The following relationships exist related to this presentation:

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
ljncooper@mdanderson.org

sitc
Society for Immunotherapy of Cancer

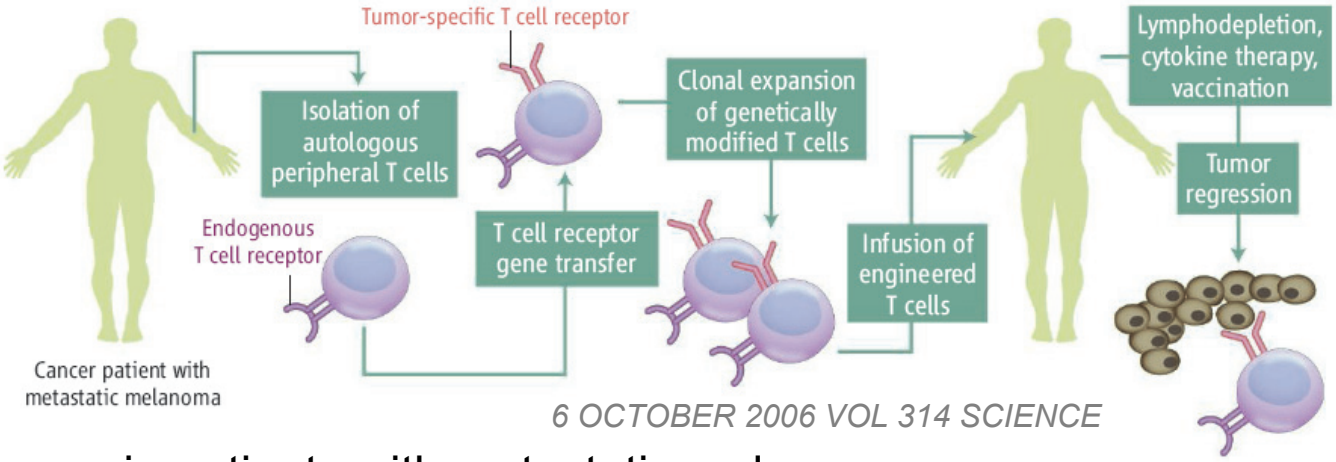
Bias of this presentation

- Combine gene therapy with T-cell therapy to over come 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 though endogenous αβ T-cell receptor (TCR) in context of MHC

Harvesting and re-expressing melanoma-specific αβTCR

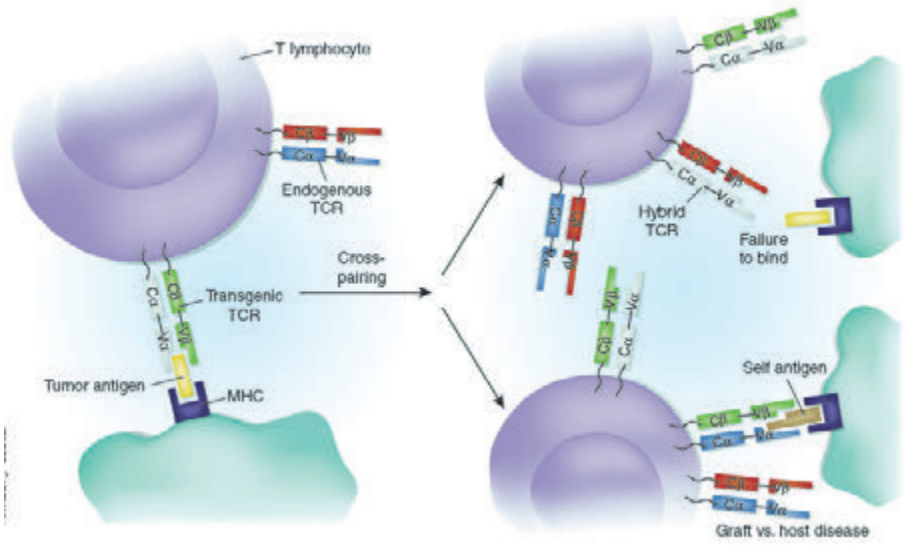


6 OCTOBER 2006 VOL 314 SCIENCE

TCR gene therapy in patients with metastatic melanoma

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

Toxicities from TCR transfer



Nature Medicine16,520–521(2010)

- GVHD due to the pairing of introduced and endogenous TCR chains in TCR gene-modified T cells in mouse model. *Nature Medicine16,565–570(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.

<u>Potential Solutions</u>

- 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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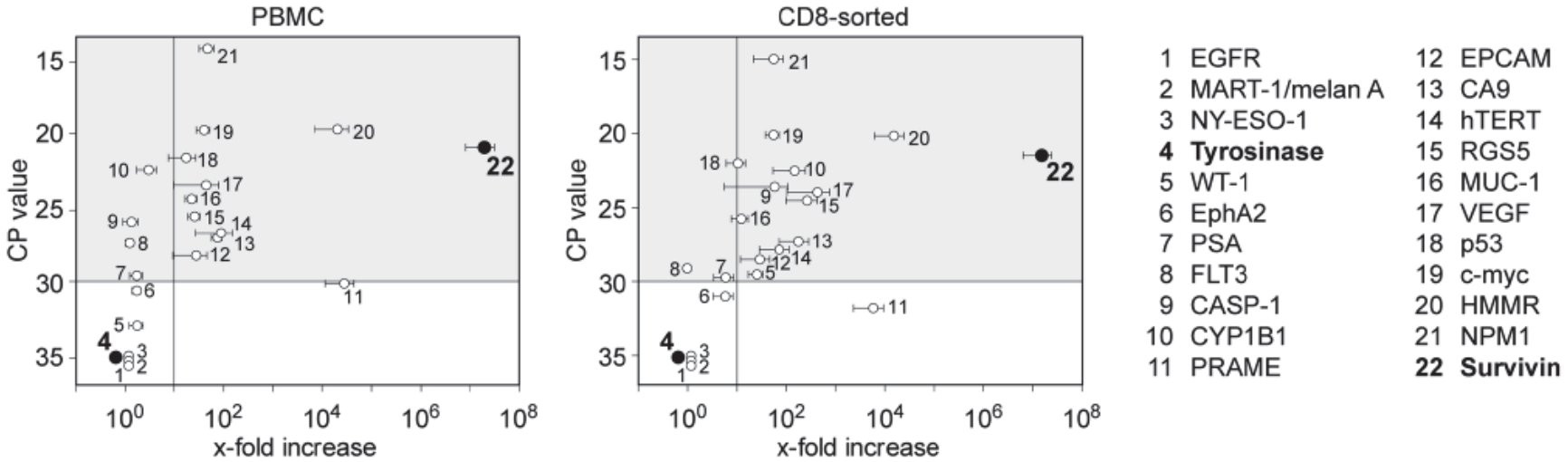
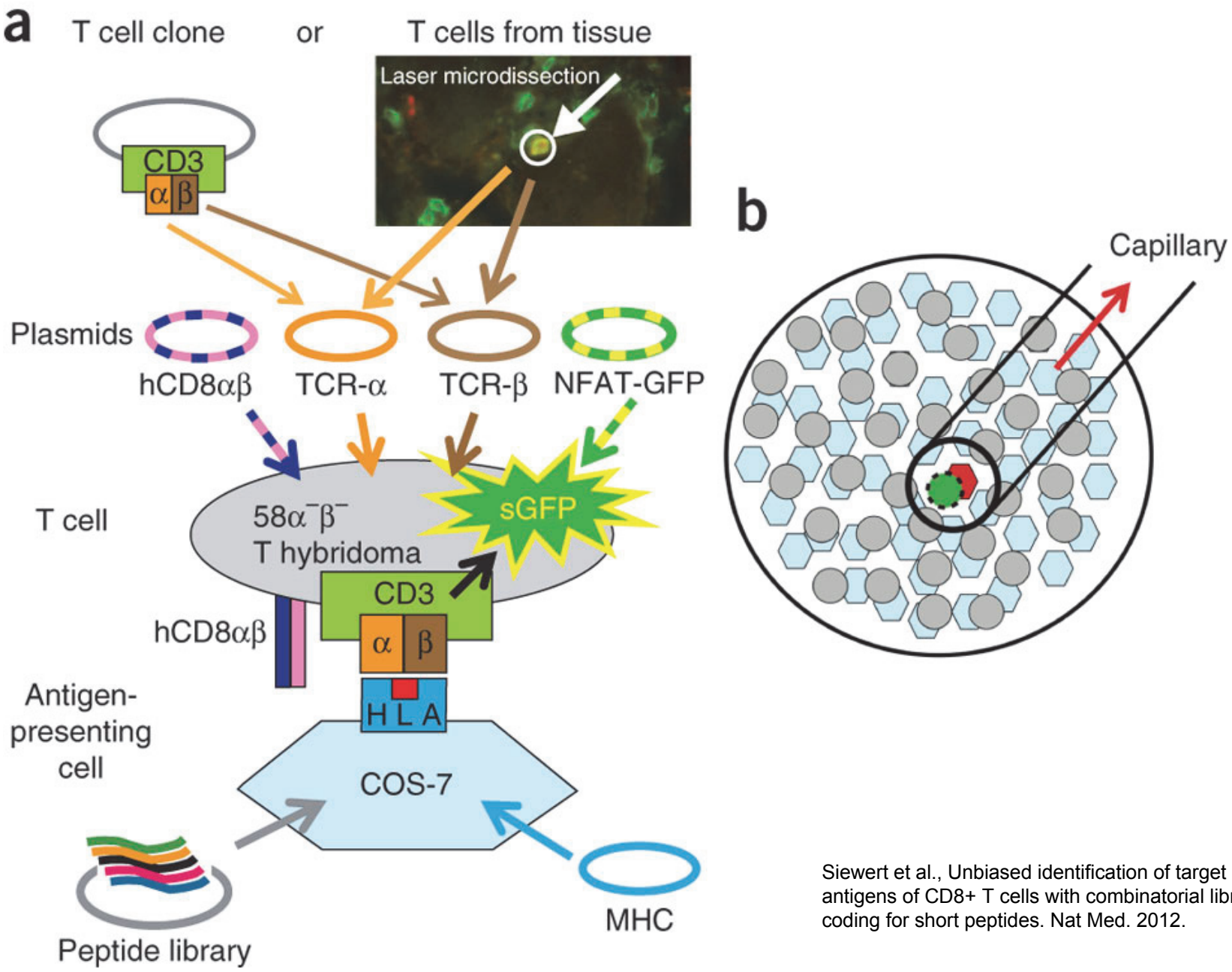


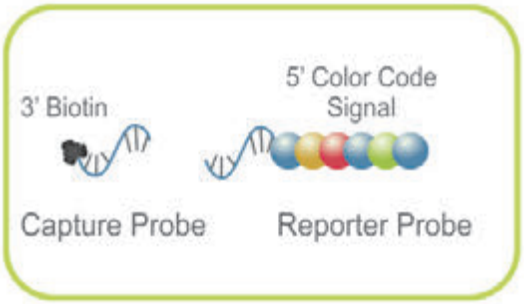
1	EGFR	12	EPCAM
2	MART-1/melan A	13	CA9
3	NY-ESO-1	14	hTERT
4	**Tyrosinase**	15	RGS5
5	WT-1	16	MUC-1
6	EphA2	17	VEGF
7	PSA	18	p53
8	FLT3	19	c-myc
9	CASP-1	20	HMMR
10	CYP1B1	21	NPM1
11	PRAME	**22**	**Survivin**

