Cancer Immunotherapy (2)

Adaptive Cell Transfer Therapy

Adoptive cell therapy (ACT) is a treatment that uses a cancer patient's own T lymphocytes with anti-tumor activity, expanded in vitro and re-infused into the patient with cancer.

Adaptive Cell Transfer Therapy

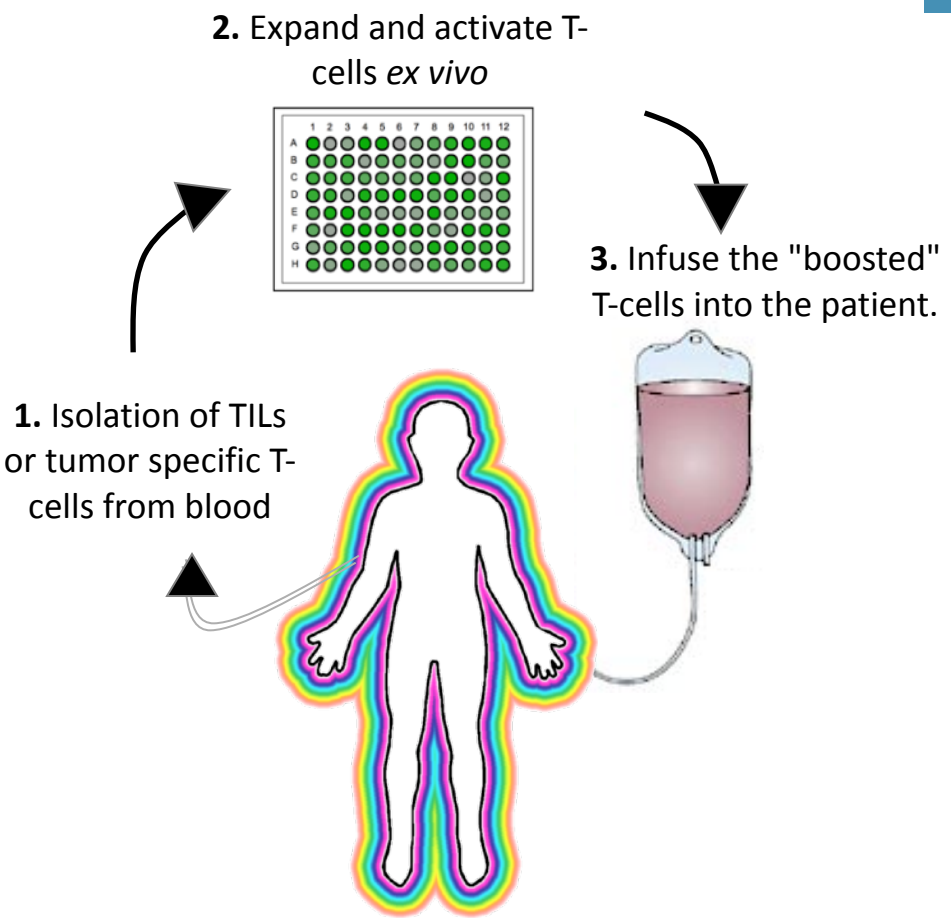
- **TIL** (Tumor infiltration T-lymphocytes therapy)

TIL

The first paper to demonstrate the regression of cancer using TIL for the immunotherapy of patients with metastatic melanoma.

Rosenberg, S. A.et al.Use of tumor infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. Preliminary report. **N. Engl. J. Med. 319, 1676–1680 (1988).**

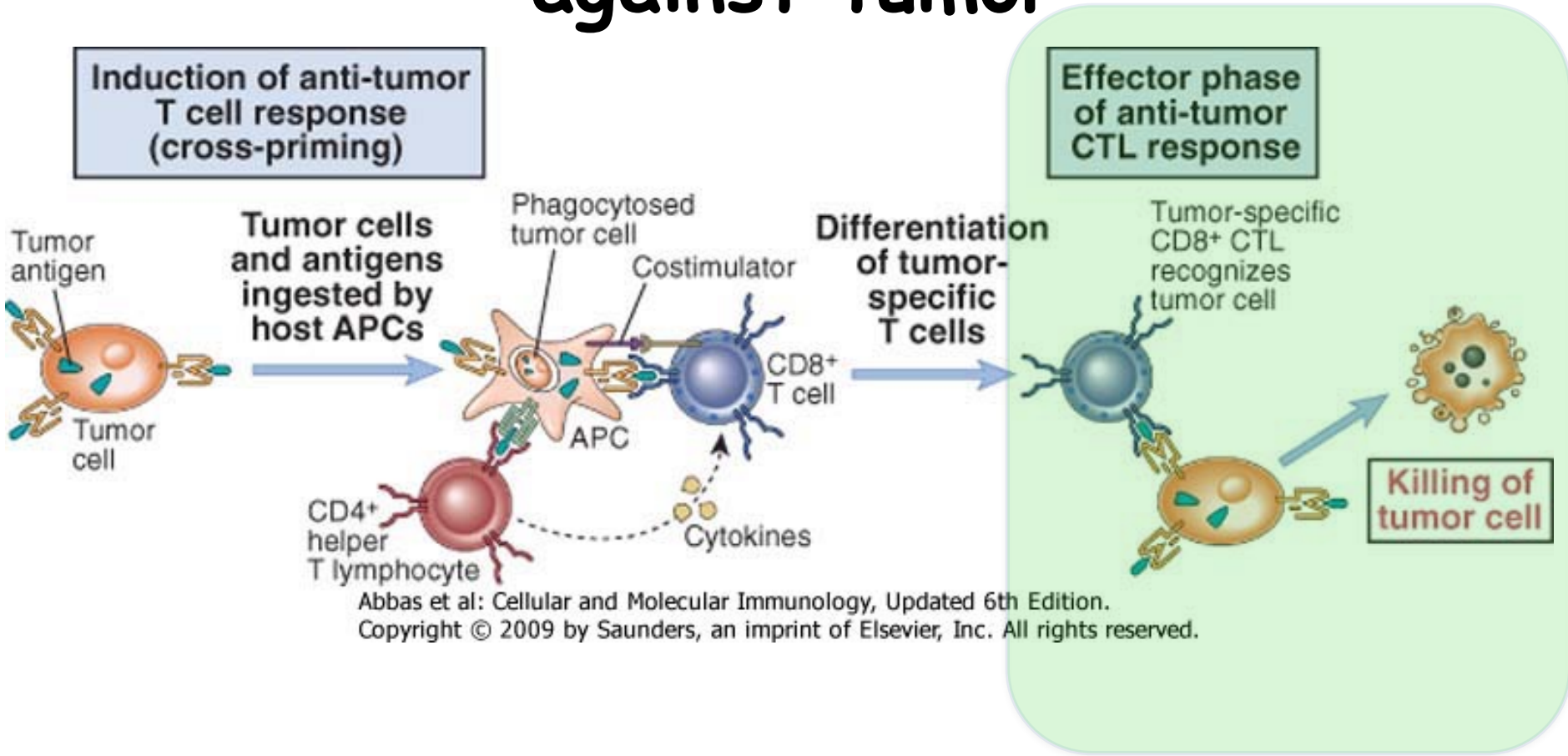
TIL

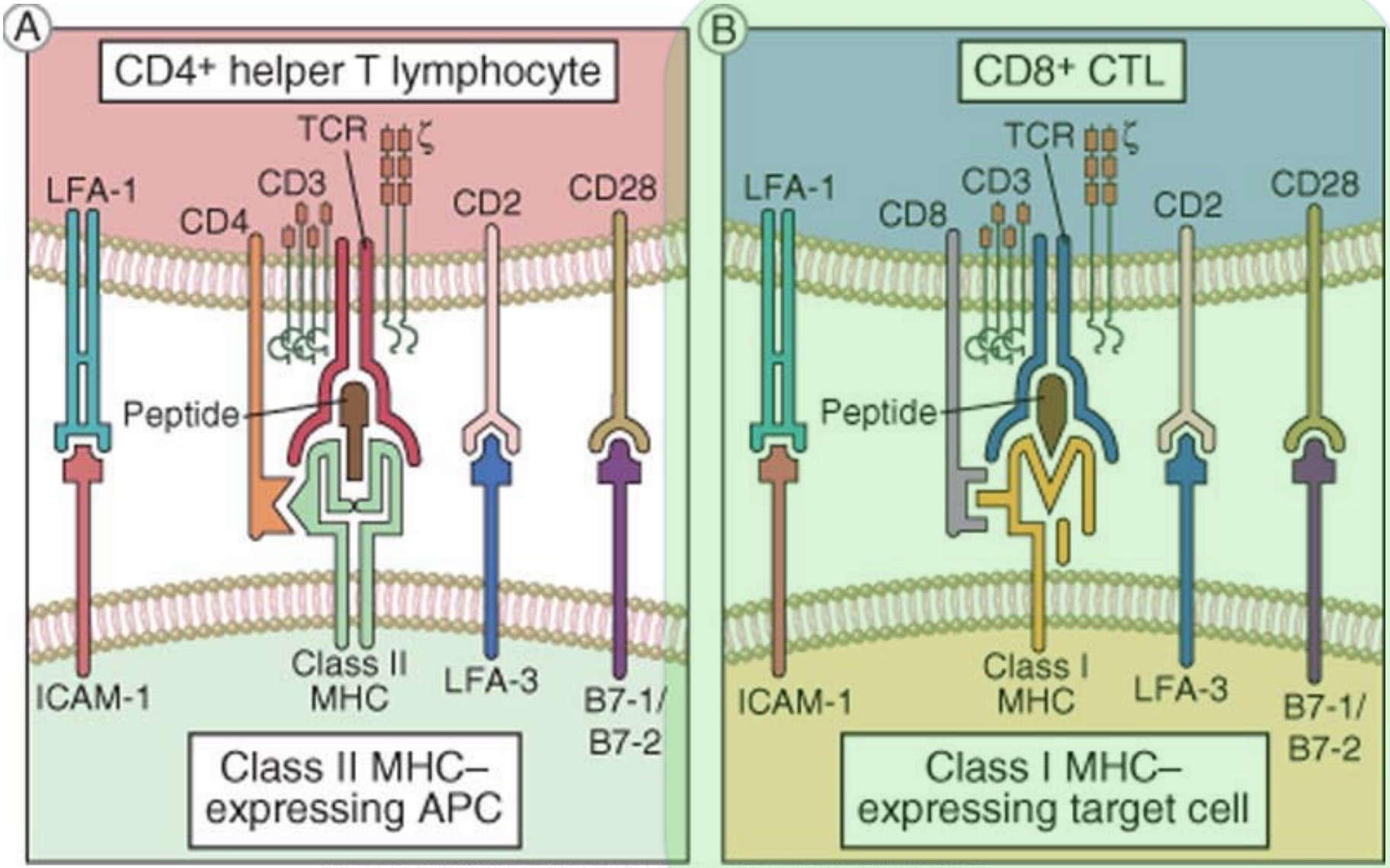


- **Target therapy with Tumor specific T cells**
  - Cancer: Melanoma
  - Autologous tumor infiltrating lymphocytes (TILs); “Live drug”
- **Advantages**
  - High response rate (>50%),
  - Long-term remission,
  - Less toxic & gentler to the patient
- **Limitation:**
  - Extraction of TILs,
  - Cell manufacturing

Rosenberg SA & Dudley ME 2009 Current Opinion of Immunology

Induction of a T-response against tumor



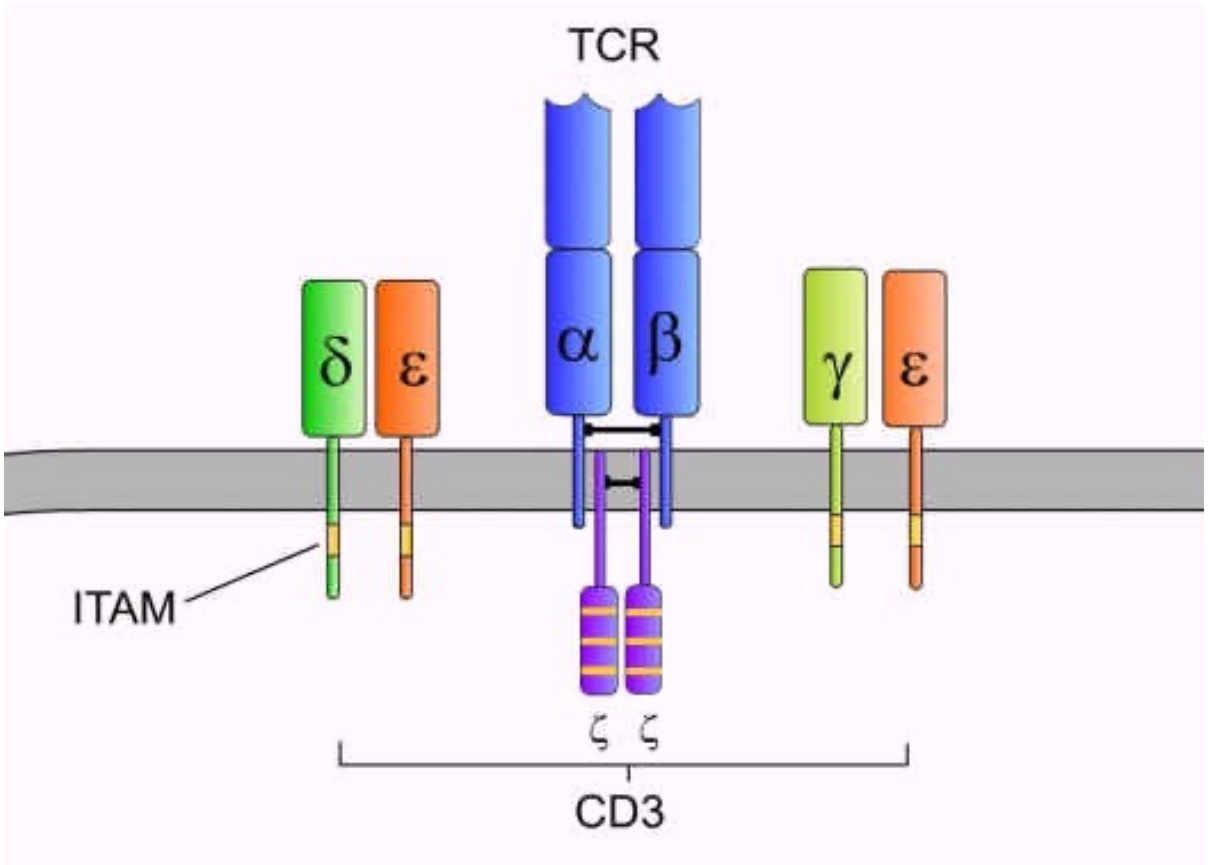


Low number of TIL

Adaptive Cell Transfer Therapy

- **TIL** (Tumor infiltration T-lymphocytes therapy)
- **TCR** (T-cell receptor therapy)

