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NCI Awardee Skills Development Consortium

Adoptive T cell Therapies

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Department of Medicine, Memorial Hospital

Memorial Sloan Kettering Cancer Center

Financial disclosures:

» The Sadelain lab has collaborative research agreements with Takeda Pharmaceuticals, Fate Tx, and Atara Bio.

» The Sadelain lab has licensed CAR technologies to Juno Tx (Celgene/BMS), Takeda Pharmaceuticals, Fate Tx, Atara Bio and Mnemo Tx.

Reference materials

The journey from discoveries in fundamental immunology to cancer immunotherapy

Jacques Miller and Michel Sadelain

Cancer Cell 27(4):439-449 (2015)

From Adoptive Immunity to CAR Therapy: An Evolutionary Perspective

Michel Sadelain

Encyclopedia of Immunobiology, Vol. 4, Elsevier Ltd (2016)

Gene therapy comes of age

Cynthia E. Dunbar, Katherine A. High, J. Keith Joung, Donald B. Kohn, Keiya Ozawa, Michel Sadelain

Science 359, eaan4672 (2018)

CAR T cells: continuation in a revolution of immunotherapy

Anurag K Singh and Joseph McGuirk

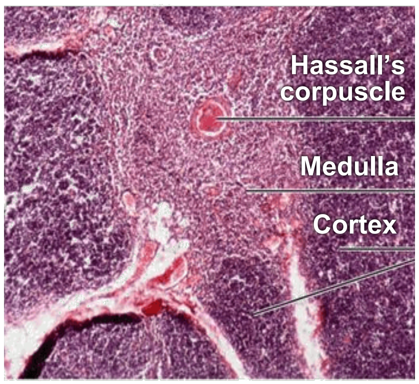
Lancet Oncol. 21(3):e168-e178 (2020)

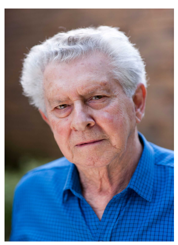
The therapeutic landscape for cells engineered with chimeric antigen receptors

Matthew MacKay, Ebrahim Afshinnekoo, Jonathan Rub, Ciaran Hassan, Mihir Khunte, Nithyashri Baskaran, Bryan Owens, Lauren Liu, Gail Roboz, Monica Guzman , Ari Melnick, Shixiu Wu and Christopher Mason

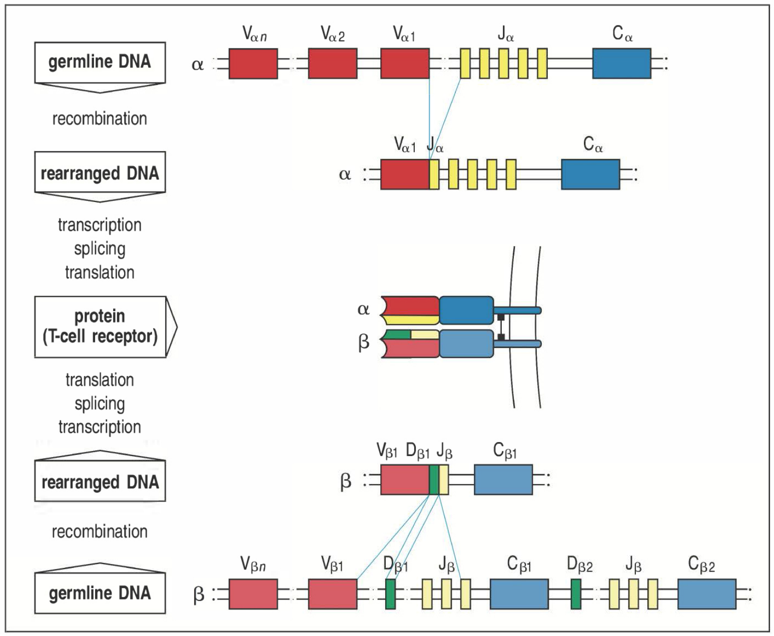
Nature Biotechnol. 39(2):233-244 (2020)

T lymphocytes: thymic origin, VDJ recombination and clonal selection



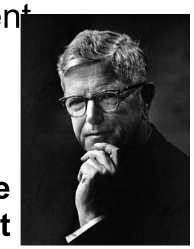


Jacques Miller



The clonal selection theory

- Each lymphocyte bears a single receptor with a unique specifity.
- Interaction between a foreign molecule and a lymphocyte receptor capable of binding that molecule with a high affinity leads to lymphocyte activation and clonal expansion.
- Lymphocytes bearing receptors specific for ubiquitous self molecules are deleted at an early stage in lymphoid cell development and are therefore absent from the repertoire of mature lymphoctyes.



F. Macfarlane Burnet

From *Adoptive Transfer*…

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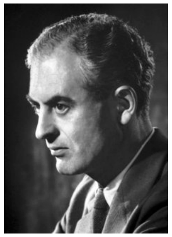
- Mitchison NA. 1953. Passive transfer of transplantation immunity. Nature 171, 267–268.
- Mitchison, N.A.. 1955. Studies on the immunological response to foreign tumor transplants in the mouse. I. The role of lymph node cells in conferring immunity by adoptive transfer. J. Exp. Med. 102, 157–177.
- Klein, G., Sjogren, H.O., Klein, E., Hellstrom, K.E., 1960. Demonstration of resistance against methylcholanthrene-induced sarcomas in the primary autochthonous host. Cancer Res. 20, 1561–1572.



Peter Medawar

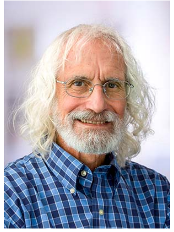
Avrion Mitchison

…to *Adoptive T Cell Therapy*

- Graft-versus-Leukemia (GVL) effect of bone marrow transplantation
- Donor leukocyte infusion (DLI)
- Lymphokine-activated killer (LAK) cell therapy
- Tumor-infiltrating lymphocytes (TILs)
- Virus-specific T cells (VSTs)

Steve Rosenberg

Hans Kolb



Phil Greenberg

and many more!

T cells fighting cancer: a complex history

Stutman O.
Science 1976

Tumor Development after 3-Methylcholanthrene in Immunologically Deficient Athymic-Nude Mice

Abstract. *Athymic-nude* (nu/nu) *mice and normal* (nu/+) *mice showed no differences in either latent period or incidence of local sarcomas or lung adenomas within 120 days after administration of 3-methylcholanthrene at birth. However,* nu/nu *mice were incapable of rejecting allogeneic skin grafts for the duration of the experiment. These results argue against an active role of thymus-dependent immunity as a surveillance mechanism preventing tumor development.*

Rolf Kiessling

R. Kiessling+, Eva Klein+, H. Pross+ and H. Wigzell+°

Department of Tumor Biology, Karolinska Institute, Stockholm+ and Department of Immunology, Uppsala University, Uppsala°

Kiessling et al., Eur J Immunol 1975

"Natural" killer cells in the mouse

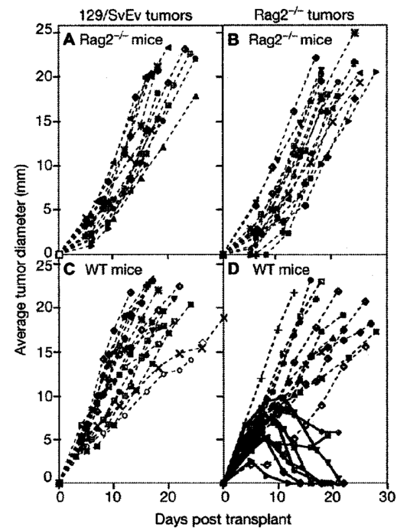
II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell*

Normal mice contain cytolytic cells with specificity for *in vitro* grown mouse Moloney leukemia cells. Such killer cells are most frequent in the spleens; lymph node and bone marrow contain less and thymus virtually no killer activity. Peak activity is found around one to three months of age. Spleen cells from genetically athymic mice are as active killer cells as those from normal mice of the same strain.

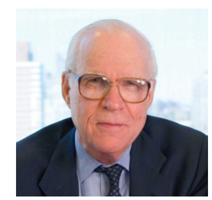
THE THREE ES OF CANCER IMMUNOEDITING

Annu Rev Immunol, 2004. 22:239

Gavin P. Dunn,[1] Lloyd J. Old,[2] and Robert D. Schreiber[1]



Bob Schreiber



Lloyd Old

Tummor Immunology – From Adoptive Immunity to CAR Therapy: An Evolutionary Perspective

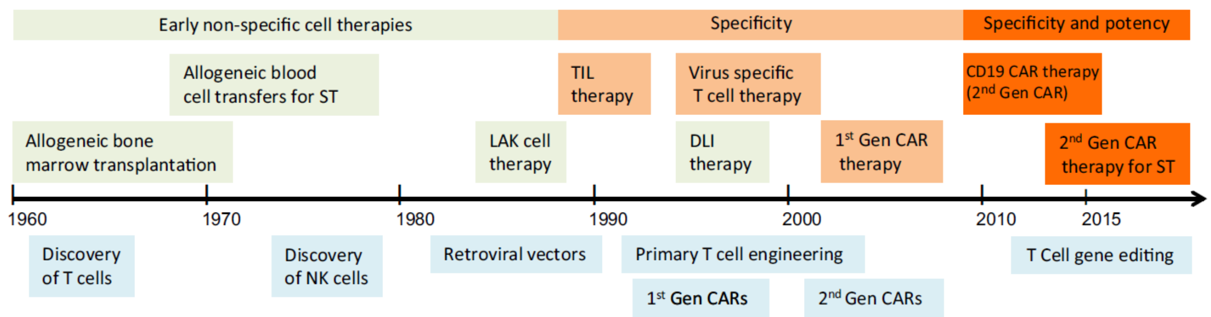
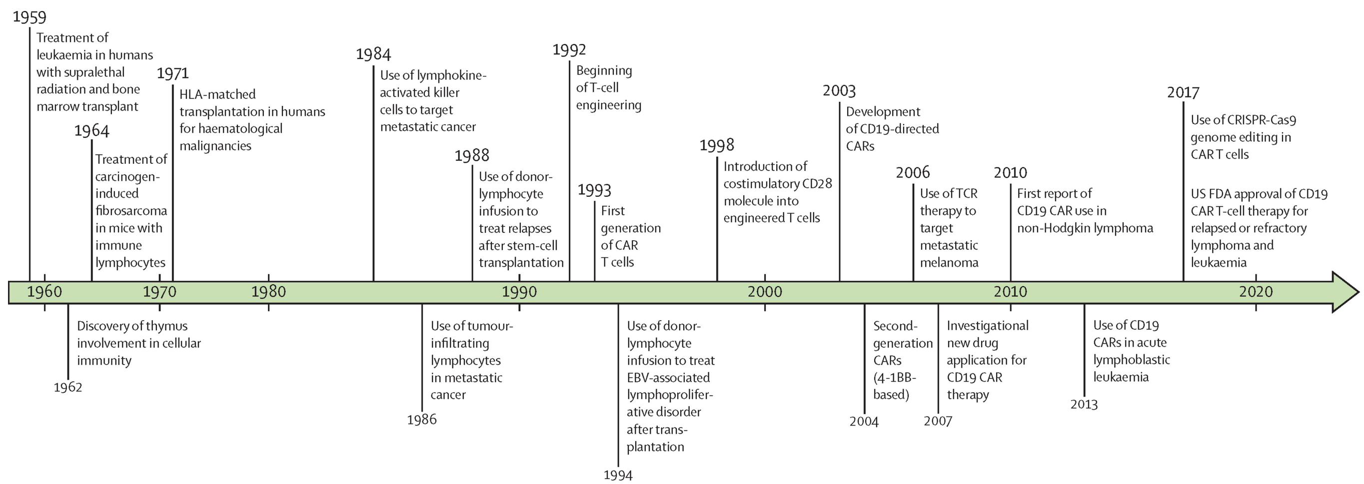


Figure 2 From the adoptive transfer of cellular immunity to CAR therapy. Scientific (below) and clinical (above) milestones.

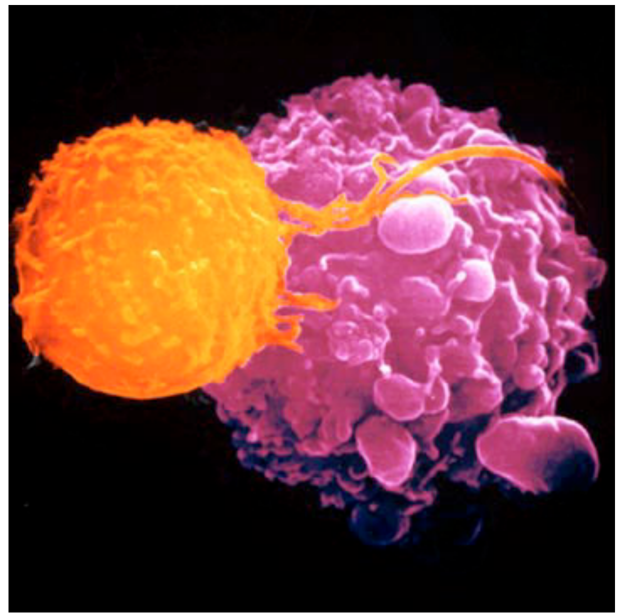


Sadelain, M. From Adoptive Immunity to CAR Therapy: An Evolutionary Perspective. Encyclopedia of Immunobiology, Vol. 4. 2016 (Elsevier)

Singh AK. McGuirk JP. Lancet Oncol. 2020 Mar;21(3):e168-e178.

