CAR design

RAC September 10, 2013

Michel Sadelain, MD, PhD
Director, Center for Cell Engineering
Molecular Pharmacology and Chemistry Program
Departments of Medicine and Pediatrics
Memorial Sloan-Kettering Cancer Center
New York, New York

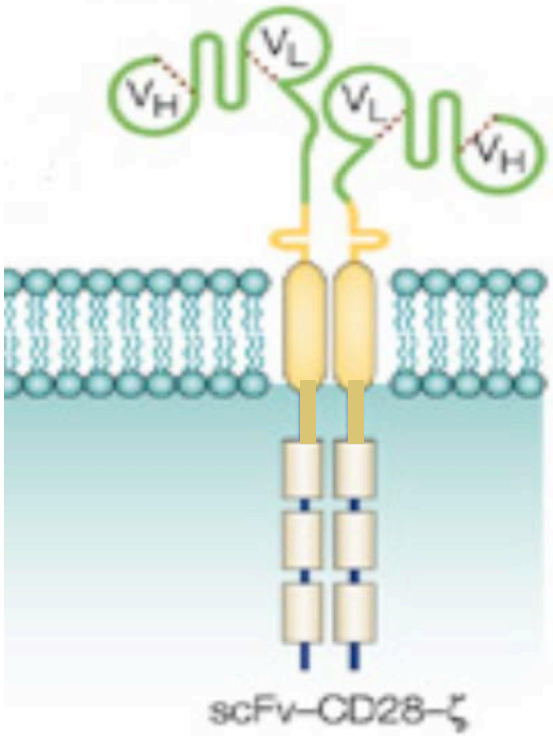
Modern CARs

(aka second generation)

scFv

Costimulation

CD3z



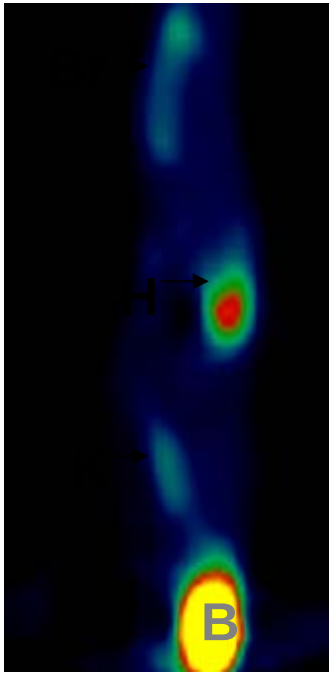
Nature Reviews | Cancer

Antigen recognition

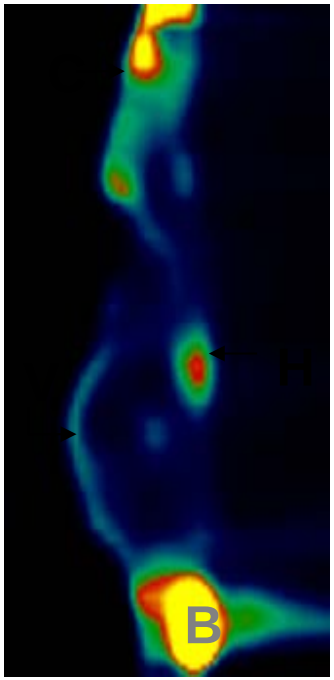
Persistence/potency

T cell activation

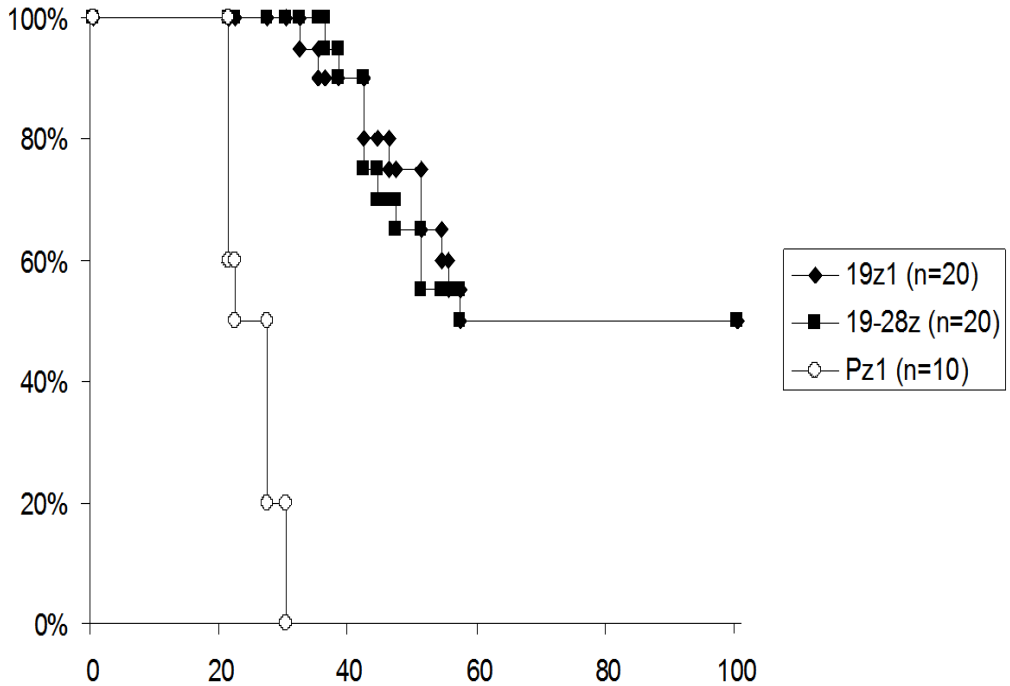
19z1+ T cells expanded on CD19+CD80+IL15+ AAPCs eradicate established systemic Raji in SCID-beige mice



Tumor Free

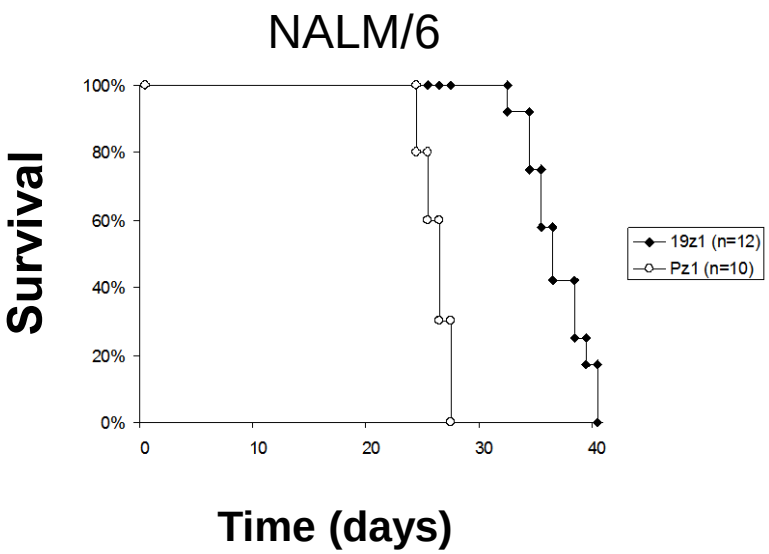


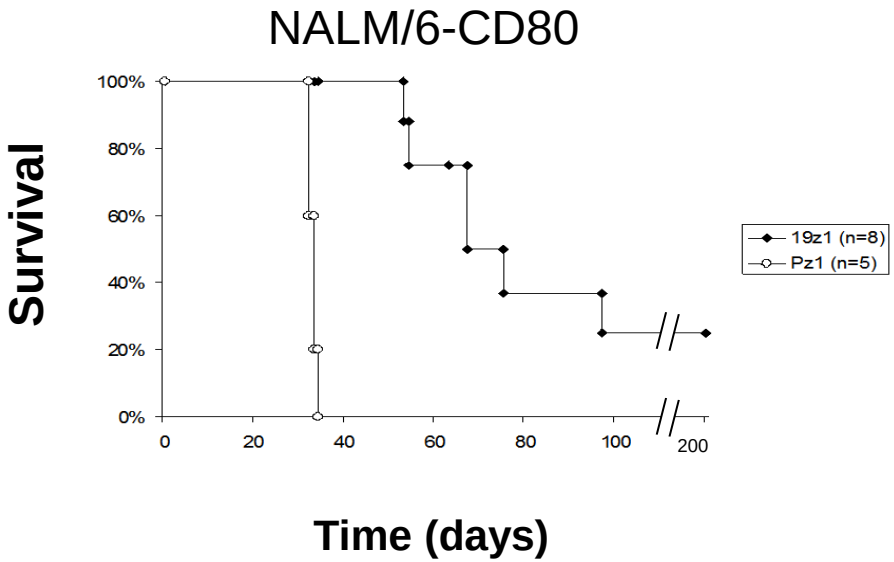
Untreated
4 weeks



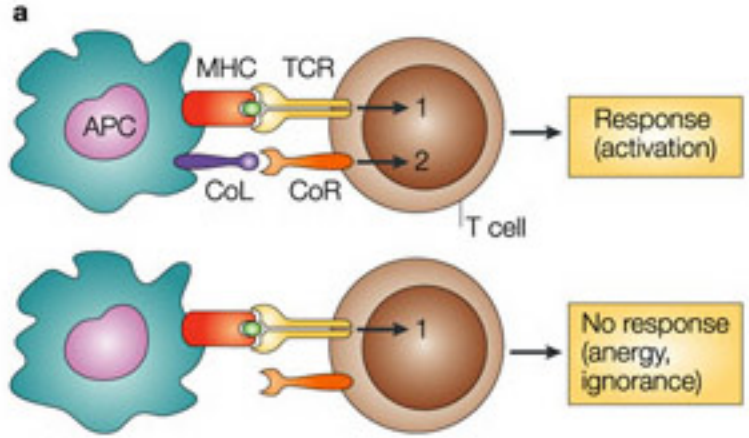
Brentjens et al, *Nat Med*, 2003

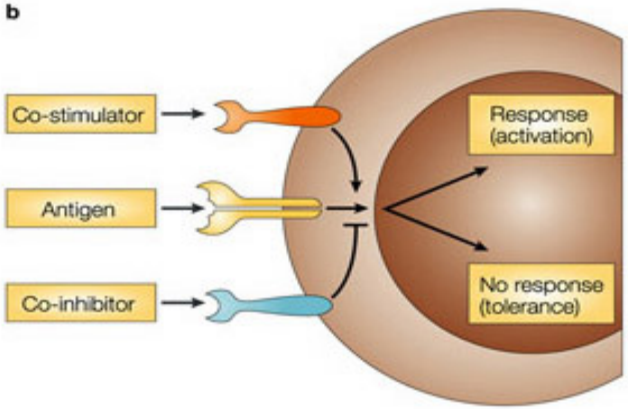
Potency - the importance of providing agonistic costimulation to adoptively transferred T cells