TCR



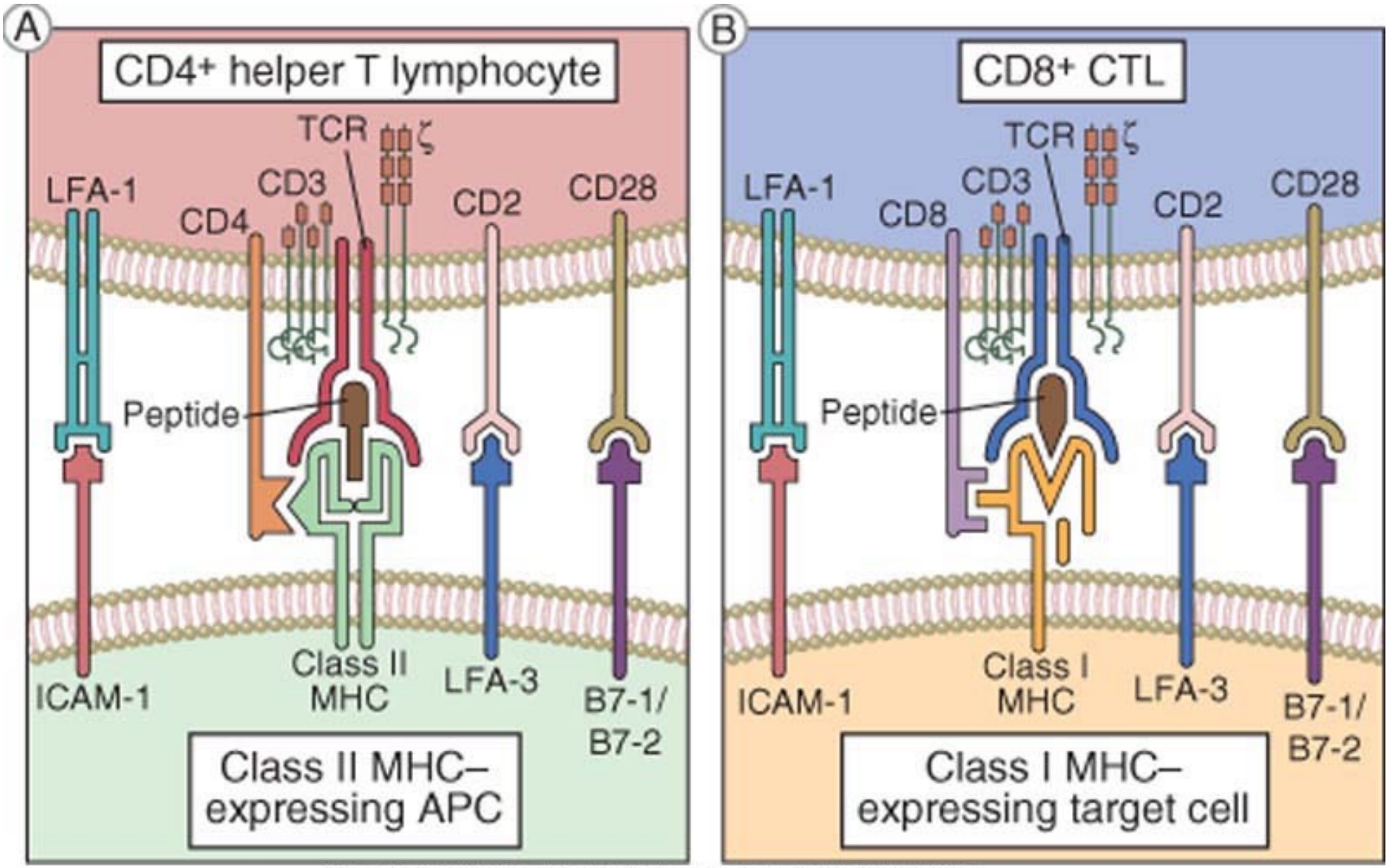
TCR complex :TCR, CD3, ζ

ITAM: immunoreceptor tyrosine-based activation motif

TCR

The first paper demonstrating the adoptive cell transfer of lymphocytes transduced with a retrovirus encoding TCRs that recognize a cancer antigen can mediate anti-tumour responses in patients with metastatic melanoma.

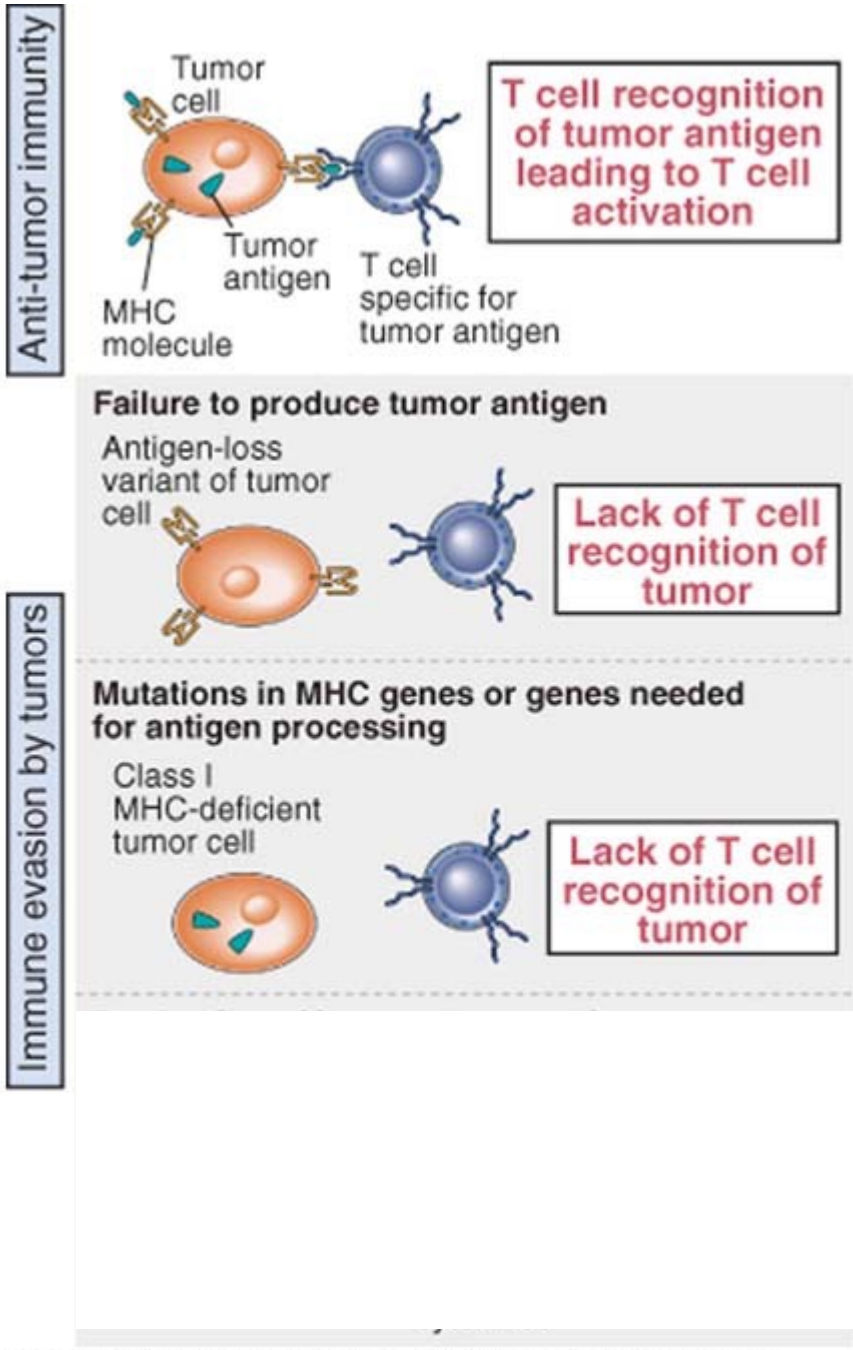
Morgan, R. A.et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. **Science 314, 126–129 (2006).**



HLA-restricted response – tumor escape

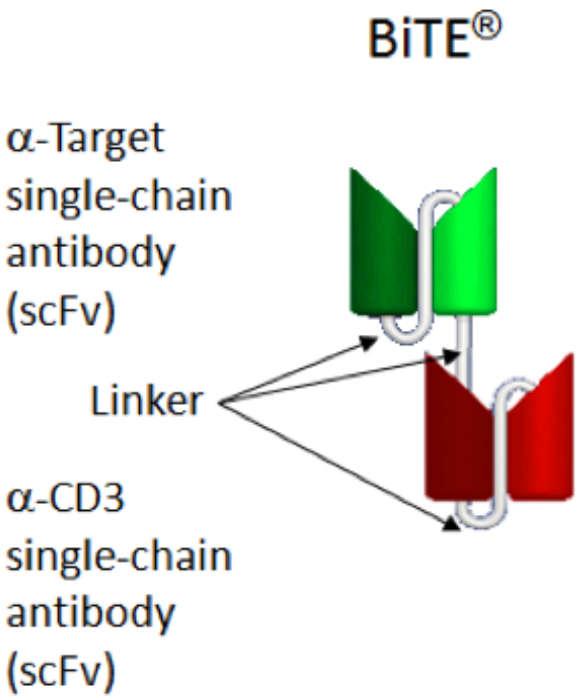
Tumor escape (4)

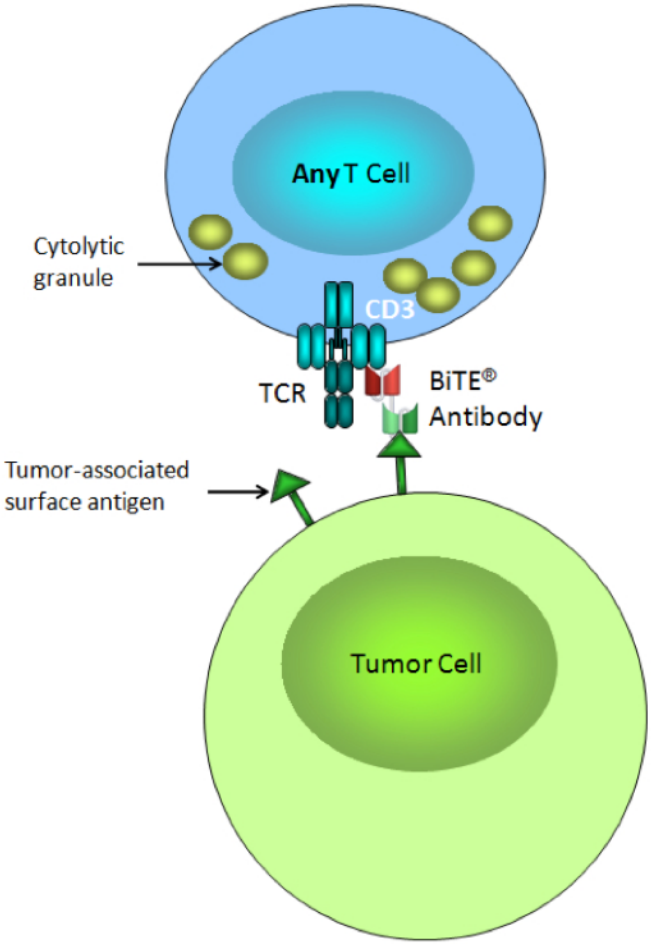
- Some tumor cells reduce the expression of MHC I



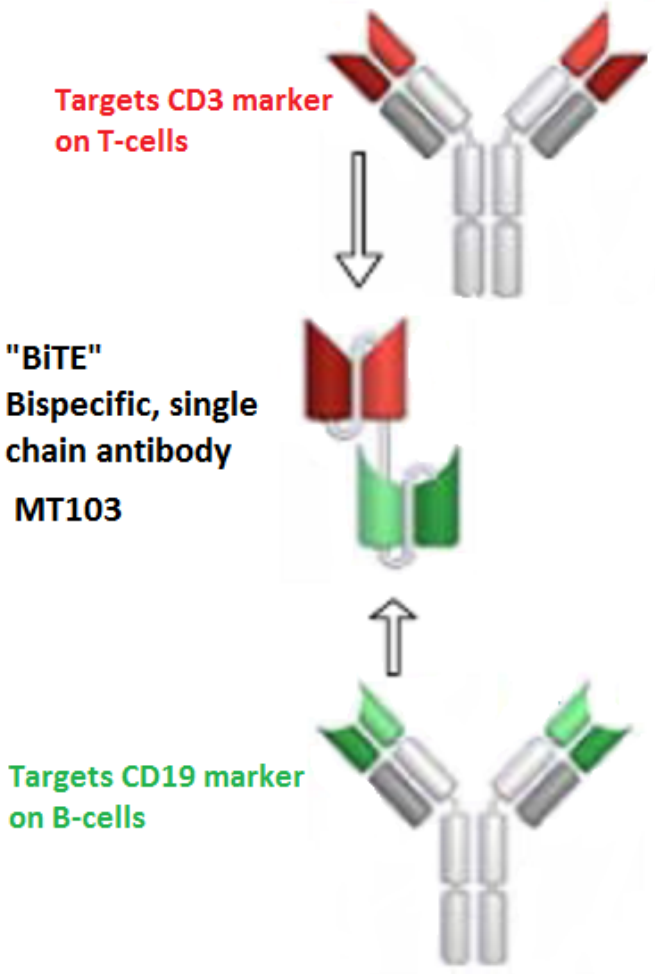
Any possibility to design an immunotherapeutic approach able to work independently from class I MHC?

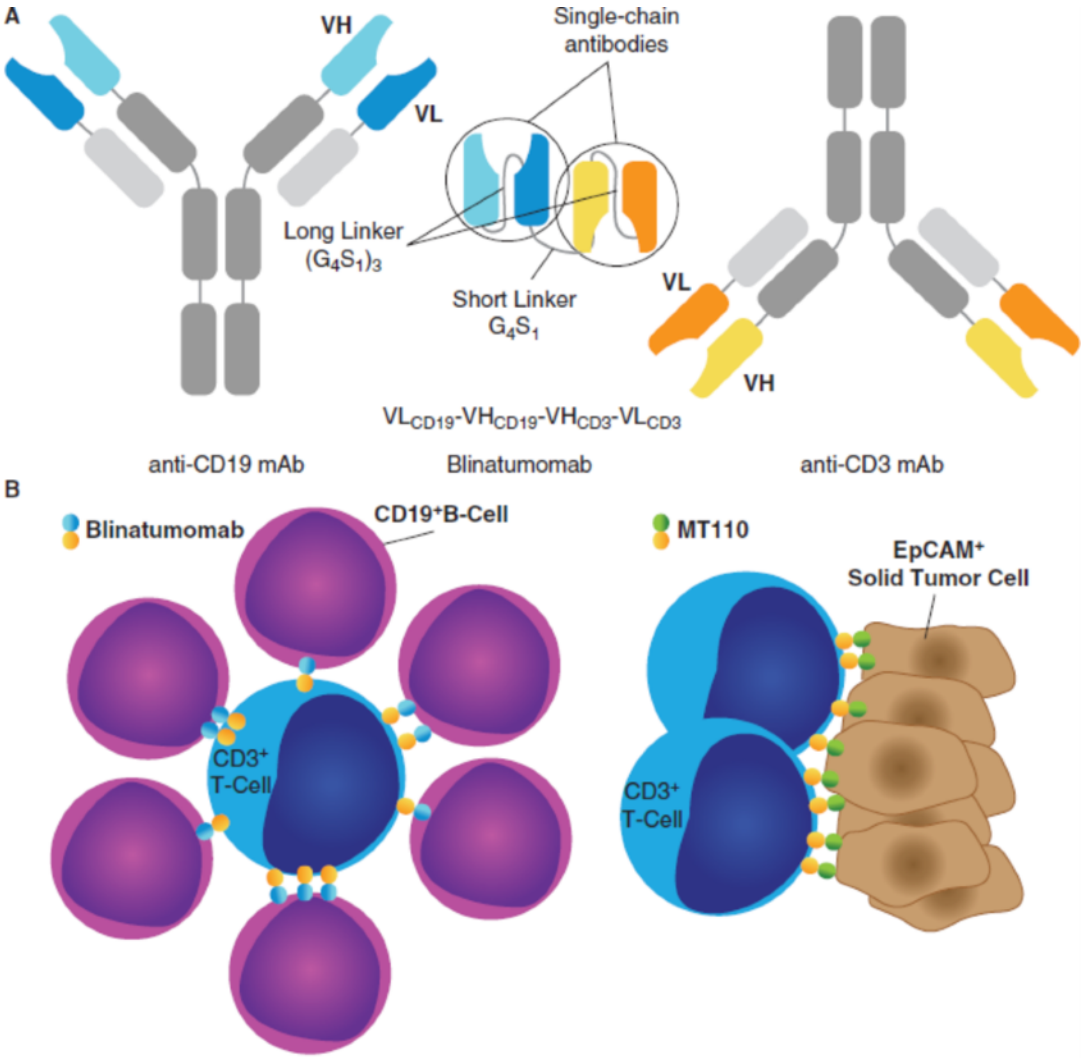
Bispecific T-Cell Engaging (BiTE) Antibody