The rise of engineered T cells as cancer drugs

- A major limitation of many current cancer therapeutics is their lack of curative potential
- Immunotherapy must harness T cell specificity, persistence and potency to achieve

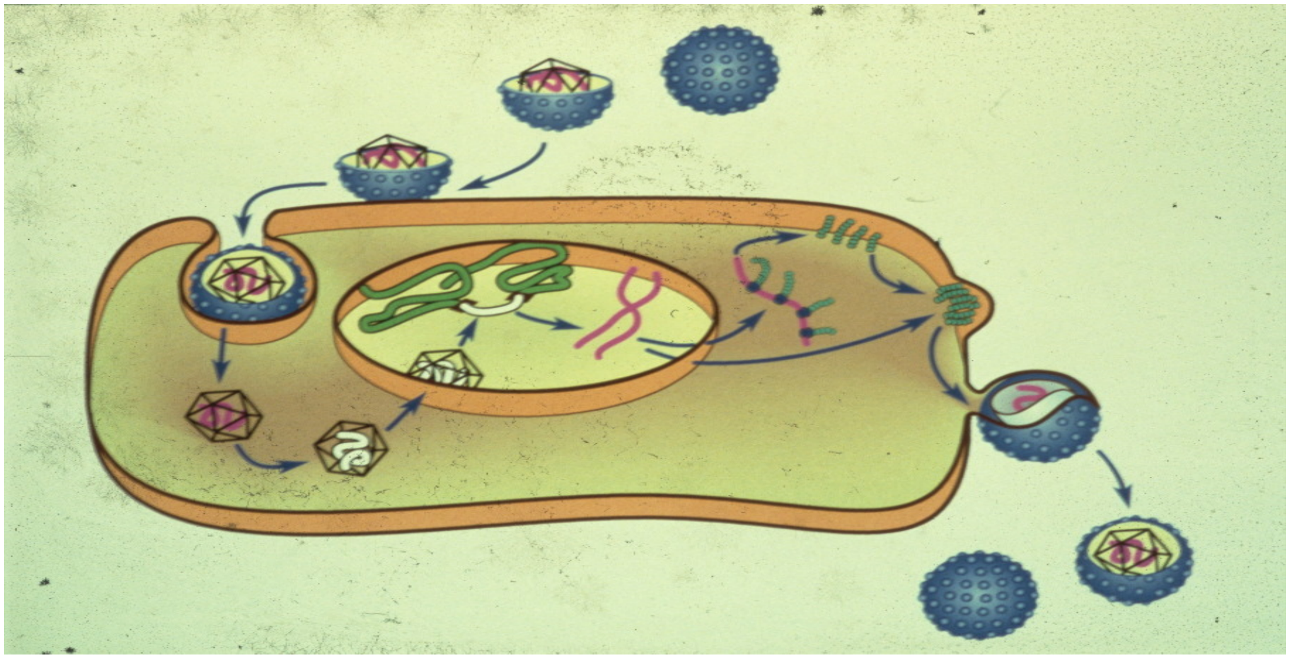


- **Safety**
- **Efficacy**

↕

- **Specificity**
- **Long-acting**
- **Potency**

Retroviral Vectors (MLV, HIV, FV)

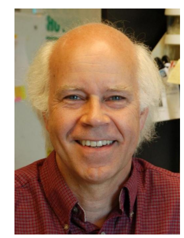


Reverse transcription (1975)

David Baltimore

Howard Temin

Packaging Cell Lines (Late 80's)



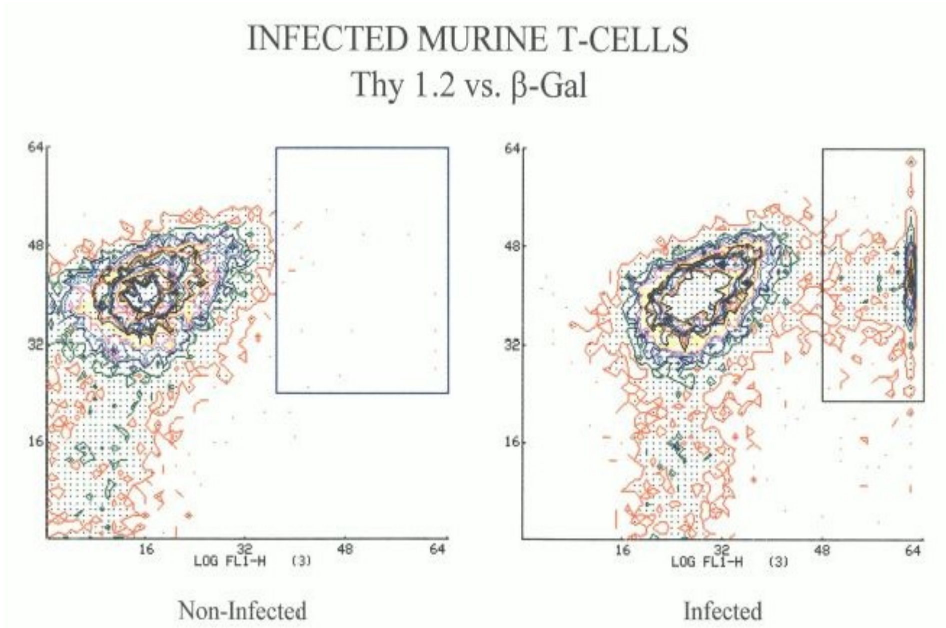
Dusty Miller

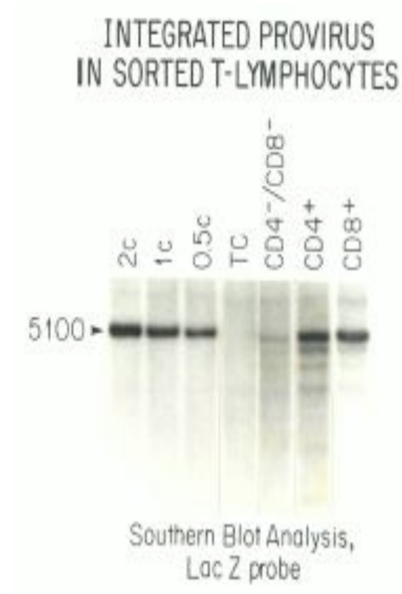
Richard Mulligan

First steps in primary T cell engineering

» Retroviral vectors (γRV, LV)

» Transposons (Sleeping Beauty)

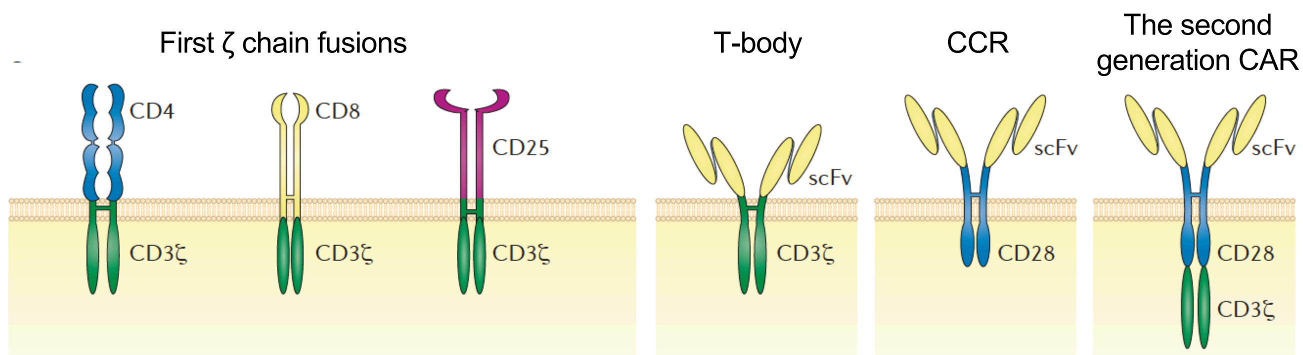




Sadelain and Mulligan, ICI, 1992

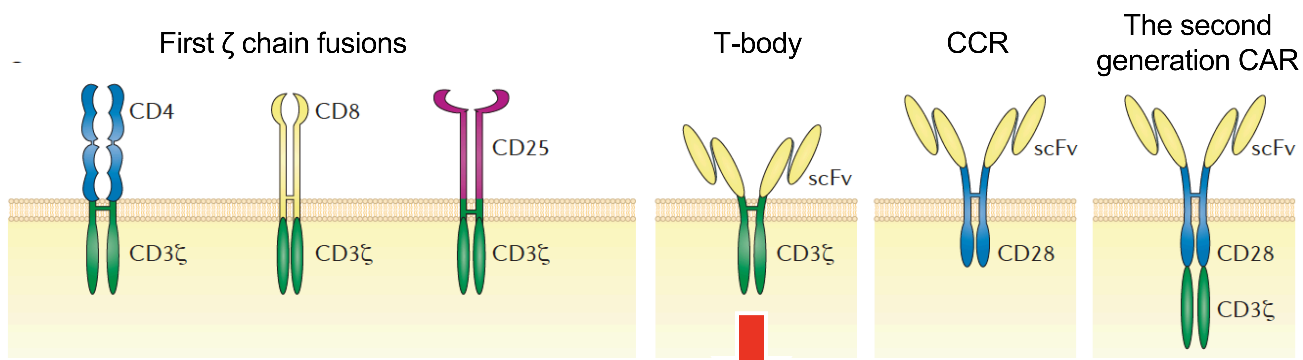
The beginning of CAR design

Van der Stegen, Nat Rev Drug Dev, 2015



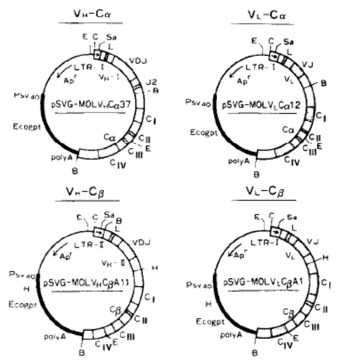
The beginning of CAR design

Van der Stegen, Nat Rev Drug Dev, 2015



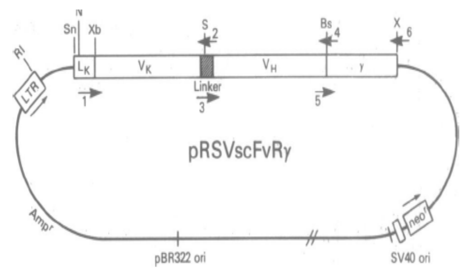
Zelig Eshhar

Thomas Brocker



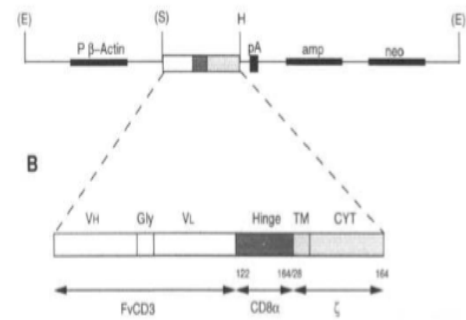
Construction of VLVH chimeric genes.

Y Kuwana et al, BBRC, 1987



Scheme of the chimeric scFvR expression vector.

Z Eshhar et al, Proc Natl Acad Sci, 1993



A schematic representation of the chimeric FvCD3ζ construct.

T Brocker et al, Eur J Immunol, 1993

The beginning of CAR design

Van der Stegen, Nat Rev Drug Dev, 2015

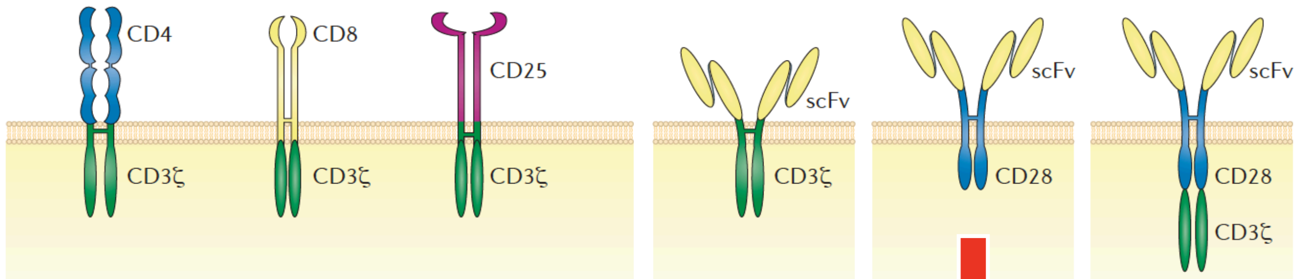
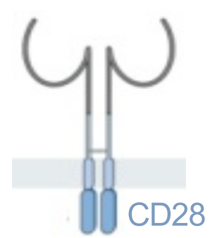


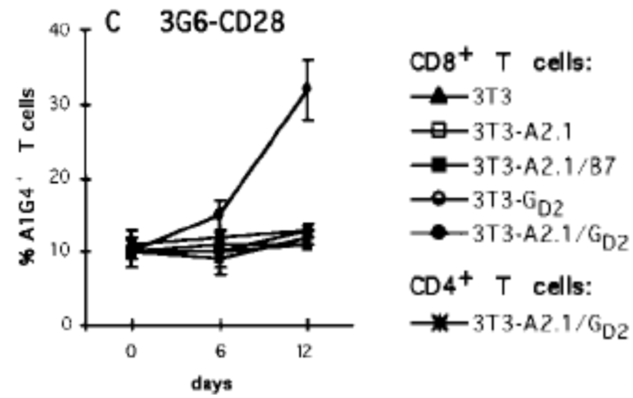
Fig 4. Cell-bound GD2 provide costimulatory signals to 3G6-CD28 CCR-transduced alloreactive human primary T lymphocytes.

