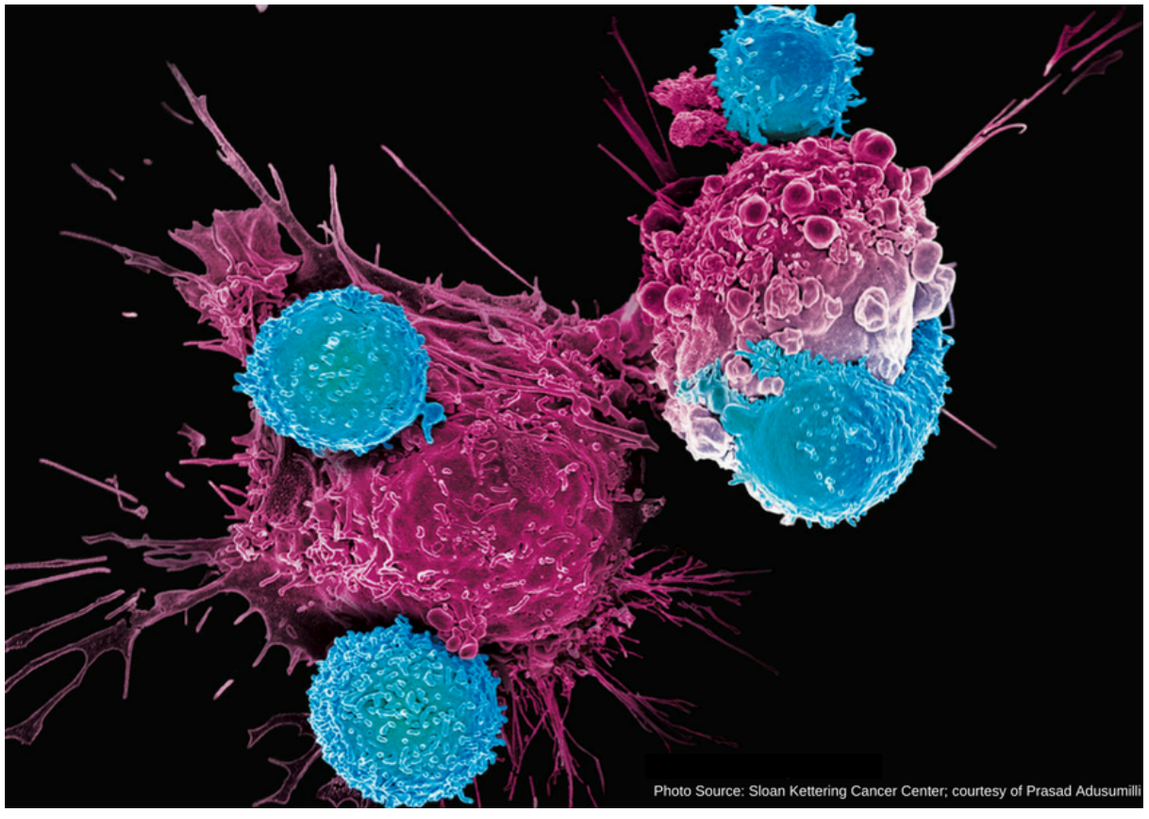
CAR T cell Therapy



Gabrielle Lovett

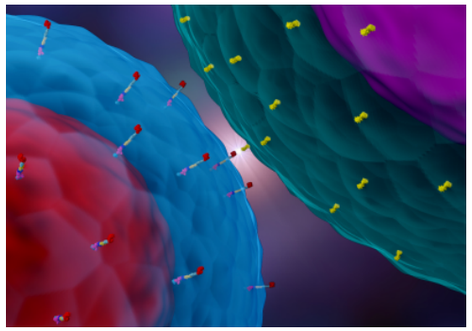
MacMillan Group Meeting

March 25, 2020

Outline

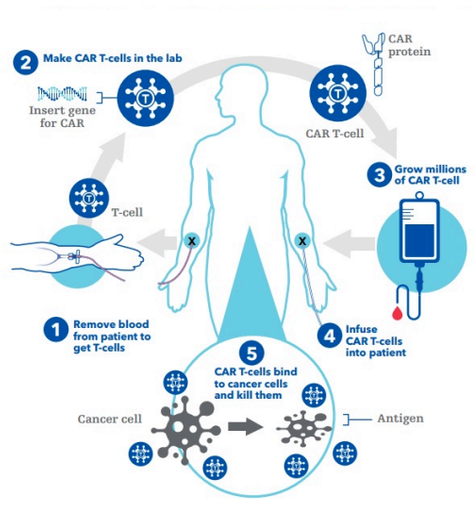
Introduction to CAR T cell Therapy

- current cancer treatment options
- introduction to immunotherapy
- definition of a CAR T cell
- building a CAR



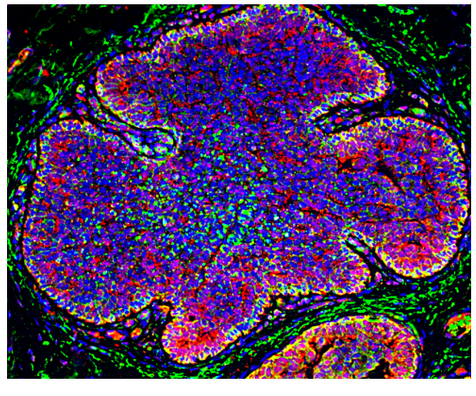
CARs in the Clinic

- how CARs are prepared and administered
- preclinical studies
- clinical trials leading to FDA approvals

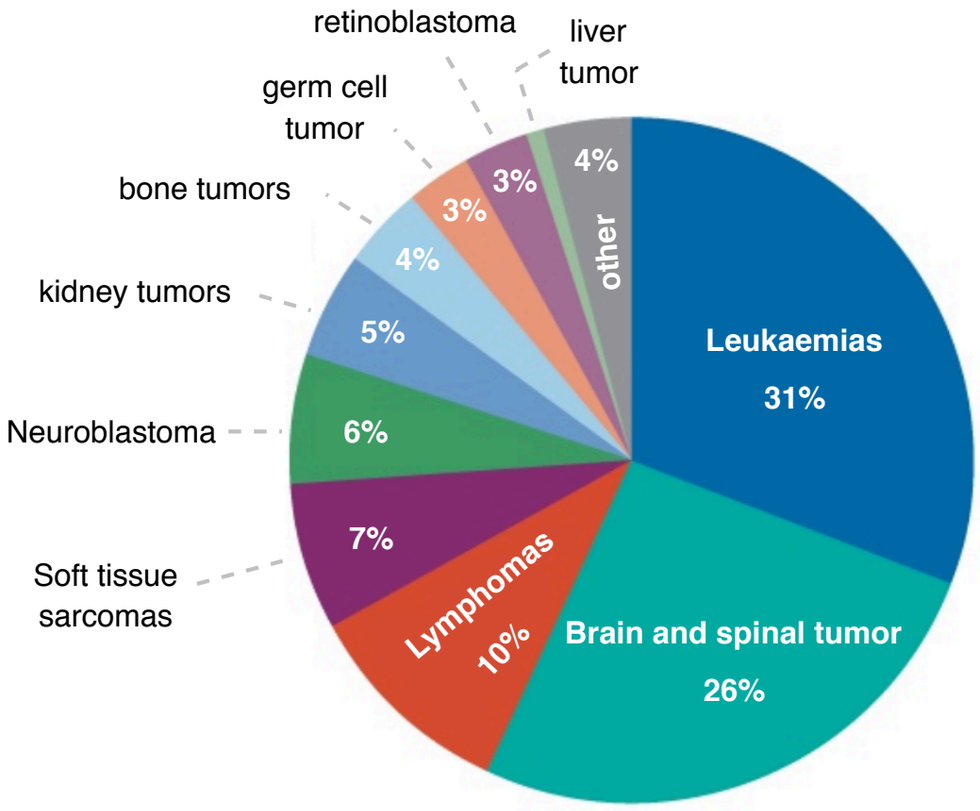


Current Limitations and Moving Forward

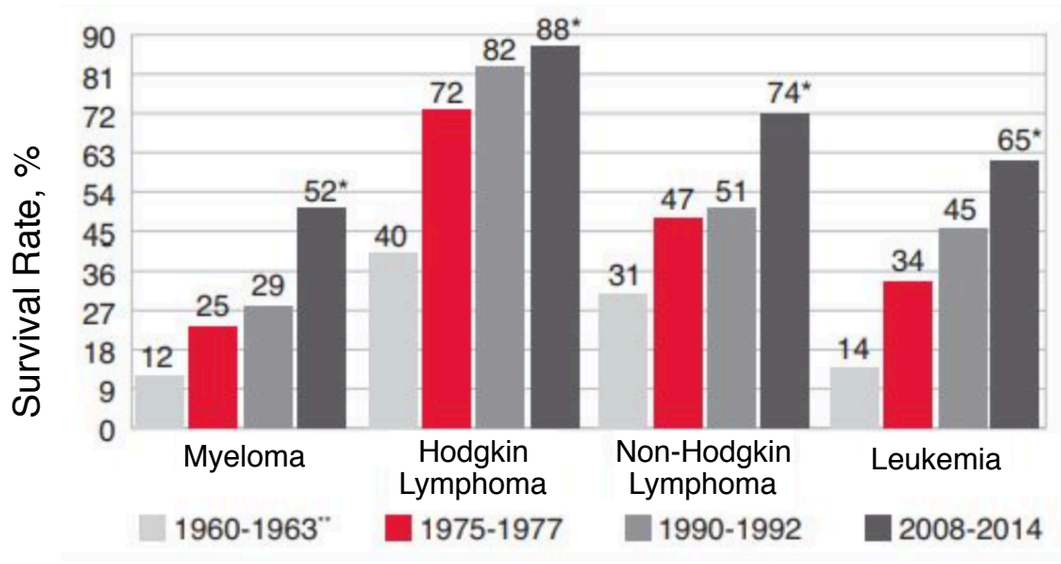
- toxicity
- difficulties extending to solid tumors
- CARs beyond cancer



Blood Cancer Survival Rates Over Time



Cancer Incidence Rates: Patients 0–14 yrs
(2009–2012)



significant increases in survival rate over time:

better diagnostics and treatment options

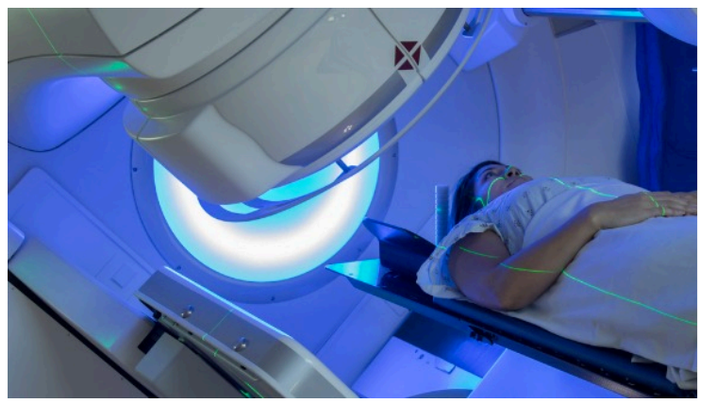
Surveillance, Epidemiology, and End Results (SEER), *Cancer Statistics Review, 1975-2015*, National Cancer Institutute, **2018**

Common Treatment Options for Acute Lymphocytic Leukemia

typically first course of action

chemotherapy

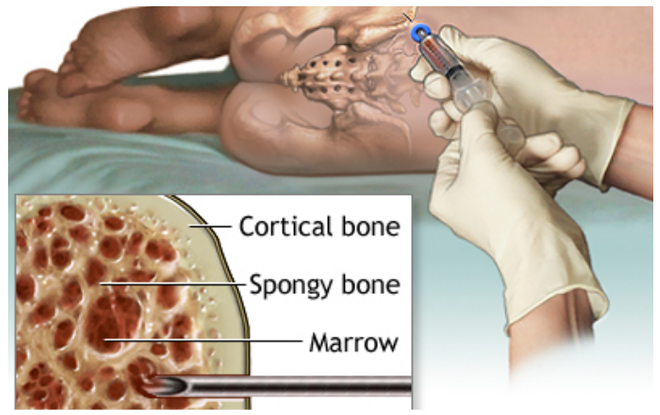
- ex: imatinib (Gleevec), dasatinib (Sprycel)
- total treatment time: ~2 years
- poor selectivity → toxicity, including for healthy immune cells



radiation therapy

- primarily used if cancer spreads to CNS
- often given in addition to chemo

not primary treatment for ALL



bone marrow transplant

- patients that may require high doses of chemo
- if nonresponsive to treatment
- can have severe side effects

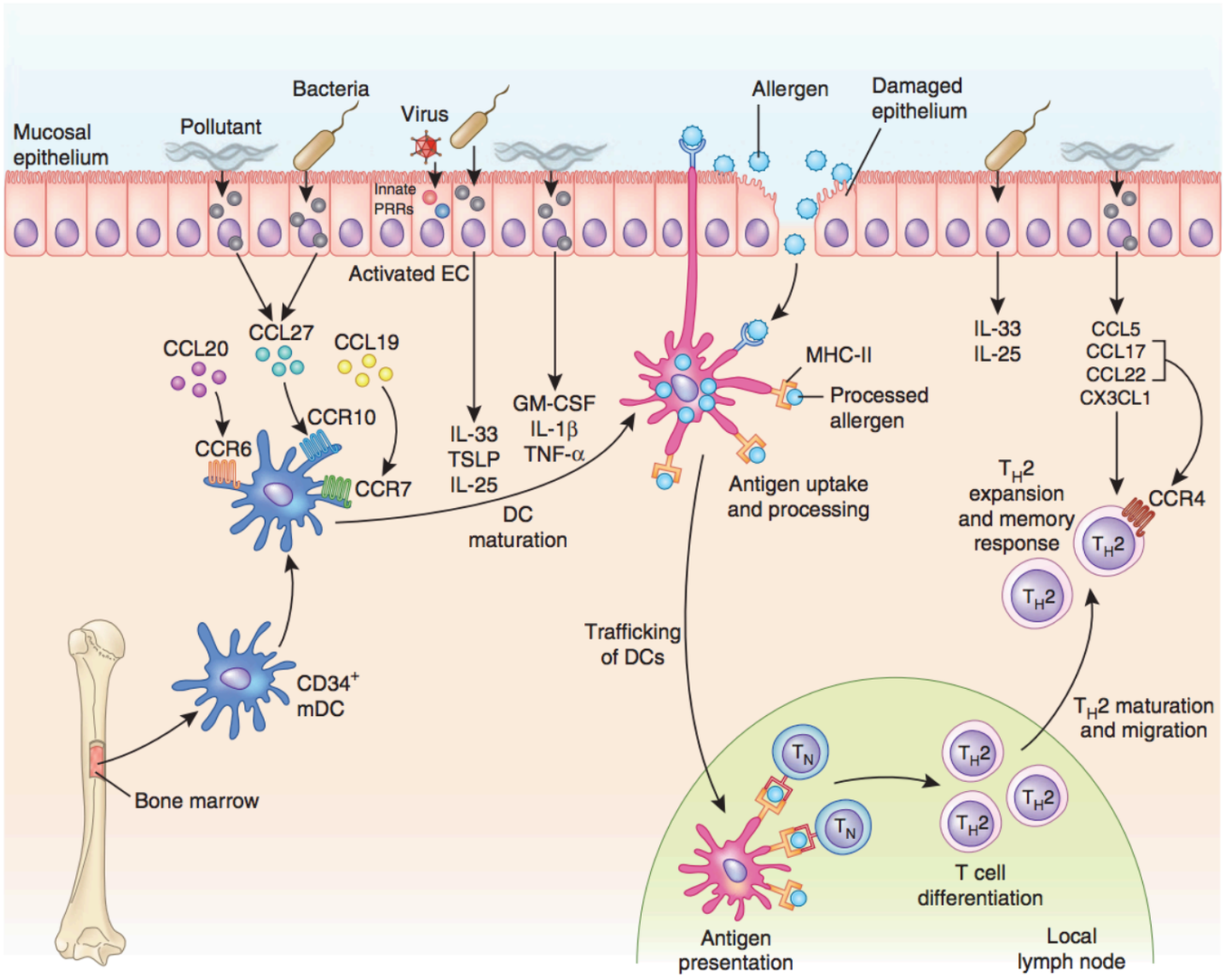
while 80–90% of adults go into complete remission, about half of the patient relapse

Sources: American Cancer Society, Leukemia & Lymphoma Society

Can we use our own immune system to fight cancer?

An (very brief) Overview of Normal Immune Response to "Invader"

Example: Innate and Adaptive Immune Responses in Asthma



a complicated and complex immune response is deployed onto "invaders"

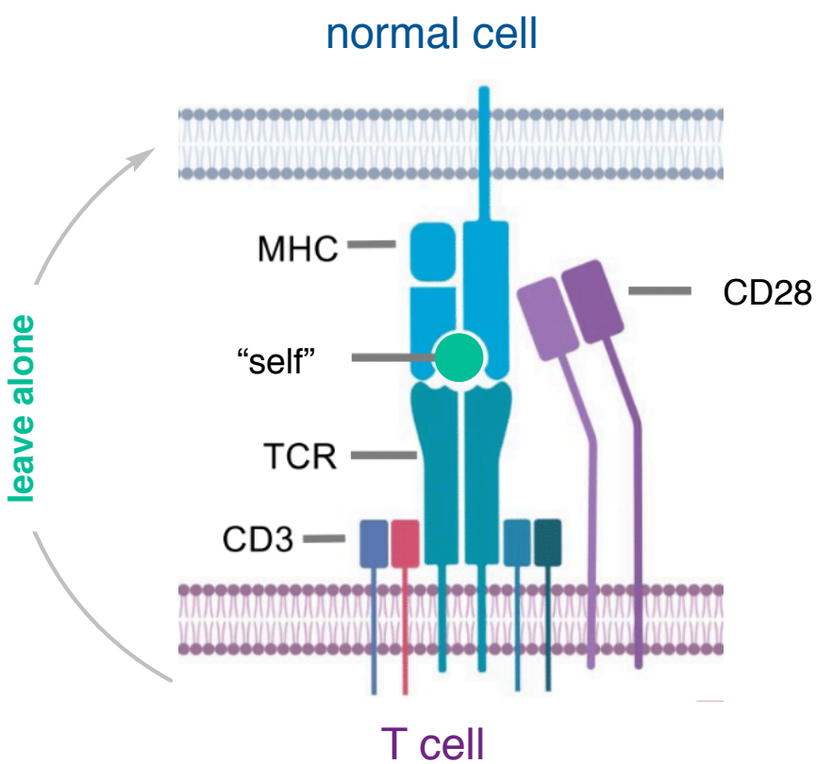
Holgate, S. T. *Nat. Med.* **2012**, *18*, 673.

An (very brief) Overview of Normal Immune Response to "Invader"

MHC class I

present on most cell surfaces

am I "self" or "invader"?



MHC: Major histocompatibility complex, TCR: T cell receptor

Sadelain, M.; Rivière, I.; Riddell, S. *Nat. Rev.* **2017**, *545*, 423.

An (very brief) Overview of Normal Immune Response to "Invader"

MHC class I

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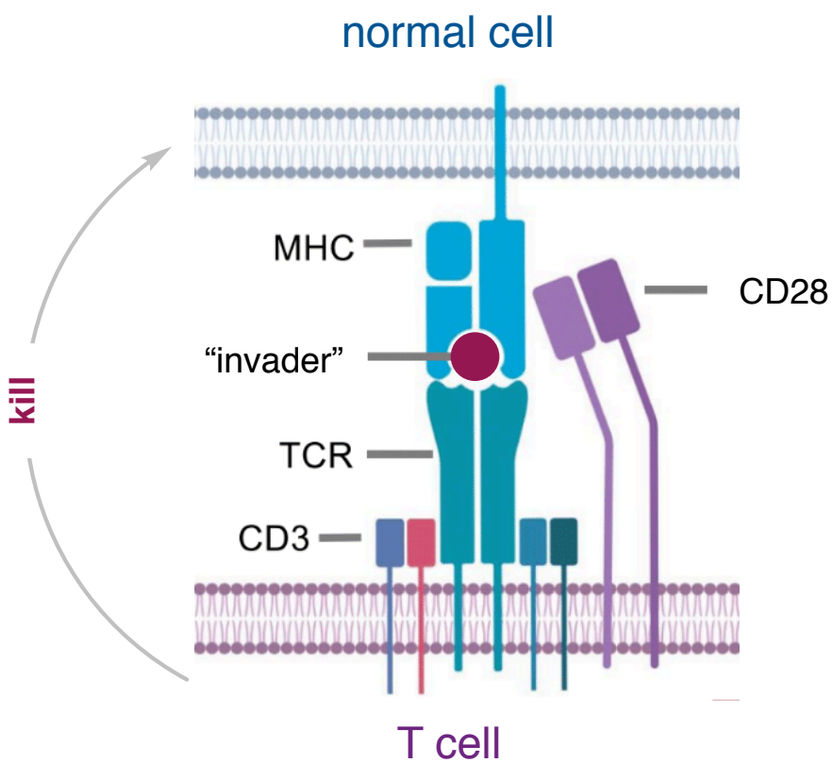


MHC: Major histocompatibility complex, TCR: T cell receptor

Sadelain, M.; Rivière, I.; Riddell, S. *Nat. Rev.* **2017**, *545*, 423.