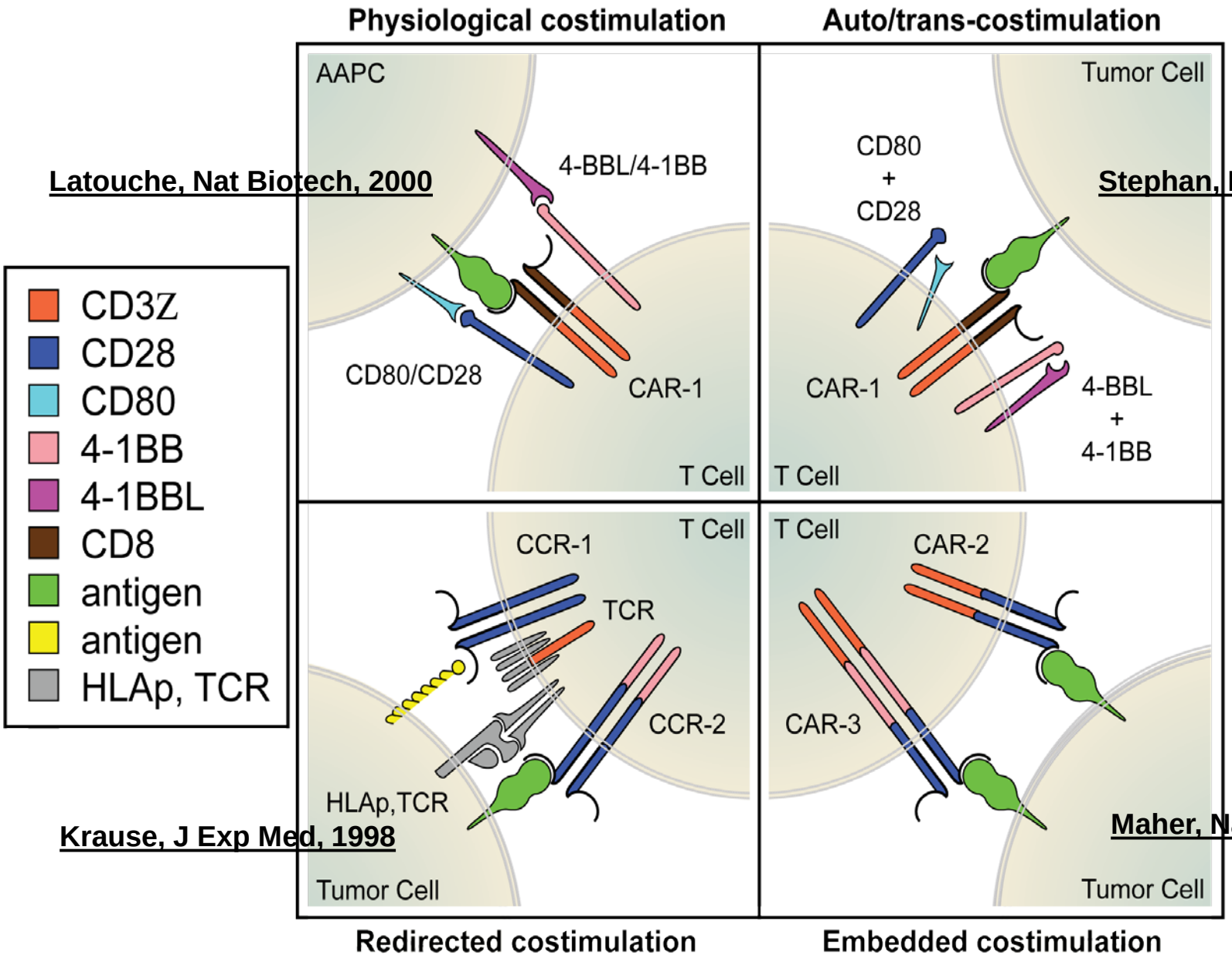




Brentjens et al., *Nat Med*, 2003





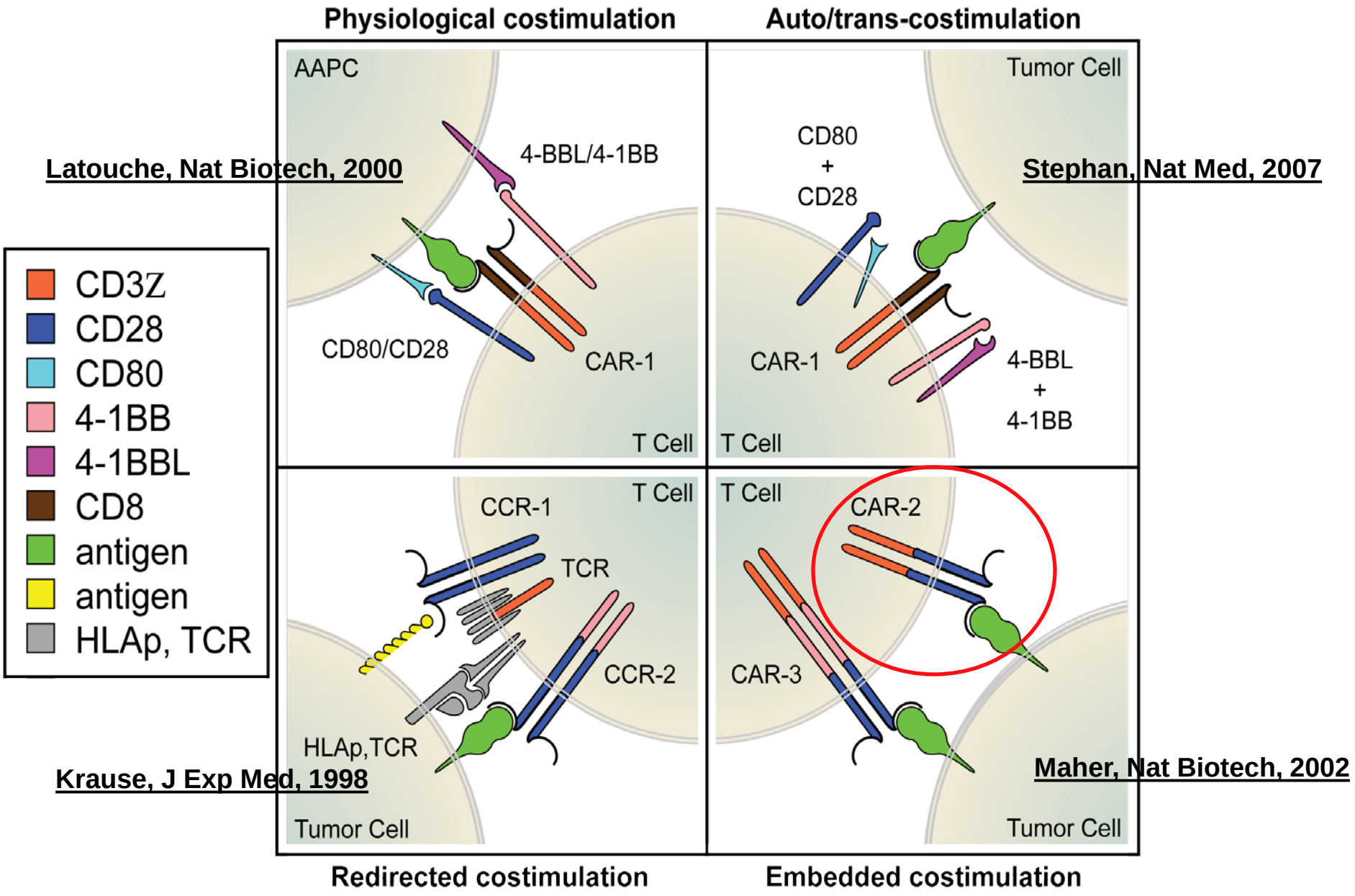


Latouche, Nat Biotech, 2000

Stephan, Nat Med, 2007

Krause, J Exp Med, 1998

Maher, Nat Biotech, 2002


Physiological costimulation
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Stephan, Nat Med, 2007
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CAR-1
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Tumor Cell
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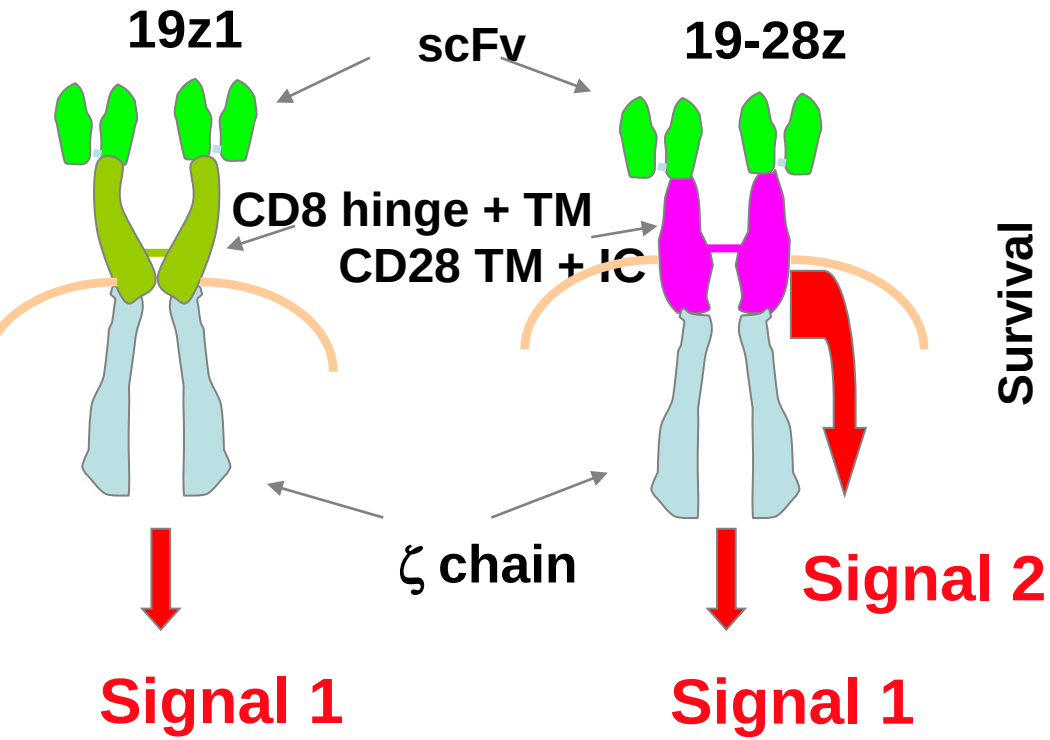
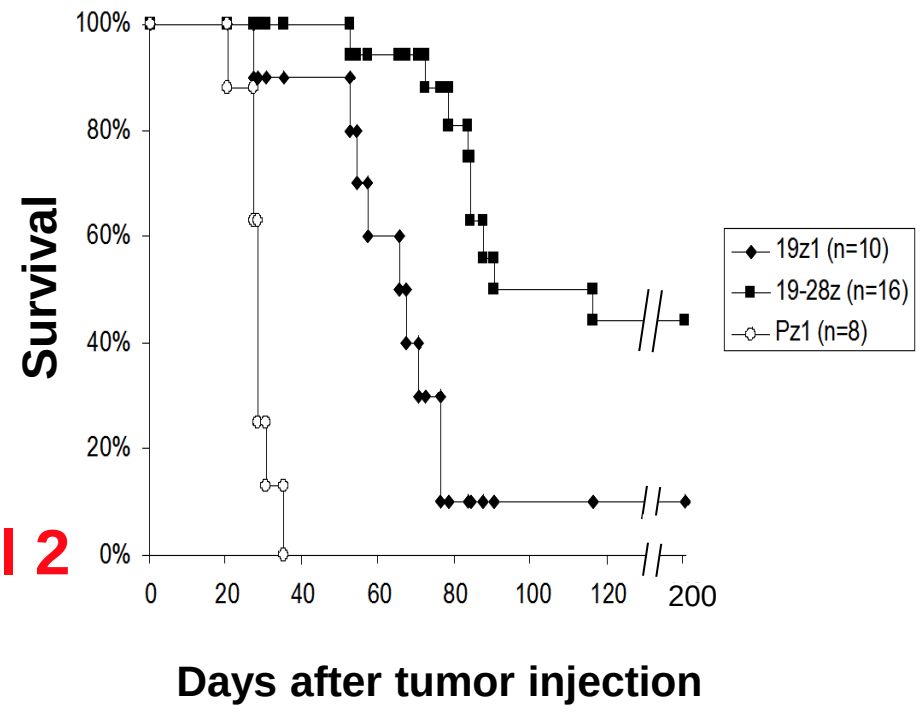
Redirected costimulation
Embedded costimulation

