CAR-T cells and bispecific antibodies: mechansisms of action and toxicities

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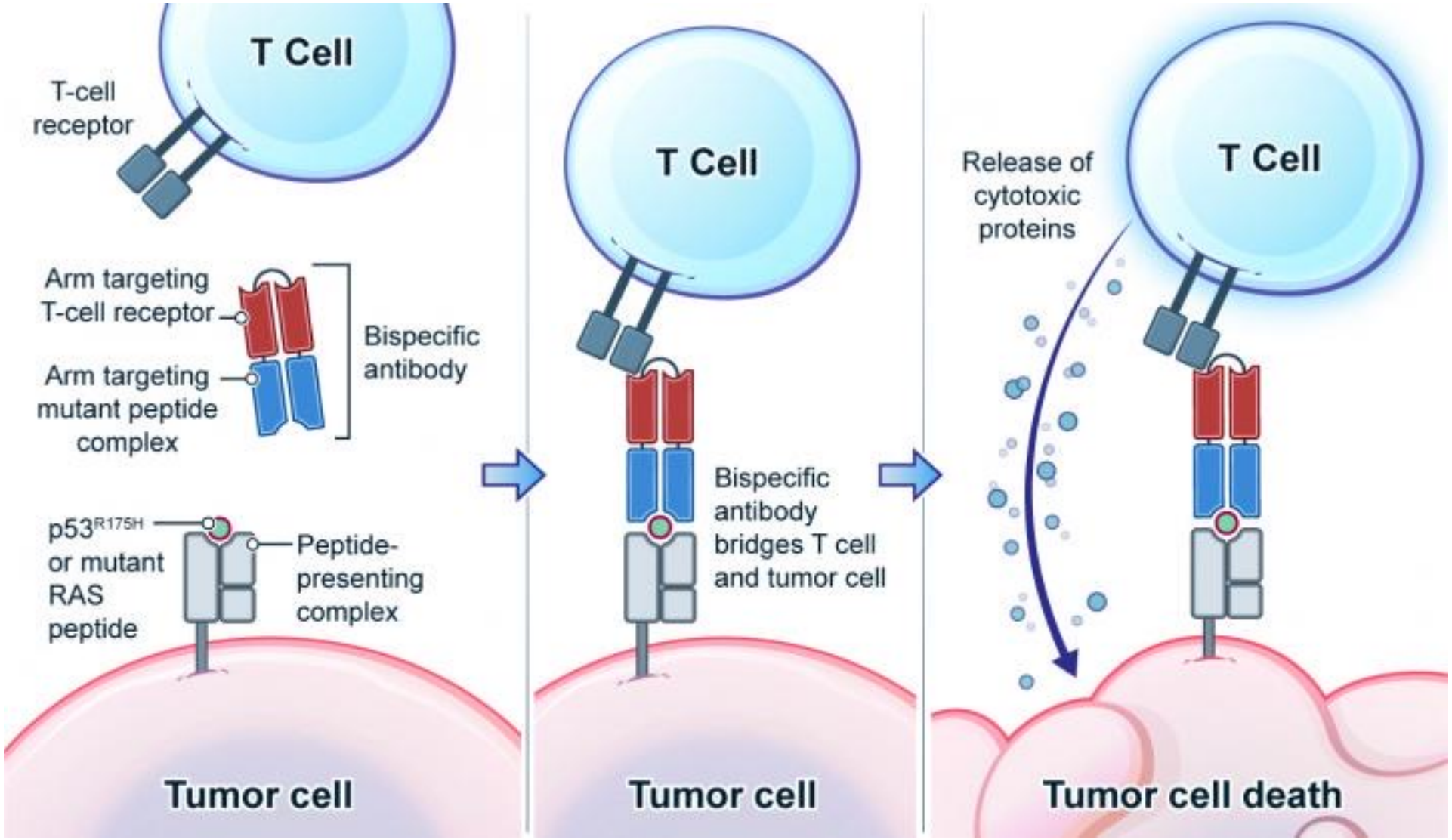
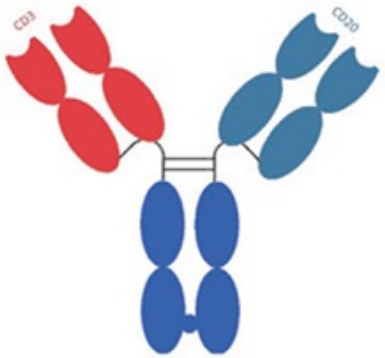
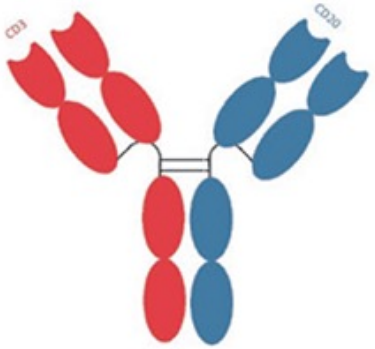
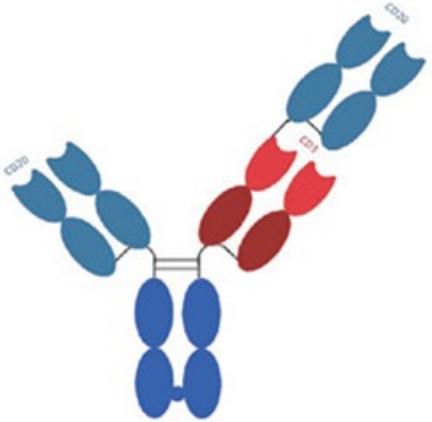
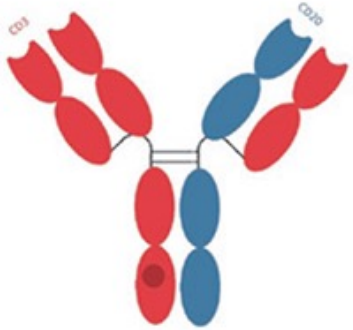
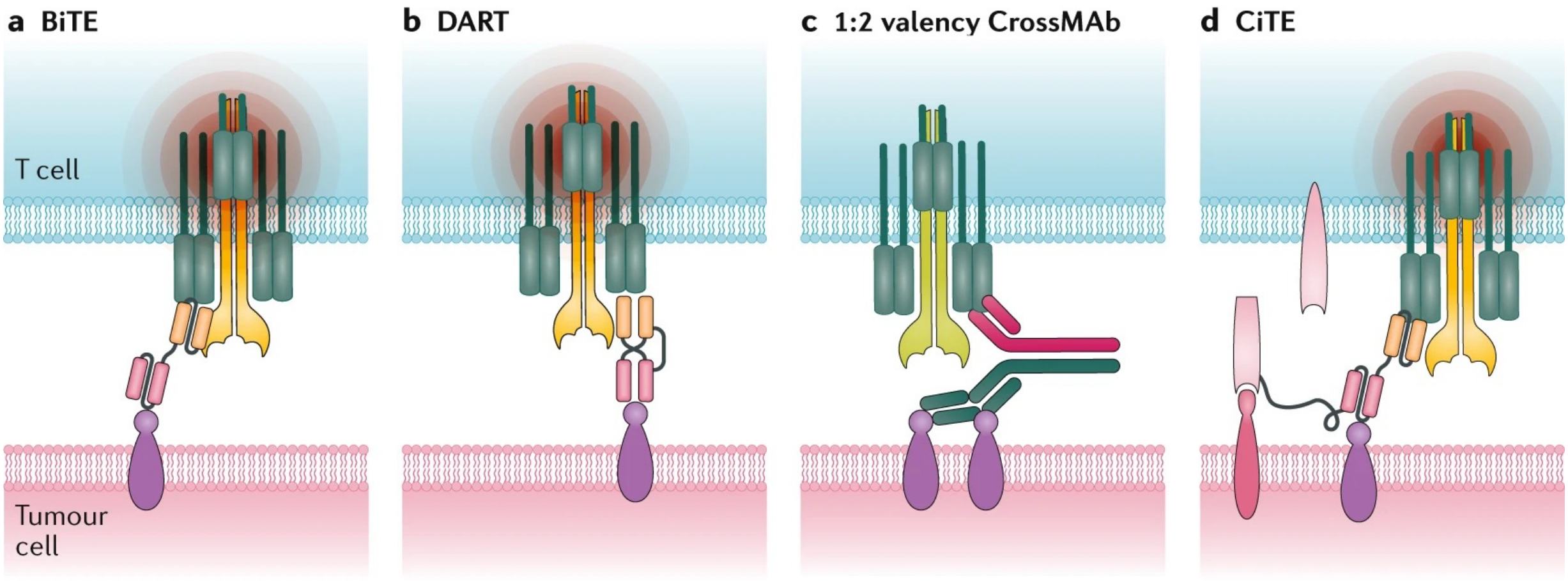
Mechanisms of Action of Bispecific Antibodies



Bi-specific antibodies

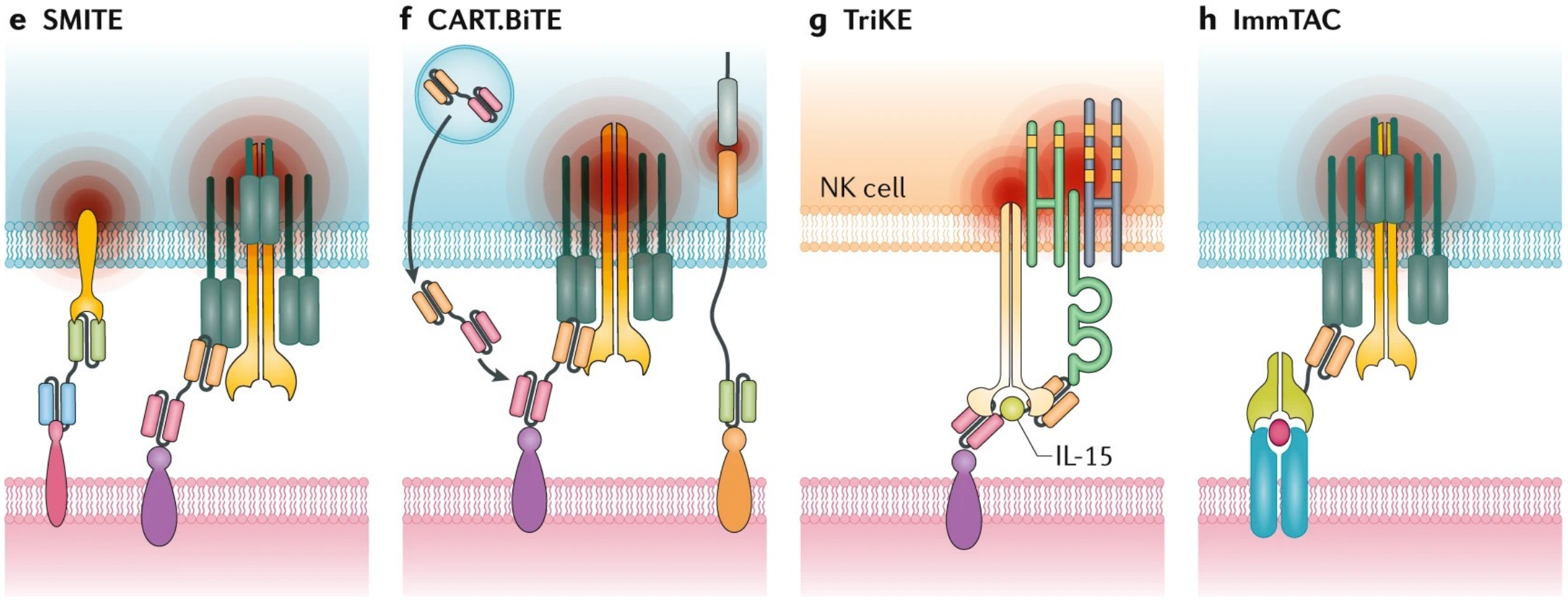
	BLINATUMOMAB	MOSUNETUZUMAB	EPCORITAMAB	GLOFITAMAB	ODRONEXTAMAB
TARGET	CD3xCD19	CD3xCD20	CD3xCD20	CD3x$(CD20)_2$	CD3xCD20
DESIGN					
	• Monovalent CD3 and monovalent CD19 binding • Two murine scFv-joined by a glycine–serine linker	• Monovalent CD3 and monovalent CD20 binding • Humanized mouse IgG1-based antibody	• Monovalent CD3 and monovalent CD20 binding • Humanized mouse IgG1-based antibody	• Monovalent CD3 and bivalent CD20 binding • Humanized mouse IgG1-based antibody	• Monovalent CD3 and monovalent CD20 binding • Fully human IgG4-based antibody

Innovative bispecific Antibodies



Goebeler Nat Rev Clin Oncol, 2020

Innovative bispecific Antibodies

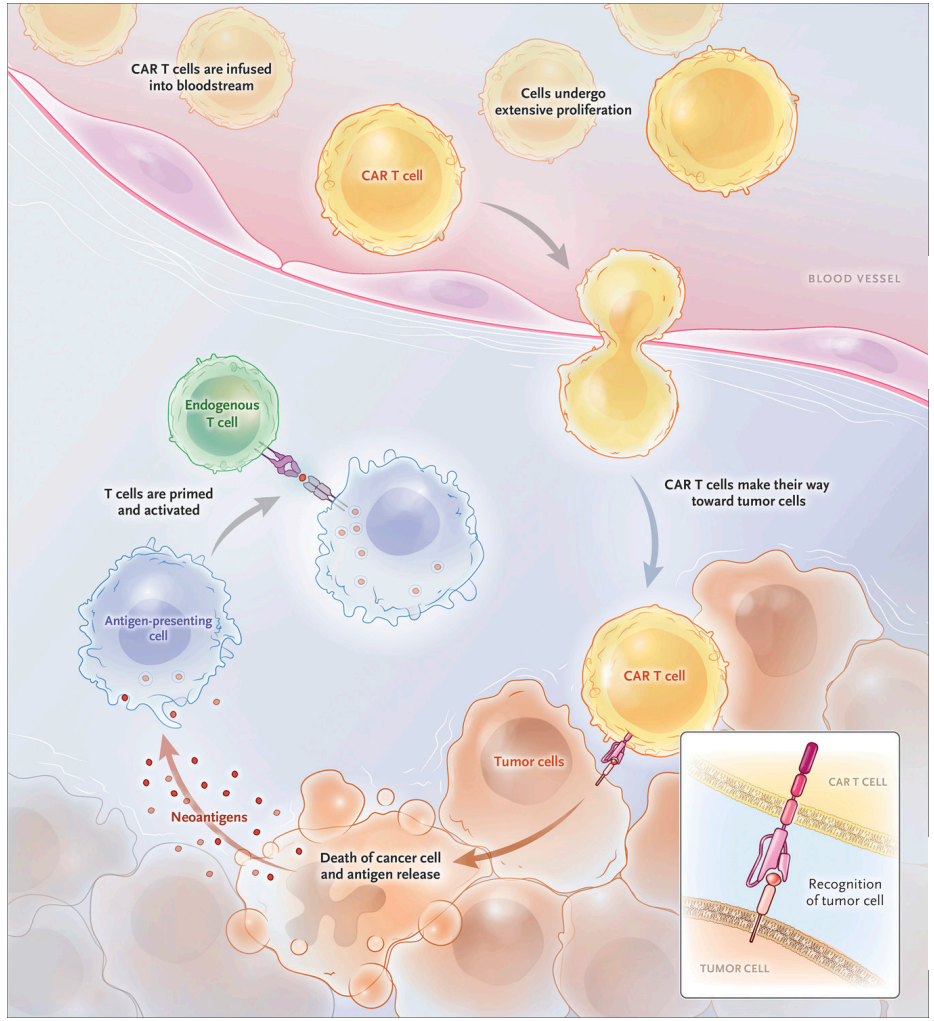


Goebeler Nat Rev Clin Oncol, 2020

Approved Bispecific Antibodies

Approved Bispecific Antibodys					
Company	Trade Name	Drug Name	Targets	First Approval	Indications
Trion Pharma	Removab	Catumaxomab	CD20/EpCAM	2009 (withdrawn In 2017 ,EMA)	Malignant ascites
Amgen	Blincyto	Blinatumomab	CD3/CD19	2014 (FDA)	R/R precursor B-cell acute lymphoblastic leukemia (ALL)
Roche	Hemlibra	Emicizumab	FIXa/FX	Nov 2017 (FDA)	Bleeding due to hemophilia A
Janssen	Rybrevant	Amivantamab-vmjw	EGFR/cMet	May 2021 (FDA)	Non-small cell lung cancer
Immunocore	Kimmtrak	tebentafusp-tebn	GP100/CD3	Jan 2022 (FDA)	unresectable or metastatic uveal melanoma
Genentech	Vabysmo	Faricimab-svoa	Ang-2/VEGF-A	Jan 2022 (FDA)	Wet AMD and DME
Roche	Lunsumio	Mosunetuzumab	CD20/CD3	Jun 2022 (EMA)	R/R follicular lymphoma (FL)
Akeso	开坦尼®	Cadonilimab	PD-1/CTLA-4	Jun 2022 (NMPA)	relapsed or metastatic cervical cancer
Janssen	Tecvayli	Teclistamab	BCMA/CD3	Aug 2022 (EMA)	R/R multiple myeloma

Living Drugs



CH June, M Sadelain. N Engl J Med 2018;379:64-73.

FDA News Release

FDA approval brings first gene therapy to the United States

CAR T-cell therapy approved to treat certain children and young adults with B-cell acute lymphoblastic leukemia

August 30, 2017

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Approved: August 22, 2018

FDA News Release

FDA approves CAR-T cell therapy to treat adults with certain types of large B-cell lymphoma

Yescarta is the second gene therapy product approved in the U.S.