Identification of target antigens for CD8$^+$ T cells

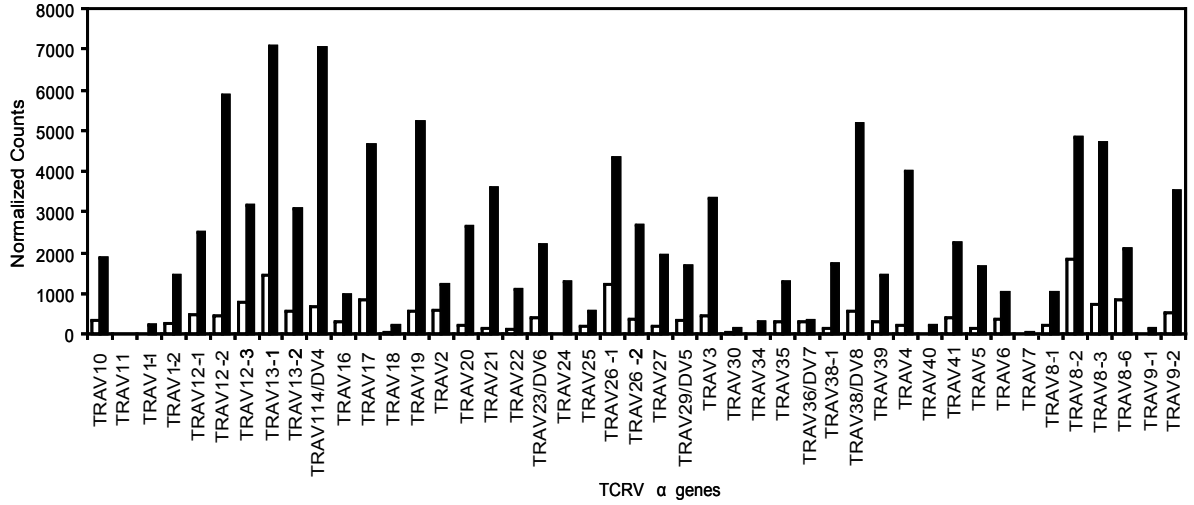


Siewert et al., Unbiased identification of target antigens of CD8+ T cells with combinatorial libraries coding for short peptides. Nat Med. 2012.

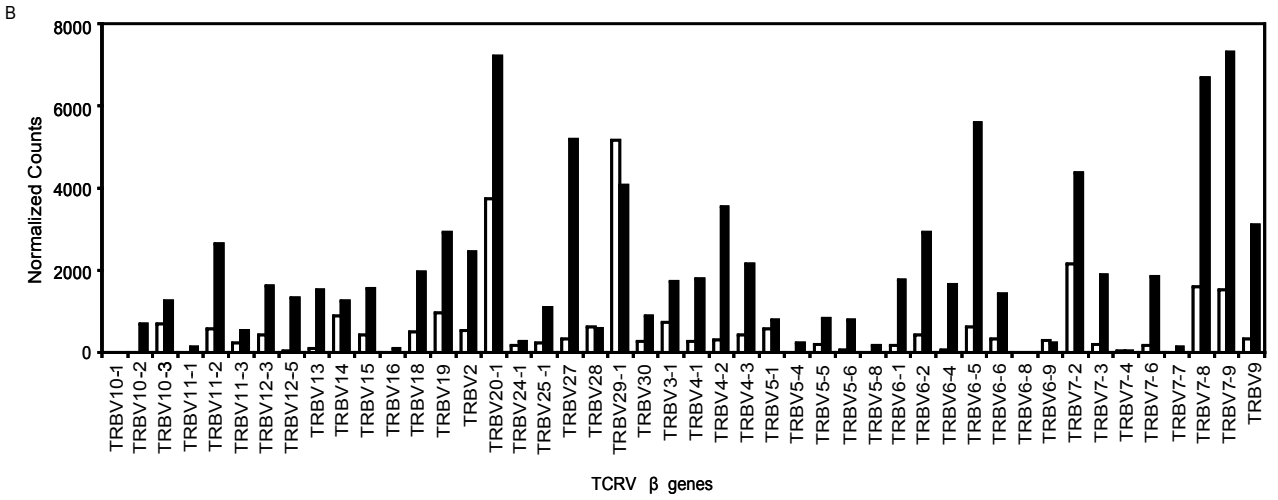
Oligoclonality



A



B

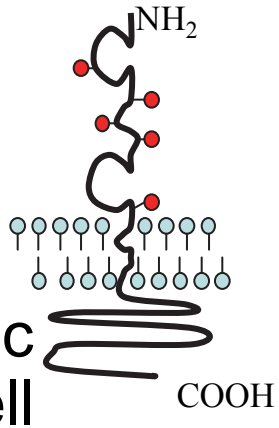


Tumor-specific T cells

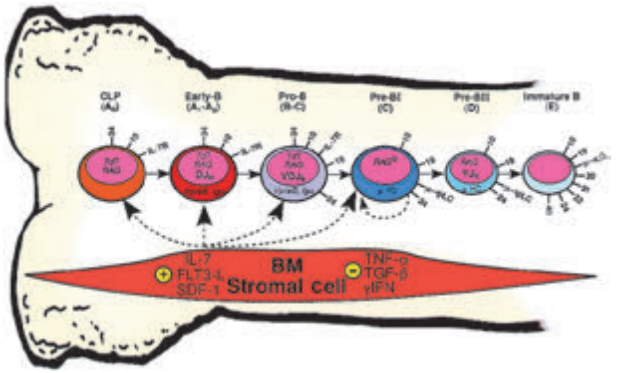
- T cells that recognize tumor-associated antigen (TAA) through introduced chimeric antigen receptor independent of MHC
- T cells that recognize TAA though endogenous αβ T-cell receptor (TCR) in context of MHC

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.

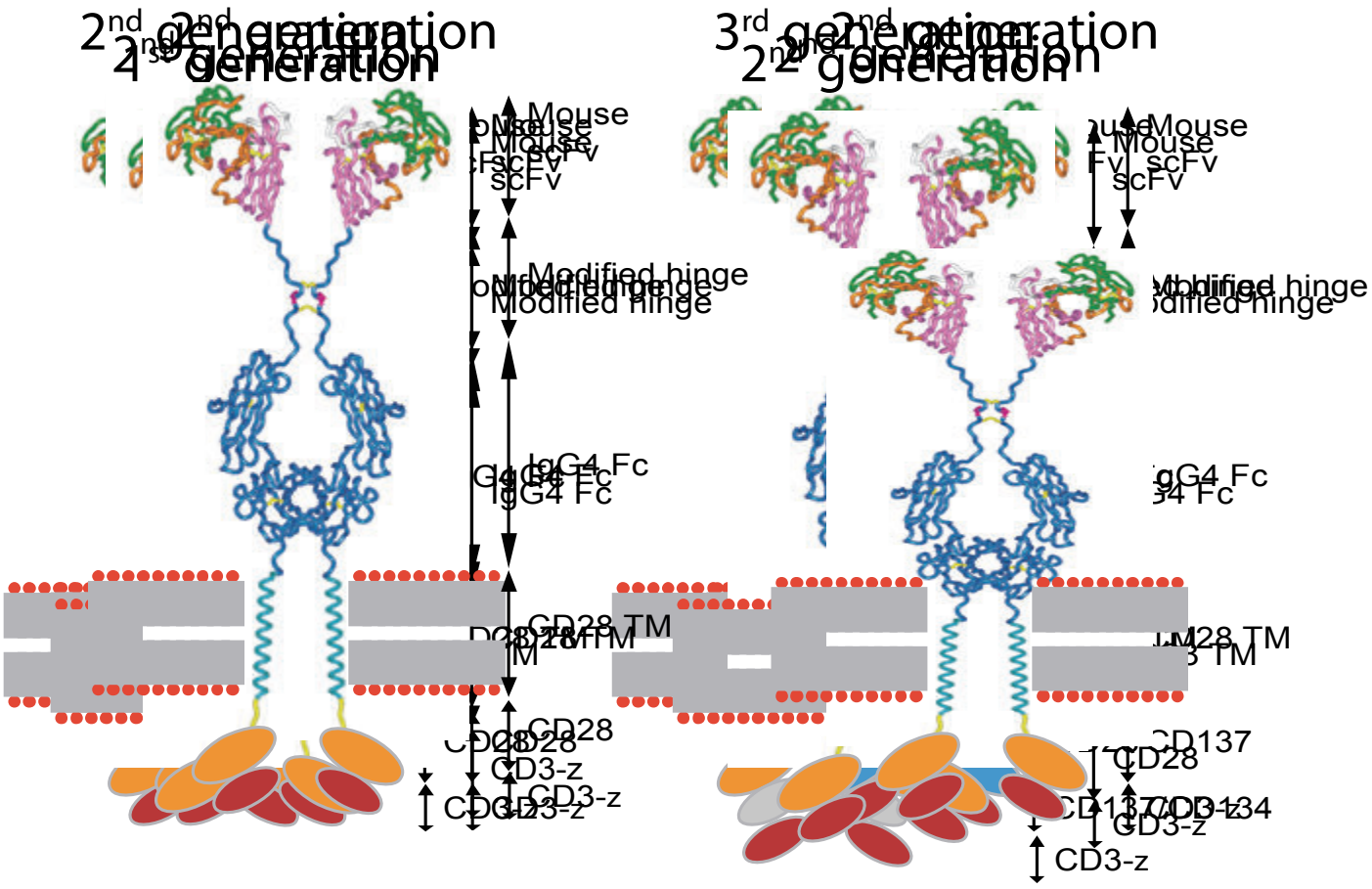


Clinical trials in USA infusing CAR$^+$ T cells

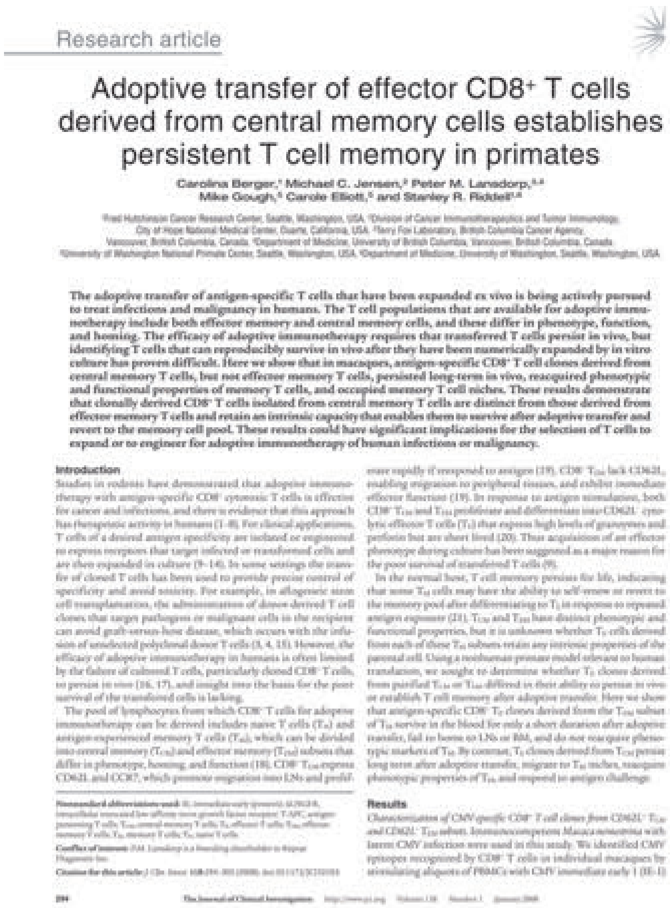
	Antigen	Tumor target	Viral-specific T cell	Lympho-depletion	CAR generation	ClinicalTrial.gov identifier	Enrolling	SAE	Gene transfer
1	Kappa light chain	B-NHL and B-CLL	No	Yes	First and second	NCT00881920	Yes	TBM	Virus
2	CD19	Lymphoma/leukemia (B-NHL) and CLL	No	No	First and second	NCT00586391	Yes	TBM	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-13Rα2	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.