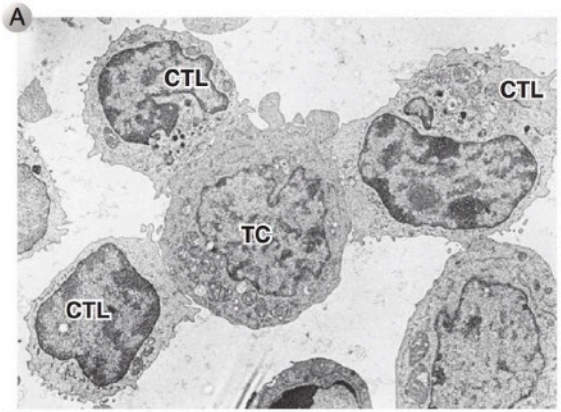
Blinatumumab

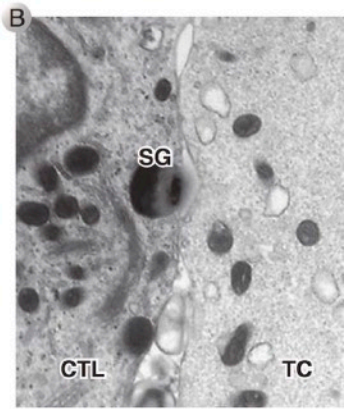


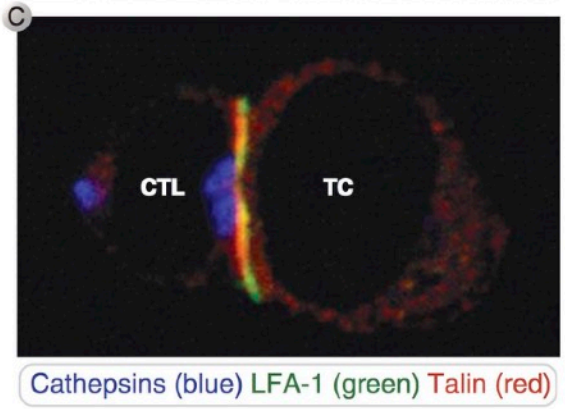


Structure and function of blinatumomab. A. The structural features of blinatumomab (MT103, AMG103) arise from monoclonal antibodies (mAbs) directed against CD19 and CD3. Single-chain antibodies are constructed from the light and heavy variable immunoglobulin domains (VL and VH) for each protein and connected using a long amino acid linker $(Gly_4Ser_1)_3$.[4, 12] Two single-chain antibodies are joined using a short amino acid linker $(Gly_4Ser_1)_1$.[31] B. Aggregation of T and B cells in the presence of blinatumomab. A cytotoxic T lymphocyte (blue) is associated with chronic lymphocytic leukemia cells (pink).[14] The EpCAM BiTE MT110 can facilitate T-lymphocyte interaction with solid tumor cells, which have high expression levels of the EpCAM antigen (e.g., pancreatic cancer cells[32]).

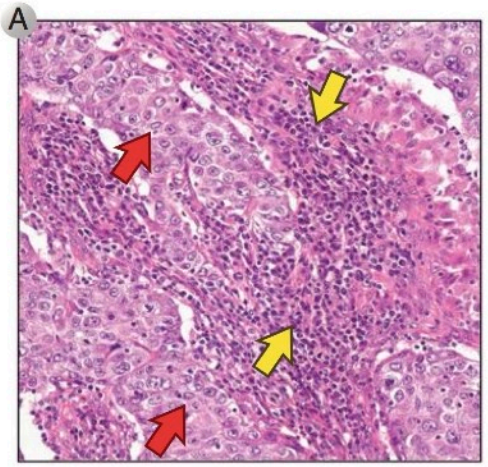
Induction of a T-response against tumor

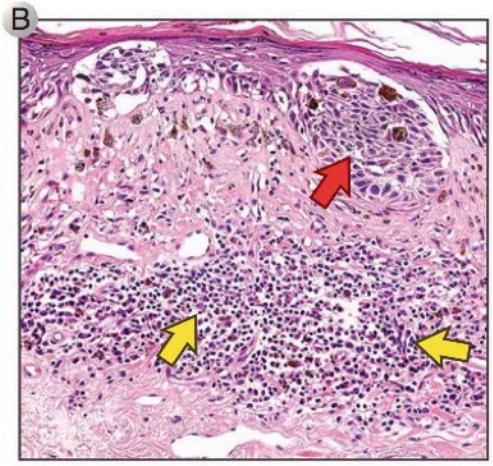


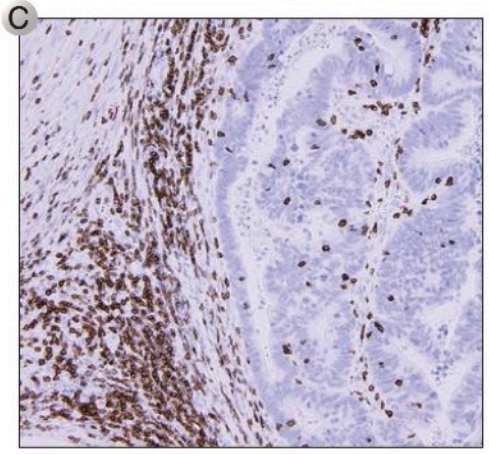


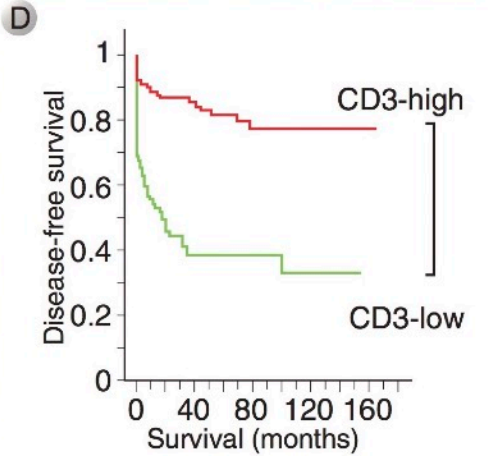


Cathepsins (blue) LFA-1 (green) Talin (red)









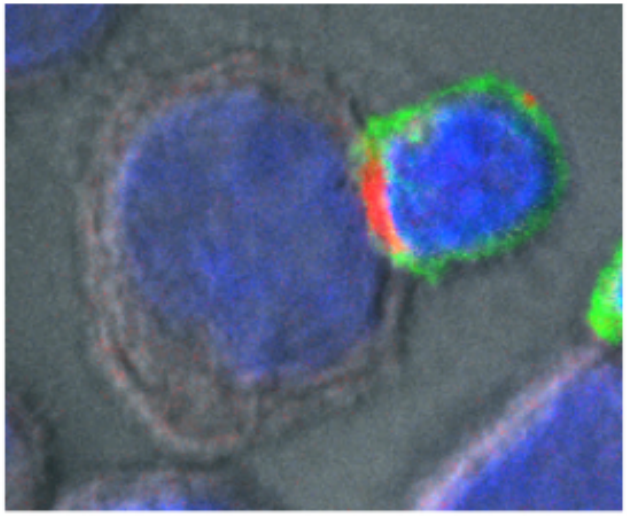


Figure 1C. Confocal microscope image displaying a cytolytic synapse between a tumor cell (with large Hoechst stained nucleus) and T cell (with small Hoechst stained nucleus and green signal recognizing CD45) in the presence of BiTE. Cytolytic synapse represented by activated PKCθ labeling (red) on T cell at tumor cell interface (courtesy of Luis Borges, Laura Smith, Padma Narayanan, and Sue Ludmann at Amgen).

Limit of BsAb (BITE)

- **Molecular weight** (have to be low MW)
- **Manufacturing** (purification)
- **Number of TIL**

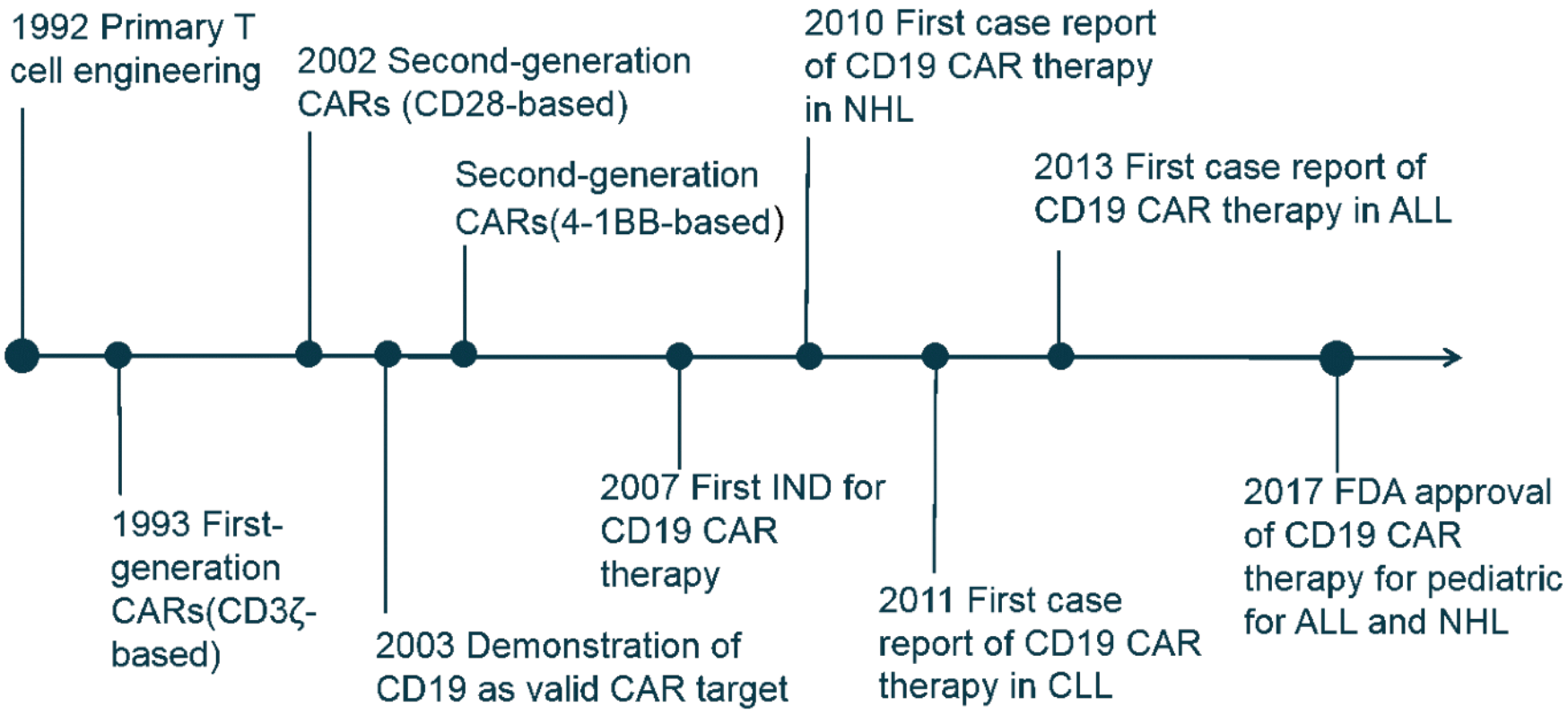
Adaptive Cell Transfer Therapy

- **TIL** (Tumor infiltration T-lymphocytes therapy)
- **TCR** (T-cell receptor therapy)
- **CAR-T** (Chimeric antigen receptor T-cell therapy)

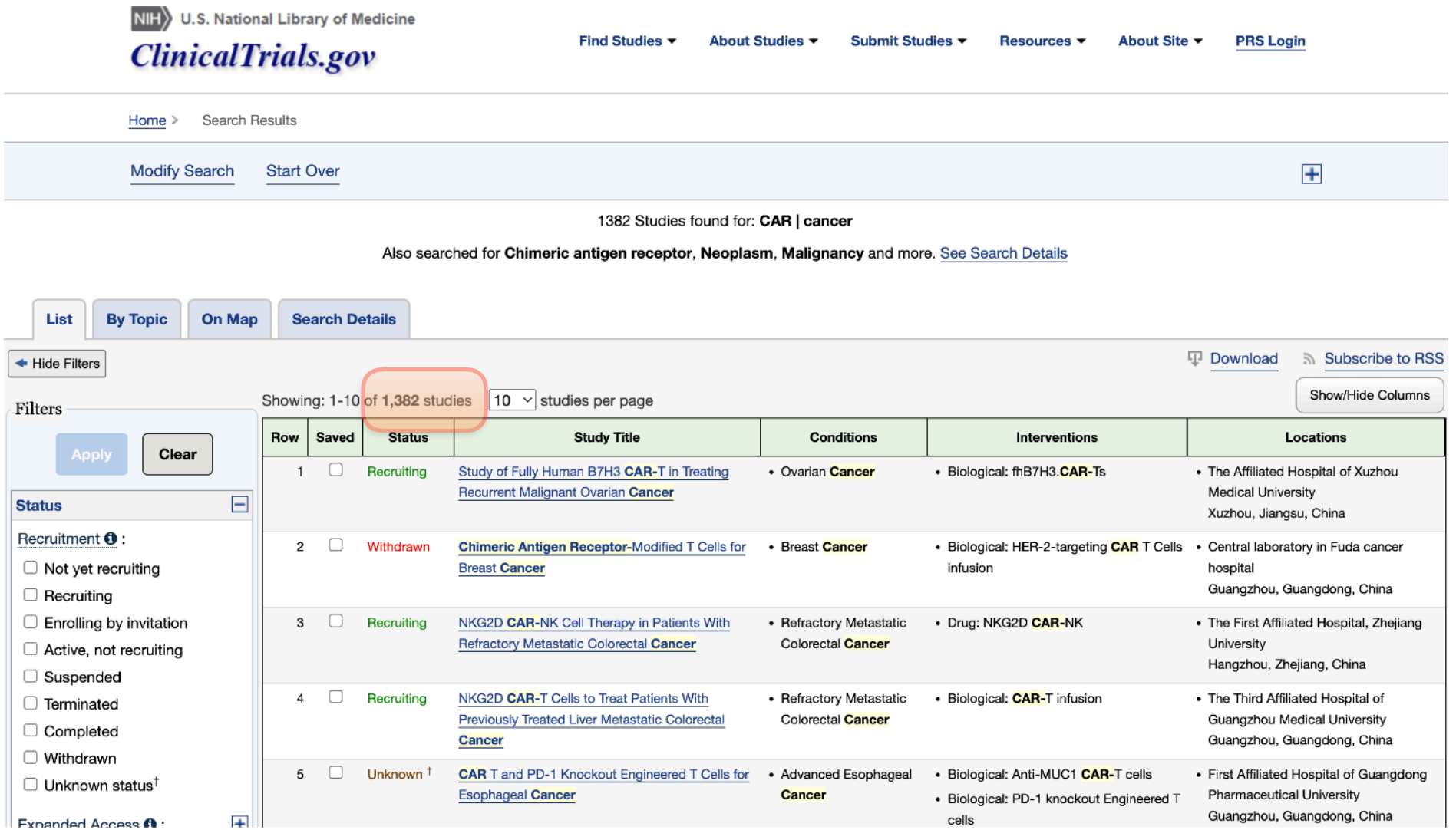
CAR-T

CAR-T cells have to recognize tumor cells independently of their expression of human leukocyte antigen (HLA) molecules, tumors that escape conventional T cells by down-regulating HLA and/or mutating components of the antigen processing machinery can be eliminated.