“Embedded” CD28 costimulation potentiates NALM/6 rejectio



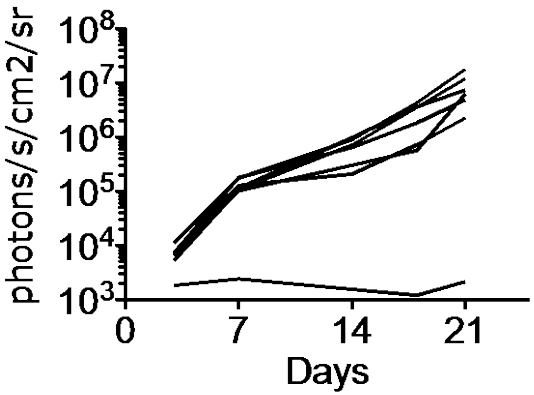
Maher, *Nat Biotechnol*, 2002



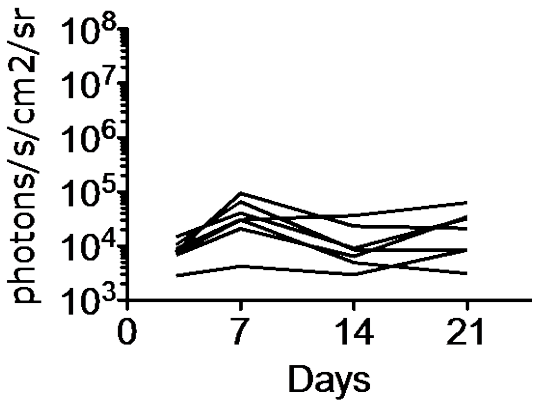
Brentjens, *Clin Can Res*, 2007

All CARs within a given category are not equivalent…

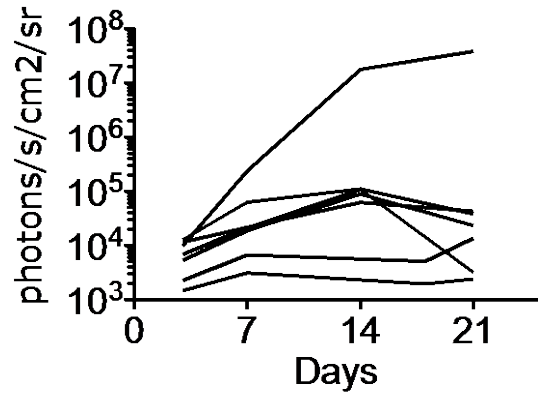
19bbz-3-LNGFR



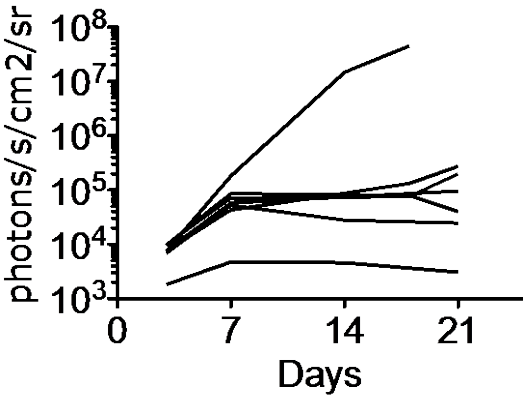
19bbz-4-LNGFR



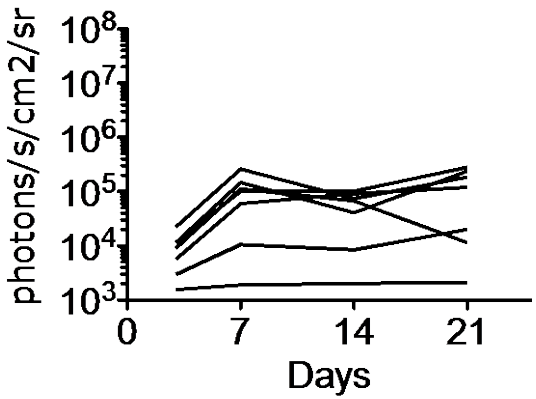
19bbz-5-LNGFR



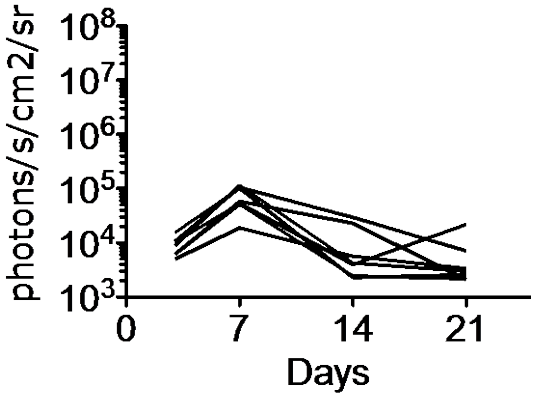
19bbz-6-LNGFR



19bbz-7-LNGFR

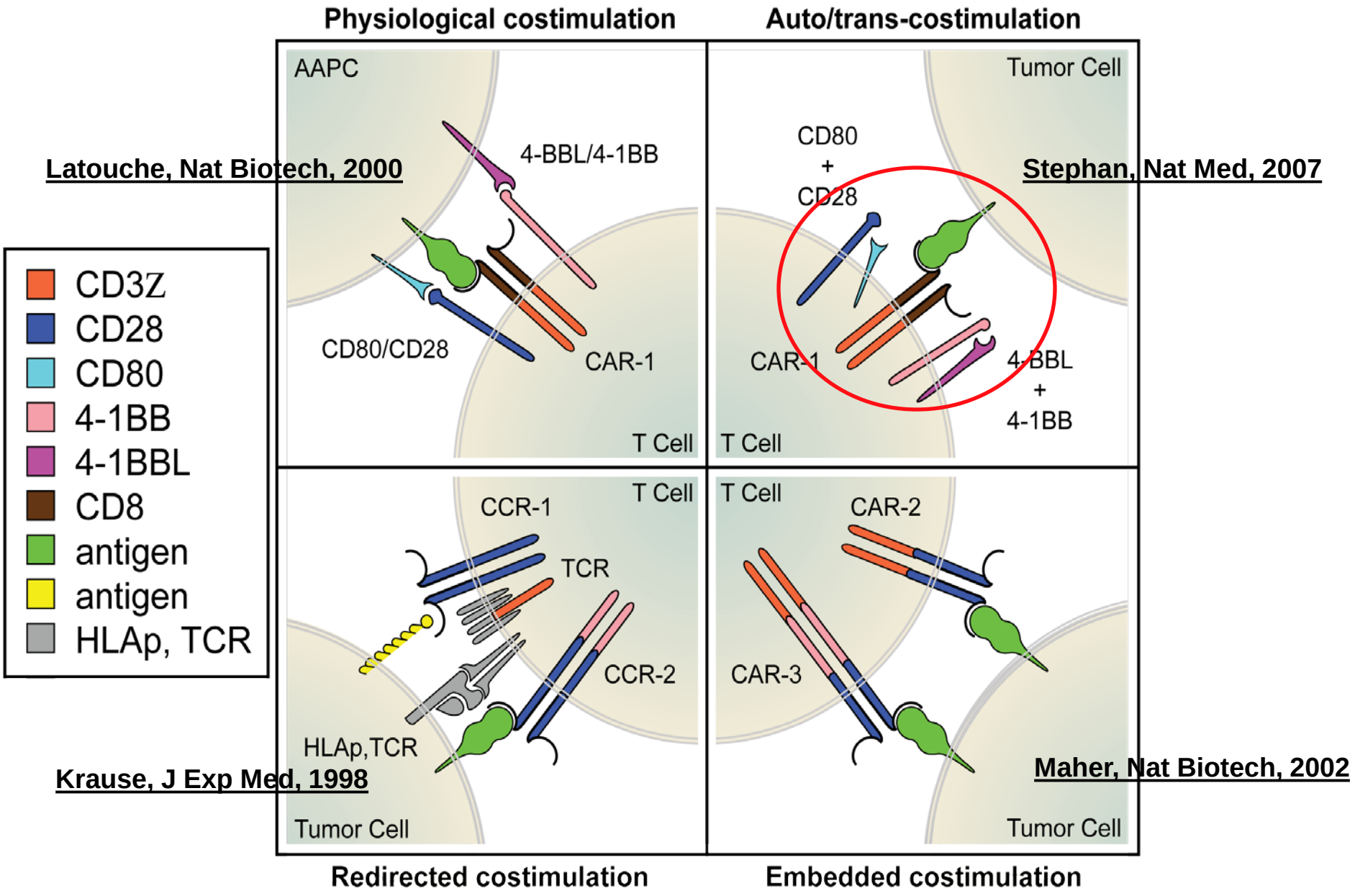


1928z-LNGFR



0.2 E6 CAR+ T cells; 0.5 E6 NALM/6 day -4

Maud Condomines, Jason Plotkin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Krause, J Exp Med, 1998
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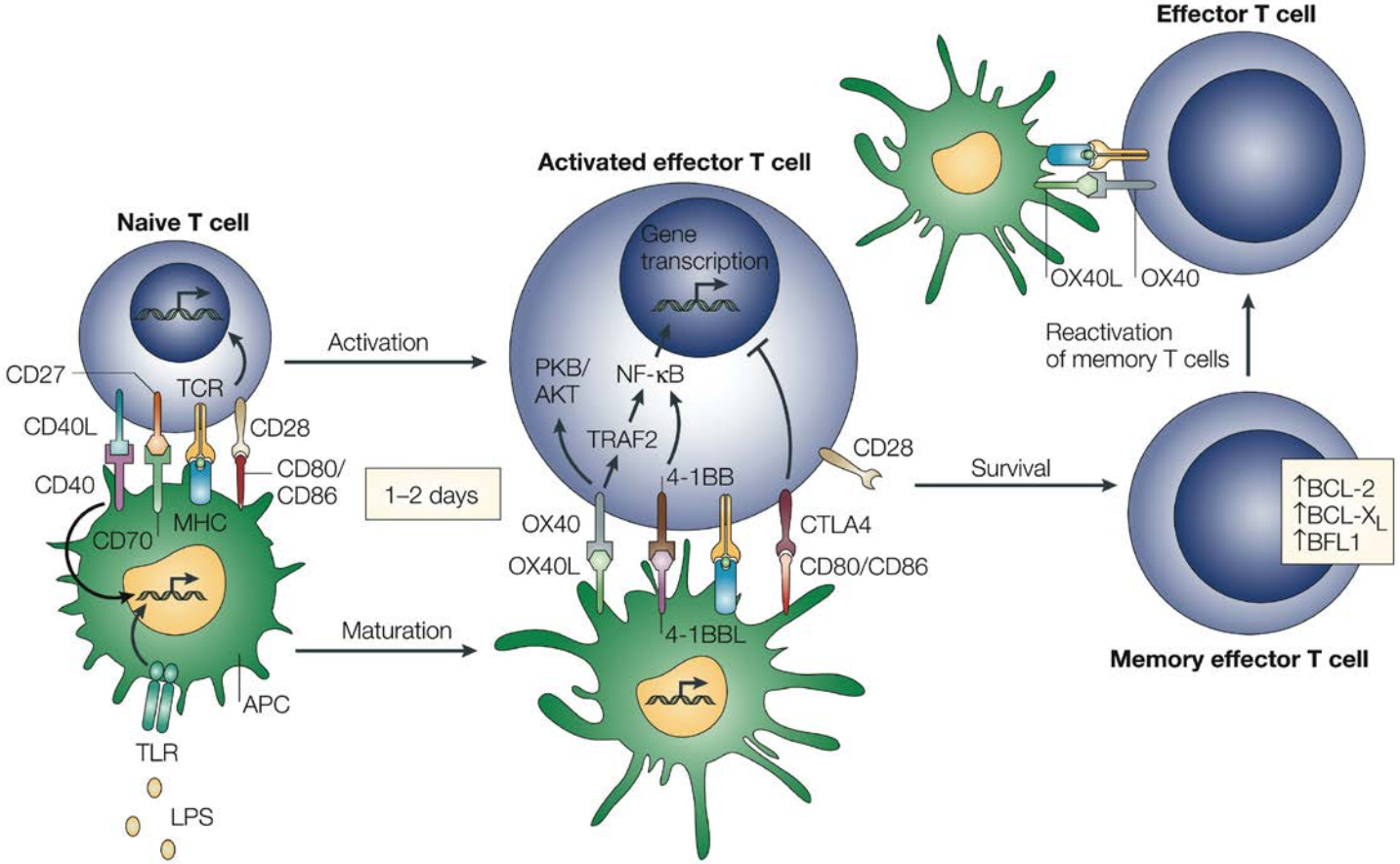
Redirected costimulation
Embedded costimulation

