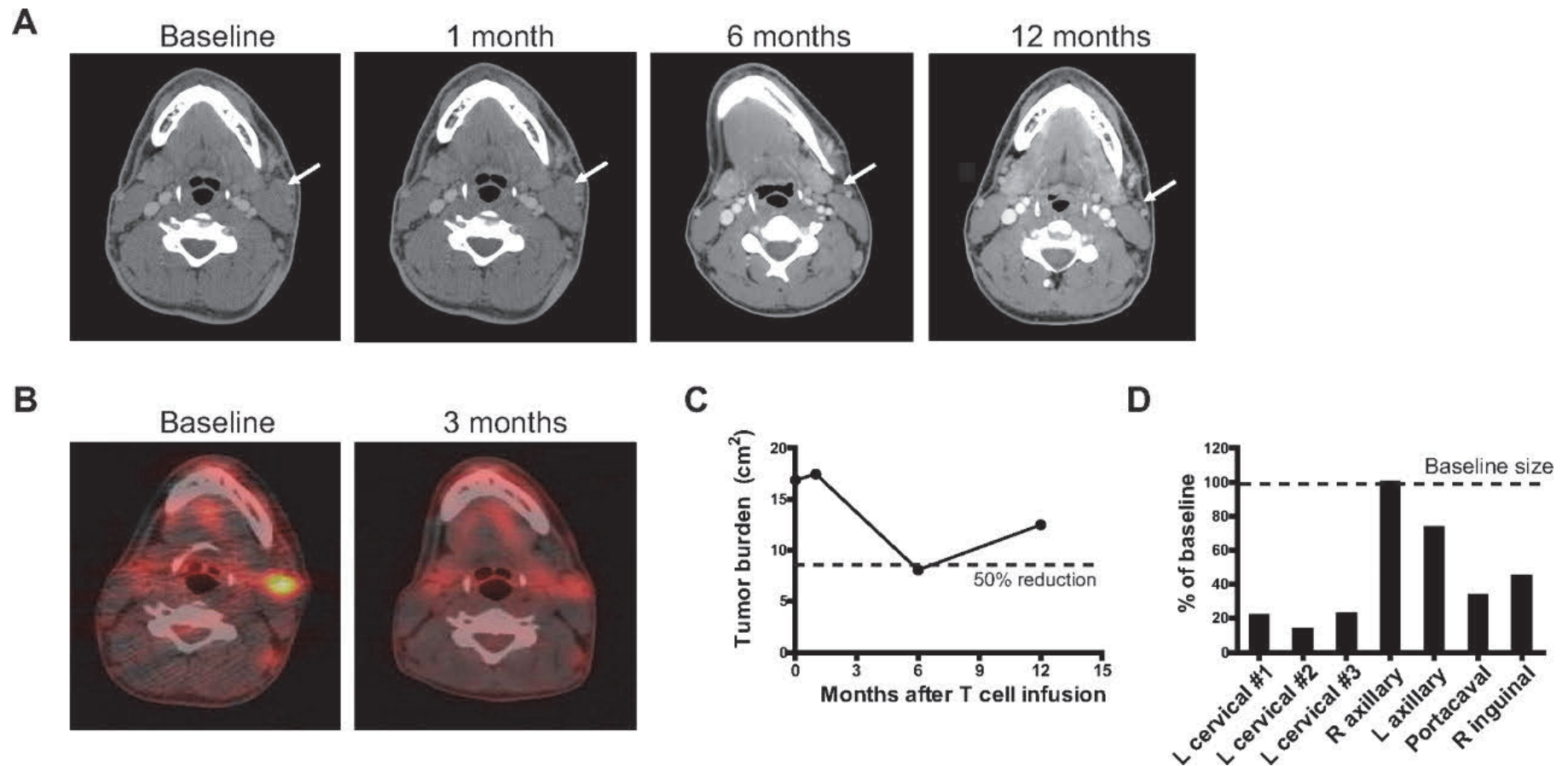


Louis C U et al. Blood 2011;118:6050-6056

CD19-targeted CAR⁺ T cells in patients with B-cell malignancies



CD20-targeted CAR⁺ T cells in patients with B-cell malignancies

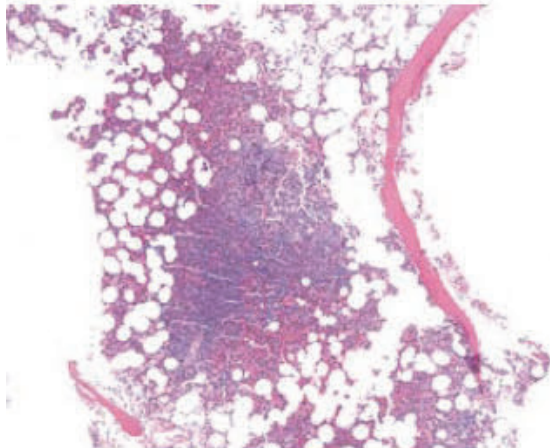


Till B G et al. Blood 2012;119:3940-3950

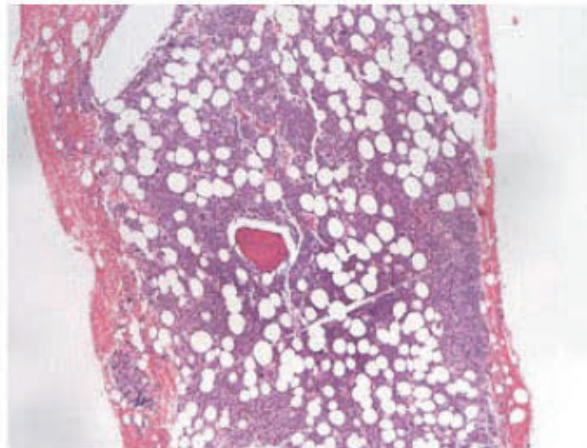
CD19-targeted CAR⁺ T cells in patients with B-cell malignancies