CAR- T Cells Clinical Trials



CAR- T Cells Clinical Trials



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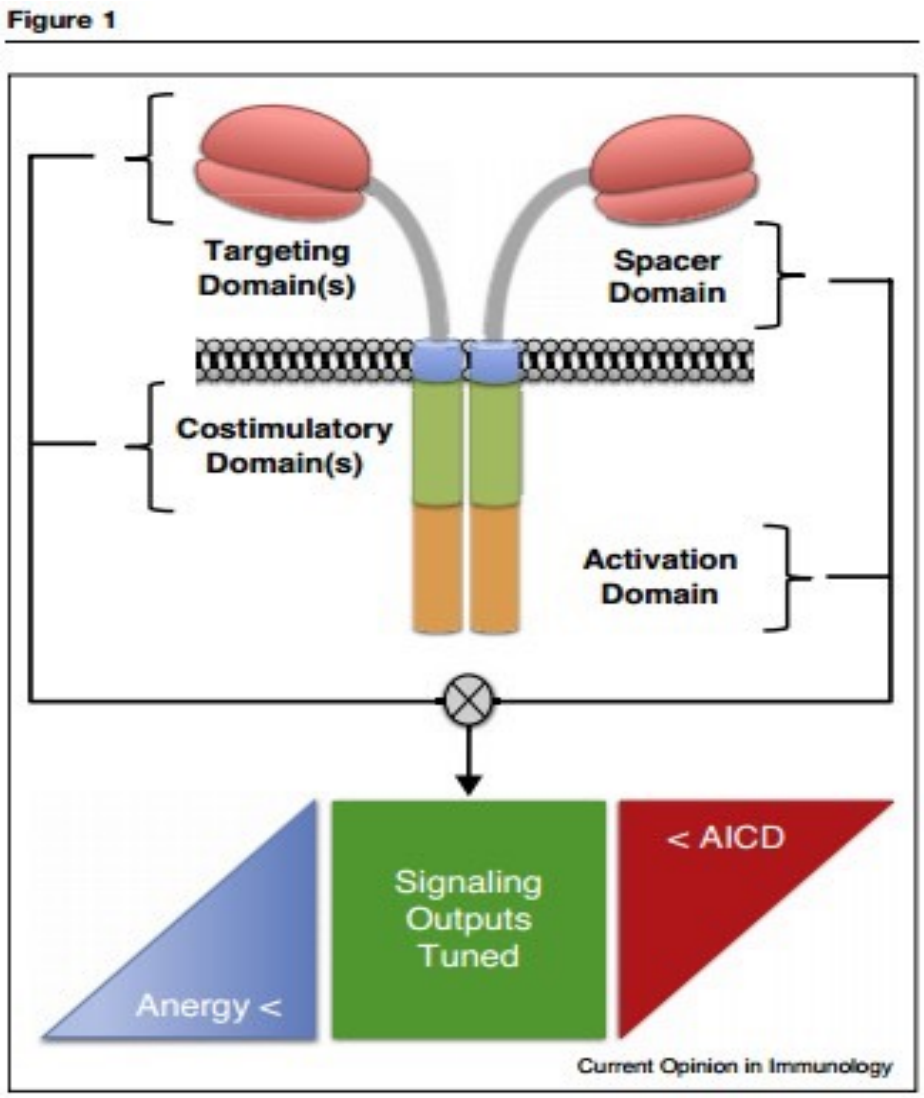
- Recruiting
- Enrolling by invitation
- Active, not recruiting
- Suspended
- Terminated
- Completed
- Withdrawn
- Unknown status†

Expanded Access :

Row	Saved	Status	Study Title	Conditions	Interventions	Locations
1		Recruiting	Study of Fully Human B7H3 **CAR**-T in Treating Recurrent Malignant Ovarian **Cancer**	• Ovarian **Cancer**	• Biological: fhB7H3.**CAR**-Ts	• The Affiliated Hospital of Xuzhou Medical University Xuzhou, Jiangsu, China
2		Withdrawn	**Chimeric Antigen Receptor**-Modified T Cells for Breast **Cancer**	• Breast **Cancer**	• Biological: HER-2-targeting **CAR** T Cells infusion	• Central laboratory in Fuda cancer hospital Guangzhou, Guangdong, China
3		Recruiting	NKG2D **CAR**-NK Cell Therapy in Patients With Refractory Metastatic Colorectal **Cancer**	• Refractory Metastatic Colorectal **Cancer**	• Drug: NKG2D **CAR**-NK	• The First Affiliated Hospital, Zhejiang University Hangzhou, Zhejiang, China
4		Recruiting	NKG2D **CAR**-T Cells to Treat Patients With Previously Treated Liver Metastatic Colorectal **Cancer**	• Refractory Metastatic Colorectal **Cancer**	• Biological: **CAR**-T infusion	• The Third Affiliated Hospital of Guangzhou Medical University Guangzhou, Guangdong, China
5		Unknown †	**CAR** T and PD-1 Knockout Engineered T Cells for Esophageal **Cancer**	• Advanced Esophageal **Cancer**	• Biological: Anti-MUC1 **CAR**-T cells • Biological: PD-1 knockout Engineered T cells	• First Affiliated Hospital of Guangdong Pharmaceutical University Guangzhou, Guangdong, China

CAR-T



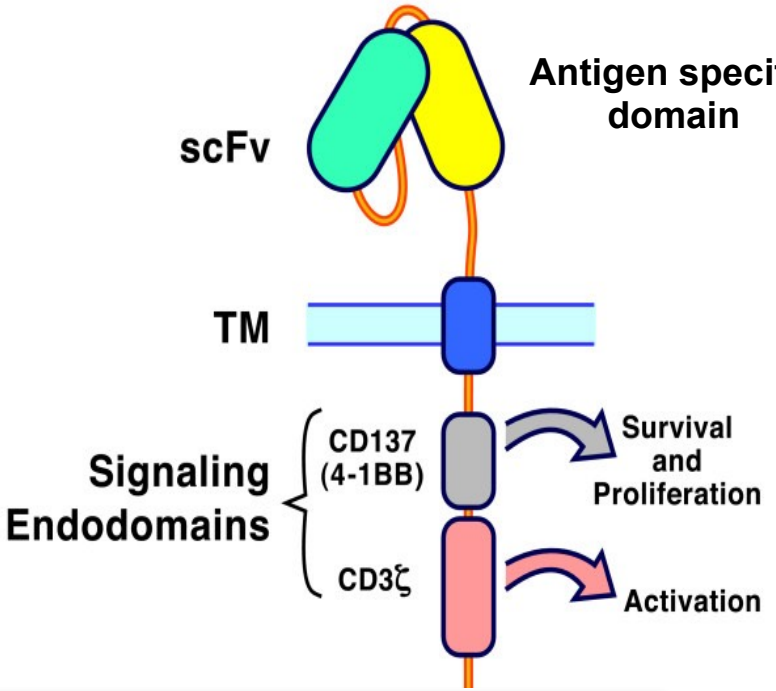
CARs consist of fusion molecules and are typically comprised of:

1. an extracellular single chain variable fragment (scFv) of a monoclonal antibody (mAb) specific for a **surface** molecule on the tumor cell,
2. a spacer domain that provides flexibility and optimizes T cell and target cell engagement,
3. a transmembrane domain,
4. signaling modules that trigger T cell effector functions.

Michael et al, Designing chimeric antigen receptors to effectively and safely target tumors. Current Opinion in Immunology 2015

CAR-T

- T cells transduced with tumor-specific CAR
- CAR: Single fusion molecule with antigen specificity plus signaling domain
- Cancer: Solid tumor & hematological malignancies

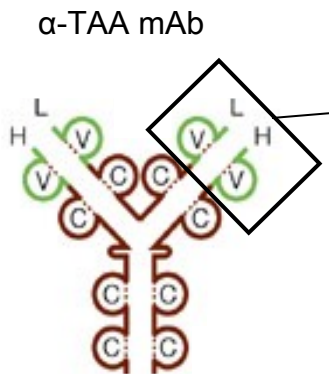


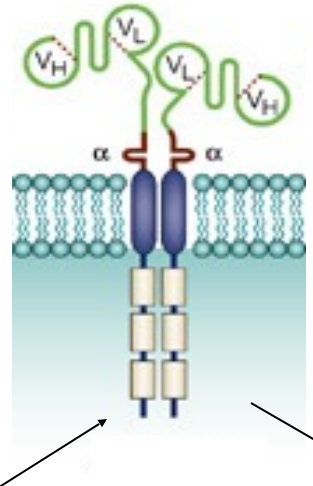
Advantages of CAR-T cells

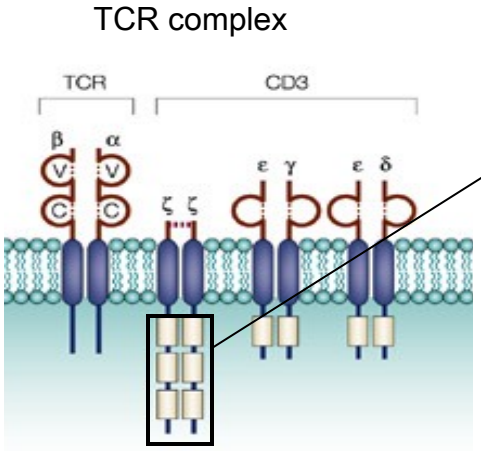
"Live drug"	Tumor recognition independent of HLA (no HLA typing needed)	Multiple anti-tumor immuno-modulators can be engineered	Target variety of antigens (self-protein, carbohydrate, glycolipid)

Maus M V et al. Blood 2014;123:2625-2635

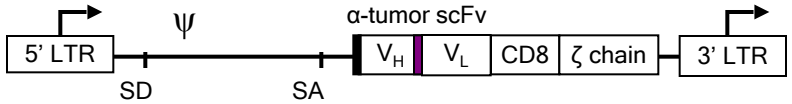
Generation of a tumor targeted chimeric antigen receptor (CAR)







α-TAA scFv—CD8-ζ

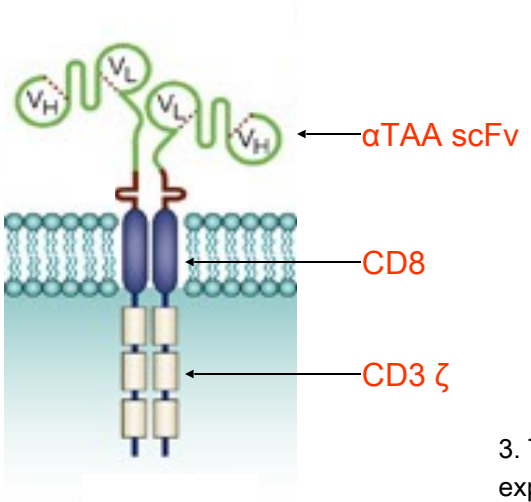


CAR retroviral vector

(??why a retrovirus??)