An (very brief) Overview of Normal Immune Response to "Invader"

MHC class I

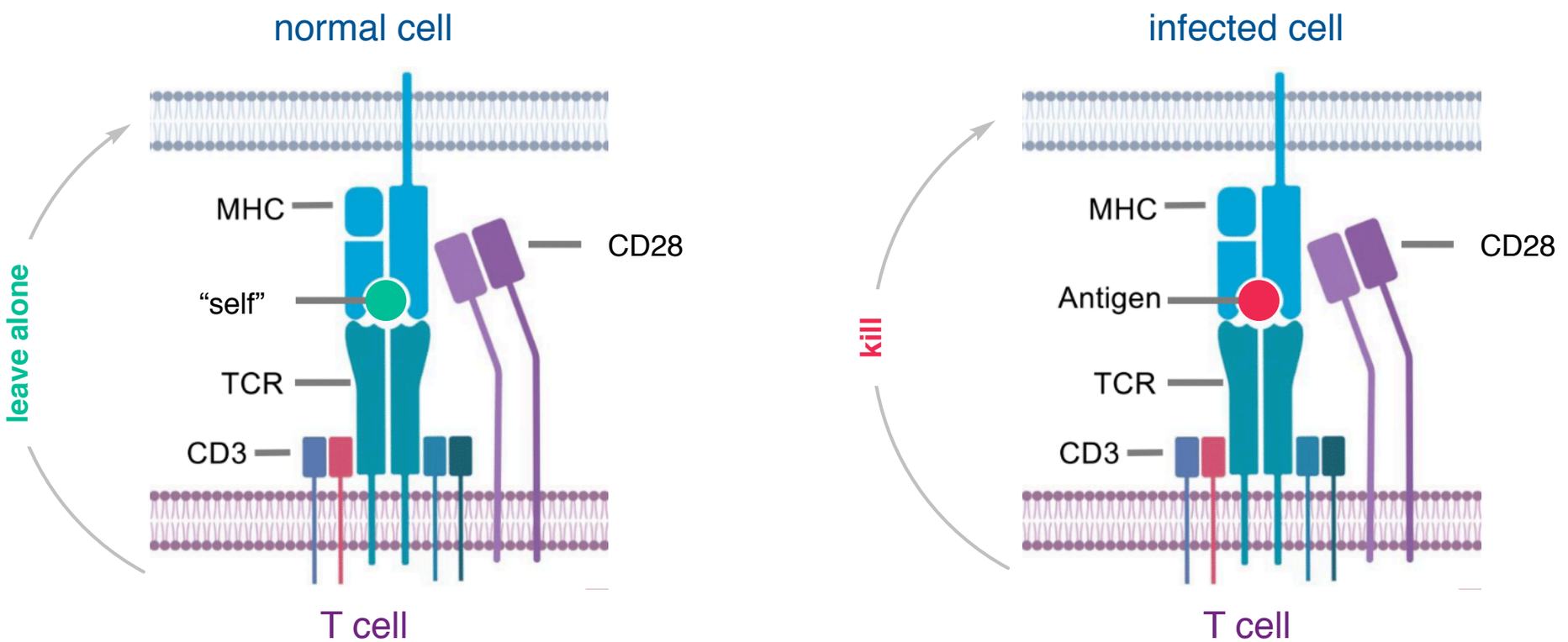
present on most cell surfaces

am I "self" or "invader"?

MHC class II

present on Antigen Presenting Cells (APC)

display antigen to the immune system



MHC: Major histocompatibility complex, TCR: T cell receptor

if MHC presents "invader" (antigen) peptide on cell surface

the TCR can recognize as "invader" and create immune response

Sadelain, M.; Rivière, I.; Riddell, S. *Nat. Rev.* **2017**, *545*, 423.

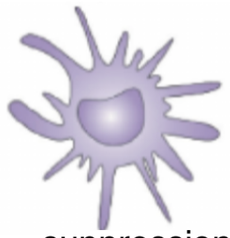
Ability of Cancer Cell to Evasion the Immune System

disrupt presentation of tumor antigens to immune system

tumor cell

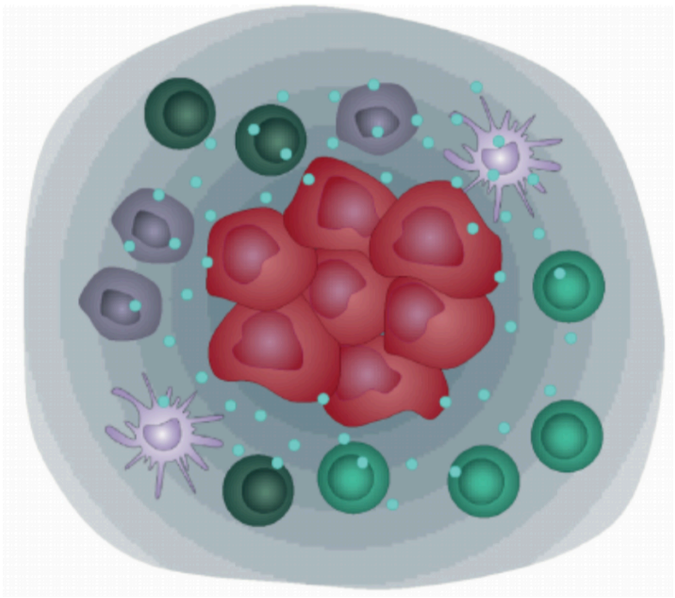
downregulation of MHC expression

APC

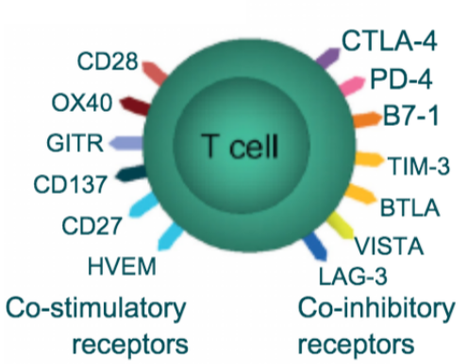


suppression of APC

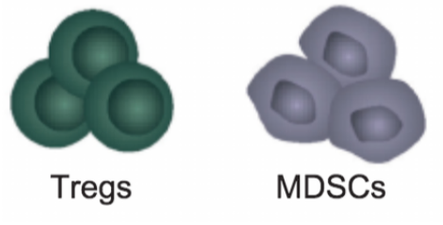
tumor microenvironment



T cell checkpoint dysregulation



recruit immunosuppressive cells



cancer cells can evade or suppress immune response

release immunosuppressive factors

Davies, M. *Cancer Management and Research*, **2014**, *6*, 63.

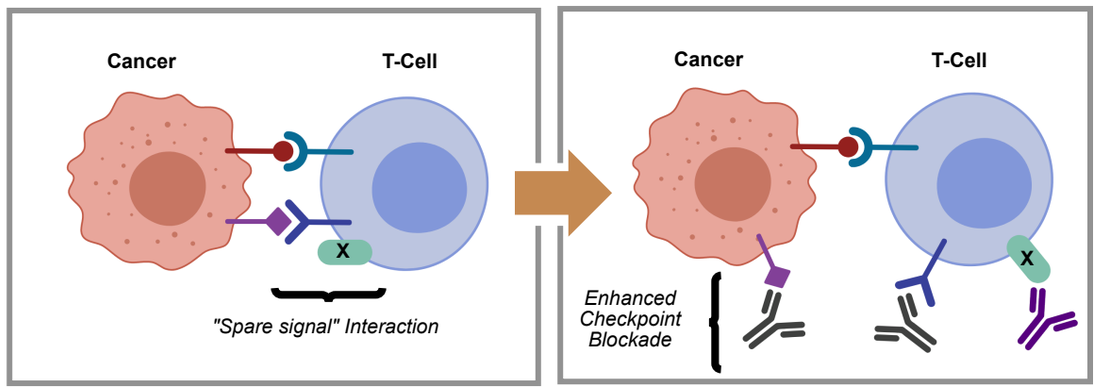
Can we use our own immune system to fight cancer?

Yes, by outsmarting the cancer cells

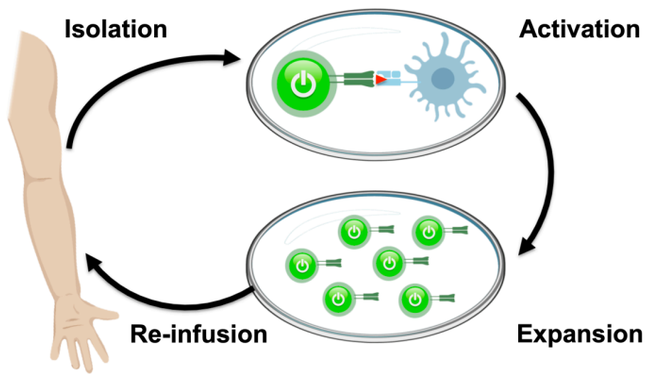
Immunotherapy Overview

Immunotherapy: recruit the body's own immune system to target and eliminate cancer

Immune Checkpoint Blockade

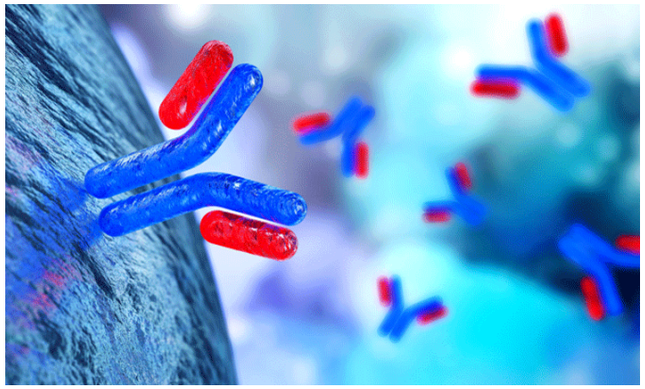


Adoptive Cell Transfer (ACT)



Anti-Cancer Vaccine

Tumor-Targeting Monoclonal Antibodies



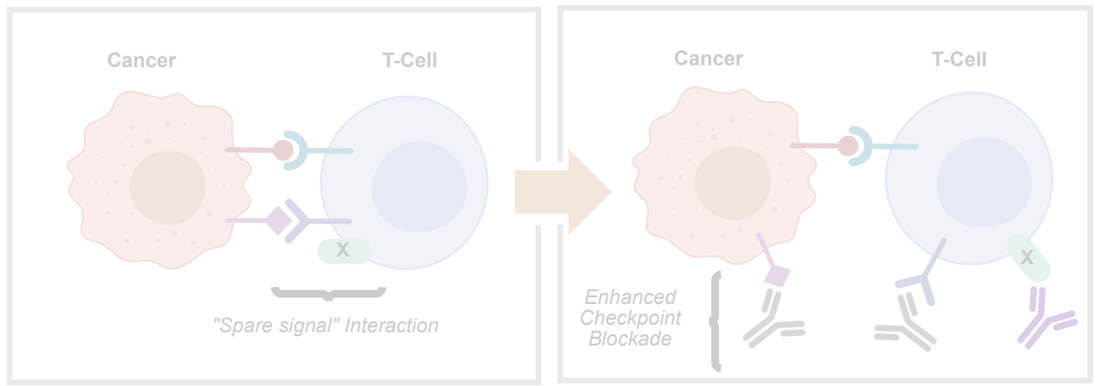
Immunostimulatory Cytokines

Subramanian, K. *Targeted Therapies in Oncology*, **2019**.

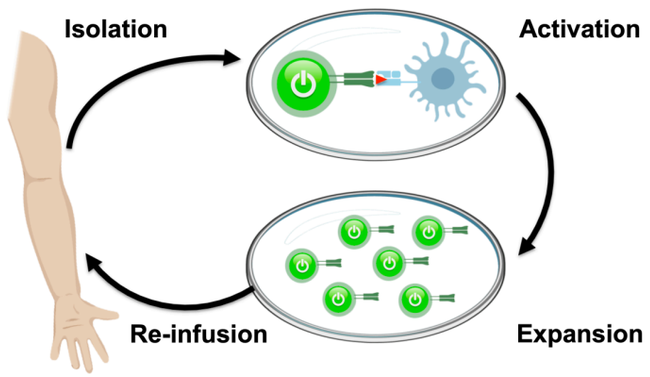
Immunotherapy Overview

Immunotherapy: recruit the body's own immune system to target and eliminate cancer

Immune Checkpoint Blockade



Adoptive Cell Transfer (ACT)



Anti-Cancer Vaccine

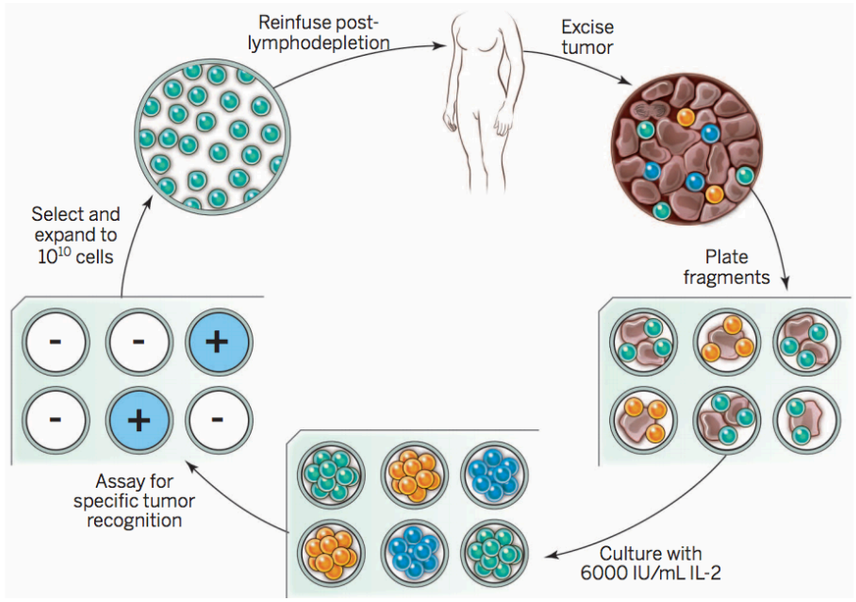
Tumor-Targeting Monoclonal Antibodies

Immunostimulatory Cytokines

Subramanian, K. *Targeted Therapies in Oncology*, **2019**.

Types of Adoptive Cell Transfer (ACT)

TILs, TCR T cells, and CAR T Cells



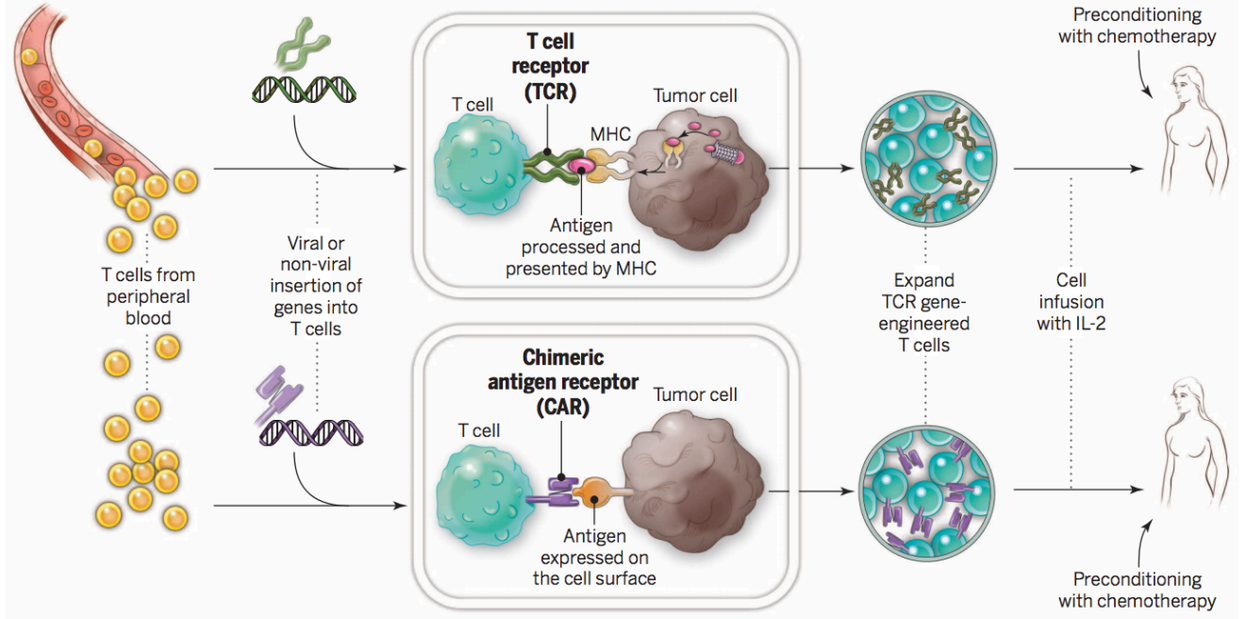
tumor infiltrating lymphocytes (TILs)

extract and identify lymphocytes

capable of tumor cell recognition,

expand, and reinfuse

T cell receptor (TCR) T cells



chimeric antigen receptor (CAR) T cells

extract T cells and engineer the receptor

to identify antigen (TCR) or design receptor (CAR)

to identify (CAR) the antigen, expand, and reinfuse

a "living therapy": T cells can expand in vivo after administration

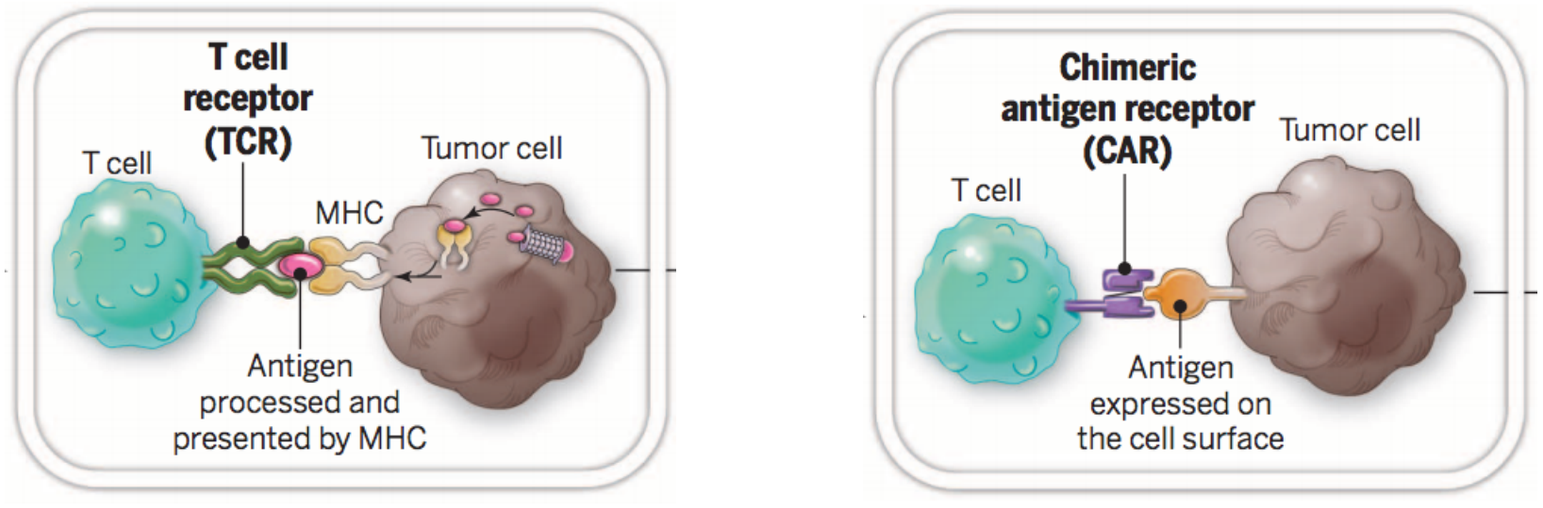
Rosenberg, S. A.; Restifo, N. P. *Science* **2015**, *348*, 62.

June, C. H.; O'Connor, R. S.; Kawalekar, O. U.; Ghassemi, S.; Milone, M. C. *Science* **2018**, *359*, 1361.