Bone Marrow–Biopsy Specimens

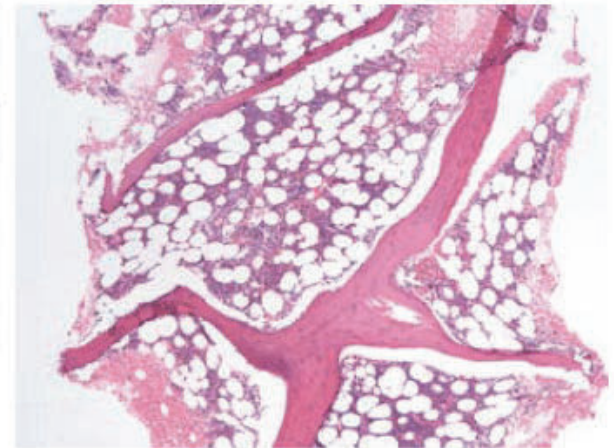
Day -1 (baseline)



Day 23



6 Mo

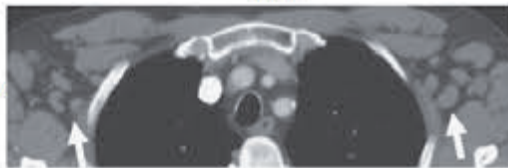


Contrast-Enhanced CT

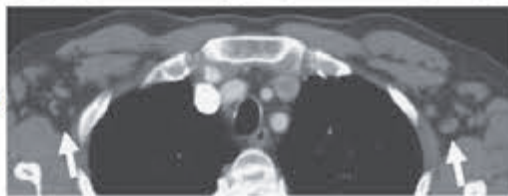
Axial

Coronal

Before Therapy



1 Mo of Treatment

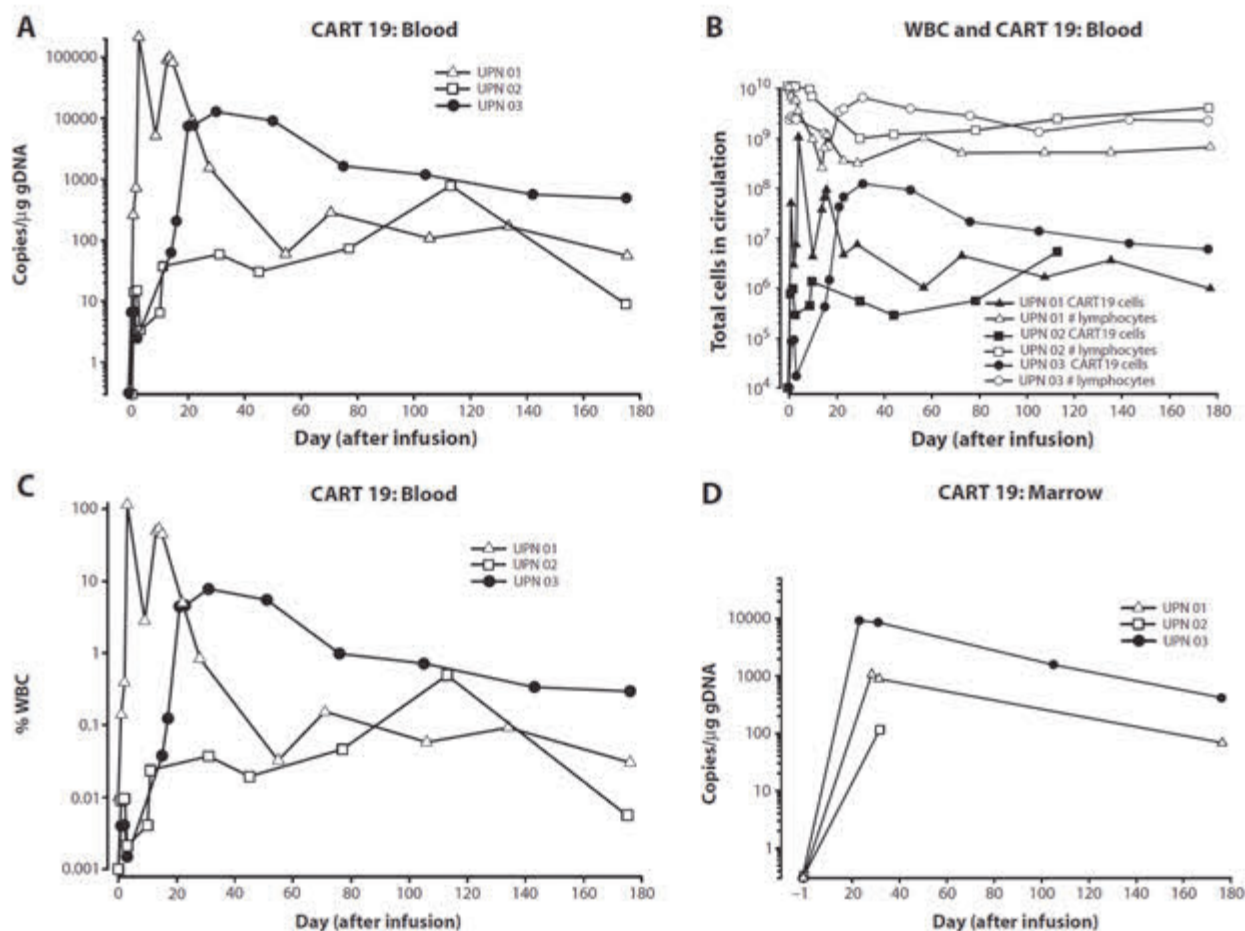


3 Mo of Treatment



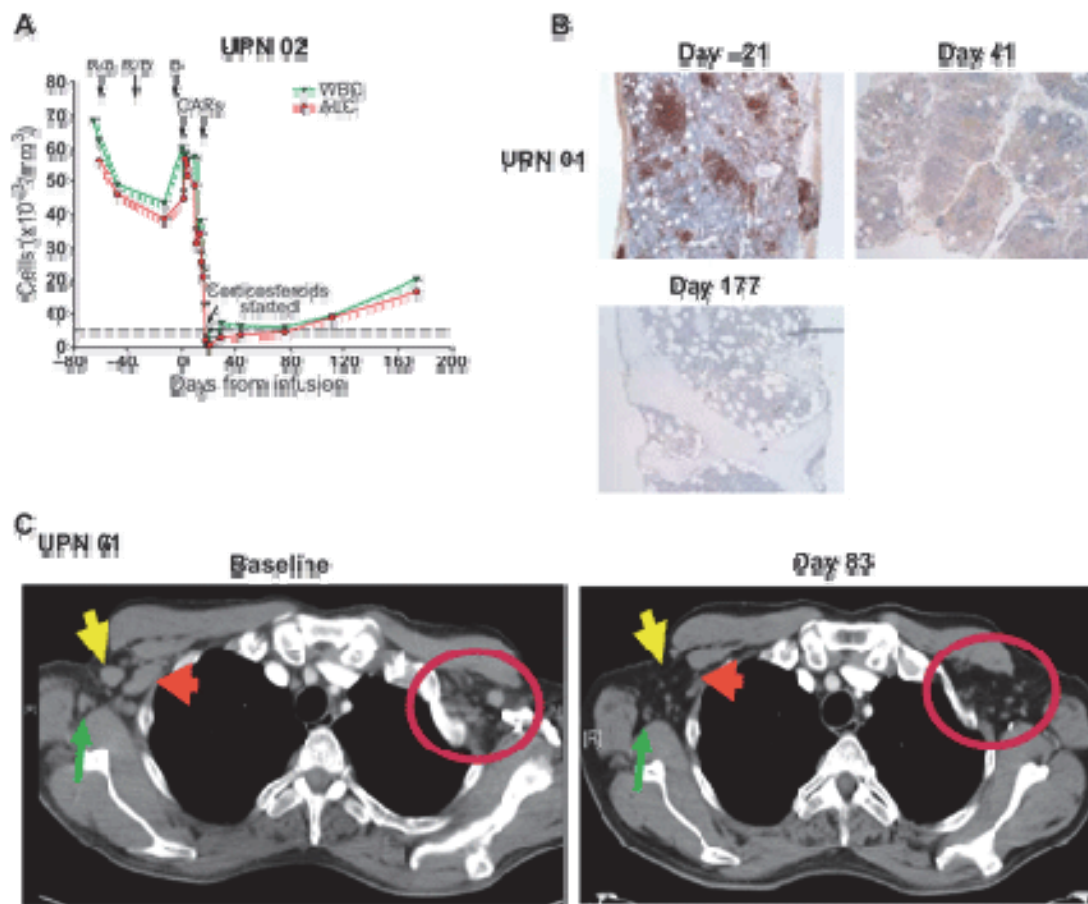
Porter et al., NEJM
2011; 365:725-33.

CD19-targeted CAR⁺ T cells in patients with B-cell malignancies



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

CD19-targeted CAR⁺ T cells in patients with B-cell malignancies



CD19-targeted CAR⁺ T cells in patients with B-cell malignancies