A Krause et al, J Exp Med, 1998

3G6-CD28



G_{D2}-Specific CCR



The beginning of CAR design

Van der Stegen, Nat Rev Drug Dev, 2015

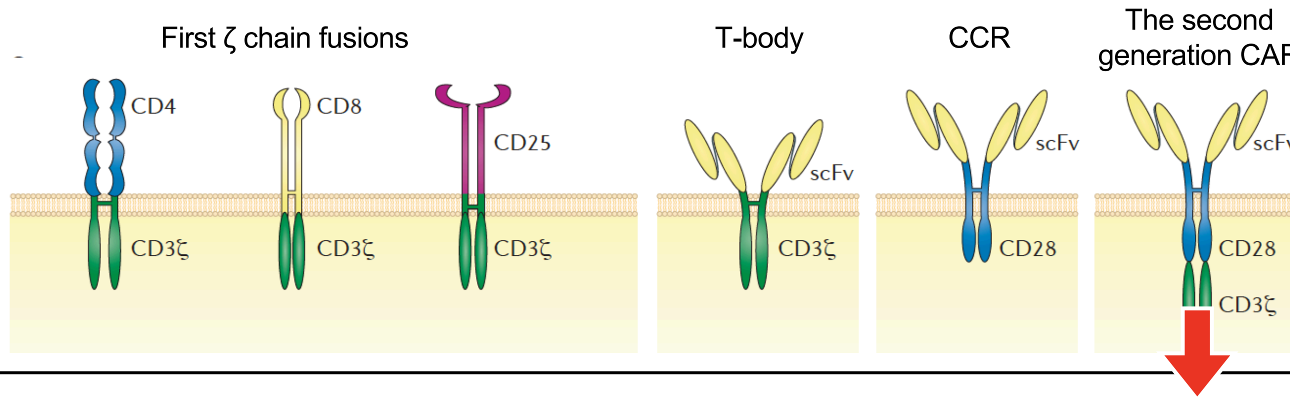
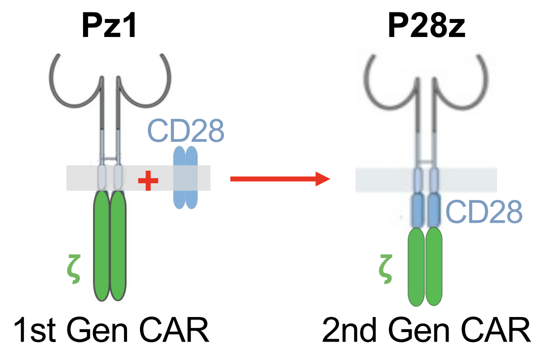
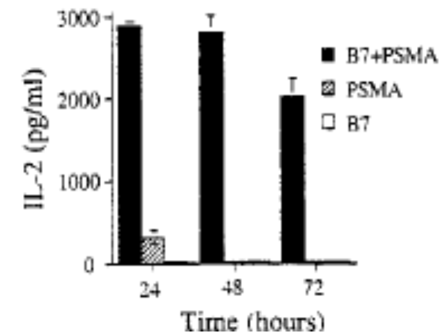


Fig 4. NIH3T3 fibroblasts coexpressing human PSMA and B7.1 (CD80) direct cytokine release by Pz-1-transduced T cells. (Pz-1;
a PSMA-specific CAR).

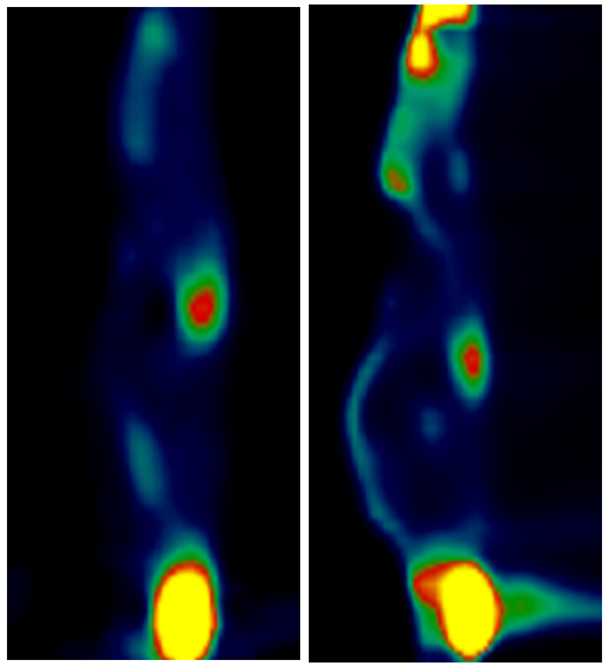
M Gong et al, Neoplasia, 1999



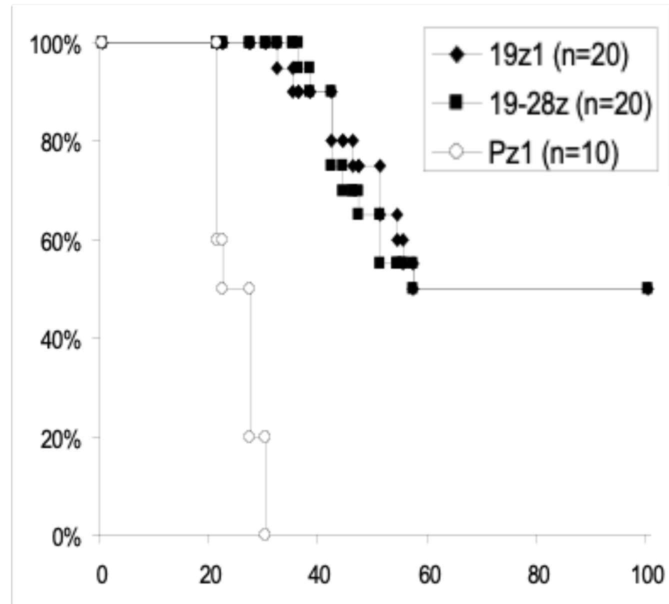


Identification of CD19 as an effective CAR target

CD19 CAR T cells eradicate systemic B cell malignancies in mice

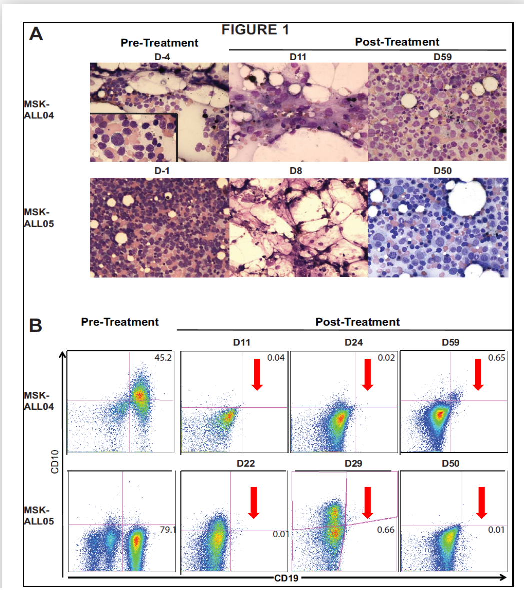


Tumor Free **Untreated 4 weeks**



Brentjens et al, Nat Med, 2003

Rapid and complete eradication of refractory leukemia by 19-28z CAR T cells



Brentjens, Davila, Rivière et al,
Science Transl Med, March 2013

Breakthrough of the year
Science, December 2013

Table 1. Reponses to CAR T-Cell Therapy.*

Disease	Response Rate	Comments	Reference
	percent		
Leukemia			
B-cell acute lymphoblastic leukemia (in adults)	83–93	High initial remission rates; unresolved issue is whether CAR T-cell therapy is definitive therapy or should be followed by allogeneic hematopoietic stem-cell therapy	Park et al.,[35] Davila et al.,[36] Turtle et al.[37]
B-cell acute lymphoblastic leukemia (in children)	68–90	Approximately 25% of patients reported to have a relapse with CD19-negative or CD19-low leukemia; CD22 CAR T cells may improve survival among some patients with CD19 relapses	Maude et al.,[34] Maude et al.,[38] Fry et al.,[39] Lee et al.[40]
Chronic lymphocytic leukemia	57–71	Relapse is rare in patients who have a complete response; ibrutinib appears to increase response rates	Porter et al.,[41] Turtle et al.[42]
Lymphoma			
Diffuse large B-cell lymphoma	64–86	Approximately 40–50% of patients reported to have a durable complete response	Turtle et al.,[43] Kochenderfer et al.,[44] Schuster et al.,[45] Neelapu et al.[46]
Follicular lymphoma	71	At a median follow-up of 28.6 mo, the response was maintained in 89% of patients who had a response	Schuster et al.[45]
Transformed follicular lymphoma	70–83	A total of 3 of 3 patients with transformed follicular lymphoma had a complete response	Turtle et al.,[43] Schuster et al.,[45] Neelapu et al.[46]
Refractory multiple myeloma	25–100	B-cell maturation antigen CAR T cells; stringent complete response in approximately 25% of patients	Ali et al.,[47] Fan et al.,[48] Berdeja et al.[49]
Solid tumors			
Glioblastoma	ND	{q4}In case report from phase 2 study, complete response on magnetic resonance imaging after intravenous and cerebrospinal fluid administration of CAR T cells; complete response lasted 7.5 mo	Brown et al.[50]
Pancreatic ductal adenocarcinoma	17	In one patient with liver metastasis, CAR T-cell treatment produced a complete metabolic response in the liver but was ineffective against the primary pancreatic tumor	Beatty et al.[51]

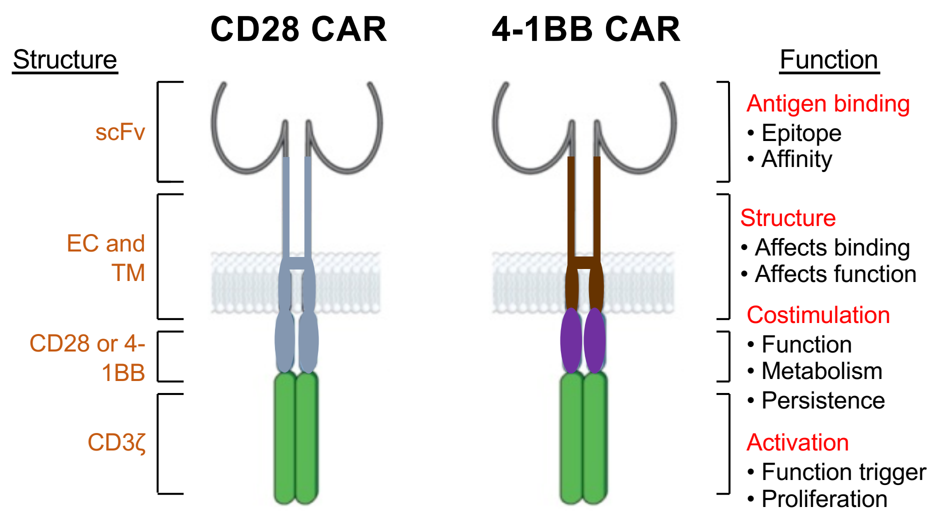
June and Sadelain,
N Engl J Med, 2018

Rapid and complete eradication of refractory leukemia by 19-28z CAR T cells

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

Breakthrough of the year
Science, December 2013

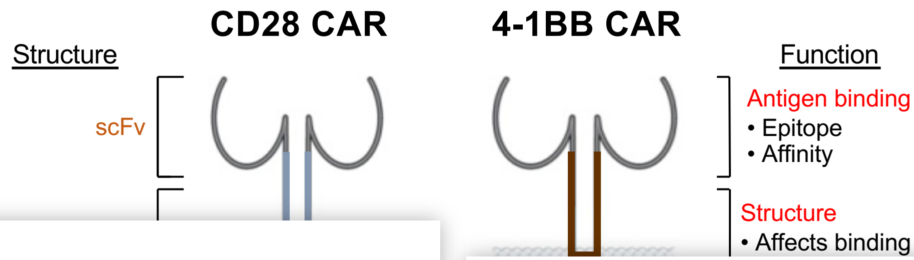
June and Sadelain,
N Engl J Med, 2018



FDA approved in 2017

Axicabtagene Ciloleucel (Yescarta)	Tisagenlecleucel (Kymriah)

Prototypic CD19 CARs



Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRζ /CD28 receptor

John Maher, Renier J. Brentjens, Gertrude Gunset, Isabelle Rivière, and Michel Sadelain*

Maher et al, Nat Biotechnol. 2002

Leukemia (2004) 18, 676-684
© 2004 Nature Publishing Group All rights reserved 0887-6924/04 $25.00
www.nature.com/leu

Chimeric receptors with 4-1BB signaling capacity provoke potent cytotoxicity against acute lymphoblastic leukemia

C Imai[1], K Mihara[1], M Andreansky[1], IC Nicholson[2], C-H Pui[1,3,4], TL Geiger[3] and D Campana[1,3,4]

