SITC 2016

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NOVEMBER 9-13, 2016

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Primer on Adoptive T cell Therapy

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University of Pennsylvania

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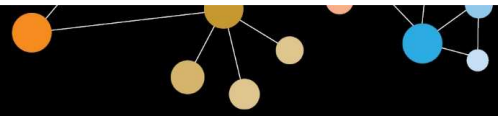
Presenter Disclosure Information

Saar Gill

The following relationships exist related to this presentation:

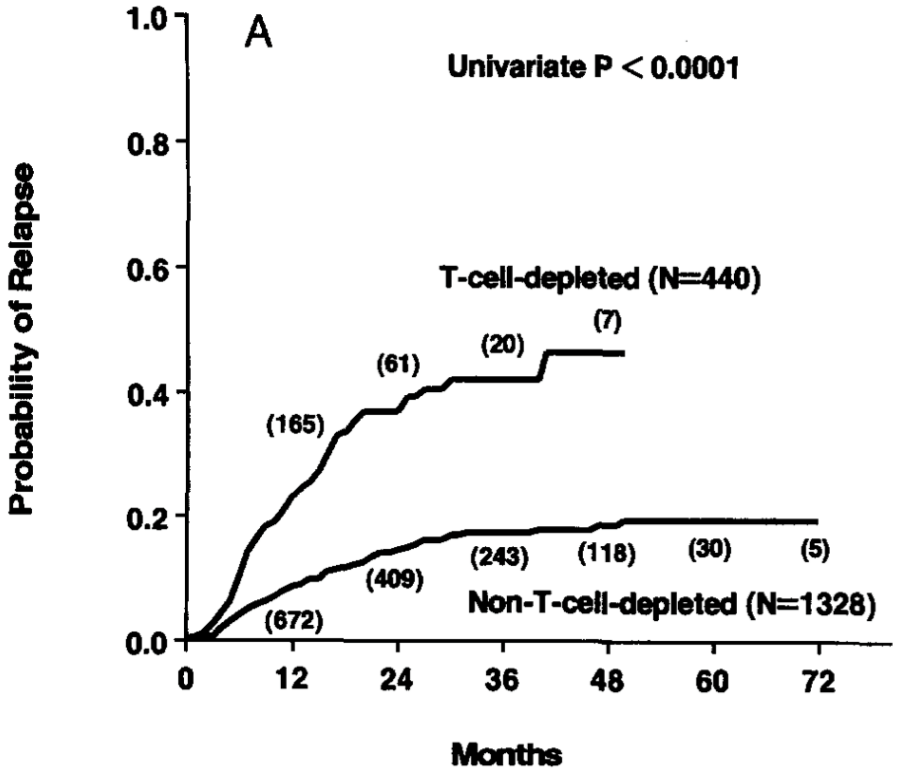
Novartis, Research funding



Learning Objectives

- Describe the key requirements for successful adoptive T cell therapy
- Describe the different types of T cell-based immunotherapy

Why T cells?

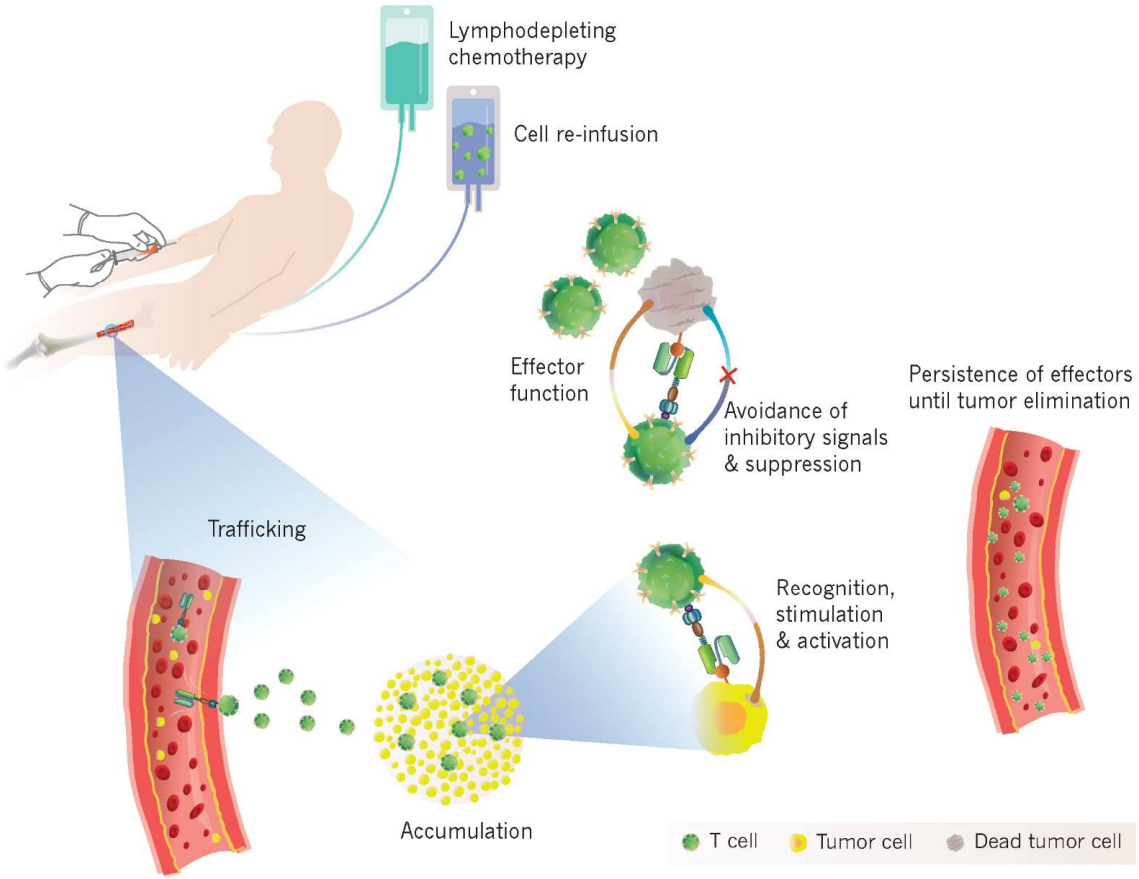


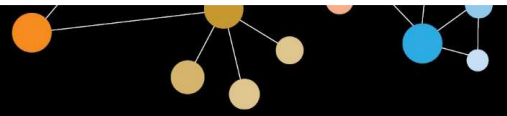
Marmont *et al*. Blood 1991

- Increased relapses in leukemia patients given T cell depleted bone marrow transplants
- BMT from syngeneic donors have more relapses than BMT from Allogeneic donors
- Immunodeficiency-associated malignancies

If T cell depletion decreases the anti-tumor effect, does T cell "supplementation" increase the anti-tumor effect?

What is required for successful adoptive T cell therapy?



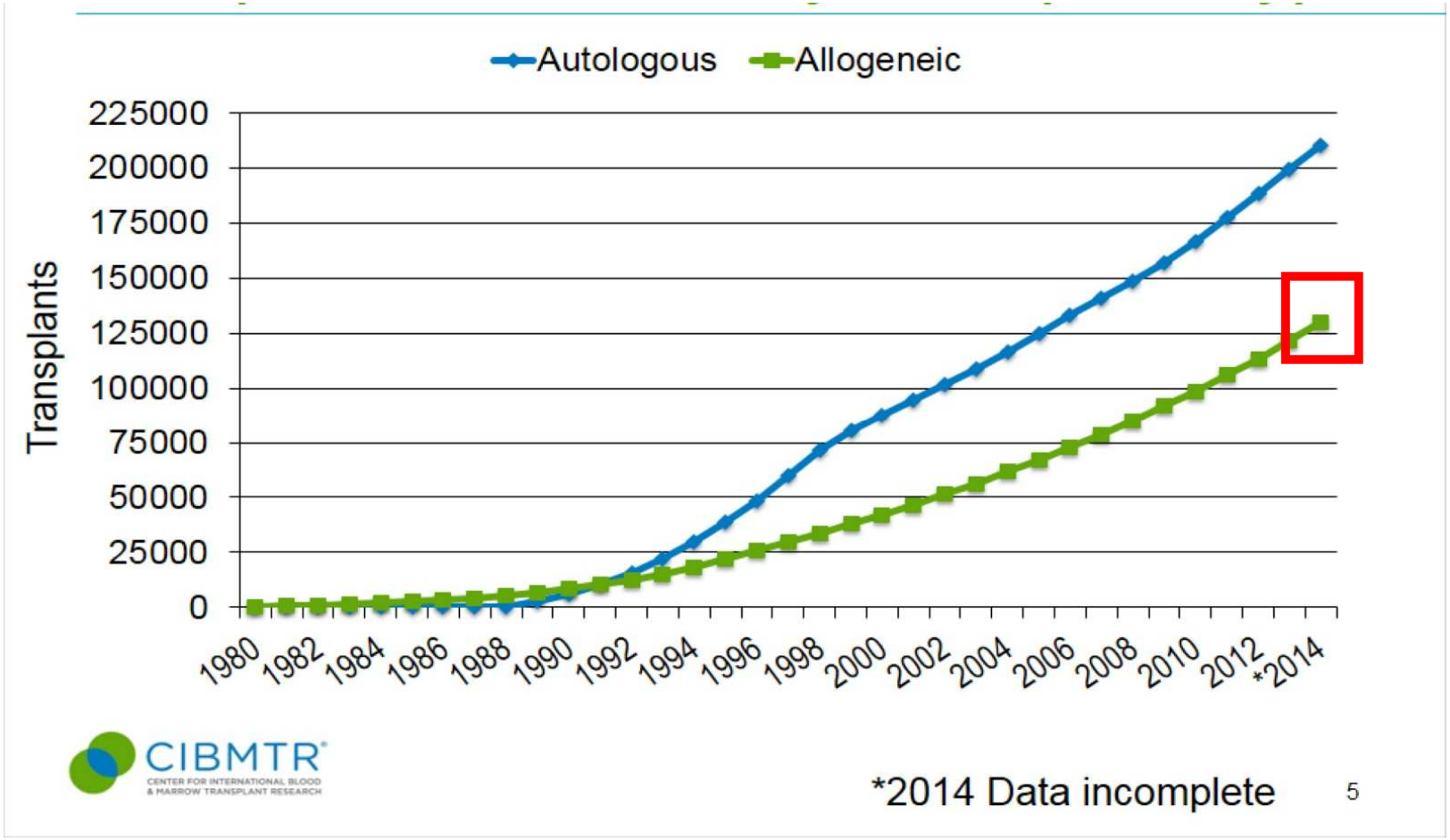


Forms of ACT

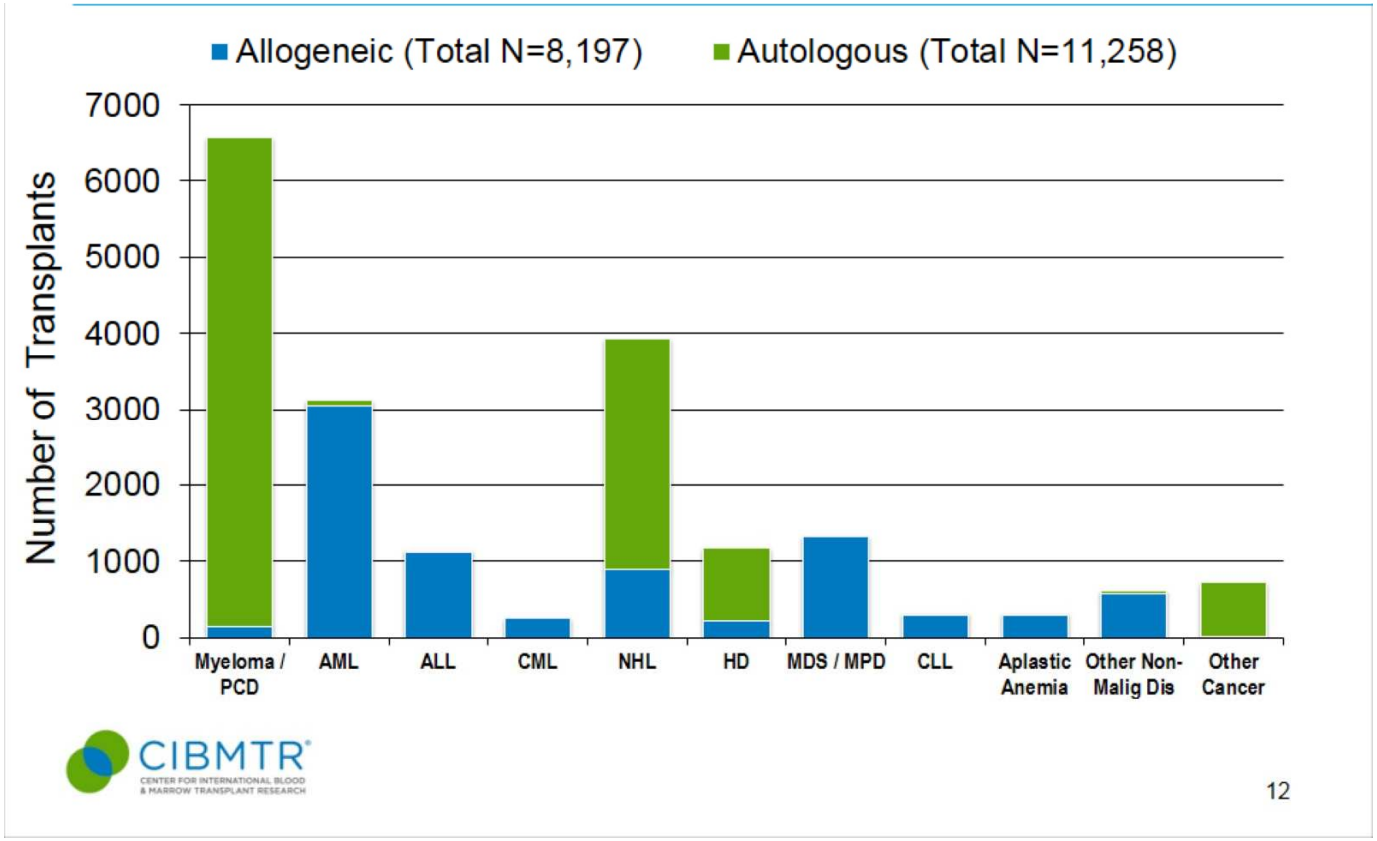
- Allogeneic hematopoietic cell transplantation (HCT) and donor lymphocyte infusion
- Tumor-specific T cells (tumor infiltrating, TIL; or circulating anti-tumor T cells)
- TCR transgenic T cells
- Chimeric antigen receptor T cells