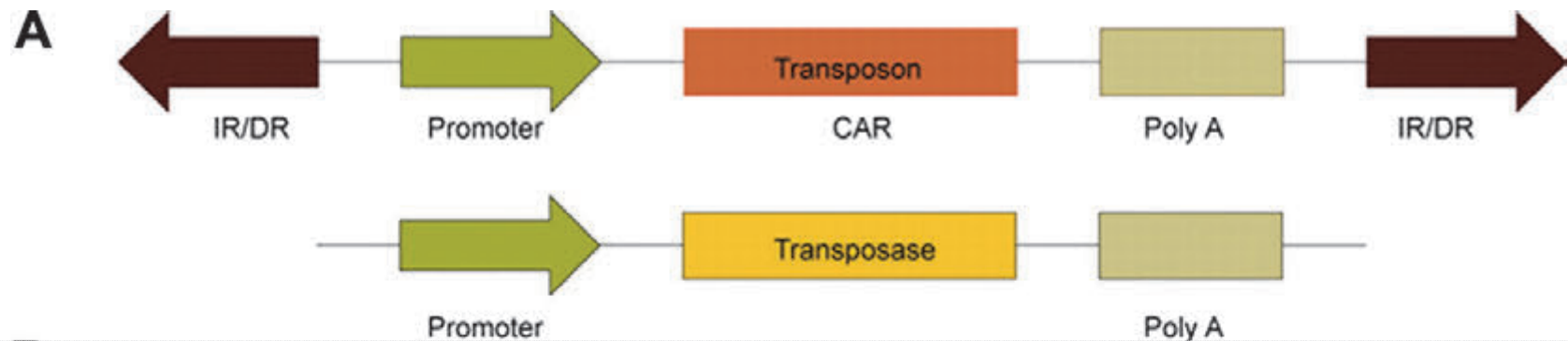
Diagnosis-Patient	Adverse events	Grade	Related
CLL-1	Febrile neutropenia	3	Probable
	Rigors, chills	2	Probable
CLL-2	Fever, rigors, chills	2	Probable
	Chest pain	2	Probable
CLL-3	Fever, rigors, chills	1	Probable
CLL-4	Fever	2	Probable
	Rigors, chills, dyspnea	1	Probable
	Hypotension, renal failure	5	Possible
CLL-5	Fever	2	Probable
	Rigors, chills	1	Probable
	Hyponatremia	1	Possible
CLL-6	Fever	1	Probable
	Hypotension	2	Possible
	Febrile neutropenia	3	Possible
CLL-7	Febrile neutropenia	3	Probable
CLL-8	Fever	2	Probable
ALL-1	Neutropenia	4	Possible
	Diarrhea	2	Possible
	Hypotension	3	Possible