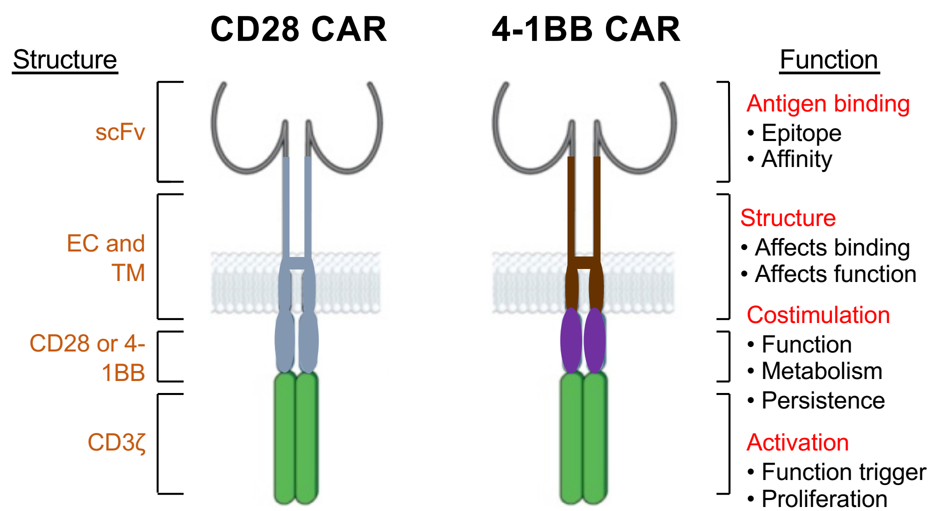
Imai et al, Leukemia, 2004

FDA approved in 2017

Axicabtagene Ciloleucel (Yescarta)	Tisagenlecleucel (Kymriah)

Prototypic CD19 CARs

Dario Campana



FDA approved in 2017

Axicabtagene Ciloleucel (Yescarta)	Tisagenlecleucel (Kymriah)

Prototypic CD19 CARs

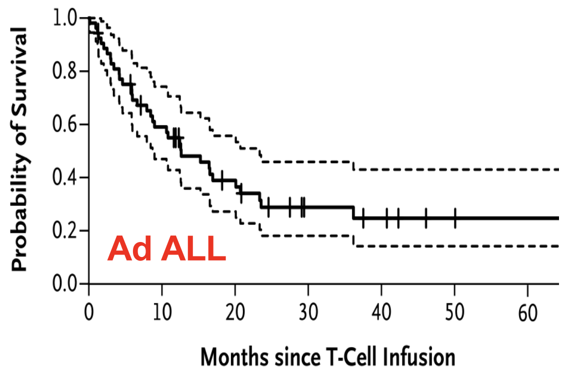
CD19 CAR therapy in R/R ALL and NHL – overall survival

Jae Park

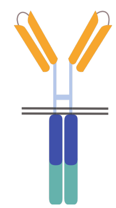
Marco Davila

Renier Brentjens

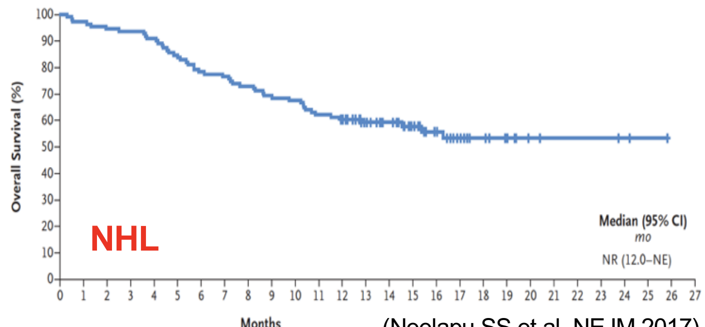
Overall survival



CD28 CAR

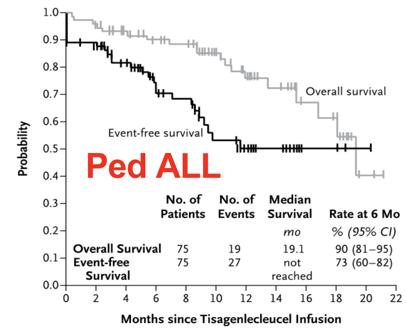


(Park JH et al, NEJM 2018)



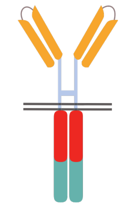
(Neelapu SS et al, NEJM 2017)

Event-free and Overall Survival

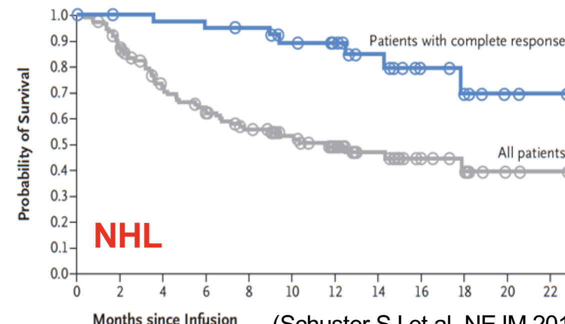


	No. of Patients	No. of Events	Median Survival	Rate at 6 Mo
			mo	*% (95% CI)*
Overall Survival	75	19	19.1	90 (81–95)
Event-free Survival	75	27	not reached	73 (60–82)

4-1BB CAR



(Maude SL et al, NEJM 2018)



(Schuster SJ et al, NEJM 2018)

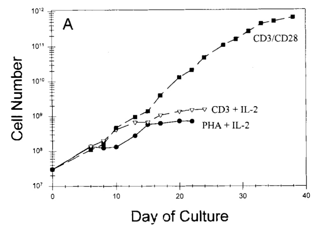
Tribute to the "Manufacturers"

Effects of CD28 Costimulation on Long-Term Proliferation of CD4+ T Cells in the Absence of Exogenous Feeder Cells[1]

Bruce L. Levine,[2]* Wendy B. Bernstein,[†] Mark Connors,[‡] Nancy Craighead,[§] Tullia Lindsten,[¶] Craig B. Thompson,[¶] and Carl H. June*

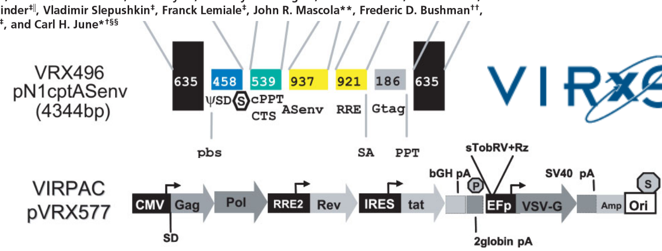
J Immunol 1997; 159:5921-5930

Carl June

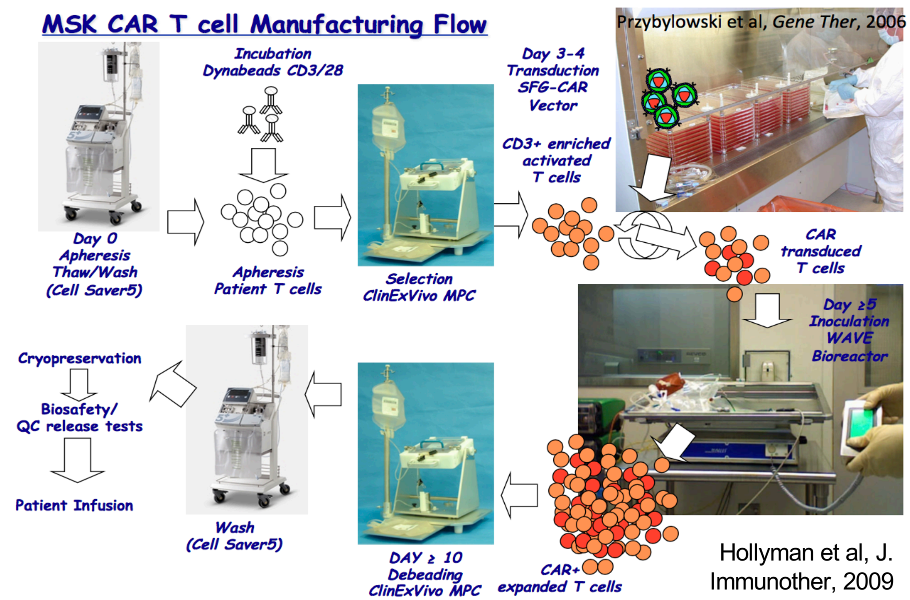


Gene transfer in humans using a conditionally replicating lentiviral vector

Bruce L. Levine*[†], Laurent M. Humeau[‡], Jean Boyer[†], Rob-Roy MacGregor[§], Tessio Rebello[‡], Xiaobin Lu[‡¶], Gwendolyn K. Binder[‡‖], Vladimir Slepushkin[‡], Franck Lemiale[‡], John R. Mascola**, Frederic D. Bushman[††], Boro Dropulic[‡,‡‡], and Carl H. June*[†§§]



VIRXSYS



Hollyman et al, J. Immunother, 2009

Levine, Penn

Dudley, NCI

Rivière, MSK

Building on the success of CD19 CAR therapy

- 500 trials worldwide (clinicaltrials.gov)
- >20,000 infused patients
- 145 biotech/pharma CAR programs

The evolution of cell therapy trials for cancer since 1993

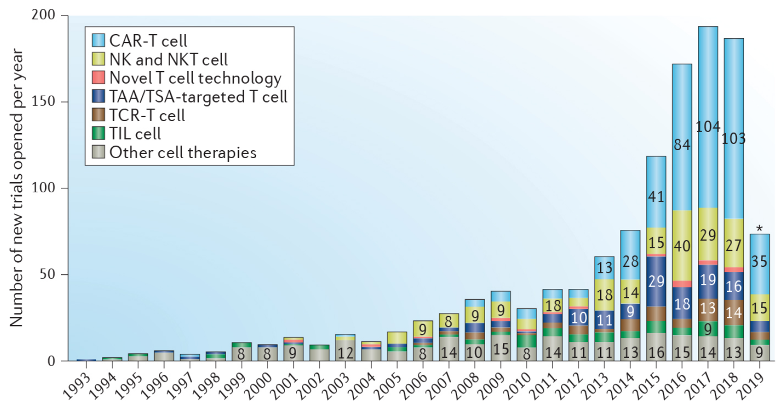
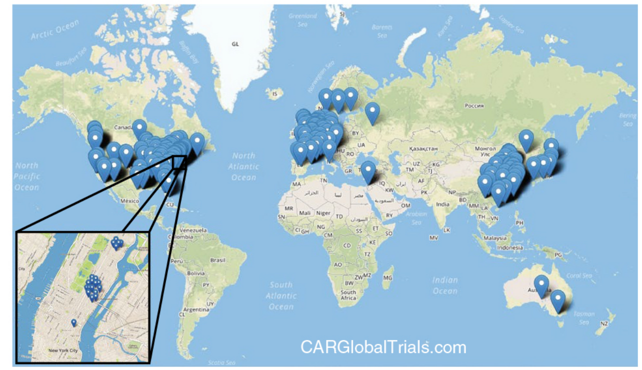


Fig. 2 | **The evolution of cell therapy trials for cancer since 1993**. Both currently active and inactive trials are included for this analysis, which is based on data extracted from ClinicalTrials.gov in March 2019, and so the data for this year is incomplete, indicated by an asterisk.