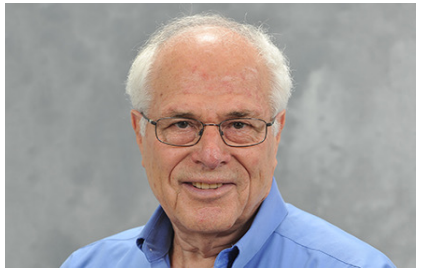
October 18, 2017

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

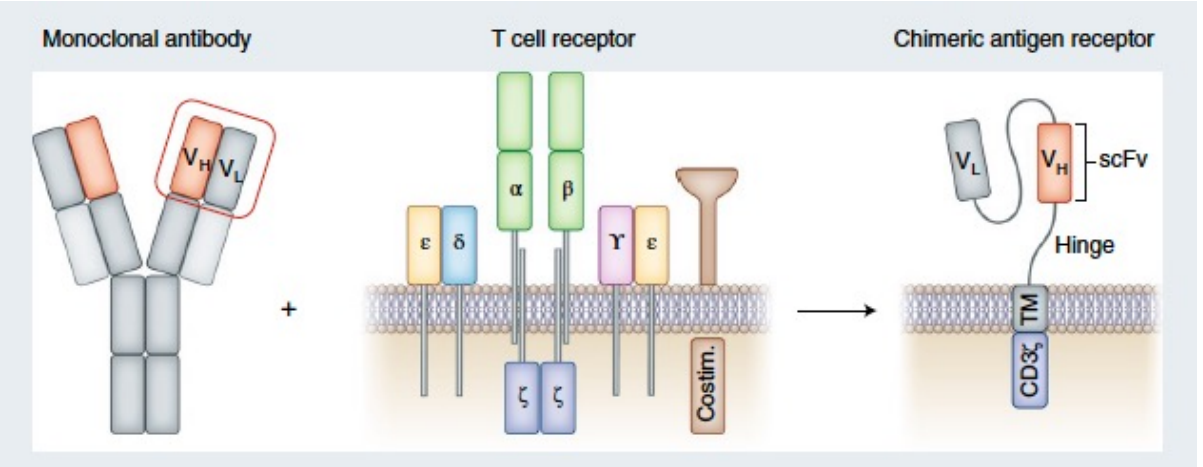
Approved: August 23, 2018

The NEW ENGLAND JOURNAL of MEDICINE

Chimeric antigen receptors



Eshhar Z et al,
PNAS 1993

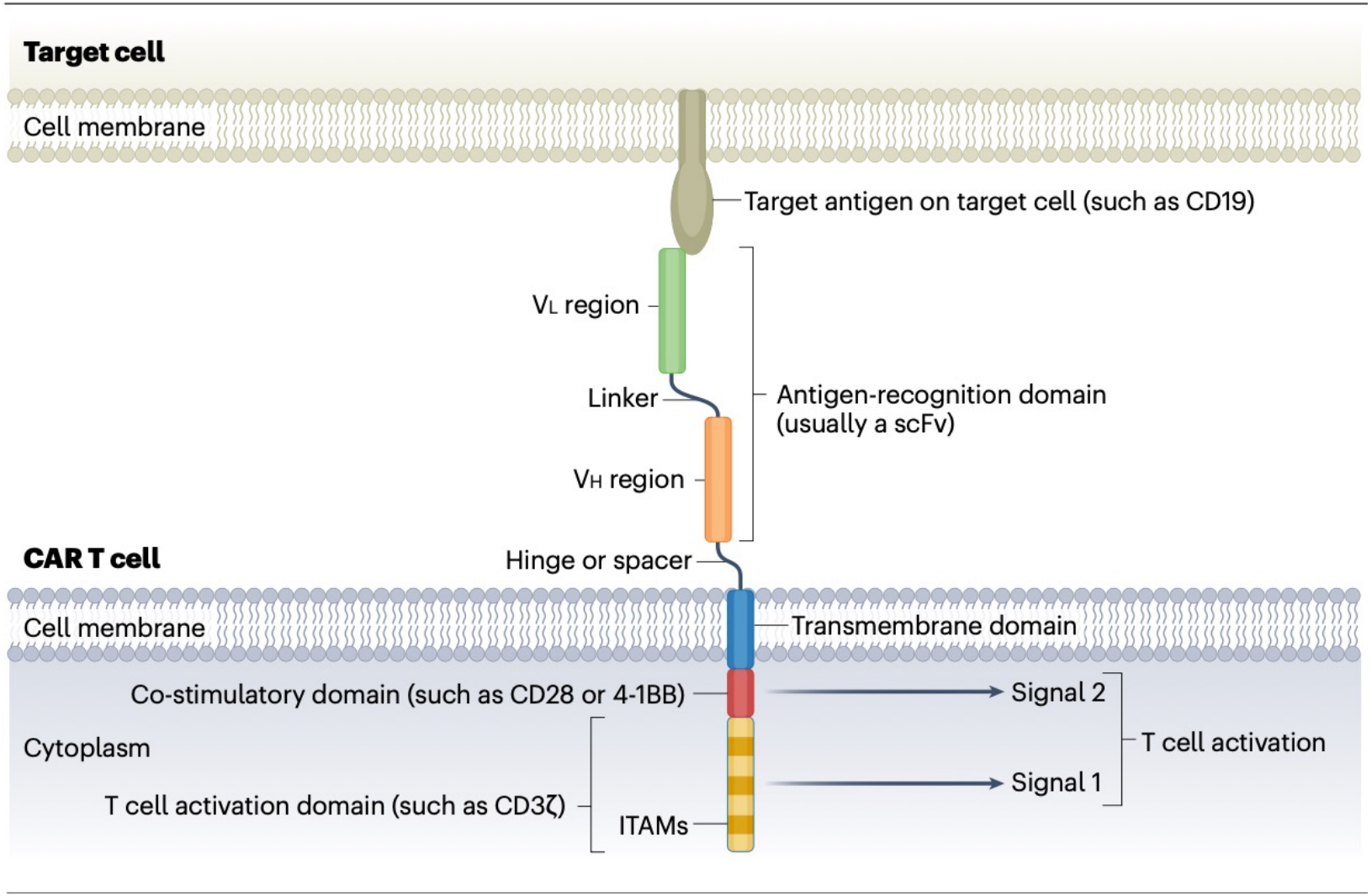


Majzner, et al Nat Med 2019

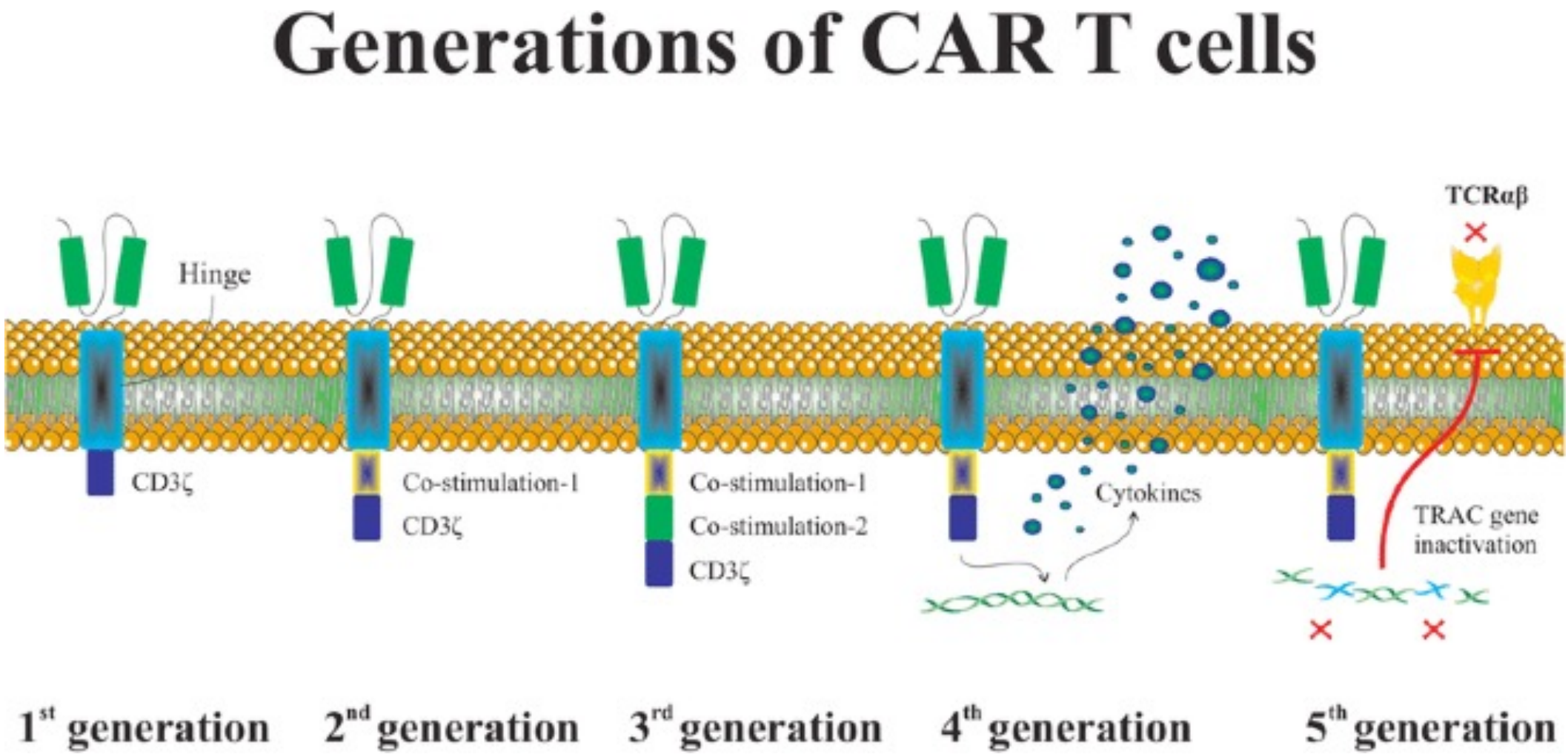
Key advantages

- Independency from MHC restriction
- Targeting of proteins, sugars, lipids
- Multiple effector mechanisms
- Living drugs (expansion, memory)

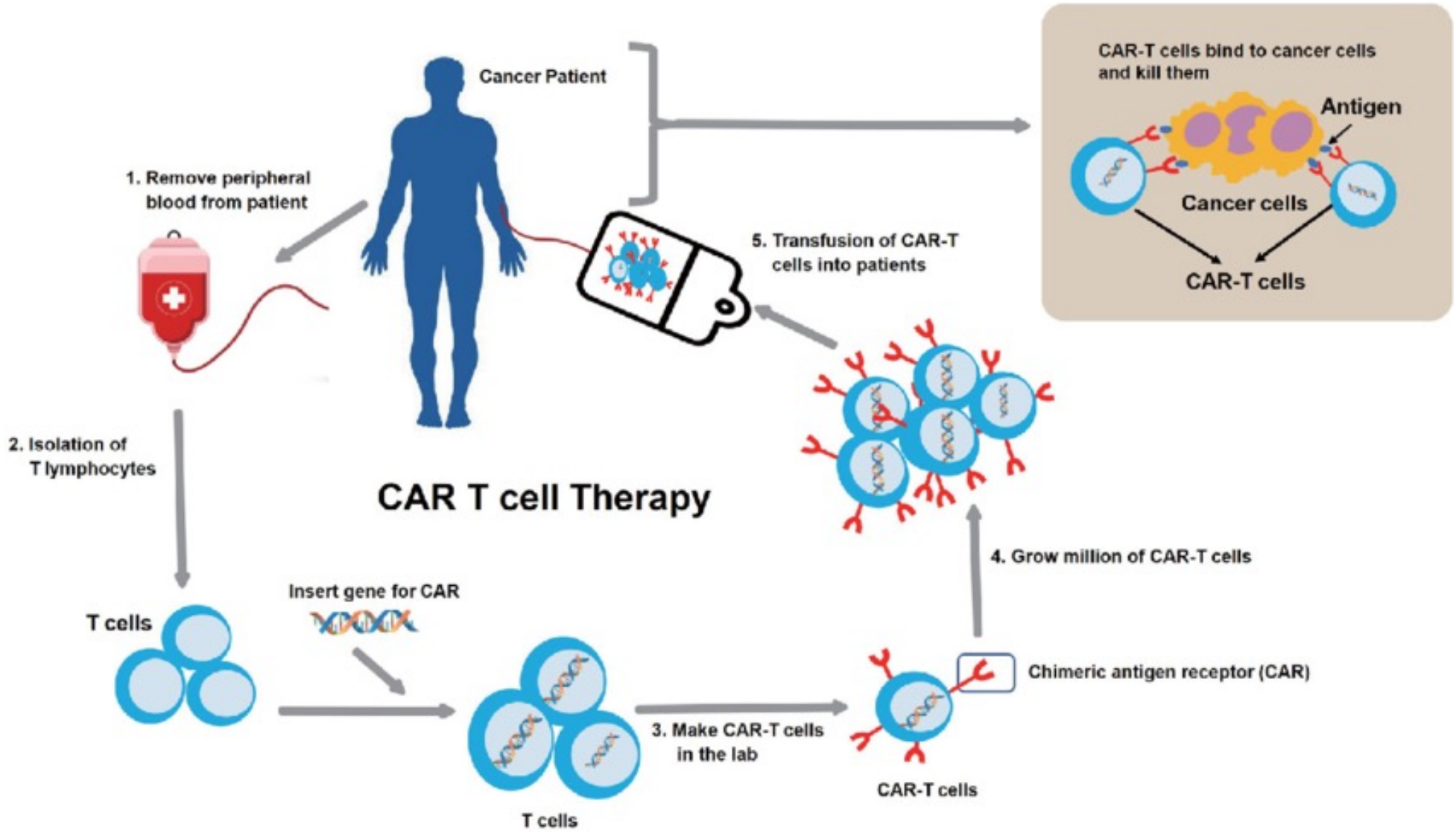
Chimeric Antigen Receptor (CAR)



CAR Generations



Therapy with CAR-T cells

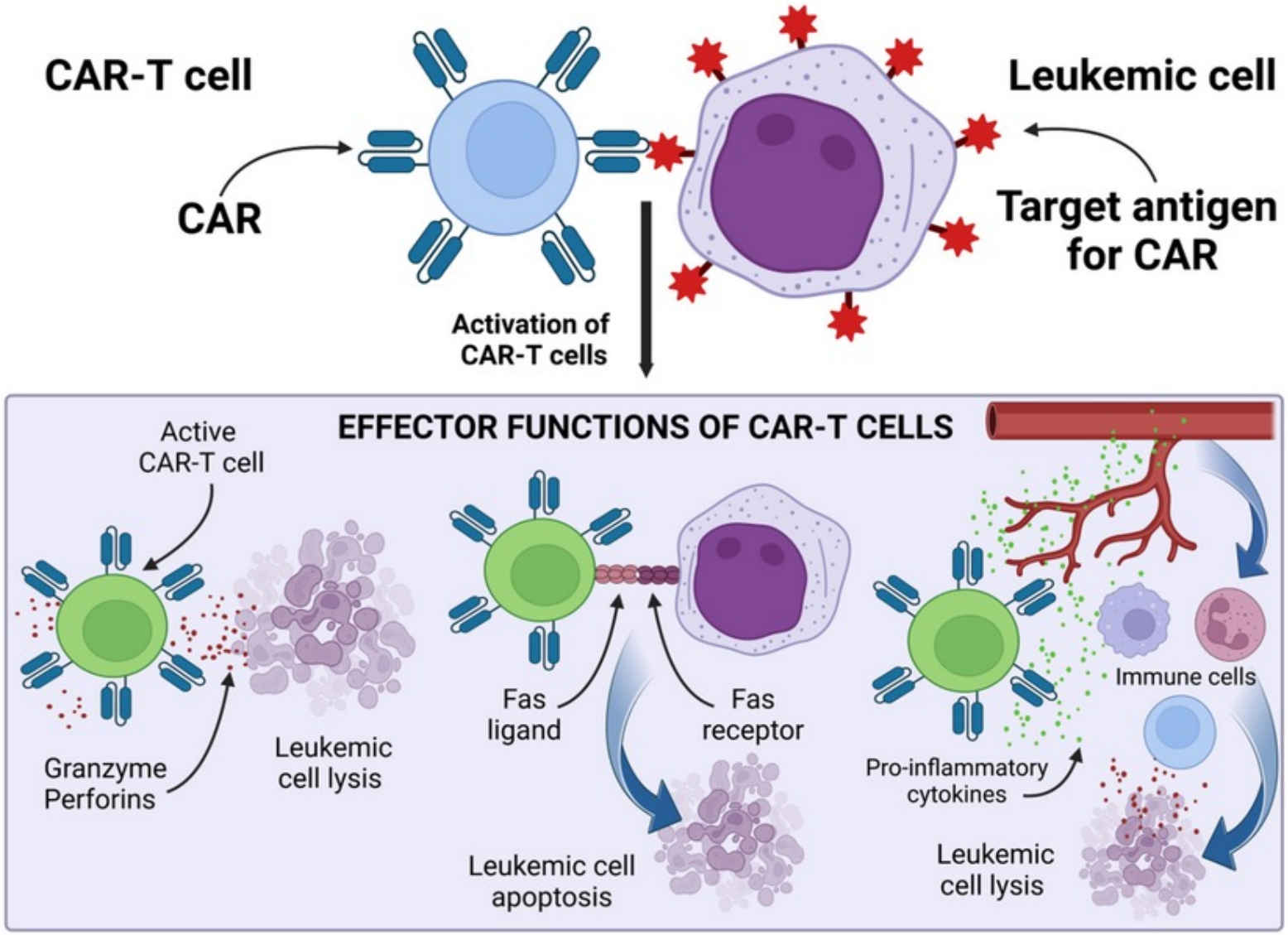


CAR-T vs. BITEs

	Bispecific Antibodies	CAR T Cell
Production	"Off-the-shelf": No need for manufacturing time, allowing for immediate treatment of the patient	Individual manufacturing for each patient, starting with autologous lymphapheresis Approach: Allogeneic CAR T cells under development
Administration	Continuous intravenous infusion Approach: extended half-life bispecific antibodies	Punctual infusion of the product (dose is sometimes split up into several days to reduce AEs)
T cell phenotype and effector function	Binding of endogenous $CD8^+$ and $CD4^+$ T cells, which have a superior cytotoxic function than naïve T cells	The product is mostly composed of naïve $CD8^+$ and $CD4^+$ T cells; these cells have higher self-renewal, survival, and penetration in lymphoid tissues

AE: Adverse events; CAR: Chimeric antigen receptor.

Mechanism of action of CAR-T



Approved CAR-T cell therapies

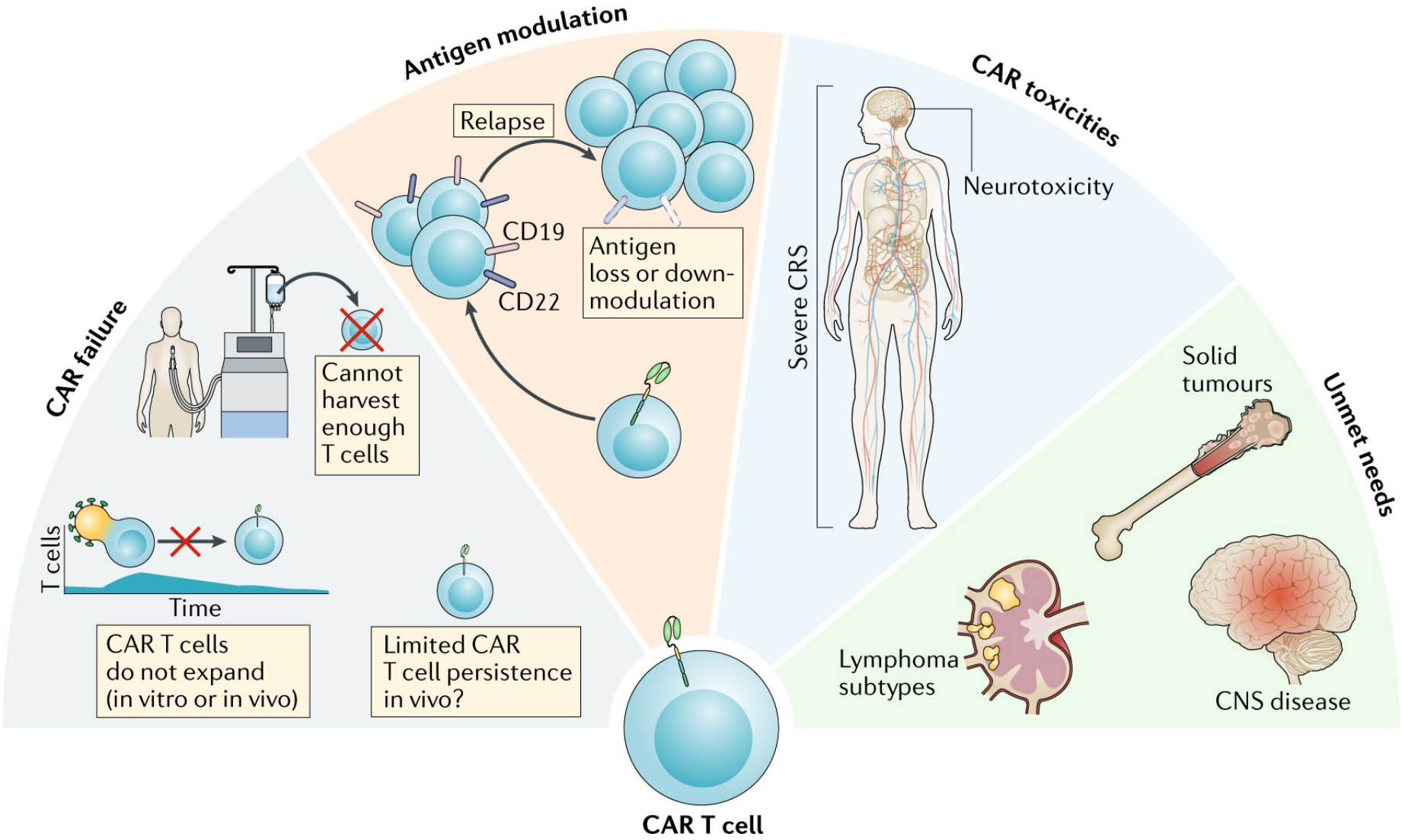
FDA-Approved CAR T-Cell Therapies

Generic Name	Target Antigen	Targeted Disease	Patient Population
Tisagenlecleucel	CD19	B-cell acute lymphoblastic leukemia (ALL)	Children and young adults with refractory or relapsed B-cell ALL
		B-cell non-Hodgkin lymphoma (NHL)	Adults with relapsed or refractory B-cell NHL
Axicabtagene ciloleucel	CD19	B-cell non-Hodgkin lymphoma (NHL)	Adults with relapsed or refractory B-cell NHL
		Follicular lymphoma	Adults with relapsed or refractory follicular lymphoma
Brexucabtagene autoleucel	CD19	Mantle cell lymphoma (MCL)	Adults with relapsed or refractory MCL
		B-cell acute lymphoblastic leukemia (ALL)	Adults with refractory or relapsed B-cell ALL
Lisocabtagene maraleucel	CD19	B-cell non-Hodgkin lymphoma (NHL)	Adults with relapsed or refractory B-cell NHL
Idecabtagene vicleucel	BCMA	Multiple myeloma	Adults with relapsed or refractory multiple myeloma
Ciltacabtagene autoleucel	BCMA	Multiple myeloma	Adults with relapsed or refractory multiple myeloma

B-cell lymphoma: around 50% of CR in relapsed/refractory patients

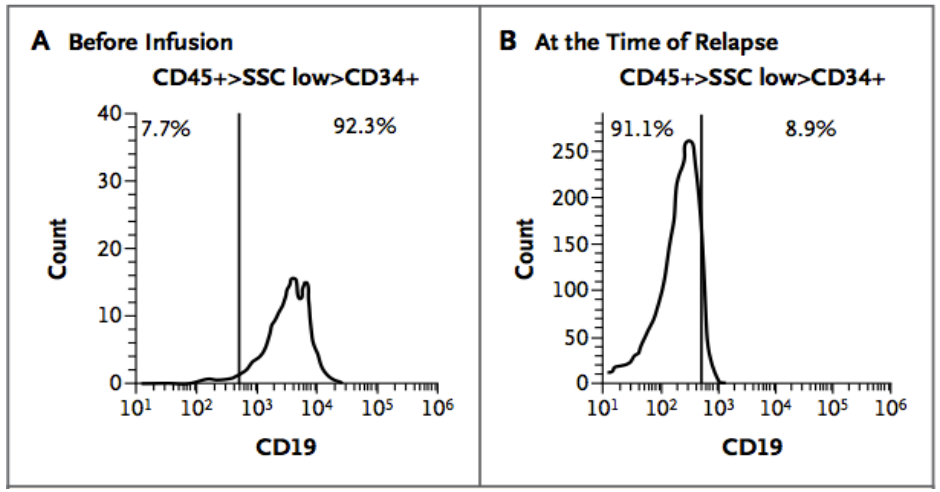
Strong interaction between academia and industries

CAR-T cell therapy “challenges”



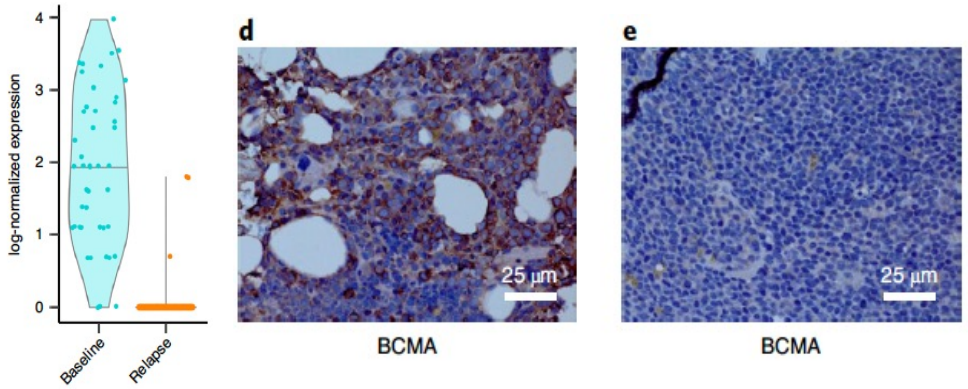
Antigen loss

CD19



Grupp et al, NEJM 2013

BCMA



De Via et al, Nat Med 2021

- Confirms the strong immune pressure exerted by CAR T cells
- Highlights that malignant cells can find ways of evading this pressure