↑ Engineering / Synthetic biology

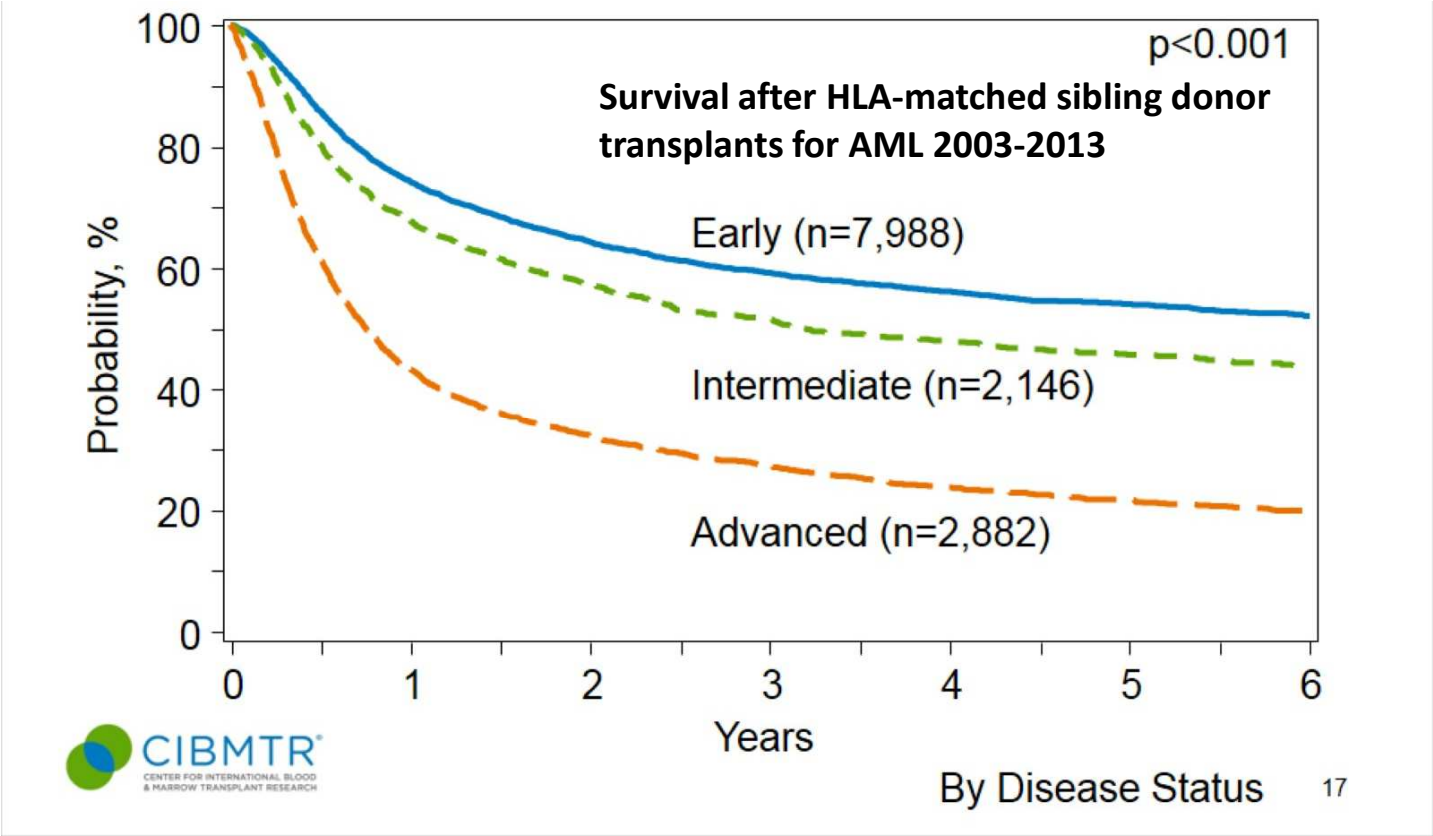
Forms of ACT: allogeneic hematopoietic cell transplantation



Forms of ACT: allogeneic hematopoietic cell transplantation

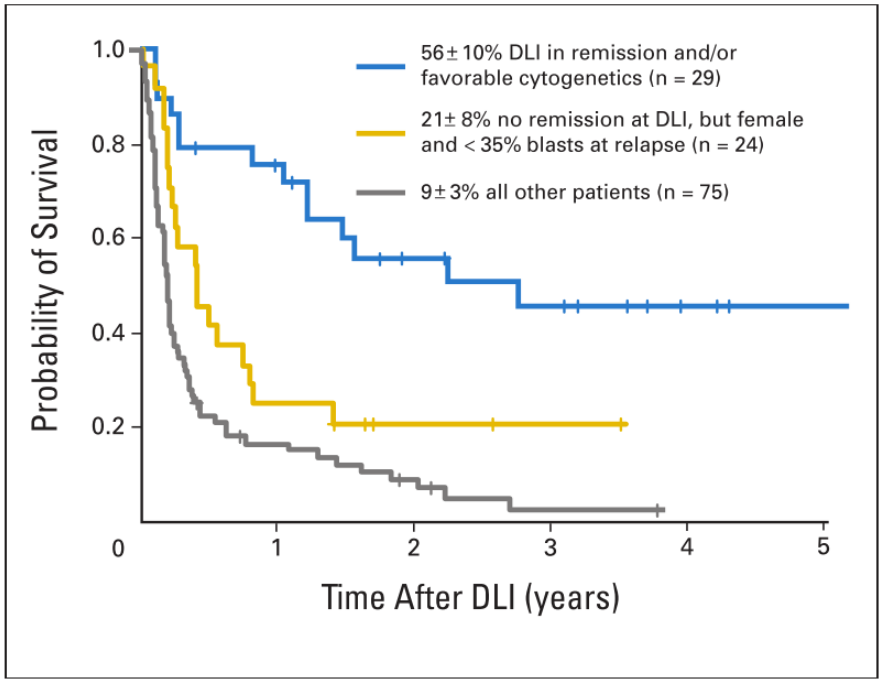


Forms of ACT: allogeneic hematopoietic cell transplantation



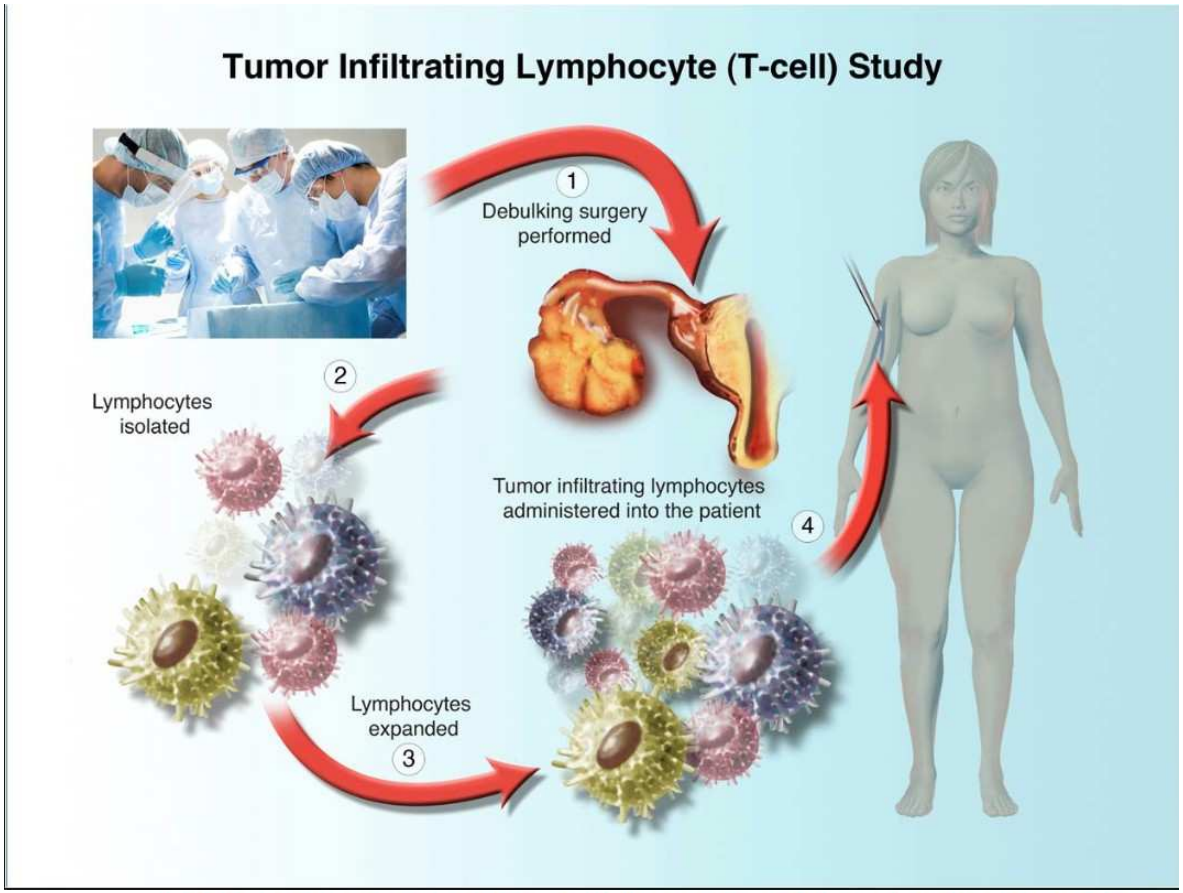
Forms of ACT: allogeneic hematopoietic cell transplantation

- Donor lymphocyte infusion is effective, but only in low disease burden
- DLI associated with significant incidence of GVHD
- AlloHCT (and DLI) not effective in solid tumors



Schmid et al, J Clin Oncol 2007

Forms of ACT: tumor-infiltrating lymphocytes (TILs)



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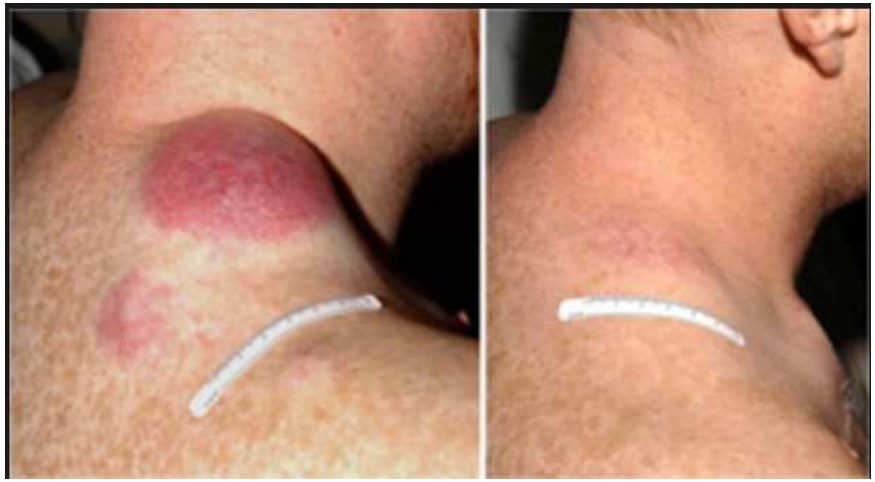
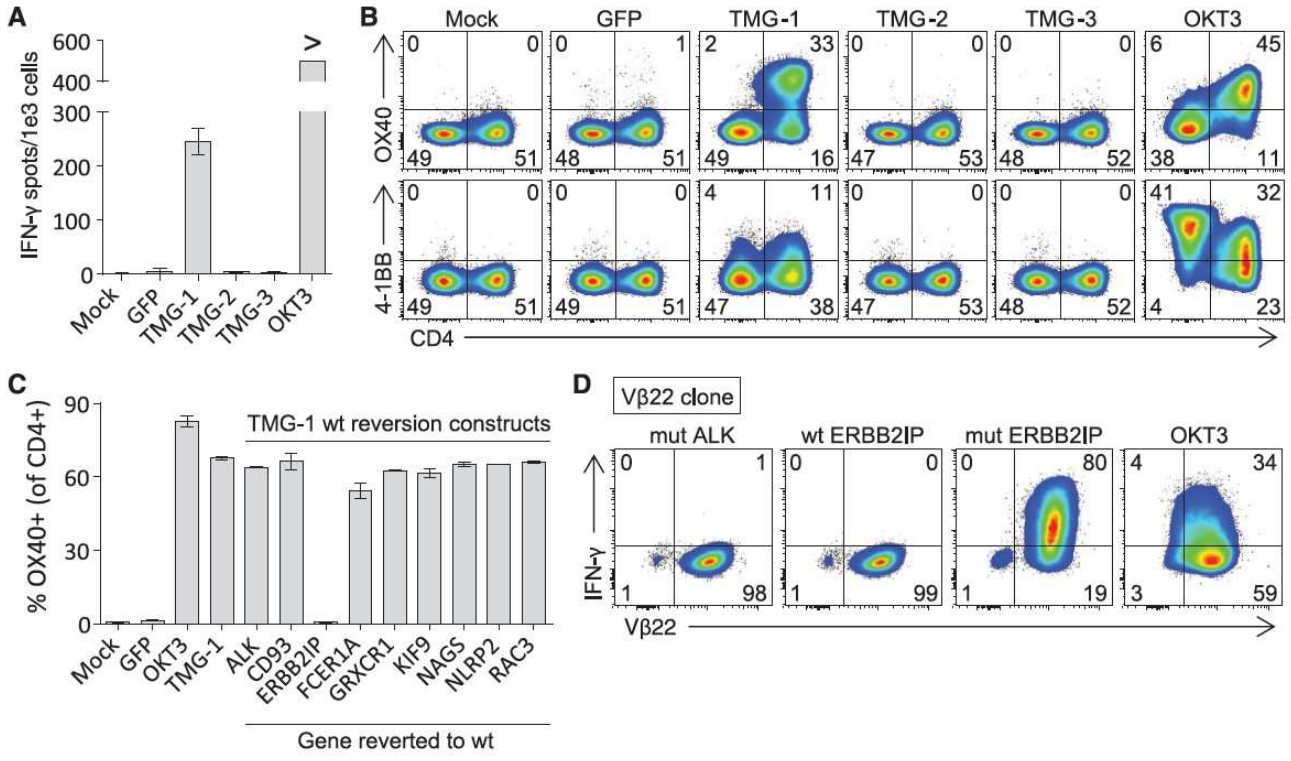


1. **Tumor-specific T cells can be found in a tumor biopsy –** *Tran et al Science 2014*

2. **Tumor-specific T cells can be found in the blood -** *Cohen et al, J Clin Invest 2015*

Smart TILs: successful adoptive T cell therapy based on mutation-specific T cells