Jia Xin Yu et al. Nat Rev Drug Discov. 2019

Interventional CAR clinical trials by country (< 2019)

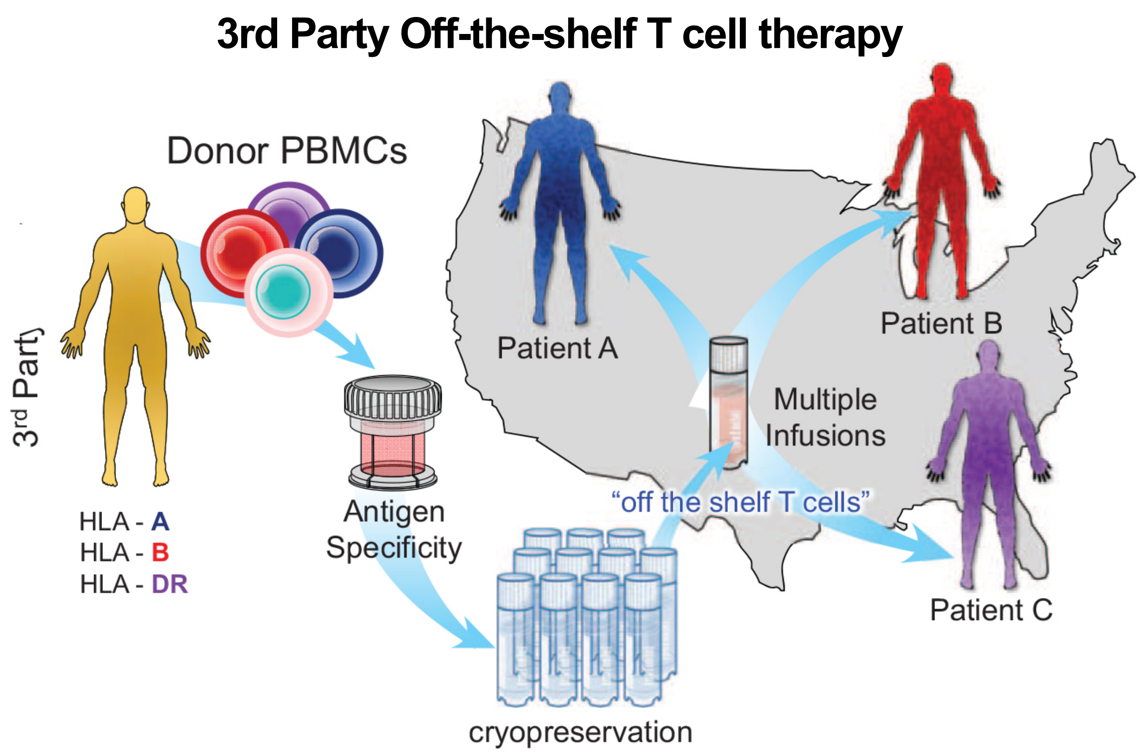


The therapeutic landscape for cells engineered with chimeric antigen receptors

Matthew MacKay[1,2,3,4], Ebrahim Afshinnekoo[1,2,3], Jonathan Rub[1,3], Ciaran Hassan[5], Mihir Khunte[5], Nithyashri Baskaran[5], Bryan Owens[5], Lauren Liu[5], Gail J. Roboz[6], Monica L. Guzman[6], Ari M. Melnick[6], Shixiu Wu[7,8]* and Christopher E. Mason[1,2,3,9]*

Matthew MacKay et al. Nat Biotechnol. 2020

Generation and distribution of off-the-shelf VSTs



Baugh et al. Curr Opin Infect Dis. 2018

Future targets for VST therapy

Potential VST targets	Targets/antigen sources	Clinical studies	Reference
HSV	A02-restricted T cells expanded	None	Ma et al[82]
VZV	VZV vaccine	Used in prophylaxis post-BMT	Ma et al[80]
HPV	E6, E7 peptide libraries	Prior use against HPV-associated cancers	McCormack et al[83]
Respiratory viruses			
Influenza A	Nucleocapsid protein, Matrix protein 1 peptide libraries	None	Vasileiou et al[87]
Respiratory syncytial virus	Nucleoprotein and glycoprotein F0 protein peptide libraries	None	Vasileiou et al[87]
Human metapneumovirus	Nucleocapsid, fusion protein peptide libraries	None	Tzannou et al[32]
Human parainfluenza virus	HPIV3 matrix protein	None published/NCT03180216	McLaughlin et al[81]
Enteric viruses			
Norovirus	VP1, NS6 peptide libraries	None	Hanajiri et al
Mycobacteria spp.	Ag85B, PPE68, ESAT6, CFP10 peptide libraries (*Mycobacteria tuberculosis*)	None	Patel et al[85]
Mucormycosis	*Rhizopus oryzae* extract	None	Castillo et al[86]

BMT, bone marrow transplantation; HPV, human papillomavirus; VZV, varicella-zoster virus.

Keller et al. Blood. 2020

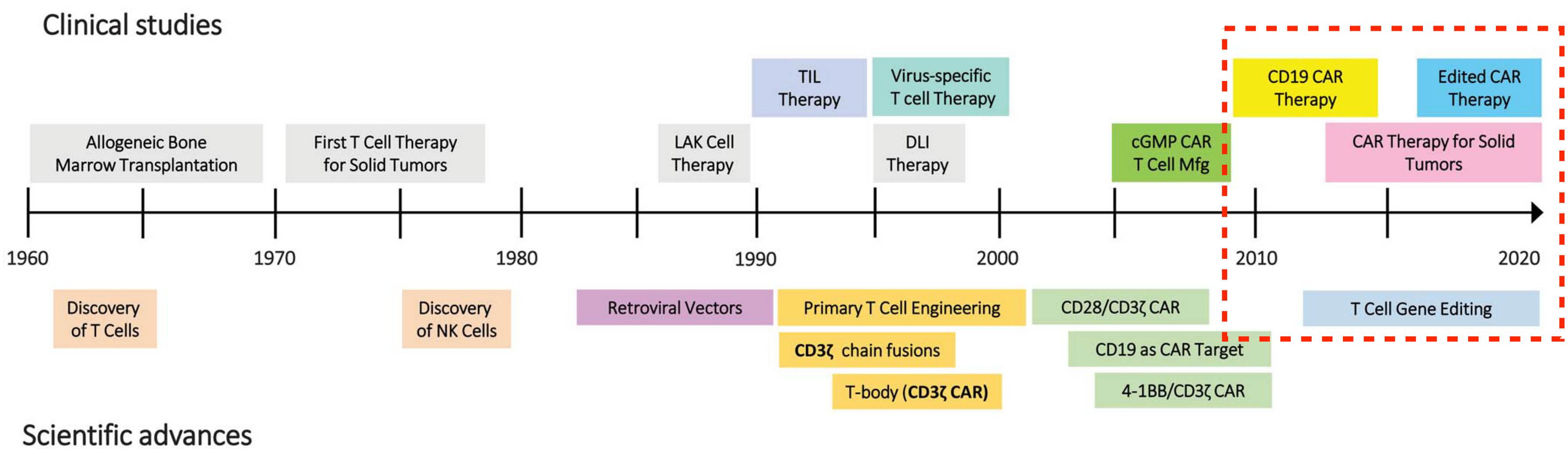
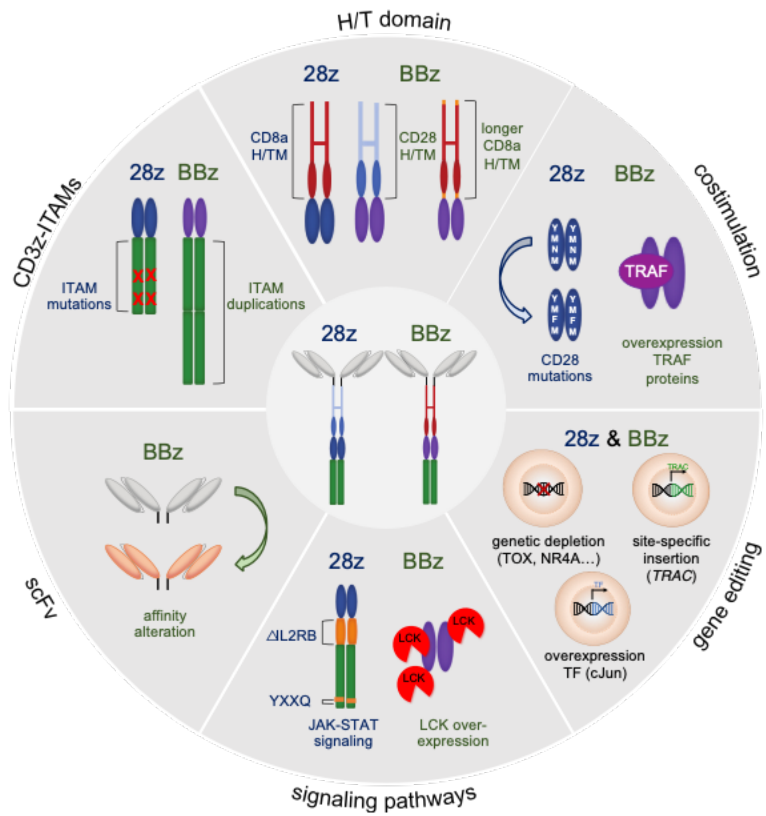


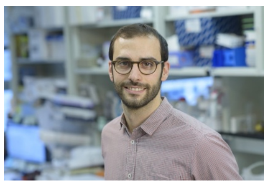
Fig. 3. Historical overview of CAR-T cell therapy. CAR: chimeric antigen receptor; cGMP: current good manufacturing practices; DLI: donor leukocyte infusion; LAK: lymphokine-activated killer; Mfg: Manufacturing; NK: natural killer; TIL: tumor-infiltrating lymphocytes.

Dunbar et al., Science 359, eaan4672 (2018) 12 January 2018



Feucht et al. IOTech, 2020

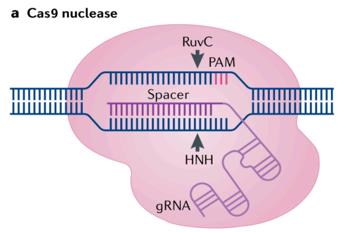
CRISPR/Cas9-targeted integration into the TRAC locus