Generation of TAA-targeted T cells for treatment of Cancer

1. Construct a chimeric antigen receptor (CAR)

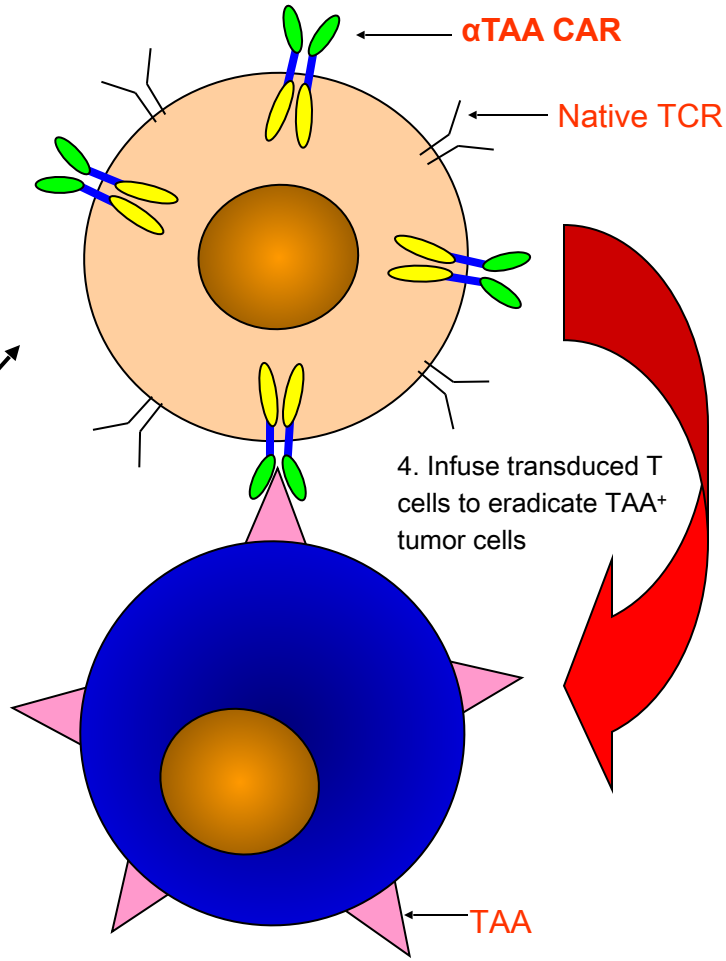


TAA CAR

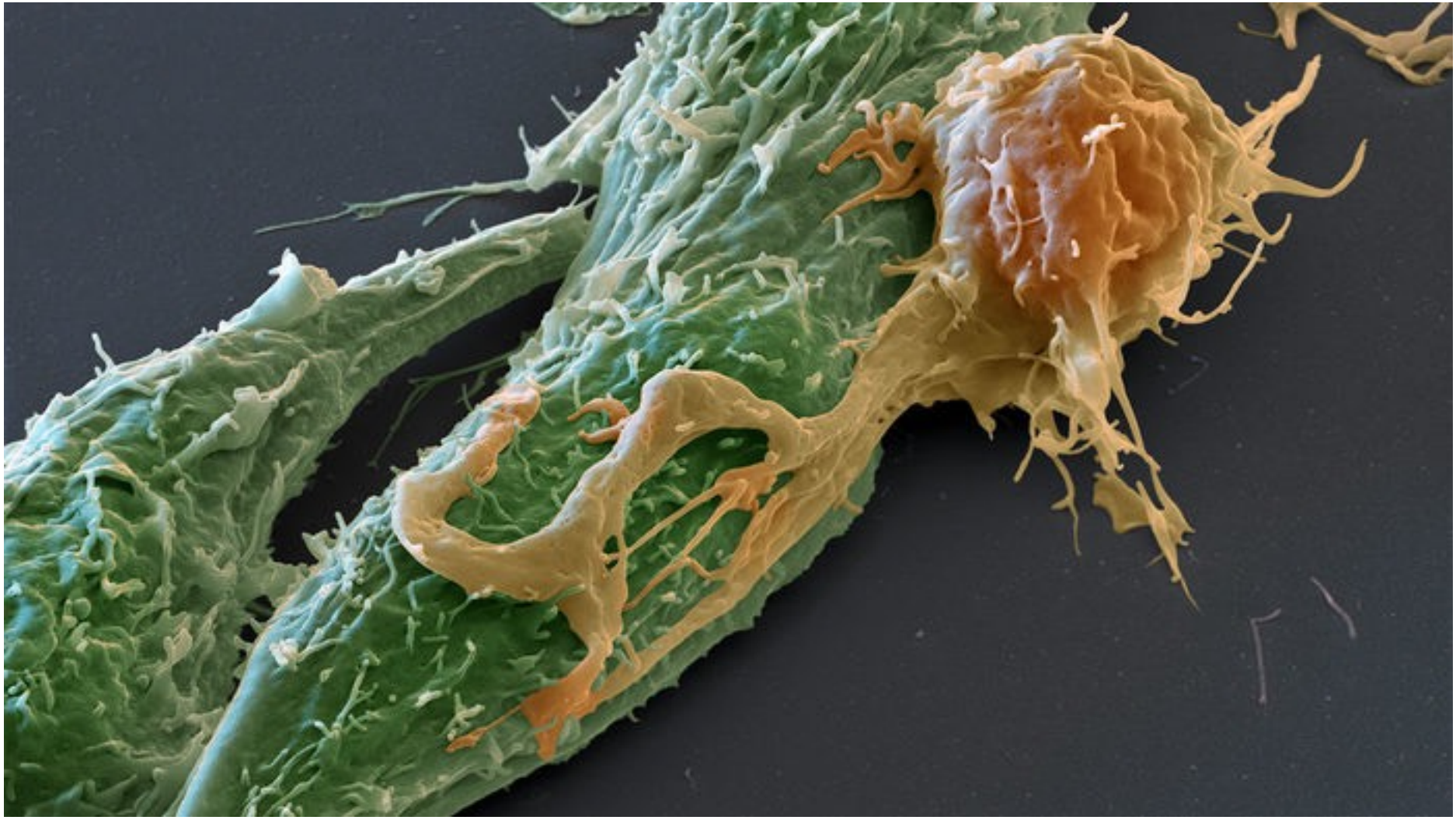
2. Subclone CAR gene into a retroviral vector (SFG)

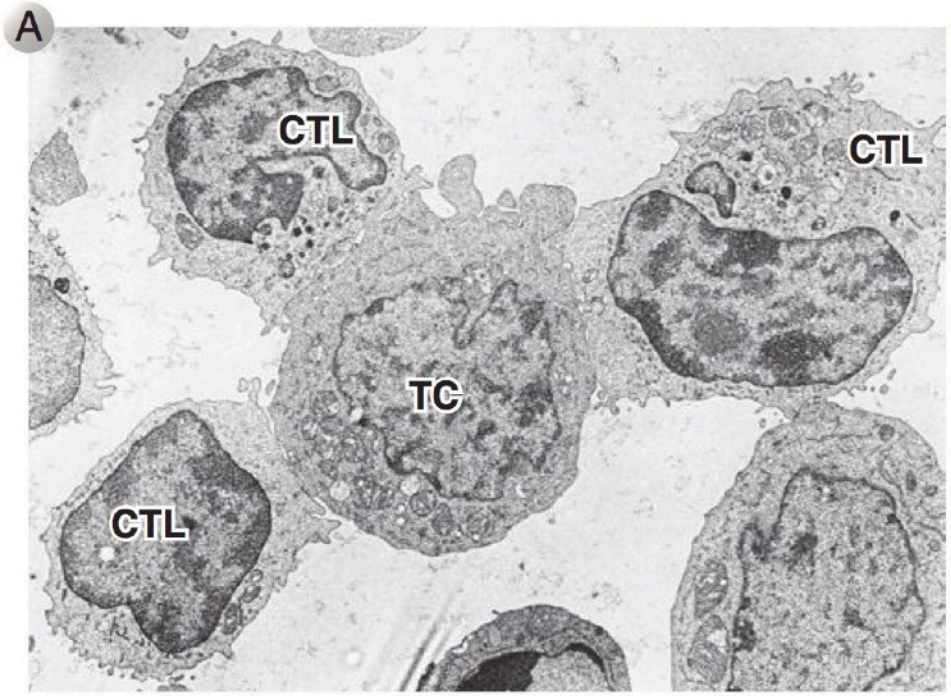
SFG-CAR

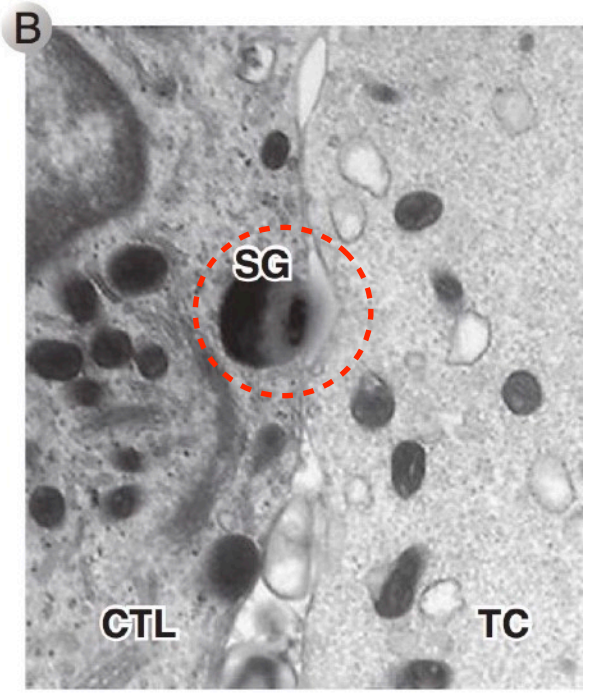
3. Transduce and expand patient T cells ex vivo

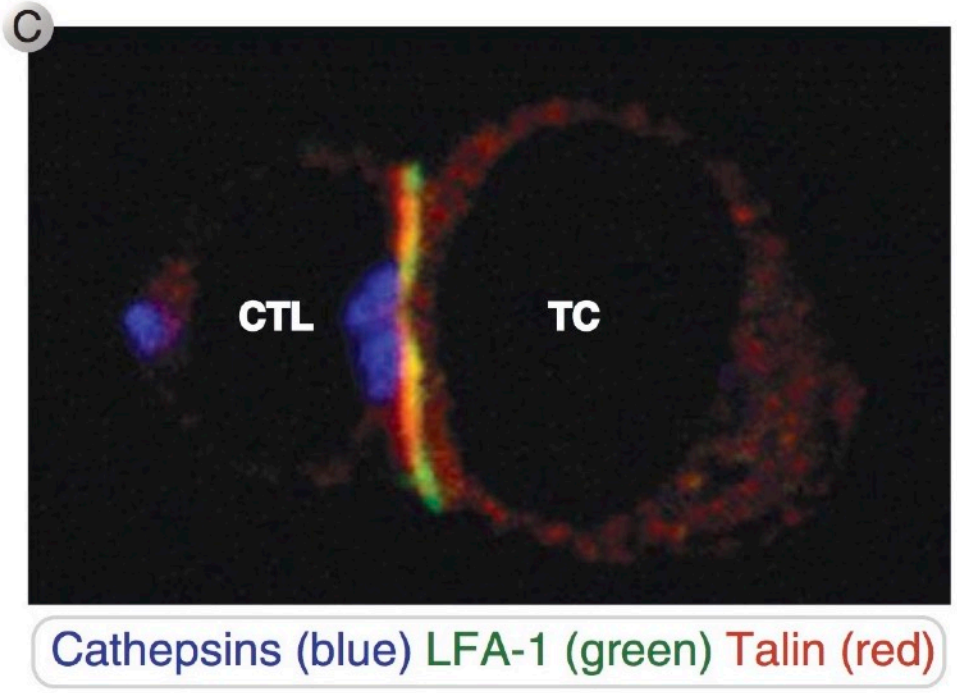


4. Infuse transduced T cells to eradicate TAA$^+$ tumor cells

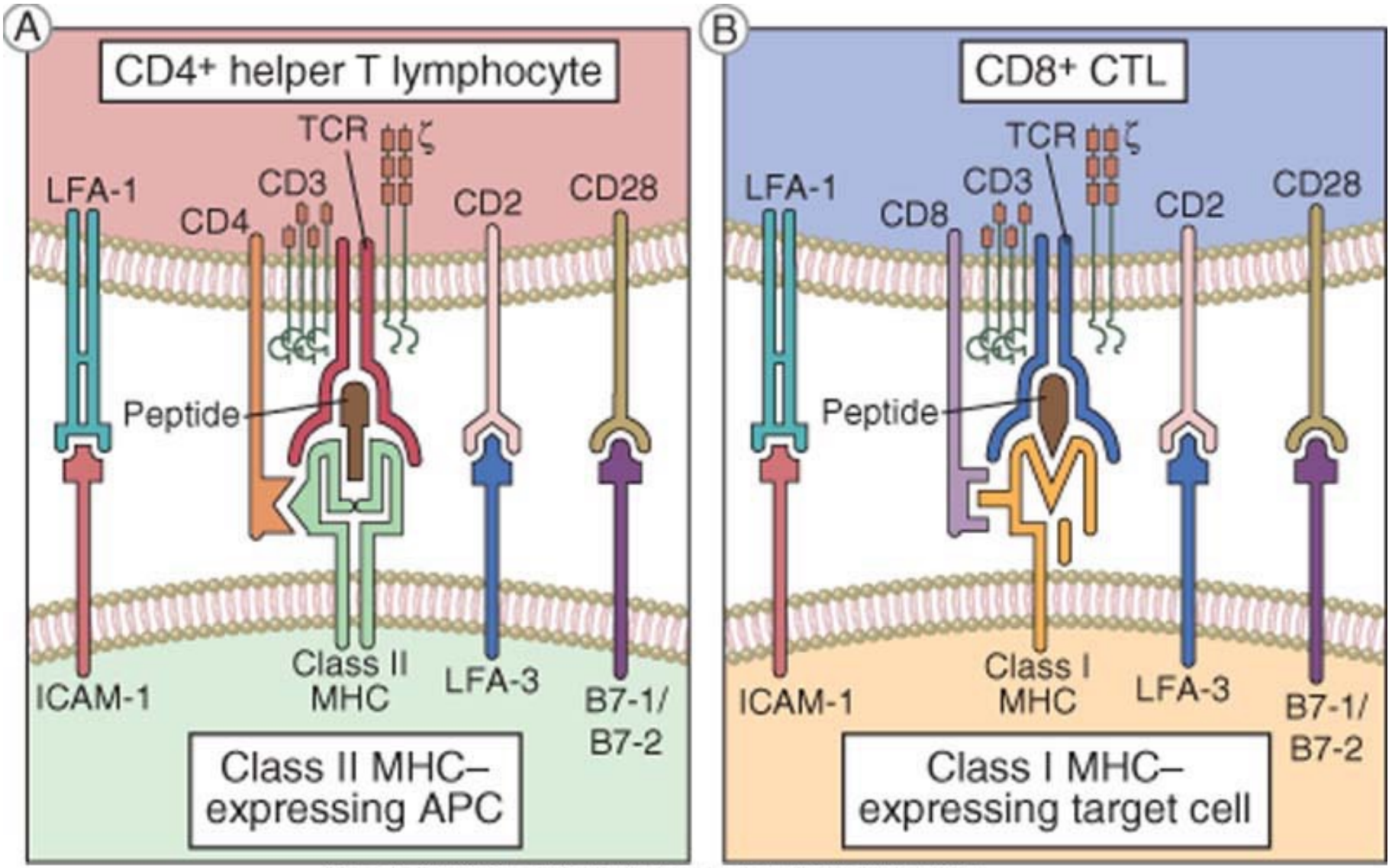








T-response against tumor



T-cell response independent from the expression of MHC, CD80/86, etc

Challenges in CAR designing

1. Target selection

CAR-T cells: target selection

Target	CAR	Cancer	Objective response
CD19	CAR:CD28-CD3ζ	Lymphoma and CLL	N=7: 1CR, 5 PR & 1SD
	CAR:CD137-CD3ζ	ALL	2CR
	CAR:CD28-CD3ζ	ALL	5CR
CD20	CAR:CD137-CD28-CD3ζ	NHL	N=3: 1PR, 2NED
CEA	CAR-CD3ζ (1st gen)	Colorectal & breast	N=7: minor responses in two patients
GD2	CAR-CD3ζ (1st gen)	Neuroblastoma	N=19: 3CR
ERBB2	CAR:CD28-CD137-CD3ζ	Colorectal cancer	N=1, patient died

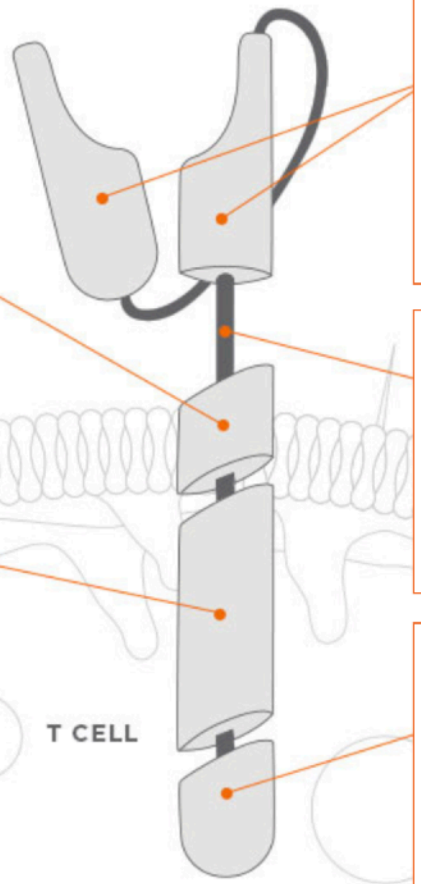
Kershaw et. al. Nature Reviews Cancer, 2013

TRANSMEMBRANE DOMAIN

The transmembrane domain traverses the cell membrane, anchors the CAR to the cell surface, and connects the extracellular domain to the intracellular signaling domain, thus impacting expression of the CAR on the cell surface.[2]

COSTIMULATORY DOMAIN

The costimulatory domain is derived from the intracellular signaling domains of costimulatory proteins, such as CD28 and 4-1BB, that enhance cytokine production.[1, 3]



TARGET ELEMENT

The single-chain variable fragment (scFv) is expressed on the surface of a CAR T cell and confers antigen specificity. The scFv is derived from the portion of an antibody that specifically recognizes a target protein.[2, 4]