Brentjens et al., 2011. Blood 118:4817-4828

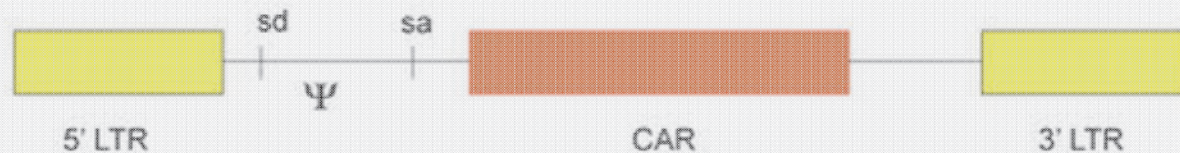
Patient	Toxicities*†
1‡	Fatigue, herpes zoster with secondary otitis externa 6 months after treatment
2	<i>Escherichia coli</i> bacteremia, died with influenza pneumonia, dyspnea, hypoxemia, nonbacterial thrombotic endocarditis, cerebral infarction, elevated liver enzymes
3	Hypotension, acute renal failure, hypoxemia, hyperbilirubinemia, capillary leak syndrome
4	Diarrhea, fatigue
5	Fever, fatigue, hypotension
6	Hypotension, capillary leak syndrome, hypoalbuminemia
7	Obtundation, acute renal failure, hyperbilirubinemia, capillary leak syndrome, anorexia, elevated liver enzymes, electrolyte abnormalities
8	Hypotension, obtundation, acute renal failure, capillary leak syndrome, headache, pleural effusion, electrolyte abnormalities

Kochenderfer et al., Blood. 2012 119: 2709-2720

Vector systems to express CAR transgenes used in clinical trials



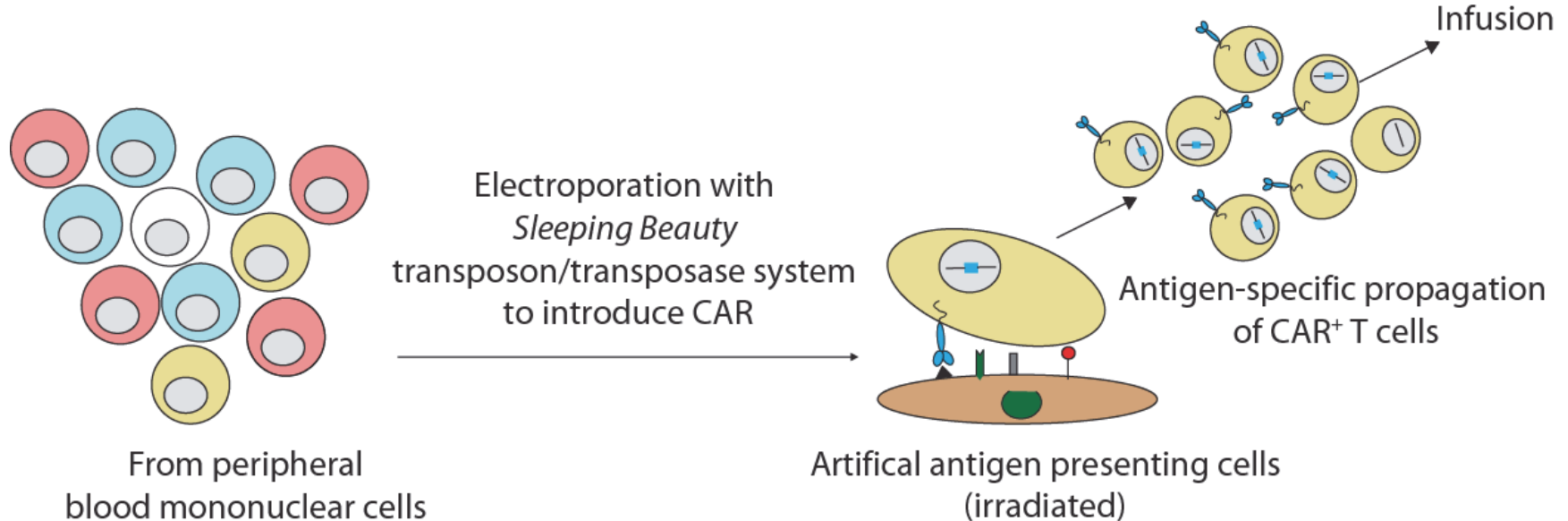
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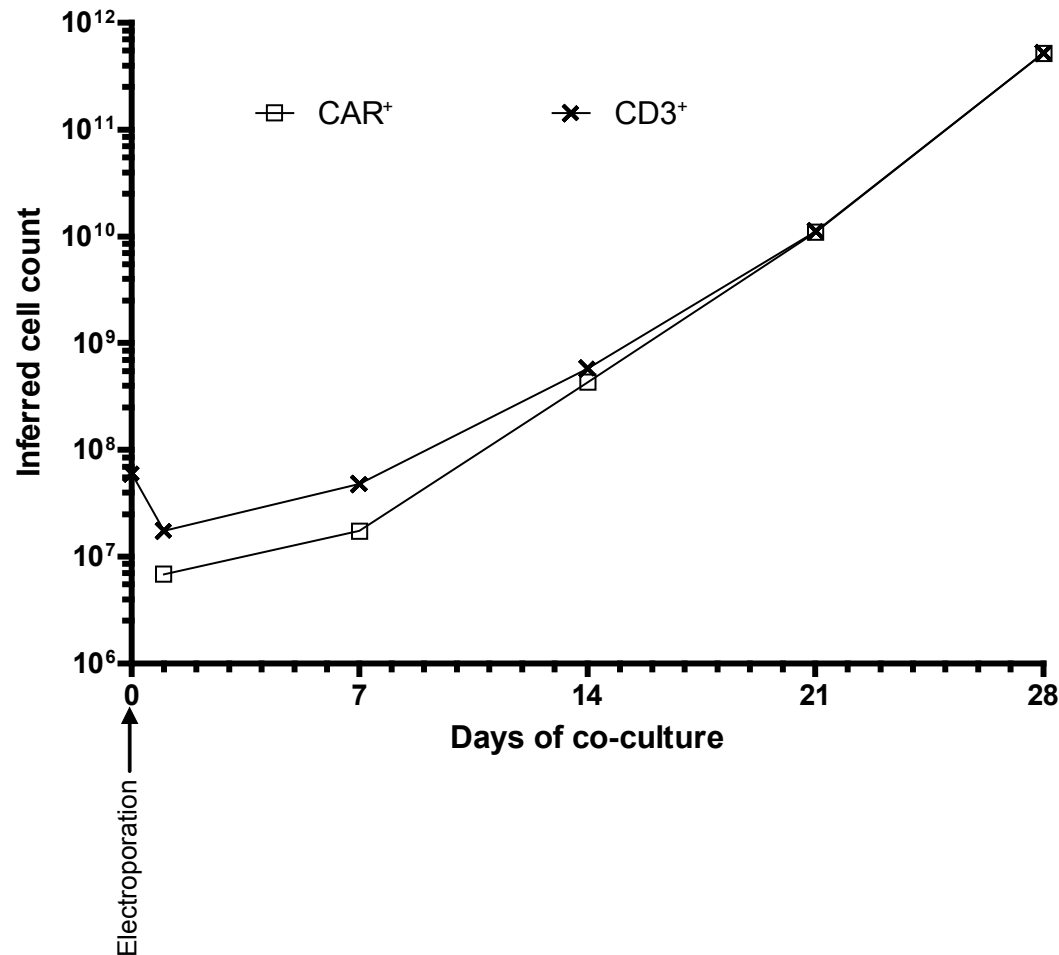
C