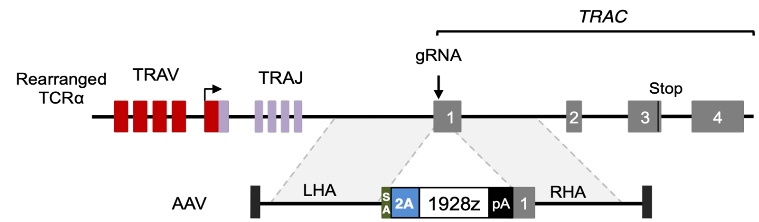


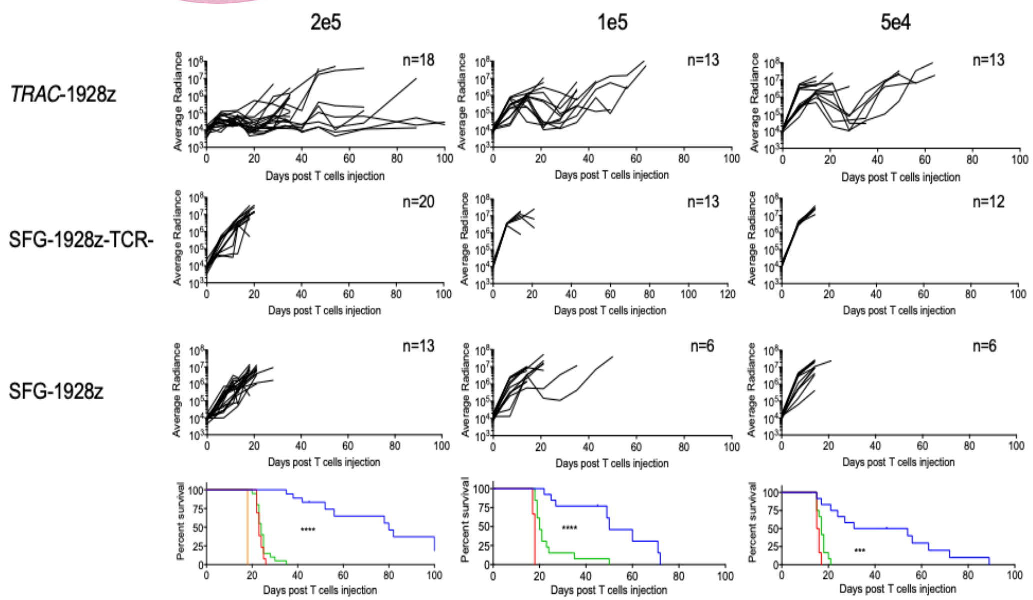
Justin Eyquem, PhD

J. Mansilla-Soto, PhD

Eyquem, Mansilla-Soto et al, Nature, 2017

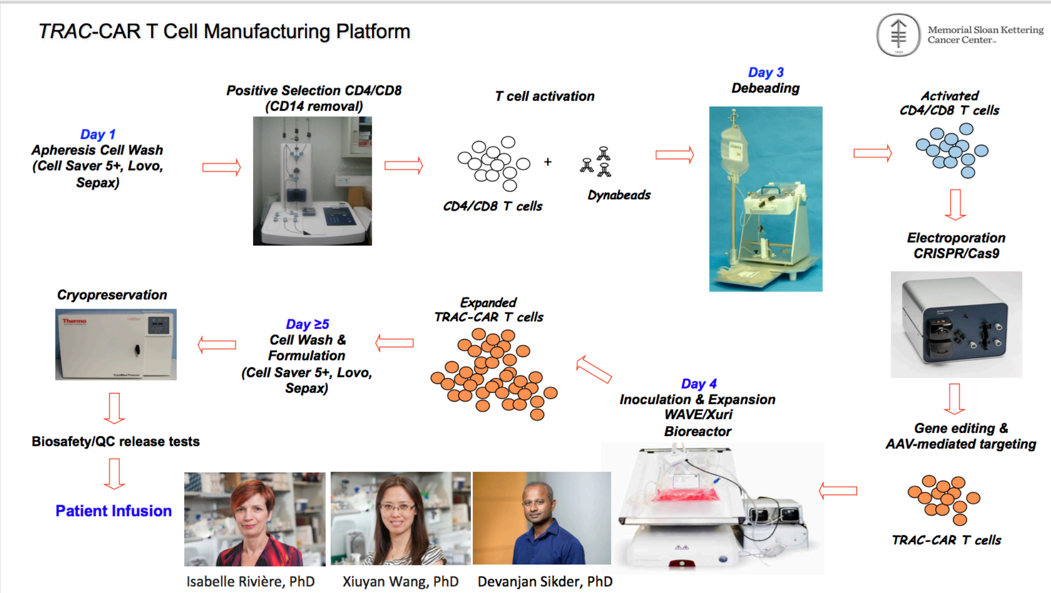






Genome-edited CAR T cells

TRAC-CAR: CAR Knock-in



Triple Knock-out + LV-TCR

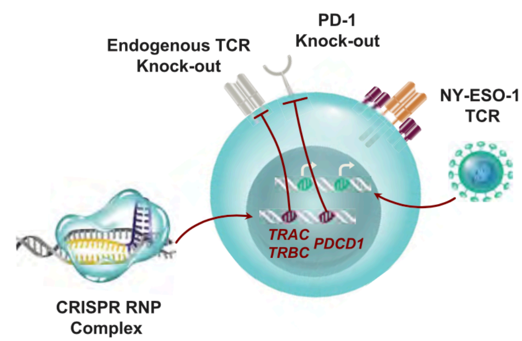
Science

CRISPR-engineered T cells in patients with refractory cancer

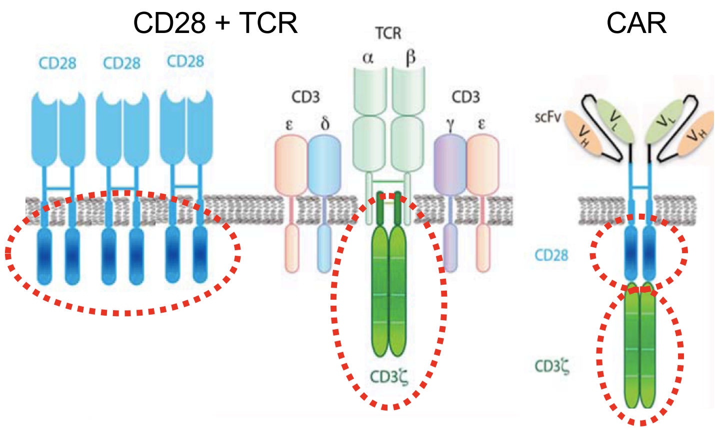
Edward A. Stadtmauer, Joseph A. Fraietta, Megan M. Davis, Adam D. Cohen, Kristy L. Weber, Eric Lancaster, Patricia A. Mangan, Irina Kulikovskaya, Minnal Gupta, Fang Chen, Lifeng Tian, Vanessa E. Gonzalez, Jun Xu, In-young Jung, J. Joseph Melenhorst, Gabriela Plesa, Joanne Shea, Tina Matlawski, Amanda Cervini, Avery L. Gaymon, Stephanie Desjardins, Anne Lamontagne, January Salas-Mckee, Andrew Fesnak, Donald L. Siegel, Bruce L. Levine, Julie K. Jadlowsky, Regina M. Young, Anne Chew, Wei-Ting Hwang, Elizabeth O. Hexner, Beatriz M. Carreno, Christopher L. Nobles, Frederic D. Bushman, Kevin R. Parker, Yanyan Qi, Ansuman T. Satpathy, Howard Y. Chang, Yangbing Zhao, Simon F. Lacey and Carl H. June

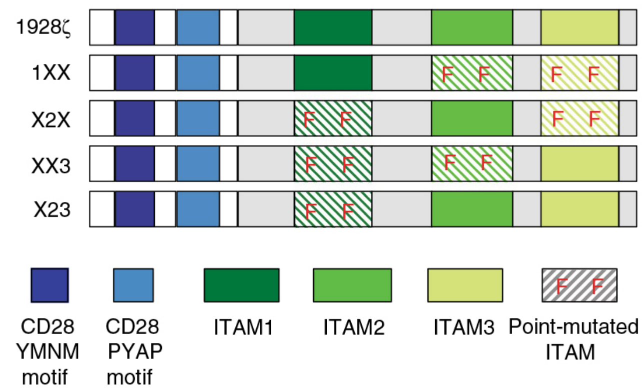
Science **367** (6481), eaba7365.
DOI: 10.1126/science.aba7365originally published online February 6, 2020

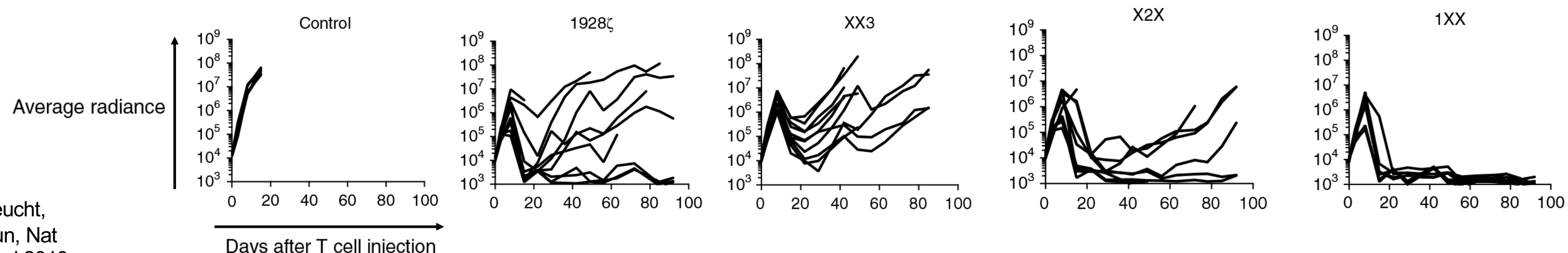
CRISPR-Cas9 NYCE T cell



ITAM-based calibration of activation strength in CD28/CD3ζ CARs



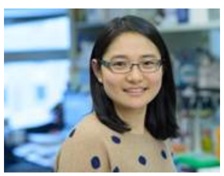




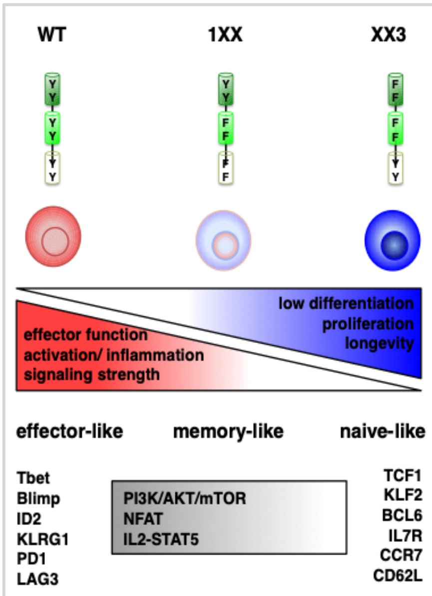
Feucht, Sun, Nat Med 2019

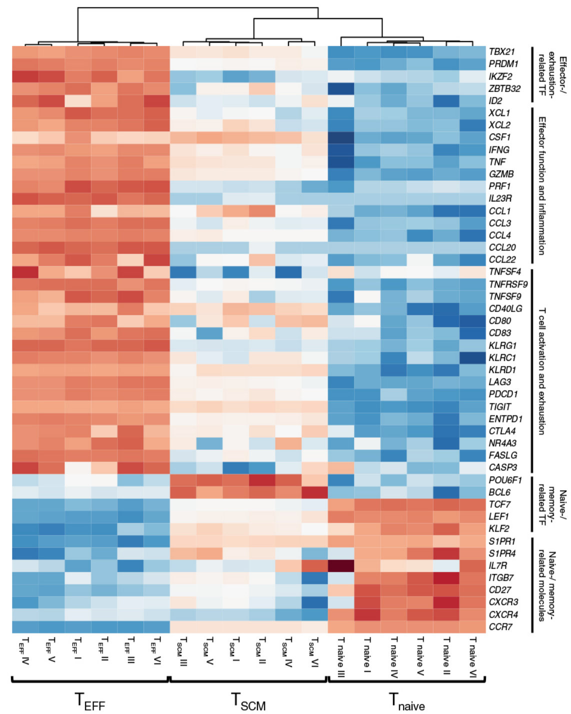
CAR ITAM-calibration directs T cell fate

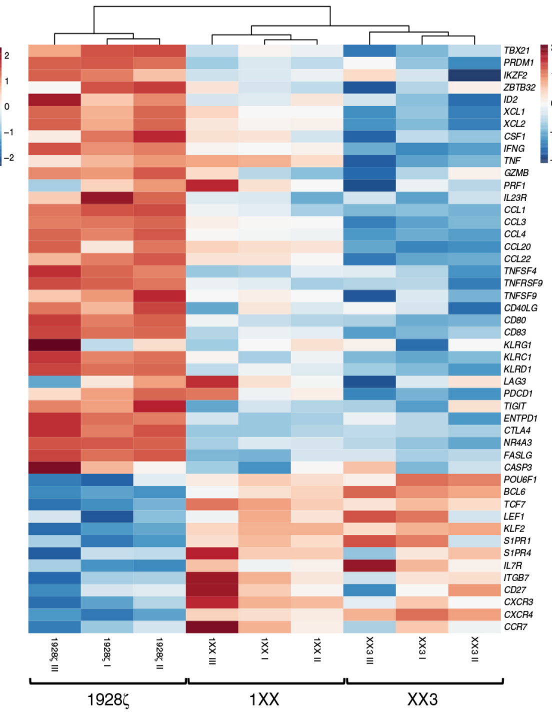
Judith Feucht, MD



Sun Jie, PhD

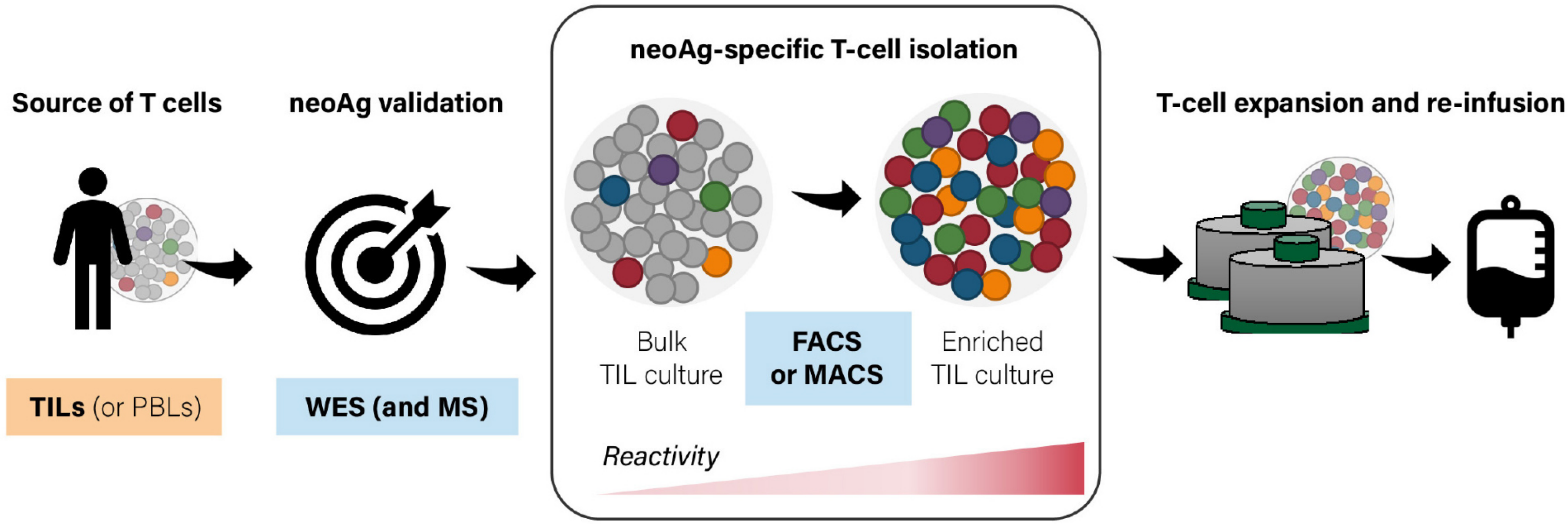






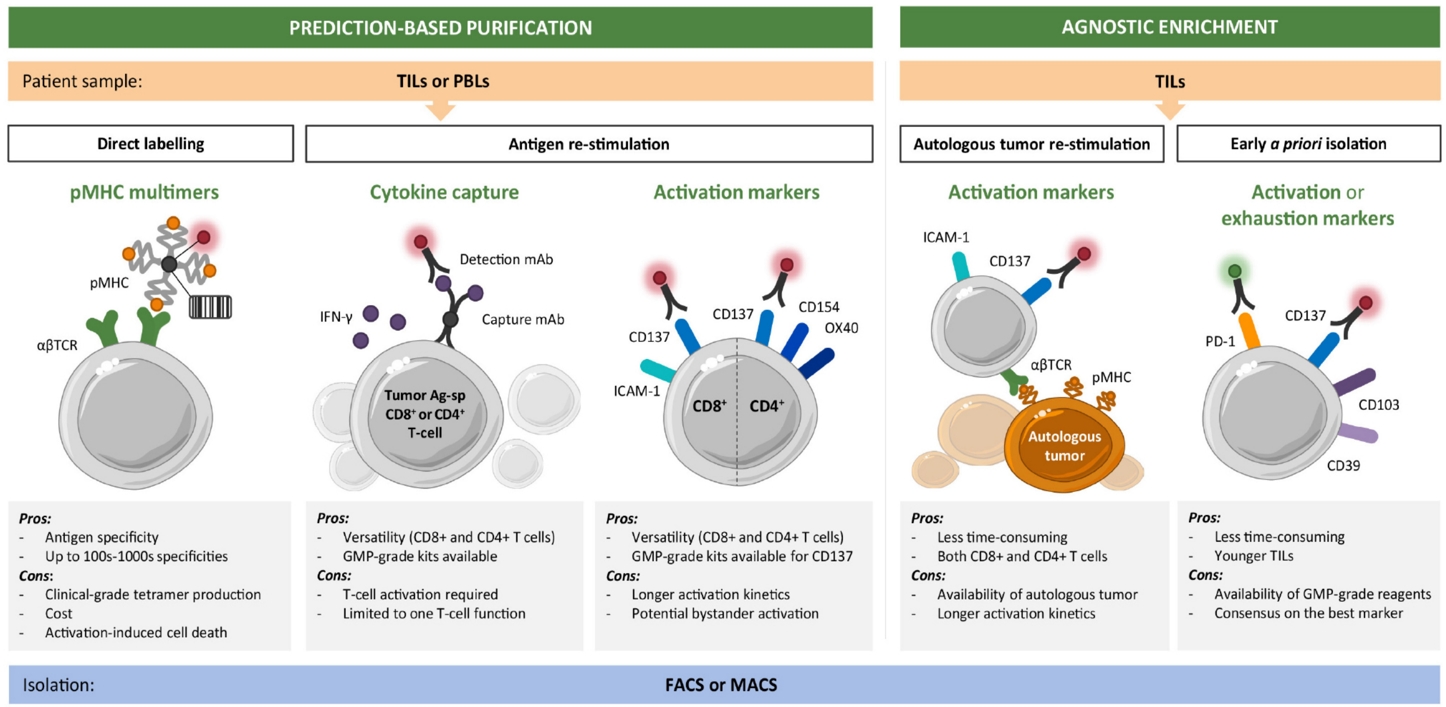
Feucht, Sun, Nat Med 2019

General workflow for personalized enrichment of antigen-specific T-cells from bulk tumor-infiltrating lymphocyte (TIL) [or peripheral blood lymphocyte (PBL)] cultures