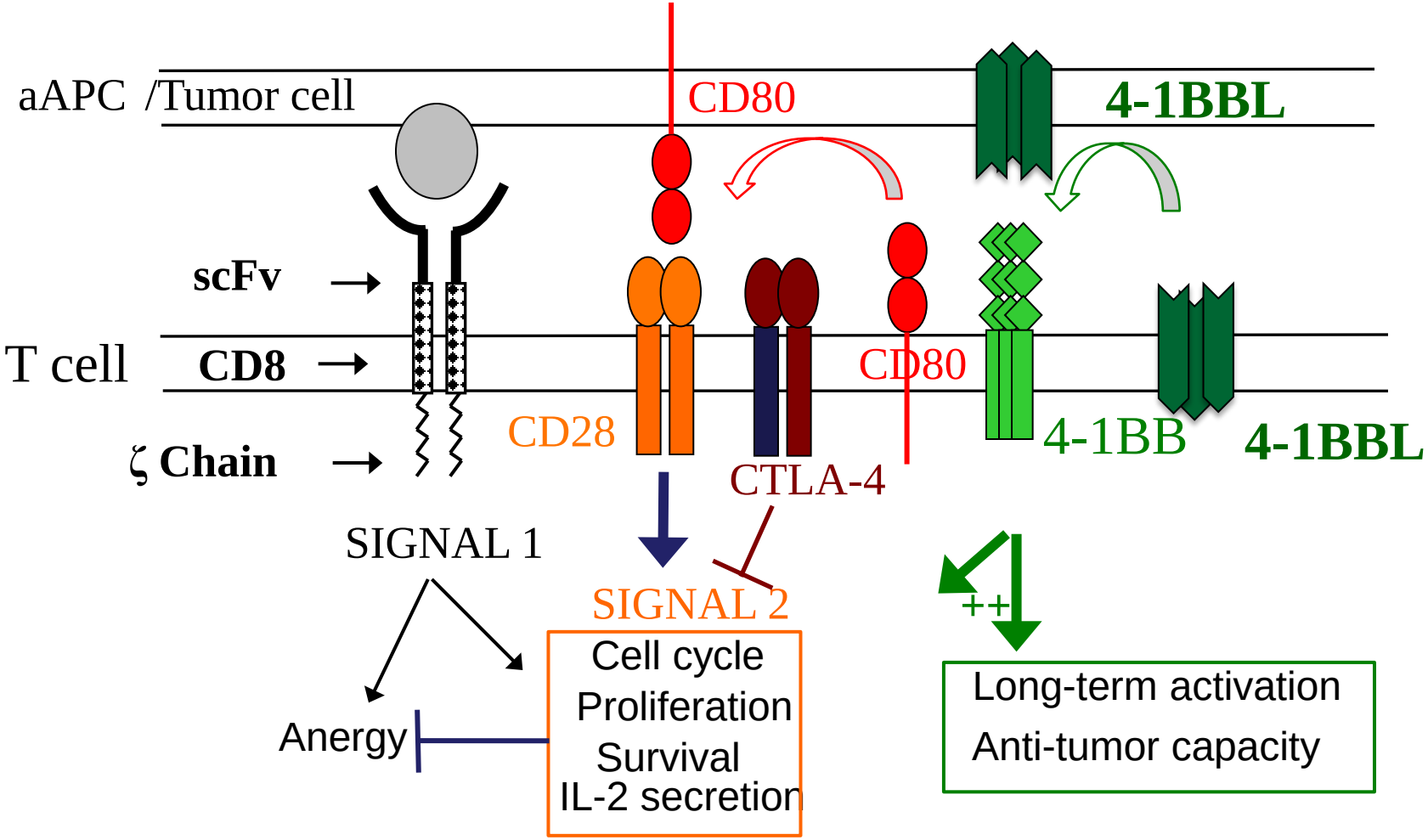
COMPLEXITY OF COSTIMULATION IN T-CELL FUNCTION



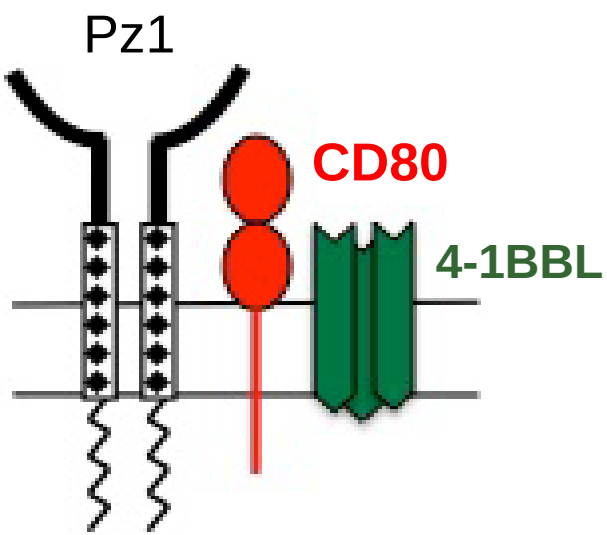
NATURE REVIEW IMMUNOLOGY, JUNE 2004, VOL 4

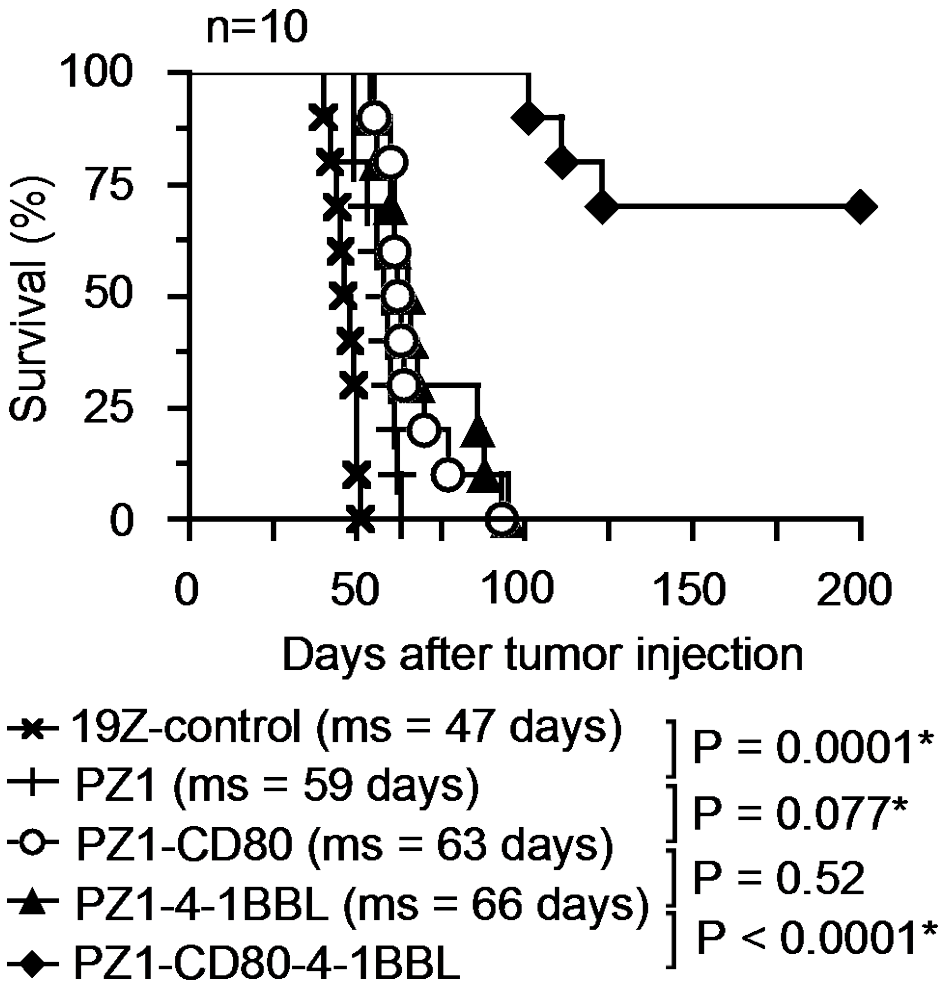
CD28/CTLA4/CD80 and 4-1BB/4-1BBL in T cell activation

T cell-encoded CD80 and 4-1BBL induce auto- and transcostimulation, resulting in potent tumor rejection. Stephan et al., *Nat Med*, 13(12):1440-9, 2007.



PSMA-targeted CD80^{+}4-1BBL^{+} T cells eradicate multi-focal PC3 tumors

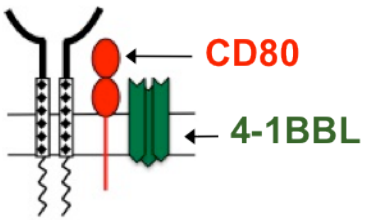


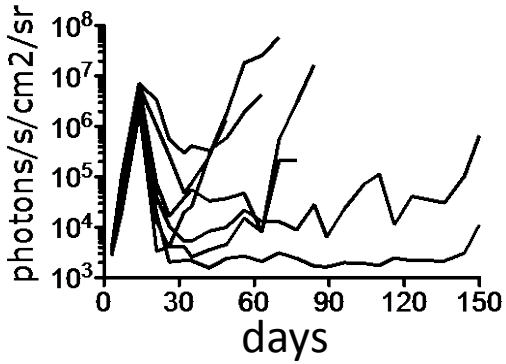


Stephan, *Nature Medicine*, 2007

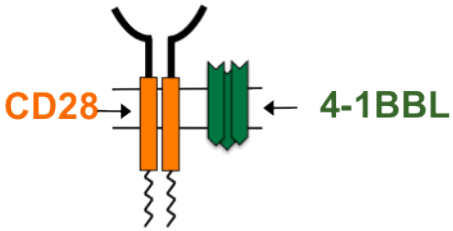
Superior potency of 1928z$^+$4-1BBL$^+$ T cells over 19z1+CD80$^+$4-1BBL$^+$ T cells