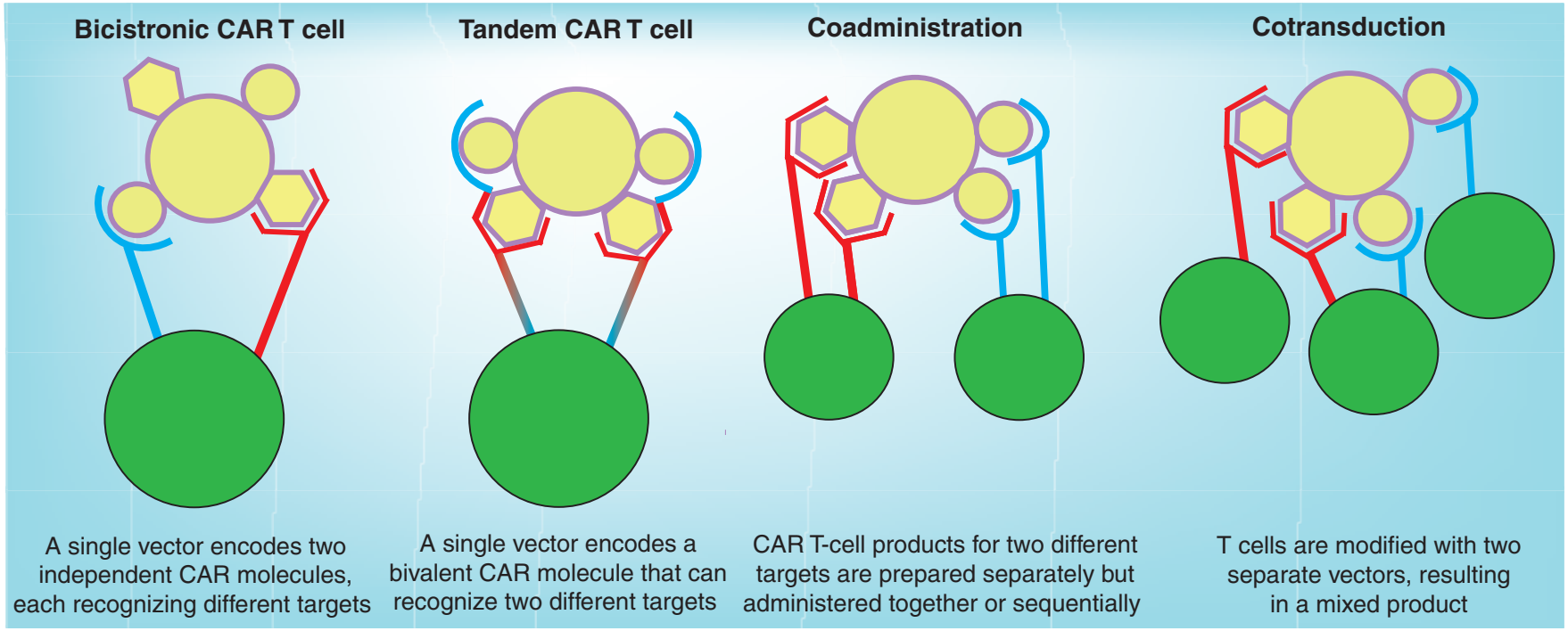
Mechanisms of CD19 loss/decrease

1. Genomic alterations
   Mutations impeding surface exposure
2. Alternative splicing
   Exon 2 skipping impeding surface exposure
3. Lineage switch
   Myeloid conversion with loss of B-cell markers
4. Trogocytosis
   Antigen transfer to CAR-T cells that lead to post-transcriptional down-modulation in tumor cells

Orlando et al, Nat Med 2018; Sotillo et al, Cancer Discovery 2015; Grupp et al, NEJM 2013; Jacoby et al, Nature Comm 2016; Hamieh et al, Nat Med 2019

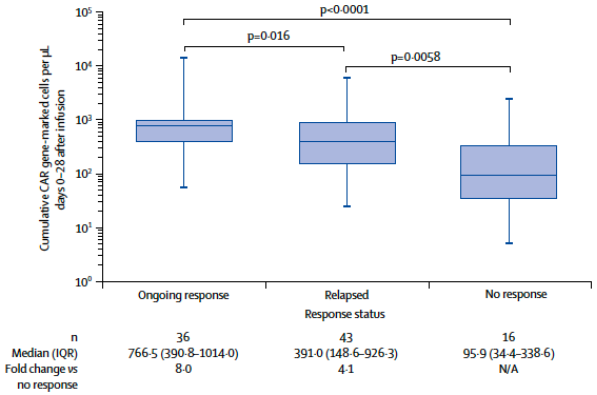
How can we overcome antigen loss?

Combinatorial targeting of multiple antigens at once

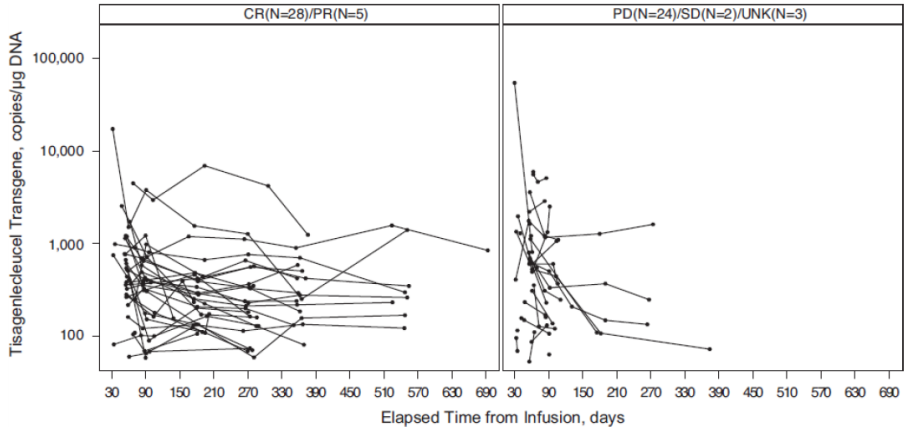


Efficacy determinants

- Choice of the target antigen
- CAR-T cell functionality, expansion and persistence

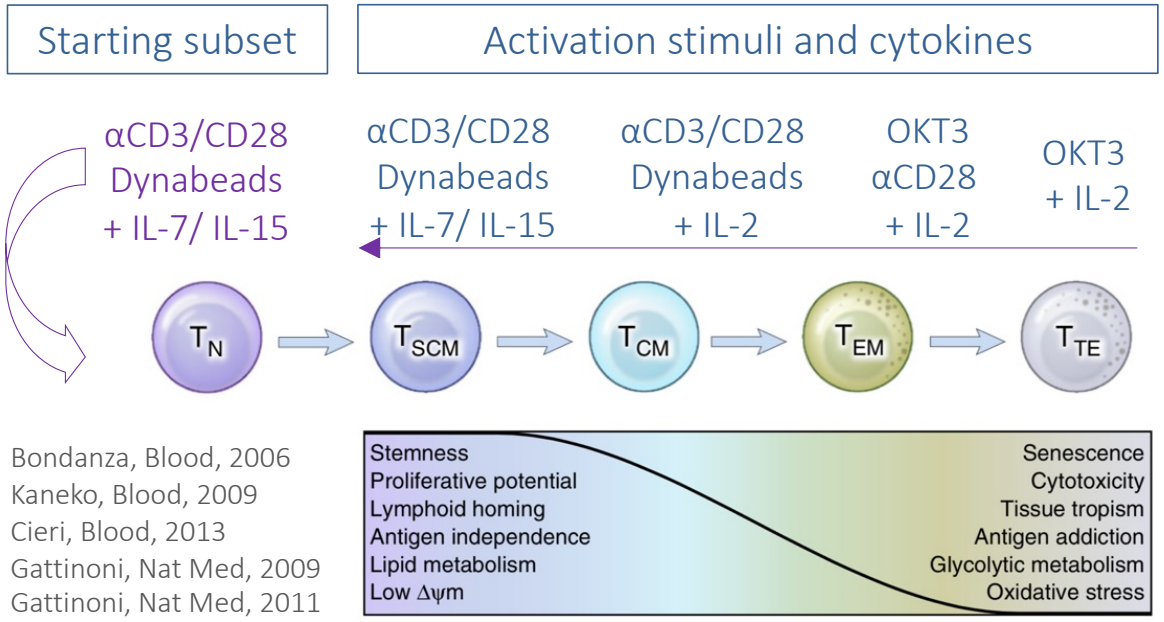


Locke et al, NEJM 2019



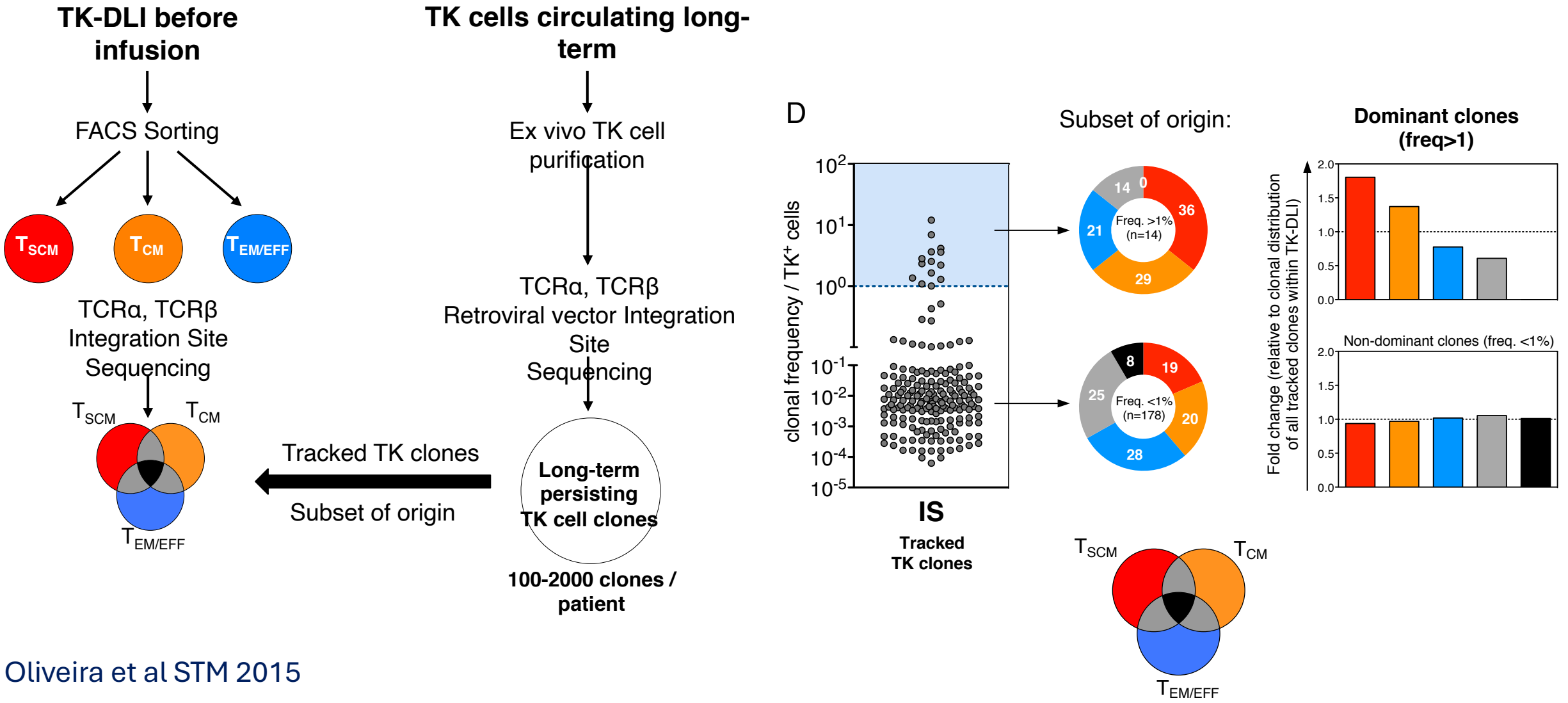
Schuster, NEJM 2019

CAR-T cell manufacturing



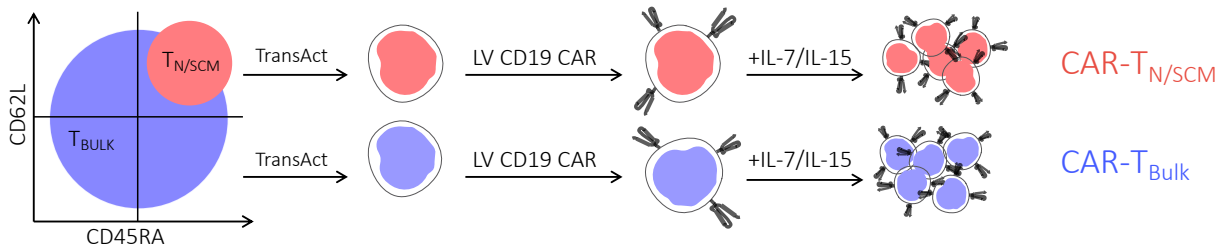
Bondanza, Blood, 2006
Kaneko, Blood, 2009
Cieri, Blood, 2013
Gattinoni, Nat Med, 2009
Gattinoni, Nat Med, 2011

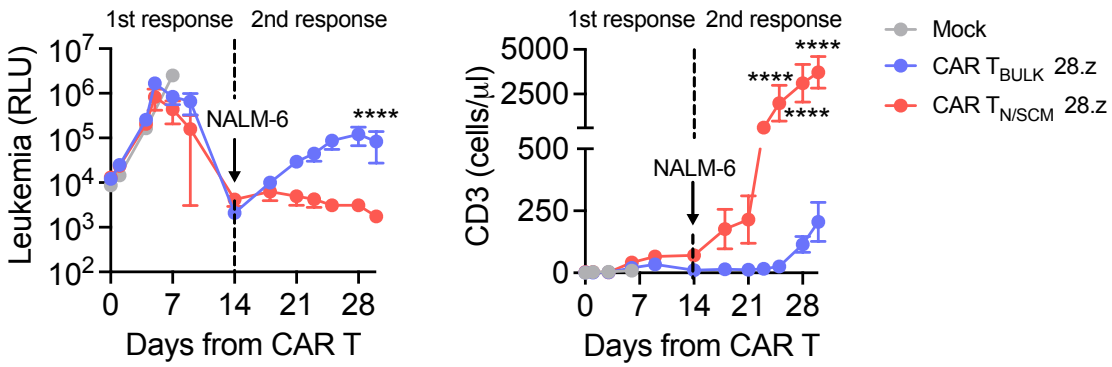
Dominant long-term persisting clones preferentially originate from infused T_{SCM}

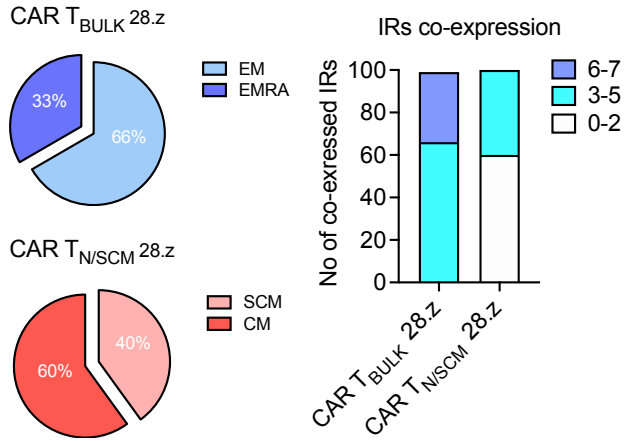


Oliveira et al STM 2015

Early memory CAR-T cell products



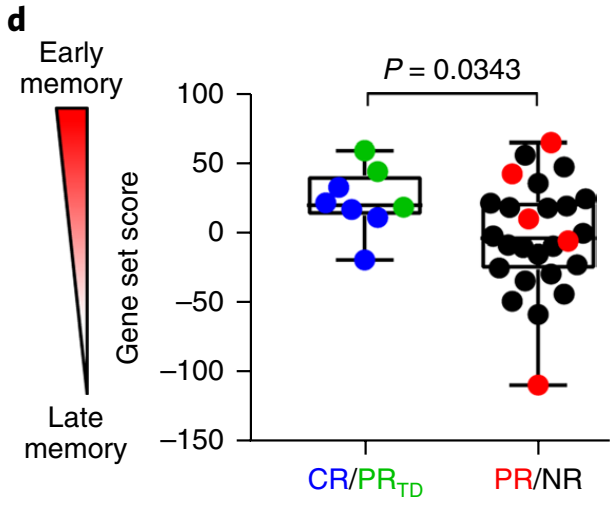




Arcangeli, Bove, Mazzanotte et al, JCI 2022

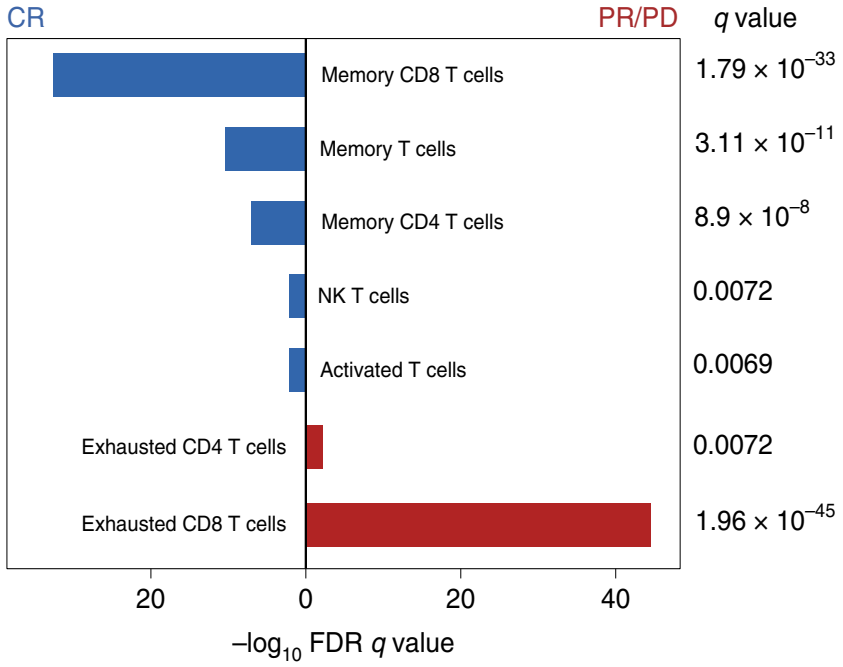
Early memory T cells

CLL



Fraietta et al, Nat Med 2018

Lymphoma

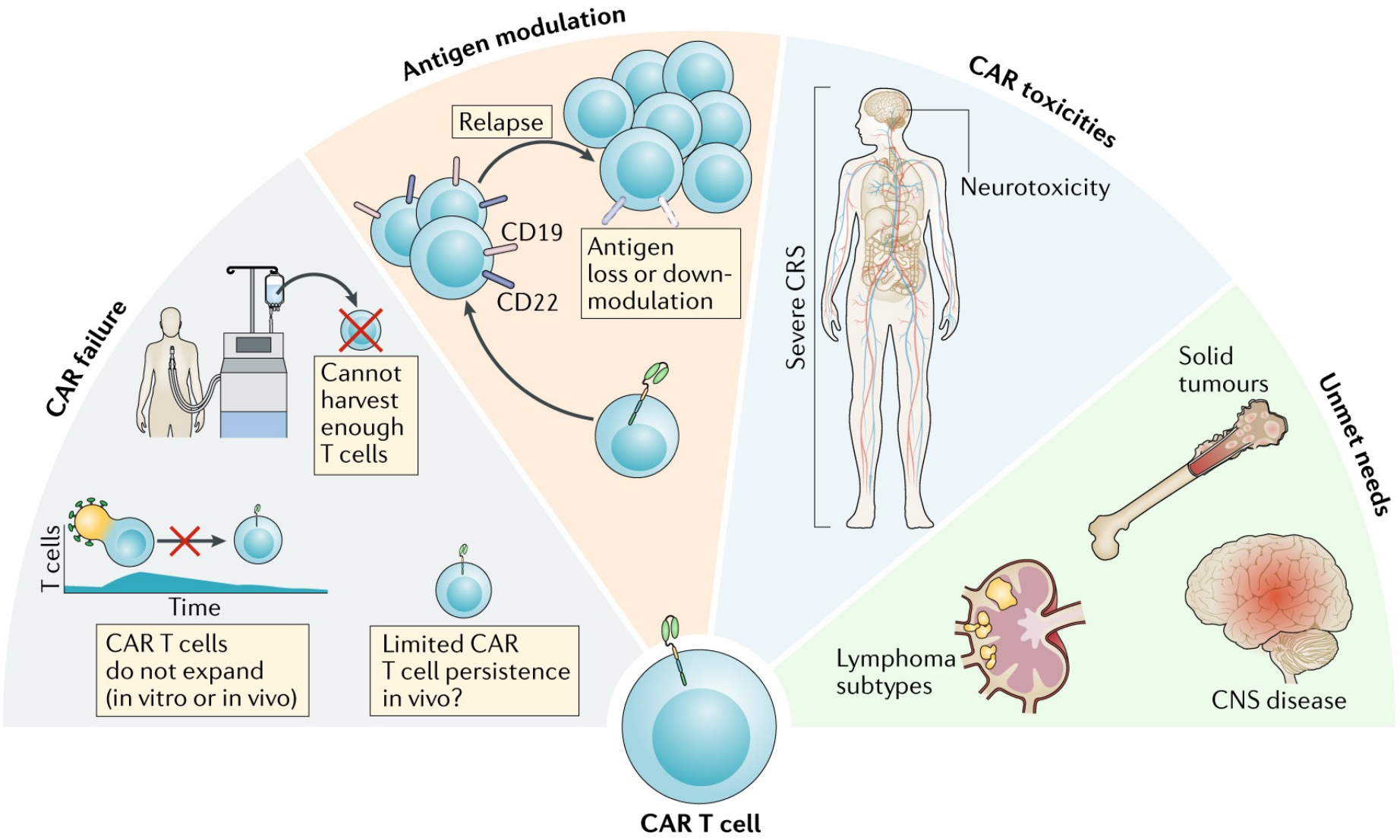


Deng et al, Nat Med 2020

Efficacy determinants

- Choice of the target antigen (antigen loss)
- CAR-T cell functionality, expansion and persistence in patients
  - CAR endocostimulation: presence, type and position
  - CAR design: signal strength, tonic signaling, anti-CAR responses
  - CAR-T cell memory differentiation status
  - CAR-T cell CD4/CD8 ratio
  - CAR-T cell exhaustion: expression of inhibitory/senescence markers
  - Presence and frequency of CAR-Tregs
  - Lymphodepleting chemotherapy
  - Intrinsic T-cell defects: underlying disease and previous treatments)
  - Immunosuppressive tumor microenvironment

CAR-T cell therapy “challenges”