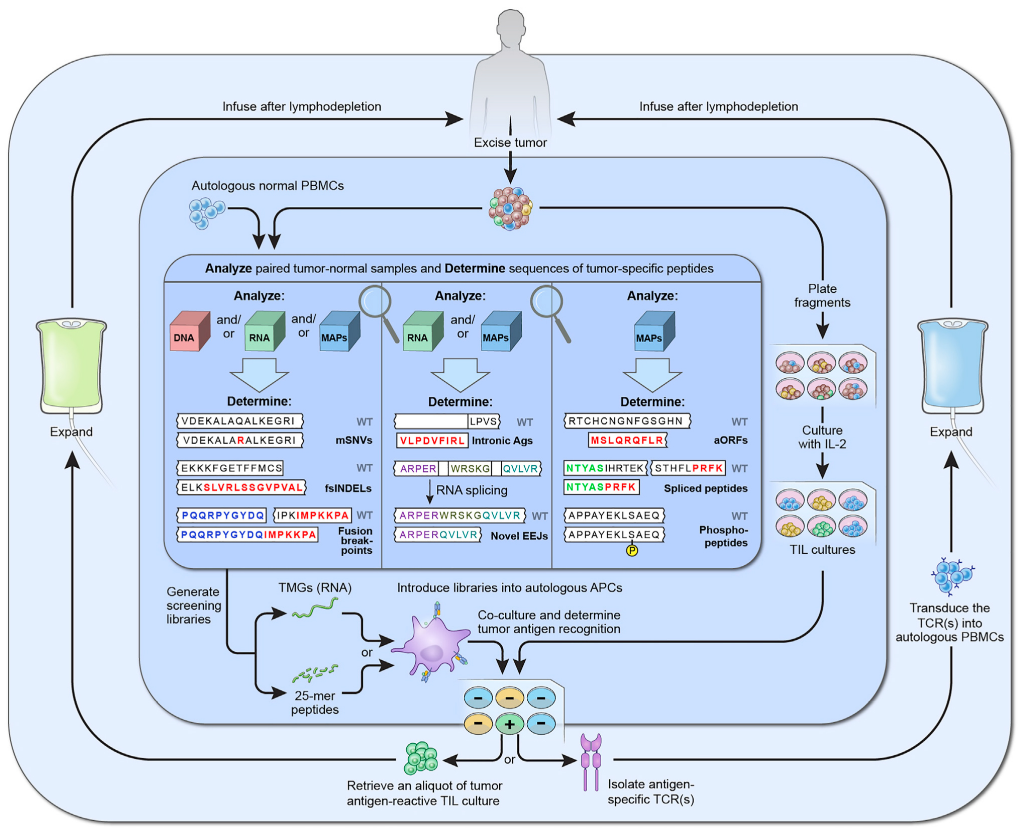
Bianchi et al. Front Immunol. 2020

Toolset for personalized enrichment of T-cell infusion products



Bianchi et al. Front Immunol. 2020

A Suggested Approach for Comprehensive Identification of Tumor Neoantigen-Reactive T Cells and Their Use for Personalized Immunotherapy



Leko et al. Cancer Cell. 2020

Table 2. Characteristics of Different Tumor Antigen Types

Tumor Antigen Class	Tumor Antigen Type	Role in Oncogenesis	Expression in Normal Tissues	Structural Similarity to Normal Proteins	Shared Among Patients?	Clinical Experience with Targeting?
TAAs	CGAs	uncertain	limited (germ cells, placenta)	high	yes	yes
	HERVs	uncertain	variable (type dependent)	low	yes	limited
	TDAs	uncertain	yes	high	yes	yes
	overexpressed antigens	uncertain	yes	high	yes	yes
TSAs	mSNVs	rarely drivers	no	moderate	rarely	yes
	INDELs	rarely drivers	no	low	rarely	limited
	gene fusions	rarely drivers	no	low	rarely	limited
	viral oncoproteins	drivers	no	low	yes	yes
UCAs	splice variants alternative ORFs post-translational modifications	unexplored	unexplored; some are expressed in normal cells	variable	unexplored	no

TSAs, tumor-specific antigens; UCAs, unconventional antigens.

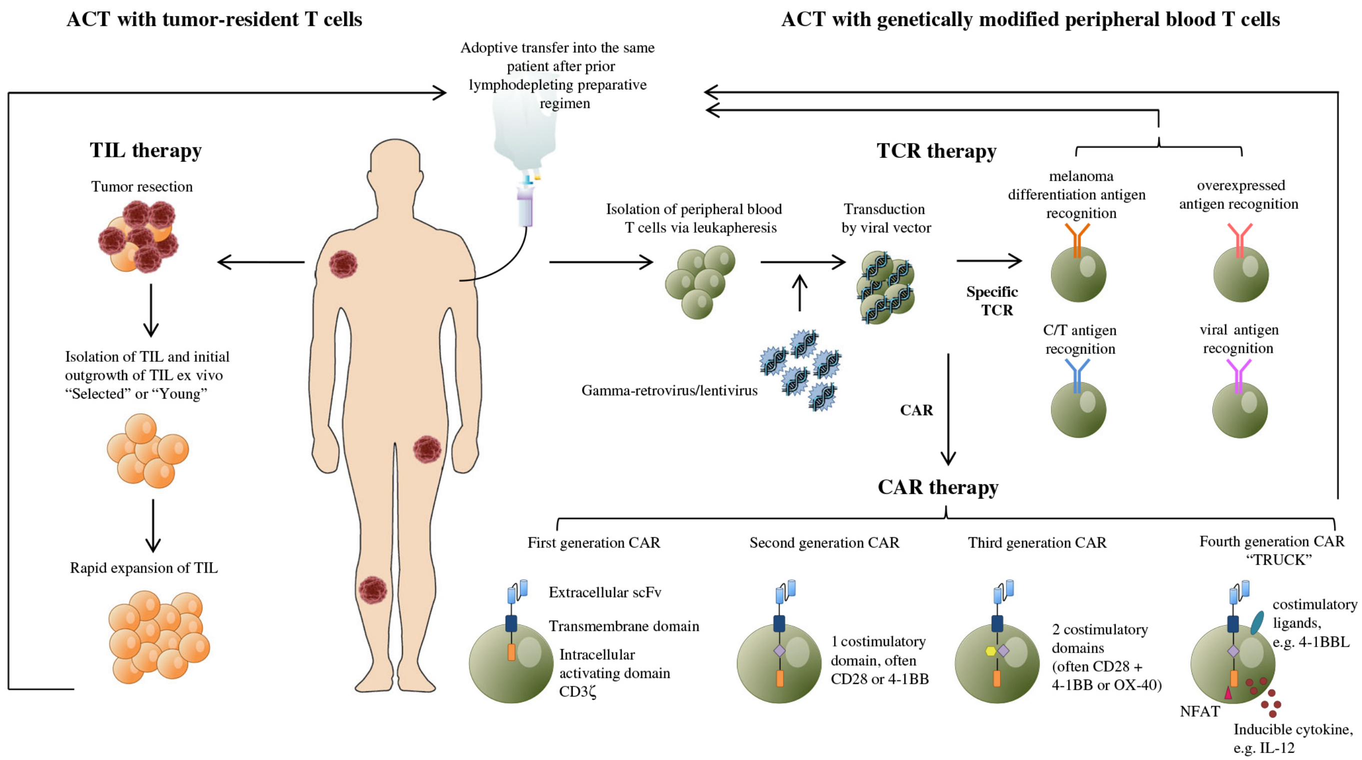
Leko et al. Cancer Cell. 2020

Table 3. Neoantigens Recognized by TILs from Patients with Select Epithelial Cancers

Cancer Type	# Of Patients Screened	# Of Patients with Neoantigen Reactivity (%)	# Of Mutations Screened	# Of Immunogenic Mutations (%)
All gastrointestinal	75	62 (83)	7,654	124 (1.6)
Colorectal	51	45 (88)	5,833	94 (1.6)
Biliary	12	8 (67)	866	12 (1.4)
Pancreatic	7	5 (71)	352	8 (2.3)
Gastric	3	2 (67)	378	6 (1.6)
Esophageal	2	2 (100)	225	4 (1.8)
Ovarian	7	5 (71)	1714	8 (0.5)

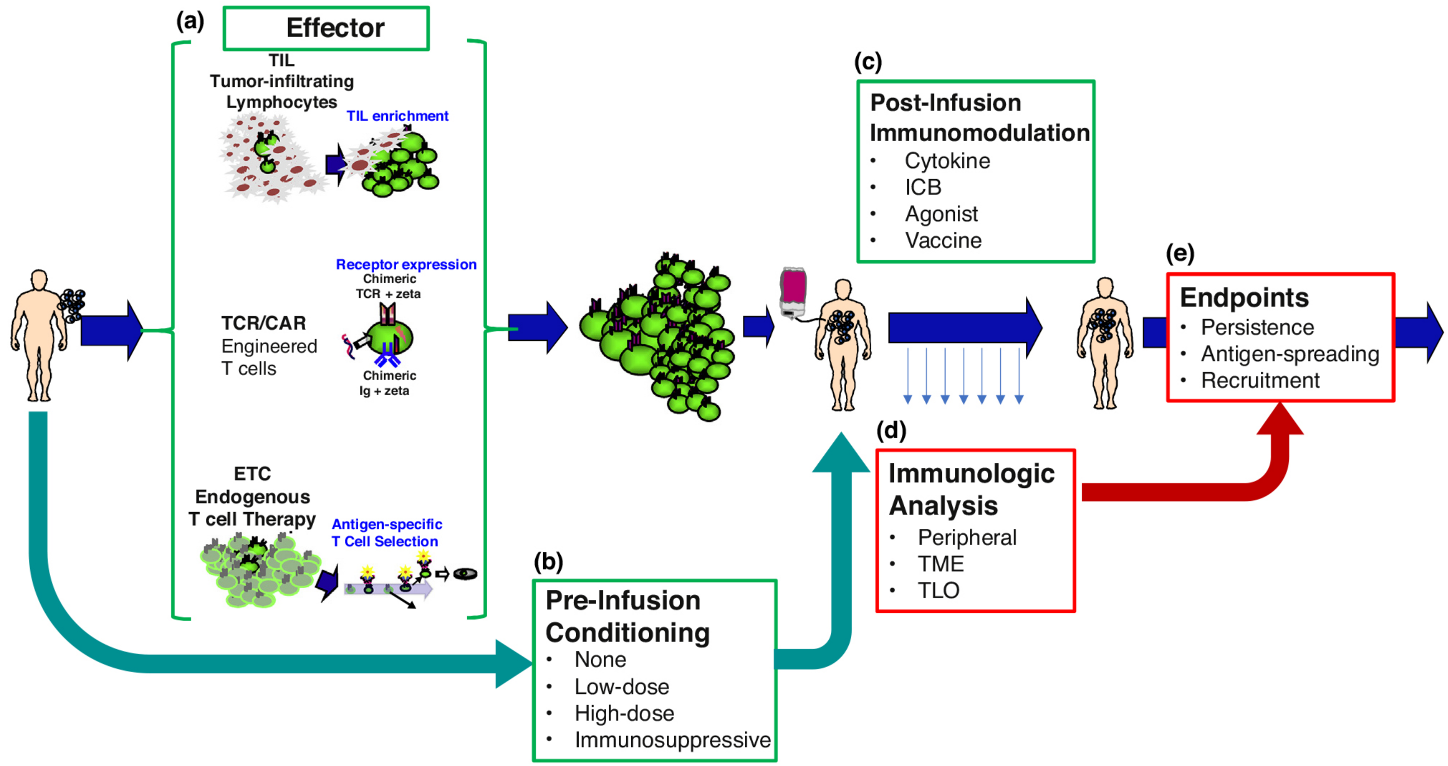
Leko et al. Cancer Cell. 2020

Schematic overview of the processes for adoptive cell therapy (ACT) of tumor-infiltrating lymphocytes (TIL), ACT with T cell receptor (TCR) gene therapy and ACT with chimeric antigen receptor (CAR)-modified T cells