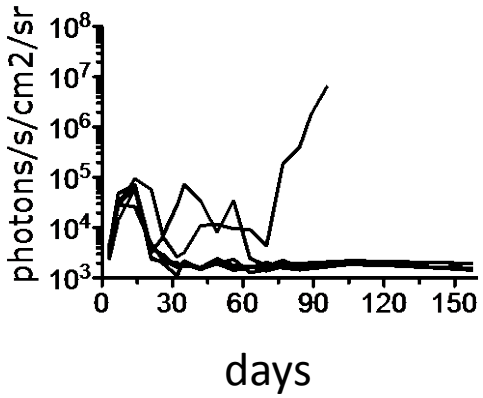
19z1$^+$ CD80$^+$ 4-1BBL$^+$

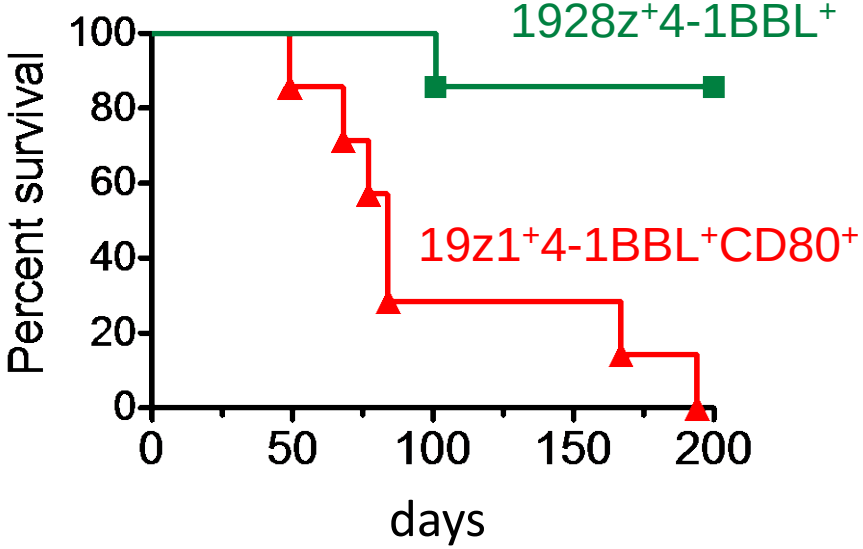




1928z$^+$ 4-1BBL$^+$

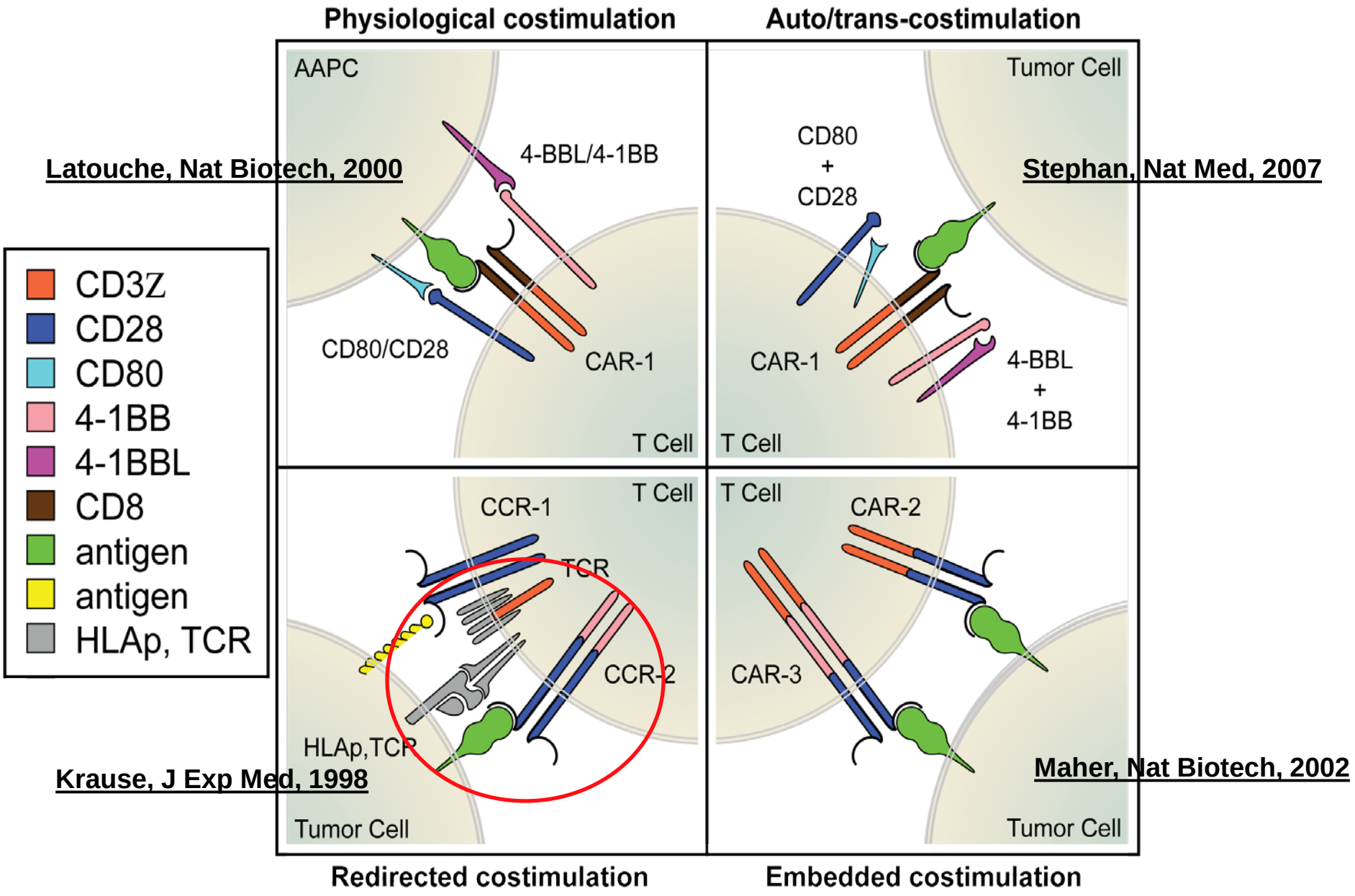






0.05 E6 T cells, 0.5 E6 NALM/6 day -4

Maud Condomines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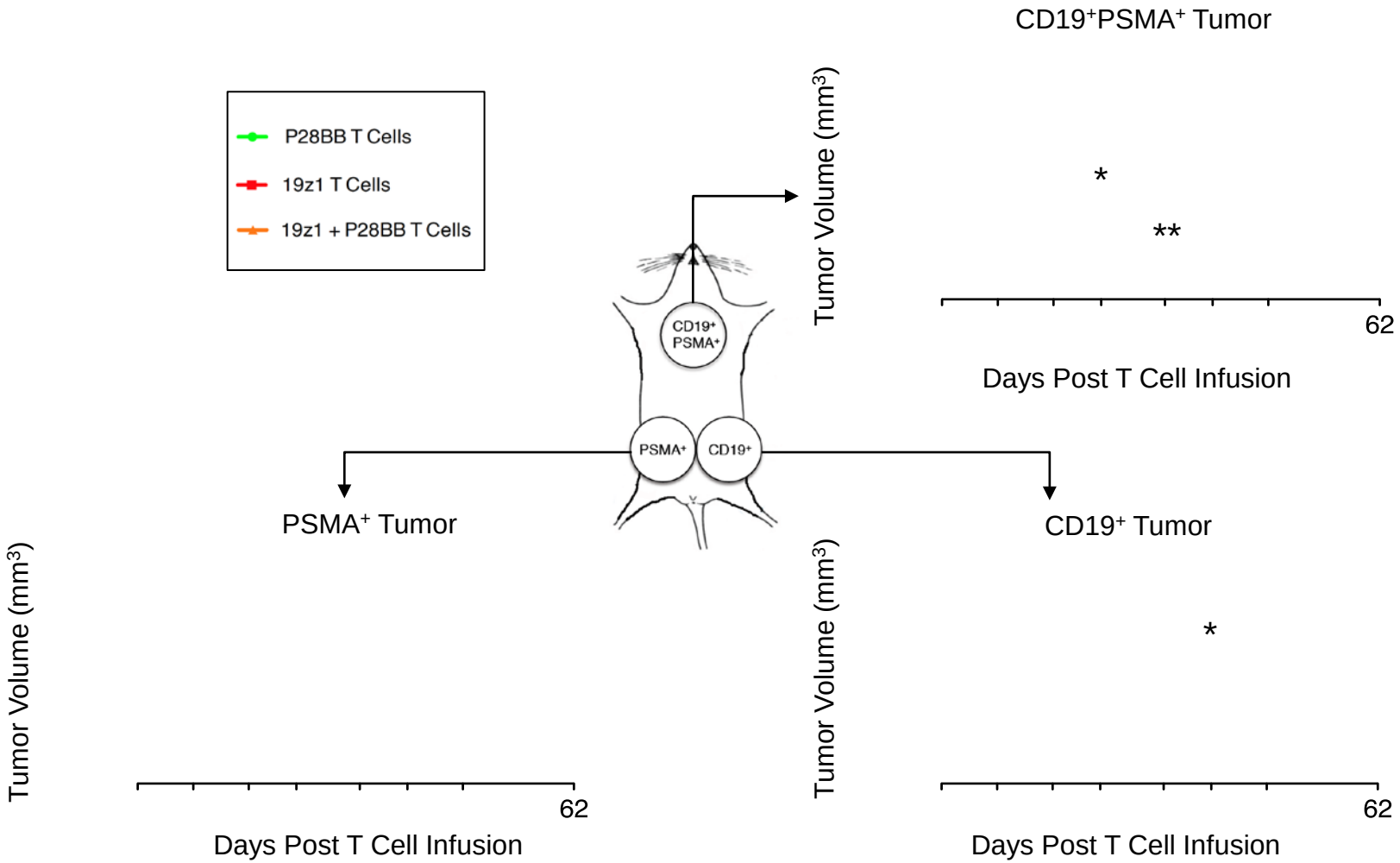
Redirected costimulation
Embedded costimulation

CAR + CCR targeted T cells show enhanced dual-ag+ tumor killing but their reactivity is not restricted to co-expressed antigens



Chris Kloss

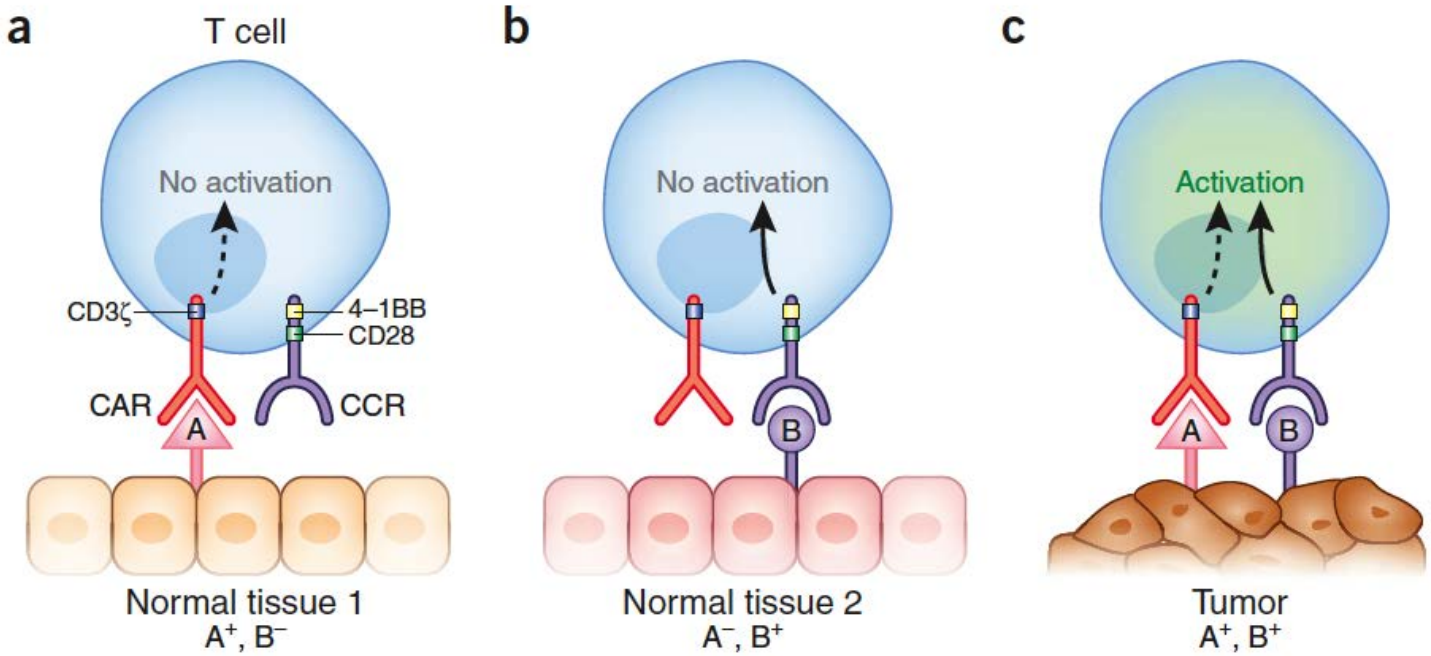
Tumor selectivity in the absence of a tumor unique antigen