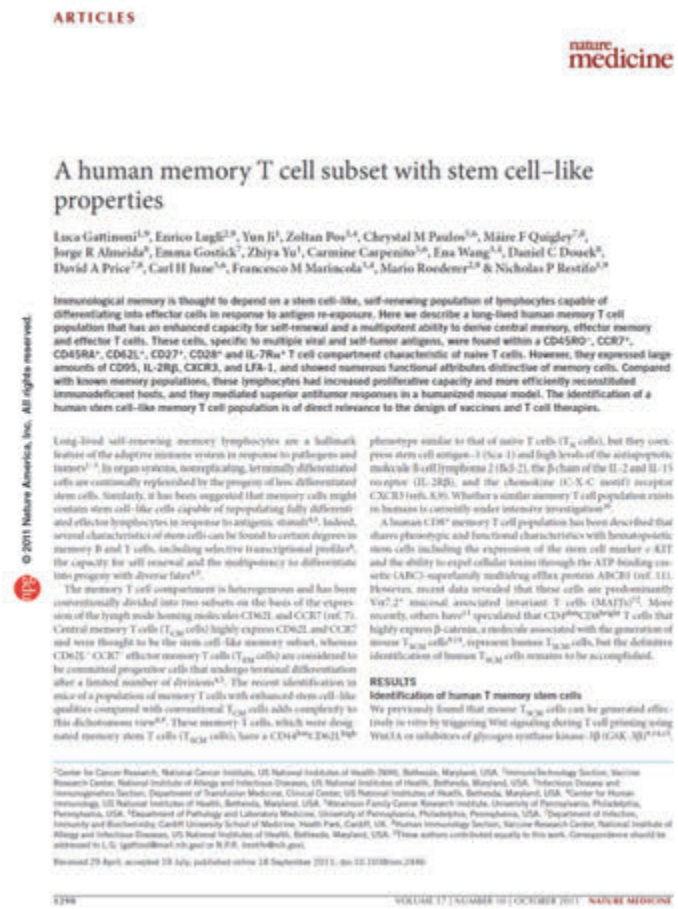
CAR design


2nd generation
4th generation
Mouse scFv
Modified hinge
IgG4 Fc
CD28 TM
CD28
CD3-z
3rd generation
2nd generation
Mouse scFv
Modified hinge
IgG4 Fc
CD28 TM
CD137
CD28
CD3-z

Which T-cell sub-population to genetically modify?



Which T-cell sub-population to genetically modify?



ARTICLES

nature medicine

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

Luca Gattinoni[1,5], Enrico Lugli[2,5], Yun Ji[1], Zoltan Pos[3,4], Chrystal M Paulos[1,6], Máire F Quigley[7,8], Jorge R Almeida[9], Emma Gostick[7], Zhiya Yu[1], Carmine Carpenito[3,6], Ena Wang[3,4], Daniel C Douek[9], David A Price[7,8], Carl H June[3,6], Francesco M Marincola[3,4], Mario Roederer[2,5] & Nicholas P Restifo[1,5]

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-7$R\alpha^+$ T cell compartment characteristic of naive T cells. However, they expressed large amounts of CD95, IL-2Rβ, CXCR3, 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 repopulating 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 profiles[6], the capacity for self-renewal and the multipotency to differentiate into progeny with diverse fates[4,5].

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[4,5]. 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 view[8,9]. These memory T cells, which were designated memory stem T cells (T_{SCM} cells), have a $CD44^{low}CD62L^{high}$ 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 B cell lymphoma 2 (Bcl-2), the β chain of the IL-2 and IL-15 receptor (IL-2Rβ), and the chemokine (C-X-C motif) receptor CXCR3 (refs. 8,9). Whether a similar memory T cell population exists in humans is currently under intensive investigation[10].

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 c-KIT and the ability to expel cellular toxins through the ATP binding cassette (ABC)-superfamily multidrug efflux protein ABCB1 (ref. 11). However, recent data revealed that these cells are predominantly $Vα7.2^+$ mucosal associated invariant T cells (MAITs)[12]. More recently, others have[13] speculated that $CD45^{RO}CD8^{beta+}$ T cells that highly express β-catenin, a molecule associated with the generation of mouse T_{SCM} cells[8,9], 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-3β (GSK-3β)[8,9,14,15].

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Cell PRESS

Immunity
Article

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

Cameron J. Turtle,[1,2,*] Hillary M. Swanson,[1] Nobuharu Fujii,[1] Elihu H. Estey,[1,2] and Stanley R. Riddell[1,2]
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[2]Department of Medicine, University of Washington, Seattle, WA 98109, USA
[*]Correspondence: cturtle@fhcrc.org
DOI 10.1016/j.immuni.2009.09.015

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 nonefluxing 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 (Murali-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 (Judge 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 insults that cause lymphocytopenia have not been extensively studied.

$CD8^+$ memory T cells are required to control reactivations of cytomegalovirus (CMV) and Epstein Barr virus (EBV) (Smiets et al., 2002; Walter et al., 1995). Patients with acute myeloid leukemia (AML) receive repeated cycles of chemotherapy that induce severe but transient lymphocytopenia, yet rarely develop clinical infection with CMV or EBV either during the lymphocyte nadir or after recovery of lymphocyte numbers (Sung et al., 2009). The absence of infection in AML patients undergoing chemotherapy suggests that sufficient virus-specific memory T cells survive chemotherapy and reconstitute functional, long-lived immunity thereafter.

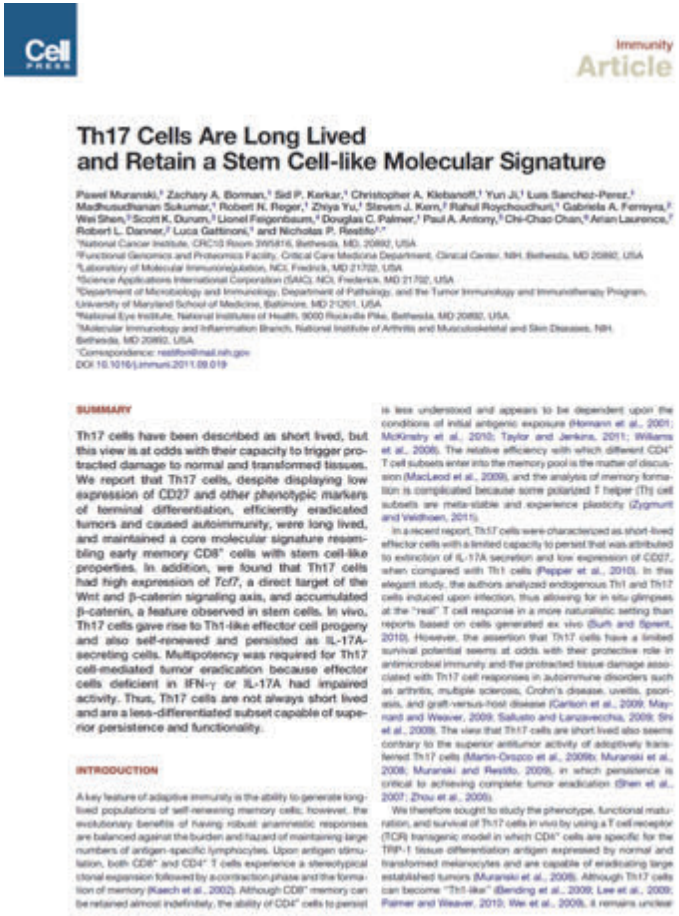
Two broad subsets of memory T cells, termed central memory (Tcm) and effector memory (Tem) cells, have been identified that differ in phenotype and function (Sallusto et al., 1999). In humans, these subsets have considerable heterogeneity, which could potentially include subpopulations that serve a distinct 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 Roninson, 1991; Gottesman et al., 2002; Mizutani 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 Tcm and Tem 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 AML treated with chemotherapy that includes ABCB1 substrates such as daunorubicin and idarubicin develop profound transient bone marrow hypoplasia and peripheral lymphocyte depletion (Figure 1A) (Berman 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

Which T-cell sub-population to genetically modify?



Cell

Immunity

Article

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

Pawel Muranski,[1] Zachary A. Borman,[1] Sid P. Kerkar,[1] Christopher A. Klebanoff,[1] Yun Ji,[1] Luis Sanchez-Perez,[1] Madhusudhanan Sukumar,[1] Robert N. Reger,[1] Zhiya Yu,[1] Steven J. Kern,[2] Rahul Roychoudhuri,[1] Gabriela A. Ferreyra,[2] Wei Shen,[3] Scott K. Durum,[3] Lionel Feigenbaum,[4] Douglas C. Palmer,[1] Paul A. Antony,[5] Chi-Chao Chan,[6] Arian Laurence,[7] Robert L. Danner,[2] Luca Gattinoni,[1] and Nicholas P. Restifo[1,*]

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[2]Functional Genomics and Proteomics Facility, Critical Care Medicine Department, Clinical Center, NIH, Bethesda, MD 20892, USA
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DOI 10.1016/j.immuni.2011.09.019

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-renewing 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 (Homann et al., 2001; McKinstry et al., 2010; Taylor and Jenkins, 2011; Williams et al., 2006). 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 Veldhoen, 2011).

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 (Surh and Sprent, 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 disorders such as arthritis, multiple sclerosis, Crohn's disease, uveitis, psoriasis, and graft-versus-host disease (Carlson et al., 2009; Maynard and Weaver, 2009; Salluzzo and Lanzavecchia, 2009; 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-Orozco 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., 2005).