Tran et al, Science 2014;344:641

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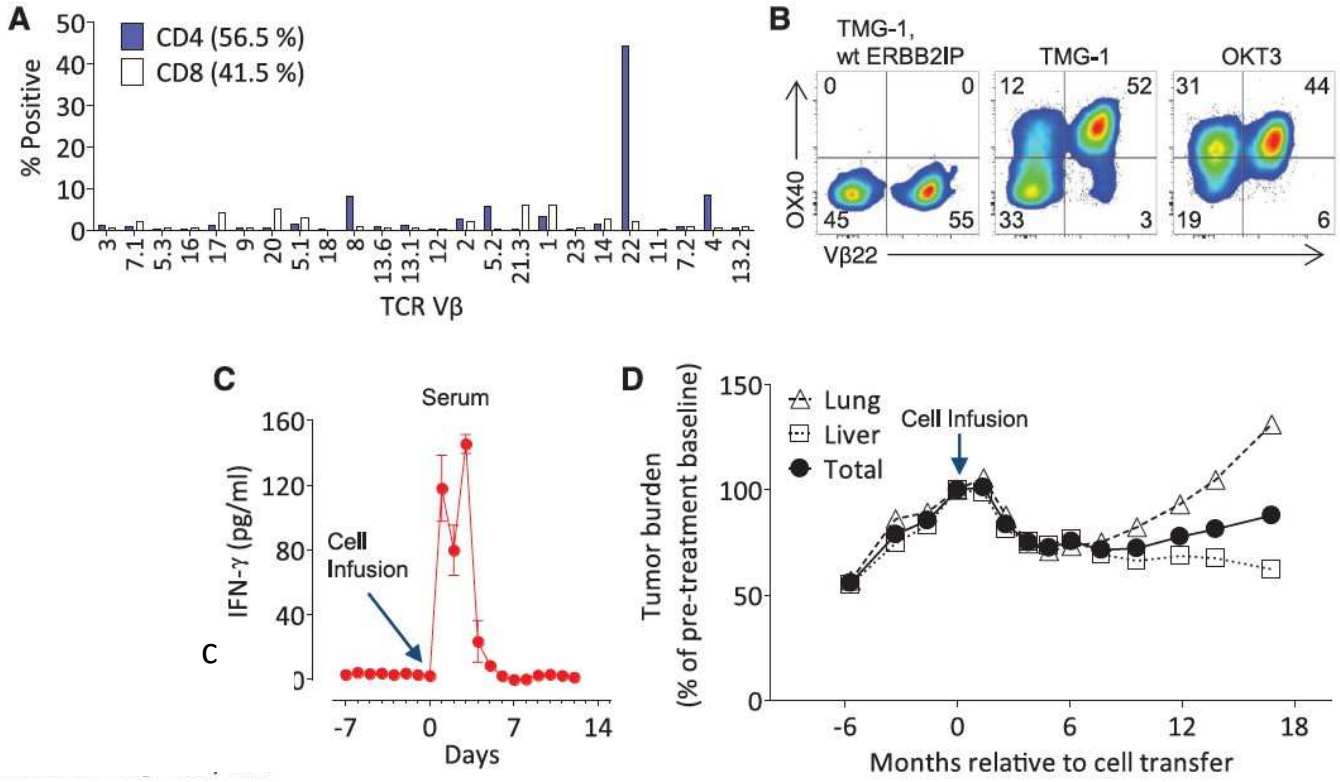
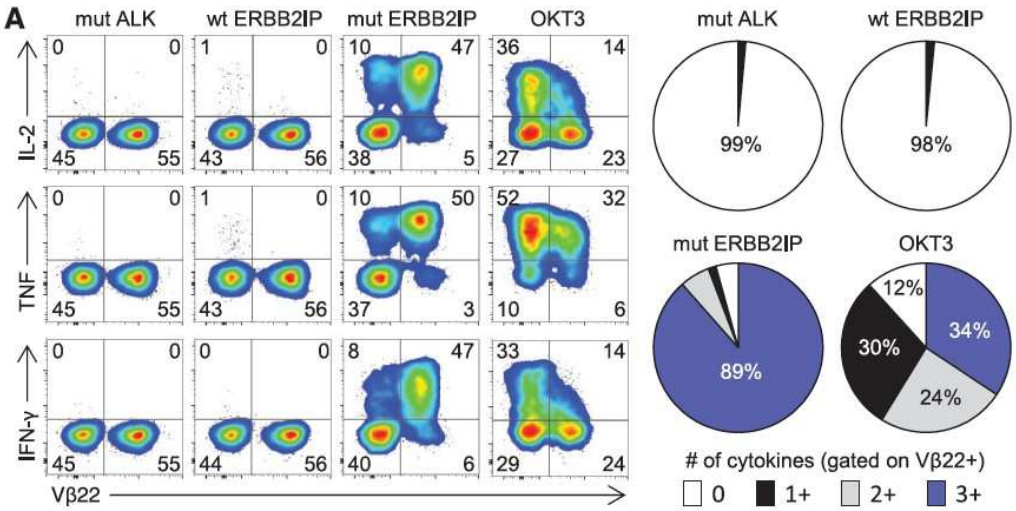
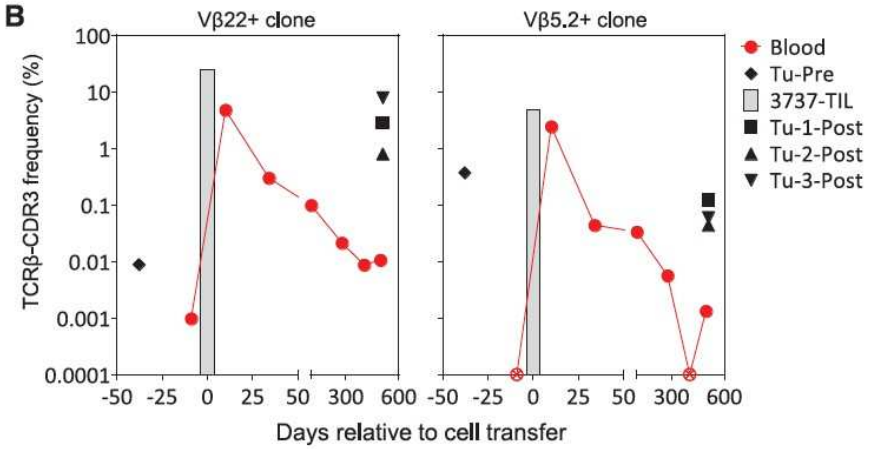


Tran et al, Science 2014;344:641

Smart TILs: successful adoptive T cell therapy based on mutation-specific T cells





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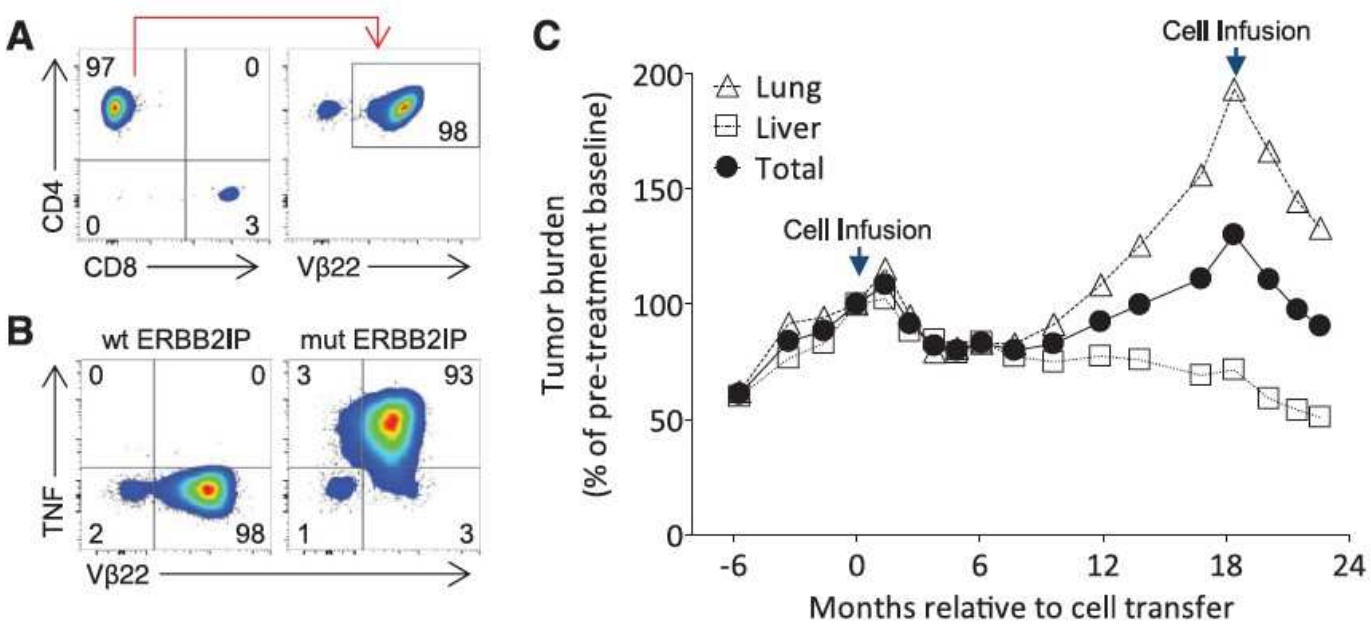
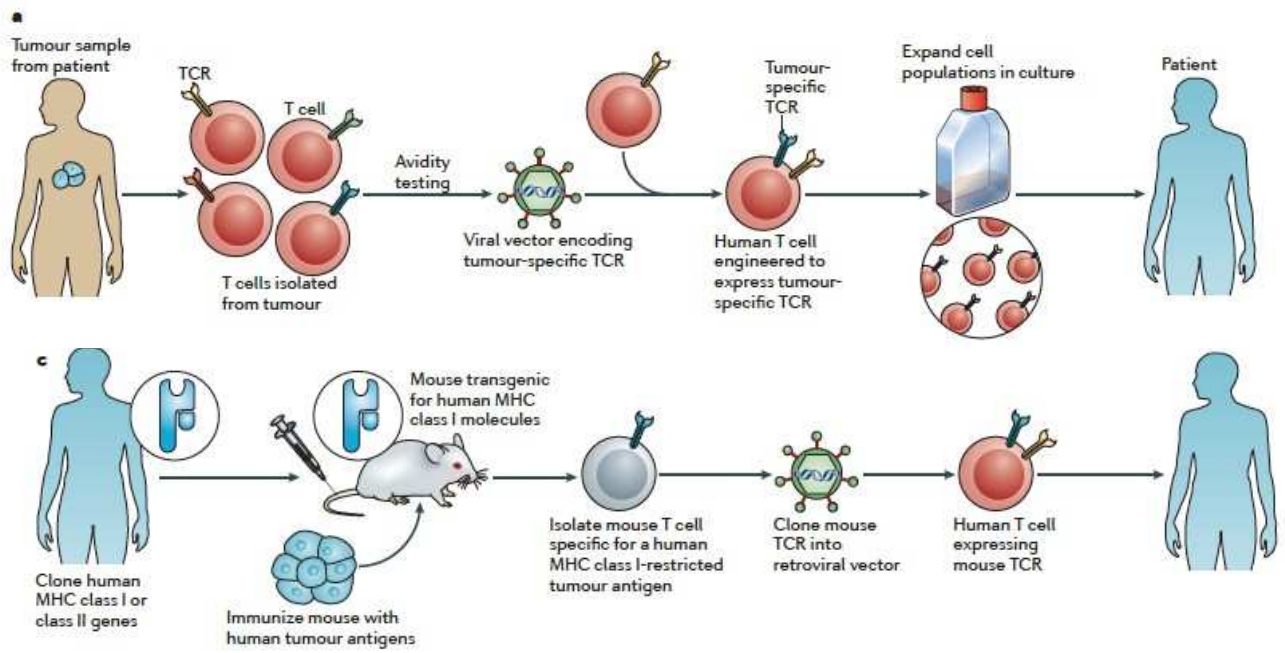
Smart TILs: successful adoptive T cell therapy based on mutation-specific T cells



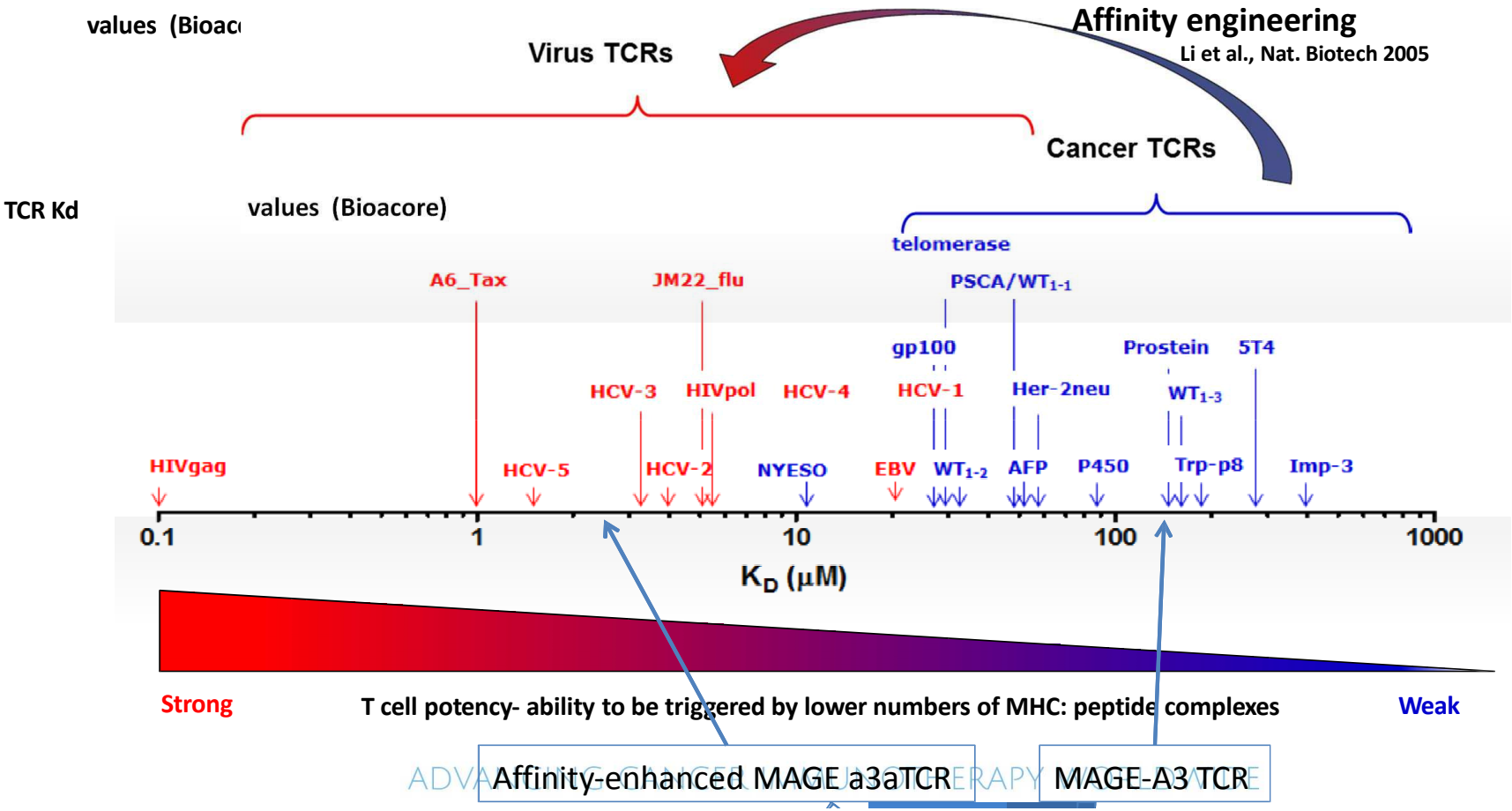
Tran et al, Science 2014;344:641

Forms of ACT: T cell receptor (TCR) transgenics



Restifo et al, Nat Rev Immunol 2012

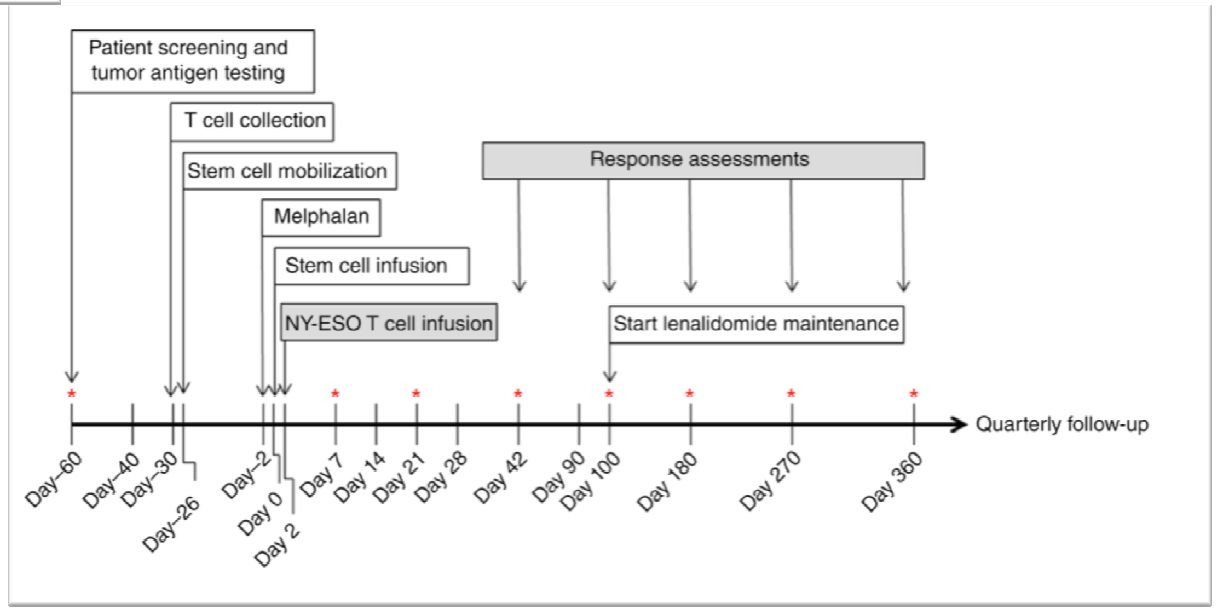
(TCR) transgenics: affinity engineering can impart viral-like affinity to cancer-specific TCRs