Chimeric Antigen Receptor (CAR) T Cells

CAR Concept

Design a receptor that will directly bind to an antigen or cancer biomarker



An advantage of CAR T cells: does not rely on MHC to present antigen

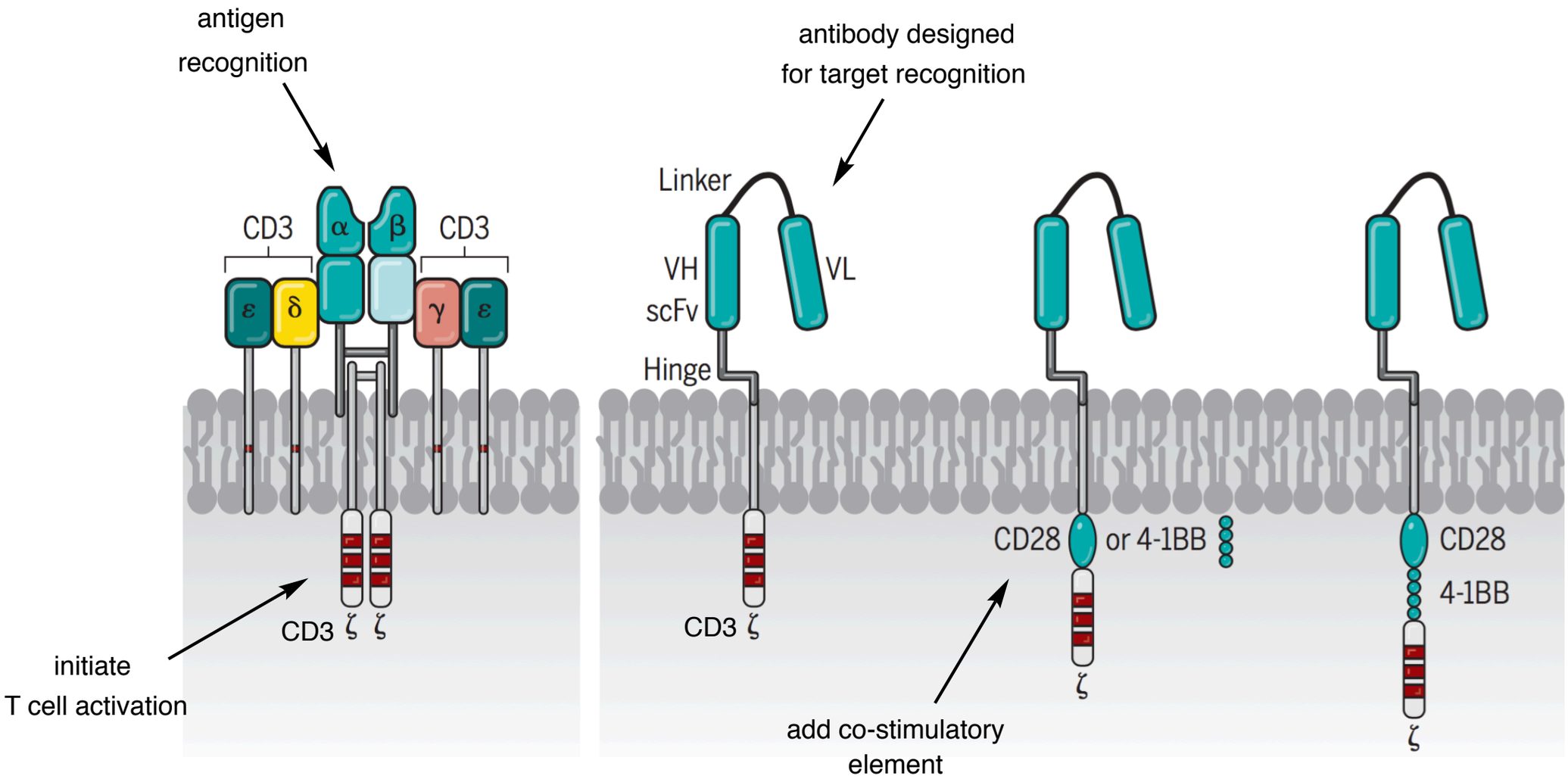
**rememer the cancer cells can downregulate MHC expression!*

how do we go about designing a receptor?

Rosenberg, S. A.; Restifo, N. P. *Science* **2015**, *348*, 62.

Chimeric Antigen Receptor (CAR) T Cells: Lessons from Biology

How does nature design functional T cells?



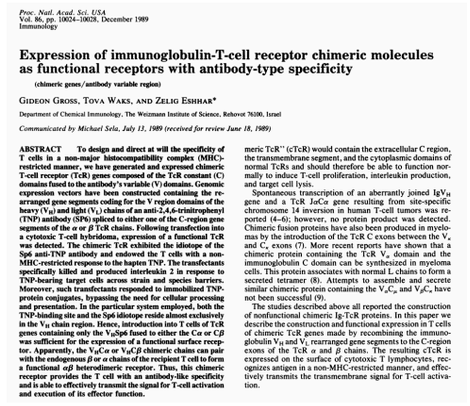
1st gen CARs aim mimic TCR recognition/activation, though activation isnt sufficient for in vivo persistence

2nd/3rd gen CARS: add costimulatory elements to promote long-term persistence and proliferation

June, C. H.; O'Connor, R. S.; Kawalekar, O. U.; Ghassemi, S.; Milone, M. C. *Science* **2018**, *359*, 1361.

Chimeric Antigen Receptor (CAR) T Cells: A Timeline

1989 - design of first chimeric receptor



Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 10024–10028, December 1989
Immunology

Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity

(chimeric genes/antibody variable region)

GIDEON GROSS, TOVA WAKS, AND ZELIG ESHHAR*

Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel

Communicated by Michael Sela, July 13, 1989 (received for review June 18, 1989)

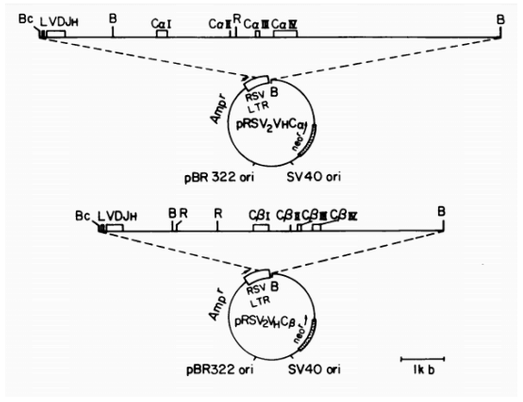
ABSTRACT **To design and direct at will the specificity of T cells in a non-major histocompatibility complex (MHC)-restricted manner, we have generated and expressed chimeric T-cell receptor (TcR) genes composed of the TcR constant (C) domains fused to the antibody's variable (V) domains. Genomic expression vectors have been constructed containing the rearranged gene segments coding for the V region domains of the heavy (V_H) and light (V_L) chains of an anti-2,4,6-trinitrophenyl (TNP) antibody (SP6) spliced to either one of the C-region gene segments of the α or β TcR chains. Following transfection into a cytotoxic T-cell hybridoma, expression of a functional TcR was detected. The chimeric TcR exhibited the idiotope of the Sp6 anti-TNP antibody and endowed the T cells with a non-MHC-restricted response to the hapten TNP. The transfectants specifically killed and produced interleukin 2 in response to TNP-bearing target cells across strain and species barriers. Moreover, such transfectants responded to immobilized TNP-protein conjugates, bypassing the need for cellular processing and presentation. In the particular system employed, both the TNP-binding site and the Sp6 idiotope reside almost exclusively in the V_H chain region. Hence, introduction into T cells of TcR genes containing only the V_HSp6 fused to either the Cα or Cβ was sufficient for the expression of a functional surface receptor. Apparently, the V_HCα or V_HCβ chimeric chains can pair with the endogenous β or α chains of the recipient T cell to form a functional $\alpha\beta$ heterodimeric receptor. Thus, this chimeric receptor provides the T cell with an antibody-like specificity and is able to effectively transmit the signal for T-cell activation and execution of its effector function.**

meric TcR" (cTcR) would contain the extracellular C region, the transmembrane segment, and the cytoplasmic domains of normal TcRs and should therefore be able to function normally to induce T-cell proliferation, interleukin production, and target cell lysis.

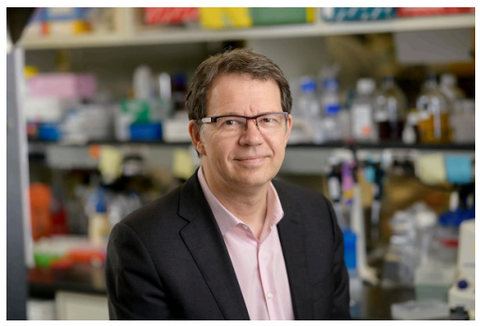
Spontaneous transcription of an aberrantly joined IgV_H gene and a TcR JαCα gene resulting from site-specific chromosome 14 inversion in human T-cell tumors was reported (4–6); however, no protein product was detected. Chimeric fusion proteins have also been produced in myelomas by the introduction of the TcR C exons between the V_κ and C_κ exons (7). More recent reports have shown that a chimeric protein containing the TcR V_α domain and the immunoglobulin C domain can be synthesized in myeloma cells. This protein associates with normal L chains to form a secreted tetramer (8). Attempts to assemble and secrete similar chimeric protein containing the $V_\alpha C_\alpha$ and $V_\beta C_\gamma$ have not been successful (9).

The studies described above all reported the construction of nonfunctional chimeric Ig-TcR proteins. In this paper we describe the construction and functional expression in T cells of chimeric TcR genes made by recombining the immunoglobulin V_H and V_L rearranged gene segments to the C-region exons of the TcR α and β chains. The resulting cTeR is expressed on the surface of cytotoxic T lymphocytes, recognizes antigen in a non-MHC-restricted manner, and effectively transmits the transmembrane signal for T-cell activation.

Dr. Zelig Eshhar
Weizman Institute



1990's - T cell engineering, 1st and 2nd gen CARs (main players:)



Dr. Michel Sadelain
Memorial Sloan Kettering

2000's

successful preclinical studies with anti-CD19 CARs

Dr. Carl June
University of Pennsylvania

2010's

successful clinical studies with anti-CD19 CARs in humans

Dr. Steven Rosenberg
National Cancer Institute

2017

First CART therapies approved: Kymriah and Yescarta

June, C. H.; O'Connor, R. S.; Kawalekar, O. U.; Ghassemi, S.; Milone, M. C. *Science* **2018**, *359*, 1361.

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- how CARs are prepared and administered
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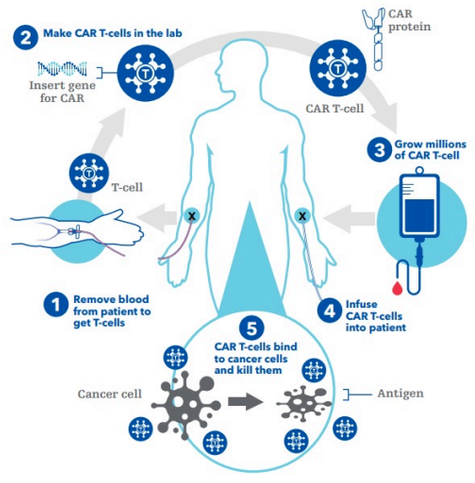
- clinical trials leading to FDA approvals



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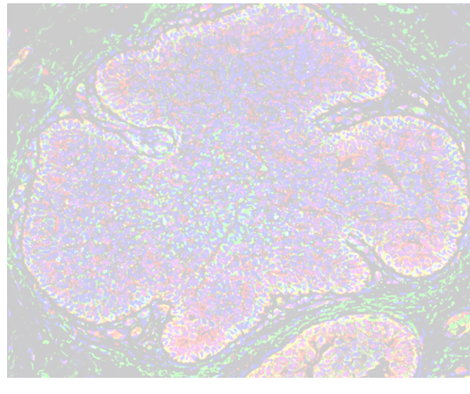
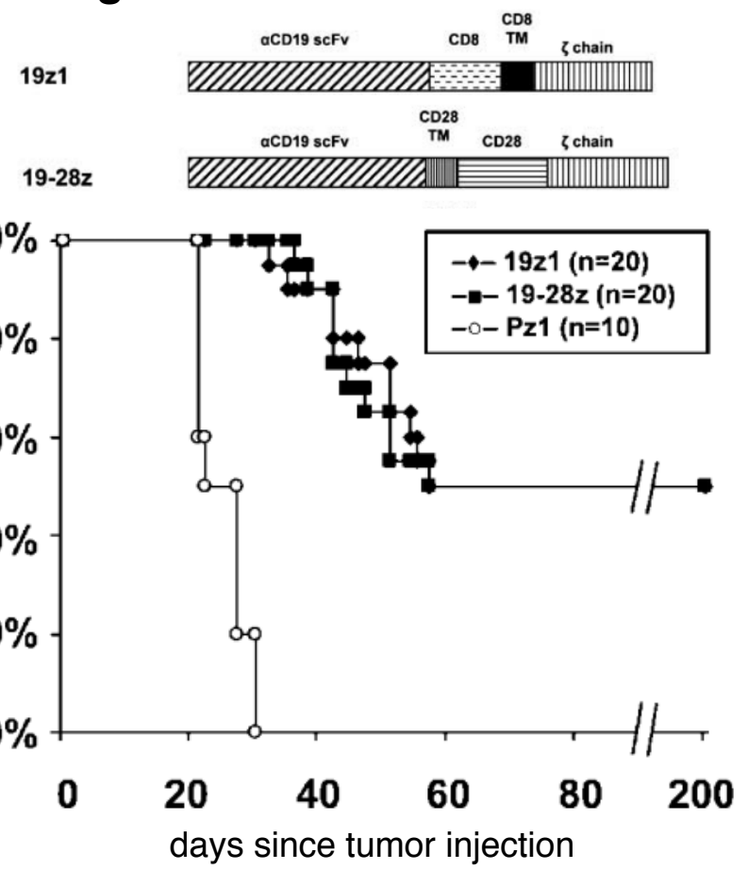


CAR T Cells: Preclinical Studies with CD19-targeted CARs in mice

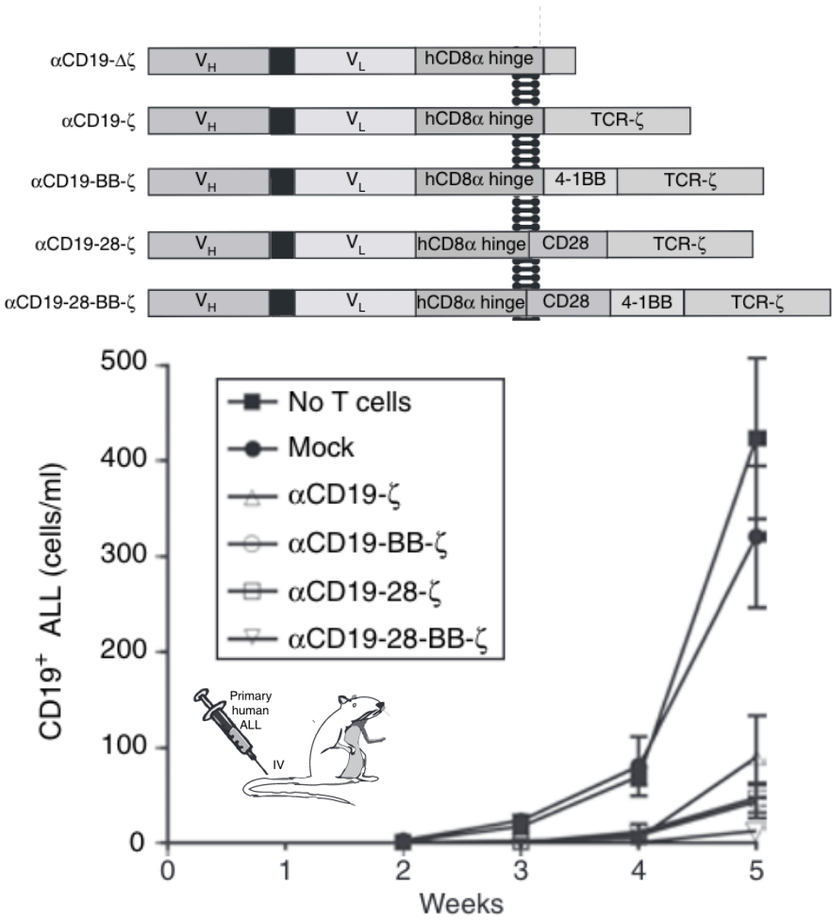
CD19 as attractive target

- expressed on most B cell malignancies: including ALL, CLL, and non-Hodkin's lymphoma
  - also expressed on normal B cells (which are temporarily dispensible)

CD19-targeted CARs for treatment of ALL



(mouse model)



adding co-stimulatory domains conferred persistence and led to increased anti-leukemic efficacy

Milone, M. C. et al, June, C. H. *Mol. Ther.* **2009**, *17*, 1453.

Brentjens, R. J. et al., Sadelain, M. *Clin. Cancer Res* **2007**, *13*, 5426.

Trials Leading to FDA Approval of Kymriah

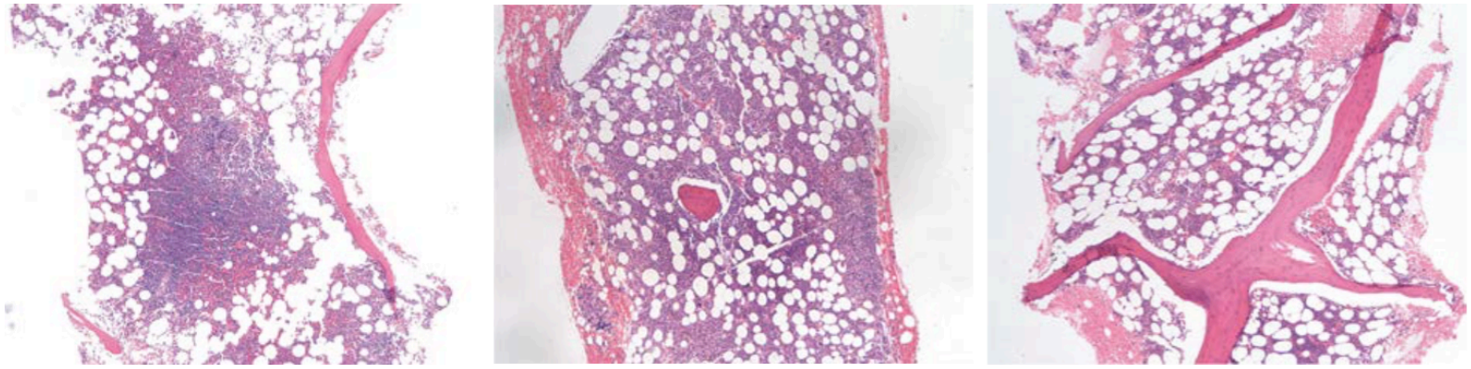
Pilot Clinical Trial with 3 Patients with Relapsed or Refractory Chronic Lymphoid Leukemia (CLL)

Patient 1:

diagnosed stage I CLL 1996 → 2 cycles chemo 2002 → 4 cycles chemo 2006 → 4 cycles chemo 2009

bone marrow biopsy

day 1 (baseline) | day 23 | 6 months



no evidence of CLL in the bone marrow

CART19 cells (with 41-BB co-stimulatory domain) persisted for at least 6 months

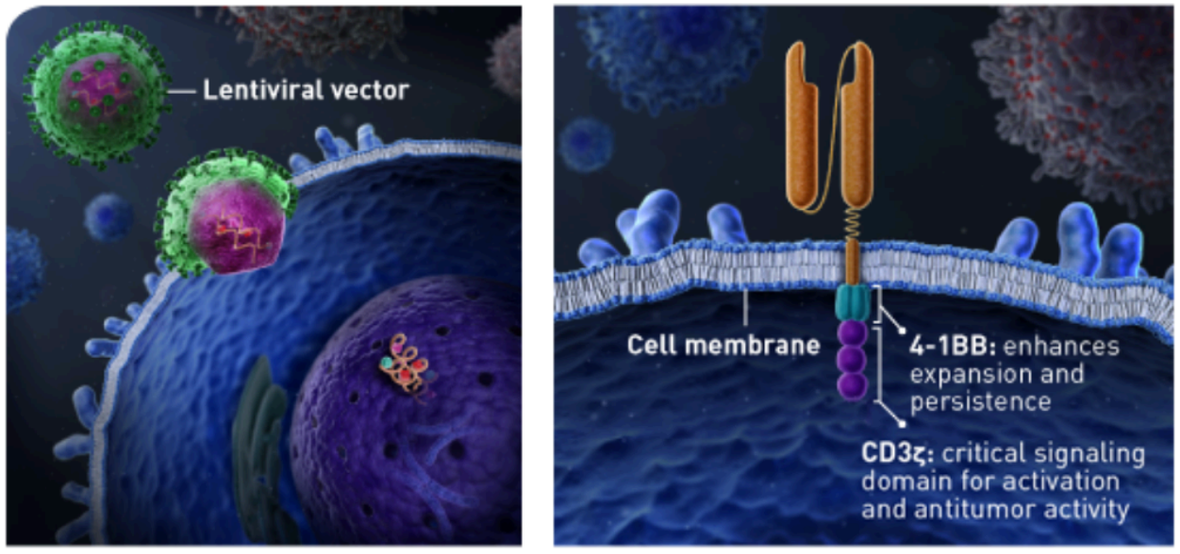
no normal B cells detected after treatment for at least 6 months after treatment

major toxicity: tumor lysis syndrome

Porter, D. L.; Levine, B. L.; Kalos, M.; Bagg, A.; June, C. H. *N. E. Engl. J. Med.* **2011**, *35*, 725.

Trials Leading to FDA Approval of Kymriah

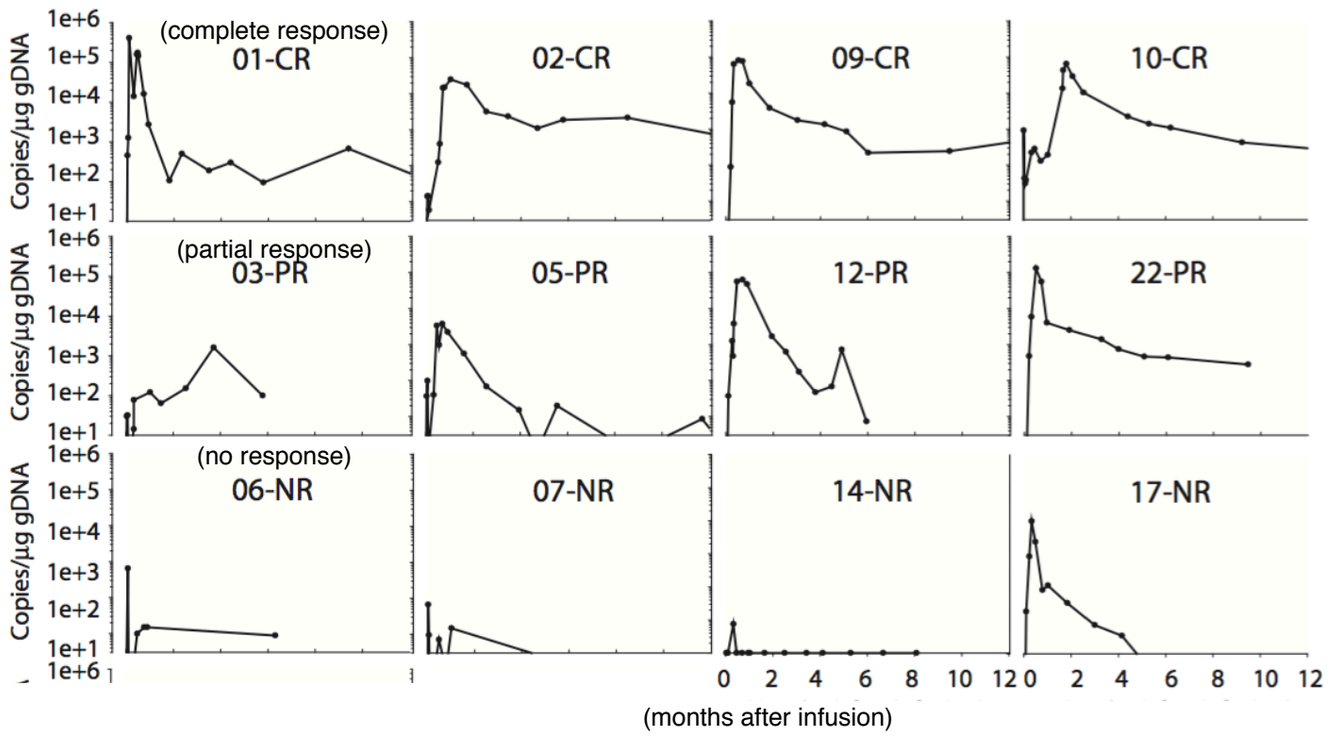
5 Year Data on Pilot Clinical Study with 14 Patients with Relapsed Refractory CLL with Novartis



June and coworkers

use anti-CD19 CARs

with 4-1BB co-stimulatory domain



CAR T cell expansion

14– 49 months

in patients with CR

B cell aplasia

cytokine release syndrome

Porter, D. L. et al.; June, C. H. *Sci. Transl. Med.* **2015**, *7*, 1.