NEWS AND VIEWS

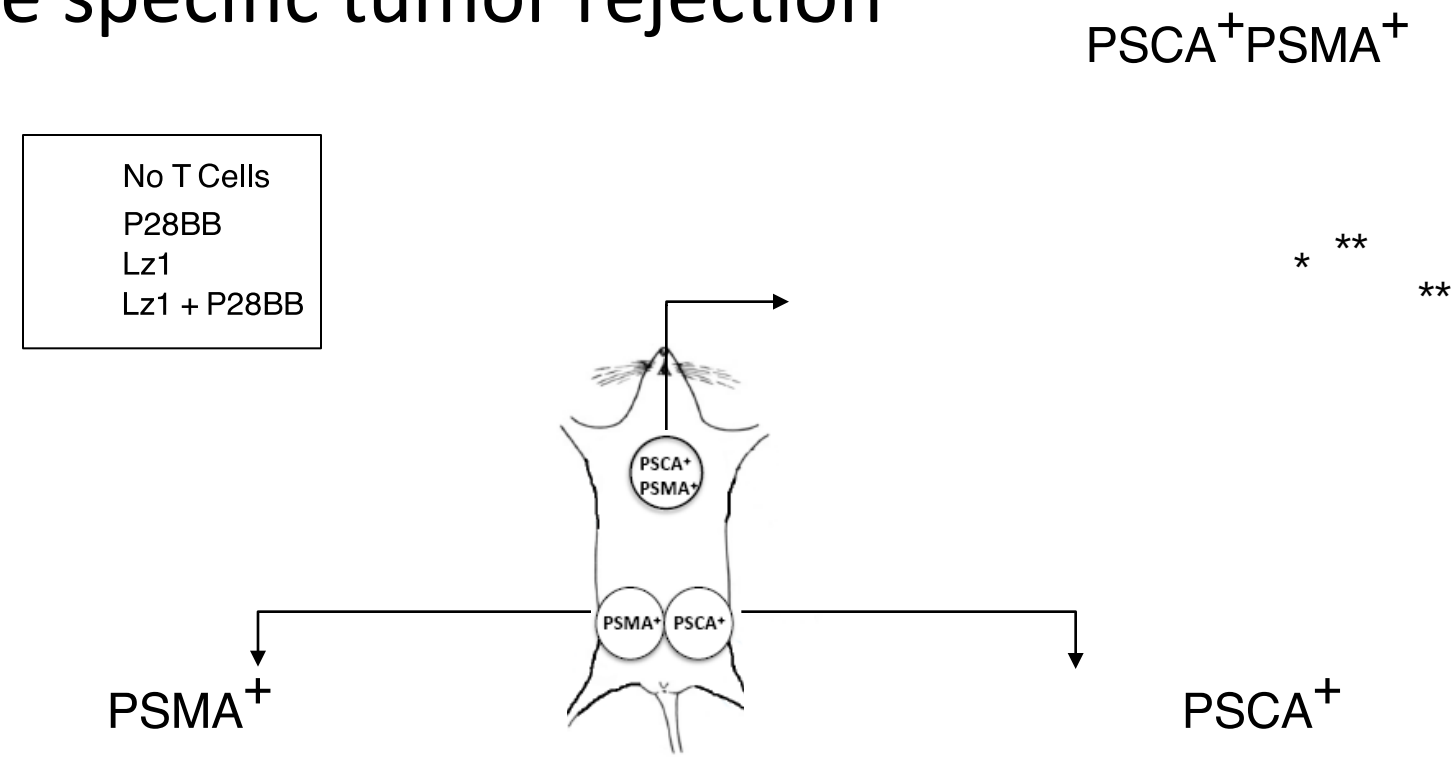
Double or nothing on cancer immunotherapy

Ken-ichi Hanada & Nicholas P Restifo

Engineered T cells expressing two receptors distinguish malignant cells from healthy cells even in the absence of a tumor-specific antigen.

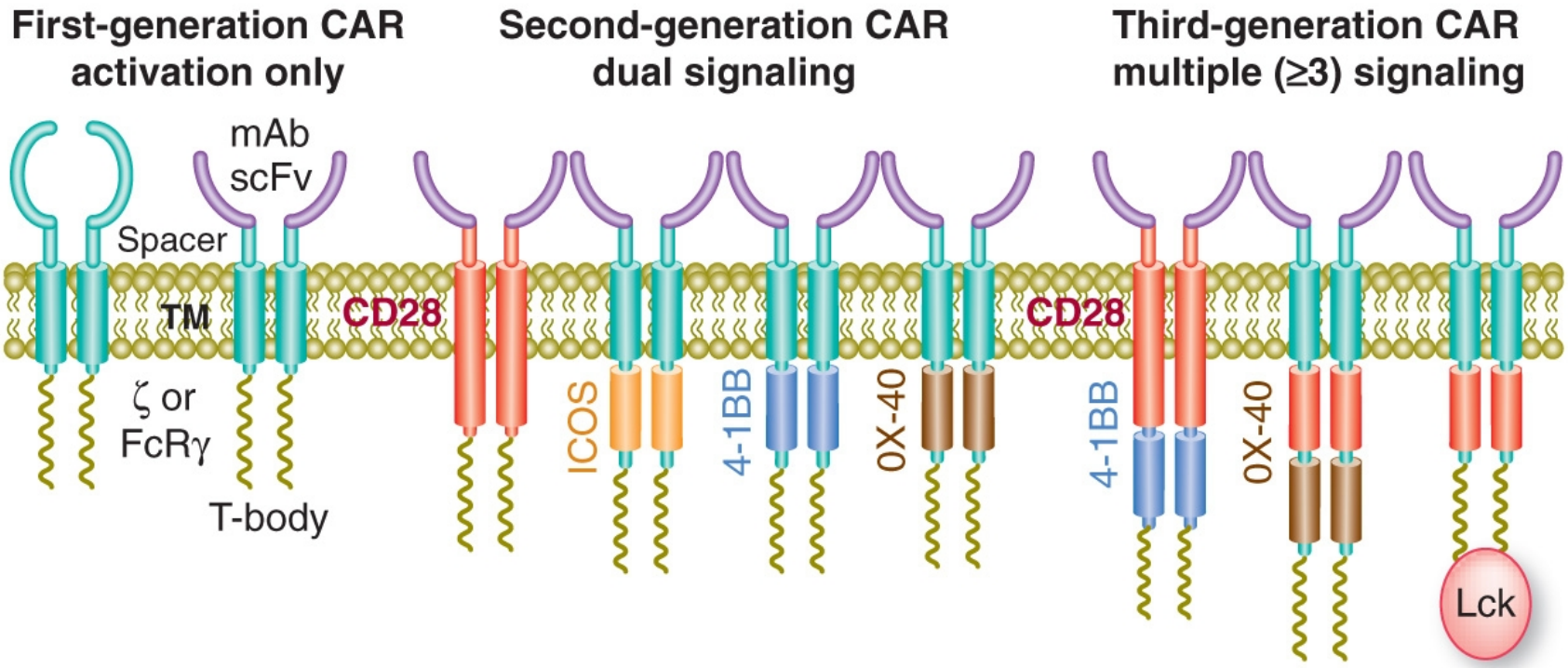


Dual antigen-targeted, calibrated CAR/CCR signals enable specific tumor rejection



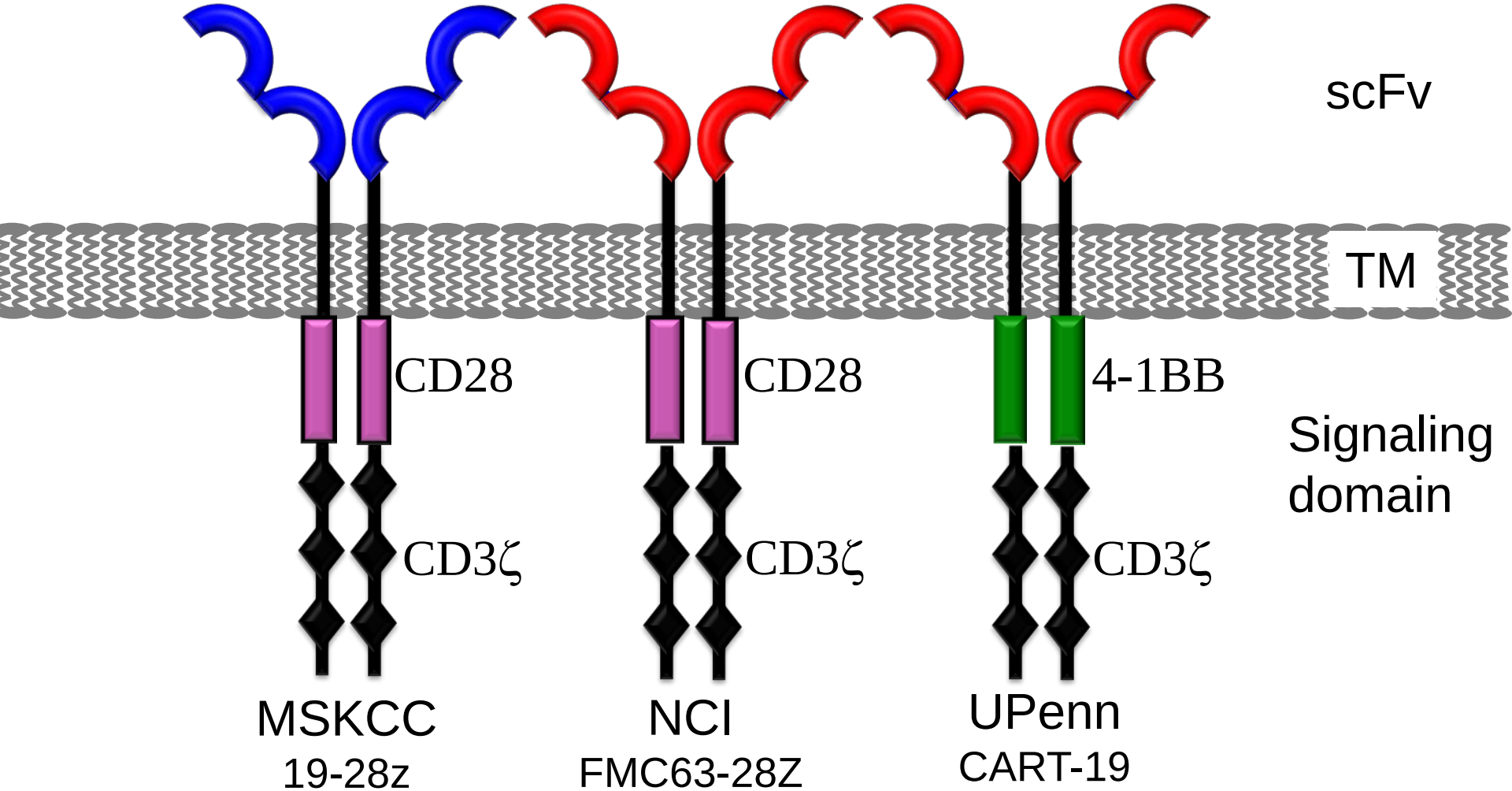
Kloss, *Nat Biotechnol*, 2013

Chimeric Antigen Receptors (CARs)