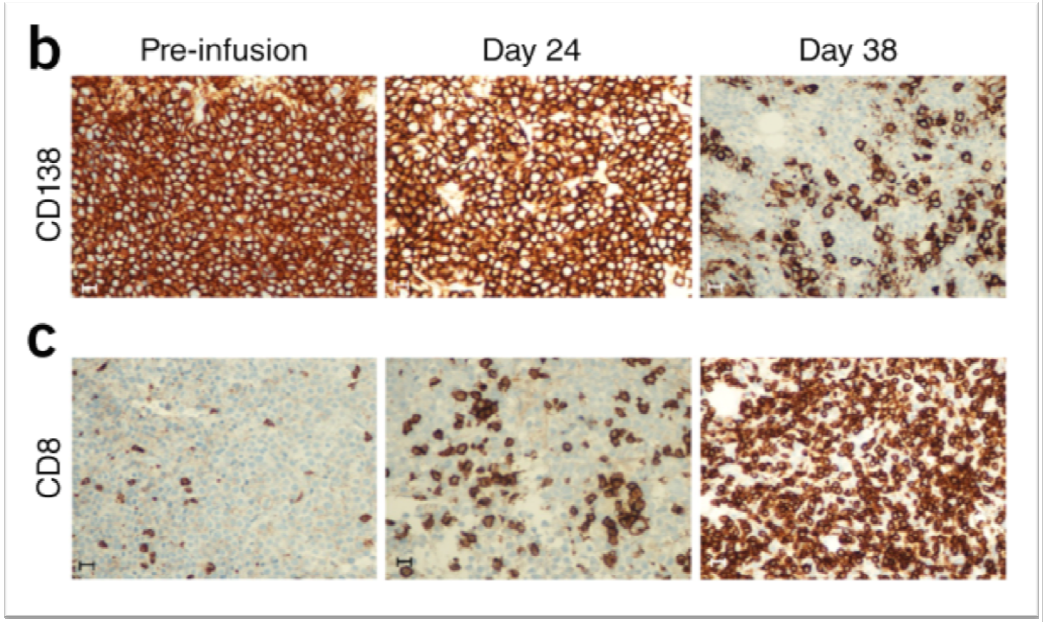
(TCR) transgenics: NY-ESO1

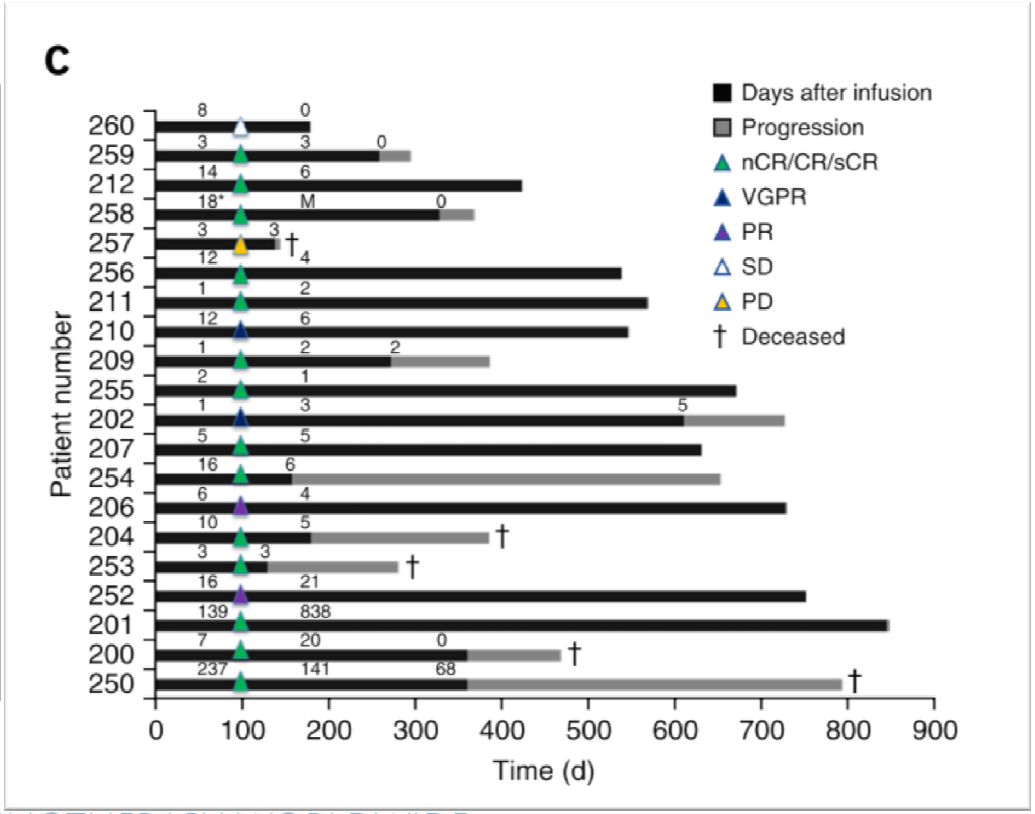
NY-ESO-1–specific TCR–engineered T cells mediate sustained antigen-specific antitumor effects in myeloma

Aaron P Rapoport[1,8], Edward A Stadtmauer[2,8], Gwendolyn K Binder-Scholl[3,8], Olga Goloubeva[1,4], Dan T Vogl[2], Simon F Lacey[2,5], Ashraf Z Badros[1], Alfred Garfall[2], Brendan Weiss[2], Jeffrey Finklestein[4,5], Irina Kulikovskaya[2,5], Sanjoy K Sinha[6], Shari Kronsberg[1,4], Minnal Gupta[2,5], Sarah Bond[7], Luca Melchiori[3], Joanna E Brewer[3], Alan D Bennett[3], Andrew B Gerry[3], Nicholas J Pumphrey[3], Daniel Williams[3], Helen K Tayton- Martin[3], Lilliam Ribeiro[3], Tom Holdich[3], Saul Yanovich[1], Nancy Hardy[1], Jean Yared[1], Naseem Kerr[5], Sunita Philip[1], Sandra Westphal[1], Don L Siegel[2,5], Bruce L Levine[2,5], Bent K Jakobsen[3], Michael Kalos[2,5,8] & Carl H June[2,5]

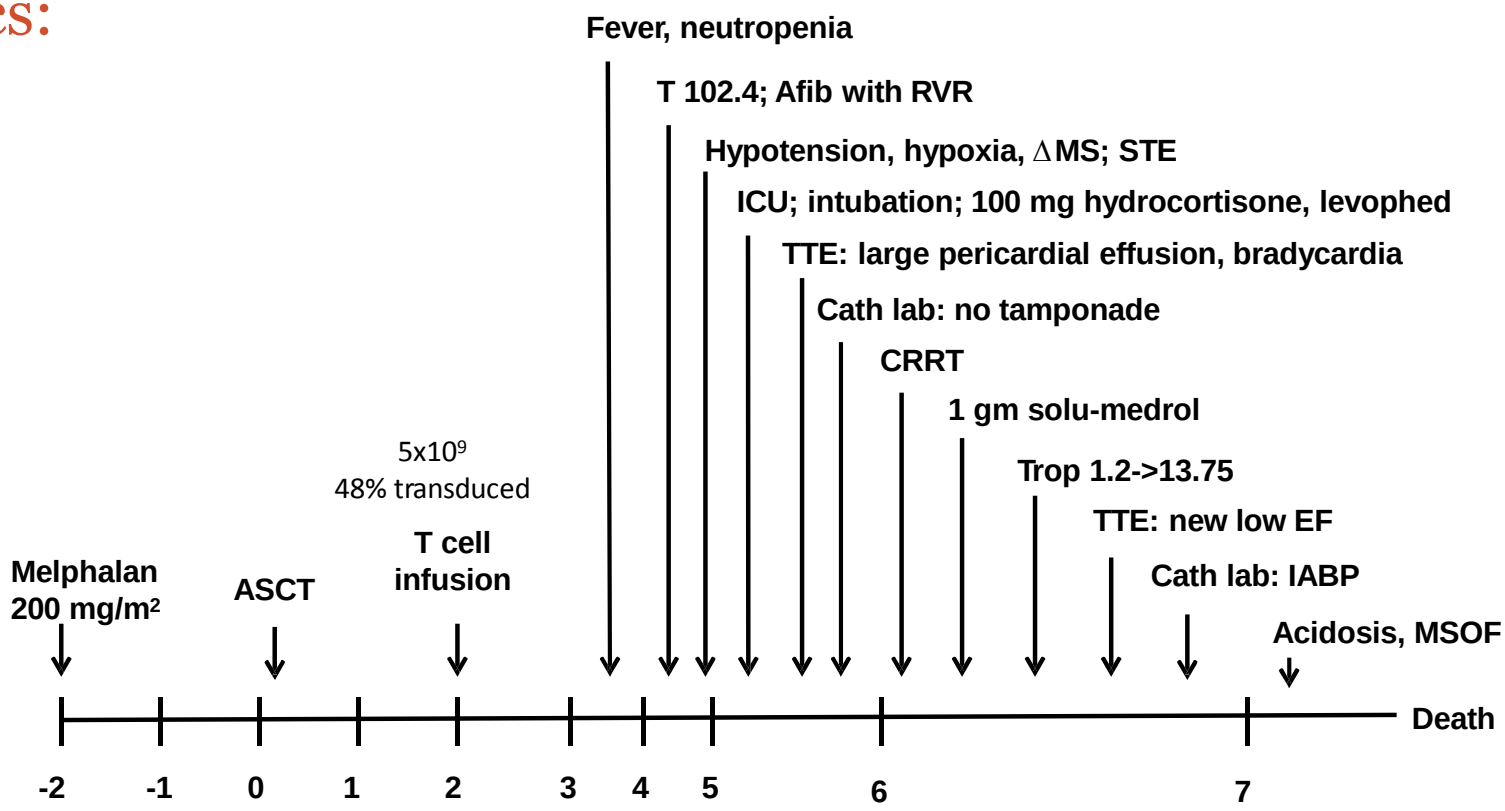


(TCR) transgenics: NY-ESO1





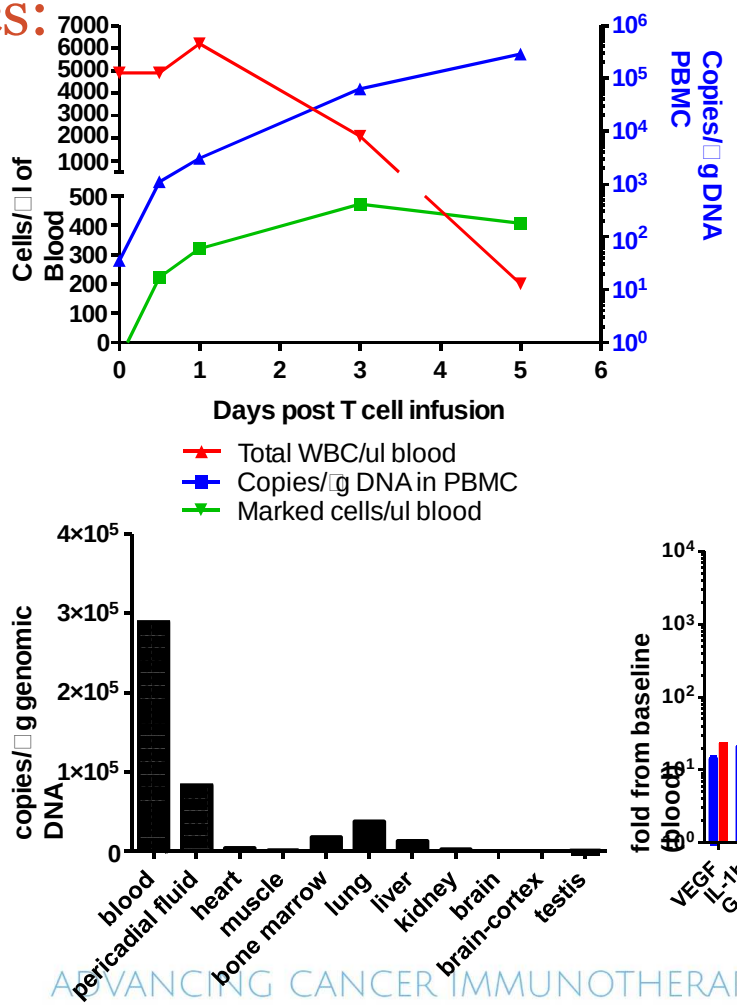
(TCR) transgenics: MAGE-A3

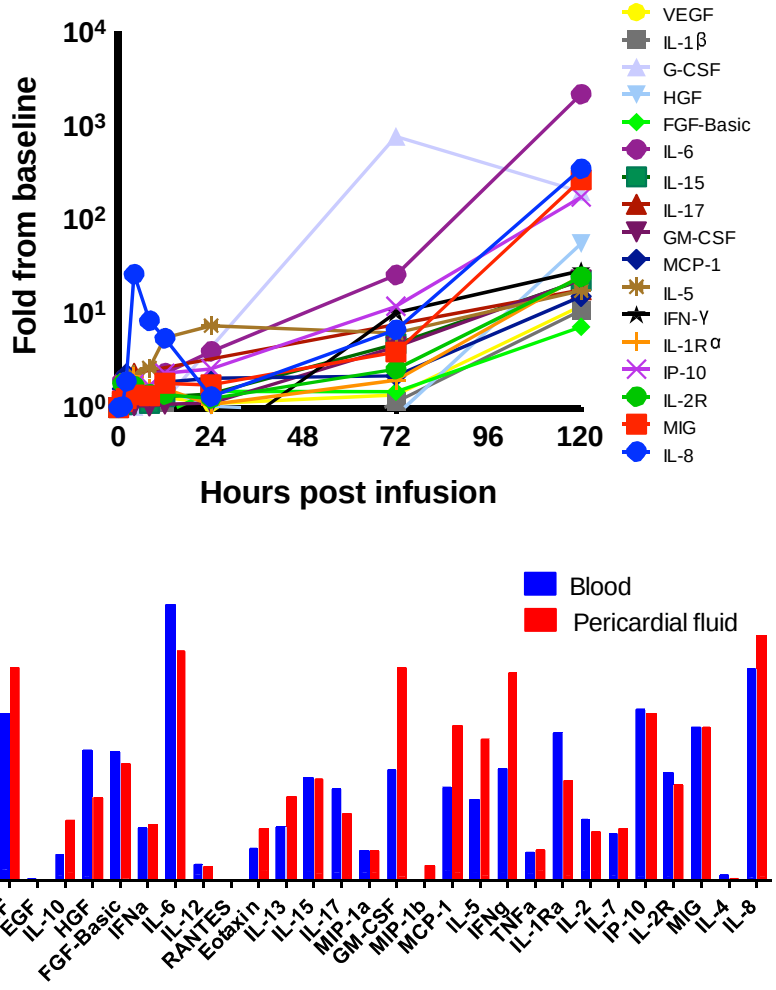


57 year old man with multiple myeloma
Prior treatments: Radiation, lenalidomide, bortezomib, dexamethasone, D-PACE
PMH: rate-controlled Afib, hypertrophic CM without outflow obstruction, normal stress test