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., 2009; Lee et al., 2009; Palmer and Weaver, 2010; Wei et al., 2009), it remains unclear

RESEARCH ARTICLE

IMMUNOLOGY

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

Chrystal M. Paulos,[1]* Carmine Carpenito,[1] Gabriela Plesa,[2] Megan M. Suhoski,[2] Angel Varela-Rohena,[2] Tatiana N. Golovina,[2] Richard G. Carroll,[1†] James L. Riley,[1] Carl H. June[1]*
(Published 27 October 2010; Volume 2 Issue 55 55ra78)

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, naïve $CD4^+$ 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 (1). In contrast, T_{reg} cells suppress effector T cell functions and are essential for regulating autoimmune responses (6), 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 tumor immunity varies, showing the potential for both antitumorigenic and protumorigenic 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-1β, IL-21, and IL-23] and transcription factors (such as RORC2 and RORα) in human T_H17 cell development are distinct from T_H1 and T_H2 effector cells (11–14). 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 co-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 cell 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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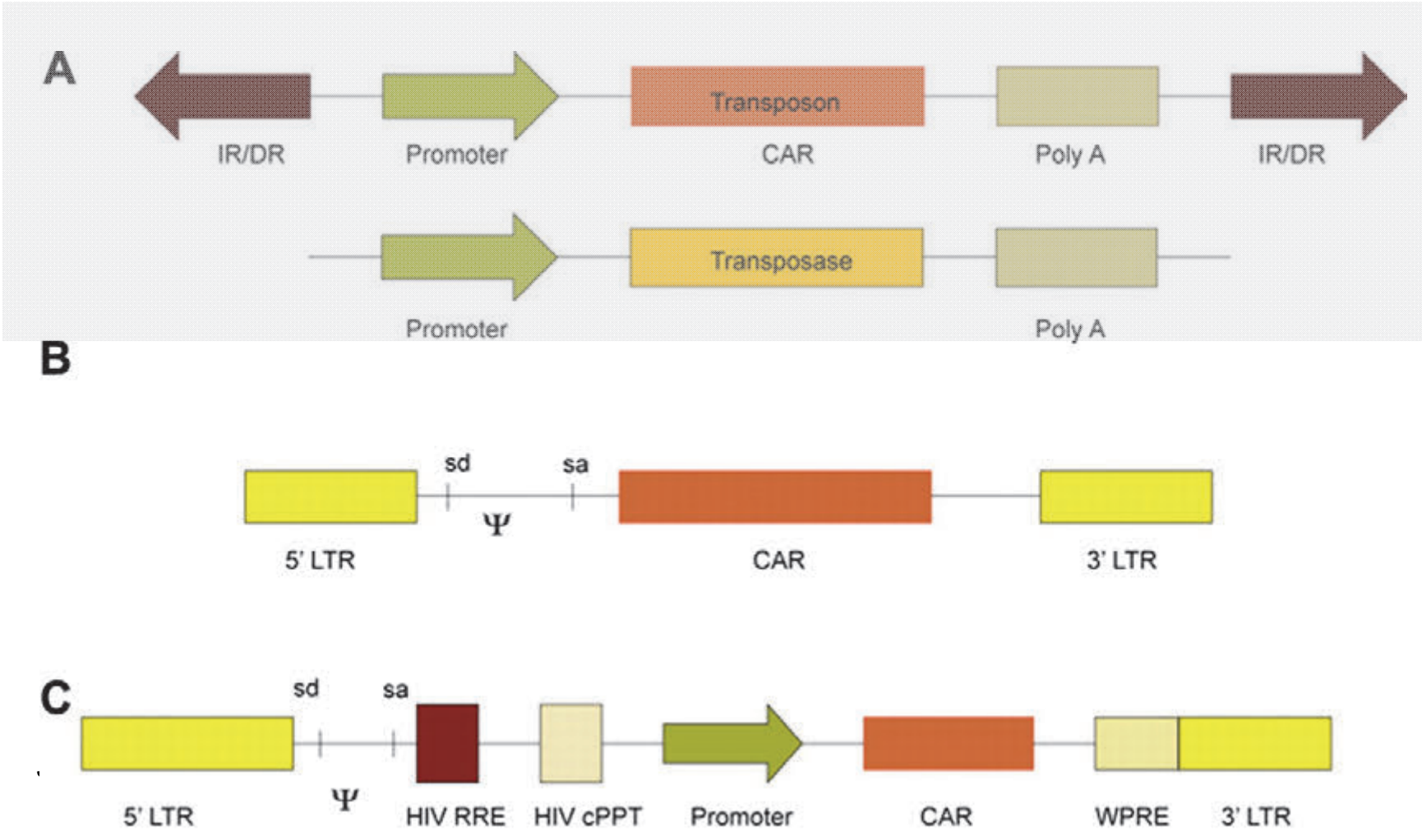
†Deceased.

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

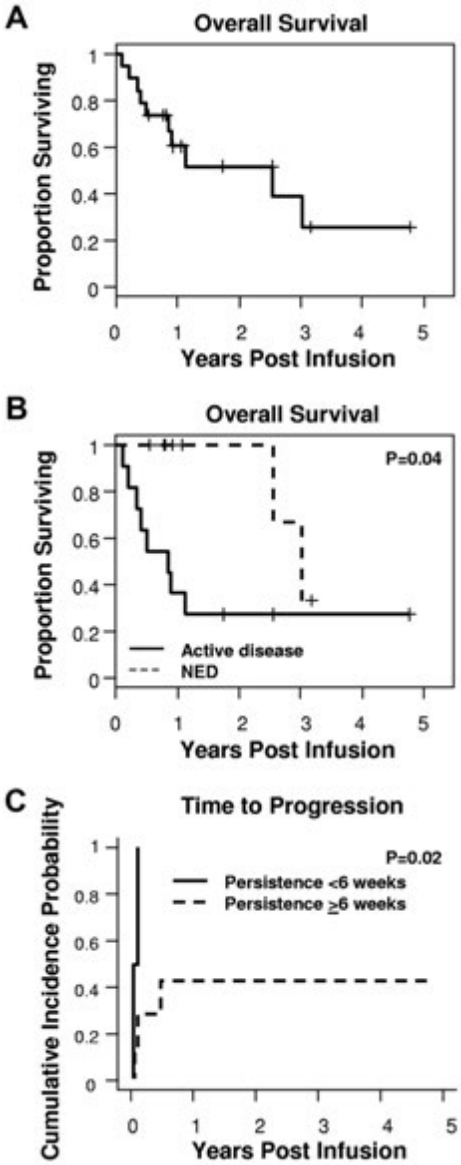
Institute	CD19$^+$ Disease	Clinical Trial.gov Identifiers	Chemotherapy prior to T-cell infusion	IL-2 after T-cell infusion	Gene transfer approach	CAR scaffold to append scFv	Mouse mAb clone used to derive scFv	CAR signaling endodomain(s)	Loss of normal B cells?
U Penn	CLL	NCT01029366	Yes	No	Lentivirus	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_2CH_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



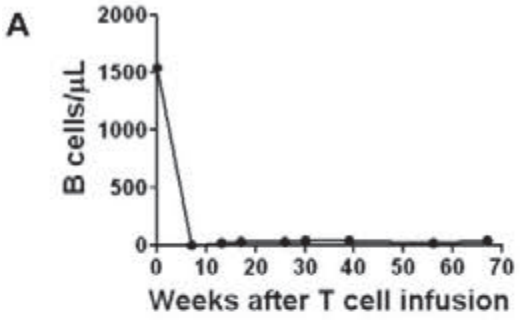
GD_2-targeted CAR^+ T cells in patients with neuroblastoma

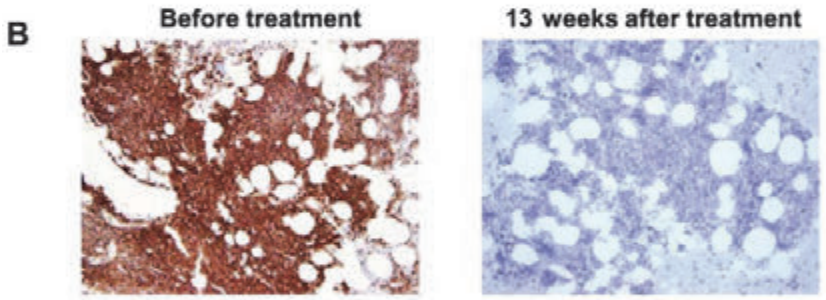


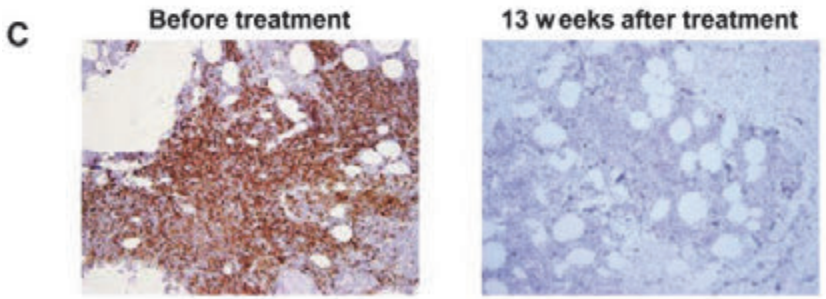
Antitumor activity and long-term fate of CAR^+ T cells in patients with neuroblastoma

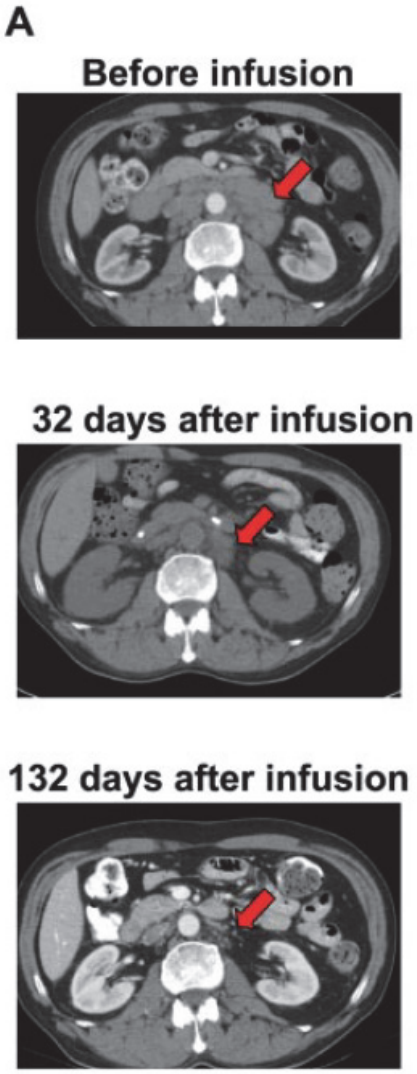
Louis C U et al. Blood 2011;118:6050-6056