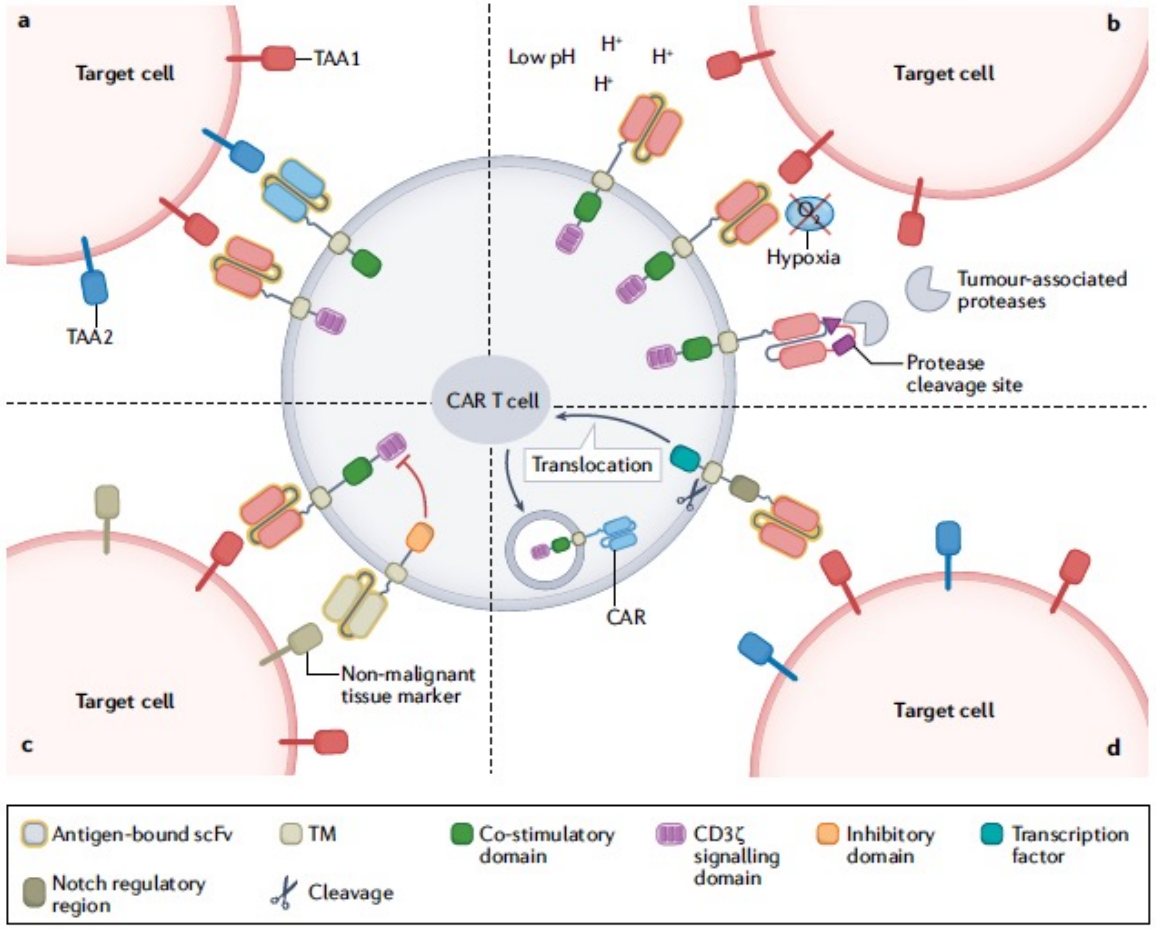
On-target off-tumor toxicity

- Damage of heathy tissues expressing the target antigen
- Relevant: tumor-specific antigens are rare
- Severity depends on how vital, accessible, widespread the tissue is
- Particularly dangerous for solid tumors

Logic gating strategies

AND
Split CAR

NOT
Inhibitory CAR



IF, THEN (TME-based)
pH, hypoxia, cleavage…

IF, THEN (TAA-based)
Syn-Notch circuits

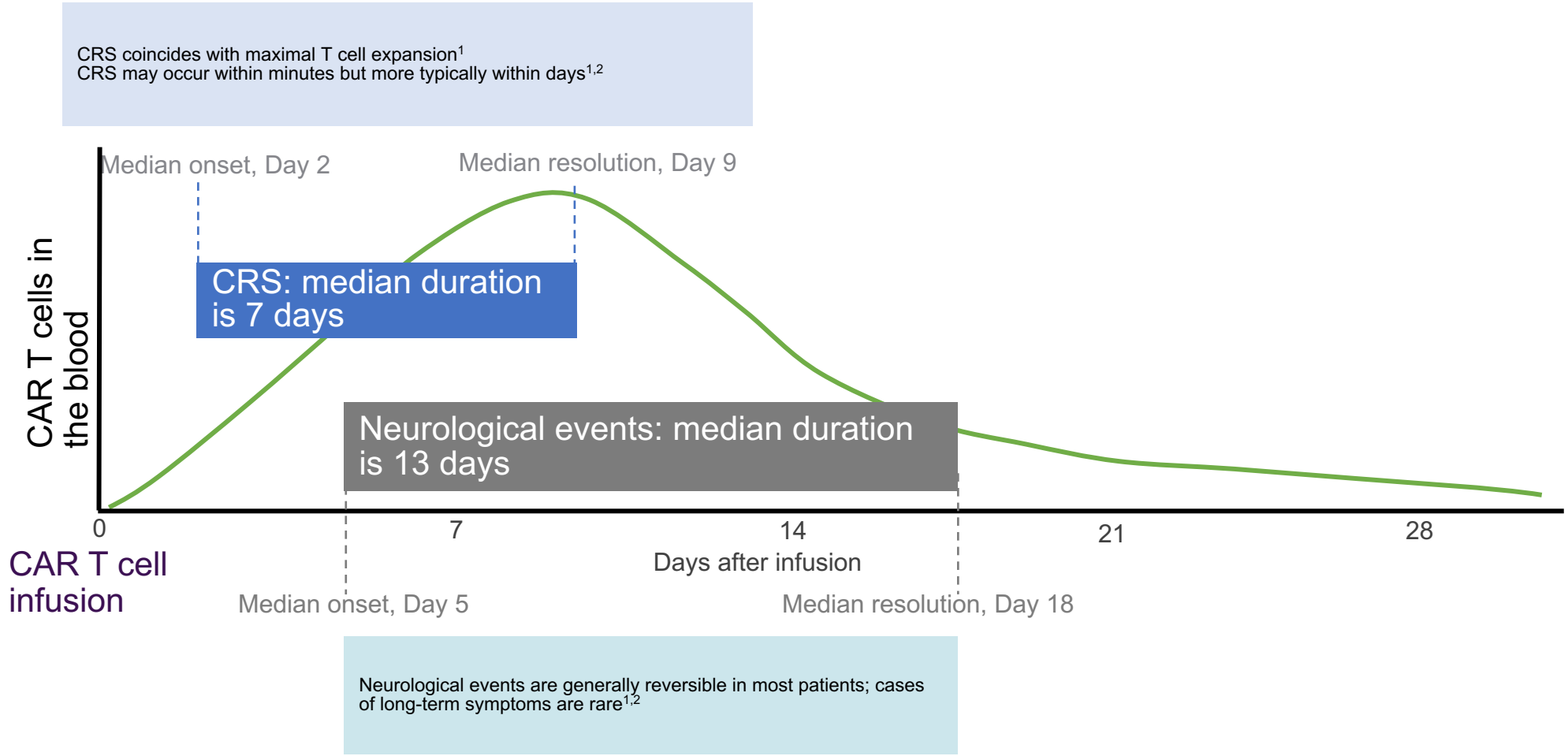
Flugel CL et al, Nat Rev Clinical Oncol 2023

Cytokine release syndrome

- Systemic inflammatory reaction
  fever, hypotension, hypoxia, capillary leak, coagulopathy
- Rapid onset within a few days after CAR-T cell infusion
- Reported with different CARs and tumor types
- Potentially life-threatening
- Its severity is associated with high tumor burdens

Teachey et al. Cancer Discovery 2016
Hay et al. Blood 2018

Kinetics of AEs associated with CAR T cell therapy



1. Lee DW, *et al*. *Blood* 2014; 124:188–195. 2. Yescarta SmPC (May 2019; available at www.ema.europa.eu).

CRS pathophysiology

- Initiated by CAR-T cells activation upon antigen engagement
- Which other cellular compartments are involved?

→ Development of animal models recapitulating CRS development

nature medicine ARTICLES
https://doi.org/10.1038/s41591-018-0036-4

Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells

Margherita Norelli[1,2], Barbara Camisa[1], Giulia Barbiera[3], Laura Falcone[1], Ayurzana Purevdorj[1], Marco Genua[3], Francesca Sanvito[4], Maurilio Ponzoni[4], Claudio Doglioni[4], Patrizia Cristofori[5], Catia Traversari[6], Claudio Bordignon[2,6], Fabio Ciceri[2,7], Renato Ostuni[3], Chiara Bonini[2,8], Monica Casucci[1] and Attilio Bondanza[1,2*]

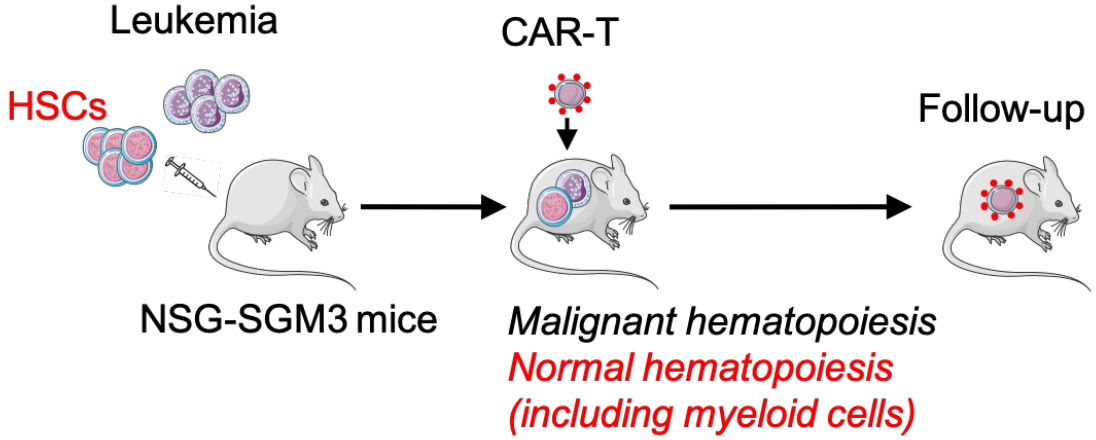
nature medicine LETTERS
https://doi.org/10.1038/s41591-018-0041-7

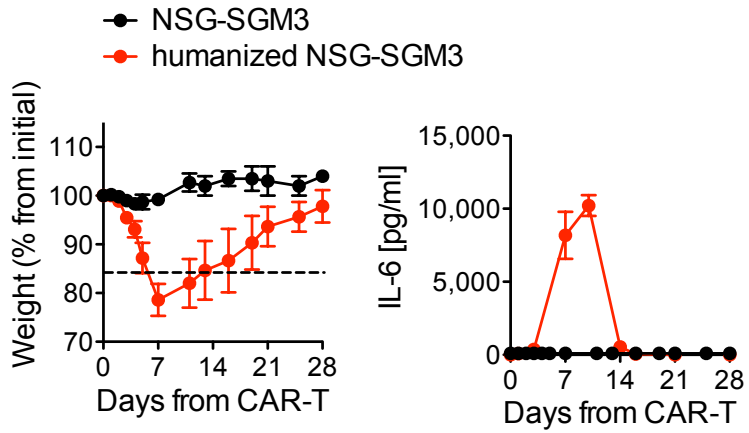
CAR T cell–induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade

Theodoros Giavridis[1], Sjoukje J. C. van der Stegen[1], Justin Eyquem[1], Mohamad Hamieh[1], Alessandra Piersigilli[2] and Michel Sadelain[1*]

Humanized model for CAR-T

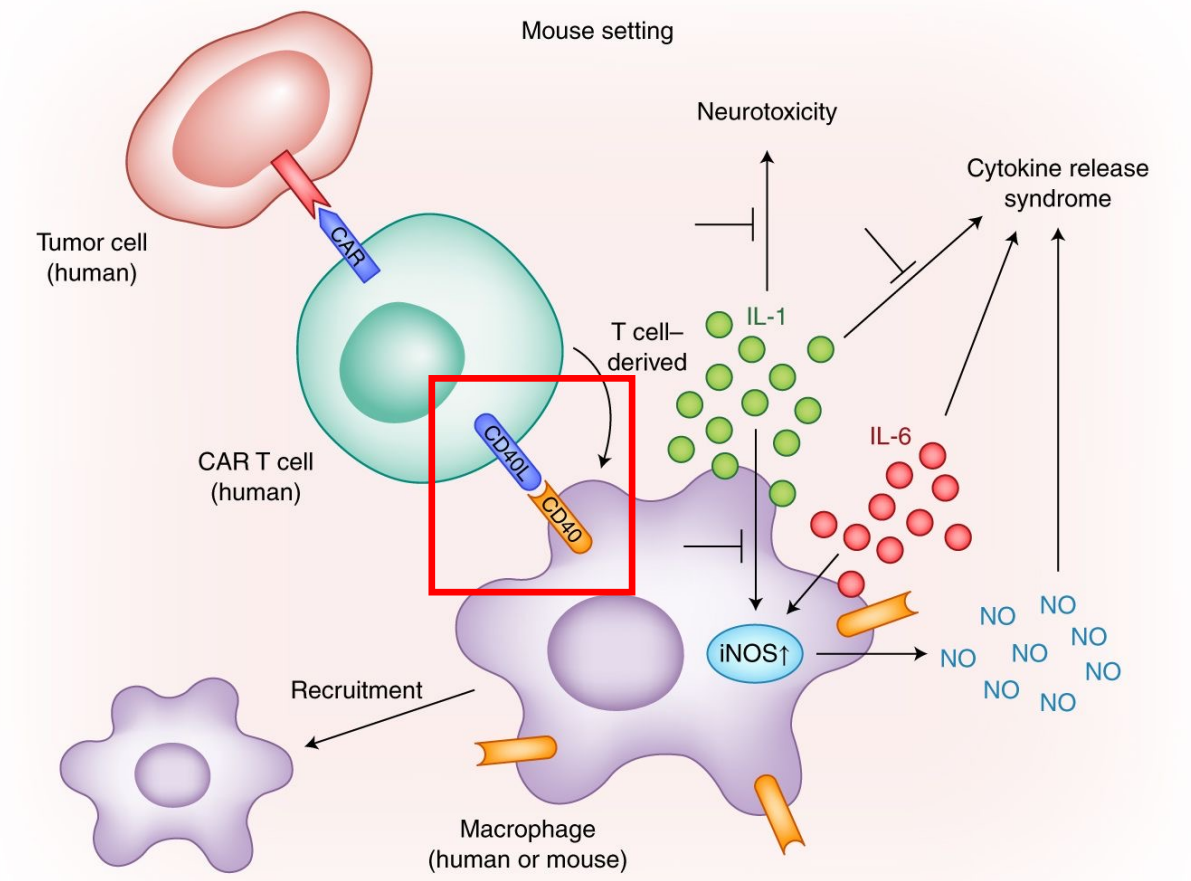
Efficacy and CAR-related Toxicities





Adapted from Norelli M et al, Nat Med 2018

CRS initiating cascade



Ronney and Sauer, Nat Med 2018

CAR-T cells release perforin to form pores, leading to the entry of granzyme B into target tumor cells, which causes the subsequent activation of GSDME and pyroptosis (programmed necrotic cell death)

Proptosis supernatants contain ATP and HMGB1 that induce macrophages to release IL-1b and IL-6, respectively

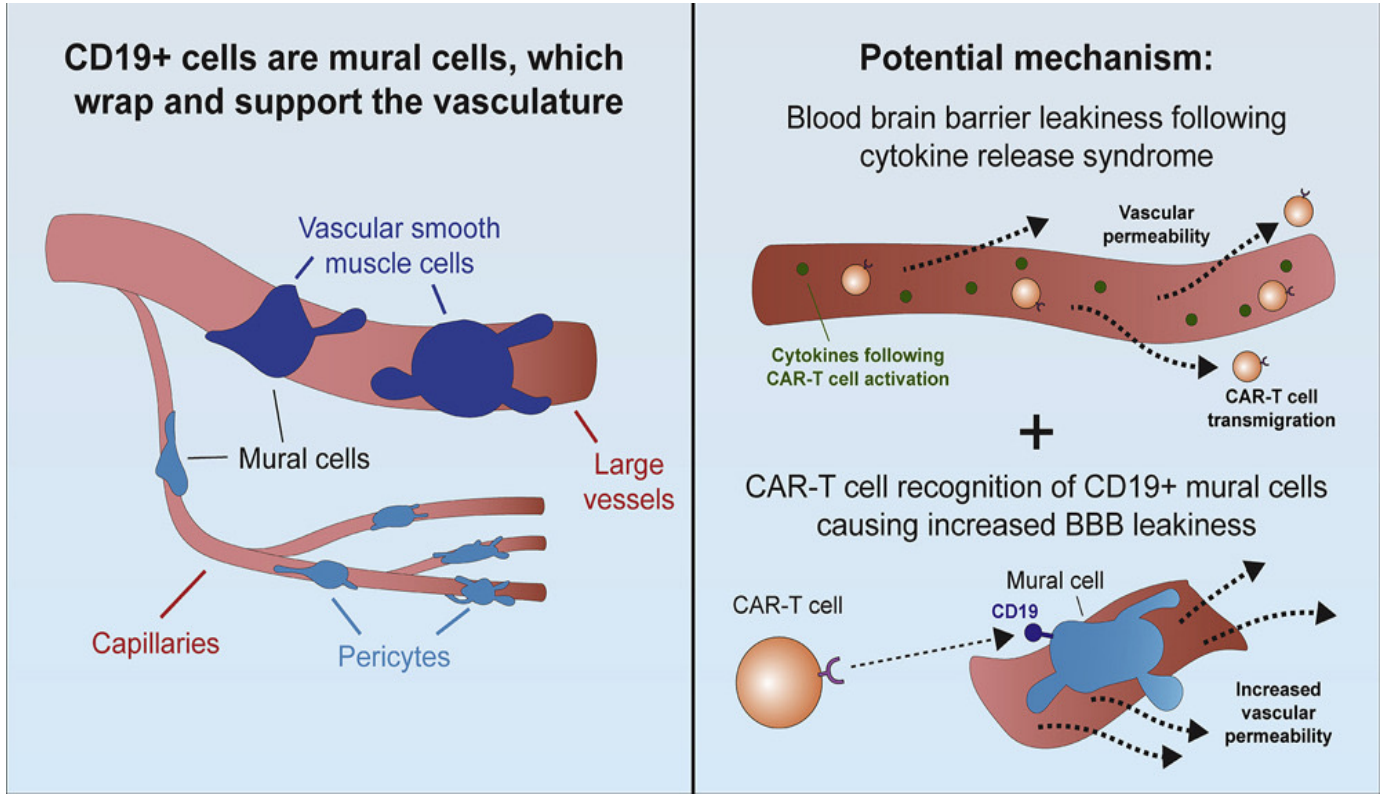
Liu Science Immunol 2020

Neurotoxicity (NTX)

"*Disorder in which the involvement of the central nervous system that follows any immune therapy that results in the activation or engagement of endogenous or infused T cells and/or other immune effector cells*"

- Reported with different CARs and tumor types
- Potentially life-threatening (cerebral hemorrhage and edema)
- It is strictly related to CRS (development and severity)

Neurotoxicity pathophysiology



1. Well accepted

2. Still debated

CRS and neurotoxicity management

- Tocilizumab
  Anti-IL-6R antibody
  Active against CRS
  Unable to control neurotoxicity in most of patients
- Corticosteroids
  At high-doses can be detrimental for efficacy

The search for strategies
to mitigate these toxicities is extremely active

Mitigating CRS and neurotoxicity

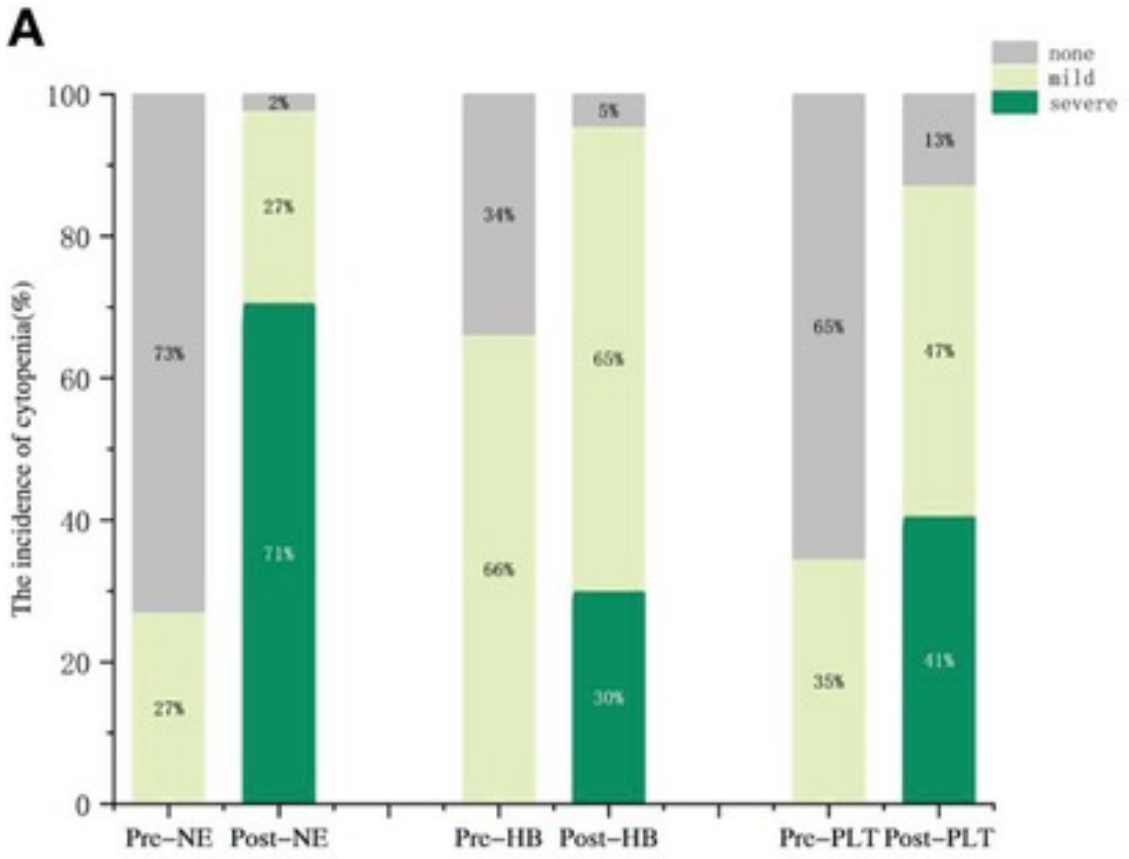
1. Early intervention in patients at risk of developing severe toxicities