Sadelain, Brentjens and Riviere, *Cancer Discovery*, 2013

scFv and signaling domain combinations in three CD19 CARs



Davila, *Oncoimmunology*, 2012

blood

CLINICAL TRIALS AND OBSERVATIONS

Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias

*Renier J. Brentjens,[1-3] *Isabelle Rivière,[1-4] Jae H. Park,[1,2] Marco L. Davila,[1,2] Xiuyan Wang,[2-4] Jolanta Stefanski,[2-4] Clare Taylor,[2-4] Raymond Yeh,[1,2] Shirley Bartido,[2,3] Oriana Borquez-Ojeda,[2-4] Malgorzata Olszewska,[2-4] Yvette Bernal,[1] Hollie Pegram,[1,2] Mark Przybylowski,[2-4] Daniel Hollyman,[2-4] Yelena Usachenko,[1,2] Domenick Pirraglia,[2-4] James Hosey,[2-4] Elmer Santos,[3,5] Elizabeth Halton,[1] Peter Maslak,[1] David Scheinberg,[1-3] Joseph Jurcic,[1] Mark Heaney,[1] Glenn Heller,[6] Mark Frattini,[1] and Michel Sadelain[1-3]

[1]Department of Medicine, [2]Center for Cell Engineering, [3]Molecular Pharmacology and Chemistry Program, [4]Cell Therapy and Cell Engineering Facility, [5]Department of Radiology, and [6]Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, NY

Table 6. Summary of patient responses

Patient ID	Response to T-cell infusions
CLL-1	No objective response
CLL-2	No objective response
CLL-3	No objective response
CLL-4	Not evaluable
CLL-5	Marked reduction in lymphadenopathy at 3 months, subsequently stable for 6 months
CLL-6	Progressive disease
CLL-7	Stable disease, lasting 4 months
CLL-8	Stable disease, lasting > 8 weeks
ALL-1	Persistent B-cell aplasia in BM and peripheral blood

No conditioning

CY conditioning

blood

Plenary paper

B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor–transduced T cells

James N. Kochenderfer,[1] Mark E. Dudley,[2] Steven A. Feldman,[2] Wyndham H. Wilson,[3] David E. Spaner,[4] Irina Maric,[5] Maryalice Stetler-Stevenson,[6] Giao Q. Phan,[2] Marybeth S. Hughes,[2] Richard M. Sherry,[2] James C. Yang,[2] Udai S. Kammula,[2] Laura Devillier,[2] Robert Carpenter,[1] Debbie-Ann N. Nathan,[2] Richard A. Morgan,[2] Carolyn Laurencot,[2] and Steven A. Rosenberg[2]

[1]Experimental Transplantation and Immunology Branch, [2]Surgery Branch, and [3]Metabolism Branch, National Cancer Institute (NCI), Bethesda, MD; [4]Sunnybrook Health Sciences Center, Toronto, ON; [5]Department of Laboratory Medicine, Clinical Center, National Institutes of Health, Bethesda, MD; and [6]Laboratory of Pathology, NCI, Bethesda, MD

Table 1. Patient data

Patient	Age, y	Malignancy	No. of prior therapies	Total no. of infused cells/kg, $\times 10^7$	Percentage of infused cells CAR^+	No. of infused CAR^+ cells/kg $\times 10^7$	Infused cells CD4/CD8 ratio	Doses of IL-2 administered	Response and time since treatment, mo*
1a†	47	Follicular lymphoma	4	0.5	64	0.3	29/63	8	PR (7)
1b†	48	Follicular lymphoma	5	2.1	63	1.3	19/71	10	PR (18+)
2	48	Follicular lymphoma	5	0.5	65	0.3	23/73	9	NE (died with influenza)
3	61	CLL	3	2.5	45	1.1	35/53	2	CR (15+)
4	55	Splenic marginal zone lymphoma	3	2.0	53	1.1	72/24	4	PR (12)
5	54	CLL	4	0.6	50	0.3	87/12	2	SD (6)
6	57	CLL	7	5.5	30	1.7	37/57	1	PR (7)
7	61	CLL	4	5.4	51	2.8	58/41	2	PR (7+)
8	63	Follicular lymphoma	7	4.2	71	3.0	54/43	5	PR (8+)

PR indicates partial remission; NE, not evaluable; CR, complete remission; SD, stable disease; and CLL, chronic lymphocytic leukemia.
*Time since treatment is in months; + indicates an ongoing response as of the time of writing. The body surface area of each patient is given in supplemental Table 2.
†Patient 1 was treated twice. His first treatment has been previously reported (21).

Science Translational Medicine
AAAS

LEUKEMIA

T Cells with Chimeric Antigen Receptors Have Potent Antitumor Effects and Can Establish Memory in Patients with Advanced Leukemia

Michael Kalos,[1,2]* Bruce L. Levine,[1,2]* David L. Porter,[1,3] Sharyn Katz,[4] Stephan A. Grupp,[5,6] Adam Bagg,[1,2] Carl H. June[1,2]†

Table 1. Patient demographics and response. CR, complete response; PR, partial response; N/A, not available.

Subject UPN	Age/sex karyotype	Previous therapies	CLL tumor burden at baseline			Total dose of CART19 (cells/kg)	Response day +30 (duration)
			BM (study day)‡	Blood (study day)‡	Nodes/spleen (study day)‡		
01	65/M normal	Fludarabine × four cycles (2002)	Hypercellular 70% CLL	N/A	6.2×10^{11} to 1.0×10^{12} CLL cells (day −37)	1.1×10^{9} (1.6×10^{7}/kg)	CR (11+ months)
		Rituximab/fludarabine × four cycles (2005)	2.4×10^{12} CLL cells (day −14)				
		Alemtuzumab × 12 weeks (2006)	1.7×10^{12} CLL cells (day −1)				
		Rituximab (two courses, 2008 to 2009)					
		R-CVP × two cycles (2009)					
		Lenalidomide (2009)					
		PCR × two cycles (5/18/2010 to 6/18/2010)					
		Bendamustine × one cycle (7/31/10 to 8/1/10) pre-CART19					
02	77/M del (17)(p13)*	Alemtuzumab × 16 weeks (6/2007)	Hypercellular >95% CLL	2.75×10^{11} CLL cells (day −1)	1.2×10^{12} to 2.0×10^{12} CLL cells (day −24)	5.8×10^{8} (1.0×10^{7}/kg)	PR (7 months)
		Alemtuzumab × 18 weeks (3/2009)	3.2×10^{12} CLL cells (day −47)				
		Bendamustine/rituximab:					
		7/1/2010 (cycle 1)					
		7/28/2010 (cycle 2)					
		8/26/2010 (cycle 3) pre-CART19					
03	64/M del (17)(p13)†	R-Fludarabine × two cycles (2002)	Hypercellular 40% CLL	N/A	3.3×10^{11} to 5.5×10^{11} CLL cells (day −10)	1.4×10^{7} (1.46×10^{5}/kg)	CR (10+ months)
		R-Fludarabine × four cycles (10/06 to 1/07)	8.8×10^{11} CLL cells (day −1)				
		R-Bendamustine × one cycle (2/09)					
		Bendamustine × three cycles (3/09 to 5/09)					
		Alemtuzumab × 11 weeks (12/09 to 3/10)					
		Pentostatin/cyclophosphamide (9/10/10) pre-CART19					

Differences between CD19 trials

- Different CARs - scFv, epitope, affinity, signaling
- Different enhancer promoter - expression level, silencing
- Different T cell manufacturing – CD3±CD28, media, duration
- Different cell product – cell dose, CD4/CD8
- Different conditioning – CY vs CY/Flu vs bendamustine
- Patient selection – chemosensitive, chemoresistant

blood

CLINICAL TRIALS AND OBSERVATIONS

Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias

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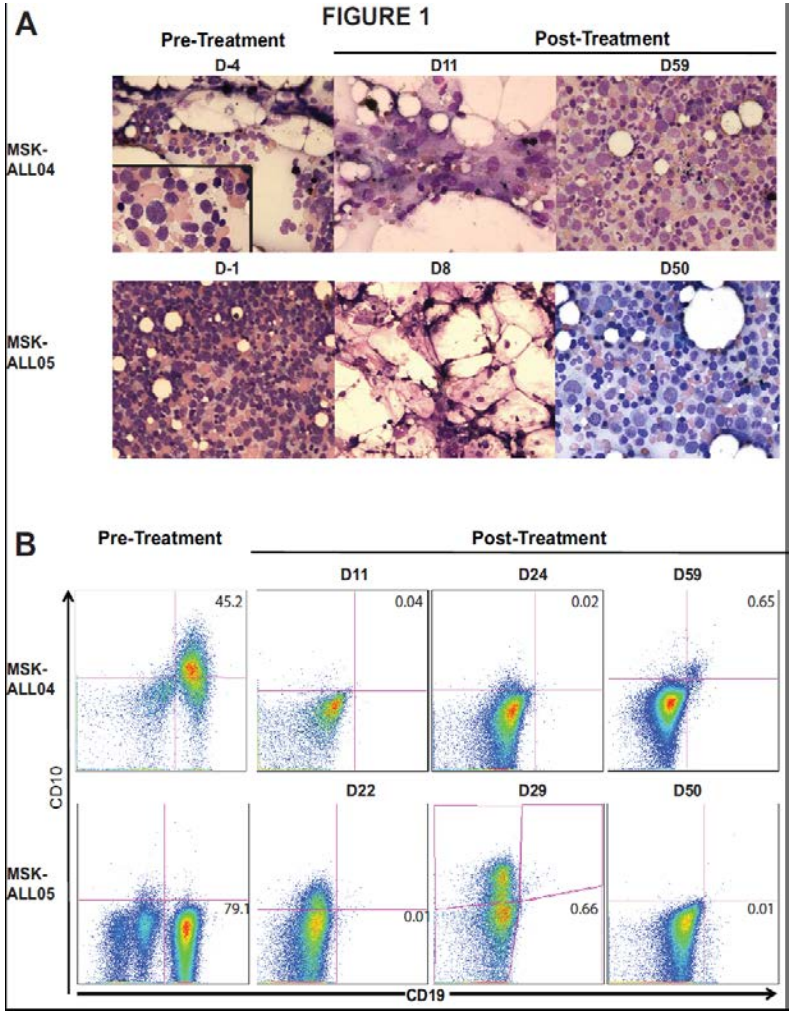
Table 6. Summary of patient responses

Patient ID	Response to T-cell infusions
CLL-1	No objective response
CLL-2	No objective response
CLL-3	No objective response
CLL-4	Not evaluable
CLL-5	Marked reduction in lymphadenopathy at 3 months, subsequently stable for 6 months
CLL-6	Progressive disease
CLL-7	Stable disease, lasting 4 months
CLL-8	Stable disease, lasting > 8 weeks
ALL-1	Persistent B-cell aplasia in BM and peripheral blood

No conditioning

CY conditioning

Rapid and complete tumor elimination mediated by 19-28z T cells



Patient ID	Day of Treatment	Total # IgH rearrangements	Total # malignant IgH rearrangements
MSK-ALL01*	-1: 55:	57,480 15,925	58 0
MSK-ALL03*	-5: 30:	1,084 0	0 0
MSK-ALL04	-4: 11: 24: 59:	2,430,058 2,407 1,144 995,563	2,426,898 1,316 637 0
MSK-ALL05*	-1: 8: 29:	3,307,494 1,880 8,270	3,300,732 0 0
MSK-ALL06	-34: 18: 39:	255,301 5,429 1,866,851	174,698 4 0