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



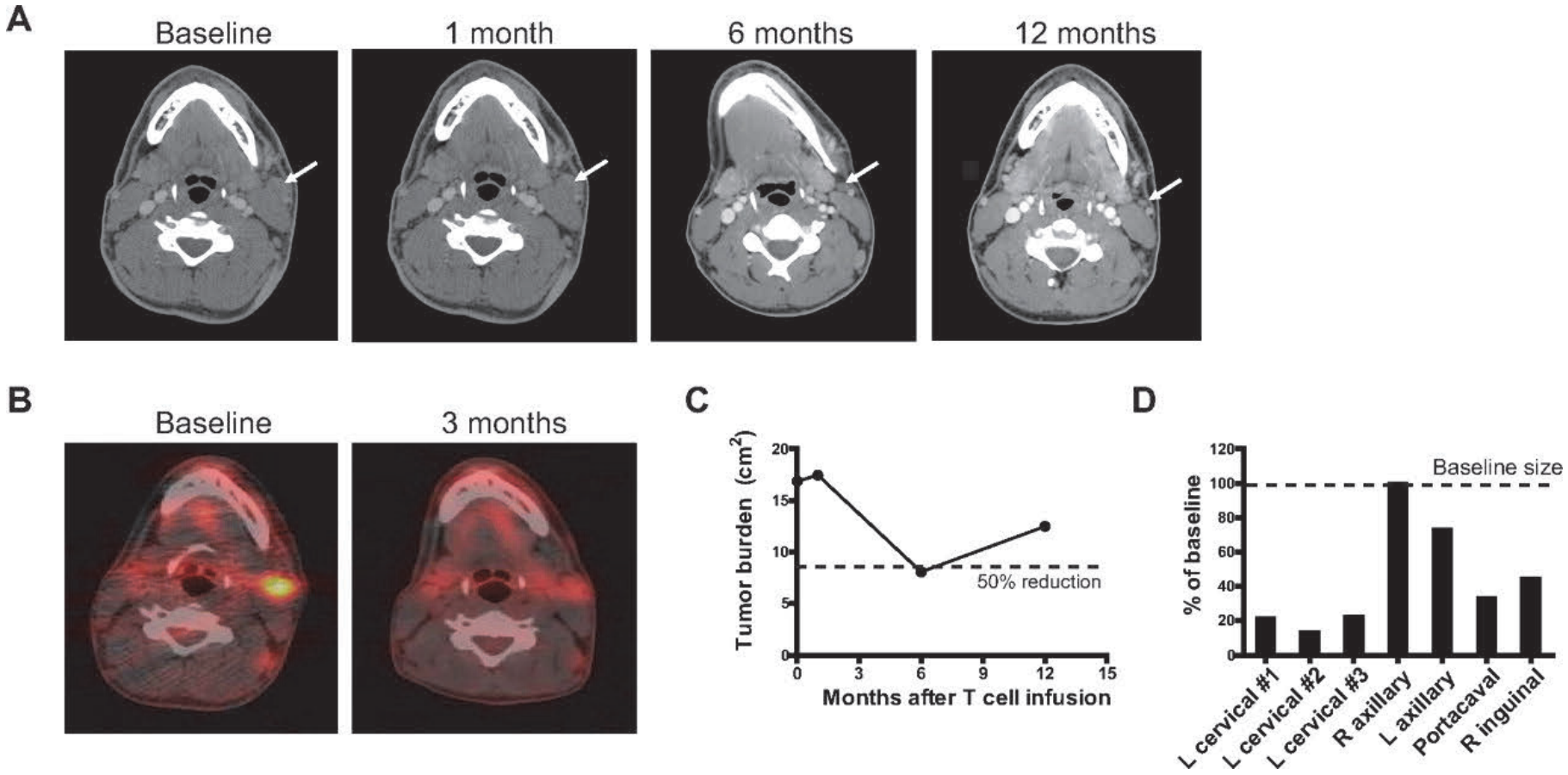






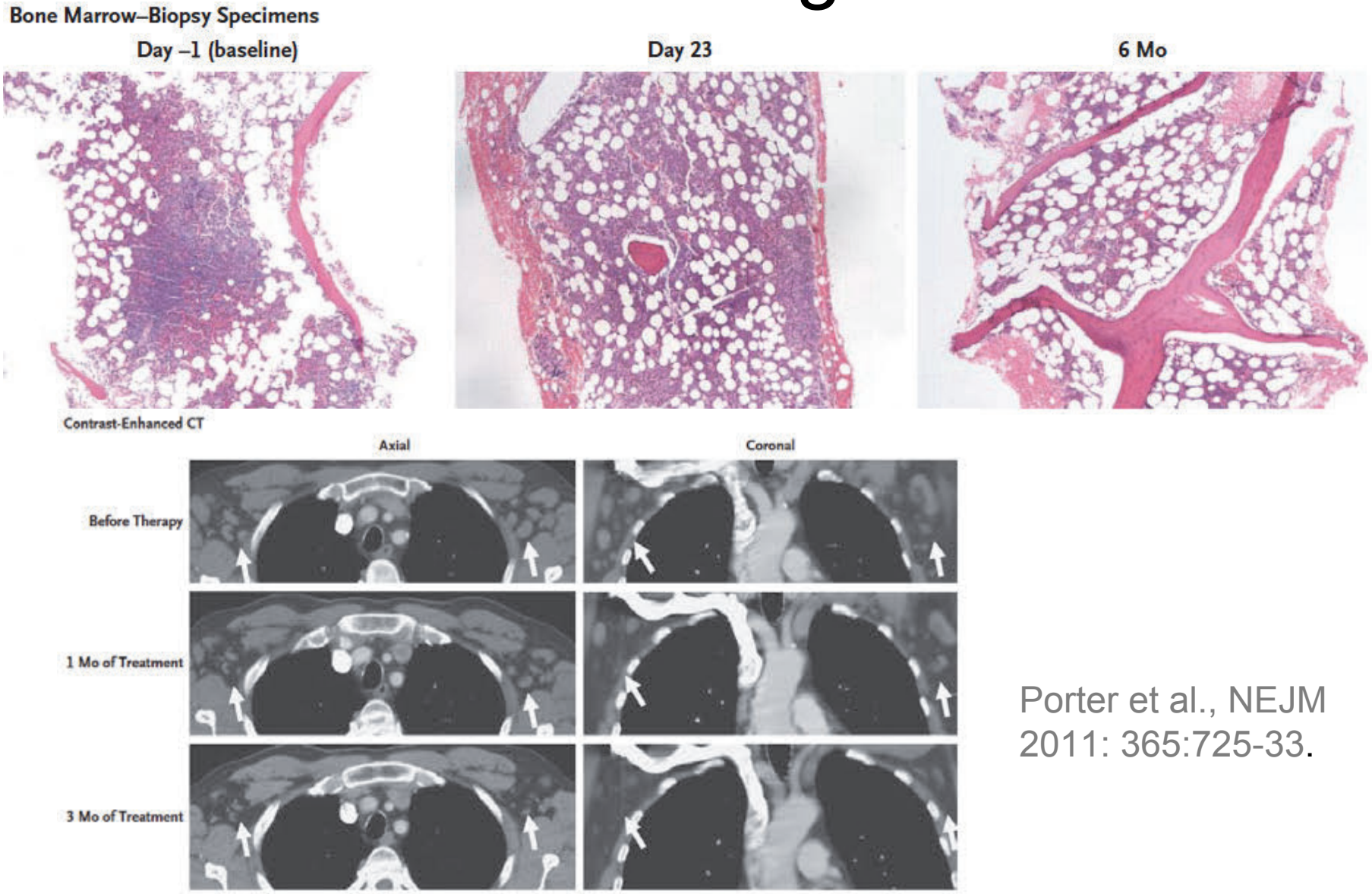
Kochenderfer et al., Blood. 2012 119: 2709-2720

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



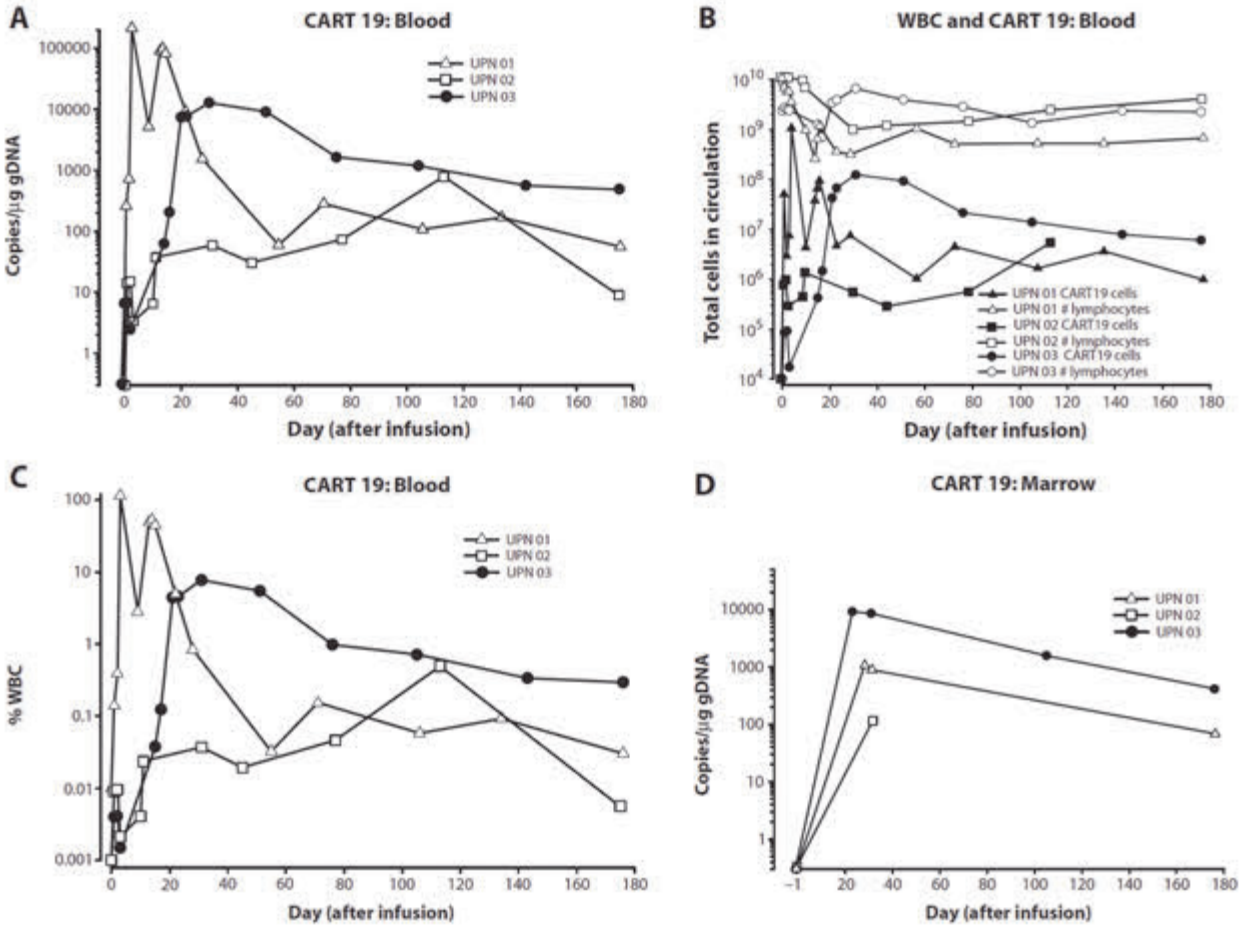
Till B G et al. Blood 2012;119:3940-3950

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



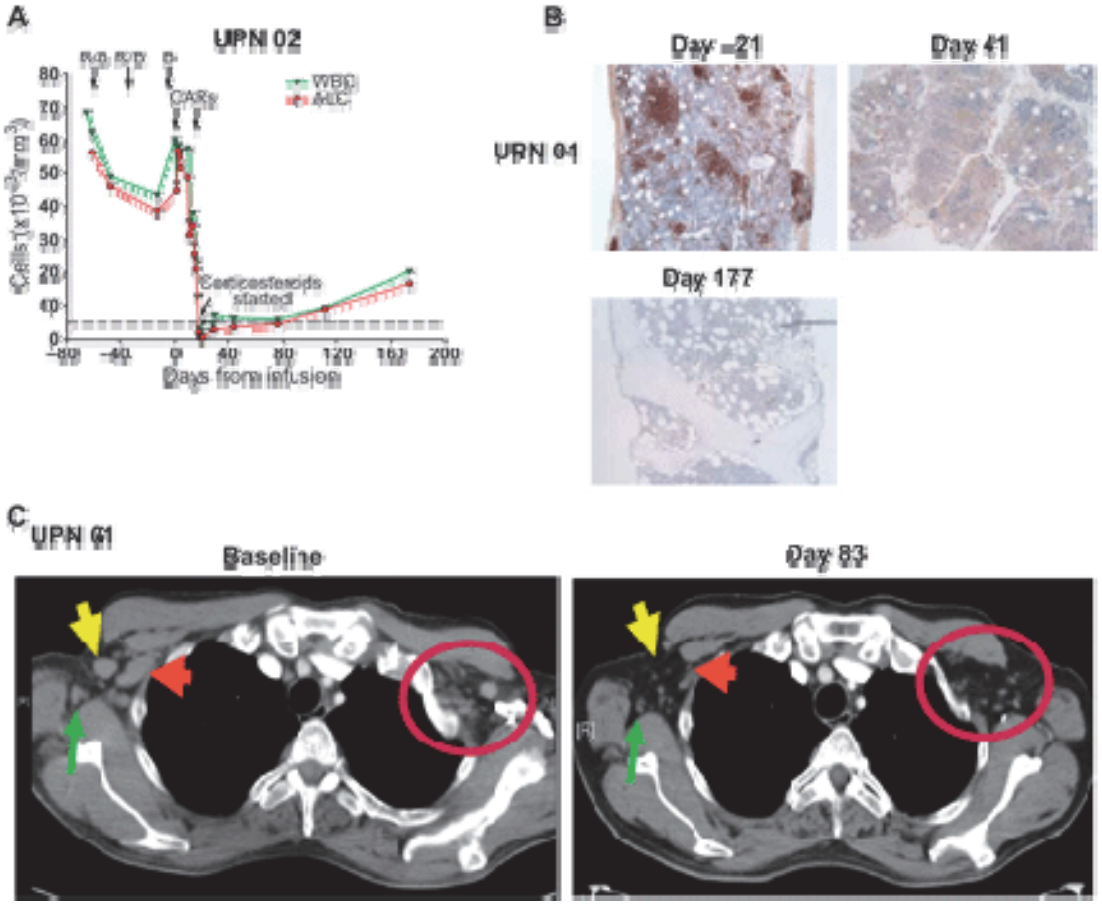
Porter et al., NEJM 2011: 365:725-33.