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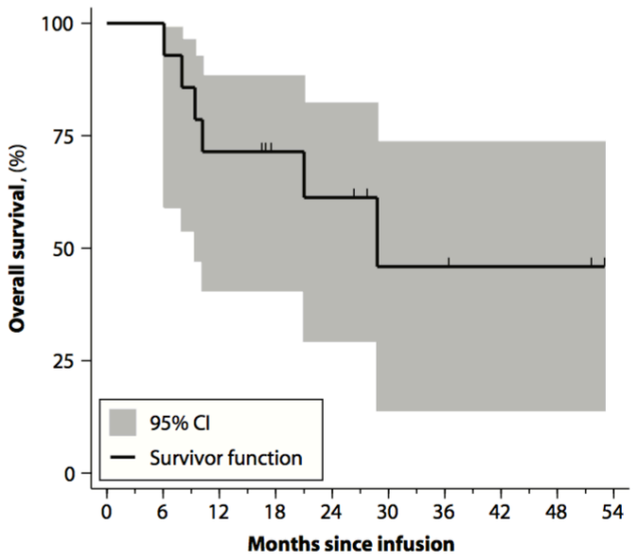
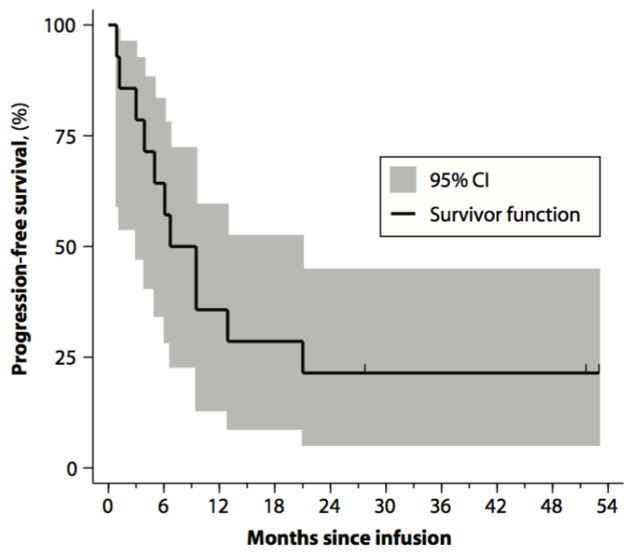
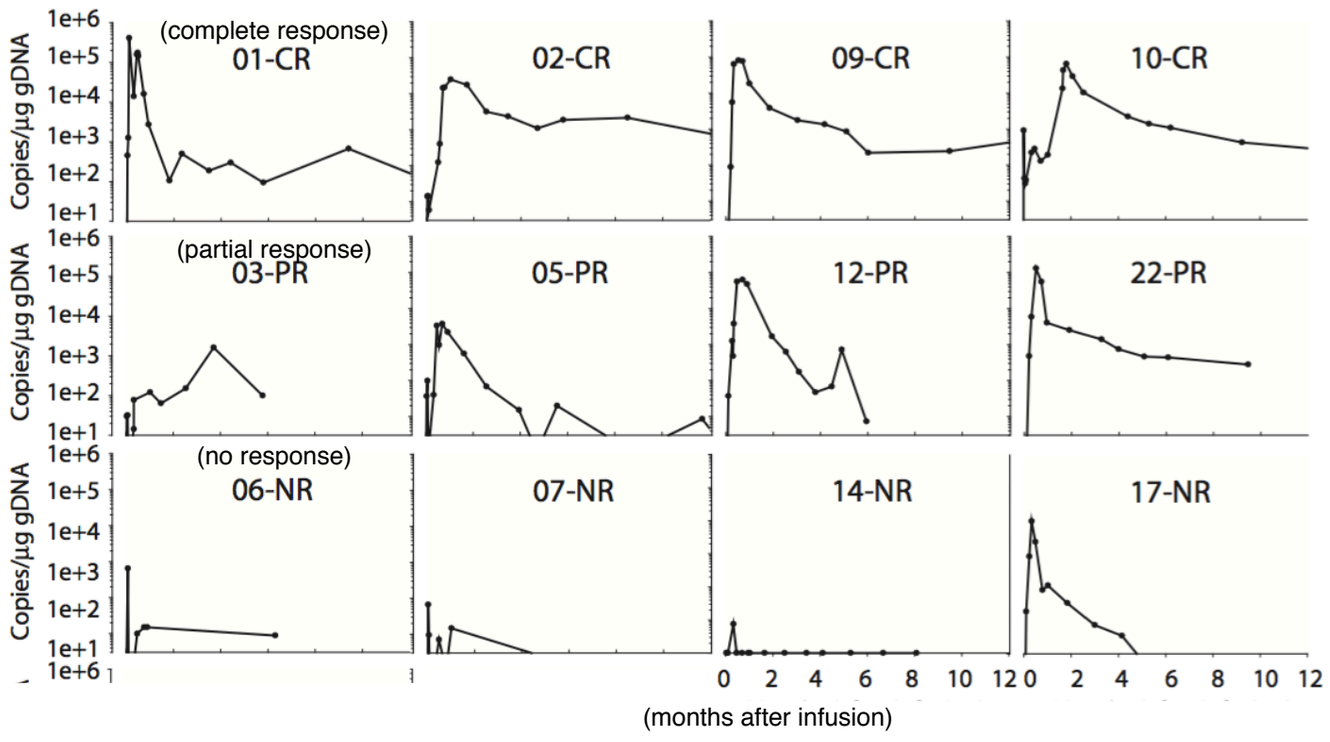






CAR T cell expansion
14– 49 months
in patients with CR

B cell aplasia

cytokine release syndrome

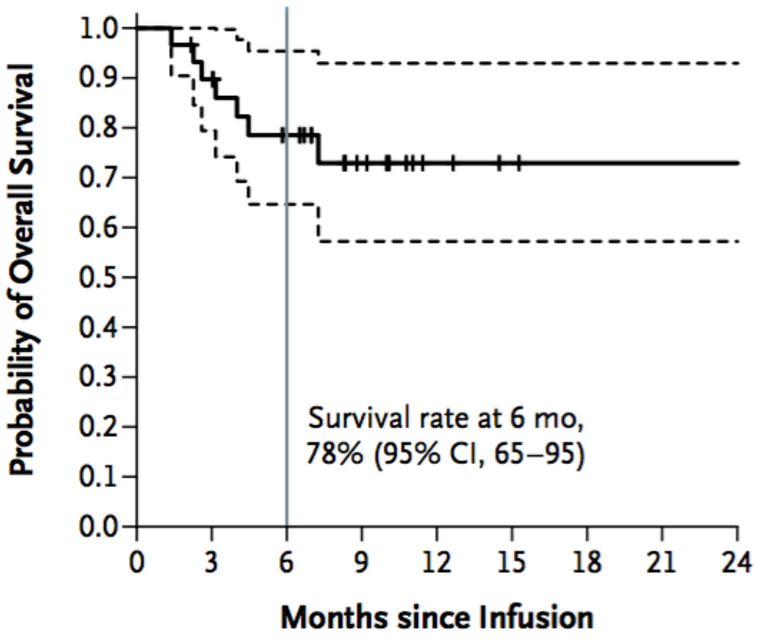
Porter, D. L. et al.; June, C. H. *Sci. Transl. Med.* **2015**, *7*, 1.

Trials Leading to FDA Approval of Kymriah

CD19 CARs for Sustained Remission in ALL with Dr. June and Novartis

trial for relapsed acute lymphoblastic leukemia (ALL)

- # of patients: 25 patients ages 5–22, 5 patients ages 26–60
- # patients in complete remission: 27 (90%) after one month, 19 remained in remission
- 6 month overall survival: 78%
- probability of CAR perisistence at 6 months: 68%
- B cell aplasia: 73%
- severe cytokine release syndrome (CRS): 27%



Orphan Drug Designation

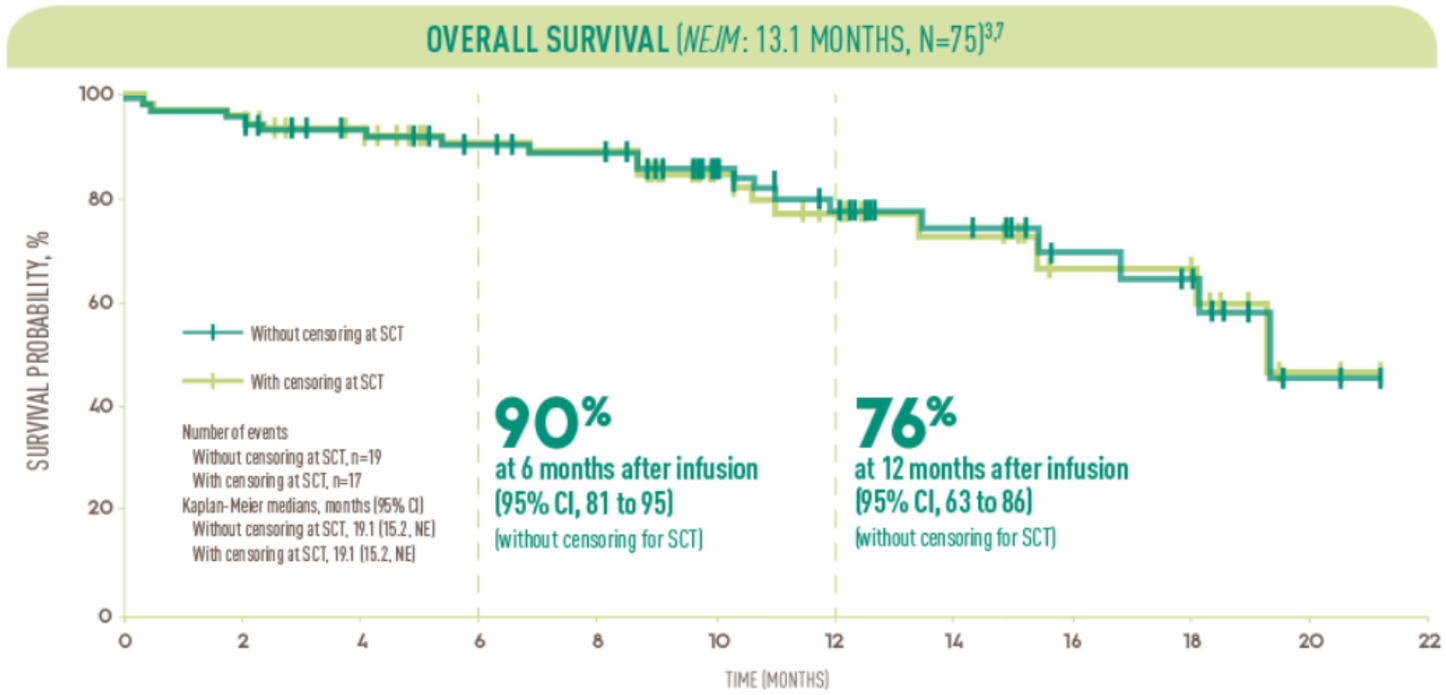
KYMRIAH® (tisagenlecleucel) Suspension for IV infusion

FDA grants orphan drug designation to Kymriah for treatment of ALL (2014)

Maude, S. L. et al. *N. Engl. J. Med.* **2014**, *371*, 1507.

Trials Leading to FDA Approval of Kymriah

Phase 2 Trial: 75 pediatric and young adult patients with relapsed or refractory B cell ALL



Adverse Events within 8 Weeks of Infusion

Type of Events	Any Grade	Grade 3	Grade 4
	number of patients (percent)		
Any adverse event of special interest	67 (89)	26 (35)	30 (40)
Cytokine release syndrome	58 (77)	16 (21)	19 (25)
Neurologic event	30 (40)	10 (13)	0
Infection	32 (43)	16 (21)	2 (3)
Febrile neutropenia	26 (35)	24 (32)	2 (3)
Cytopenia not resolved by day 28	28 (37)	12 (16)	12 (16)
Tumor lysis syndrome	3 (4)	3 (4)	0

approved Aug 30, 2017

First FDA approved cell and gene therapy (US)

KYMRIAH®
(tisagenlecleucel) Suspension for IV infusion

for patients up to 25 years old with ALL

that is refractory or in second or later relapse

Maude, S. L. et al. *N. Engl. J. Med.* **2018**, *378*, 439.

Treatment Options for Relapsed or Refractory Large B-cell Lymphomas

following diagnosis with aggressive B-cell lympoma:

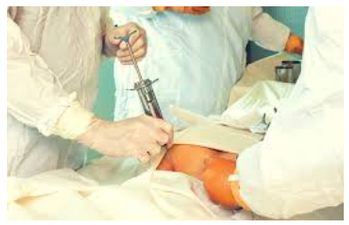
chemotherapy (round 1)

20-40% fail

intial treatment

chemo (round 2)

and/or



stem cell transplant

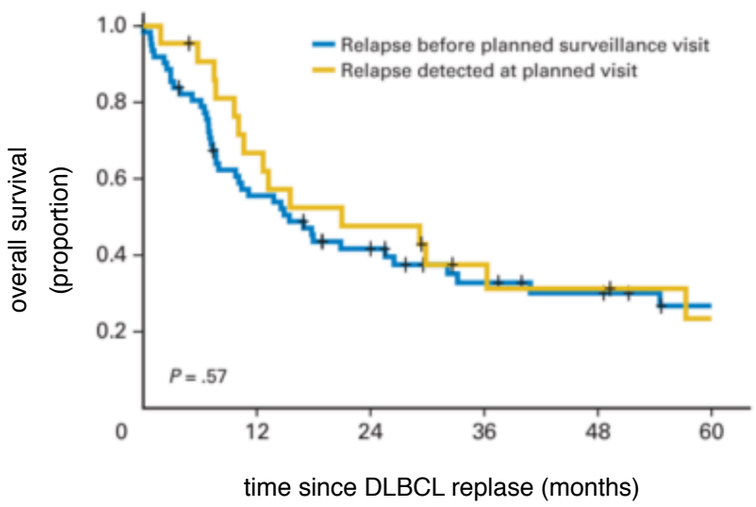
40-60%

fail treatment

limited options for relapsed or refractory patients

Diffuse Large B-cell Lymphoma (DLBCL) most common subtype of non-Hodkin lymphoma

overal survival of 112 patients with relapsed DLBCL



636 patient-study with refractory large B-cell lymphoma:

- complete reponse rate: 7%
- median overall survival: 6.3 months
- only 10–25% of patients with relapsed or refractory large B-cell lymphoma are cured

Blood **2017**, *130*, 1800.

Jacobson, C. A.; Farooq, U.; Ghobadi, A. *The Oncologist* **2020**, *25*, 138.

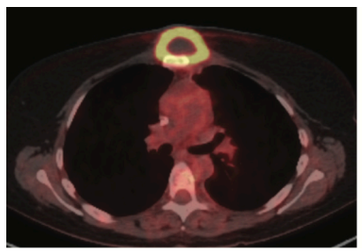
Thompson, C. A. et al. *J. Clin. Onc.* **2014**, *32*, 3506.

Trials Leading to FDA Approval of Yescarta

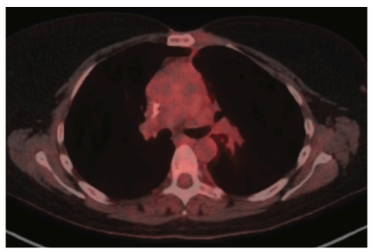
Dr. Rosenberg (NCI) with Kite Pharma using anti-CD19 CAR (with CD28 co-stimulatory domain)

study involved 15 patients with chemo-refractory B-cell malignancies: 8/15 in complete remission, 4/15 partial remission

before treatment

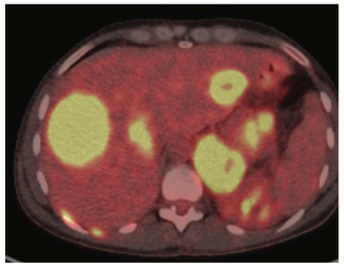


after 23 months

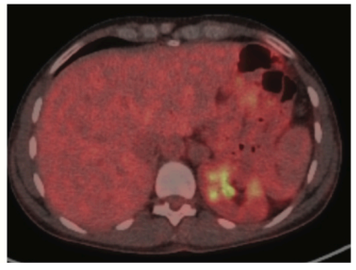


"Patient No. 2 was diagnosed with PMBCL. She underwent treatment with **six cycles** of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP)…**radiation therapy**, which resulted in a CR that lasted 5 months before relapse. Next, she received **two cycles** of … chemotherapy… Finally, she received a regimen of rituximab, cytarabine, and methotrexate, which also led to SD. Patient No. 2 was treated on the anti-CD19 CAR protocol and entered a CR that is ongoing after 22 months"

before treatment



after 9 months

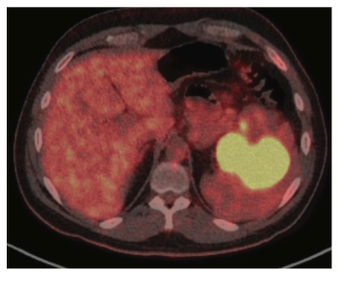


patient with primary mediastinal B-cell lymphoma (PMBLC)

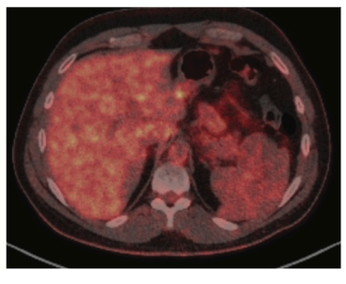
had *10 prior regiments* before CAR

ongoing complete response (CR) after 12 months

before treatment



after 5 months



patient with primary DLBCL

had two rounds of intenstive chemo before CAR

despite CR after treatment, lymphoma recurred after 6 months

acute toxicity occured in some patients, though resolved within 3 weeks

one patient died suddenly after 16 days

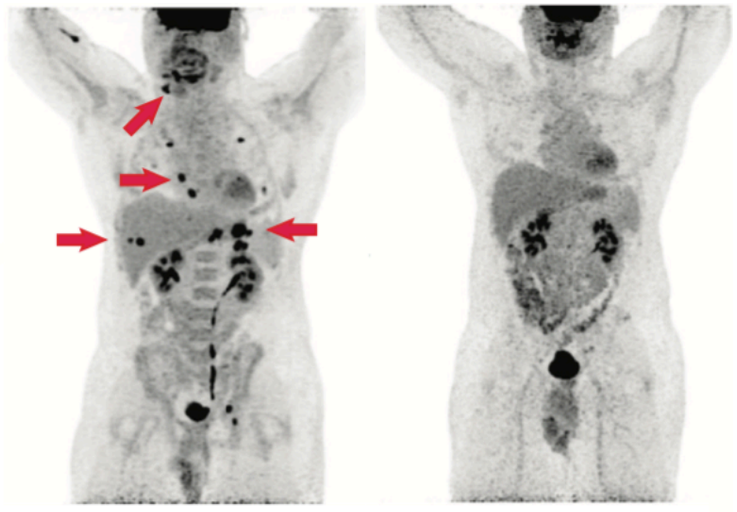
Kochenderfer, J. N. et al.; Rosenberg, S. A. *J. Clin. Oncol.* **2014**, *33*, 540.