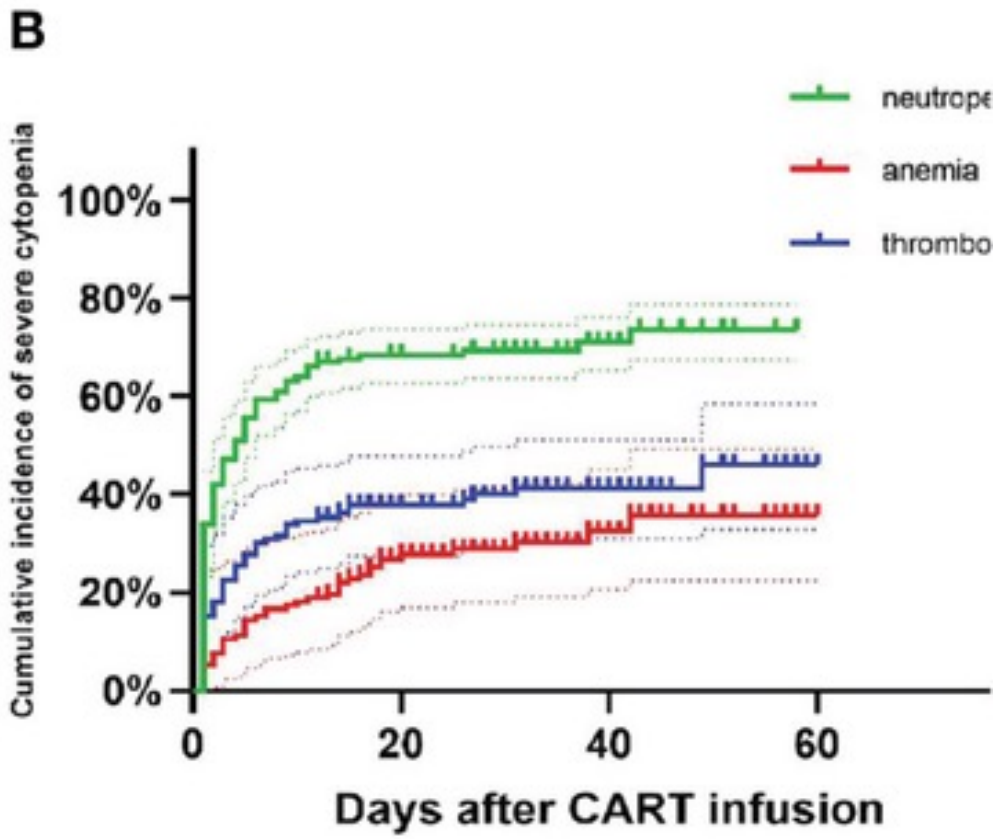
   Identification of predictive biomarkers
2. Cytokine inhibitors

   IL-6, IL-1, GM-CSF, catecholamine
3. On/off switches

   Pharmacological control over CAR T-cell activity (drugs or CAR designs)

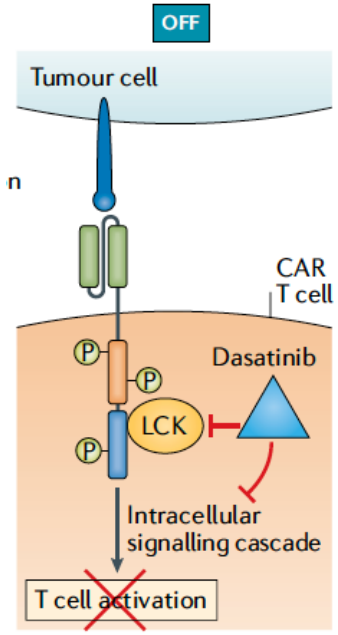
Hematopoietic toxicity(ICATH)



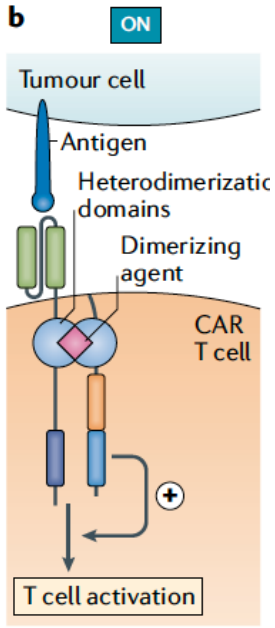


On/off switches to mitigate toxicity

Short treatment with Dasatinib can rapidly and temporary switch-off CAR T-cell function



CAR constructs able to induce full T-cell activation only upon administration of a dimerizing agent

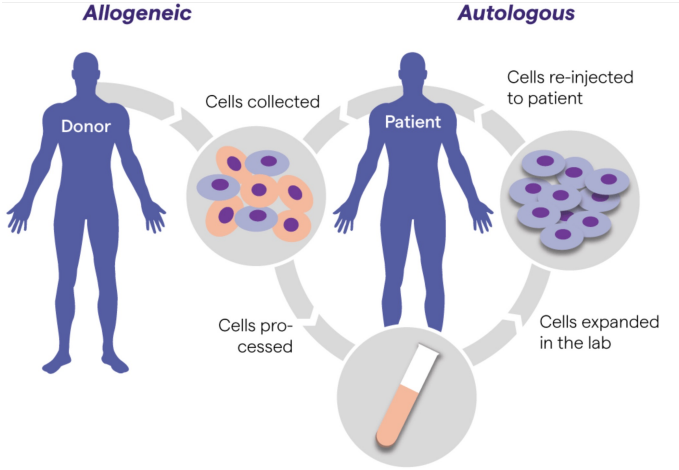


Rafiq et al, Nat Rev Clinical Oncol 2020

Allogeneic platform: pros

"Off-the-shelf" CAR products from healthy donors

- Overcome patients' T-cell defects
- Simplifying manufacturing and reducing costs
- Making the treatment accessible to lymphopenic patients
- Speeding up drug administration (rapidly-progressing diseases)

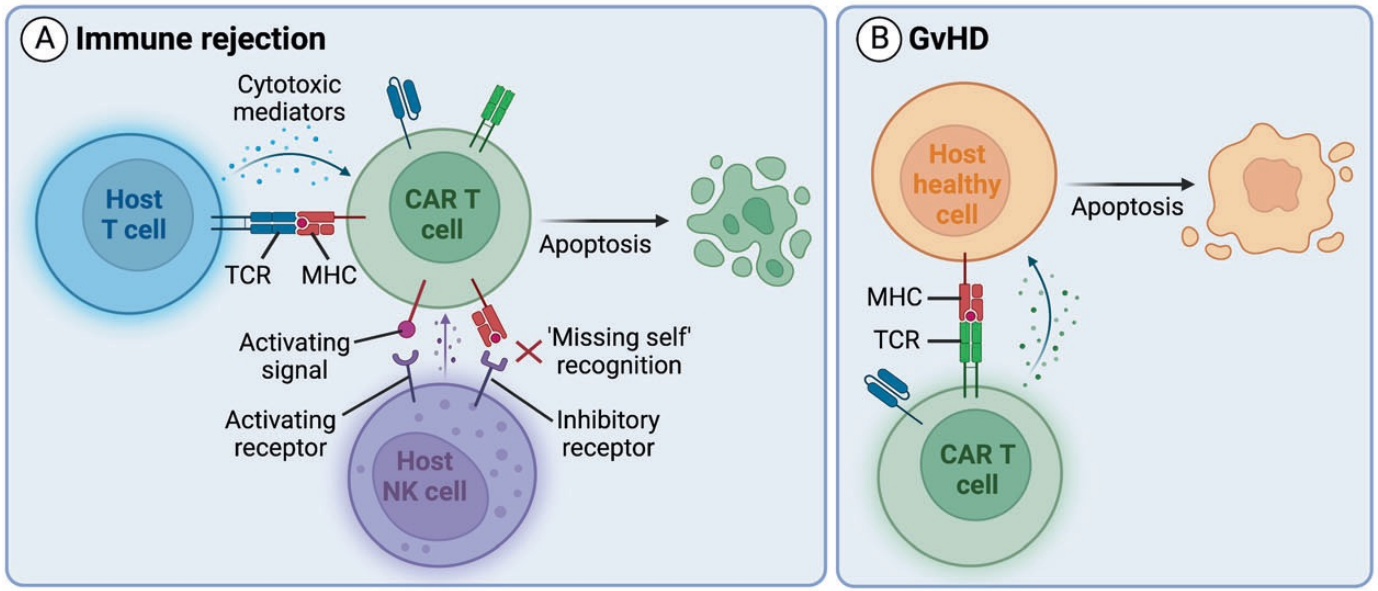


Dimitri A, Molecular Cancer 2022; Wagner DL, Nat Rev Clinical Oncol 2021; Depil S, Nat Rev Drug Discov 2020

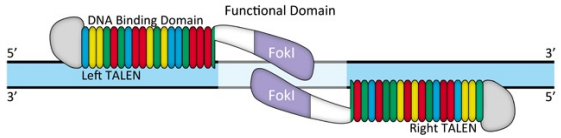
Allogeneic platform: cons

"Off-the-shelf" CAR products from healthy donors

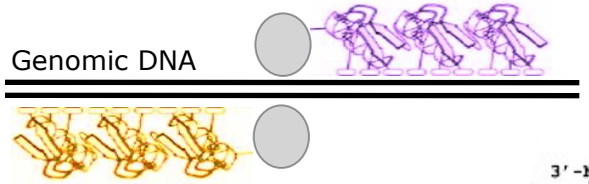
- Host-versus-Graft reaction, rejection (Problem: efficacy)
- Graft-versus-Host Disease, GvHD (Problem: toxicity)



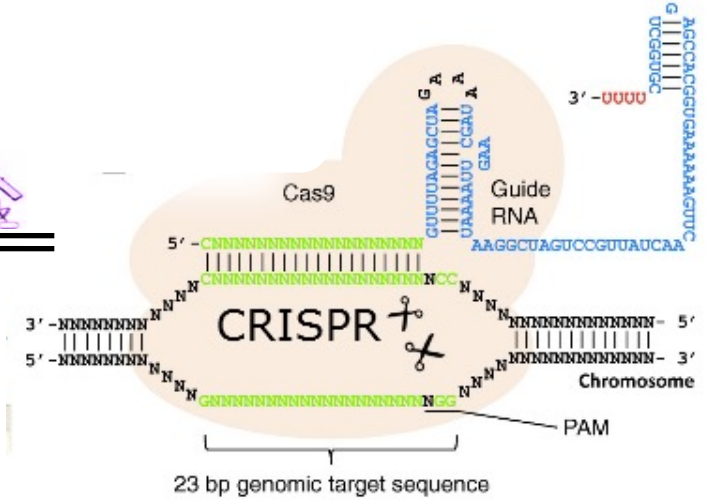
The genome editing technology applied to cancer immunotherapy

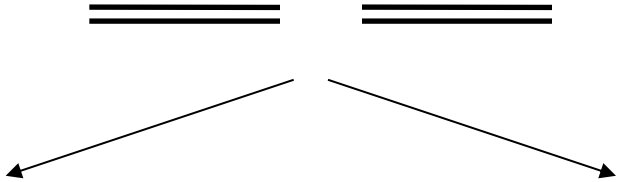


TALEN



ZFN



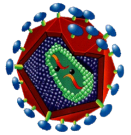


Nucleases introduce Double Strand Breaks at selected genome sites

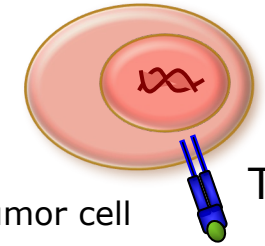
The Genome Editing Technology allows to move from “simple” gene addition to genetic knock-down and gene substitution leading to substitution of biological functions in targeted cells

TCR gene EDITING: rational

Zinc Finger Nucleases
CRISPR/Cas9

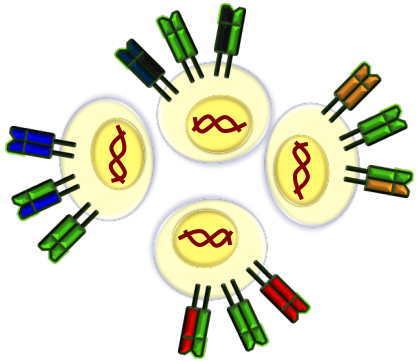


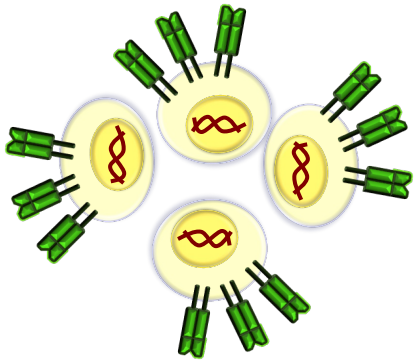
TCR-LV

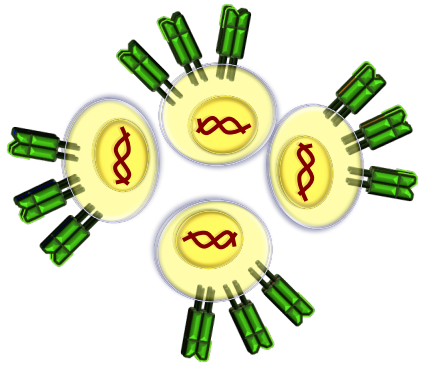


Tumor cell

Tumor antigen



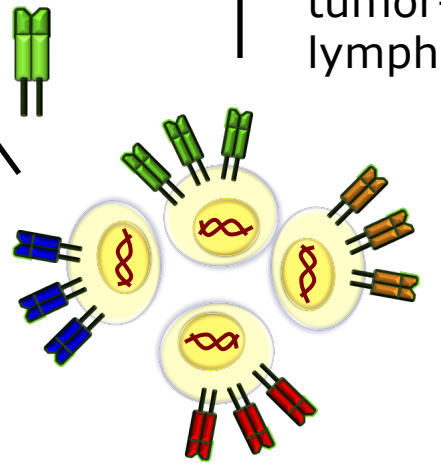




TCR gene transfer

Expansion of tumor-specific lymphocytes

TCR gene EDITING



Provasi, Genovese et al., *Nat Med* 2012
Mastaglio et al., *Blood* 2017
Ruggiero et al., *Sci Transl Med* 2022

Allogeneic platform: optimization

- Exploiting gene editing approaches:
  - TCR knockout (*GvHD*)
  - B2M knockout (*rejection*, CD8 T cells)
  - CIITA knockout (*rejection*, CD4 T cells)
  - HLA-E (*rejection*, NK)
  - CD45 (*rejection*, phagocytes)
- Intensifying lymphodepletion (*rejection*, alemtuzumab + CD52 ko in CAR-T)
- Using non-alloreactive effectors (*GvHD*, NK cells, invariant NKT, γδ T cells)

Autoimmune diseases

Diseases characterized by an aberrant inflammatory response against self-antigens that leads to tissue damage

Very heterogeneous group

In U.S. prevalence 3-7% of population

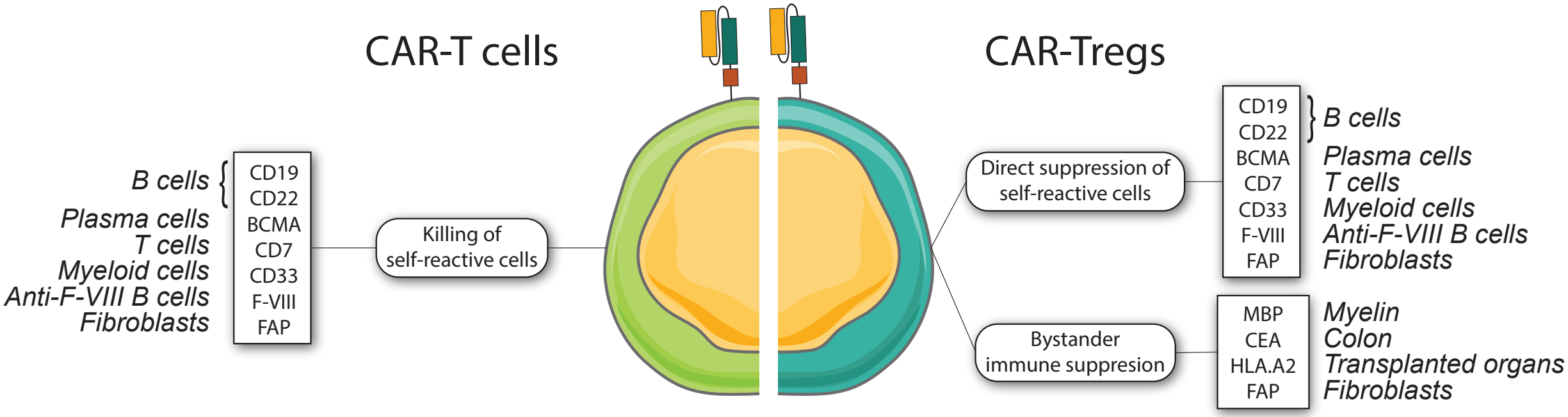
Unsatisfactory control with standard treatment

Chronic disease with recurrent flares

Immune selection
Autoreactive Tconvs

Immune tolerance
Dysfunctional Tregs

Strategies in autoimmunity



Doglio & Alexander et al. JACI 2022

CAR-T in autoimmunity

CAR	Dose and conditioning	Safety	Clinical outcome	Ref.
Systemic lupus erythematosus				
MB-CART19.1: mouse anti-CD19 scFv, CD8 hinge, TNFRSF19 transmembrane domain, 4-1BB co-stimulatory domain, CD3ζ activation domain	1×10^6 cells kg^{-1} CYC 1,000 mg $m^{-2}\times1$ day FLU 25 mg $m^{-2}\times3$ days	No CRS or ICANS	Very rapid (~1 month) and profound improvement in autoantibody levels and clinical disease activity	Mougiakakos D. et al. N. Eng. J. Med. 2021
MB-CART19.1: mouse anti-CD19 scFv, CD8 hinge, TNFRSF19 transmembrane domain, 4-1BB co-stimulatory domain, CD3ζ activation domain	1×10^6 cells kg^{-1} CYC 1,000 mg $m^{-2}\times1$ day FLU 25 mg $m^{-2}\times3$ days	Grade 1 CRS in 3 of 5 patients; no ICANS	Favourable safety, strong efficacy and durability of drug-free remission confirmed B cells returned in all patients after a median of 110 days	Mackensen A. et al. Nat Med. 2022
Anti-BCMA-CD19 compound CAR: 2-unit scFv with anti-BCMA fused to anti-CD19 by a self-cleaving P2A peptide	$1.5–3\times10^6$ cells kg^{-1} Conditioning was used (agents not specified)	Grade 1 CRS; no ICANS	Reduced autoantibodies and prolonged disease remissions IVIG was administered monthly until B cell recovery	Yuan Y. et al. Ann. Rheum. Dis. 2023
Systemic sclerosis				
MB-CART19.1: mouse anti-CD19 scFv, CD8 hinge, TNFRSF19 transmembrane domain, 4-1BB co-stimulatory domain, CD3ζ activation domain	1×10^6 cells kg^{-1} CYC 500 mg $m^{-2}\times1$ day FLU 12.5 mg $m^{-2}\times3$ days	Grade 1 CRS; no ICANS	Reduction in autoantibodies, reduction in fibroblast activation, and clinical stabilization or improvement	Bergmann C. et al. Ann. Rheum. Dis. 2023
Mouse anti-CD19 scFv, human IgG1–CH2CH3 hinge, CD28 transmembrane domain, CD28 and 4-1BB co-stimulatory domains, CD3ζ activation domain	5×10^6 cells kg^{-1} CYC 500 mg $m^{-2}\times3$ days FLU 30 mg $m^{-2}\times3$ days	Grade 1 CRS; no ICANS	Reduction in autoantibodies, reduction in fibroblast activation, and clinical improvement in skin fibrosis and lung function Prolonged CAR T cell persistence	Merkt W. et al. Ann. Rheum. Dis. 2023

CAR-T in autoimmunity

Idiopathic inflammatory myopathy				
MB-CART19.1: mouse anti-CD19 scFv, CD8 hinge, TNFRSF19 transmembrane domain, 4-1BB co-stimulatory domain, CD3ζ activation domain	1×10^6 cells kg^{-1} CYC 1,000 mg $m^{-2}\times1$ day FLU 25 mg $m^{-2}\times3$ days	Grade 1 CRS; no ICANS	Positive clinical impact with disappearance of autoantibodies and dramatic resolution of myositis and alveolitis	Muller F. et al. Lancet 2023
Anti-CD19 scFv, co-stimulatory domain, CD3ζ signalling domain	1.23×10^6 cells kg^{-1} CYC 1,000 mg $m^{-2}\times1$ day FLU 25 mg $m^{-2}\times3$ days	Grade 1 CRS; no ICANS	Efficacy and safety comparable with report by Müller et al.[13], but worsening of myalgia and CK elevation post CAR T cell treatment managed with MMF	Pecher A. et al. JAMA 2023
Systemic lupus erythematosus, systemic sclerosis or idiopathic inflammatory myopathy				
MB-CART19.1: mouse anti-CD19 scFv, CD8 hinge, TNFRSF19 transmembrane domain, 4-1BB co-stimulatory domain, CD3ζ activation domain	1×10^6 cells CYC 1 g m^{-2} FLU 75 mg	Grade 1–2 CRS (9 of 15); Grade 1 ICANS (1 of 15)	Tolerability similar for patients with any of the conditions	Taubmann J. et al. Arthritis Rheum 2023
Myasthenia gravis				
Descartes-08: RNA CAR T cell, mouse anti-BCMA scFv, CD8 HTM domains, CD28 co-stimulatory domain, CD3ζ activation domain	$3.5–52.5\times10^6$ cells kg^{-1} 2× per week–1× per month dosing No lymphodepletion	No CRS or ICANS	Clinical improvement Immunosuppressive therapy was continued and there was no clear decrease in autoantibodies or serum IgG	Granit V. et al. Lancet Neurol. 2023
KYV-101: human anti-CD19 scFv, CD8α HTM domains, CD28 co-stimulatory domain, CD3ζ activation domain	1×10^8 cells CYC 300 mg $m^{-2}\times3$ days FLU 30 mg $m^{-2}\times3$ days	No CRS or ICANS	Rapid improvement of clinical disease activity scores and reduction of acetylcholine receptor-specific autoantibodies	Haghikia A. et al. Lancet Neurol. 2023
Multiple sclerosis				
KYV-101: human anti-CD19 scFv, CD8α HTM domains, CD28 co-stimulatory domain, CD3ζ activation domain	1×10^8 cells CYC 300 mg $m^{-2}\times3$ days FLU 30 mg $m^{-2}\times3$ days	Grade 1 CRS (1 of 2); no ICANS	Acceptable safety and CAR T cell enrichment in the CSF without neurotoxicity, with reduced intrathecal antibodies in one patient	Fischbach F. et al. Med 2024