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



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

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

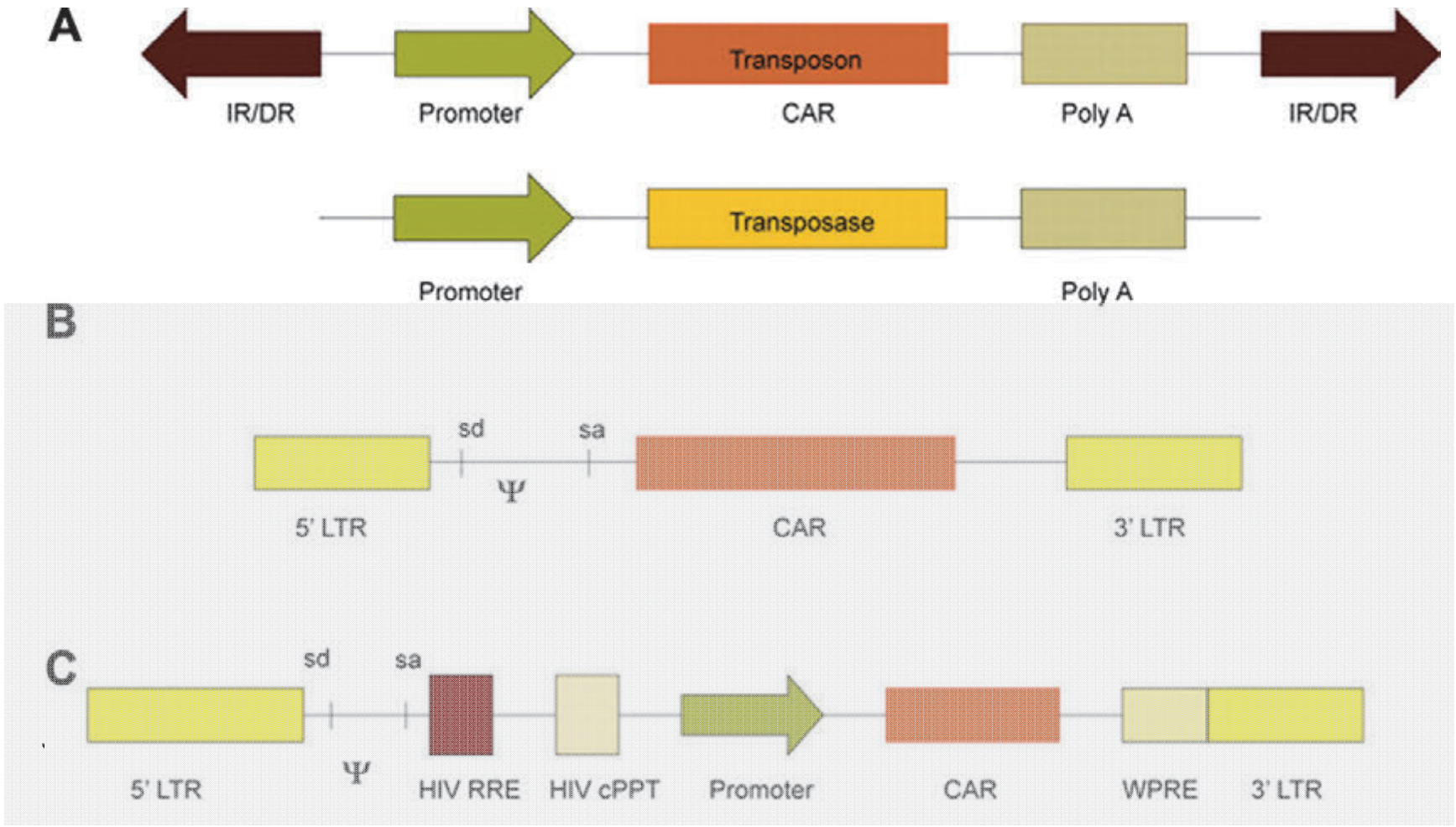
Diagnosis-Patient	Adverse events	Grade	Related
CLL-1	Febrile neutropenia	3	Probable
	Rigors, chills	2	Probable
CLL-2	Fever, rigors, chills	2	Probable
	Chest pain	2	Probable
CLL-3	Fever, rigors, chills	1	Probable
CLL-4	Fever	2	Probable
	Rigors, chills, dyspnea	1	Probable
	Hypotension, renal failure	5	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

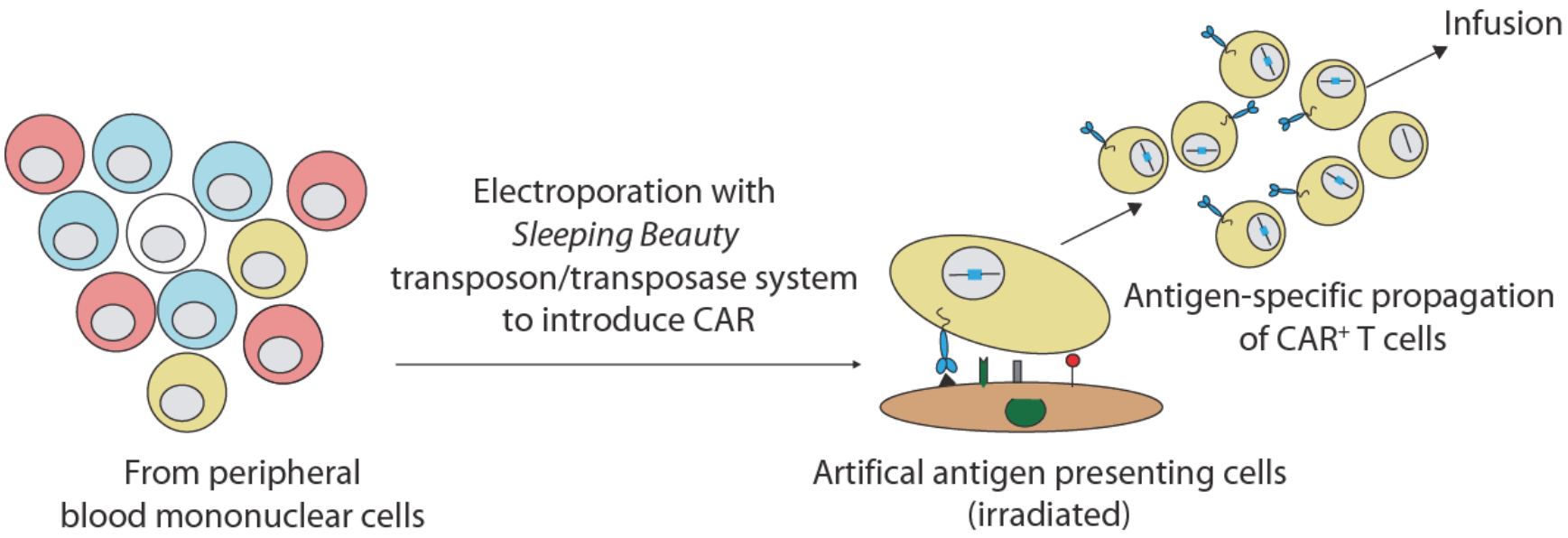
Patient	Toxicities*†
1‡	Fatigue, herpes zoster with secondary otitis externa 6 months after treatment
2	*Escherichia coli* bacteremia, died with influenza pneumonia, dyspnea, hypoxemia, nonbacterial thrombotic endocarditis, cerebral infarction, elevated liver enzymes
3	Hypotension, acute renal failure, 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

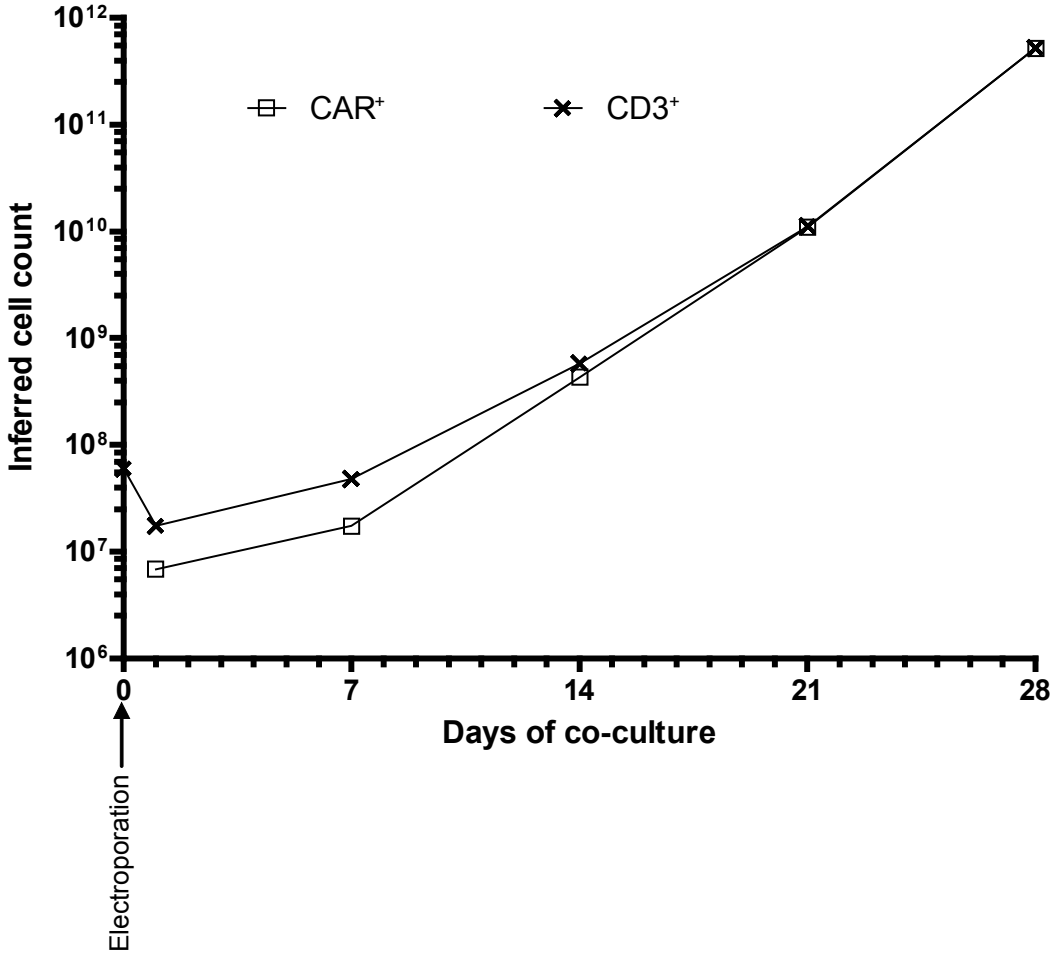
Vector systems to express CAR transgenes used in clinical trials