Trials Leading to FDA Approval of Yescarta

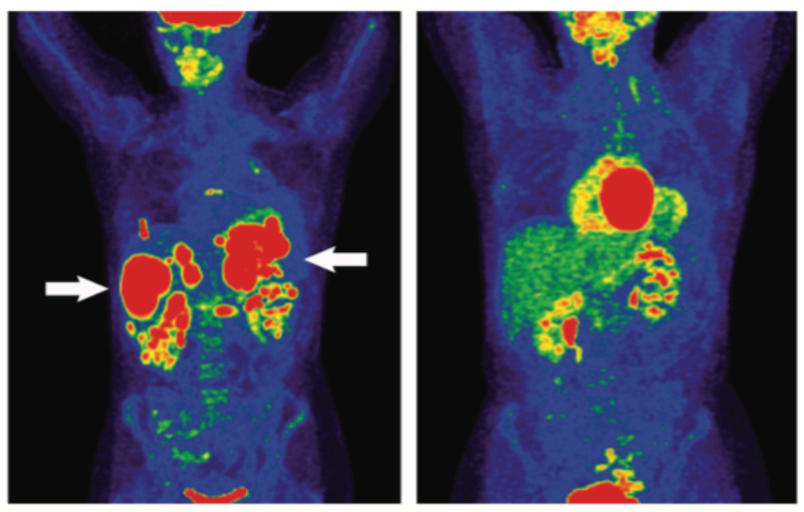
Follow Up on Long-Duration of Complete Remission from Previous Study

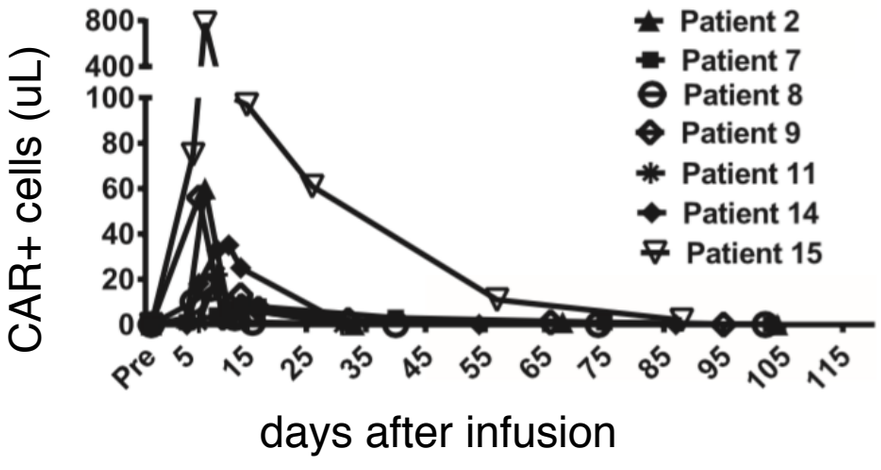
4/5 patients in complete remission had long term duration of remission > 3 years

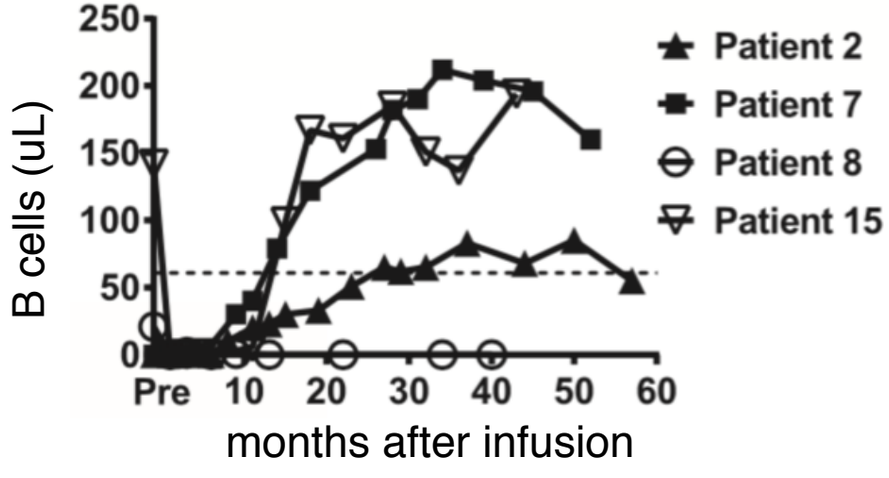
before treatment 45 months after



before treatment 39 months after







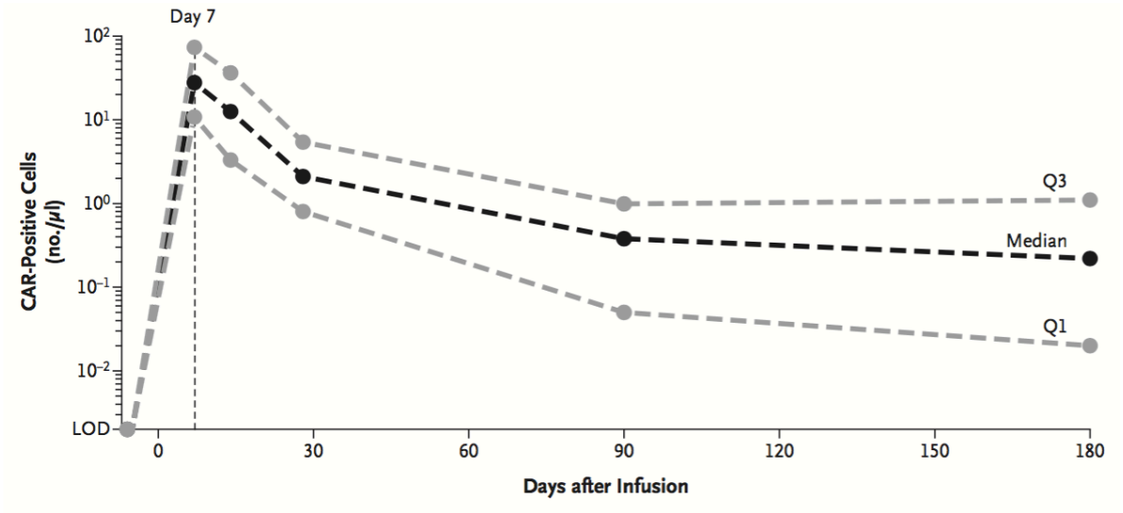
Kochenderfer, J. N. et al.; Rosenberg, S. A. *Mol. Ther.* **2017**, *25,* 2245.

Trials Leading to FDA Approval of Yescarta

Phase II Trial for Refractory Large B-Cell Lymphoma

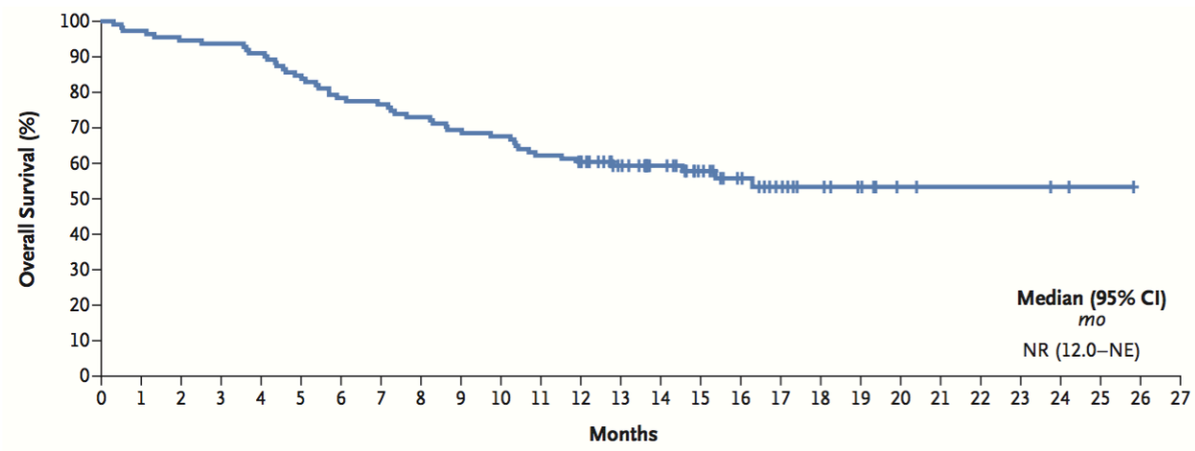
54% of the 111 patients had a complete response

overall survival after 18 months was 52%



CAR T Cell expansion

correlation found between the
# of CAR T cells and
the clinical outcome



13% had > Grade 3 cytokine release syndrome, and 28% with neurological events

3 patients died during treatment

Neepalu, S. S. et al. *N. Engl. J. Med.* **2017**, *377*, 2531.

Trials Leading to FDA Approval of Yescarta

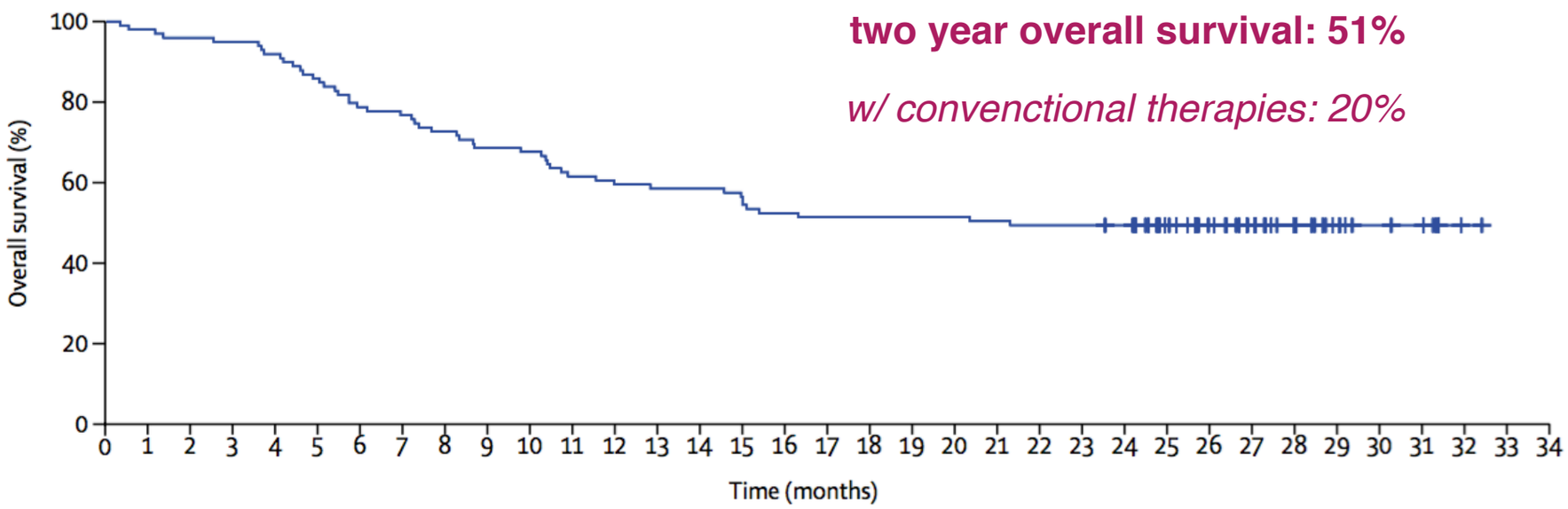
approved Oct 18, 2017

YESCARTA®
(axicabtagene ciloleucel) Suspension for IV infusion

for adults with large B-cell lymphoma
that is refractory or in second or later relapse

adverse side effect - largly similar after 2 years

- grade 3 or worse adverse events: 98%
- all events were managable and largely reversible
- grade 3 or worse CRS: 11%
- grade 3 or worse neological events: 32%



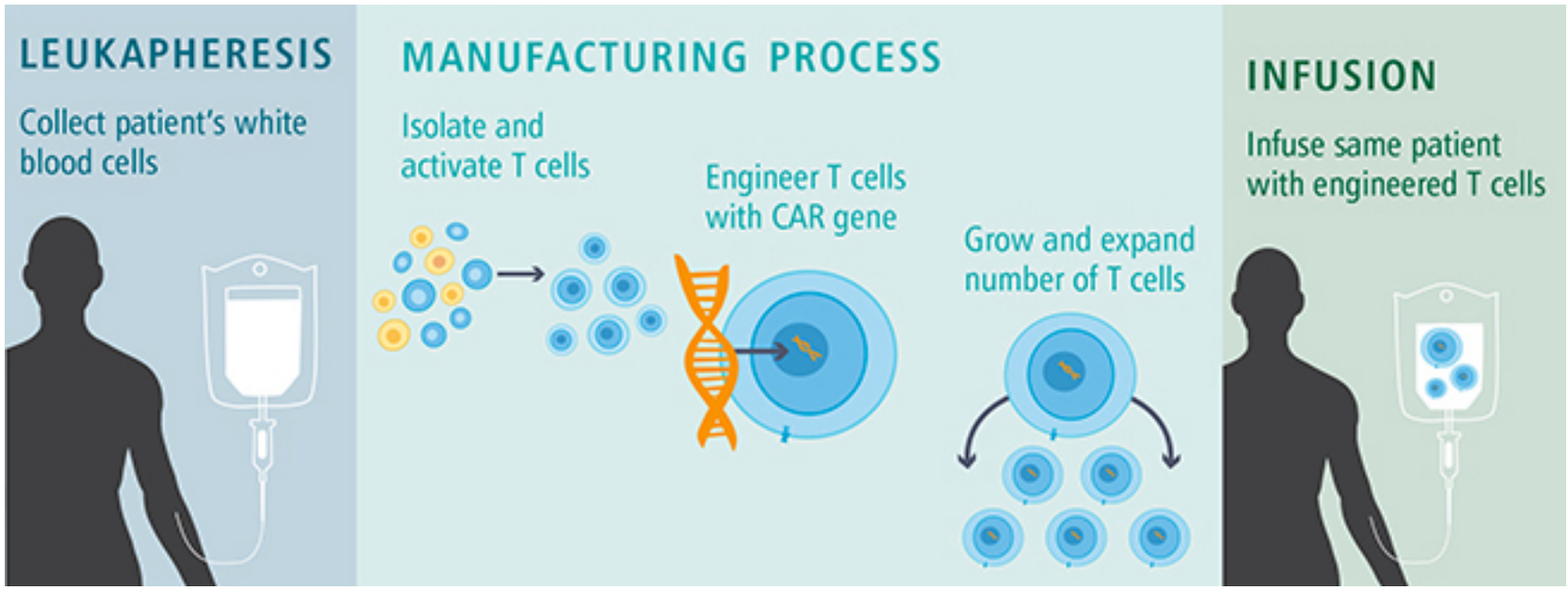
two year overall survival: 51%

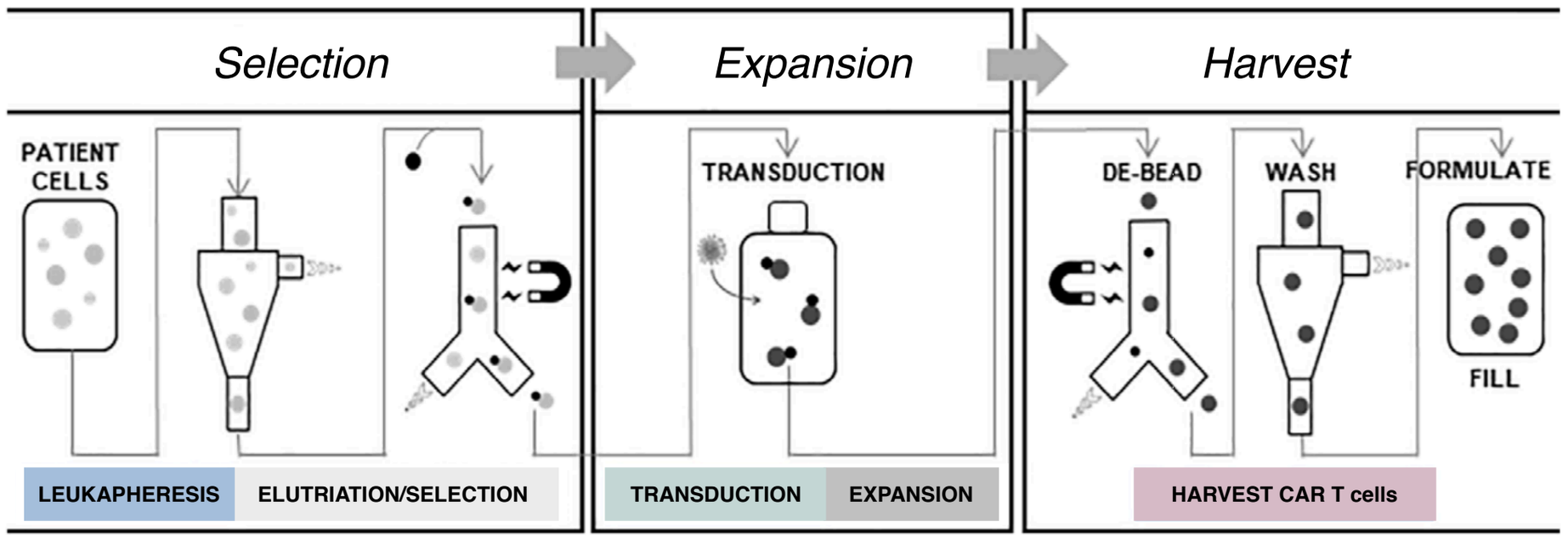
w/ convenctional therapies: 20%

Locke, F. L. et al. *Lancet Oncol.* **2019**, *20*, 31.

How is CAR T cell therapy administered?

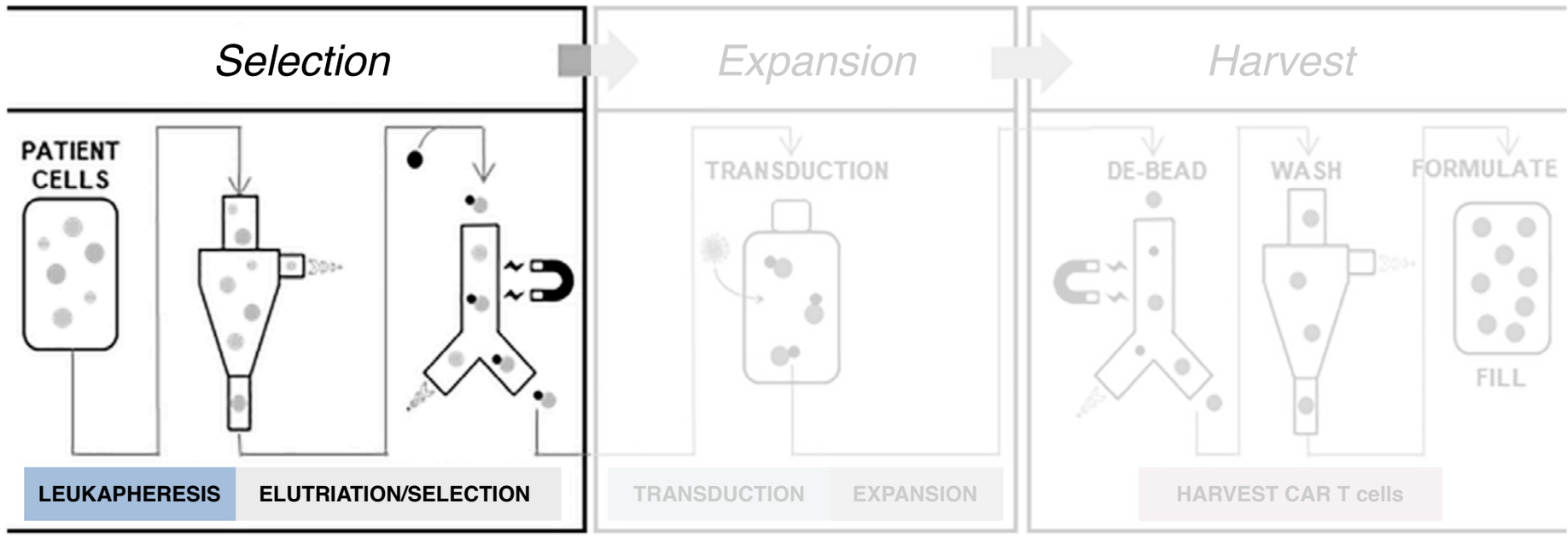
CAR T cell Therapy in the Clinic





Feins, S.; Kong, W.; Williams, E. F.; Milone, M. C.; Fraietta, J. A. *Am. J. Hematol.* **2019**, *94*, S3.

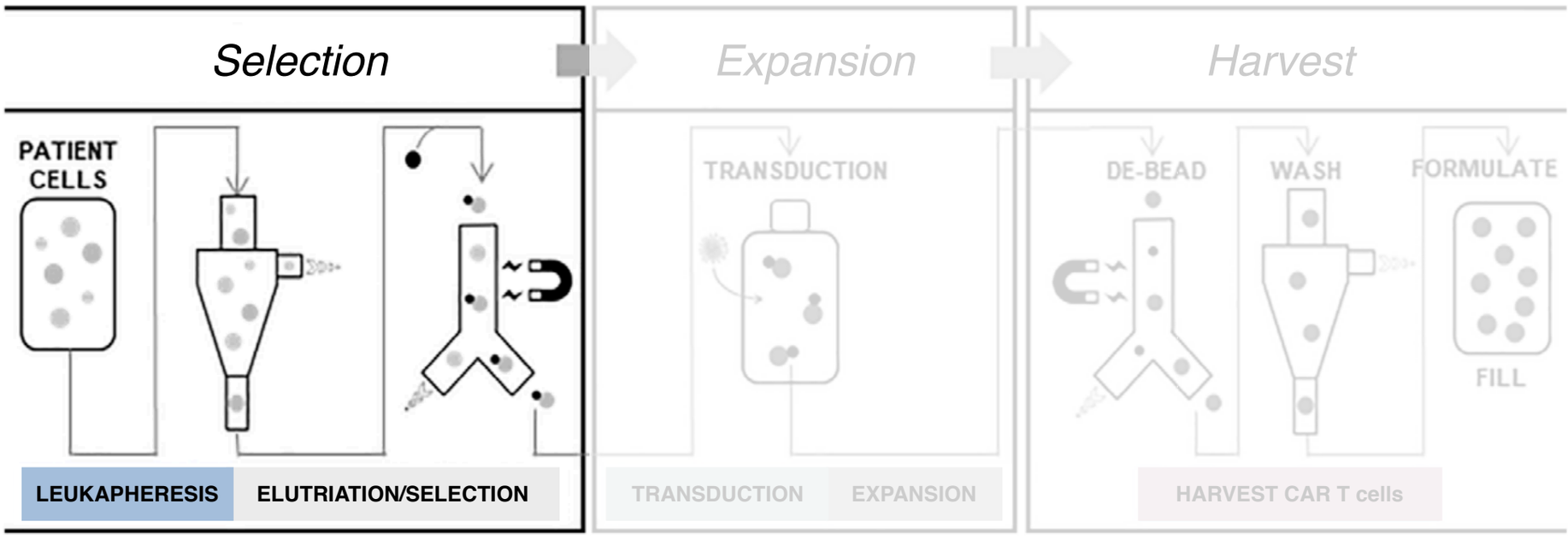
CAR T cell Therapy in the Clinic



Leukapheresis: remove cells from patient's bodies and centrifuge

Feins, S.; Kong, W.; Williams, E. F.; Milone, M. C.; Fraietta, J. A. *Am. J. Hematol.* **2019**, *94*, S3.