Genetic modification and propagation of T cells



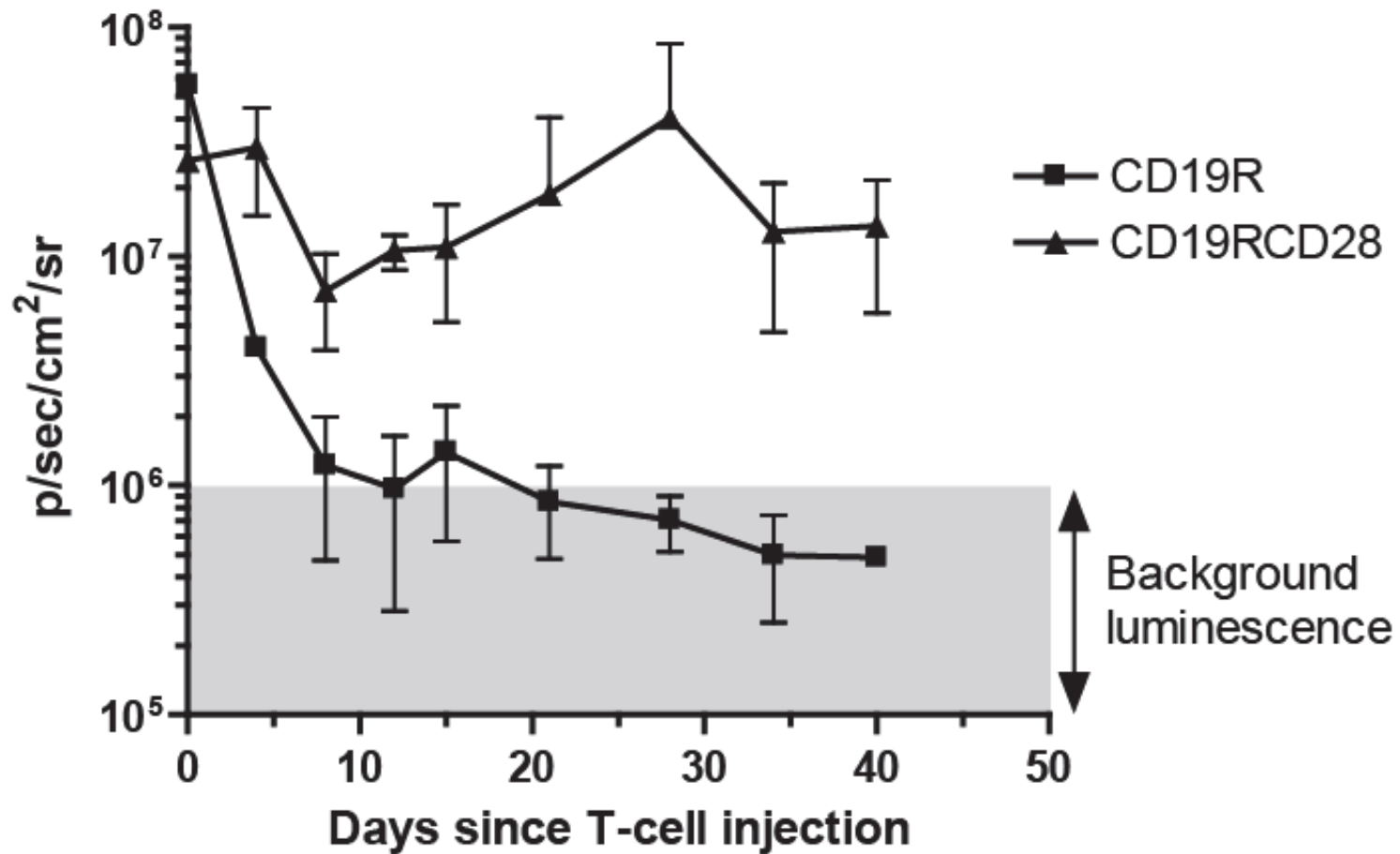
Selective outgrowth of CAR⁺ T cells on aAPC