SPACER

The spacer connects the extracellular targeting element to the transmembrane domain and affects CAR function and scFv flexibility.[4]

SIGNALING DOMAIN

The CD3 zeta domain is derived from the intracellular signaling portion of the T cell receptor, which mediates downstream signaling during T cell activation.[3, 4]

NHL CD19 : JCAR017 (Phase 1) +

NHL CD19 : JCAR014 Combinations (Phase 1) +

Pediatric ALL CD22 : JCAR018 (Phase 1) +

NHL CD22 : JCAR018 (Phase 1) +

Multiple Myeloma BCMA (Phase 1) +

AML WT1 : JTCR016 (Phase 1/Phase 2) +

NSCLC, Mesothelioma WT1 : JTCR016 (Phase 1/Phase 2) +

Pediatric Neuroblastoma L1CAM : JCAR023 (Phase 1) +

Ovarian MUC16 : JCAR020 (Phase 1) +

NSCLC, Breast ROR1 : JCAR024 (Phase 1) +

Lung Cancer LeY (Phase 1) +

CAR-T cells: target/Ab selection

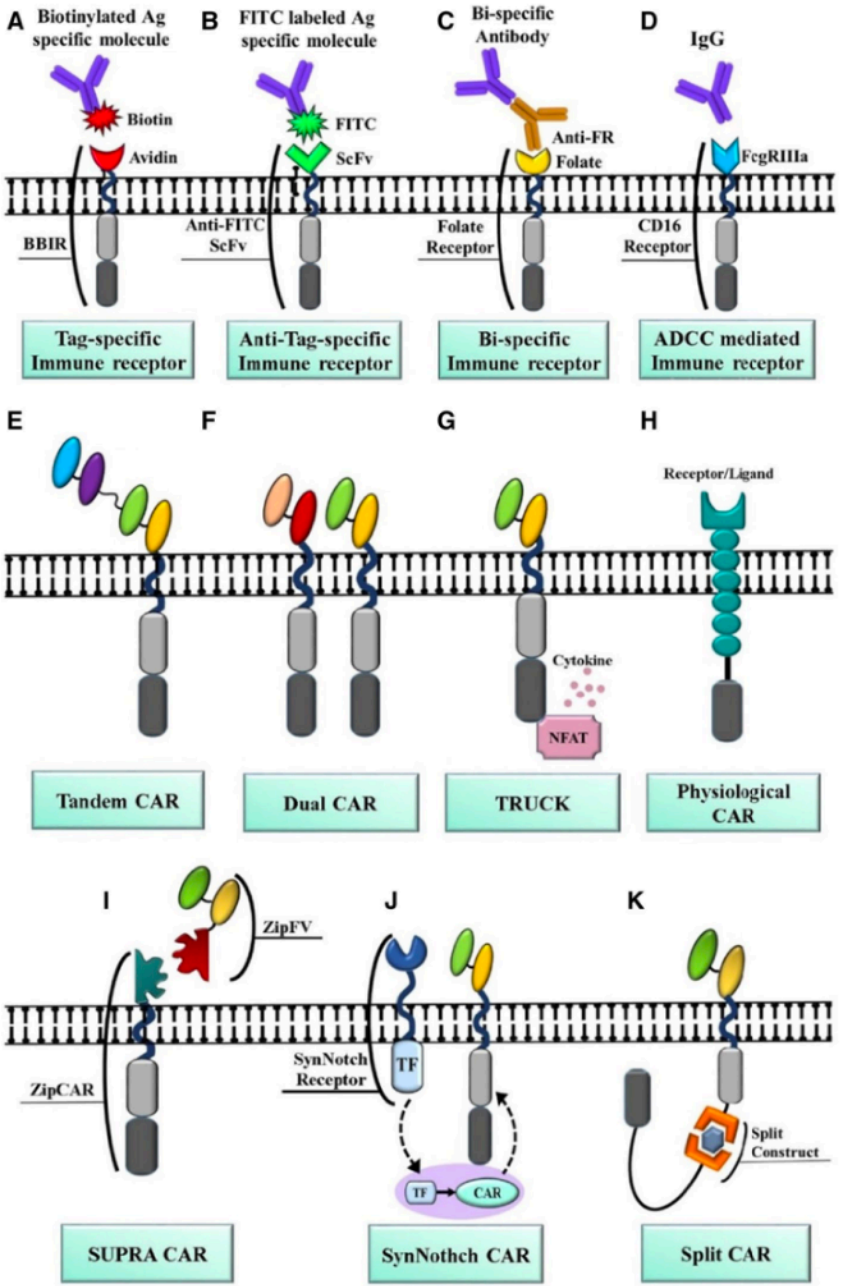


Fig. 2 Types of multiple targeting programmable CARs. (A) Tag specific immune receptor targeting the biotinylated tumor Ag through the Avidin–Biotin mechanism. (B) Anti-tag specific immune receptor targeting the FITC labeled tumor Ag via the interaction between FITC and anti-FITC ScFv. (C) Bi-specific immune receptor attaching to CD20 Ag by engaging the folate receptor and tumor antigen through the bi-specific antibody. (D) ADCC mediated receptor performing ADCC mechanism via the interaction between CD16gRIIIα receptor and Fc-IgG. (E) Tandem-CAR-T cell with two different ScFvs on a single receptor chain. (F) Dual-CAR-T Cell with two separate CARs expressing two various ScFvs. (G) TRUCK cell armored with transcription factor to produce cytokines. (H) Physiological CAR with the capability of antigen recognition based on ligand receptor. (I) SUPRA CAR with two split structures includes the antigen-binding domain (zipFV) and function domain (zipCAR). (J) SynNotch CAR with two separate portions includes antigen A-specific SynNotch receptor and antigen B-specific CAR. (K) split CAR with programmable mechanism exerted by split structure assembling through the small molecule (created by Esmaeilzadeh et al.)

Tahmasebi, et al.

Clinical and Translational Oncology

2020

CAR-T cells: target selection

Table 1 Clinical trial studies of multi-targeted and programmable CAR-T cells

Study	CAR type	Target antigen	Cancer type	References
Beijing, China	Universal CAR-T cell	CD19	Leukemia, lymphoma	NCT03166878
Henan, China		CD19	ALL–NHL	NCT03229876
New York, USA		CD123	AML	NCT03190278
USA, UK, Belgium		CD19	Children ALL	NCT02808442
France, Spain		CD19	Adult ALL	NCT02746952
USA, UK, Belgium		CD123	BPDCN	NCT03203369
France, Spain				
Houston, Texas, USA				
Beijing, China	Multi targeted CAR-T cell	CD19–CD20	Leukemia, lymphoma	NCT03097770
Beijing, China		CD19–CD20/D22	Leukemia, lymphoma	NCT03398967
Milwaukee, WI, USA		CD19–CD20	CLL/SLL/NHL	NCT03019055
Seattle, WA, USA		CD19–CD22	Leukemia, lymphoma	NCT03330691
Bethesda, MD, USA		CD19–CD22	Leukemia, lymphoma	NCT03448393
Xi'an, China		CD19–CD22	Lymphoma	NCT03593109
Shanghai, China		CD19–CD22	Lymphoma	NCT03468153
London, Manchester		CD19–CD22	Lymphoma	NCT03287817
& Newcastle, UK		CD19–CD22	Lymphoma	NCT03233854
Palo Alto, CA, USA		CD19–CD22	Leukemia	NCT03241940
Palo Alto, CA, USA		CD19–CD22	Leukemia	NCT03614858
Suzhou, China				

CAR Chimeric antigen receptor, *CD* cluster of differentiation, *ALL* acute lymphocytic leukemia, *NHL* non-Hodgkin lymphoma, *AML* acute myeloid leukemia, *BPDCN* blastic plasmacytoid dendritic cell neoplasm, *CLL* chronic lymphocytic leukemia, *SLL* small lymphocytic lymphoma

Table 2 Pre-Clinical studies of multi-targeted and programmable CAR-T cells

Study	CAR type	Target antigen	Cancer type	References
Urbanska et al., 2012	Universal	EpCAM–Meso–FRa	Ovarian cancer	[69]
Cartellieri et al., 2016	Universal	CD33–CD123	AML	[72]
Chen et al., 2017	Universal	CD20	MCL	[94]
Kim et al., 2015	Universal	FRa	Malignant tumors	[70]
Zah et al., 2016	Tandem	CD19–CD20	Lymphoma	[46]
Ruella et al., 2016	Tandem	CD19–CD123	Leukemia	[44]
Li et al., 2018	Tandem	CD19–CD133	MLL	[47]
Schneider et al., 2017	Tandem	CD19–CD20	Lymphoma	[45]
Grada et al., 2013	Tandem	HER2–CD19	B cell malignancy	[41]
Qin et al., 2018	Tandem	CD19–CD22	ALL	[48]
Hegde et al., 2016	Tandem	HER2 -IL13Rα2	Glioblastoma	[43]
Wilkie et al., 2012	Dual	ErbB2–MUC1	Breast	[34]
Lanitis et al., 2013	Dual	Meso–FRa	Ovarian	[38]
Kloss et al., 2013	Dual	PSMA–CD19	Prostate	[36]
Anurathapan et al., 2013	Dual	MUC1–PSCA	Pancreatic	[33]
Jiang et al., 2018	Dual	EGFR–EGFRvIII	Glioblastoma	[95]
Cho et al., 2018	SUPRA	HER2–Meso–Axl	Breast	[61]
Roybal et al., 2016	SynNotch	CD19–Meso–GFP	Leukemia	[58]
Roybal et al., 2016	SynNotch	CD19–HER2–GFP	Leukemia	[56]
Morsut et al., 2016	SynNotch	CD19–GFP	Lymphoma	[55]
Srivastava et al., 2019	SynNotch	EpCAM–ROR1	Breast cancer	[59]
Wu et al., 2015	On switch	CD19	Leukemia	[96]
Juillerat et al., 2016	On switch	CD19	Leukemia	[97]
Cao et al., 2016	On switch	HER2	Breast cancer	[91]
Rodgers et al., 2016	On switch	CD19	Leukemia	[77]

CAR chimeric antigen receptor, *CD* cluster of differentiation, *EpCAM* epithelial cell adhesion molecule, *Meso* mesothelin, *FRa* folate receptor a, *HER2* human epidermal growth factor receptor 2, *MUC1* Mucin 1, *PSMA* prostate-specific membrane antigen, *PSCA* prostate stem cell antigen, *EGFR* epidermal growth factor receptor, *GFP* green fluorescent protein, *ROR1* receptor-tyrosine-kinase-like orphan receptor 1, *AML* acute myeloid leukemia, *MCL* mantle cell lymphoma, *MLL* mixed-lineage leukemia, *ALL* acute lymphocytic leukemia

Tahmasebi, et al.

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CAR-T cells: target selection

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LETTER

Acute lymphoblastic leukemia

Frequent occurrence of CD19-negative relapse after CD19 CAR T and consolidation therapy in 14 *TP53*-mutated r/r B-ALL children

Jing Pan[1] · Yue Tan[2] · Biping Deng[3] · Chunrong Tong[4] · Lin Hua[5] · Zhuojun Ling[4] · Weiliang Song[4] · Jinlong Xu[4] · Jiajia Duan[4] · Zelin Wang[4] · Huilin Guo[6] · Xinjian Yu[6] · Alex H. Chang[7] · Qinlong Zheng[6] · Xiaoming Feng[2,8]

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To the Editor:

Despite high remission rates after CD19 CAR T-cell therapy in patients with refractory or relapsed B acute lymphoblastic leukemia (r/r B-ALL), relapses were commonly observed [1–4]. To improve long-term disease-free survival (DFS), our and other centers have conducted post-CD19 CAR consolidations with allogeneic hematopoietic stem cell transplantation (allo-HCT) or CD22 CAR T-cell infusion [5–9]. However, some patients still relapsed, but it is unknown which factors caused their relapses. *TP53* mutation predicts nonresponse and poor outcome in childhood B-ALL during traditional therapies. Genomic instability caused by *TP53* mutation induces leukemia cells to undergo genomic evolution to survive stress and treatment [10–12]. In our previous studies, CAR T therapy can overcome genetic adverse features including *TP53* mutation to induce remission [5, 6], but it is unknown whether the long-term outcome would be influenced by *TP53* mutation and other genetic aberrations.

We analyzed the outcome of 68 r/r B-ALL children (characteristics in Supplementary Tables. 1 and 2) treated with CD19 CAR T cells and post-CAR consolidations of allo-HCT or CD22 CAR T-cell infusion between January 2nd, 2018 and April 19th 2019 in three trials. The details of trials, clinical procedure, and analysis methods were in supplemental methods pp1-8. Sixty-six patients (97.1%) achieved complete remission. With a median follow-up of 11.3 (range, 1–21) months, a promising one-year DFS of 79.6% (95% CI, 65.9–87.8) was achieved (Supplementary Fig. 1a), but 12 patients still relapsed within a median time of 6.3 (range, 1–11.4) months (Fig. 1a and Supplementary Fig. 1b). DFS was comparable between allo-HCT ($n = 34$) and CD22 CAR T ($n = 30$) consolidation subgroups ($P = 0.232$, Supplementary Fig. 1c).

CAR-T cells: target selection

nature medicine

LETTERS

https://doi.org/10.1038/s41591-020-1081-3

Check for updates

Bispecific anti-CD20, anti-CD19 CAR T cells for relapsed B cell malignancies: a phase 1 dose escalation and expansion trial

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Chimeric antigen receptor (CAR) T cells targeting CD19 are a breakthrough treatment for relapsed, refractory B cell malignancies[1–5]. Despite impressive outcomes, relapse with CD19⁻ disease remains a challenge. We address this limitation through a first-in-human trial of bispecific anti-CD20, anti-CD19 (LV20.19) CAR T cells for relapsed, refractory B cell malignancies. Adult patients with B cell non-Hodgkin lymphoma or chronic lymphocytic leukemia were treated on a phase 1 dose escalation and expansion trial (NCT03019055) to evaluate the safety of 4-1BB–CD3ζ LV20.19 CAR T cells and the feasibility of on-site manufacturing using the CliniMACS Prodigy system. CAR T cell doses ranged from 2.5×10^5–2.5×10^6 cells per kg. Cell manufacturing was set at 14 d with the goal of infusing non-cryopreserved LV20.19 CAR T cells. The target dose of LV20.19 CAR T cells was met in all CAR-naive patients, and 22 patients received LV20.19 CAR T cells on protocol. In the absence of dose-limiting toxicity, a dose of 2.5×10^6 cells per kg was chosen for expansion. Grade 3–4 cytokine release syndrome occurred in one (5%) patient, and grade 3–4 neurotoxicity occurred in three (14%) patients. Eighteen (82%) patients achieved an overall response at day 28, 14 (64%) had a complete response, and 4 (18%) had a partial response. The overall response rate to the dose of 2.5×10^6 cells per kg with non-cryopreserved infusion ($n=12$) was 100% (complete response, 92%; partial response, 8%). Notably, loss of the CD19 antigen was not seen in patients who relapsed or experienced treatment failure. In conclusion, on-site manufacturing and infusion of non-cryopreserved LV20.19 CAR T cells were feasible and therapeutically safe, showing low toxicity and high efficacy. Bispecific CARs may improve clinical responses by mitigating target antigen downregulation as a mechanism of relapse.