CAR T cell Therapy in the Clinic



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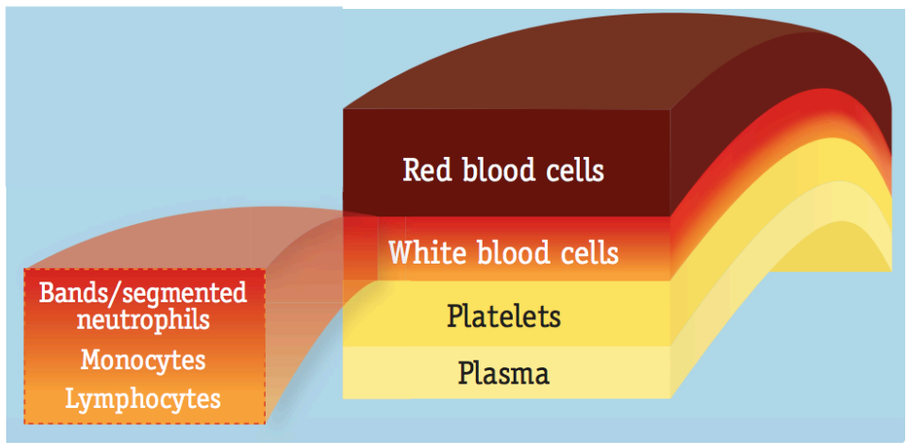
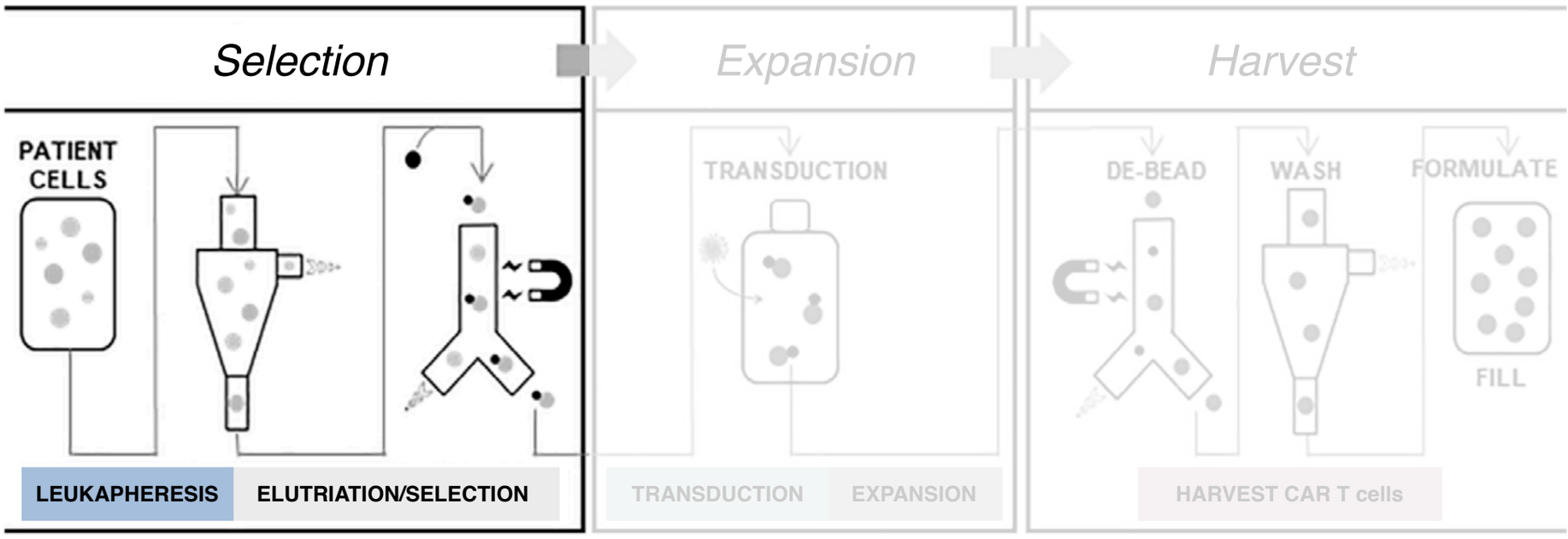


potential issues in this step:

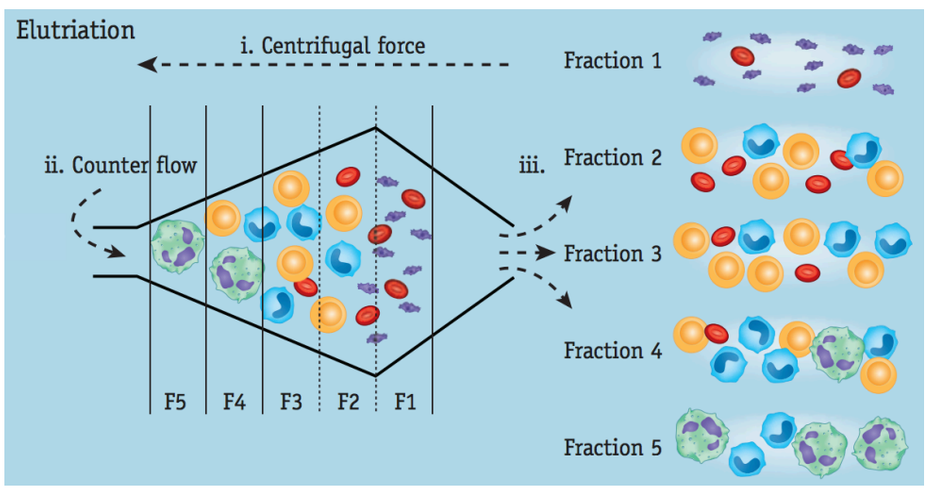
- *low lymphocyte blood count for patient treated with chemo*
- *impure samples may inhibit growth in culture or may contain tumor cells*

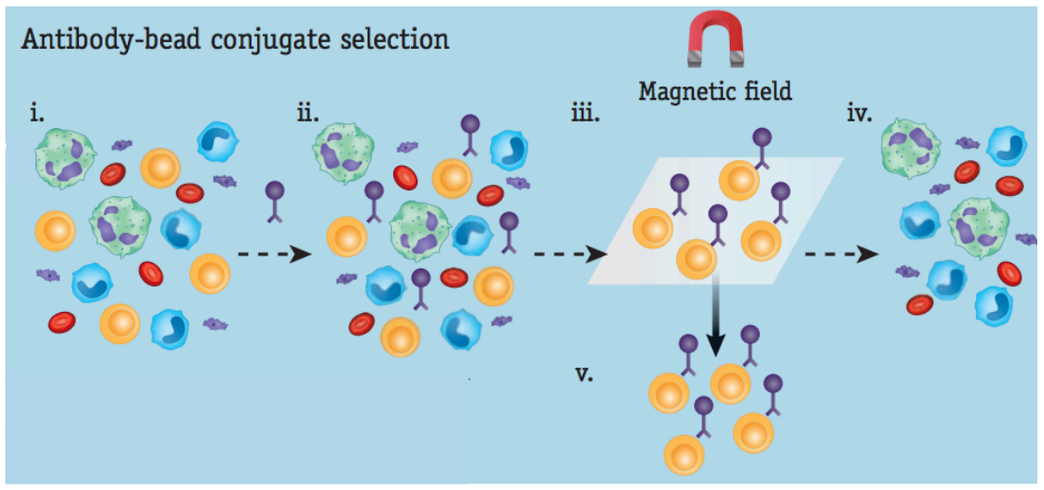
Feins, S.; Kong, W.; Williams, E. F.; Milone, M. C.; Fraietta, J. A. *Am. J. Hematol.* **2019**, *94*, S3.

CAR T cell Therapy in the Clinic



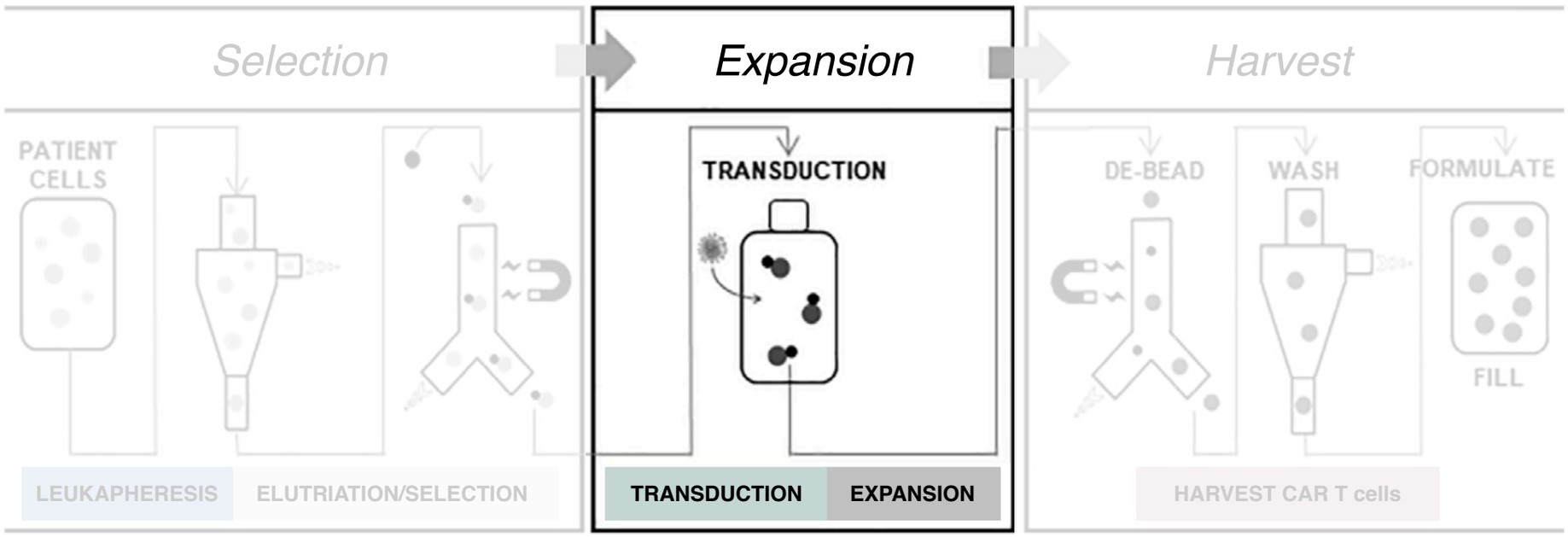
Elutriation and Selection: seperation based on cell size and density followed by immunomagnetic bead selection



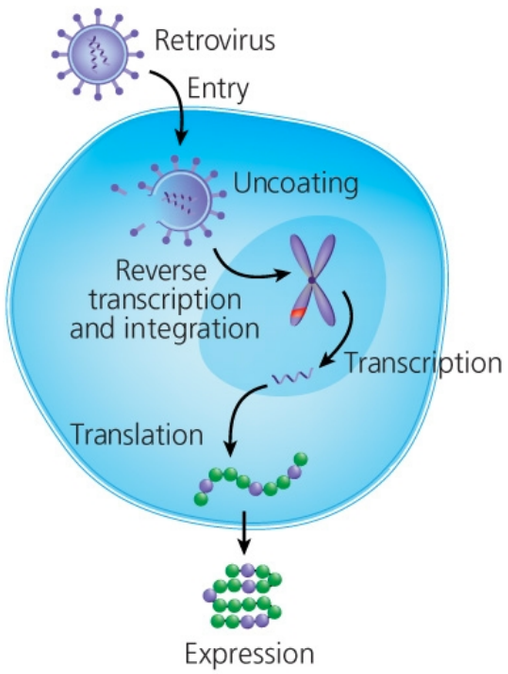


Fesnak, A. D.; Suhoski Davis, M. M.; Levine, B. L. *Nat. Prot.* **2017**

CAR T cell Therapy in the Clinic



Viral Transduction: using viral vectors from retroviruses or lentiviruses to deliver desired CAR gene

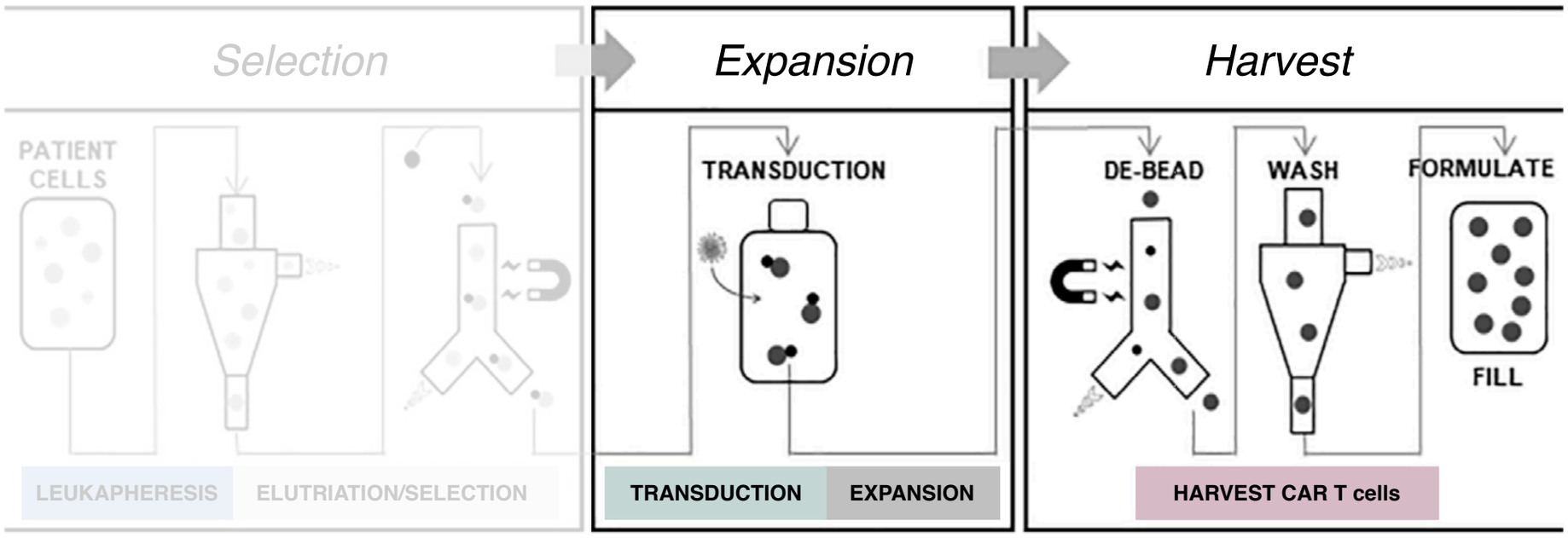


potential issues in this step:

gene modification may lead to some ex vivo cytotoxicity leading to cell loss during manufacturing

Fesnak, A. D.; Suhoski Davis, M. M.; Levine, B. L. *Nat. Prot.* **2017**

CAR T cell Therapy in the Clinic



Expansion and T cell harvest: activate T cells for proliferation, de-bead, wash and package

overall time: ~ 2 weeks

during this time: patients receive chemotherapy to kill remaining lymphocytes

Fesnak, A. D.; Suhoski Davis, M. M.; Levine, B. L. *Nat. Prot.* **2017**

Outline

Introduction to CAR T cell Therapy

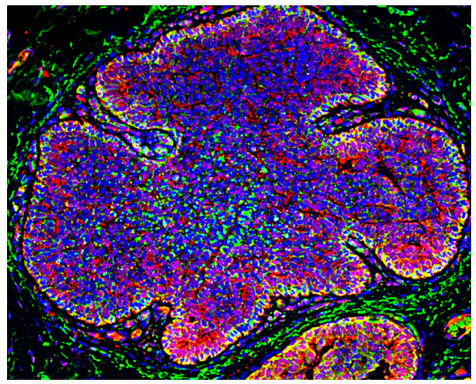
- current cancer treatment options
- introduction to immunotherapy
- definition of a CAR T cell
- building a CAR

CARs in the Clinic

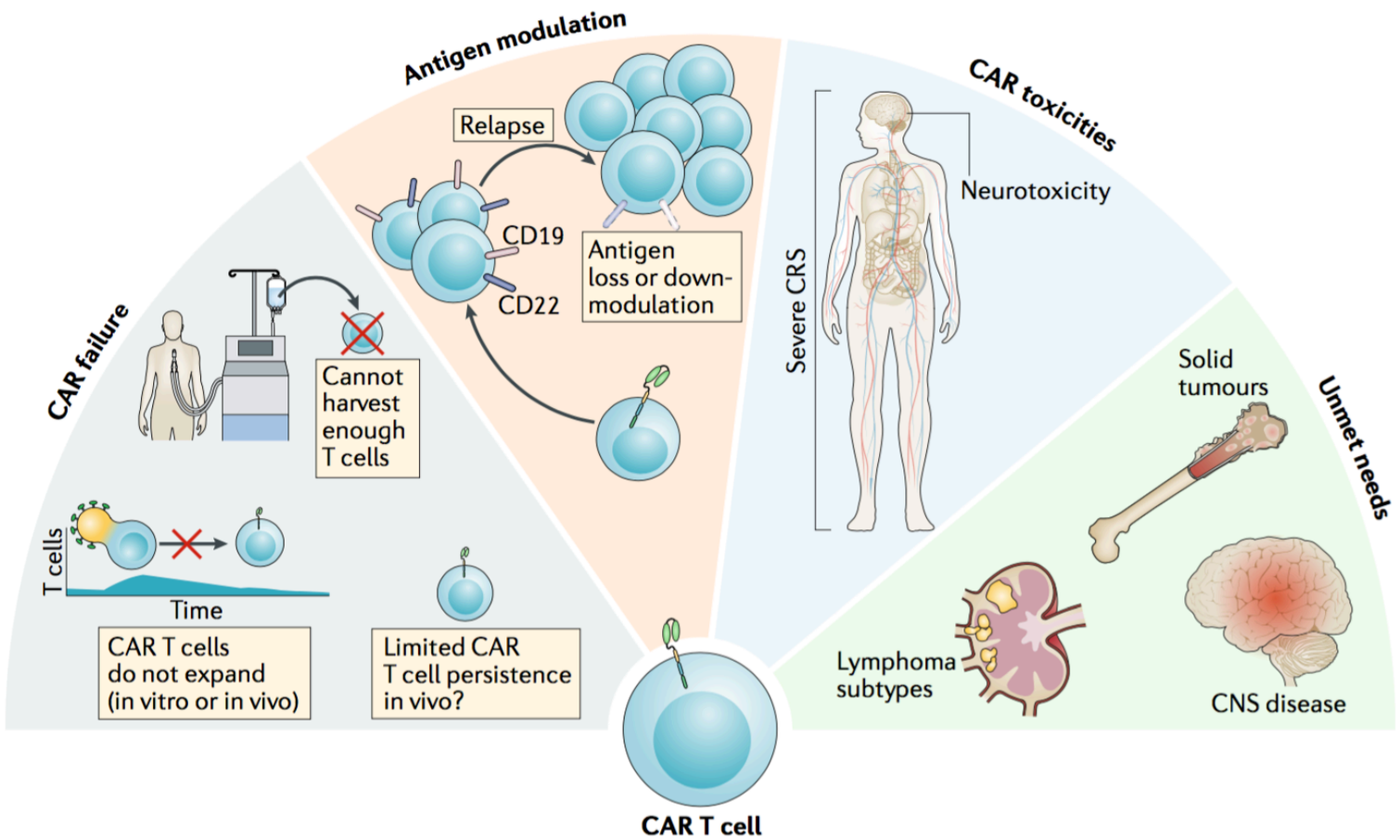
- how CARs are prepared and administered
- preclinical studies
- clinical trials leading to FDA approvals

Current Limitations and Moving Forward

- toxicity
- difficulties extending to solid tumors
- CARs beyond cancer



Current Limitations to CAR T cell Therapy



Expansion	**CAR manufacturing**	**Persistence**
CAR T cells not proliferating leads to poor response	poor quality of cells from patients w/ advanced cancer and pre-treatment	poor persistence of CARs may lead to relapse

Shah, N. N.; Fry, T. J. *Nat. Rev. Clin. Oncol.* **2020**, *16*, 372.