Systemic Lupus Erythematosus

nature medicine

ARTICLES

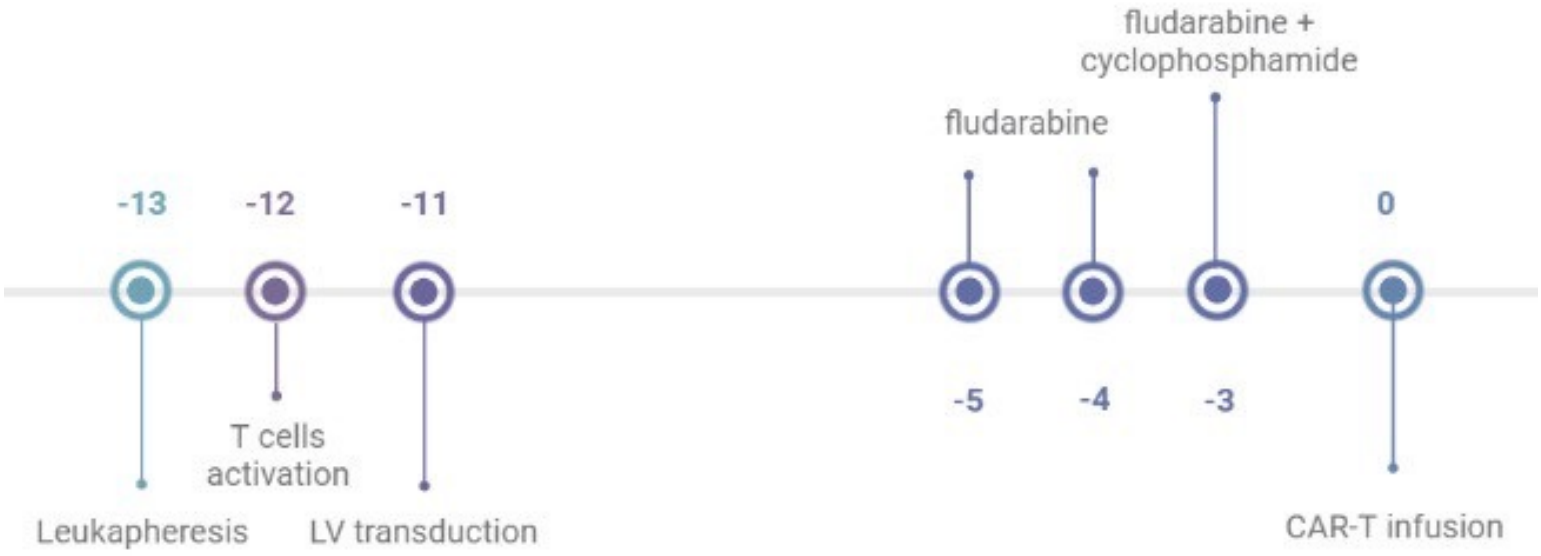
https://doi.org/10.1038/s41591-022-02017-5

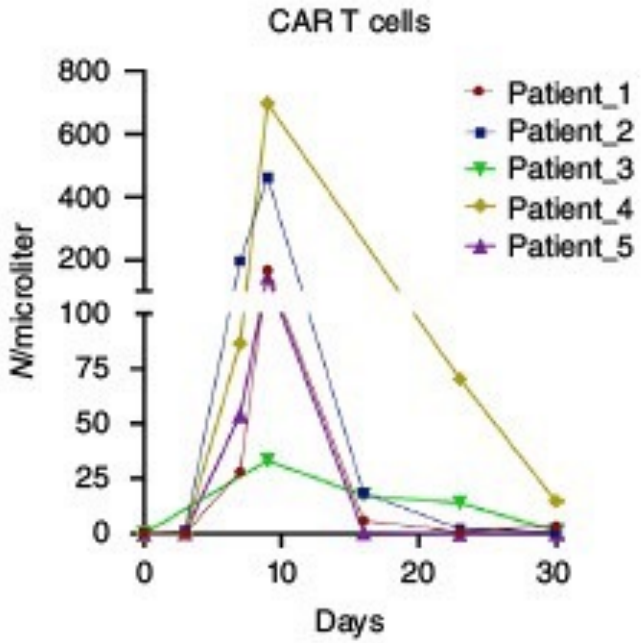
Check for updates

Anti-CD19 CAR T cell therapy for refractory systemic lupus erythematosus

Andreas Mackensen[1,2,8], Fabian Müller[1,2,8], Dimitrios Mougiakakos[1,2,3,8], Sebastian Böltz[2,4], Artur Wilhelm[2,4], Michael Aigner[1,2], Simon Völkl[1,2], David Simon[2,4], Arnd Kleyer[2,4], Luis Munoz[2,4], Sascha Kretschmann[1,2], Soraya Kharboutli[1,2], Regina Gary[1,2], Hannah Reimann[1,2], Wolf Rösler[1,2], Stefan Uderhardt[2,4], Holger Bang[5], Martin Herrmann[2,4], Arif Bülent Ekici[6], Christian Buettner[6], Katharina Maria Habenicht[7], Thomas H. Winkler[7], Gerhard Krönke[2,4,8] and Georg Schett[2,4,8] ✉

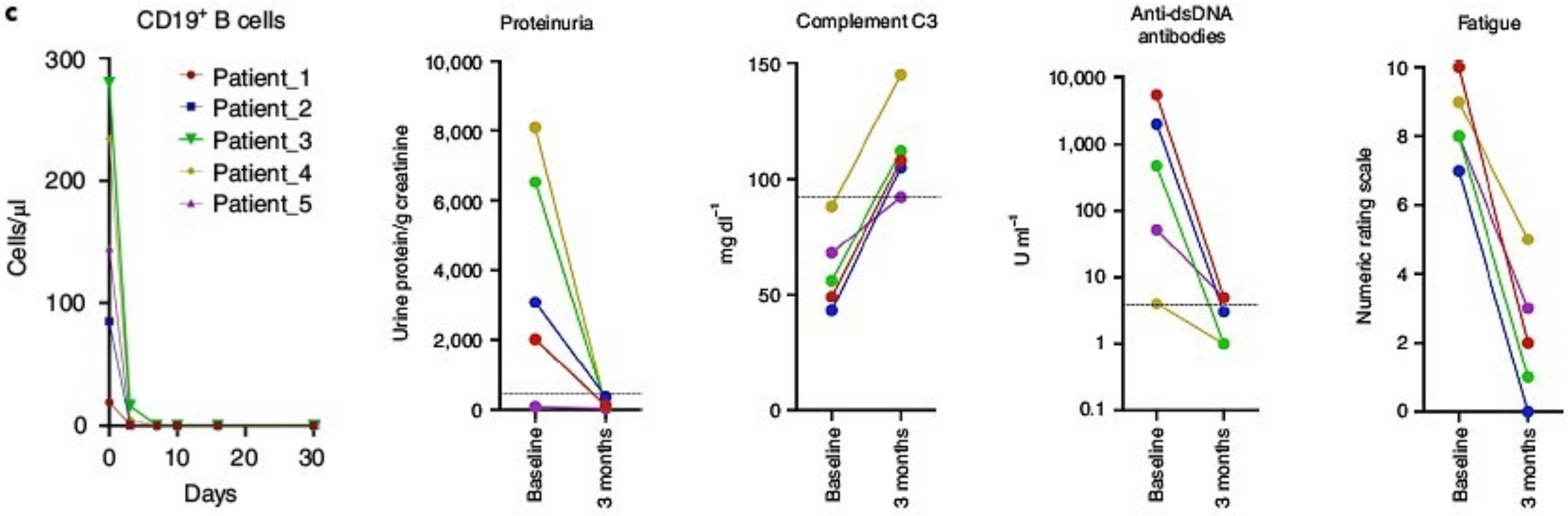
Systemic Lupus Erythematosus





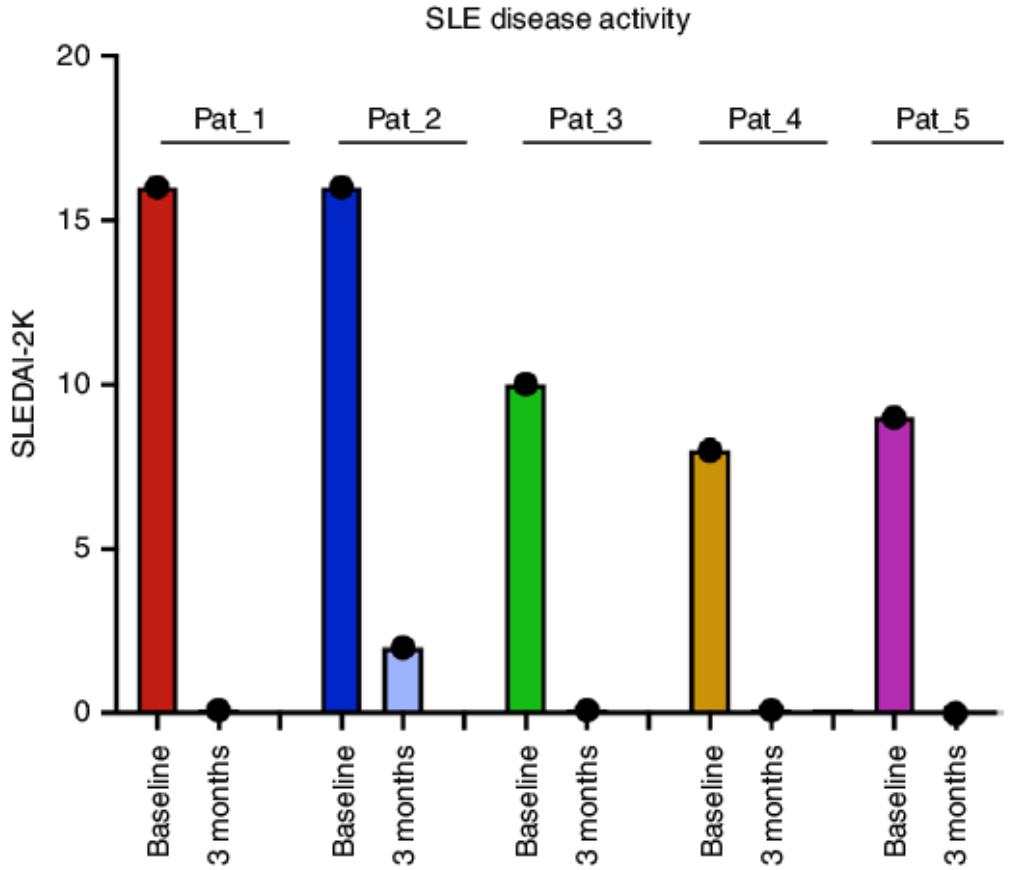
Mackensen et al. Nat Med 2022

Systemic Lupus Erythematosus



Mackensen et al. Nat Med 2022

Systemic Lupus Erythematosus

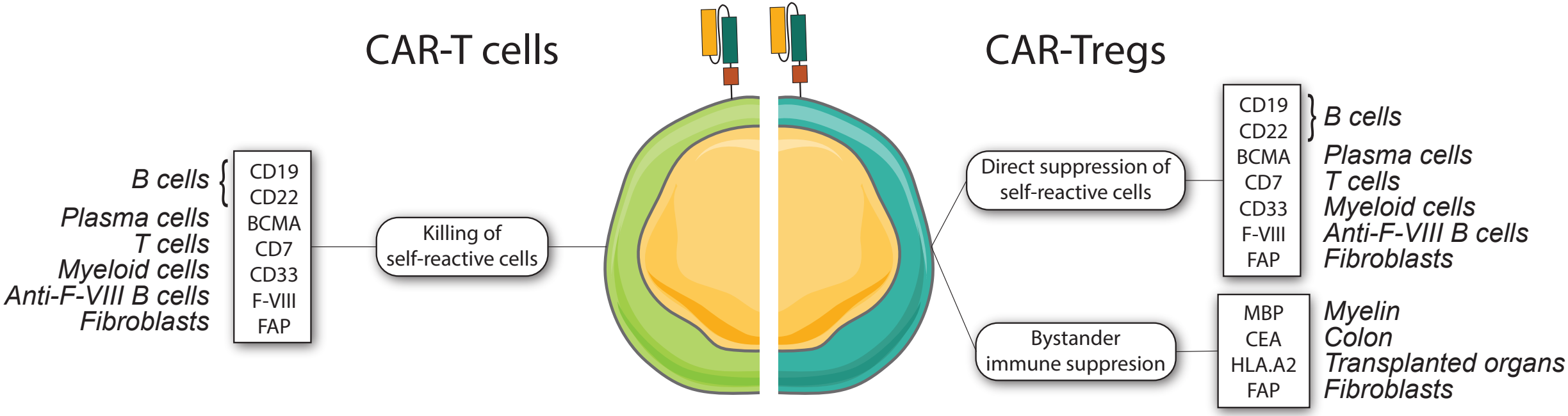


- <u>After CAR-T cells:</u>

- Reduced pro-inflammatory CKs
- Reshape of B cell compartment (immature cells)
- Normal IFN signature
- No effect on pre-existing humoral immunity

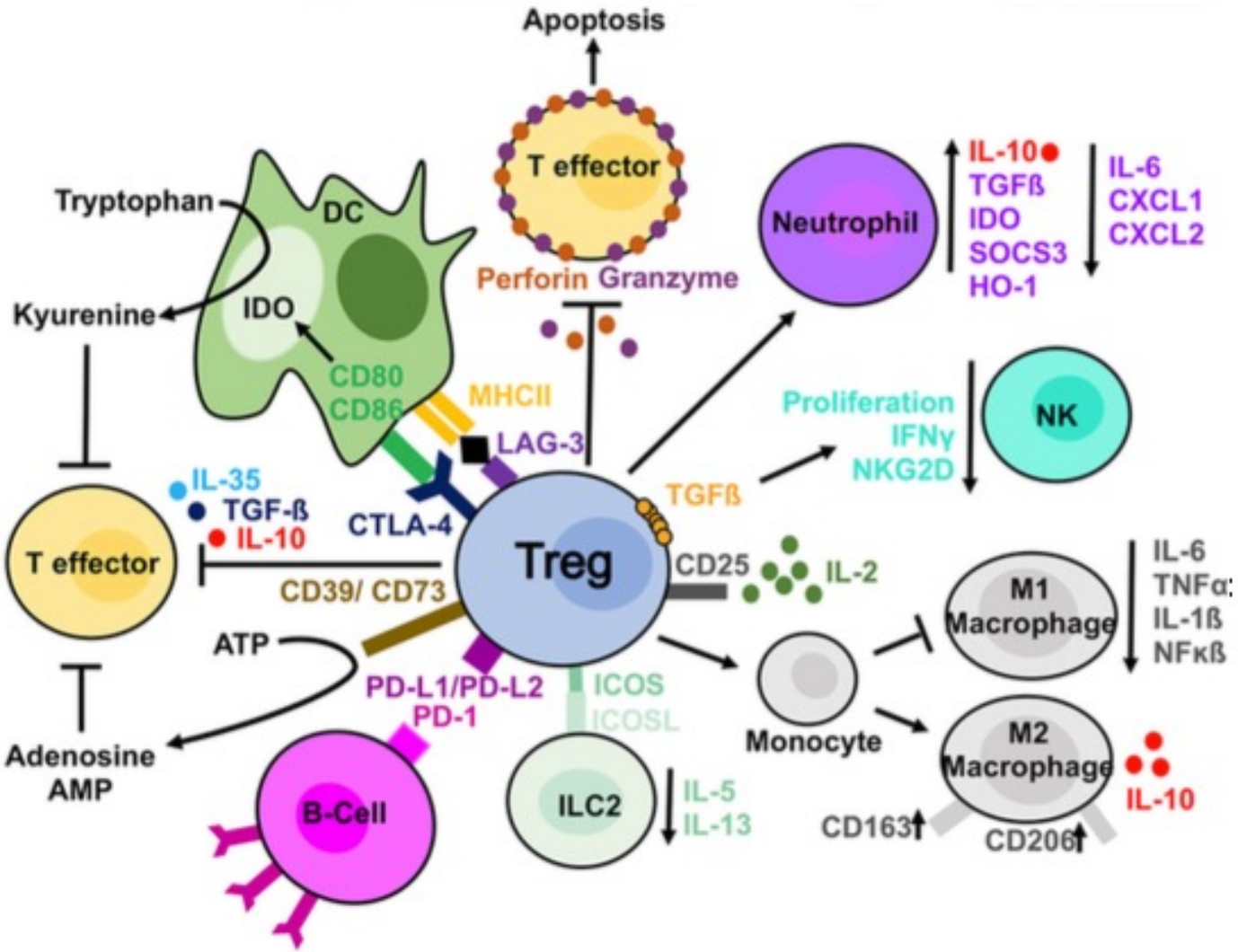
Mackensen et al. Nat Med 2022

Strategies in autoimmunity



Doglio & Alexander et al. JACI 2022

Regulatory T cells



Romano et al. Front. Imm. 2019

Treg characteristics

Forkhead box P3 (FoxP3): master regulator of Treg development

Not univocally expressed by Tregs

Transiently up-regulated by Tconvs

No unique surface markers

Combination of markers

→ Mostly identifyed as: $CD4^{+}$ $CD25^{+}$ $CD127^{low}$

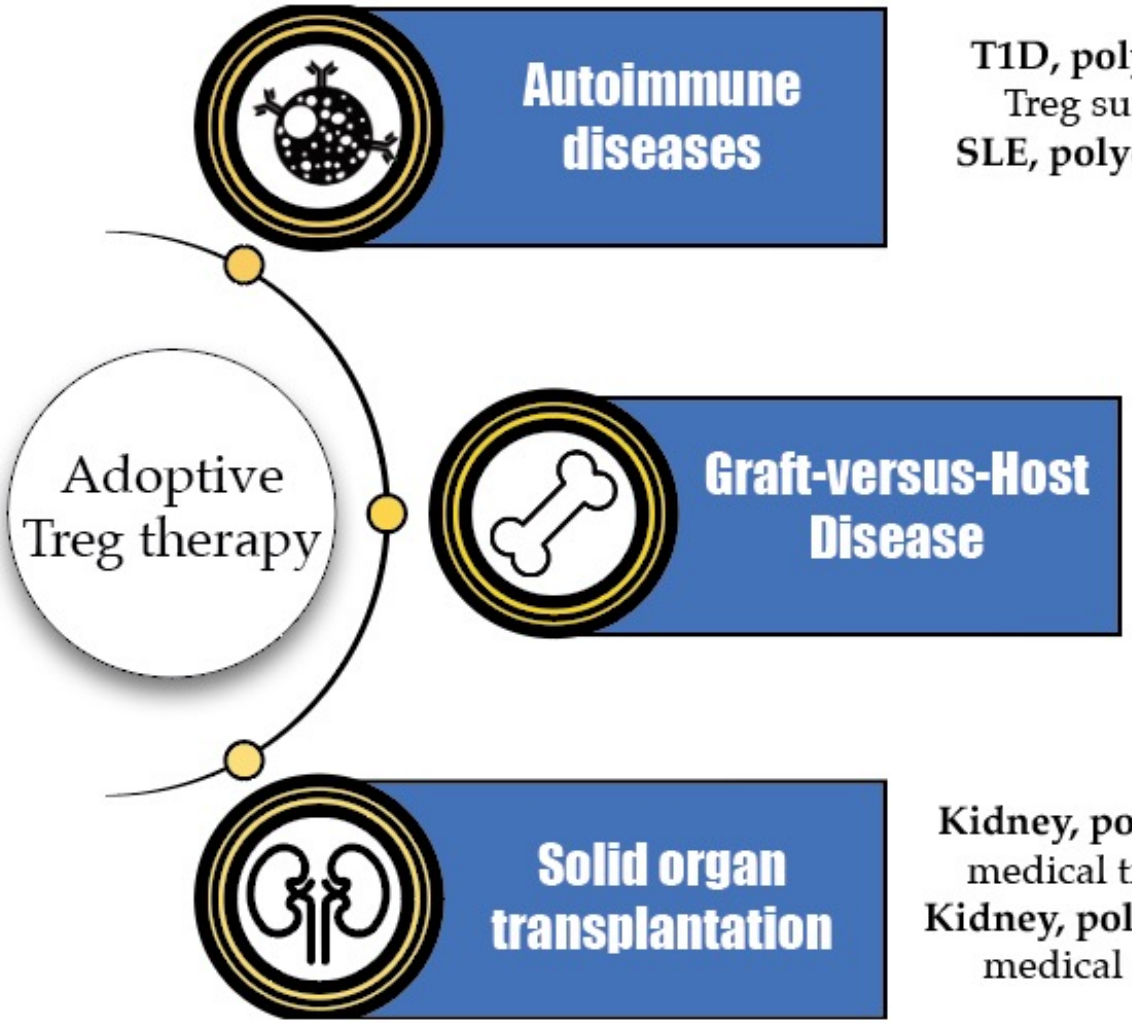
Other markers: identification of specific subsets (e.g. CD45RA, CD49b, CD15s)