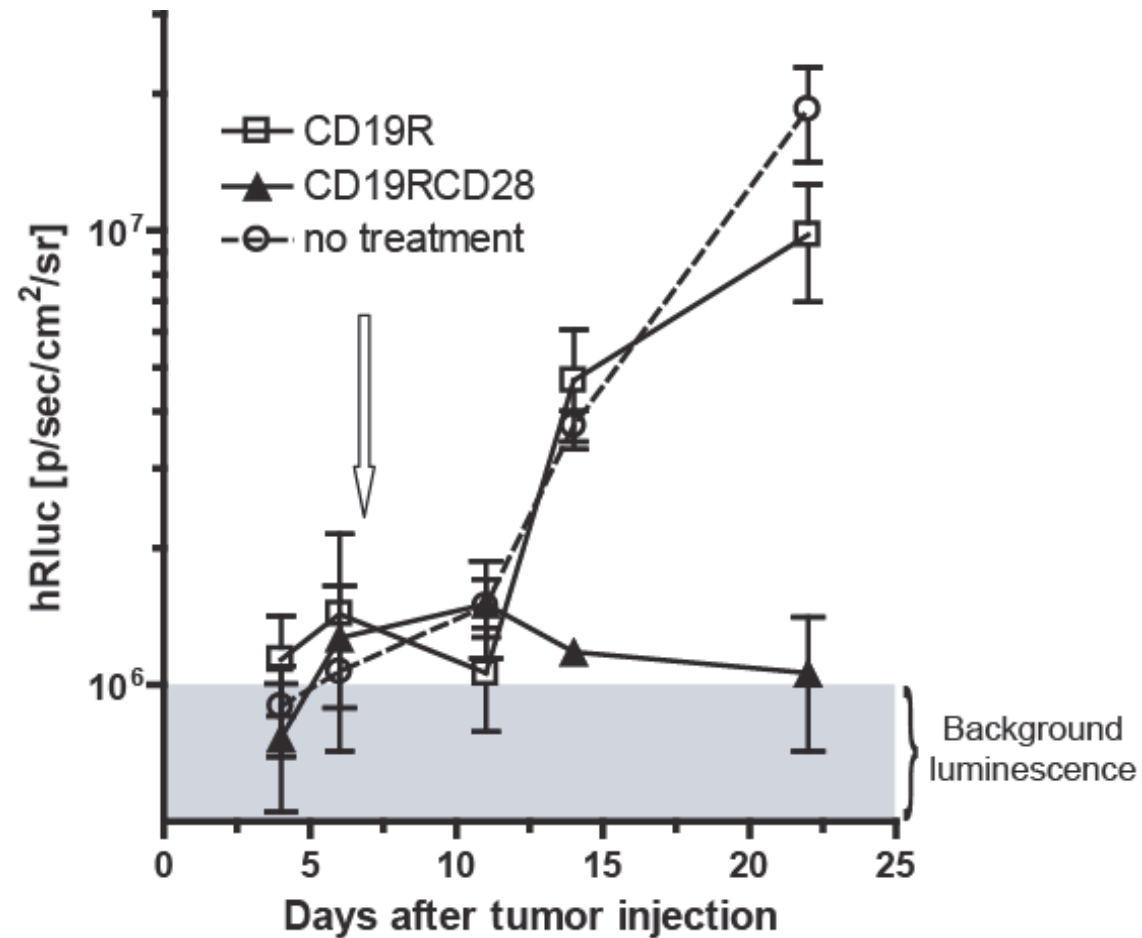
Mouse models – predictive?

- Infuse human CAR⁺ T cells into NOD/*Scid*/IL-2R γ ^{-/-} (NSG) mice
- Does this predict for toxicity or potency?