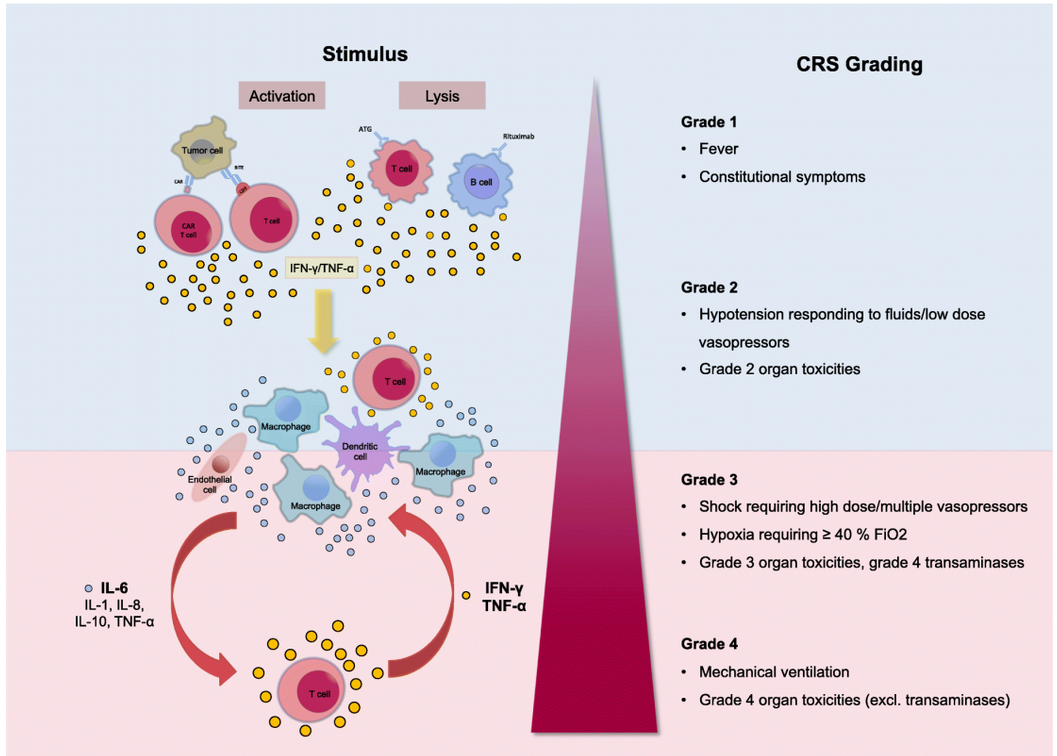
Overcoming Treatment-Related Toxicities

Toxicity related to T cell activation:

systemic release of high cytokine levels

on-target, off tumor effect

CAR interact with target on non-malignant cells



normal B-cell

cancer B-cell

Rafiq, S.; Hackett, C. S.; Brentjens, R. L. *Nat. Rev. Clin. Oncol.* **2020**, *17*, 147.

Overcoming Treatment-Related Toxicities

Toxicity related to T cell activation:

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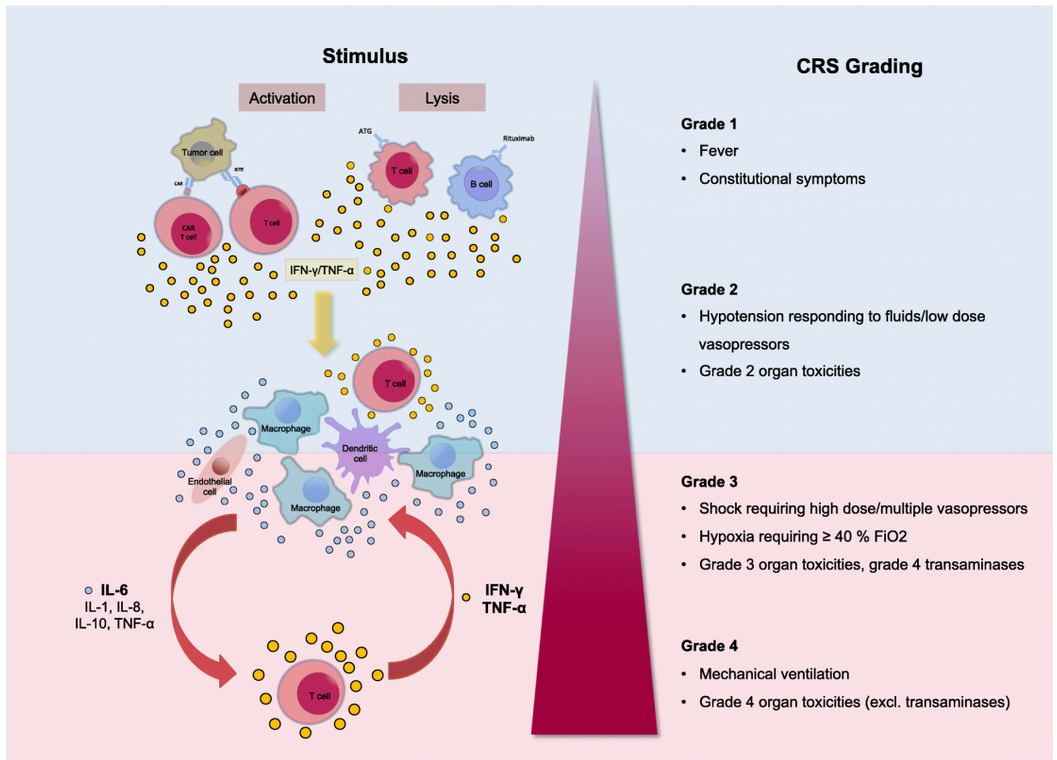


on-target, off tumor effect

CAR interact with target on non-malignant cells

Rafiq, S.; Hackett, C. S.; Brentjens, R. L. *Nat. Rev. Clin. Oncol.* **2020**, *17*, 147.

Overcoming Treatment-Related Toxicities

Cytokine release syndrome:

a large number of T cells are activated and release inflammatory cytokines which activate more T cells

Treatments:

IL-6 among core cytokines consistently elevated in CRS

treatment with anti-IL-6 antibody (tocilizumab)

Engineering Solutions: Fine-tuning the CAR

co-stimlatory domain: 4-1BB vs. CD28

CD28: more rapid onset on activitiy and subsequent exhaustion

4-1BB: lower peak levels of expansion, increased endurance, lower risk CRS

hinge and transmembrane sequence

alterations led to slower proliferation, while retaining potency: 6/11 with complete remission

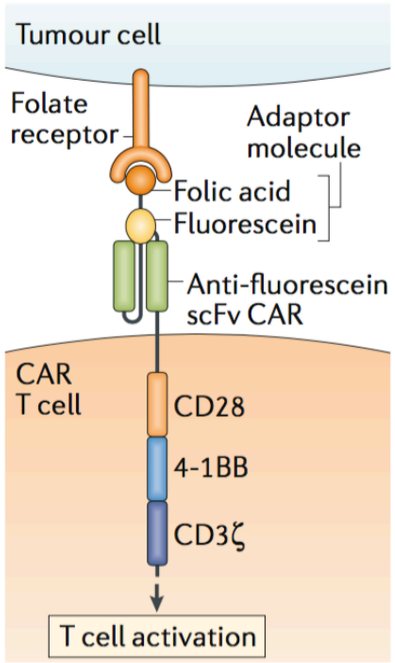
no CRS > grade 1 observed

Nat. Med. **2019**, *25*, 947

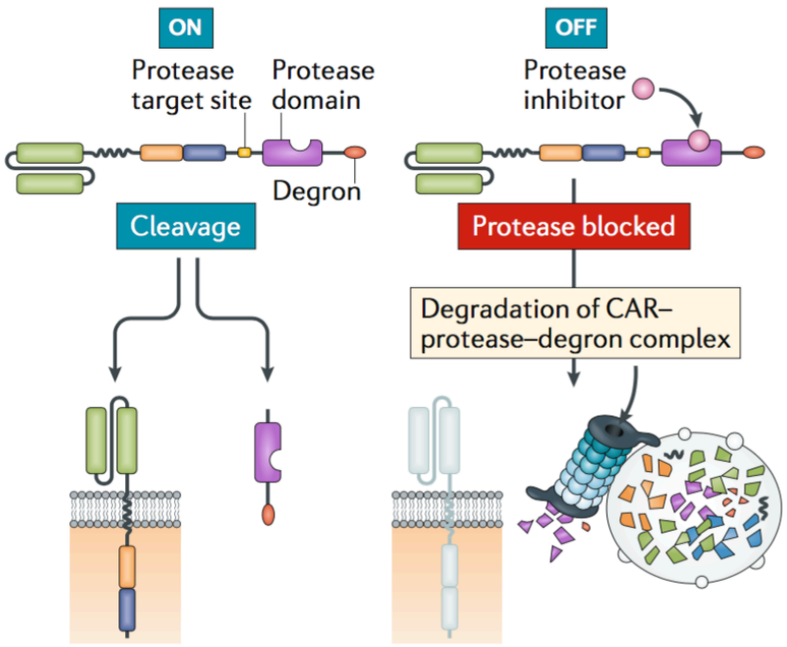
Rafiq, S.; Hackett, C. S.; Brentjens, R. L. *Nat. Rev. Clin. Oncol.* **2020***, 17*, 147.

Overcoming Treatment-Related Toxicities: Engineering Solutions

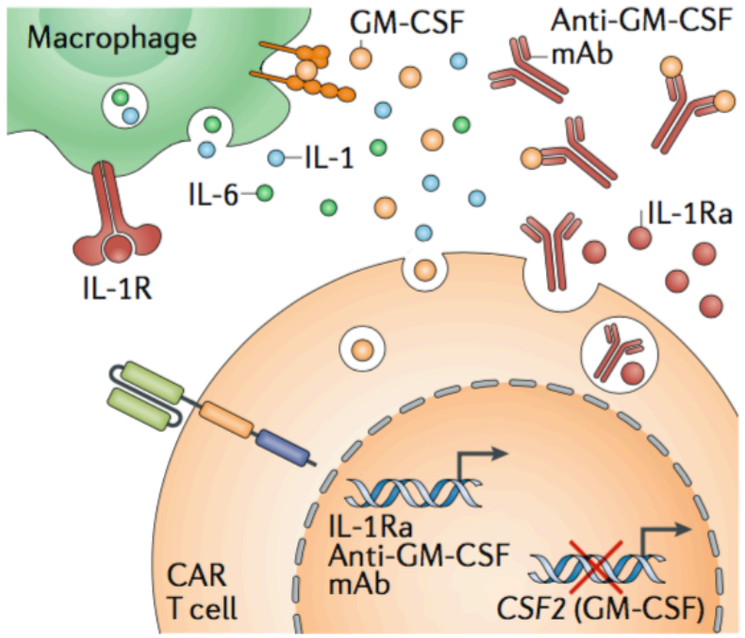
Developing On/Off switches, suicide genes, and engineering cytokine antagonists



Nat. Comm. **2019**, *10*, 1.



BMC Biotech. **2019**, *19*, 44.



J. Biol. Chem. **2019**, *294*, 5430.

knockout cytokine genes or

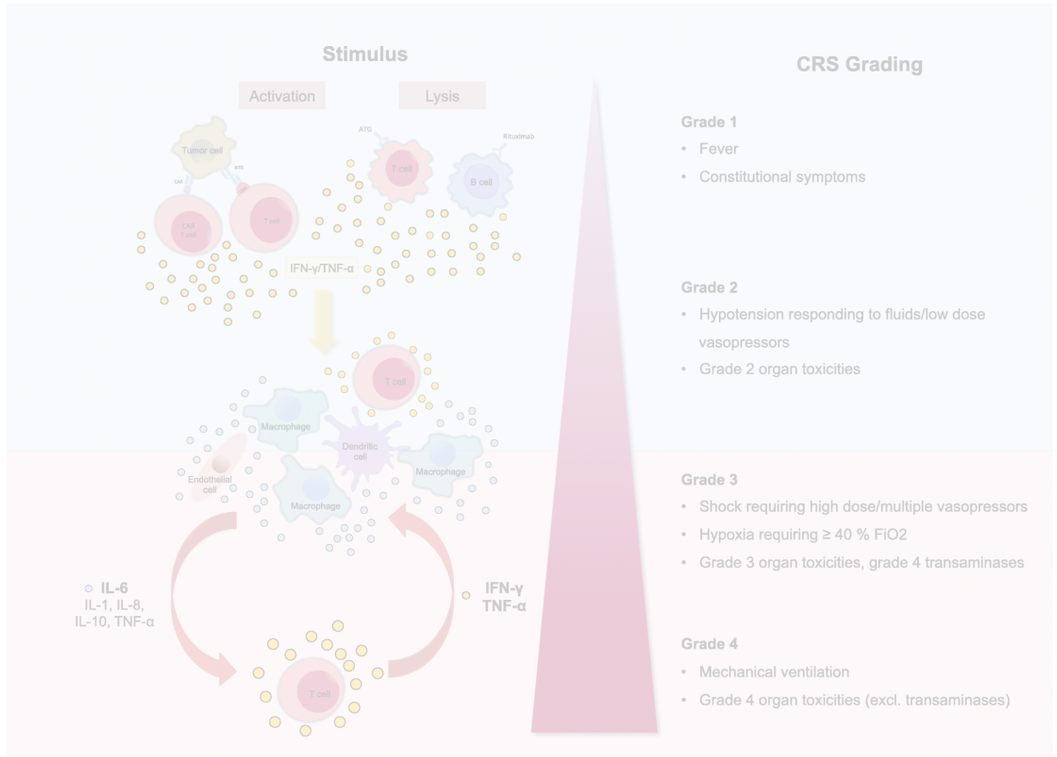
express cytokine antagonists
that neutralize relevant cytockines

Rafiq, S.; Hackett, C. S.; Brentjens, R. L. *Nat. Rev. Clin. Oncol.* **2020**, *17*, 147.

Overcoming Treatment-Related Toxicities

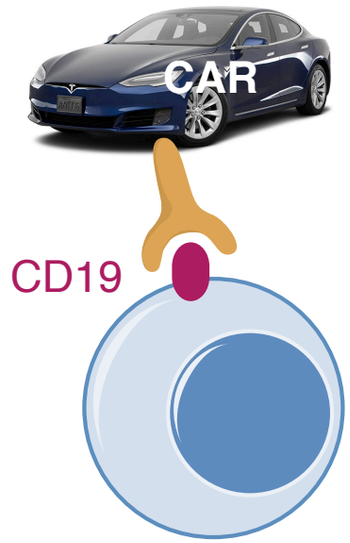
Toxicity related to T cell activation:

systemic release of high cytokine levels



on-target, off tumor effect

CAR interact with target on non-malignant cells



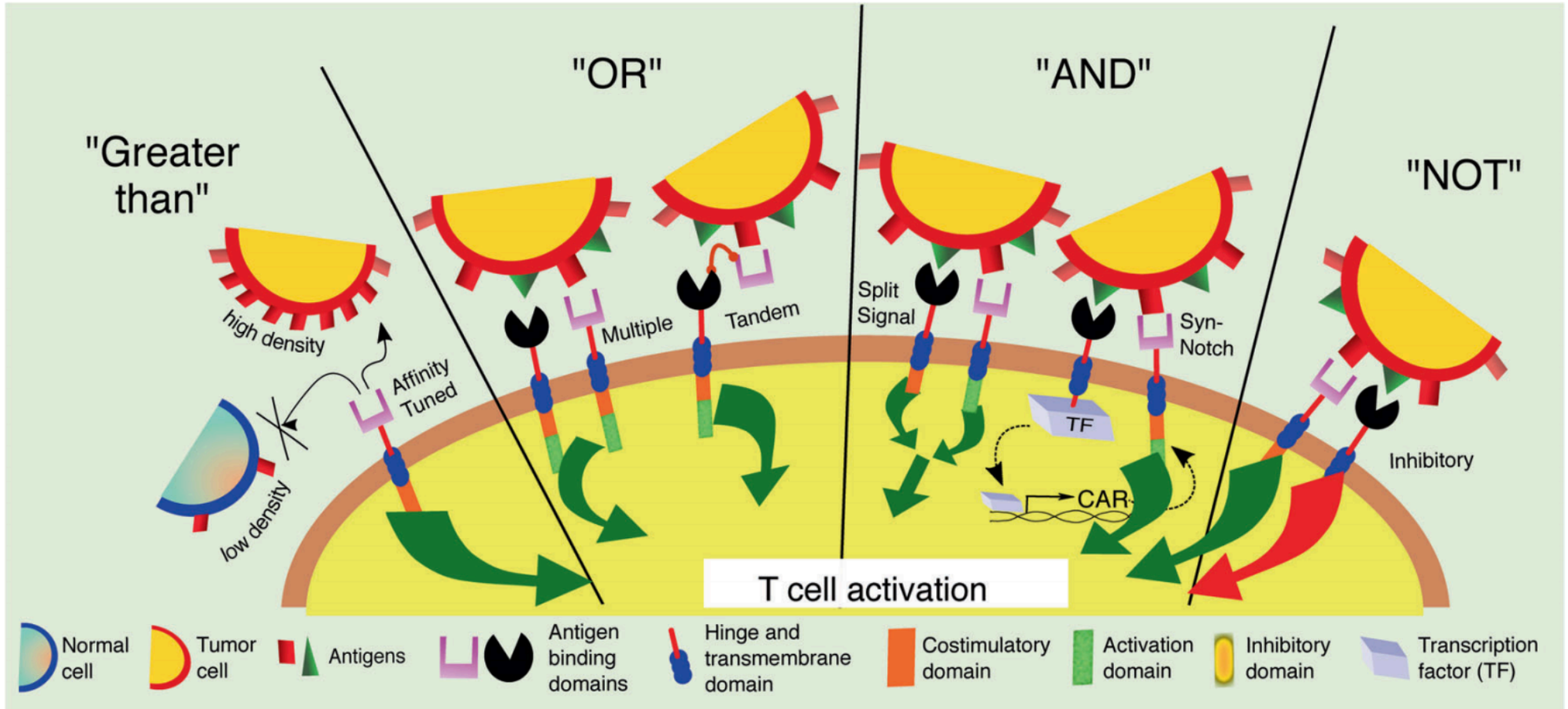
normal B-cell

cancer B-cell

Rafiq, S.; Hackett, C. S.; Brentjens, R. L. *Nat. Rev. Clin. Oncol.* **2020**, *17*, 147.

Overcoming Treatment-Related Toxicities: On-Target Off-Tumor

Methods for Better On-Target, On-Tumor Efficacy



Affinity Tuning

take advantage of antigen expression levels that are “greater than” those on normal cells

target with a lower affinity CAR

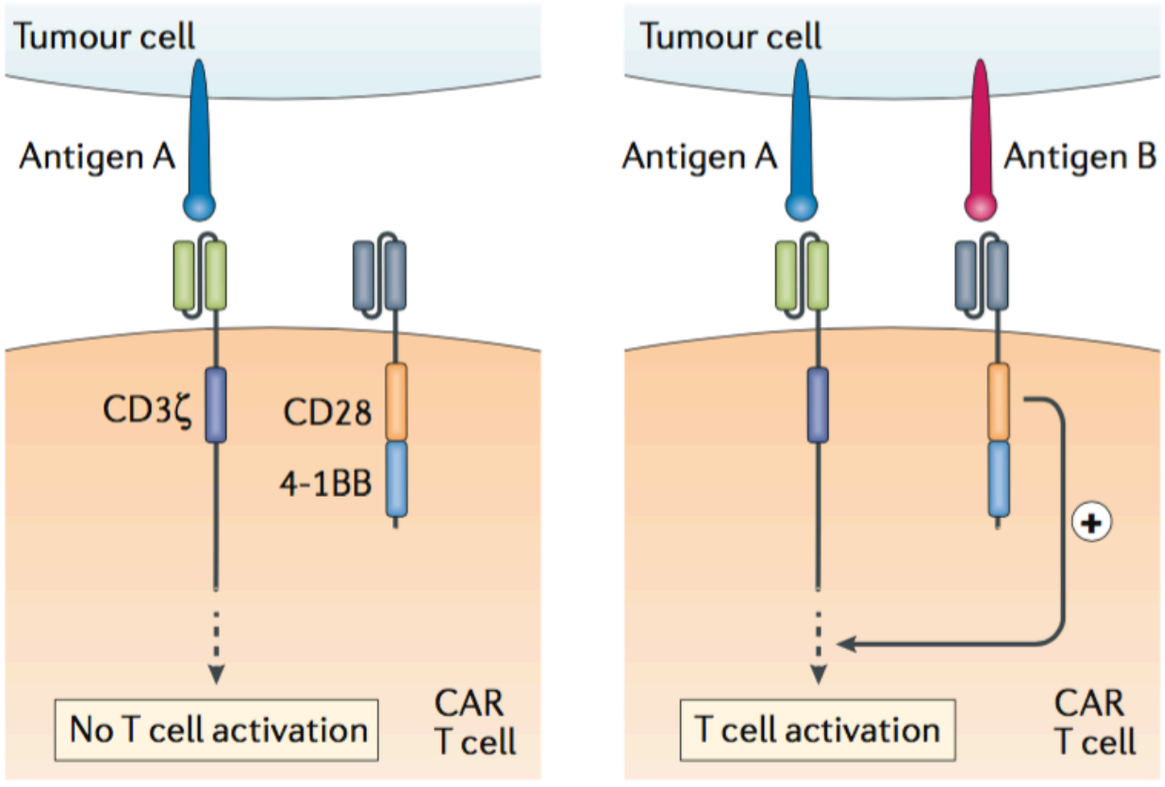
→ *downregulation of target antigen as mechanism of resistance*

Castellarin, M. et al. *Gene Ther.* **2018**, *25*, 165.

Overcoming Treatment-Related Toxicities: On-Target Off-Tumor

"AND" Logic: Two Cell-Surface Antigens Required for CAR Activation

Split CARS



Nat. Biotech. **2013**, *31*, 71. and *Canc. Immunol. Res.* **2013**, *1*, 34.