Linette et al, Blood 2013

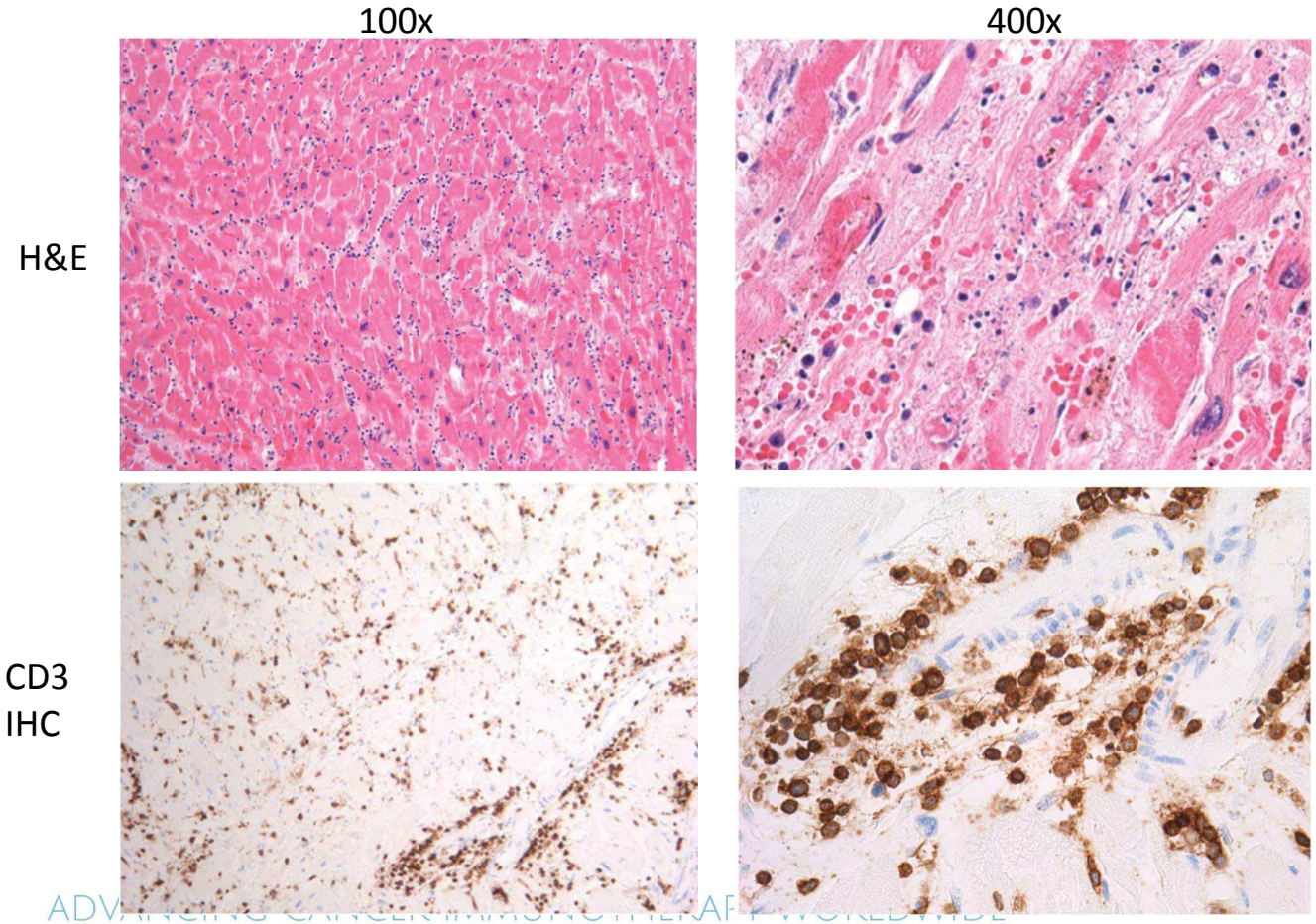
(TCR) transgenics: MAGE-A3





(TCR) transgenics: MAGE-A3

myocardium

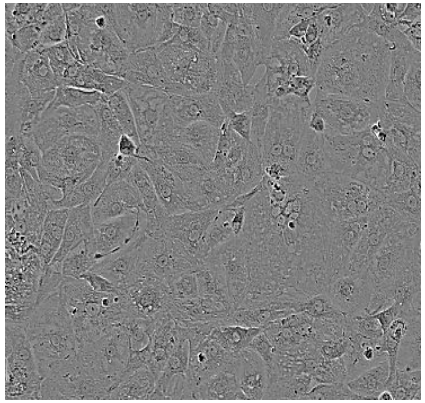


(TCR) transgenics: MAGE-A3

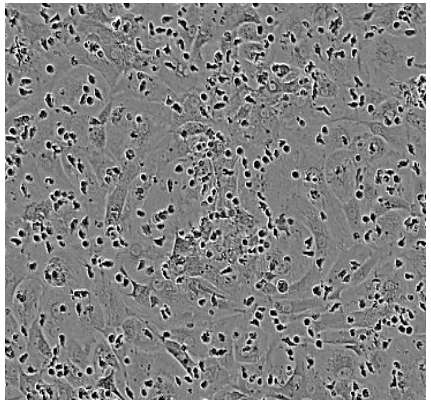
- Titin cross-reactivity
- Expressed in striated muscle
- Mutations are a/w cardiomyopathy
- Low/undetectable in most cultured cells
- Mouse titin no homology with human

MAGE-A3 specific T cells kill HLA-A1^{+} beating, iPSC- derived cardiomyocytes

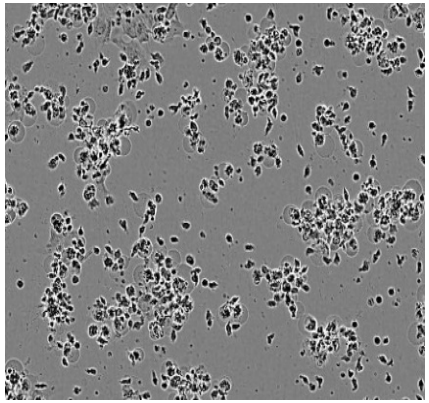
iCells alone



iCells + untransduced T cells



iCells + MAGE a3a-TCR- T cells



Cameron et al. *Science Translational Medicine 5:197ra103, 2013*

(TCR) transgenics: MAGE-A3

First example of off-target effects with TCR-engineered T cells

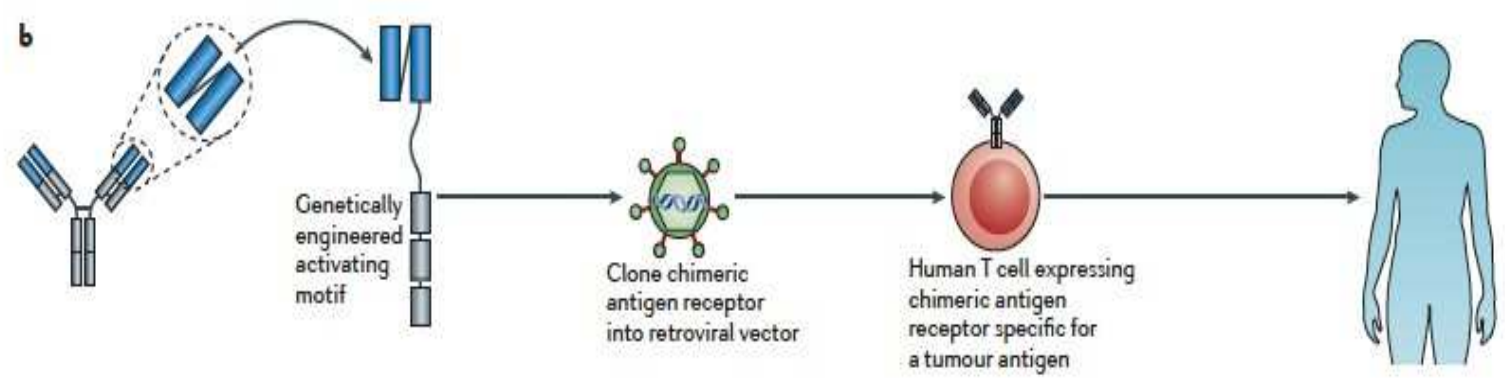
- Affinity enhanced TCR engineered T cell therapy at risk for cross-reactivity
- Biologically relevant preclinical screening of new TCRs is critical

Dose reduction may not ameliorate risk and may only delay onset of toxicity (due to in vivo T cell expansion)

Toxicity management: corticosteroids did not ablate. Would suicide systems or other forms abort toxicity?

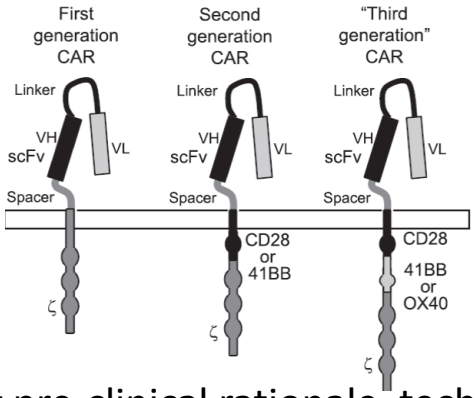
NY-ESO-1 TCRs are safe with encouraging clinical results to date

Forms of ACT: chimeric antigen receptor (CAR) T cells



Chimeric antigen receptor (CAR) T cells

Redirected T cell concept pioneered in vitro by Eshhar *et al (PNAS, 1989)*



Despite strong pre-clinical rationale, technical difficulties prevented clinical translation until recently:

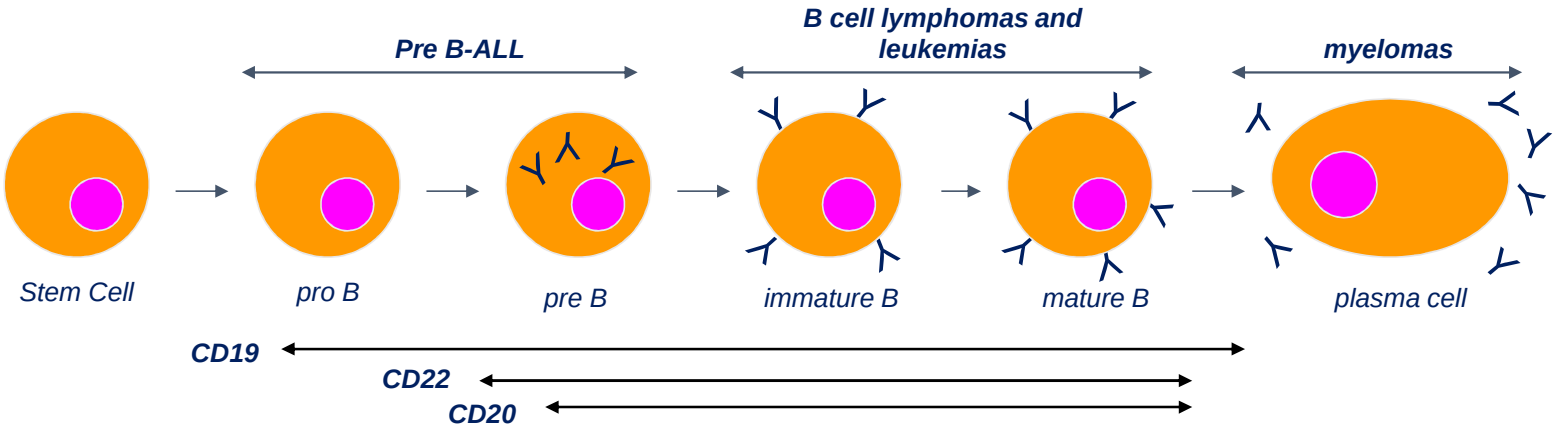
- **Efficient T cell culture systems**
- **Efficient gene transfer systems**

➢Early trials showed some promise but ultimately disappointing, due to poor T cell persistence

Anti-CD19 CAR Study	Year
Jensen, *BBMT*	**2010**
Porter, *NEJM*	**2011**
Kalos, *STM*	**2011**
Brentjens, *Blood*	**2011**
Kochenderfer, *Blood*	**2011**
Kochenderfer, *Blood*	**2012**
Cruz, *Blood*	**2013**
Brentjens, *STM*	**2013**
Grupp, *NEJM*	**2013**
Kochenderfer, *Blood*	**2013**
Davila, *STM*	**2014**
Maude, *NEJM*	**2014**
Lee, *Lancet*	**2015**
Kochenderfer, *JCO*	**2015**

Chimeric antigen receptor (CAR) T cells

CD19 is a prototypic antigen



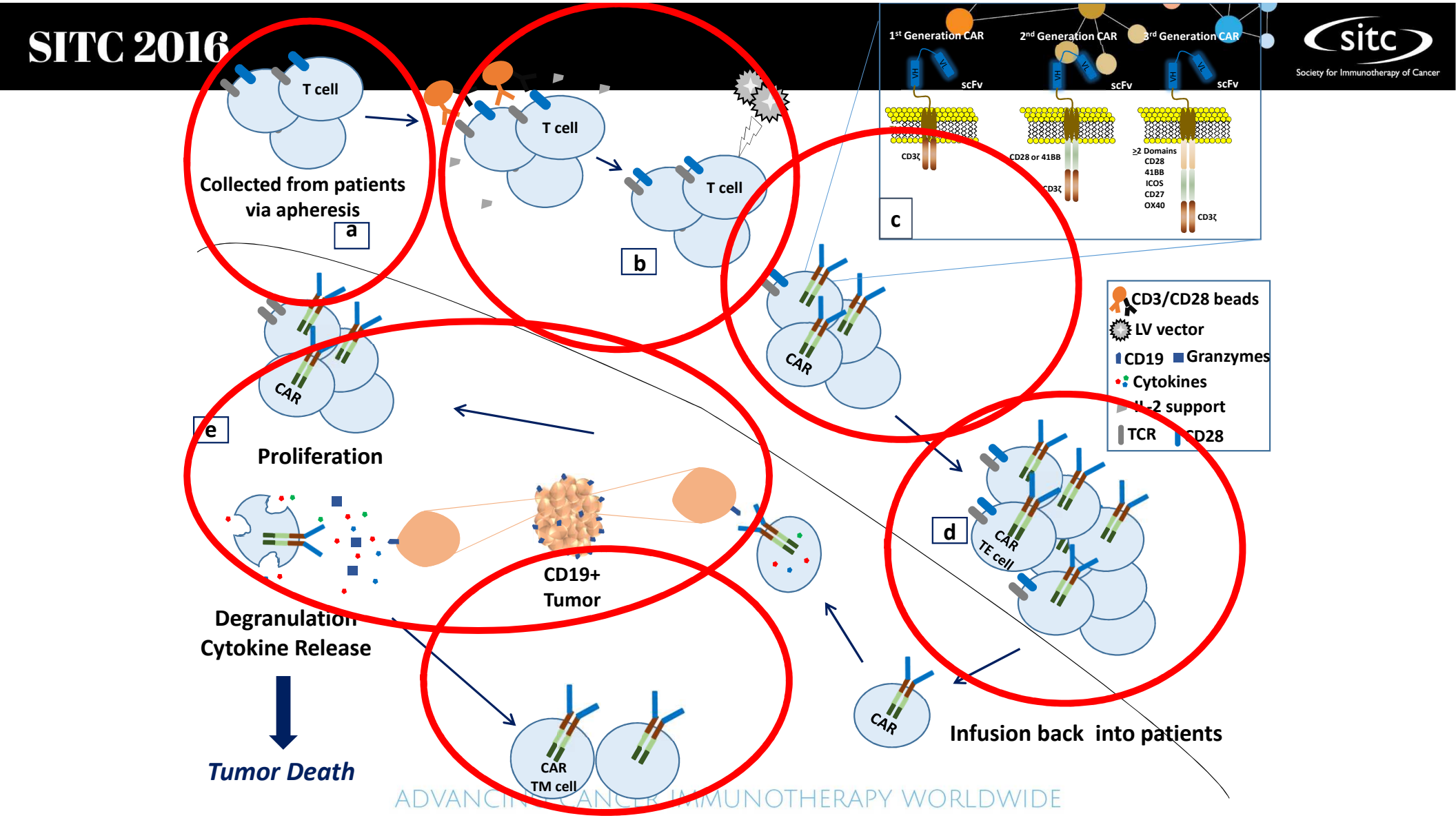
Brentjens and Sadelain

CAR T cells: what have we learned?