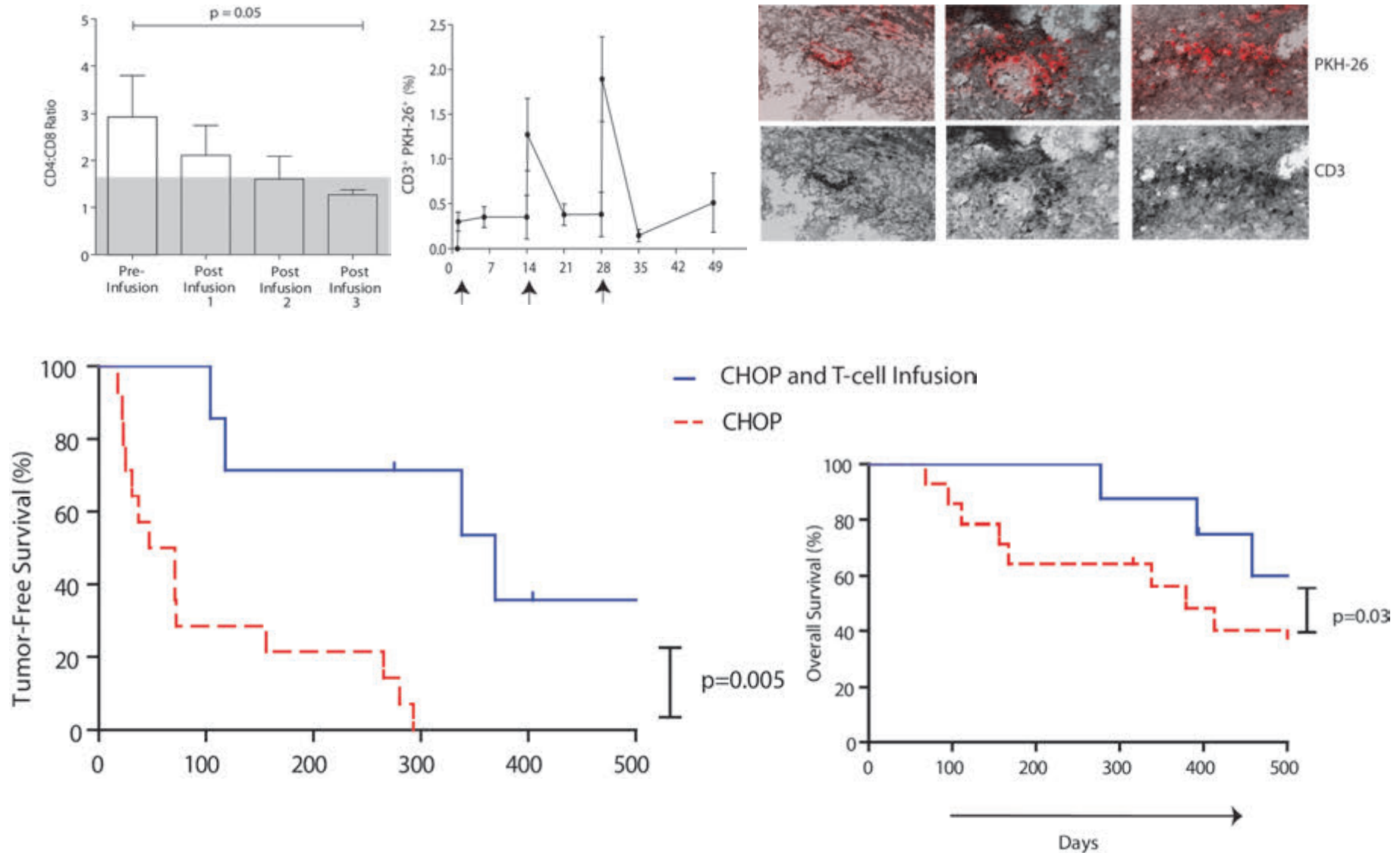
1st generation vs. 2nd generation CARs



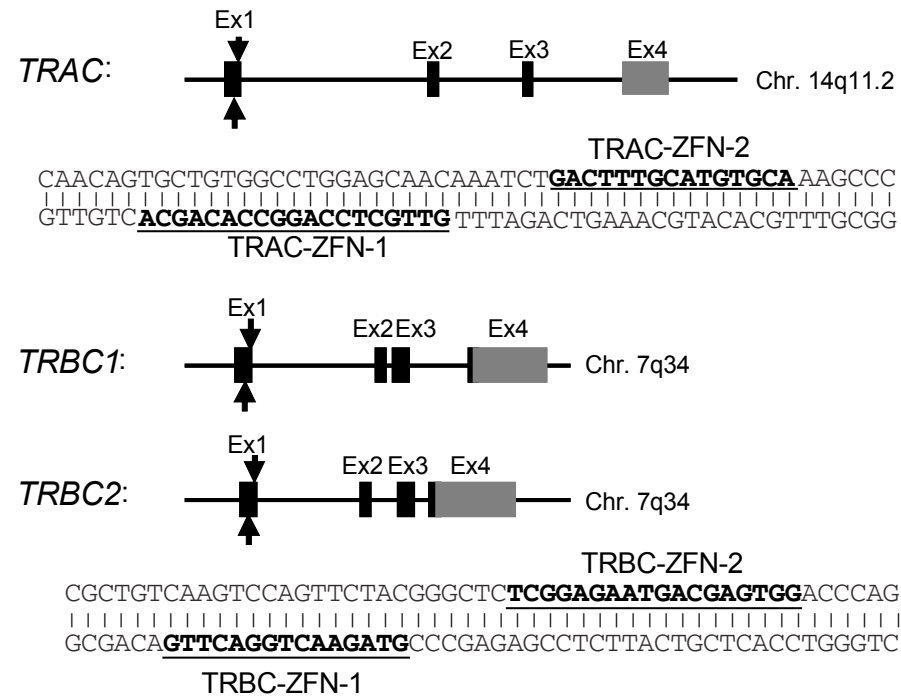
1st generation vs. 2nd generation CARs



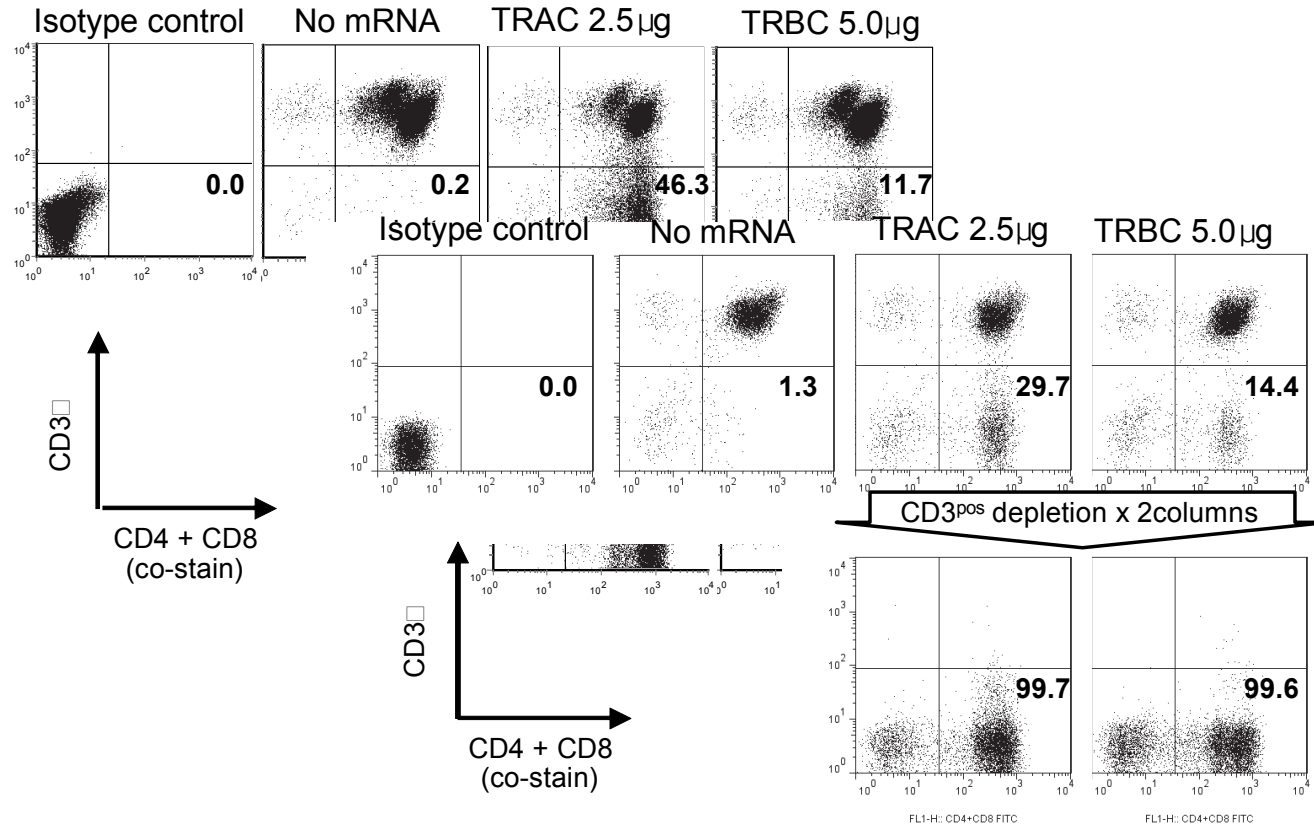
Companion canine NHL



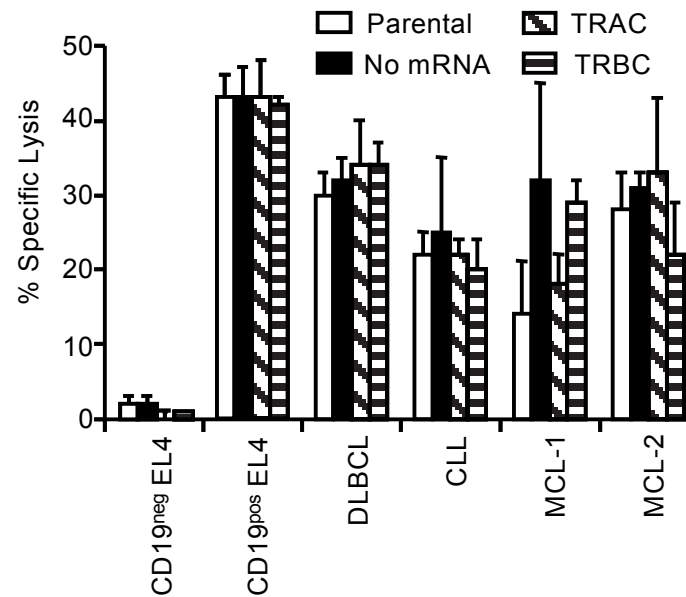
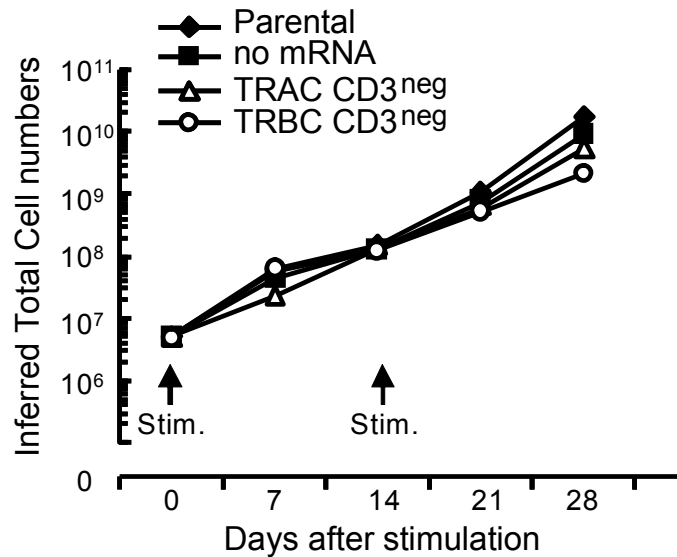
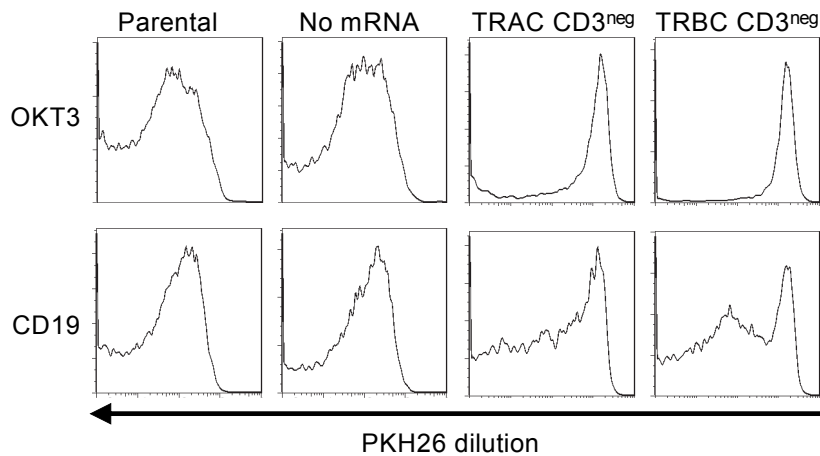
“Off-the-shelf” CAR⁺TCR^{neg} T cells



“Off-the-shelf” CAR⁺TCR^{neg} T cells



“Off-the-shelf” CAR⁺TCR^{neg} T cells



Future approach to clinical trials

- Use of DNA vectors affords opportunity to change CAR design
- aAPC may be useful for propagating subsets of genetically modified T cells
- Off-the-shelf T cells
- Role of iCaspase9 co-expressed with CAR

Thanks

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