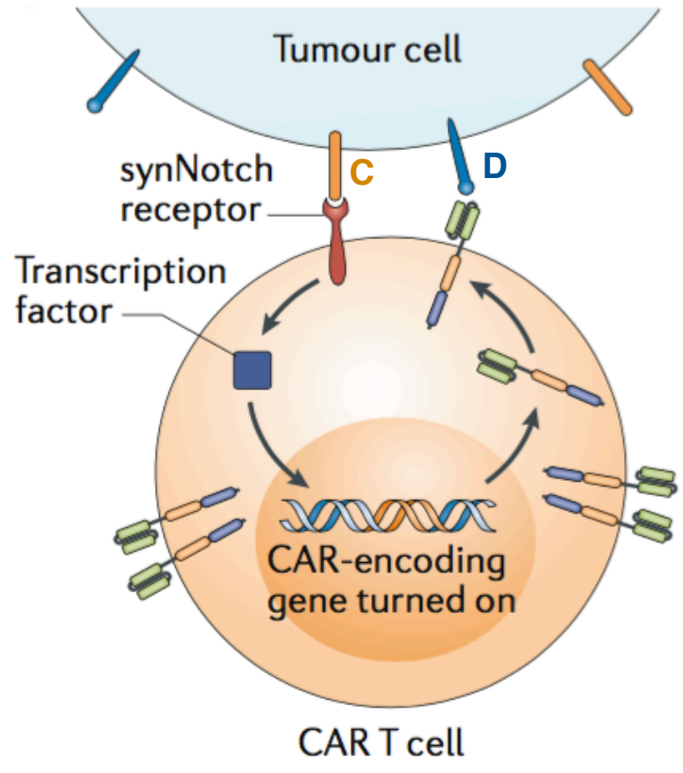
CD3ζ linked to receptor for antigen **A**

co-stimulatory domain linked to receptor for **B**

activation only occurs when both CD3ζ

and co-stimulatory domains activated

SynNotch CARs



Cell **2016**, *164*, 770.

synthetic notch receptor engineered

to recognize antigen **C**

activates TF

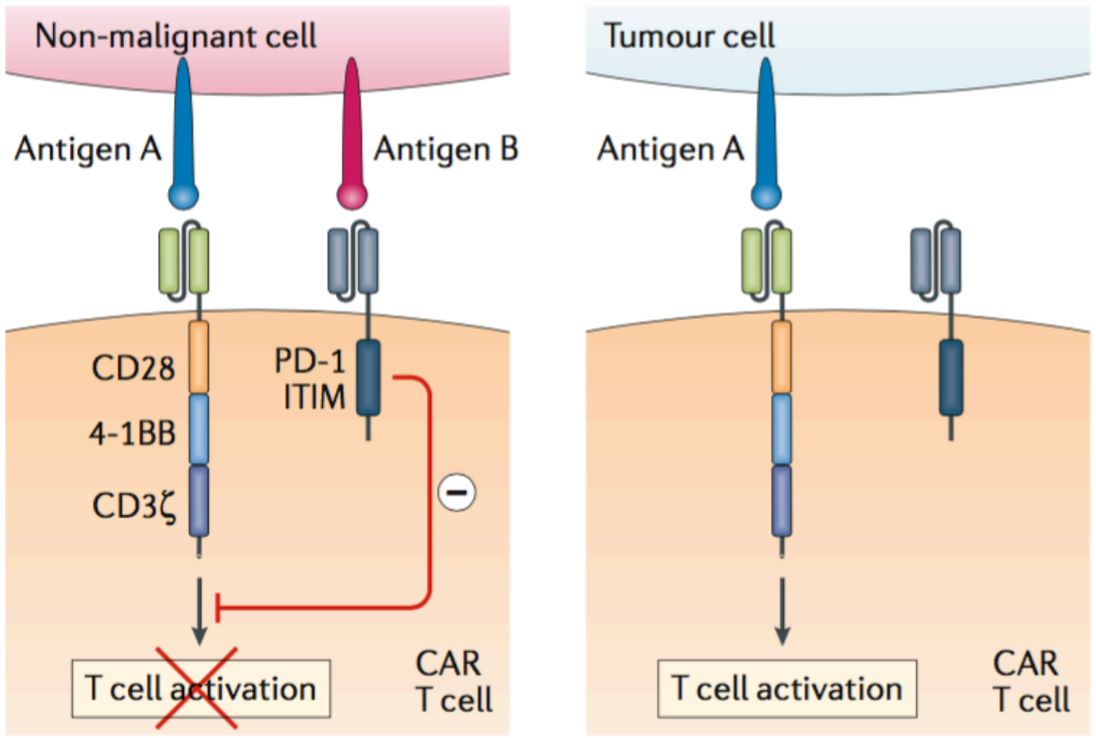
encoding a CAR for antigen **D**

Rafiq, S.; Hackett, C. S.; Brentjens, R. L. *Nat. Rev. Clin. Oncol.* **2020**, *17*, 147.

Overcoming Treatment-Related Toxicities: On-Target Off-Tumor

"NOT" Logic: Adding Inhibitory Signalling

Inhibitory CART (iCAR)



Sci. Trans. Med. **2013**, *5*, 215.

antigen **B** is expressed *only* on non-malignant cells

use inhibitory domains derived from immune-checkpoint proteins, e.g. PD-1

thereby only killing tumor cells

CRISPR/Cas9-based approach

- CD33 is a common target for acute myeloid leukemia (AML)
- CD33 is expressed on most myeloid cells but non-essential if knocked-out

knock-out CD33 from stem cells

- anti-CD33 CARs selectively target CD33+ AML without killing non-malignant cells in a mouse model

Cell **2018**, *173*, 1439.

Rafiq, S.; Hackett, C. S.; Brentjens, R. L. *Nat. Rev. Clin. Oncol.* **2020**, *17*, 147.

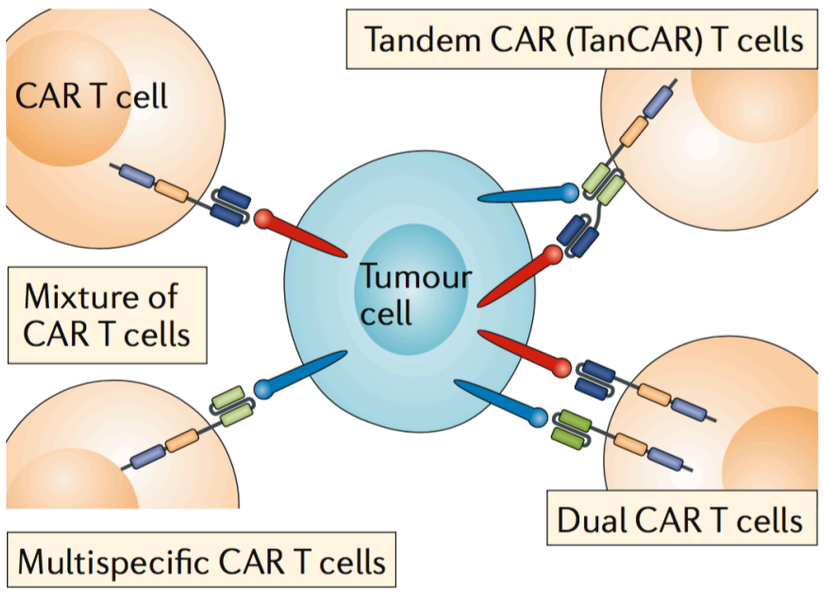
Overcoming CAR T Resistance with Multi-Target Strategy

antigen escape as resistance mechanism

complete of partial loss of target antigen expression from cancer cells

7–25% of anti-CD19 CAR patients relapse with CD19⁻ disease

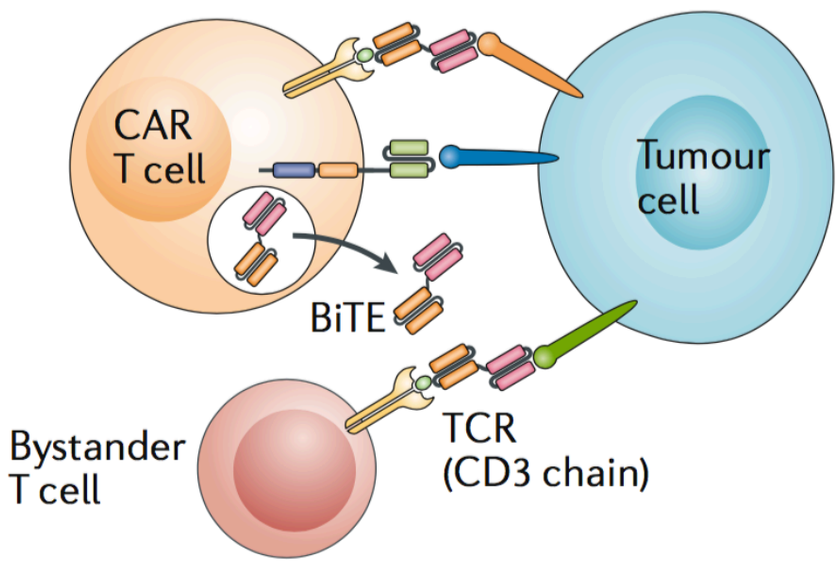
targeting multiple antigens: combinatorial strategy



numerous worldwide clinical trials

co-targeting e.g., CD19/CD20, CD19/CD22

CAR.BiTEs



modify CAR to excrete

bi-specific T cell engager (BiTE)

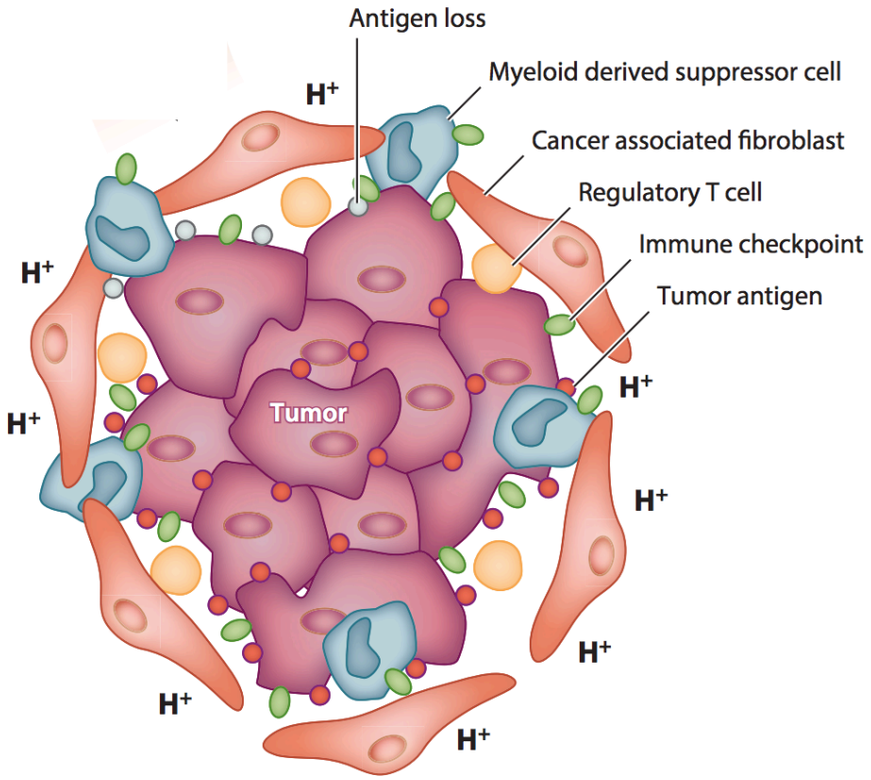
which recruit T cells to tumor

Rafiq, S.; Hackett, C. S.; Brentjens, R. L. *Nat. Rev. Clin. Oncol.* **2020**, *17*, 147.

Majzner, R. G.; Mackall, C. L. *Cancer Discov.* **2018**, *8*, 1219.

Overcoming Hostile Tumor Microenvironment

major challenge in CAR T therapy: developing succesful therapy for solid tumors



hostile tumor microenvironment

extracellular environment suboptimal for T cell function

- hypoxia
- acidification
- nutrient shortage
- immunosuppressive molecules

need to engineer new CARs to successfully move to solid tumors

Newick, K.; O'Brien, S.; Moon, E.; Albelda, S. M. *Annu. Rev. Med.* **2017**, *68*, 139.

Targeting Solid Tumors: Overcoming Hostile Tumor Microenvironment

overcoming antigen heterogeneity

can target multiple antigens:

ex: CART.BiTEs

bringing to the CAR to the solid tumor

direct injection into tumor,

(may not always be possible)

overcoming T cell inhibitory signalling

co-administer anti-PD-1 antibodies or engineer a disruption of PD-1 pathway into CAR

penetrating the solid tumor microenvironment

program the CARs to secrete tumor extracellular matrix-modifying enzymes

altering the milieu of the tumor microenvironment

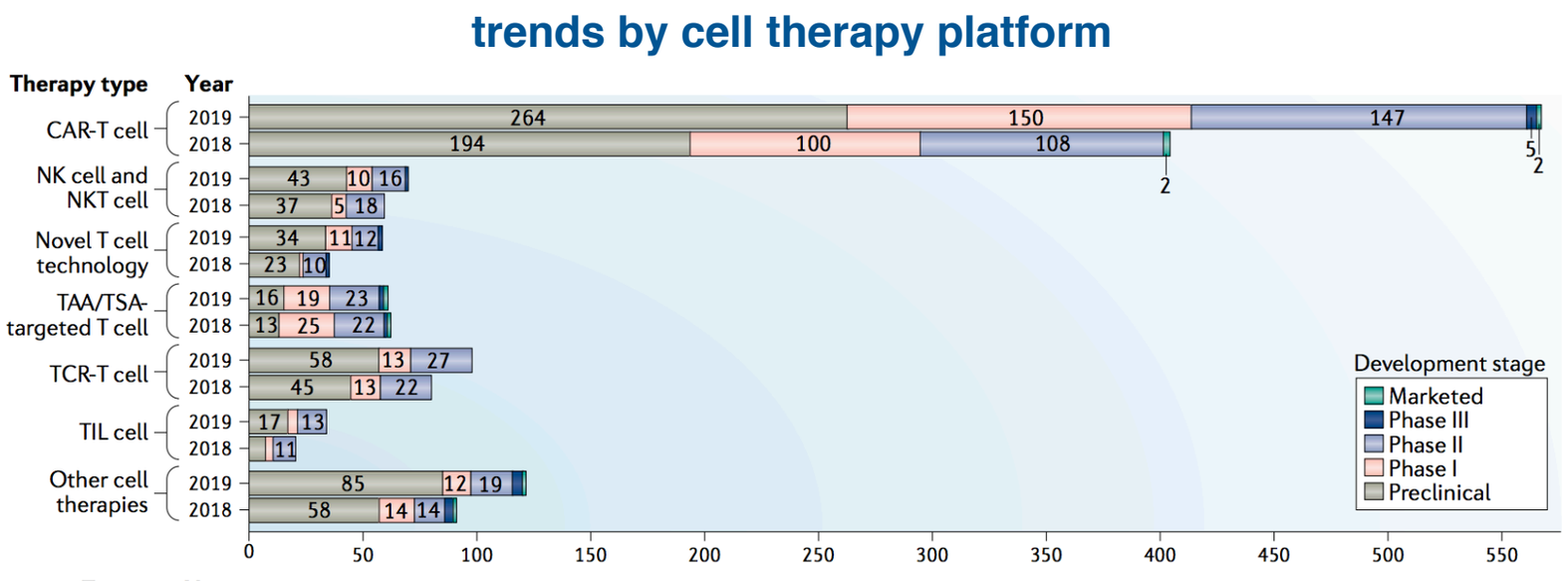
armored CARs and TRUCKS
(T cell redireted for universal cytokine killing)
T cells secrete cytokine or immunmodulatory ligand to alter inflammatory microenvironment

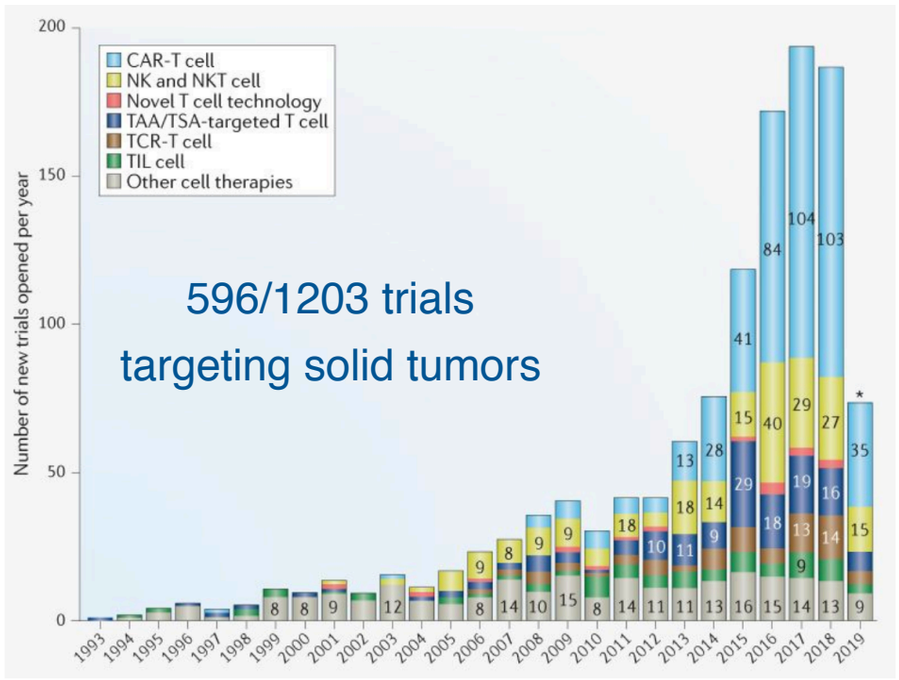
For comprehensive reviews on the area, see:

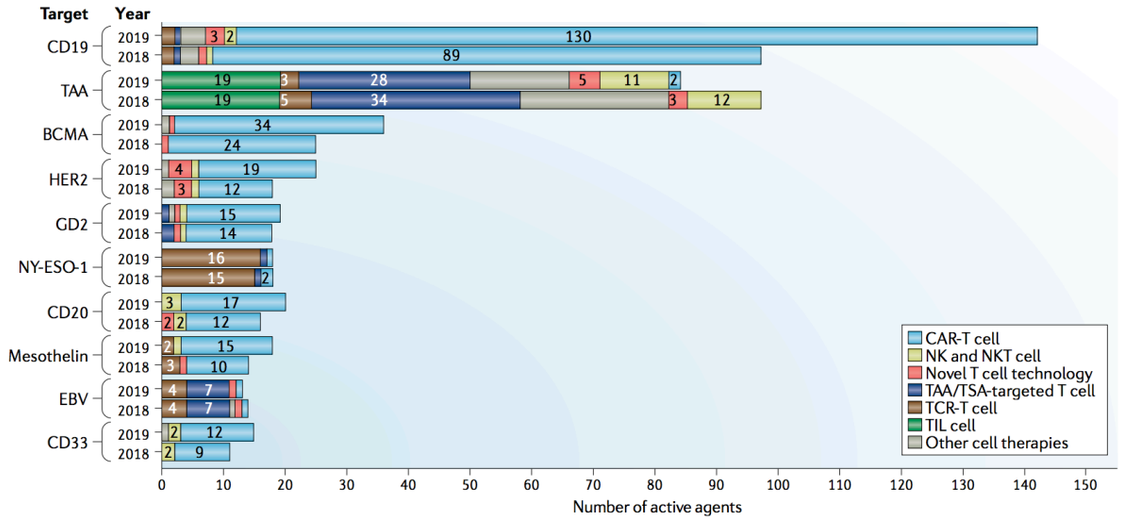
Martinez, M.; Moon, E. K. *Front. Immunol.* **2019**, *10*, 1.

Rafiq, S.; Hackett, C. S.; Brentjens, R. L. *Nat. Rev. Clin. Oncol.* **2020***, 17*, 147.

Global Clinical Trials for CAR T cell Therapy





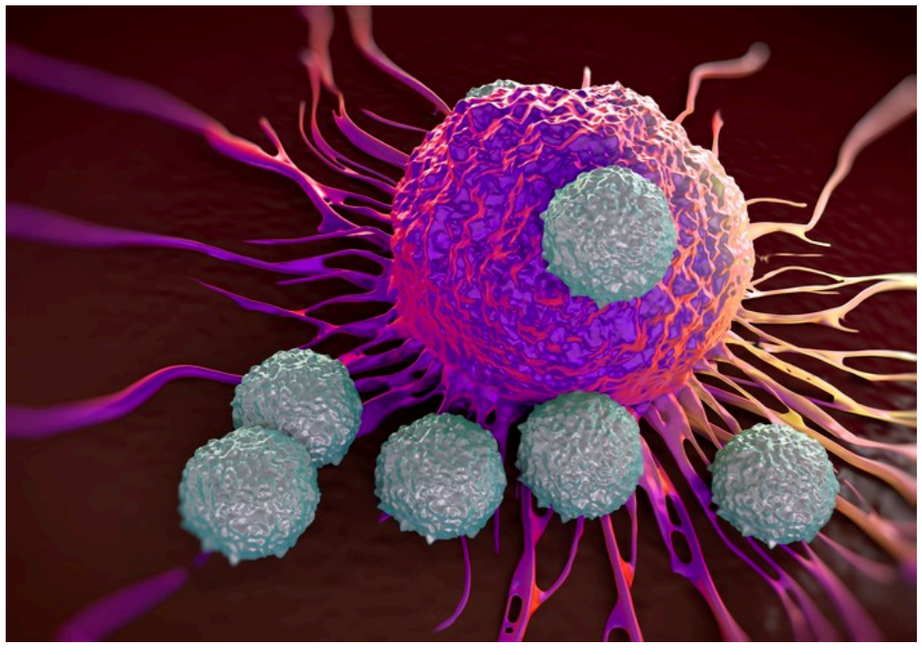


CD19 still primary target

Yu, J. X.; Hubbard-Lucey, V. M.; Tang, J. *Nat. Rev. Drug Discov.* **2019**, *18*, 820.

CARs: Looking Forward

“off-the-shelf” T cells: allogeneic cell sources (from a healthy donor) to minimize cost of cell manufacturing



CARs beyond cancer therapies: including infection diseases, HIV, autoimmune diseases

Sadelain, M.; Rivière, I.; Riddell, S. *Nat. Rev.* **2017**, *545*, 423.

Questions?