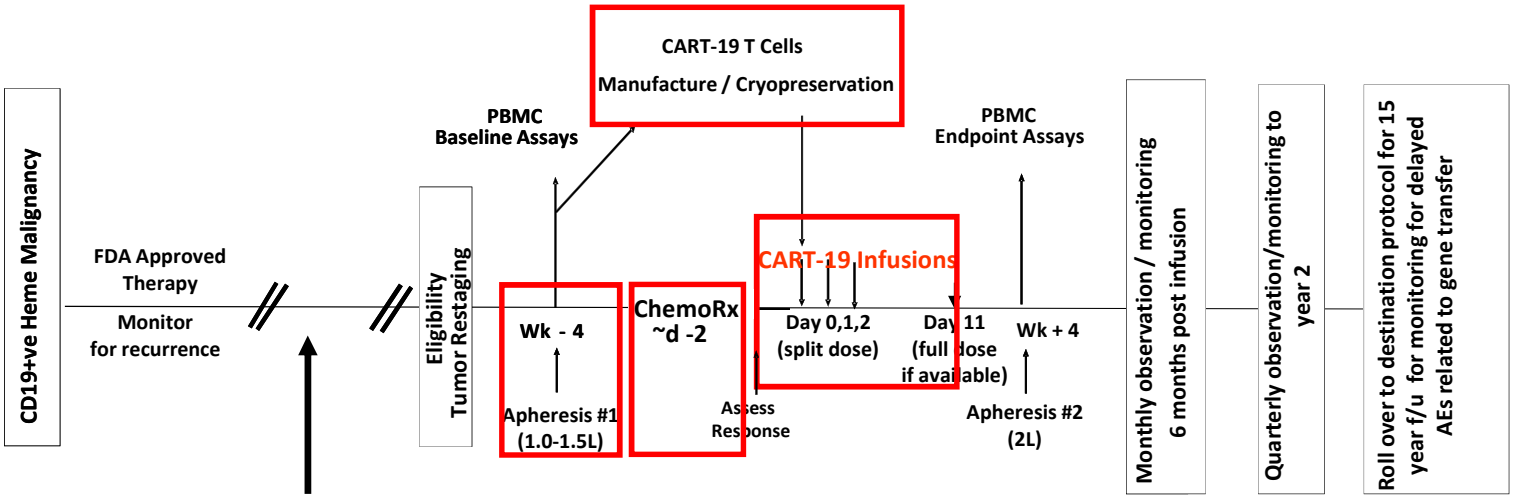
Concept	Selected Reference
Co-stimulation is important	Savoldo 2011
Lymphodepletion is important	Brentjens 2011
Persistence is important	Kalos 2011
Establishment of memory	Kalos 2011
Disease kinetics not important	many
No dose-response (probably)	unpublished
Antigen-loss (immunosurveillance)	Grupp 2013
Cytokine release / Macrophage activation	Grupp 2013
CRS correlates with antigen burden	Maude 2014
Trafficking to “immunoprivileged” sites	Grupp 2013
Encephalopathy	Davila 2014

CAR T cells: open questions in 2016

Concept
T cell manufacturing – optimal method? Optimal for what / who?
Gene transfer – LV, RV, mRNA, transposon, other
Which co-stimulatory molecule? (efficacy, toxicity)
Beyond 2nd generation?
Solid tumors – trafficking, other
CLL - ?immunosuppression
What to do when CART fail to persist?
CRS – prophylaxis or treatment?


SITC 2016
Society for Immunotherapy of Cancer
T cell
Collected from patients via apheresis
a
T cell
T cell
b
1st Generation CAR
2nd Generation CAR
3rd Generation CAR
scFv
CD3ζ
CD28 or 41BB
≥2 Domains
CD28
41BB
ICOS
CD27
OX40
c
CAR
CD3/CD28 beads
LV vector
CD19
Granzymes
Cytokines
IL-2 support
TCR
CD28
e
Proliferation
CD19+
Tumor
Degranulation
Cytokine Release
Tumor Death
CAR
TM cell
d
CAR
TE cell
CAR
Infusion back into patients
ADVANCING CANCER IMMUNOTHERAPY WORLDWIDE

CART-19 trial overview



CART-19 in CLL

RESEARCH ARTICLE

IMMUNOTHERAPY

Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia

David L. Porter,[1]* Wei-Ting Hwang,[2] Noelle V. Frey,[1] Simon F. Lacey,[3] Pamela A. Shaw,[2] Alison W. Loren,[1] Adam Bagg,[3] Katherine T. Marcucci,[3] Angela Shen,[4] Vanessa Gonzalez,[3] David Ambrose,[3] Stephan A. Grupp,[5] Anne Chew,[3] Zhaohui Zheng,[3] Michael C. Milone,[3] Bruce L. Levine,[3] Jan J. Melenhorst,[3] Carl H. June[3]*

Table 1. Summary of patient baseline characteristics (*N* = 14).

Characteristics	Statistics, *n* (%)
N	14
Age at infusion (years)	
Mean (SD)	66.9 (8.1)
Median (range)	66 (51–78)
Gender	
Male	12 (85)
Female	2 (14)
No. of previous therapies	
Mean (SD)	5.3 (2.8)
Median (range)	5 (1–11)
P53 or 17p deletion	
No	8 (57)
Yes	6 (43)
IGHV mutation	
No	9 (64)
Yes	4 (29)
Unknown	1 (7)
Lymphocyte-depleting chemotherapy	
Bendamustine	6 (43)
Fludarabine/cyclophosphamide	3 (21)
Pentostatin/cyclophosphamide	5 (36)
Lymphocytes in bone marrow at enrollment (%)*	
Mean (SD)	79.5 (17.9)
Median (range)	87.5 (40–95)
Rai stage	
1	5 (36)
4	9 (64)
Binet stage	
A	1 (7)
B	4 (29)
C	9 (64)

CART-19 in CLL

Table 2. Treatment and clinical characteristics of subjects (*N* = 14). DLBCL, diffuse large B cell lymphoma; NED, no evidence of disease; NR, no response. MRD tested by deep sequencing analysis as described in Materials and Methods.

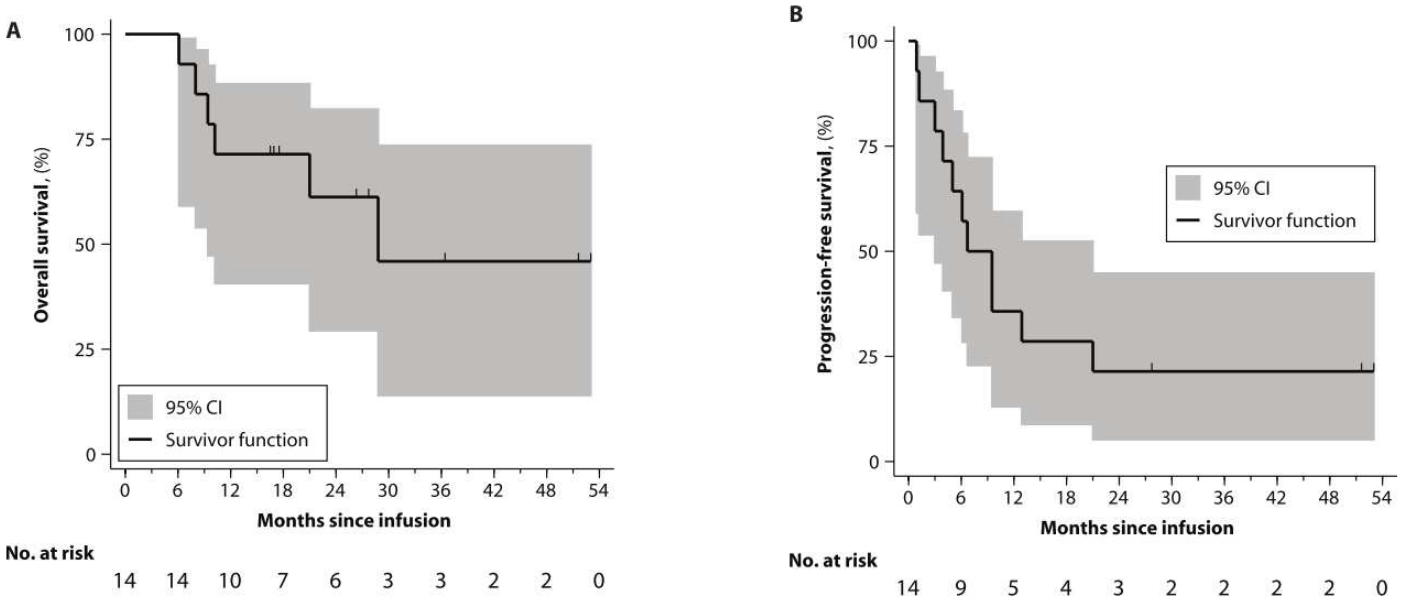
ID	Total T cells infused ($\times 10^8$)	Total CTL019 cells infused ($\times 10^8$)	Peak CTL019 expansion (% of $CD3^+$ cells)	Best overall response	Last follow-up or progression (months)	Comments, current status
01	50	11.3	N/A†	CR	53	MRD-negative; progression-free
02	3.0	0.142	N/A†	CR	52	MRD-negative; progression-free
03	25.5	5.86	N/A†	PR	5	Progression, 5 months; died of disease, 27 months
05	10.0	3.92	14.1	PR	13	Progression, 13 months; alive with disease, 36 months
06	3.0	0.646	0.2	NR	1	Died of disease, 8 months
07	1.7	0.172	0.3	NR	1	Died of complications from bone marrow transplant, 9 months
09	5.0	1.70	81.9	CR	21	MRD-negative; progression-free, 21 months; died of infection
10	30.0	5.61	34.3	CR	28	Bulky adenopathy (11 cm); MRD-negative; progression-free
12	5.0	1.18	18.3	PR	6	Bulky adenopathy (9 cm); died of pulmonary embolus
14	18.0	1.56	<0.1	NR	7	Alive with disease, 26 months
17	4.2	1.03	1.6	NR	10	Alive with disease, 18 months
18	50.0	2.77	0.2	NR	4	Alive with disease, 17 months
22	5.0	0.864	34.9	PR	10	Bulky adenopathy (9 cm); progressed 10 months with transformed CD19-dim DLBCL; died of disease at 10 months
25	20.0	2.71	2.6	NR	3	Alive with disease, 16 months

CART-19 in CLL

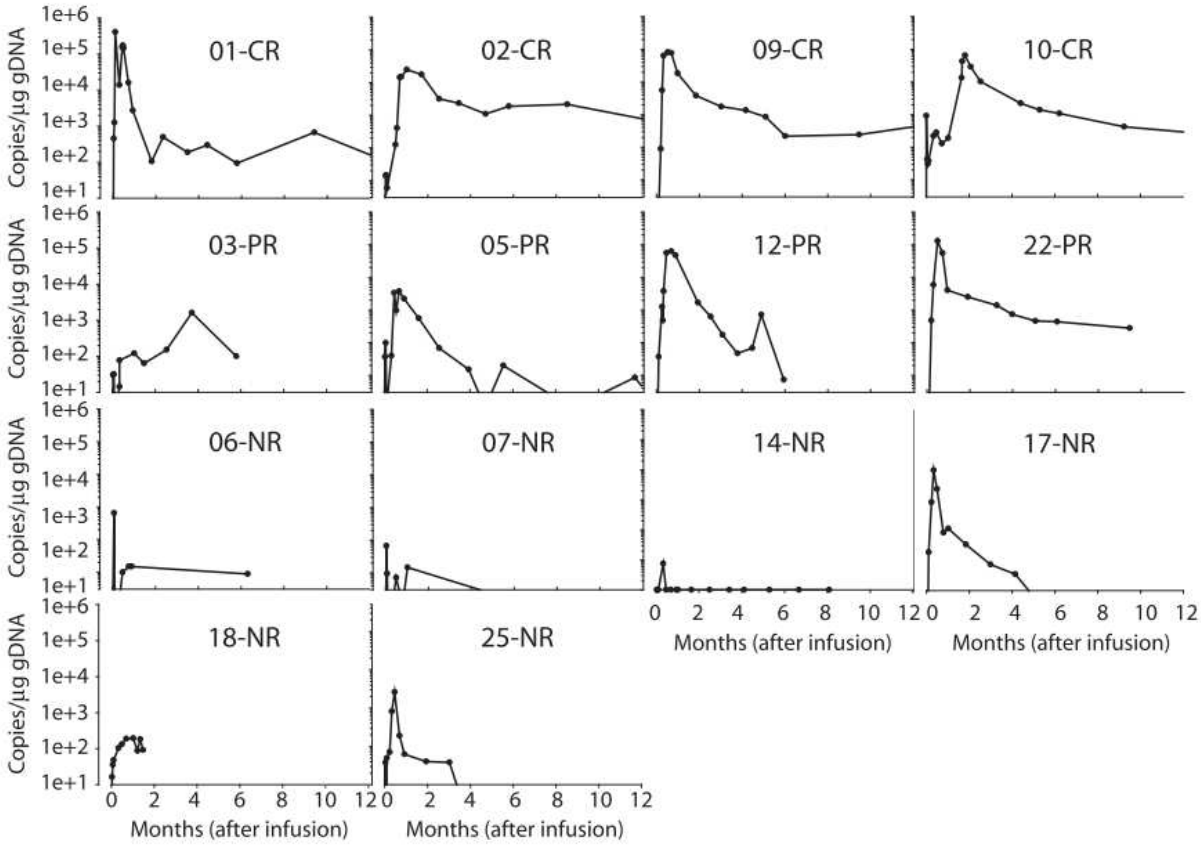
Table 3. IGH deep sequencing analysis of blood and bone marrow shows eradication of CLL and B cells for subjects 01 and 02. BM, bone marrow; PB, peripheral blood; Mo, month; Yr, year.

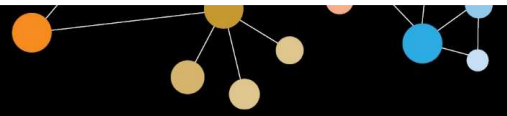
Patient UPCC04409 no.	Sample type	Time point	Cell equivalents sequenced	Total reads of IGH	Total unique IGH reads	Tumor clone reads	CLL clone (% of total)
01	PB	Baseline		408,579	48	407,592	99.76
Mo 6		285,305	7362	0	0.00		
Yr 1		41	12	0	0.00		
Yr 3	298,667	174	6	0	0.00		
Yr 3.5	350,171	123	8	0	0.00		
BM	Mo 1	408,838	179	3	0	0.00	
Mo 6		202,535	4451	0	0.00		
Mo 12		18,506	231	0	0.00		
Mo 24		88	2	0	0.00		
02	PB	Baseline		1,385,340	4544	1,285,862	92.82
Mo 6		25,041	38	0	0.00		
Mo 32	317,714	88	8	0	0.00		
Yr 3	346,057	160	8	0	0.00		
Yr 4	308,419	212	10	0	0.00		
BM	Yr 1		5	2	0	0.00	
Yr 2		601	25	0	0.00		