Genetic modification and propagation of T cells



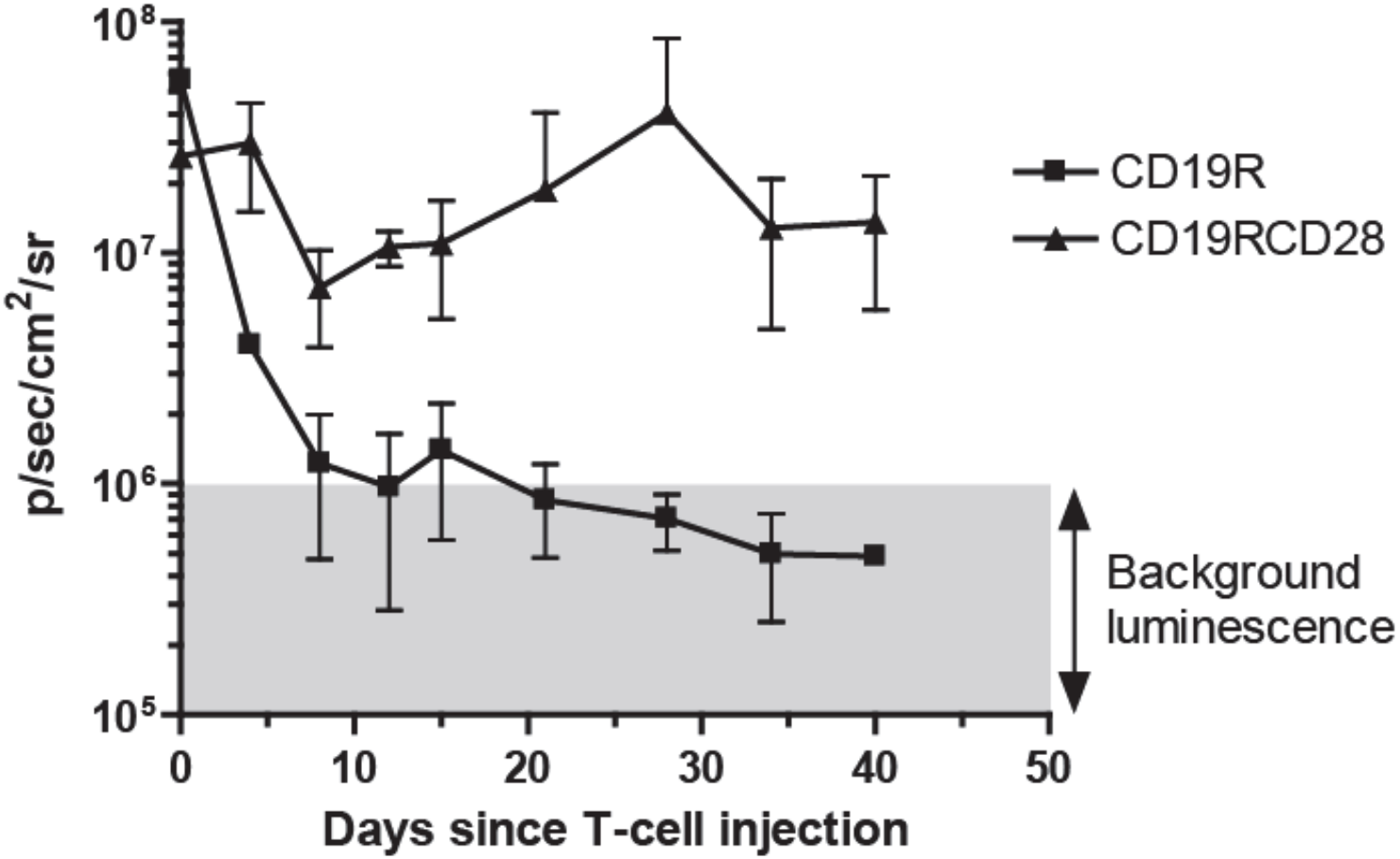
Selective outgrowth of CAR+ T cells on aAPC



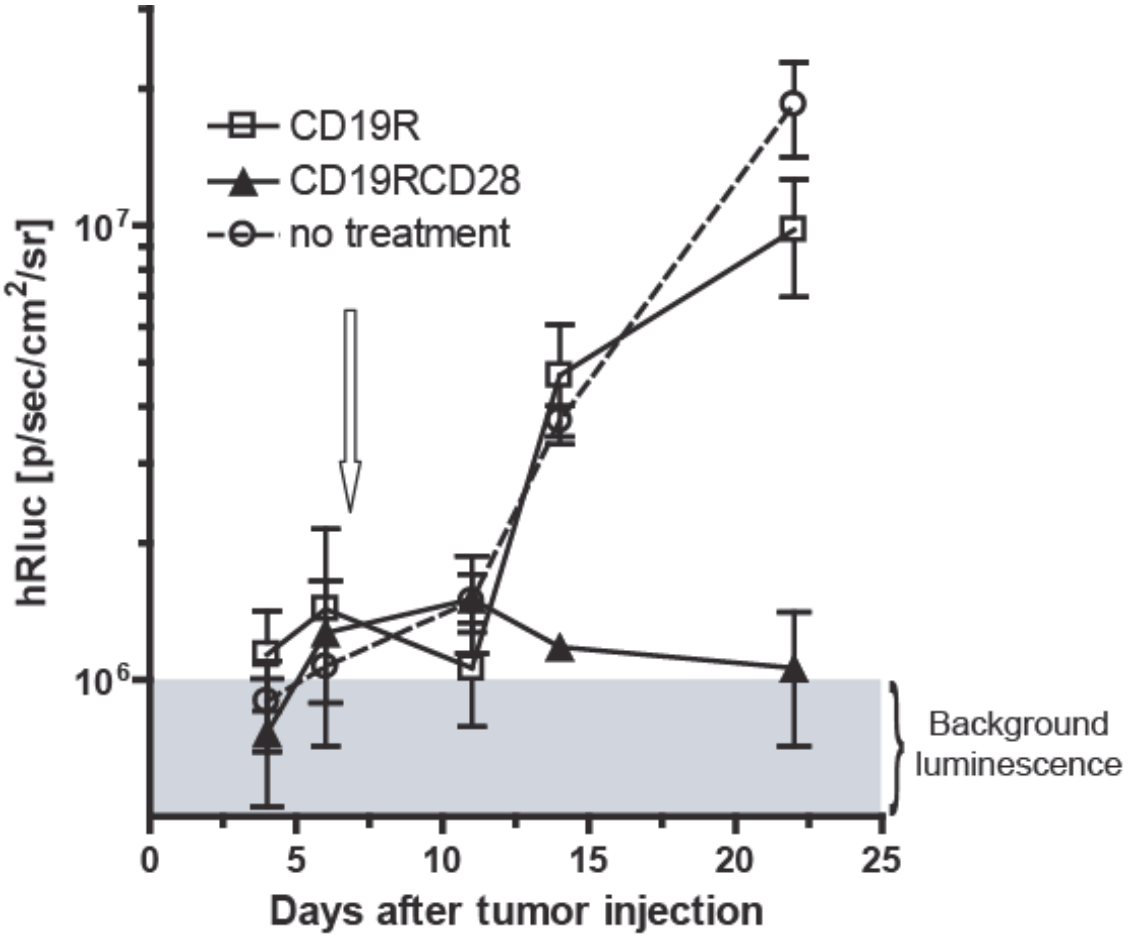
Mouse models – predictive?