Epigenetic status

Methylation of the FoxP3 locus (TSDR): very specific

Not useful for sorting live cells

Treg potential applications

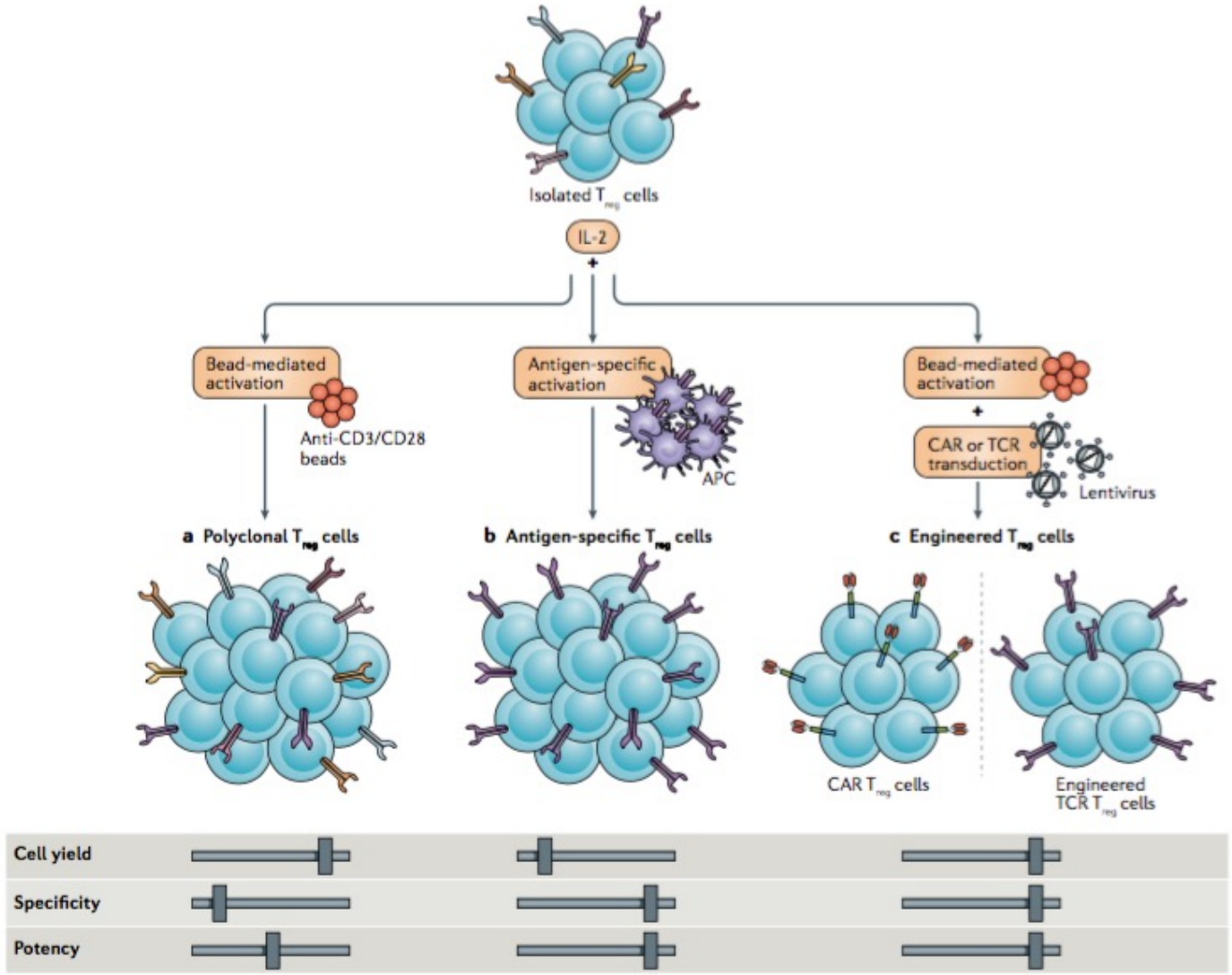


T1D, polyclonal Treg: no conclusions, hints on Treg survival (14 pt.s, Bluestone et al. 2015)
SLE, polyclonal Treg: limited skin improvement (1 pt., Dall'Era et al. 2019)

Acute, umbilical polyclonal Treg: reduced incidence (11 pt.s, Brunstein et al. 2016)
Polyclonal Treg: reduced incidence, no standard prophylaxis (43 pt.s, Martelli et al. 2014)
Chronic, polyclonal Treg: tapering of the immune suppressive regimen (5 pt.s, Theil et al. 2015)

Kidney, polyclonal Treg: no conclusions, standard medical treatment (3 pt.s, Chandran et al. 2017)
Kidney, polyclonal Treg: no graft rejection standard medical treatment (9 pt.s, Mathew et al. 2018)

Antigen-specific Tregs



Raffin et al. Nat Rev Imm 2019

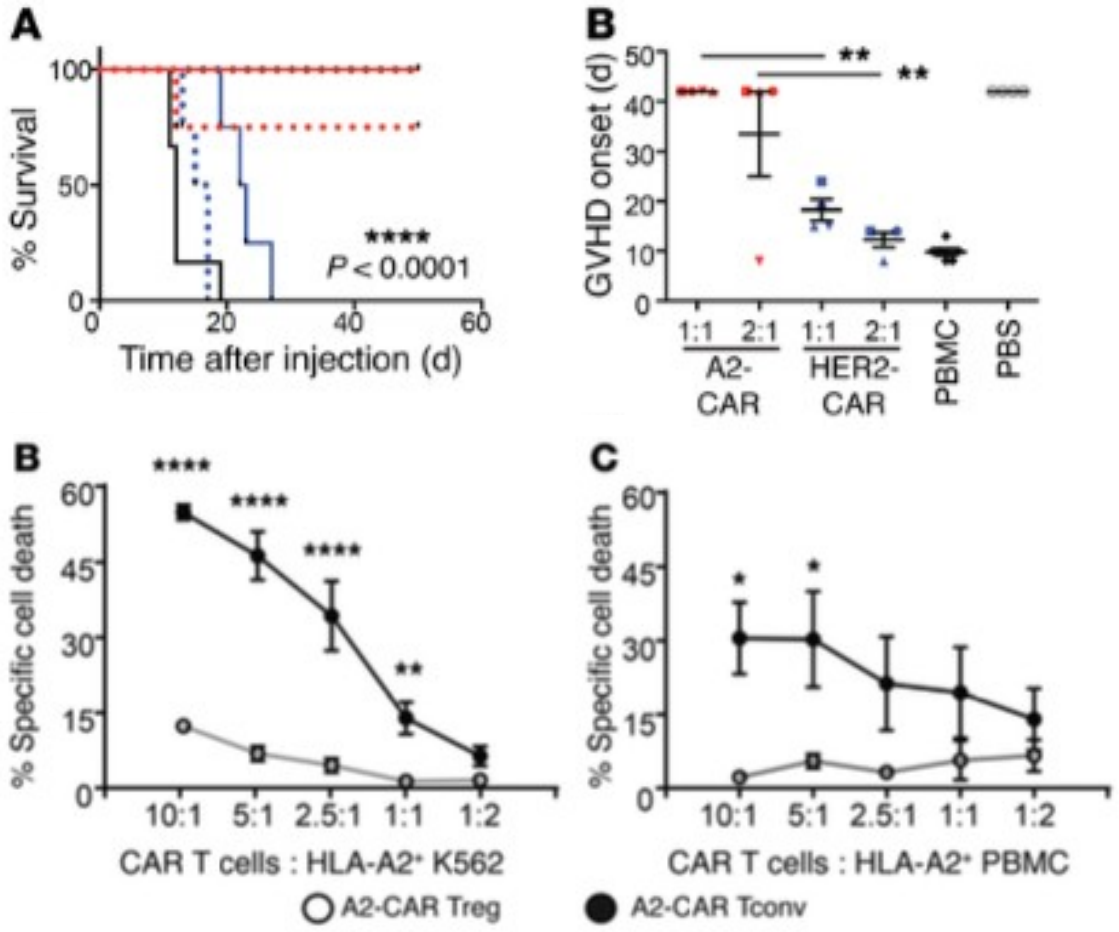
Anti-HLA.A2 CAR-Tregs

Second-generation CAR with 28z

Delay of aGvHD onset in a xenograft mouse model

STEADFAST clinical trial (ongoing)

Anti-HLA.A2 CAR-Tregs for mismatched kidney transplantation in ESRD

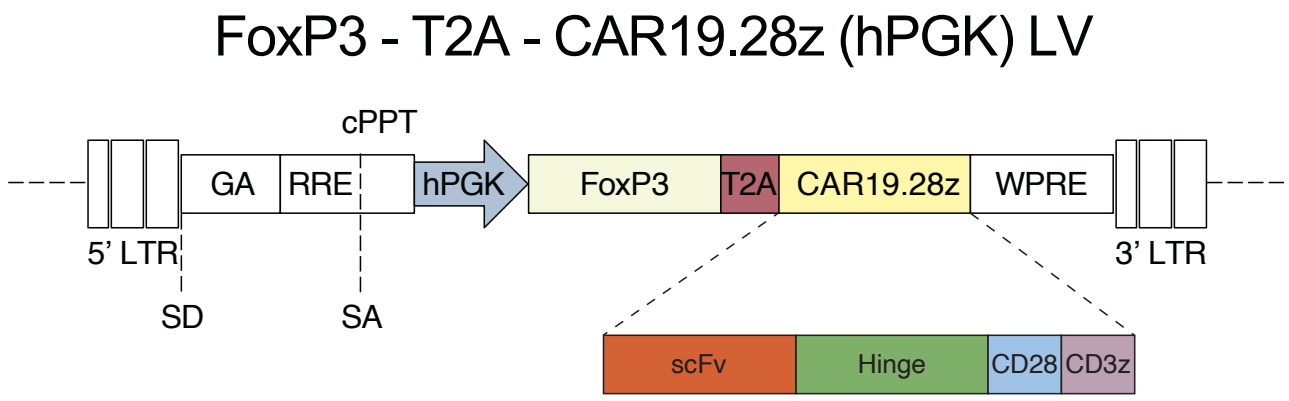


MacDonald et al. JCI 2016

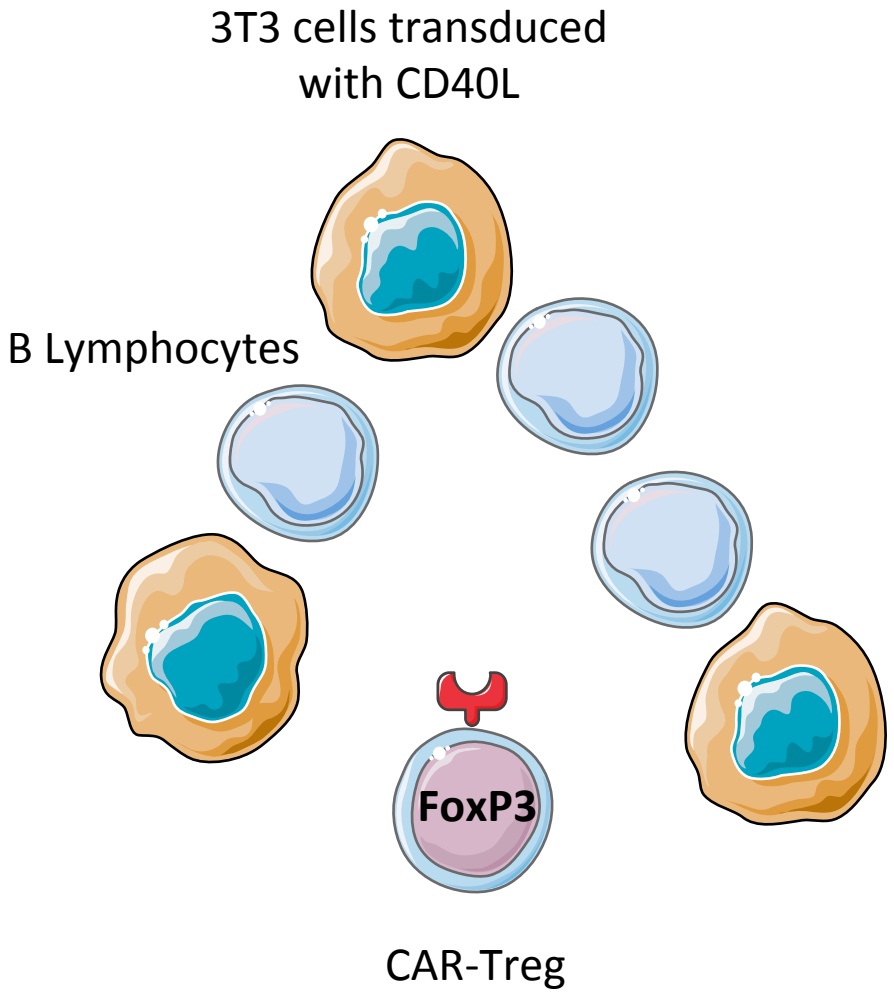
Our experience

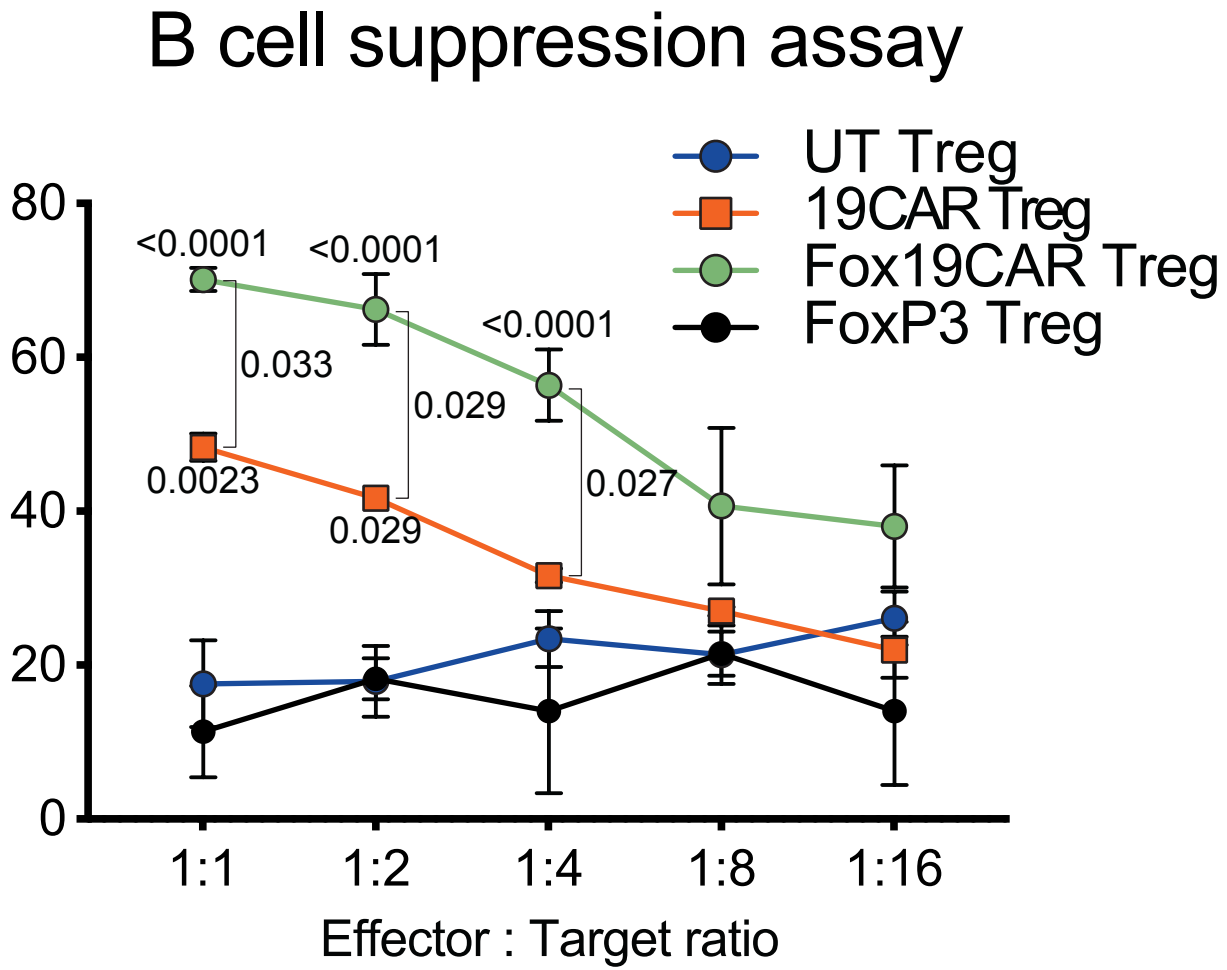
We aimed at developing a CAR-Treg product for the treatment of **Systemic Lupus Erythematosus**