CART-19 in CLL



CART-19 in CLL





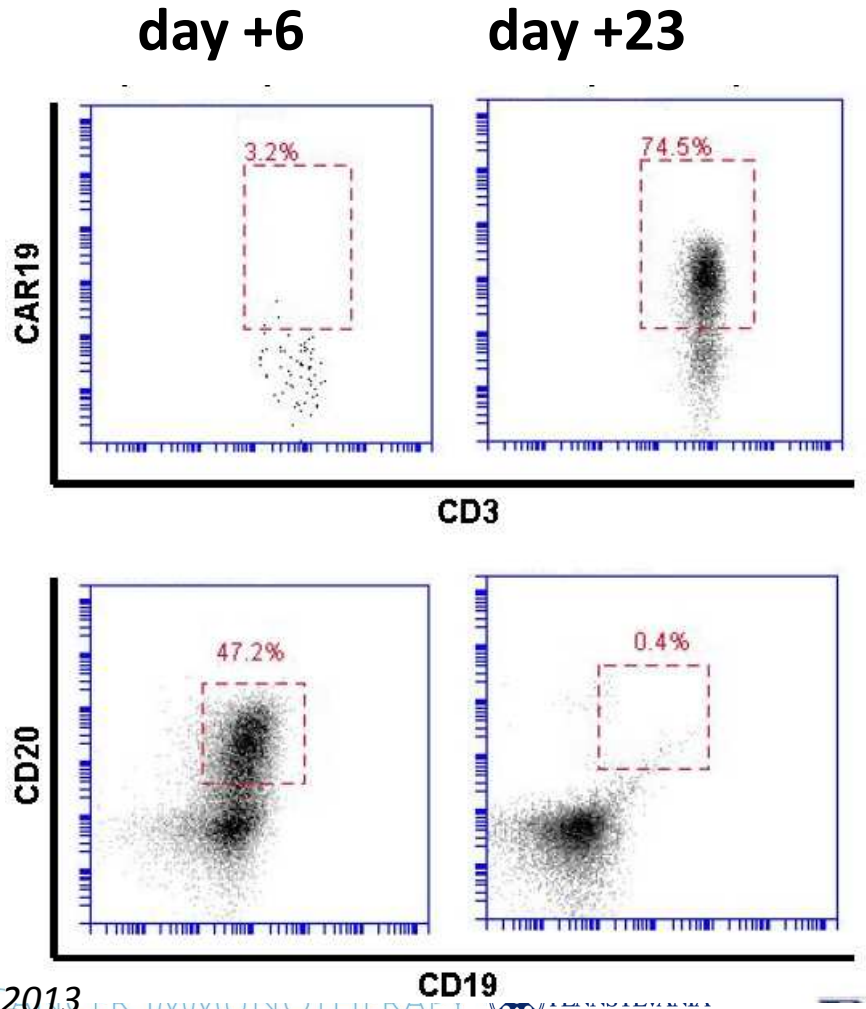
CART-19 in CLL

- Proof of concept
- 47% ORR in heavily pre-treated patients
- Cause of poorer-than-expected response rates?
- Immunosuppression in CLL
- Combination with other agents
- Immunotherapy vs small molecules, esp ABT-199

CART-19 in ALL

- N=30 (evaluable)
- 25 pediatric and 5 adult patients
- 40% female, 60% male
- Median age 14 (5-61)
- Disease status
  - Primary refractory 10%
  - 1st relapse 17%
  - ≥2nd relapse 73%

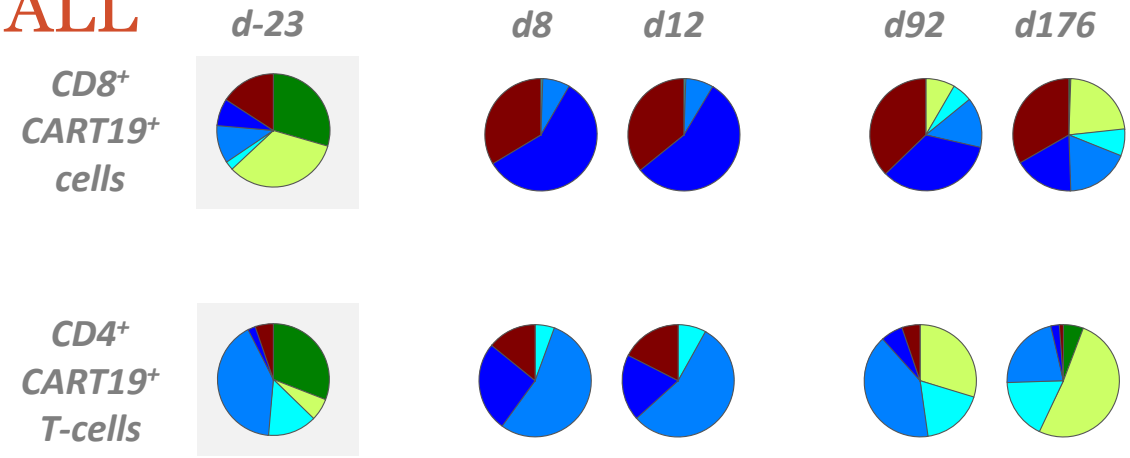
CART-19 in ALL



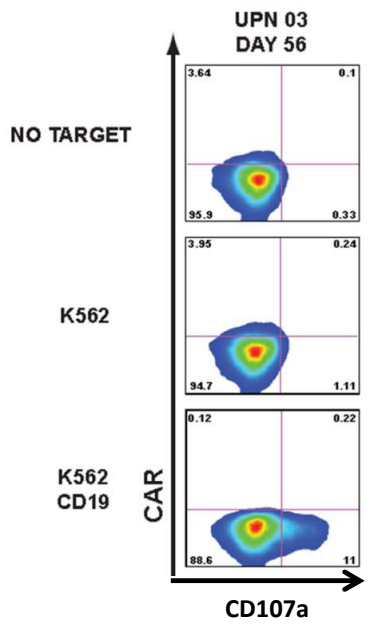
- Deep remission induced in 23 days
- Status: CR (11+)
- MRD <0.01% cells

Grupp et al, NEJM 2013

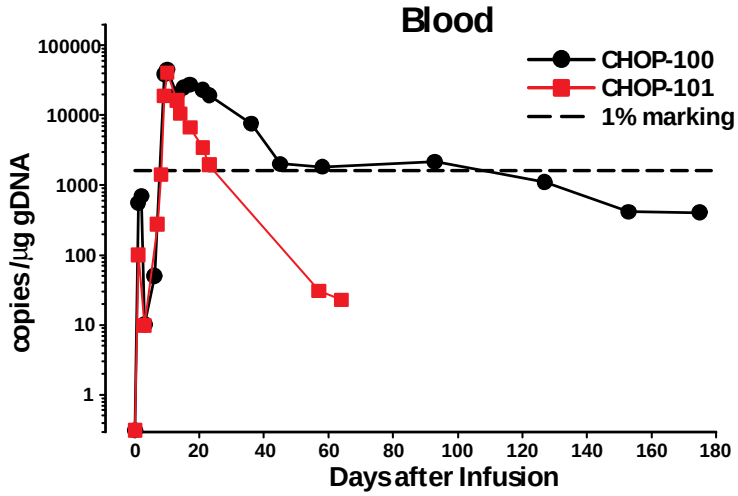
CART-19 in ALL



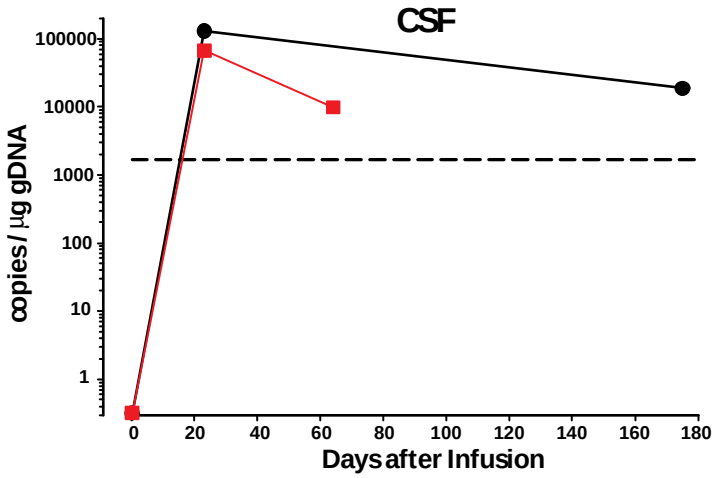
Persisting cells remain functional



CART-19 in ALL

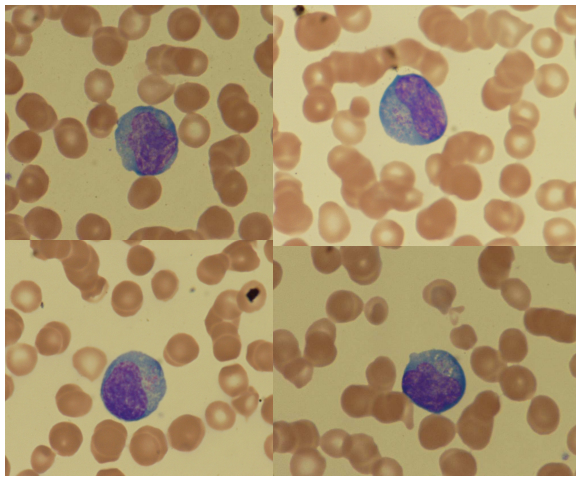


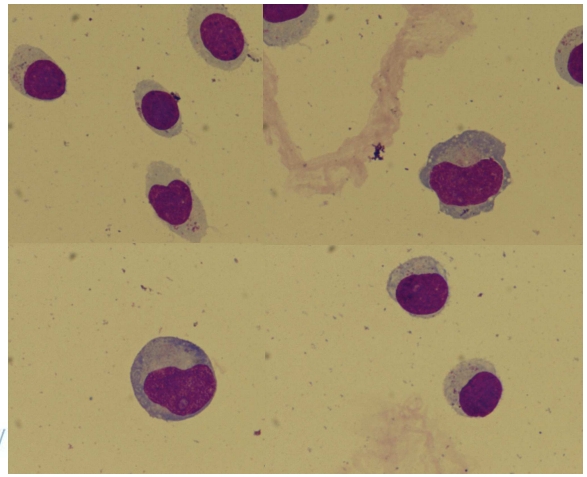
Blood
Day 10

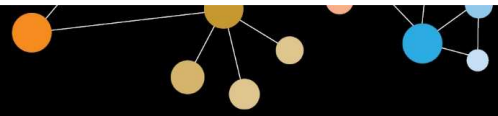


CSF
Day 23

Morphology of CARs In Vivo







CART-19 in ALL

Response	N=30	%
Complete Response	**27/30**	**90%**
No response	3/30	10%
Not evaluable (extramedullary dz (1) and short f/u (4)	5	

CART-19 in ALL

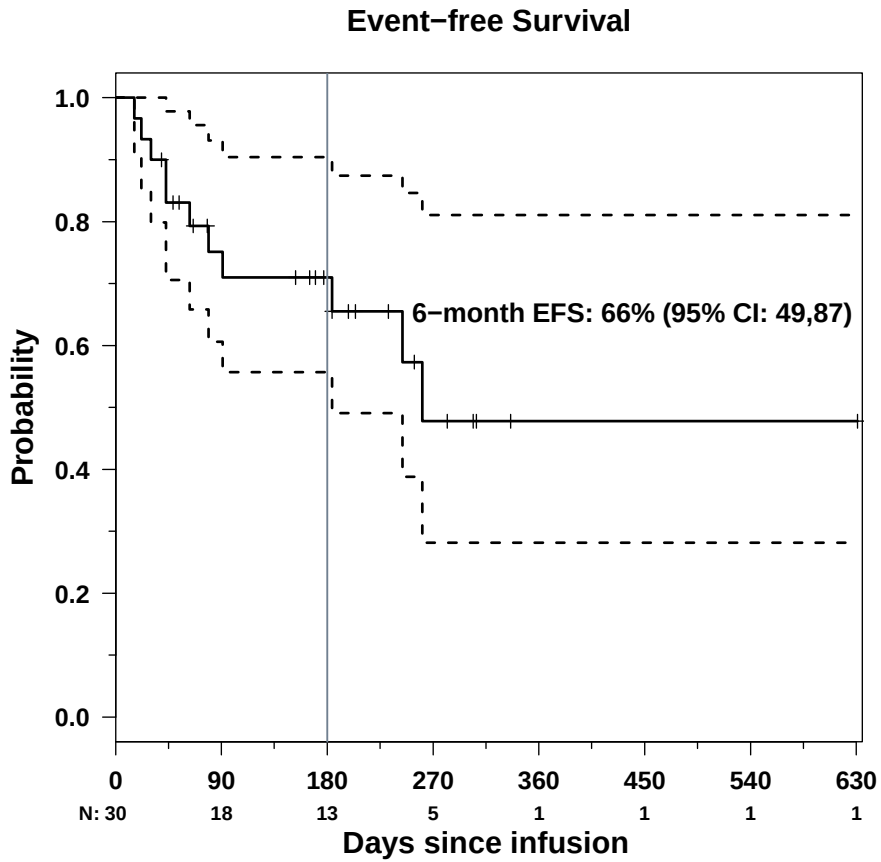
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

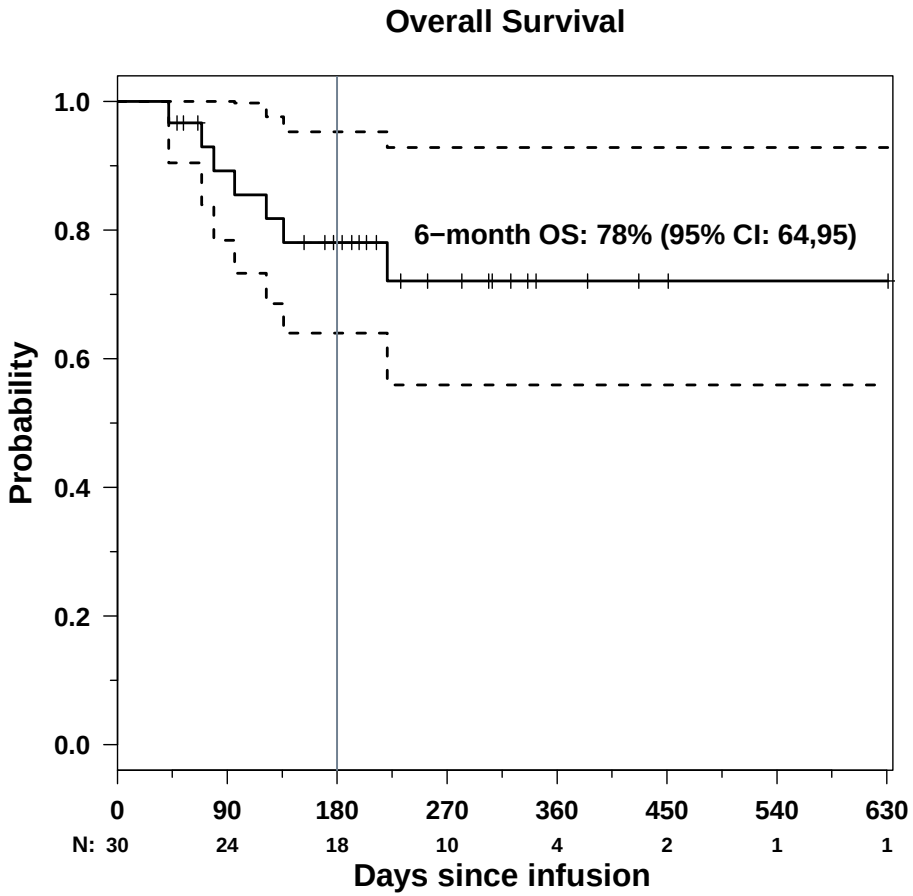
Chimeric Antigen Receptor T Cells for Sustained Remissions in Leukemia

Shannon L. Maude, M.D., Ph.D., Noelle Frey, M.D., Pamela A. Shaw, Ph.D., Richard Aplenc, M.D., Ph.D., David M. Barrett, M.D., Ph.D., Nancy J. Bunin, M.D., Anne Chew, Ph.D., Vanessa E. Gonzalez, M.B.A., Zhaohui Zheng, M.S., Simon F. Lacey, Ph.D., Yolanda D. Mahnke, Ph.D., Jan J. Melenhorst, Ph.D., Susan R. Rheingold, M.D., Angela Shen, M.D., David T. Teachey, M.D., Bruce L. Levine, Ph.D., Carl H. June, M.D., David L. Porter, M.D., and Stephan A. Grupp, M.D., Ph.D.