Anti-CD19 CAR T cell therapy is a new immunotherapeutic approach for patients with relapsed, refractory B cell malignancies[1–5]. Despite early excitement about this treatment, long-term progression-free survival (PFS) with anti-CD19 CAR T cell products ranges from 30–40% for aggressive B cell non-Hodgkin lymphoma (NHL), suggesting that most patients will either not respond or relapse after receiving this treatment[3,6,7]. A common mechanism of relapse is downregulation of the CD19 antigen and development of a CD19⁻ clone[8–11]. Biopsies obtained at relapse from patients with B cell NHL after anti-CD19 CAR T cell therapy revealed that approximately 30% of patients were CD19⁻, demonstrating the impact of clonal selection that occurs with single targeting of the CD19 antigen[12,13]. Simultaneous targeting of more than one B cell antigen has been proposed as a therapeutic strategy to reduce the risk of relapse mediated by antigen-negative clonal escape[14–16]. Preclinical studies found that tandem, bispecific CD20–CD19 lentiviral CARs can mitigate downregulation of not only the targeted receptors but also at least one non-targeted B cell receptor (that is, CD22)[17]. These data provided support for the clinical development of a phase 1 trial for tandem bispecific anti-CD20, anti-CD19 4-1BB–CD3ζ lentiviral (LV20.19) CAR T cells (Extended Data Fig. 1) for patients with relapsed, refractory B cell malignancies, including NHL and chronic lymphocytic leukemia (CLL).

Twenty-six patients with B cell NHL or CLL met eligibility criteria and underwent apheresis for LV20.19 CAR T cell production (Extended Data Fig. 2). Four patients did not meet their target doses, three of whom were treated per clause, allowing infusion outside of specified cohorts. Patient and disease characteristics for the remaining 22 patients are detailed in Table 1 (top). The median age at CAR T cell infusion was 57 years (range, 38–72 years), and the median number of lines of prior therapy was 4 (range, 2–12) (see Supplementary Table 1 for detailed patient history). Patients with mantle cell lymphoma (MCL) were particularly heavily pretreated, with a median of 8 prior lines, and all patients had experienced treatment failure with Bruton's tyrosine kinase (BTK) inhibitors. Most patients (82%) were refractory to their last line of treatment. Baseline CD19 and CD20 expression on tumor cells from biopsy material taken before infusion with LV20.19 CAR T cells is listed in Supplementary Table 2.

Twenty-six LV20.19 CAR T cell products were manufactured using a fixed 14-d process in the CliniMACS Prodigy device (Supplementary Note, CAR T cell manufacturing). The target dose of LV20.19 CAR T cells was achieved in 85% of patients (22 of 26), which met our feasibility endpoint (>75% success), with 100% successful manufacturing in CAR-naive patients. Detailed manufacturing data are presented in Extended Data Fig. 3.

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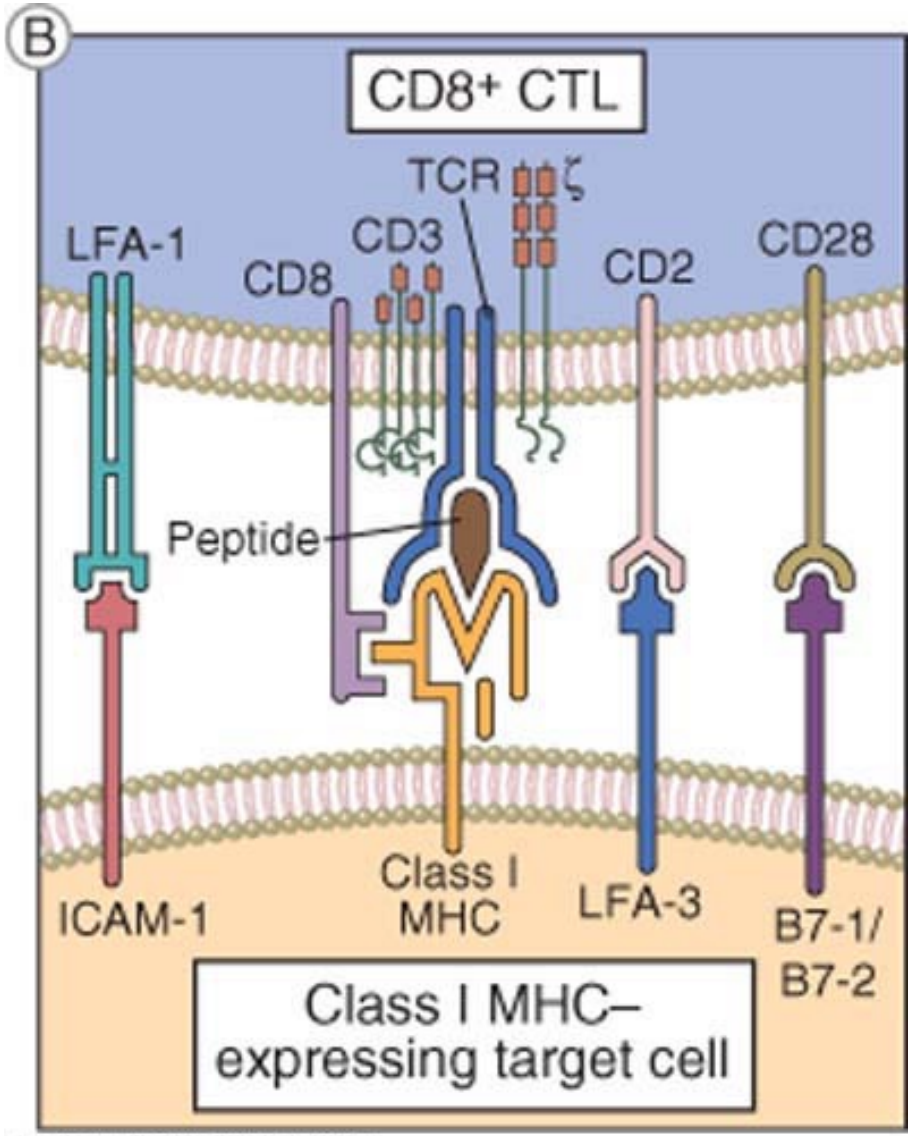
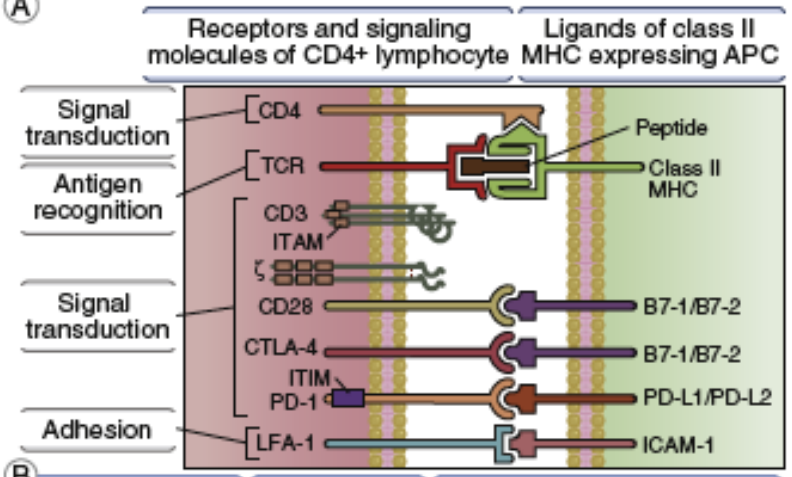
Challenges of CAR-T

1. Target selection

2. Optimize co-stimulatory signaling of T cell effector functions

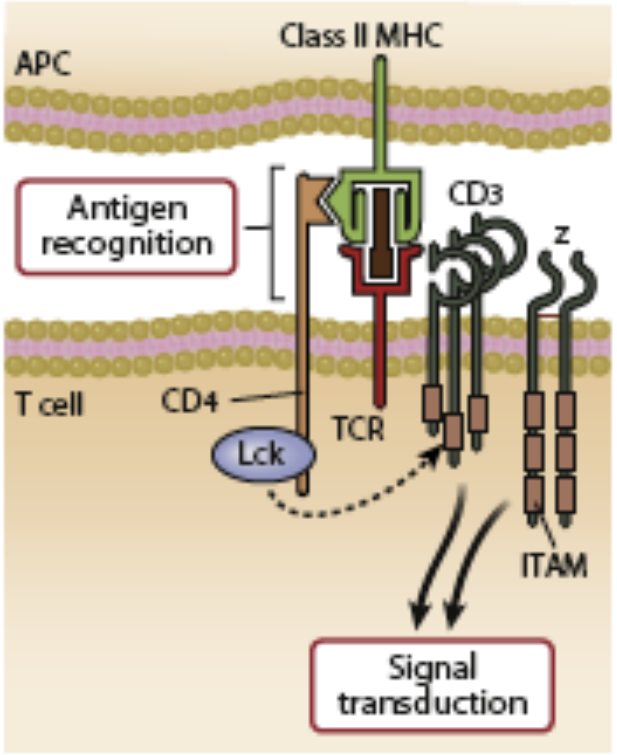


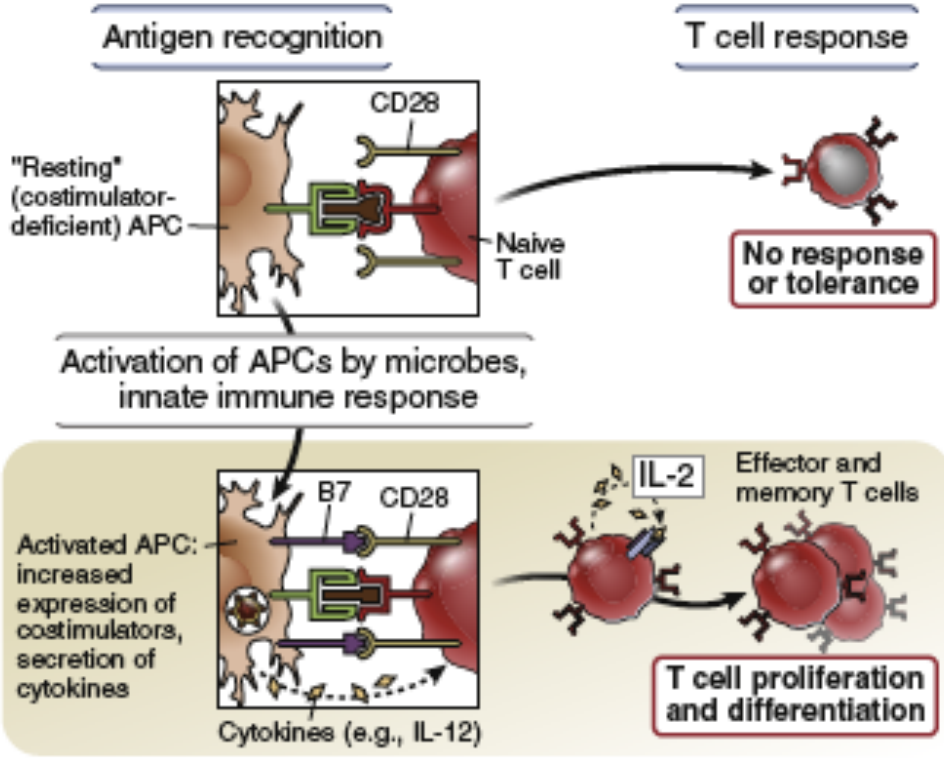
A

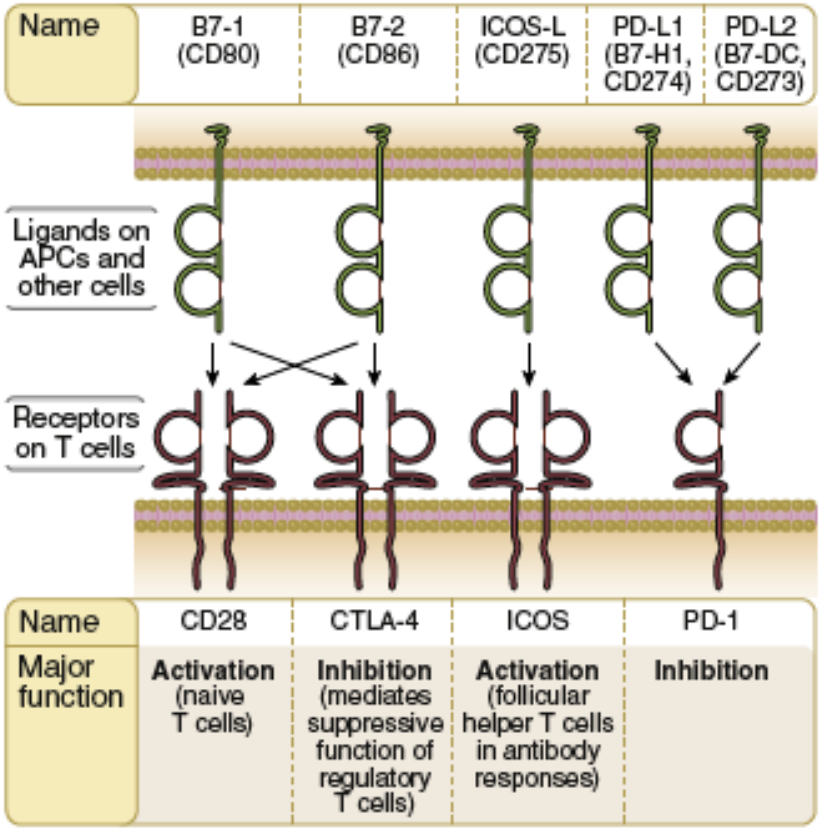


B

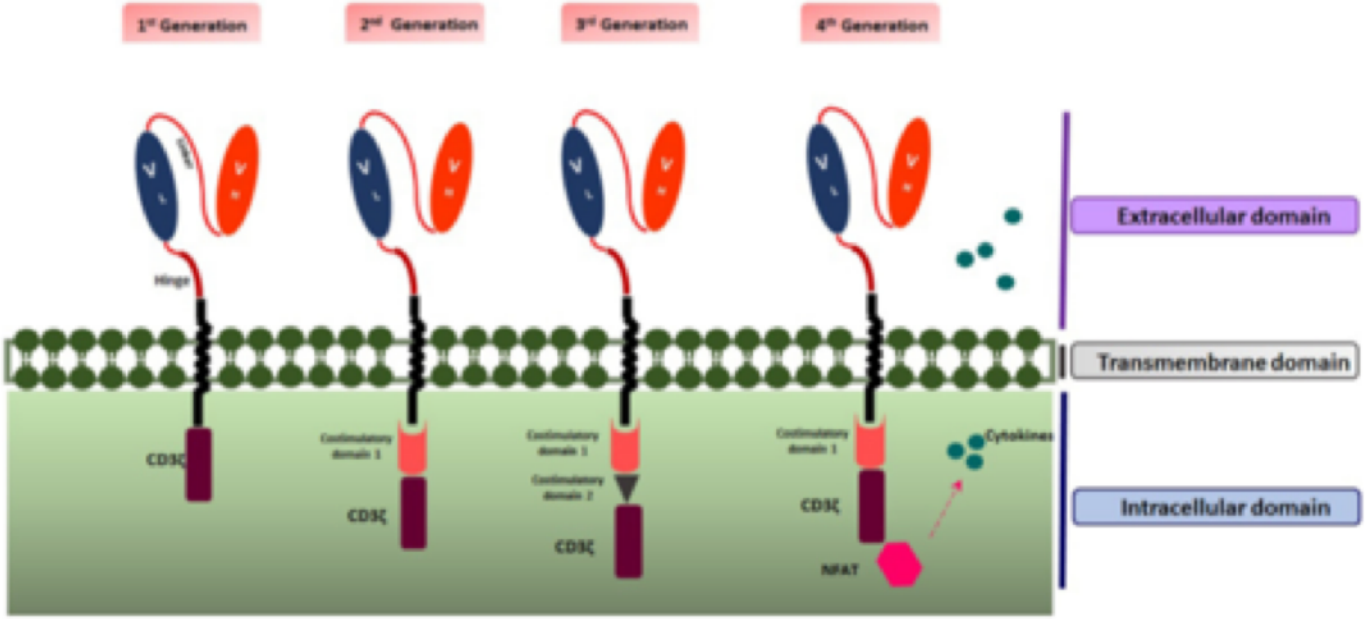
Surface molecules of T lymphocytes	Function	Ligand	
		Name	Expressed on
CD3	Signal transduction by TCR complex	None	
ζ	Signal transduction by TCR complex	None	
CD4	Signal transduction	Class II MHC	Antigen presenting cells
CD8	Signal transduction	Class I MHC	All nucleated cells
CD28	Signal transduction (costimulation)	B7-1/B7-2	Antigen presenting cells
CTLA-4	Inhibition	B7-1/B7-2	Antigen presenting cells
PD-1	Inhibition	PD-L1/PD-L2	Antigen presenting cells, tissue cells, tumor cells
LFA-1	Adhesion, signal transduction	ICAM-1	Antigen presenting cells, endothelium