Rohaan et al. Virchows Arch. 2019

Adoptive cell therapy: points to consider

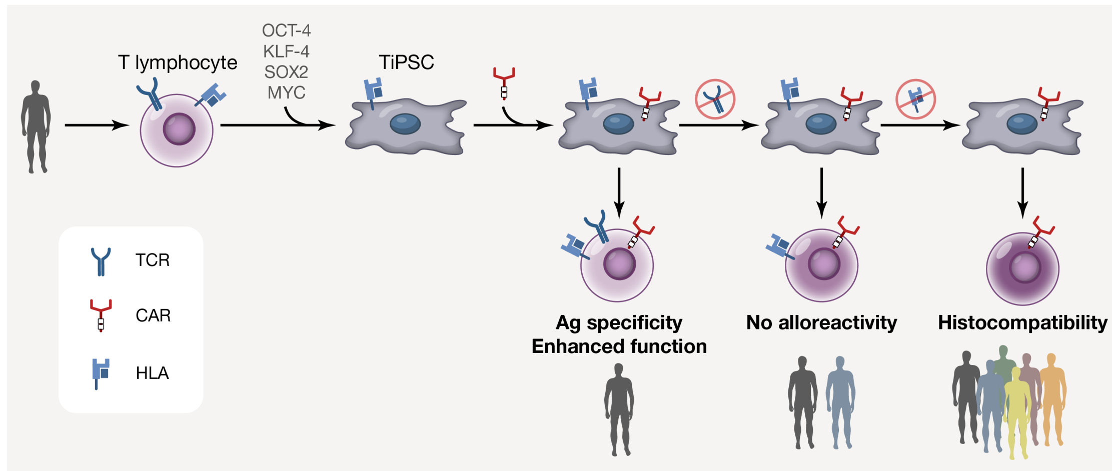


Yee et al. Curr Opin Immunol. 2018

Current Opinion in Immunology

CAR T cell sources

AutologousT cells	With TCR	» Bulk PBMCs » T cell subsets
AllogeneicT cells	With TCR	» DLI » VSTs » γδ T cells » iNKT cells
	Without TCR	» αβ-TCR–/– DLI » (NK cells)
In vitro generatedT cells	With/Without	» CB » ESC » TiPS



Themeli, Riviere & Sadelain,
Cell Stem Cells, 2015

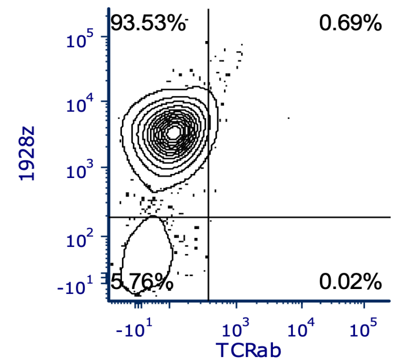
TiPS for T cell immunotherapy: a long-term aspiration for synthetic immunity

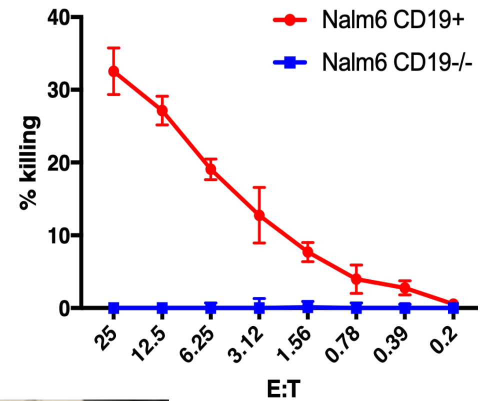
Antigen specificity
Targeted gene disruption
Targeted integration
Checkpoint modulation
Histocompatibility

Genotype selection

T_{SCM} T_N phenotype
CD8 or CD4 T cells
$\gamma\delta$ T cells
NKT cells
Tregs

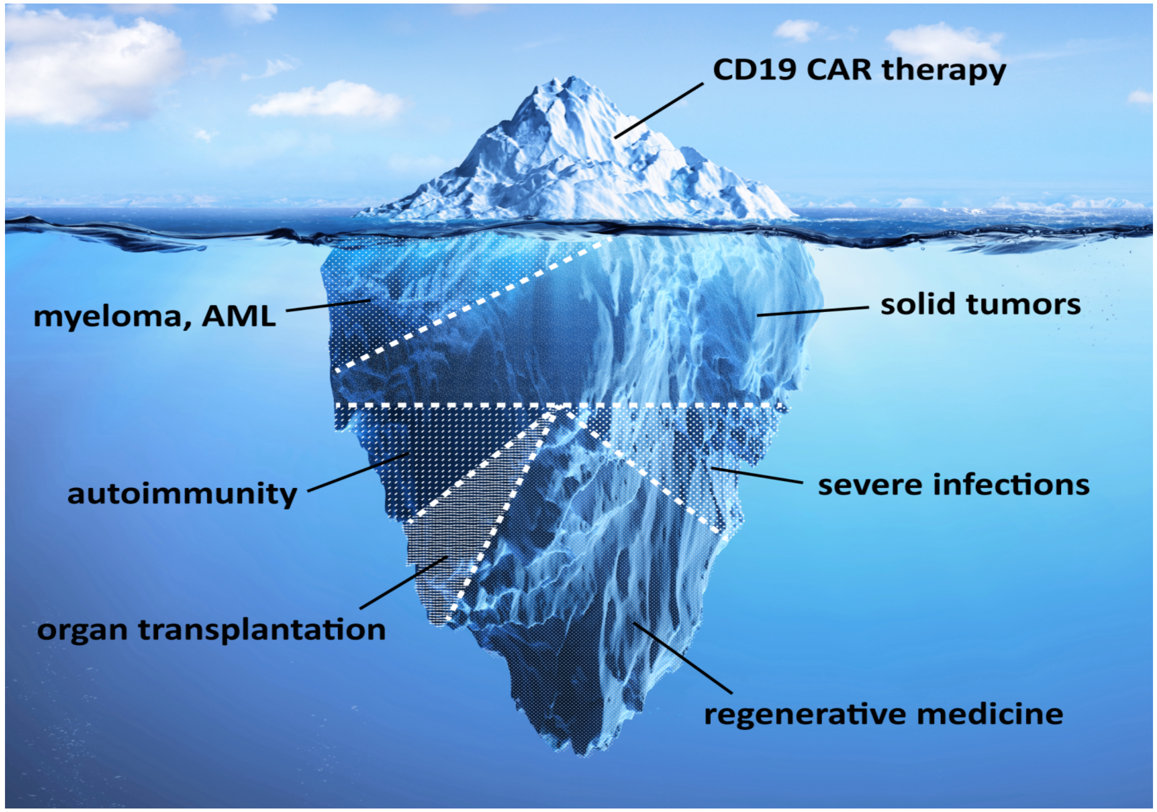
100% purity



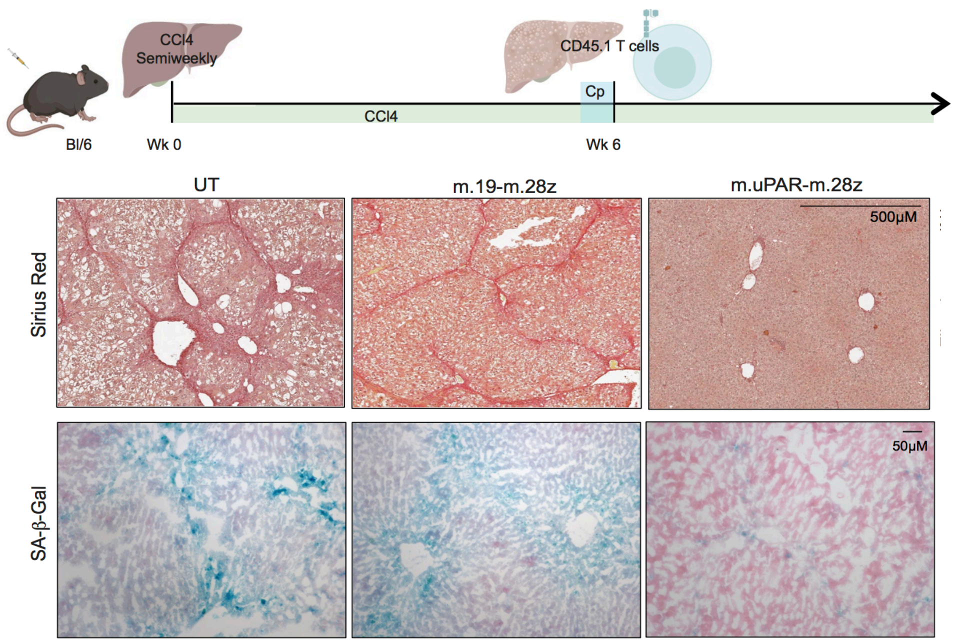


Sjoukje van der Stegen


CD19 CAR therapy


CD19 CAR therapy
myeloma, AML
solid tumors
autoimmunity
severe infections
organ transplantation
regenerative medicine

CAR T cells: beyond cancer

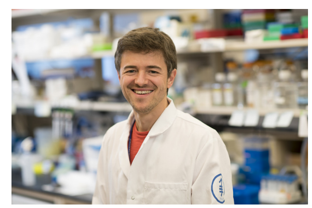


Senescence-associated pathologies

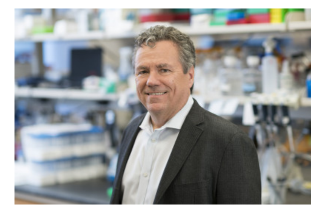
Senolytic CAR T cells
e.g., liver fibrosis, NASH

Judith C. Feucht

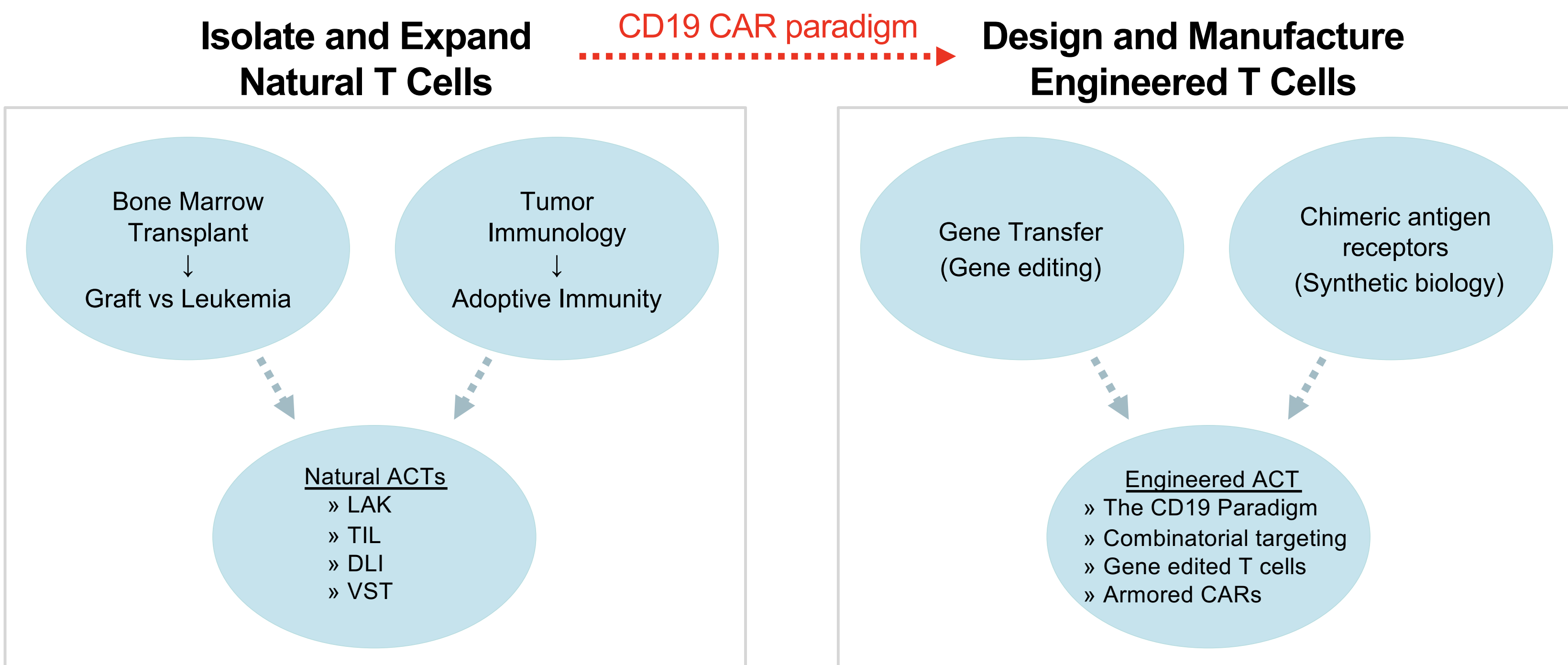
Corina A. Vegas Lowe Lab



Josef Leibold Lowe Lab



Scott Lowe



Sadelain. CARs: driving immunology toward synthetic biology, Curr Opin Immunol, 2016

NASDC

NCI Awardee Skills Development Consortium

Adoptive T cell Therapies

Questions