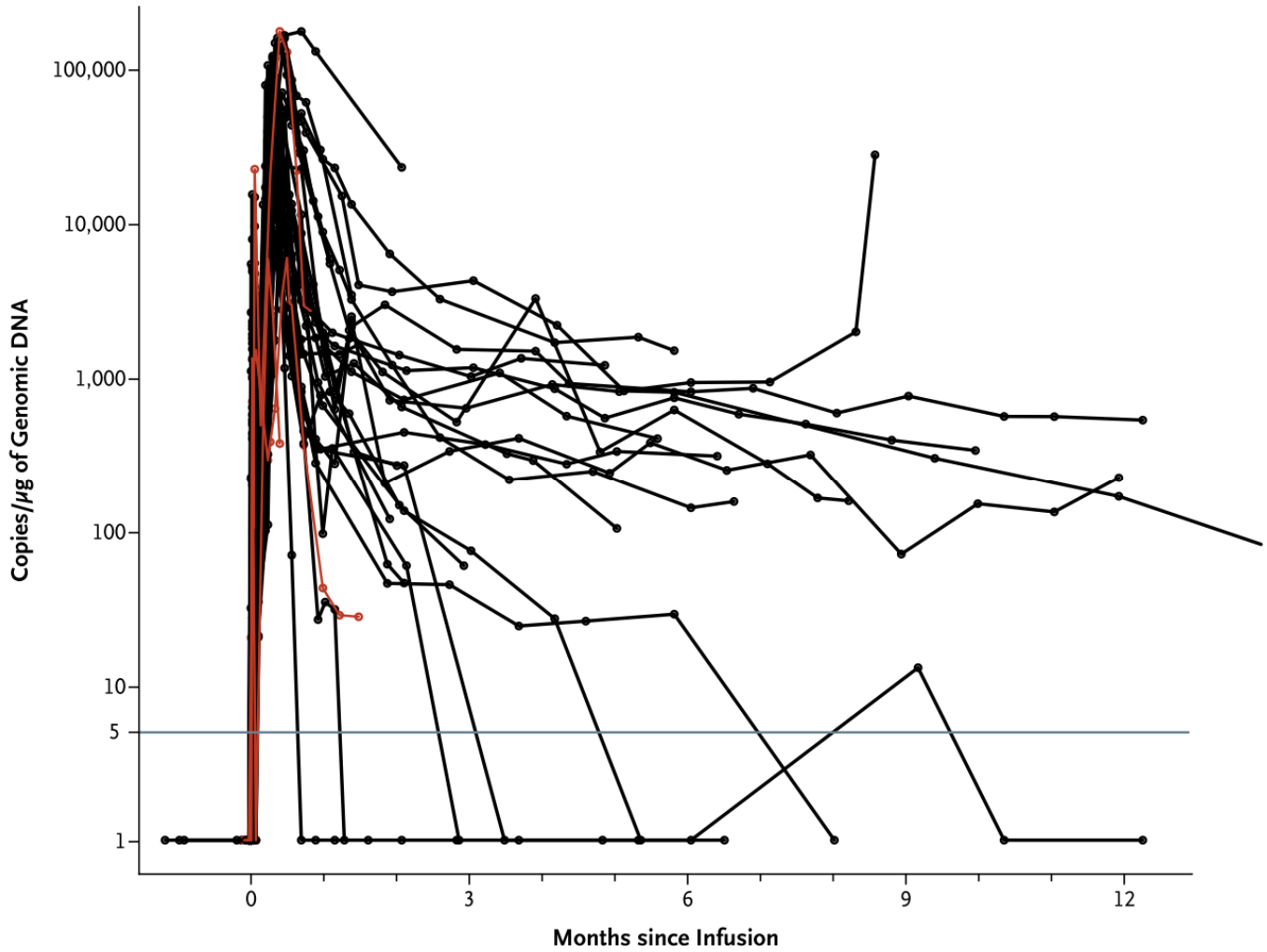
CART-19 in ALL



CART-19 in ALL

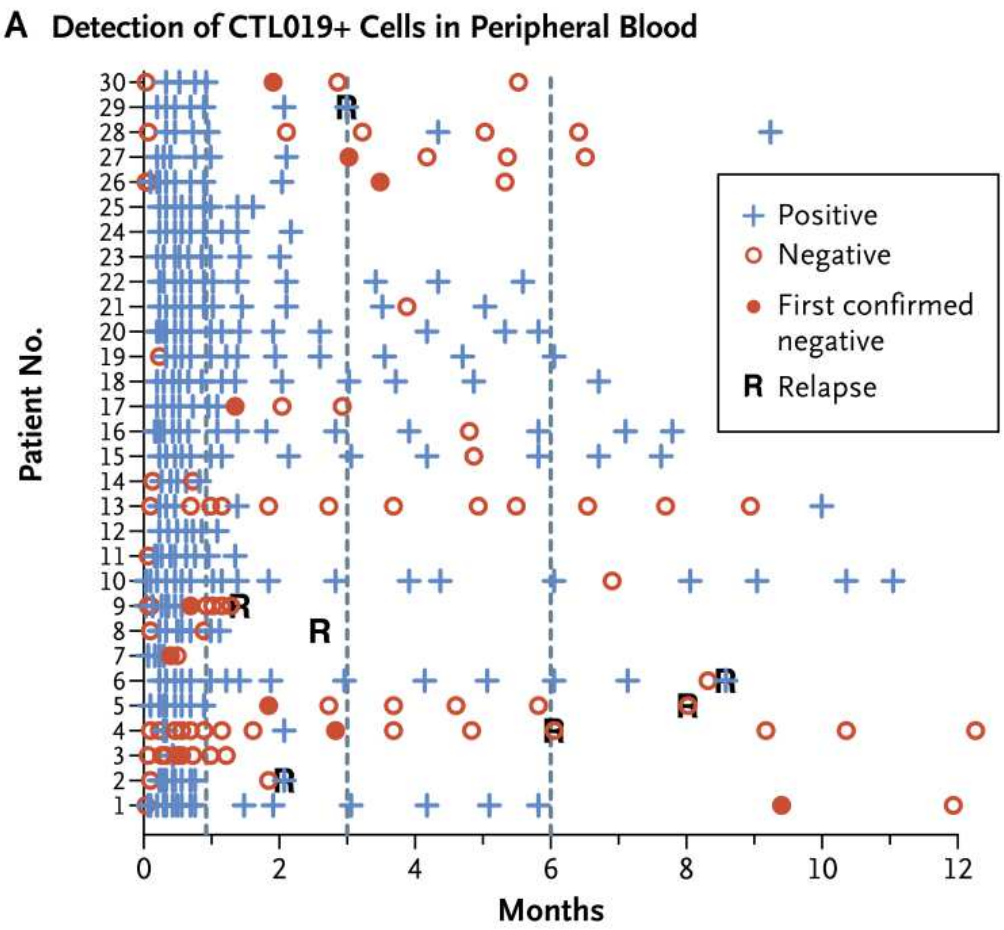


CART-19 in ALL



ADVANC

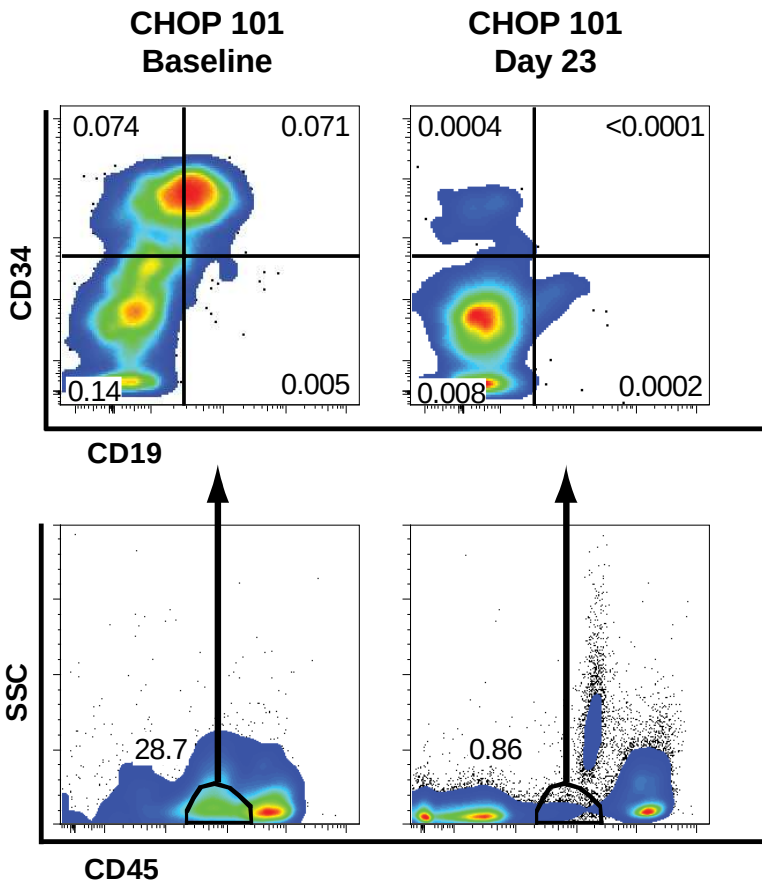
CART-19 in ALL



CART-19 in ALL

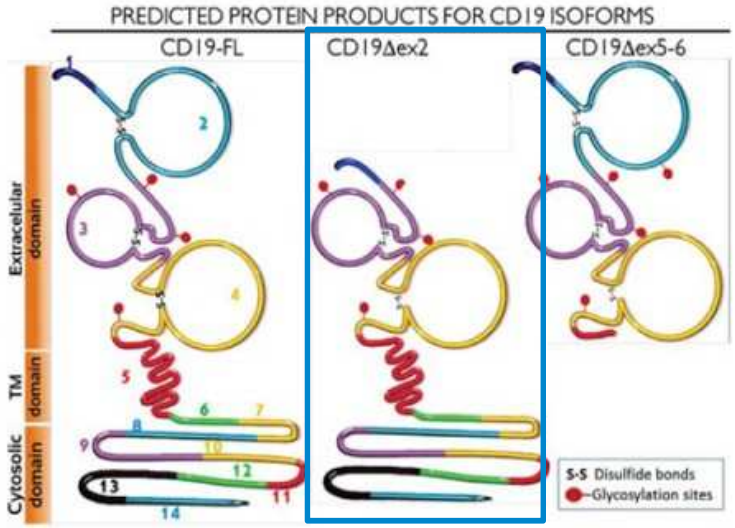
Patient	Tissue	timepoint	Cell equivalents	total productive reads	Total unique sequences	Total tumor reads	tumor clone frequency
UPN01	Blood	-1	158, 730	408,579	48	407,592	99.8
		28	158, 730	0	0	0	0
		176	79,365	285,305	7362	0	0
	Marrow	28	158,730	0	0	0	0
		176	158,730	202,535	4451	0	0
		720	279,924	261	13	0	0
UPN02	Blood	-1	61,270	1,385,340	4,534	1,231,018	88.9
		31	158, 730	0	0	0	0
		176	317,460	0	0	0	0
	Marrow	31	277,778	0	0	0	0
		176	158730	0	0	0	0
		741	222,019	707	29	0	0
CHP959-100	Blood	-1	111,340	189	6	185	97.88
		23	218,210	0	0	0	0
		87	288,152	0	0	0	0
		180	420,571	6	2	0	0
	Marrow	-1	317,460	59,791	318	59,774	99.97
		23	362,819	37	2	33	89.19
		87	645,333	10	1	10	100
		180	952,381	45	7	0	0
CHP959-101	Blood	-1	152,584	38,170	52	30,425	79.71
		23	417,371	92	5	18	19.6
	Marrow	-1	158,730	68,368	65	50,887	74.43
		23	305,067	1,414	11	946	66.9
		60	916,571	530,833	206	363,736	68.9

CART-19 in ALL



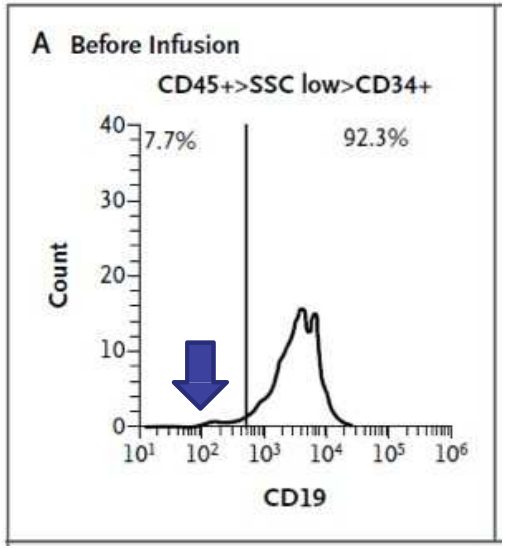
CART-19 in ALL

Sotillo E., Cancer Discovery 2015

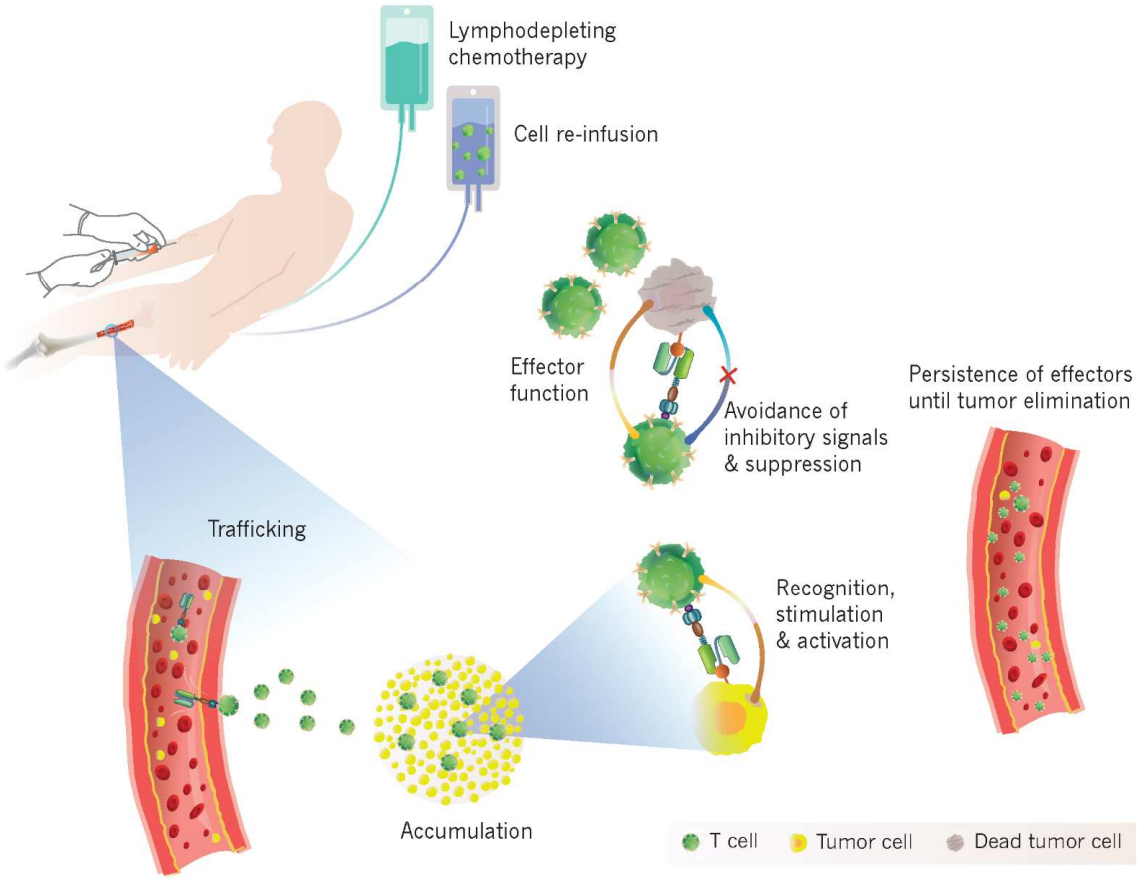


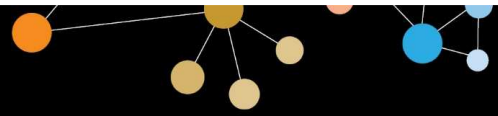
Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy

Grupp SA, NEJM, 2013



What is required for successful adoptive T cell therapy?





ACT: conclusions

- T cells may be the most potent immune cells
- Early studies suffered from lack of specificity (toxicity and lack of activity)
- TIL therapy is elegant but resource-intensive
- Genetic engineering by TCR or CAR gene transfer confers specificity
- Co-stimulatory molecule engineering leads to enhanced T cell function
- By successively addressing the requirements for ACT, it is likely that we will gradually develop a robust, predictable platform for cancer immunotherapy