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

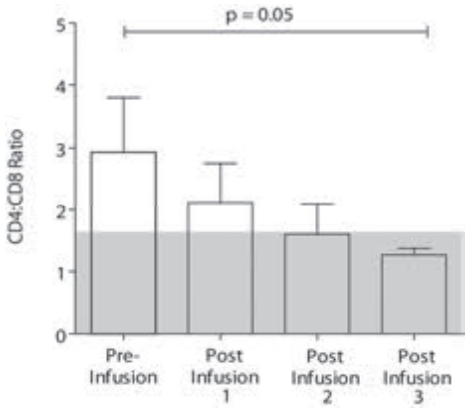
1st generation vs. 2nd generation CARs

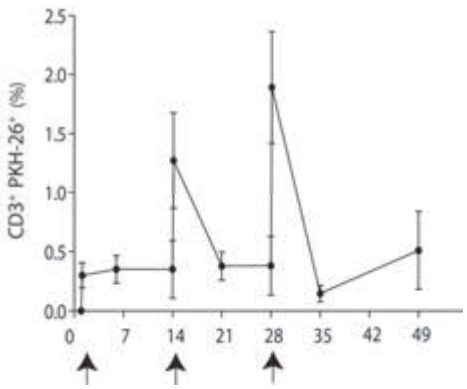


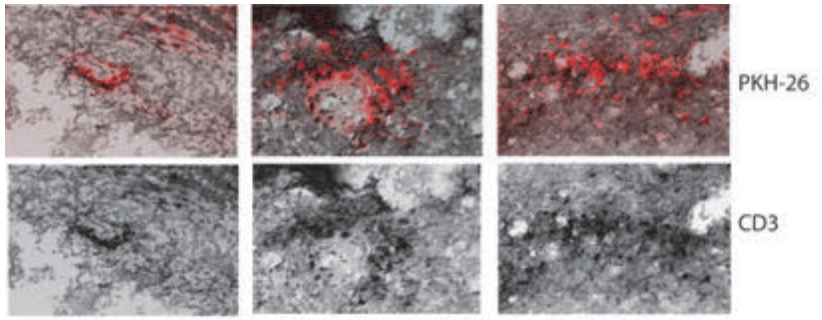
1st generation vs. 2nd generation CARs

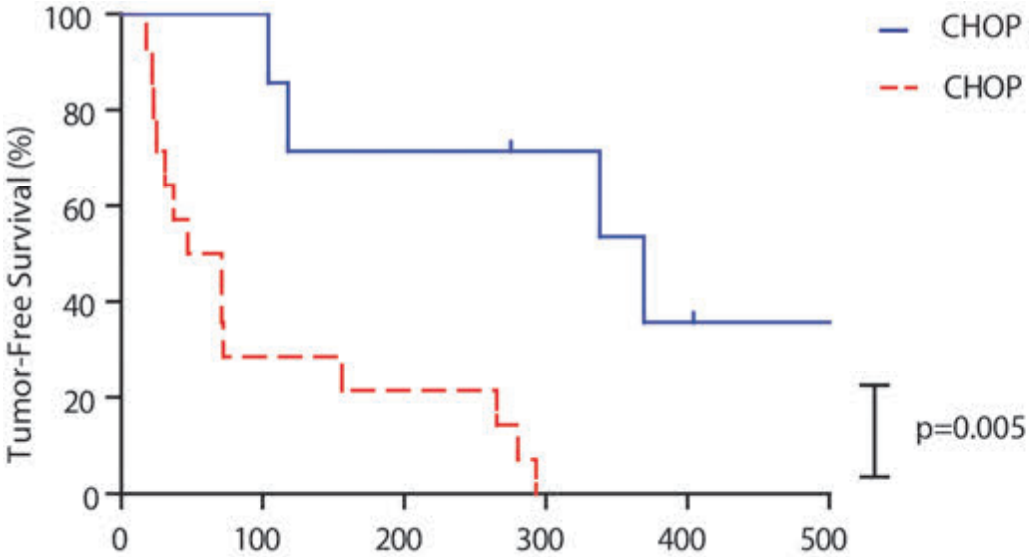


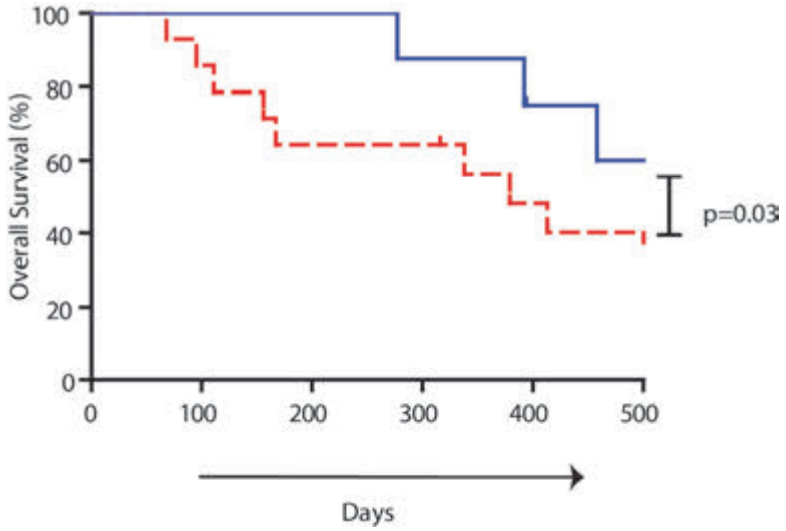
Companion canine NHL



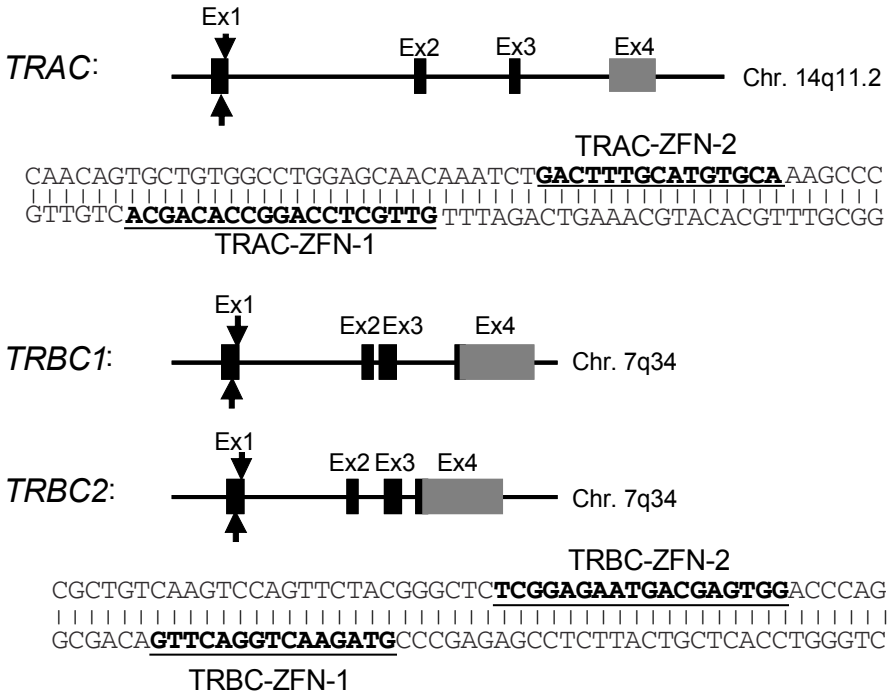








“Off-the-shelf” CAR^{+}TCR^{neg} T cells



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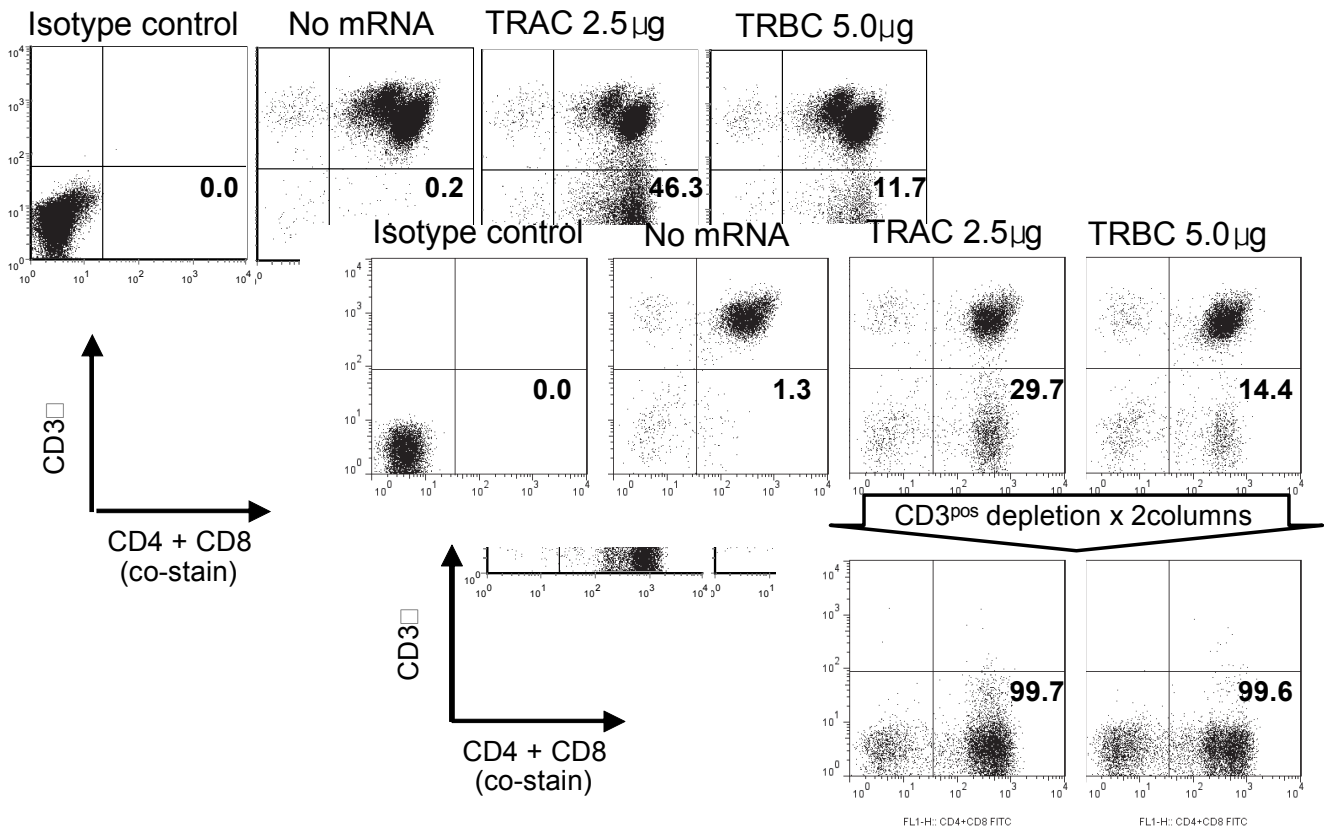

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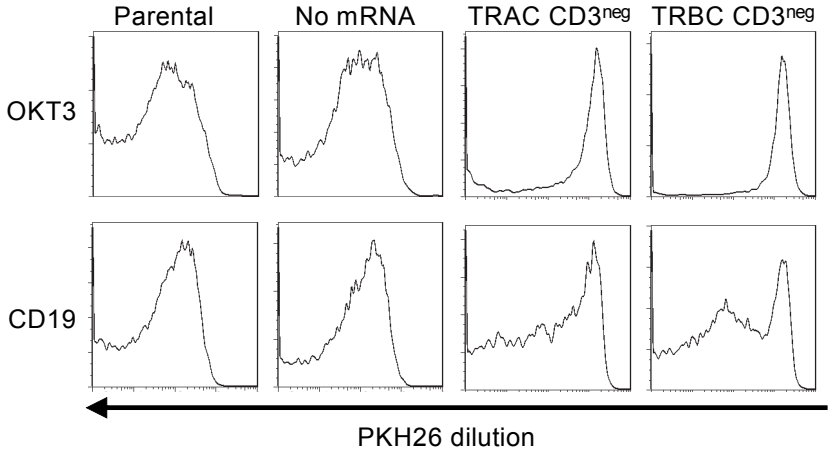

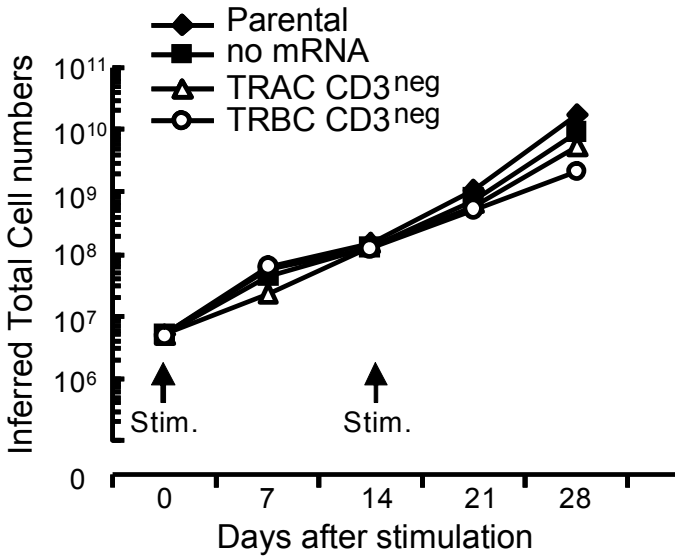

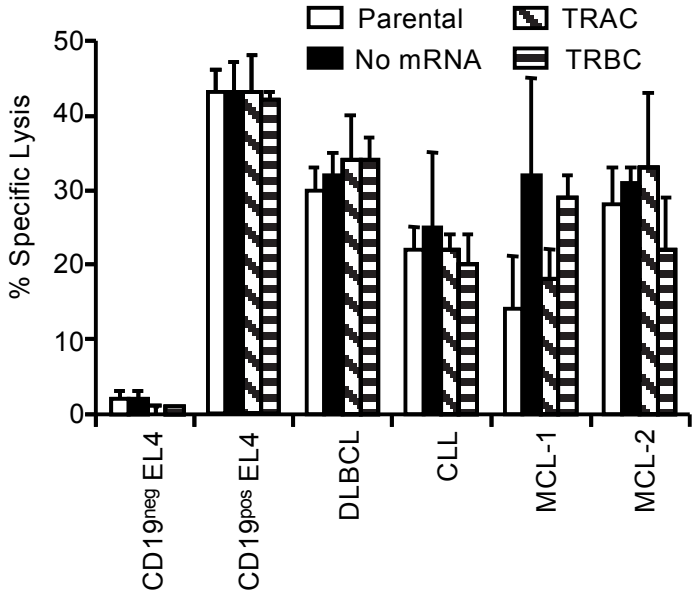

Future approach to clinical trials

- 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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