B cells play a major role in the pathogenesis



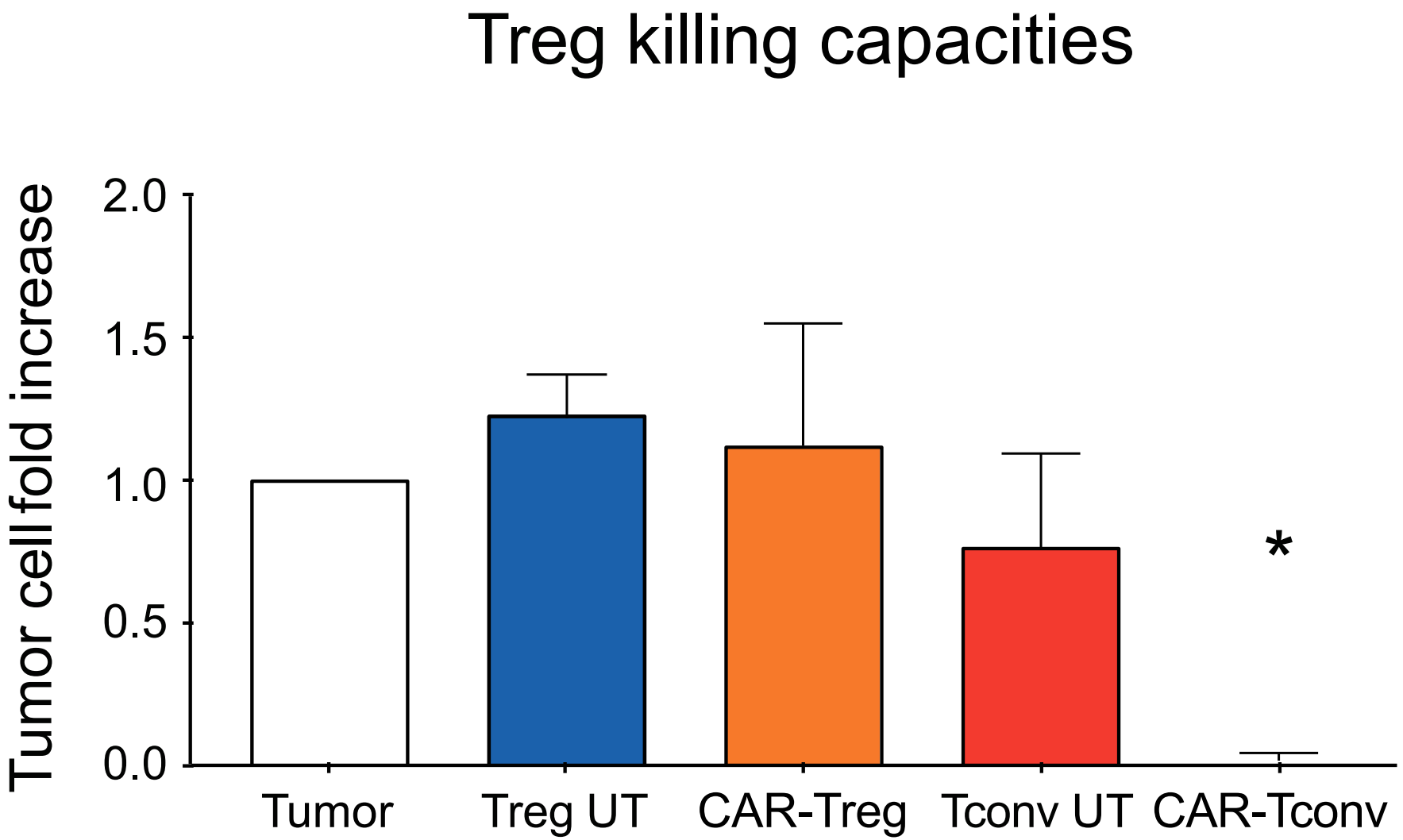
Ag-specific suppression





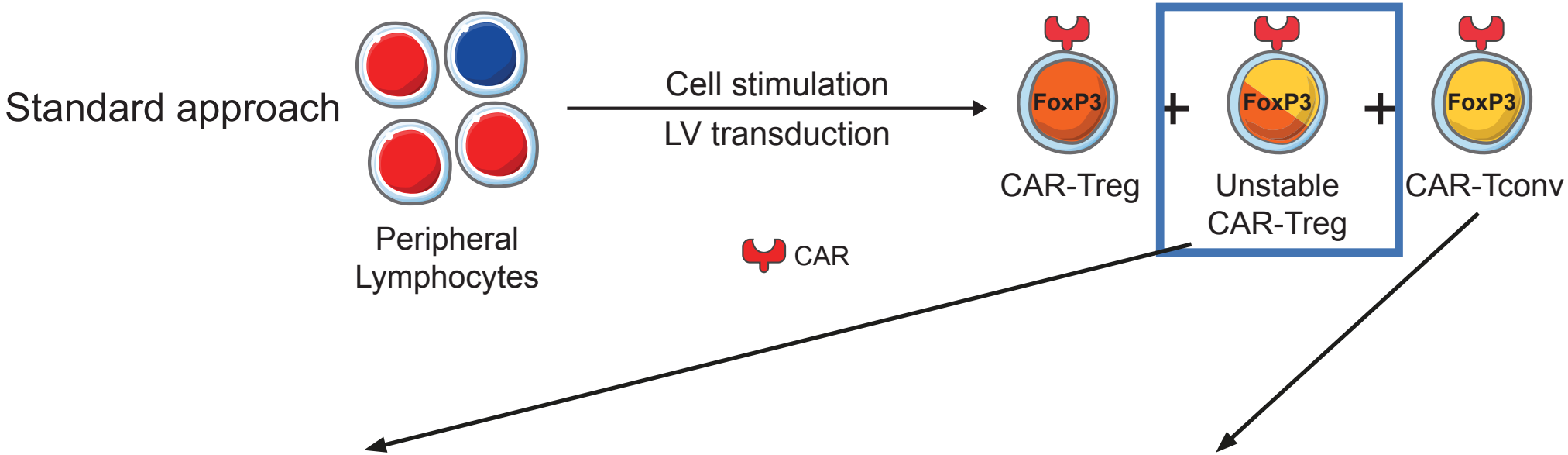
Doglio et al. Nat Comm 2024

CAR-Tregs do not kill



Doglio et al. Nat Comm 2024

Treg instability



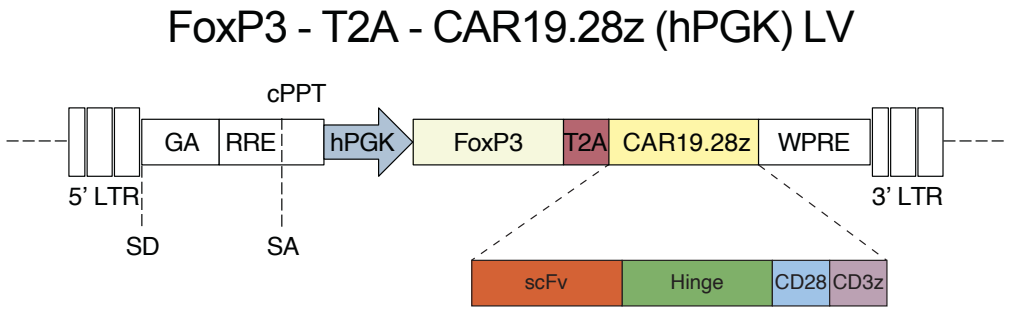
Instability

Reprogramming to pro-inflammatory cells in inflammatory environments

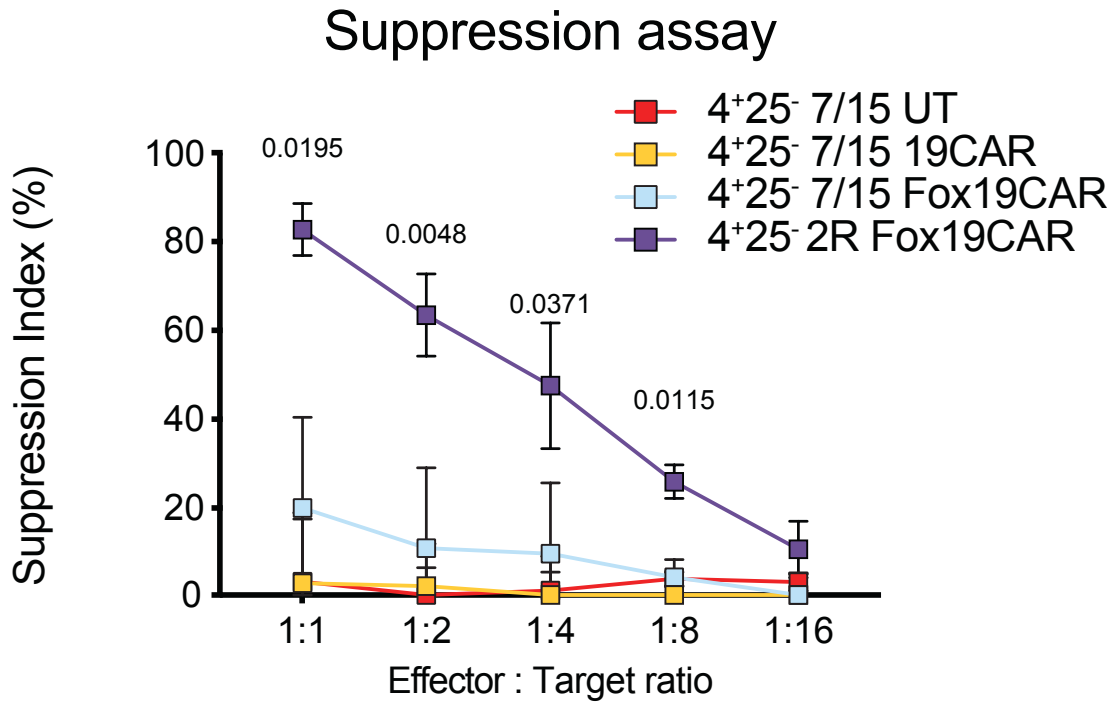
Self-reactive cells

Direct aggression with consequent damages to healthy tissues

Tconv reprogramming

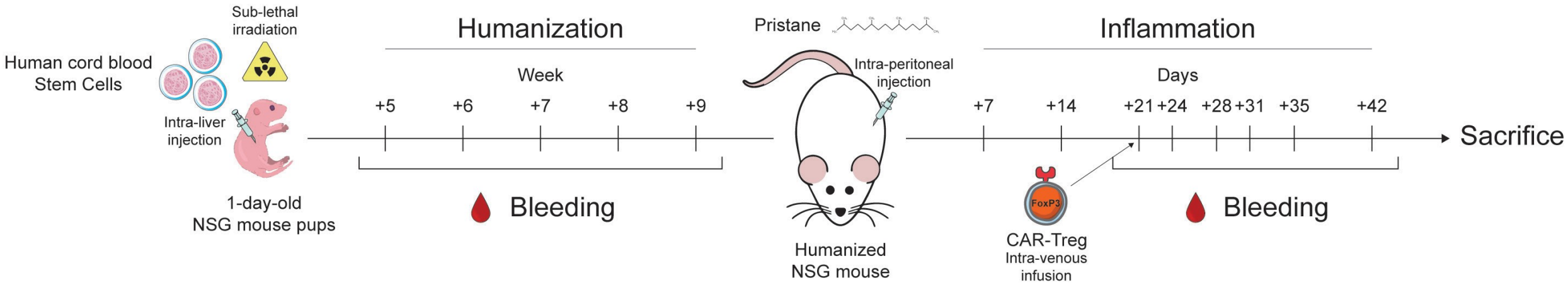


Starting population:
CD4+CD25- T cells



Doglio et al. Nat Comm 2024

In vivo evaluation



3 groups of treatment

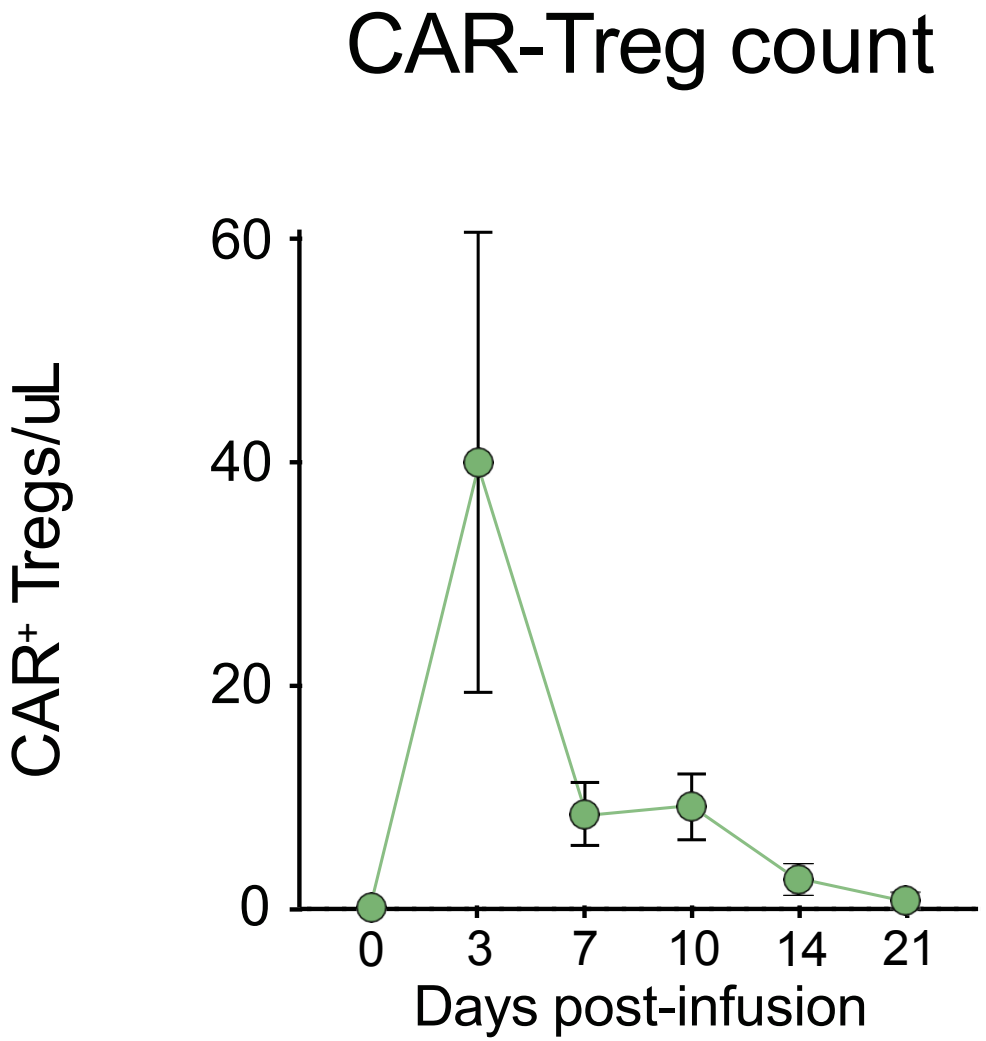
CAR-Treg

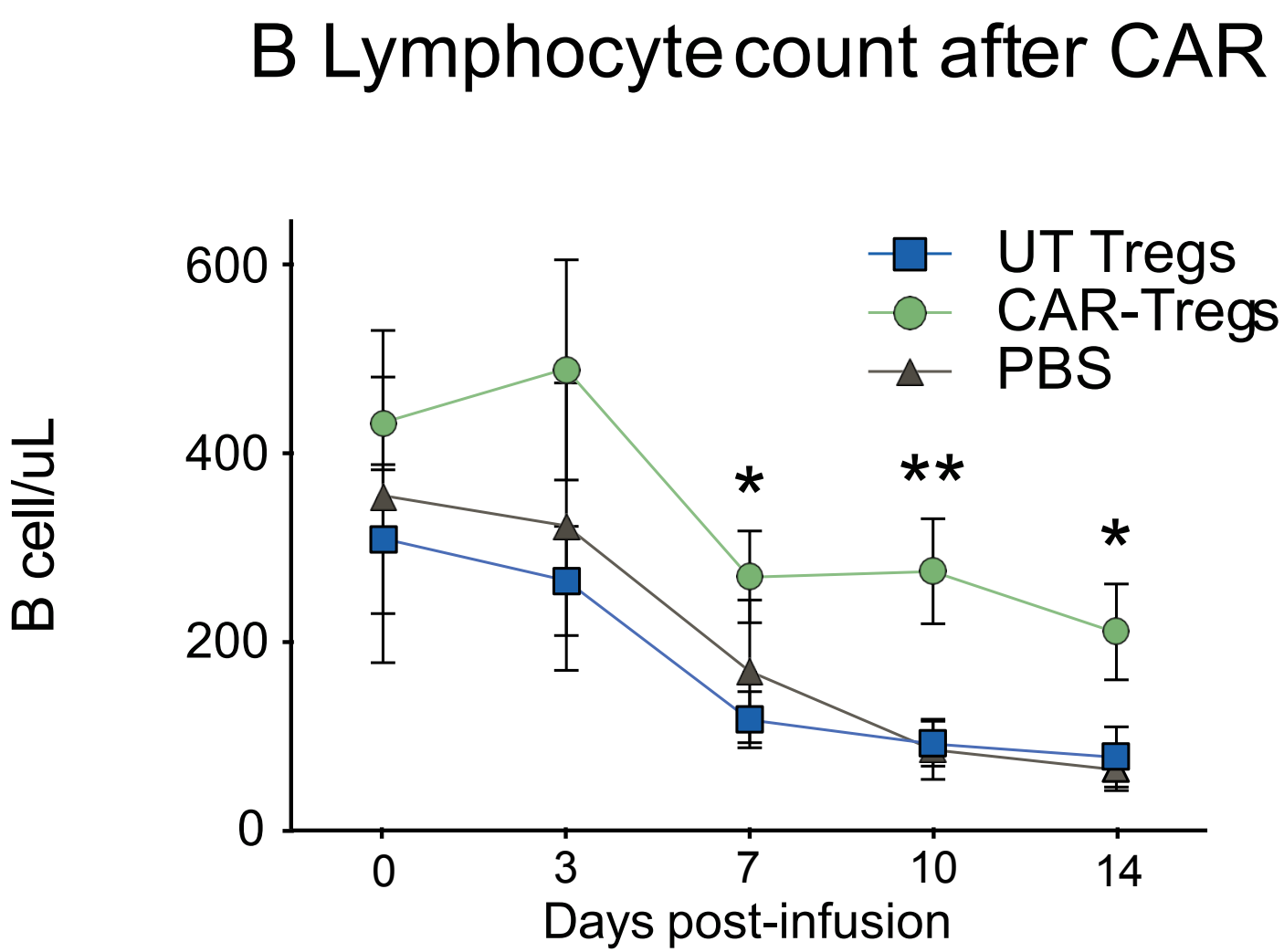
Untransduced Treg

Control

Doglio et al. Nat Comm 2024

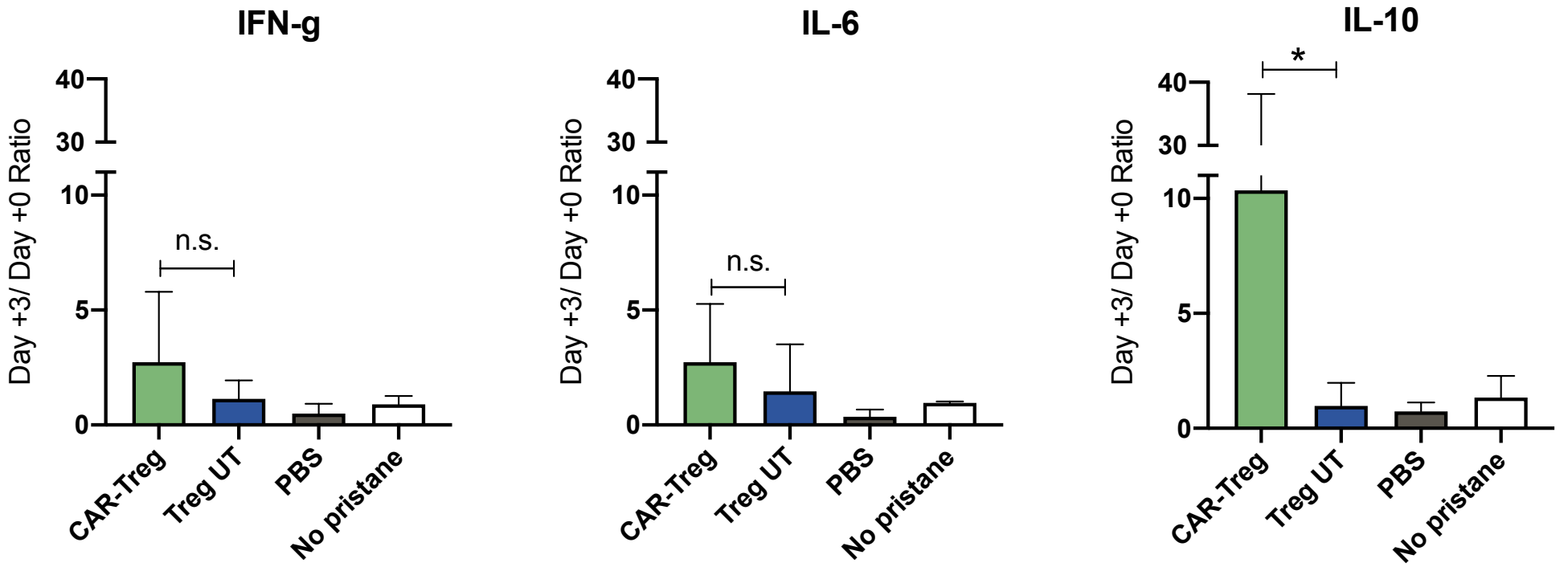
CAR-Tregs delay B lymphopenia



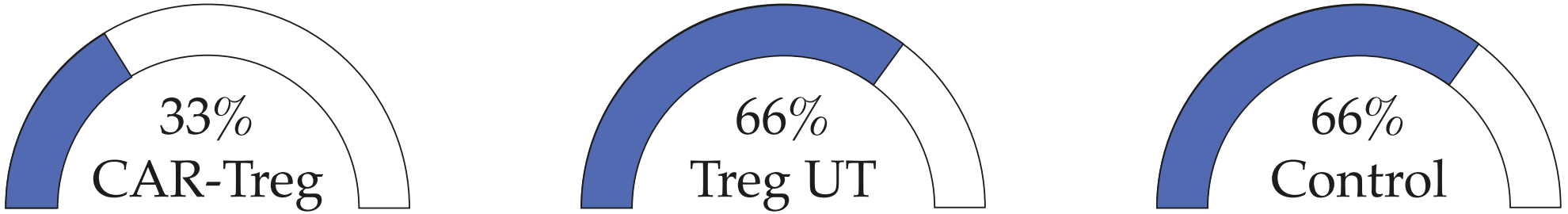


Doglio et al. Nat Comm 2024

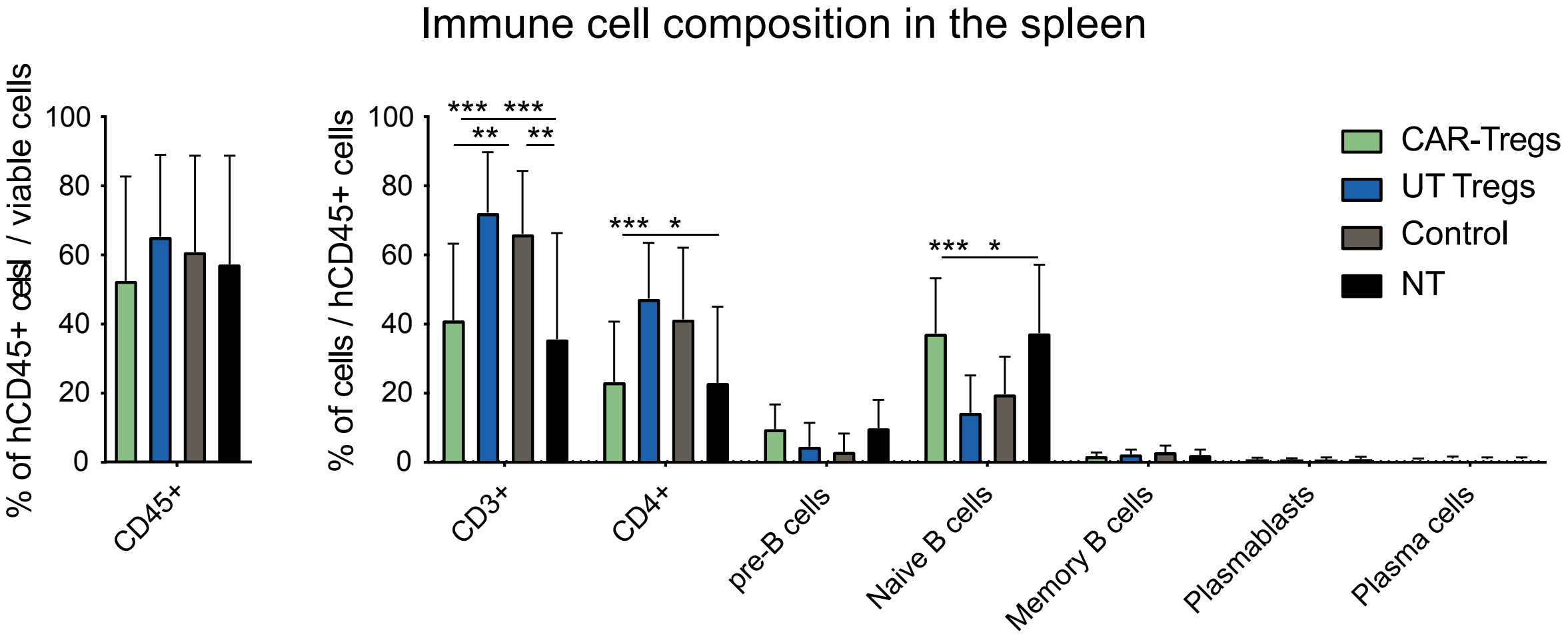
CAR-Treg immunomodulation



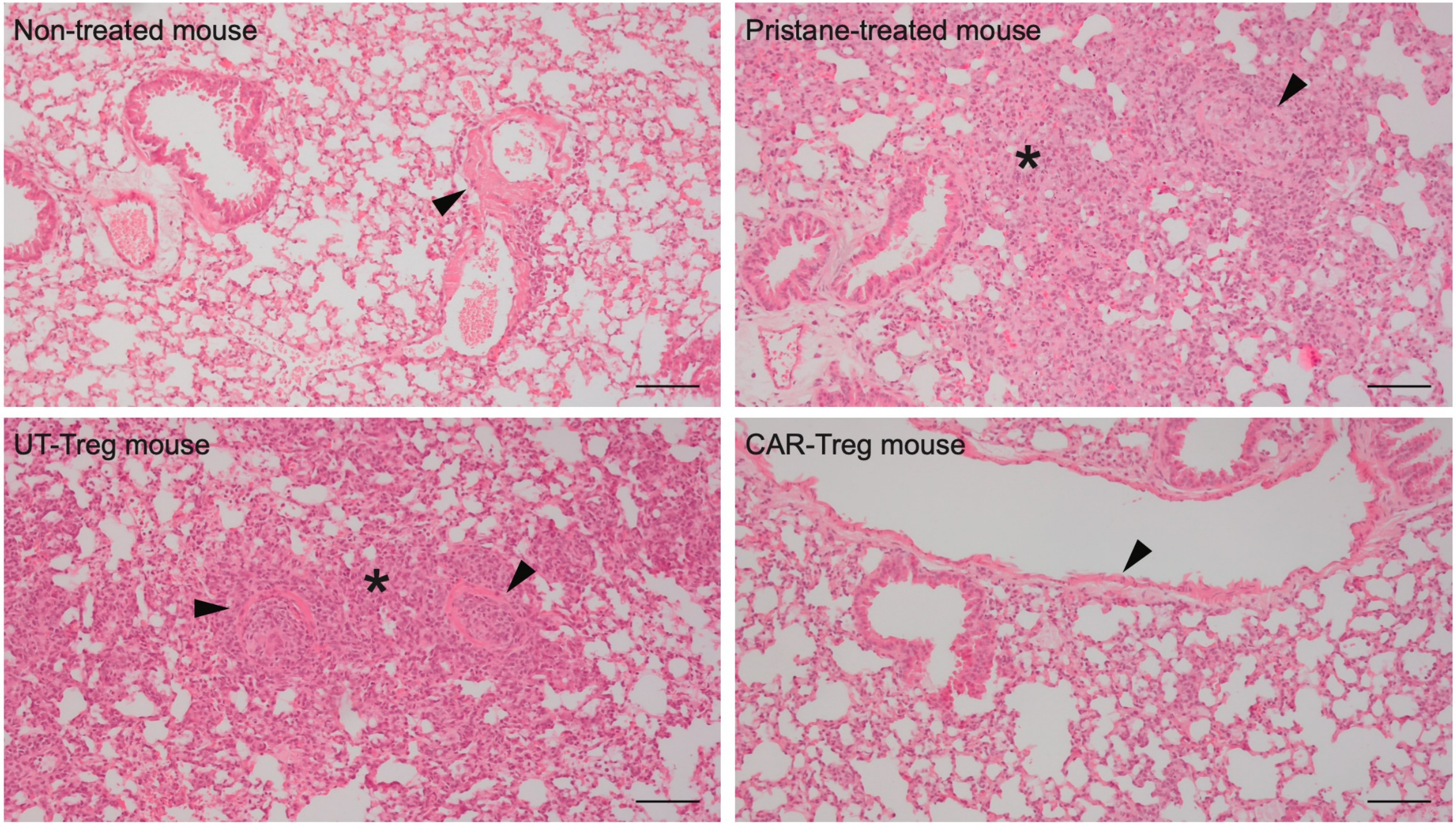
Anti-dsDNA autoantibodies



Restored immune composition



Doglio et al. Nat Comm 2024


Non-treated mouse
Pristane-treated mouse
UT-Treg mouse
CAR-Treg mouse

Conclusions

- We optimize a protocol to efficiently generate CAR-Tregs
- CAR-Tregs exert antigen-suppressive capacities without showing killing capacities
- FoxP3 over-expression reprograms Tconv to suppressive cells
- We generated a humanized mouse model of SLE
- CAR-Tregs proved safe when injected in vivo
- CAR-Tregs controled the inflammation and restore the normal composition of the human immune system in vivo

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Acknowledgments

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and all MDs, Nurses, Data Managers

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Innovative Therapy Unit
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