Brentjens, Davila, Riviere et al, *Science Transl Med*, 2013

Third generation CAR – ERBB2-28BBζ

Expression of the ERBB2 CAR

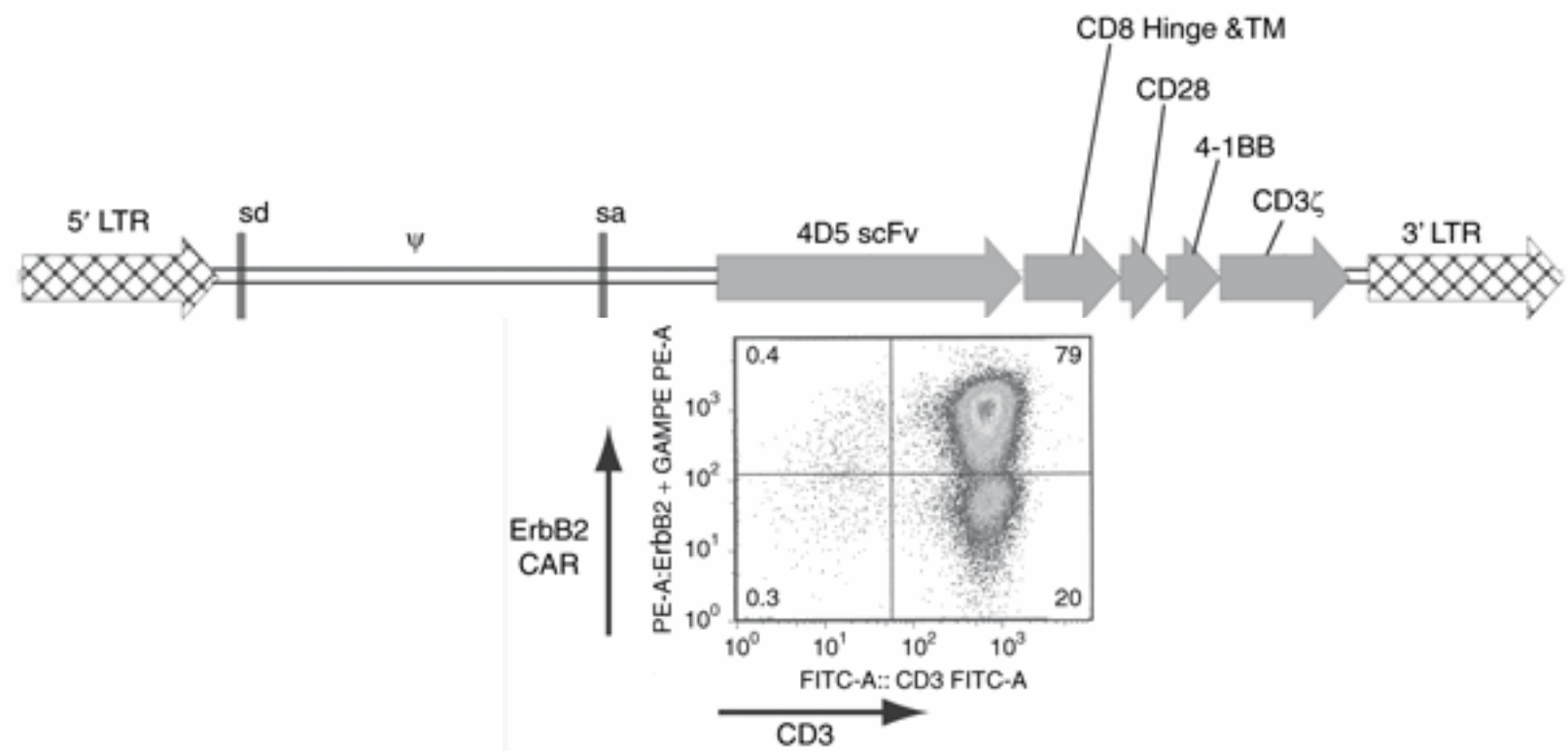
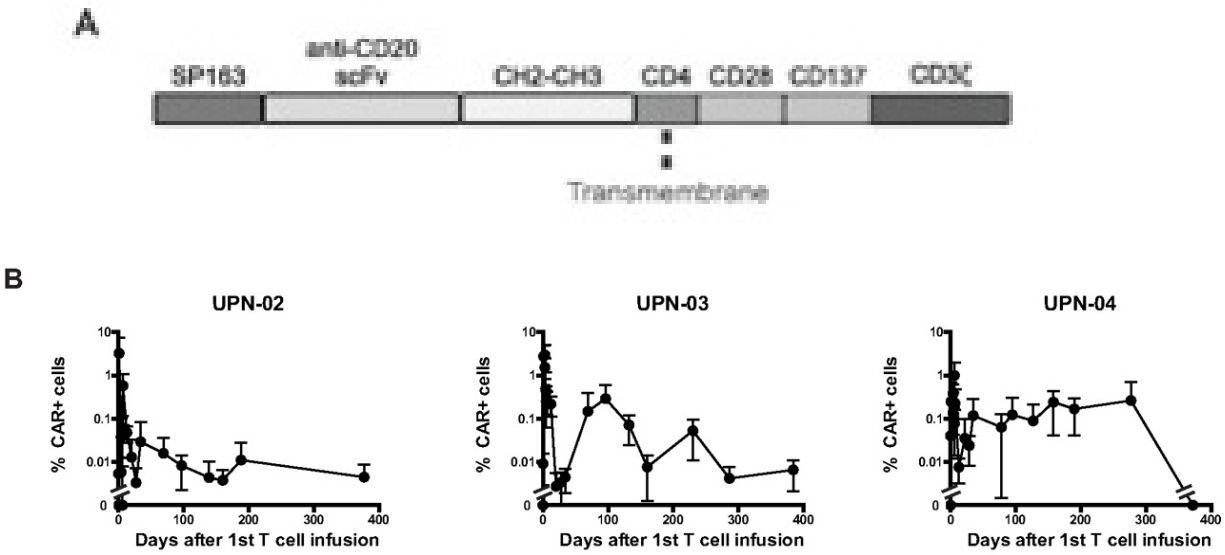


Diagram of the ERBB2-CAR vector (MSGV1-4D5-CD8-28BBZ) used in this trial is as shown on the top of the figure. As described in Materials and Methods section, patient PBMC were stimulated to induced T-cell division and then transduced with the CAR vector. Four days before cell infusion samples were removed for analysis of *ERBB2-CAR* gene expression by FACS (along with the CD3, CD4, or CD8 T-cell markers). CAR, chimeric antigen receptor; LTR, long terminal repeats; PBMC, peripheral blood mononuclear cell; scFv, single-chain Fv fragment.

Morgan et al. *Mol Ther* 2010

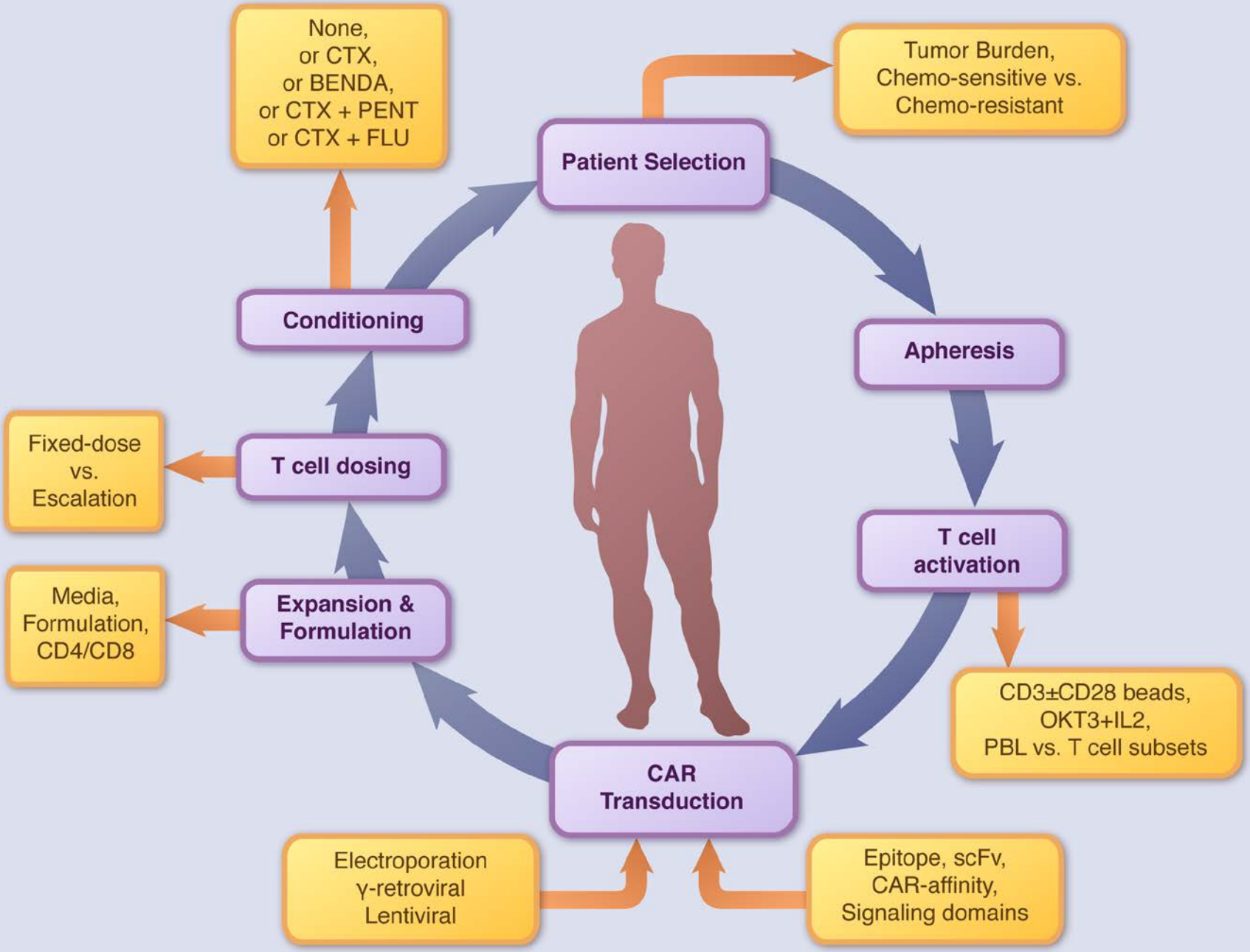
Third generation CAR - CD20-28-BB-ζ

Engraftment, persistence, and localization of modified T cells to tumor sites.



Engraftment, persistence, and localization of modified T cells to tumor sites. Quantitative real-time PCR was performed on genomic DNA harvested from study subject PBMCs collected before and at serial time points after T-cell infusions, using primers specific for the transgene. CAR^+ cells are shown as a percentage of total PBMCs for each of the treated patients over (A) the first month and (B) the first year. The same quantitative PCR assay was used to detect modified cells in genomic DNA harvested from surgically excised lymph nodes (C) or bone marrow aspirates (D) collected 24 to 48 hours after the third T-cell infusion. For patient UPN-03, 2 adjacent lymph nodes were excised and analyzed separately, and an additional bone marrow aspirate was performed 1 month after the third T-cell infusion. Data represent mean values (± SD) of at least 2 assays per time point, with each sample performed in triplicate. Arrows indicate infusions of genetically modified T cells; and black bar, the 14-day period of low-dose IL-2 injections.

Till B G et al. *Blood* 2012



Davila et al, *Oncoimmunology*, 2012