Antigen recognition
T cell response
CD28
"Resting" (costimulator-deficient) APC
Naive T cell
No response or tolerance
Activation of APCs by microbes, innate immune response
B7
CD28
IL-2
Effector and memory T cells
Activated APC: increased expression of costimulators, secretion of cytokines
T cell proliferation and differentiation
Cytokines (e.g., IL-12)

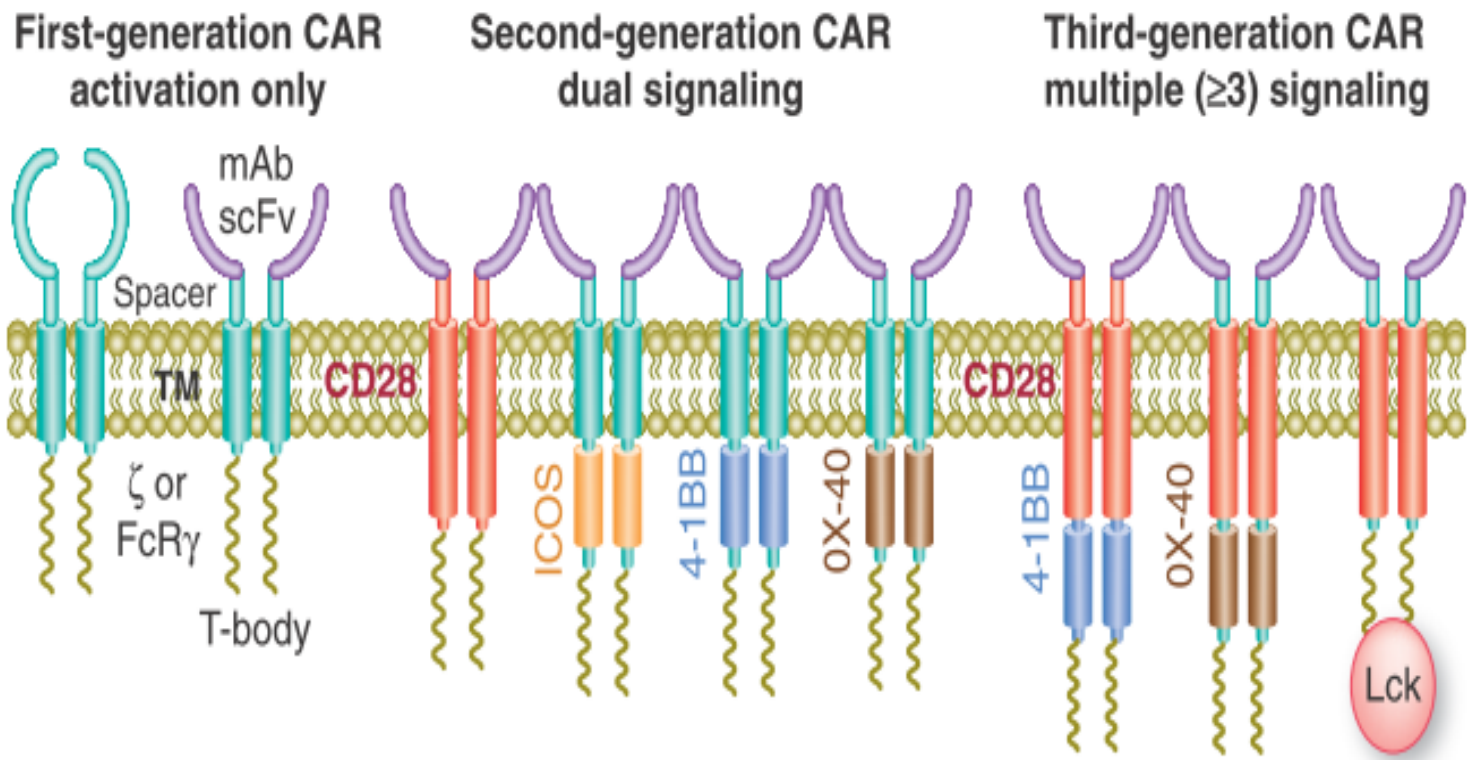

Name
B7-1 (CD80)
B7-2 (CD86)
ICOS-L (CD275)
PD-L1 (B7-H1, CD274)
PD-L2 (B7-DC, CD273)
Ligands on APCs and other cells
Receptors on T cells
Name
CD28
CTLA-4
ICOS
PD-1
Major function
Activation (naive T cells)
Inhibition (mediates suppressive function of regulatory T cells)
Activation (follicular helper T cells in antibody responses)
Inhibition

Evolution in CAR design



First-generation CARs: including activating receptors such as CD8/CD3-ζ fusion receptors;
Second-generation CARs: providing dual signaling to direct combined activating and co-stimulatory signals;
Third-generation CARs: comprising more complex structures with 3 or more signaling domains.

Evolution in CAR design



First-generation CARs: including activating receptors such as CD8/CD3-ζ fusion receptors;
Second-generation CARs: providing dual signaling to direct combined activating and co-stimulatory signals;
Third-generation CARs: comprising more complex structures with 3 or more signaling domains.

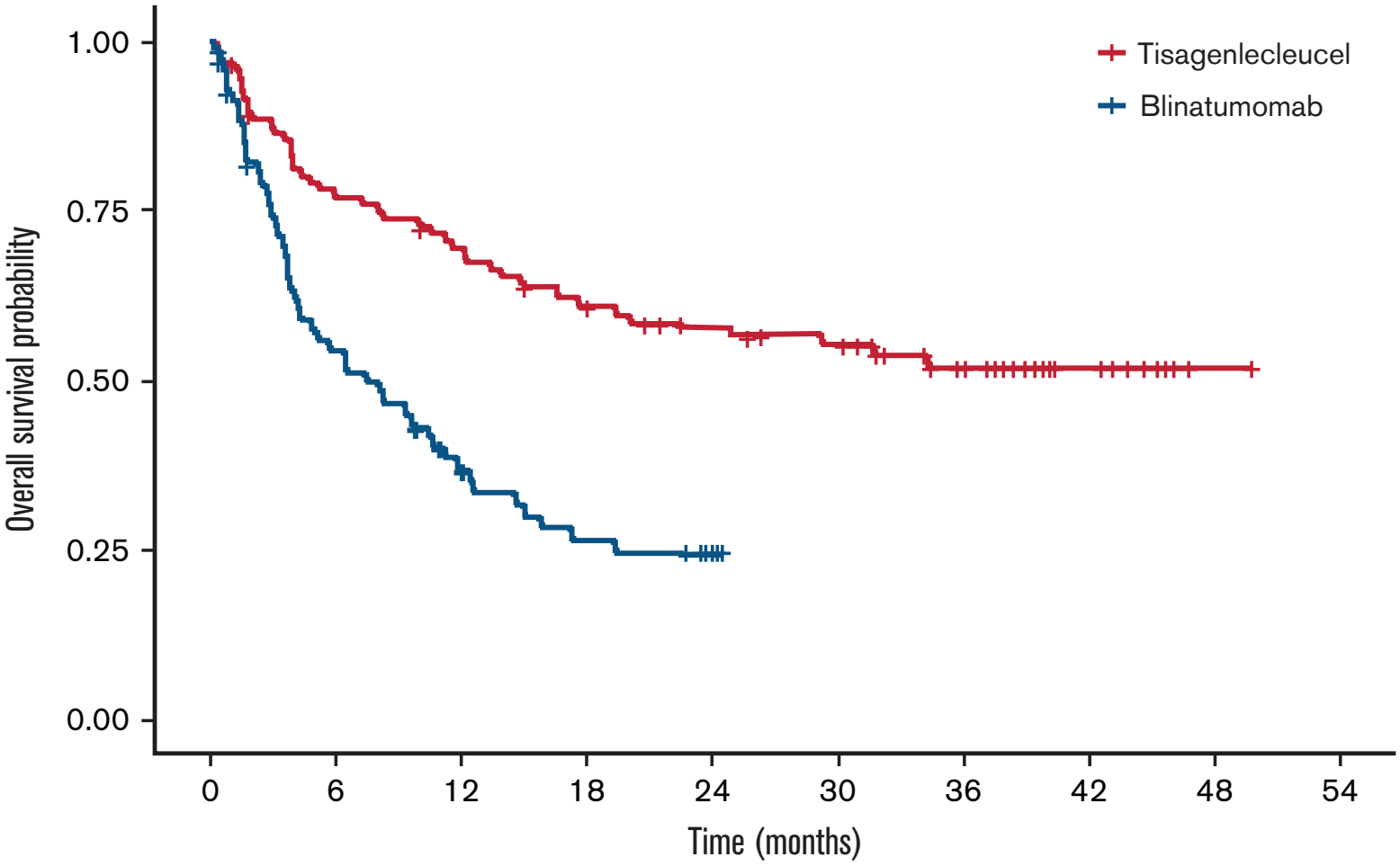
The third generation of CARs

- A third generation of CARs in which a second co-stimulatory molecule is fused in the intra-cellular motif with the co-stimulatory signals, therefore, generating triple-signaling CARs, was developed
- Third-generation CARs seem to have improved proliferation, cytokine secretion and a better persistence in circulation

- **Unfortunately, this last generation of CARs may also be dangerous and the activation can be too strong leading to cytokine storm and eventually to death**

Advantages of CAR-T cell therapy

- HLA-independent antigen recognition, therefore universal application
- Active in both $CD4^+$ and $CD8^+$ T cells
- Target antigens include proteins, carbohydrates and glycolipids
- Rapid generation of tumor specific T cells
- Minimal risk of autoimmunity or GvHD
- A living drug, single infusion



Verneris et al, Blood Advances, Dec 2021

Challenges of CAR-T

1. Target selection

2. Optimize co-stimulatory signaling of T cell effector functions

3. Toxicities (on-target but off-tumor toxicity)

(The on-target toxicities result from the inability of engineered T cells to distinguish between normal cells and cancer cells that express the targeted Ag)

Challenges of CAR-T

Toxicities

- **On target/off tumor toxicities**
  - Metastatic colon cancer patient died after 5 days of infusion of ERBB2+CAR-T cells
    - Low levels of ERBB2 express on lung epithelium (lung tox)
  - Renal cell carcinoma: 5/11 patients developed liver toxicity

Toxicities of CAR-T cells

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Toxicities following CAR-T therapy for hematological malignancies

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Toxicities of CAR-T cells

Table 1
Toxicities of FDA-approved CAR-T cell therapies.

Trial	N	Target	Costimulatory domain	Toxicity scale	Neutropenia, n (%)	Grade >=3 neutropenia, n (%)	Anemia, n (%)	Grade >=3 anemia, n (%)	Thrombopenia, n (%)	Grade >=3 thrombopenia, n (%)	CRS, n (%)	Severe CRS, n (%)	ICANS, n (%)	Severe ICANS, n (%)	CRS- / ICANS-related deaths, n
NHL															
ZUMA-1 [16,122]	108[a]	CD19	CD28	Lee; CTCAE	93 (86)[b]	86 (80)[b]	73 (68)[b]	49 (45)[b]	67 (62)[b]	43 (40)[b]	94 (93)[c]	13 (13)[c]	65 (64)[c]	28 (28)[c]	1 CRS, 1 HLH
JULIET [2]	111	CD19	4-1BB	Penn; CTCAE	38 (34)[d]	37 (33)[d]	53 (48)	42 (38)	37 (33)[e]	21 (19)[e]	64 (58)	24 (22)	23 (21)	13 (12)	0
TRANSCEND [4]	269	CD19	4-1BB	Lee; CTCAE	ND	101 (60)	ND	101 (37)	ND	72 (27)	113 (42)	6 (2)	80 (30)	27 (10)	0
MCL															
ZUMA-2 [5]	68	CD19	CD28	Lee; CTCAE	59 (87)	58 (85)	46 (68)	34 (50)	50 (74)	35 (51)	62 (91)	10 (15)	43 (63)	21 (30)	0
ALL															
ELIANA [1]	75	CD19	4-1BB	Penn; CTCAE	ND	10 (13)	ND	3 (4)	ND	11 (15)[e]	58 (77)	35 (47)	30 (40)	10 (13)	0
MM															
KarMMa [6]	128	BCMA	4-1BB	Lee; CTCAE	117 (91)	114 (89)	89 (70)	77 (60)	81 (63)	67 (52)	107 (84)	7 (5)	23 (18)	4 (3)	1 CRS
CARTITUDE-1 [7]	97	BCMA	4-1BB	CARTOX; CTCAE	93 (96)	92 (95)	79 (81)	66 (68)	77 (79)	58 (60)	92 (95)	4 (4)	20 (21)	9 (9)	1 CRS/ HLH, 1 ICANS

Abbreviations: CARTOX, chimeric antigen receptor *T*-cell therapy-associated toxicity; CTCAE, common terminology criteria for adverse events; CRS, cytokine-release syndrome; HLH, hemophagocytic lymphohistiocytosis; ICANS, immune effector cell-associated neurotoxicity syndrome; MCL, mantle cell lymphoma; MM, multiple myeloma; ND, no data; NHL, non-Hodgkin lymphoma.
[a]Phase 1, n = 7; phase 2, n = 101.
[b]Data on 108 phase 1 and 2 patients.
[c]Data on 101 phase 2 patients.
[d]Caution is warranted since "neutropenia" and "neutrophil count decreased" appear as different adverse events in supplementary material.
[e]Caution is warranted since "thrombopenia" and "decreased platelet count" appear as different adverse events in supplementary material.

Toxicities of CAR-T cells

Table 2
Risk factors for toxicities.

Risk factors	CRS	ICANS	Hematotoxicity	Infection
Patient-related				
Age	SPS:refid::bib25_bib105_bib112 [25,105,112]	[22,25,105]		[157]
ECOG	[32,37,158]	[25,105,114,158]		[91]
CIRS score	[159]			
Autologous SCT	[160]			
Allogeneic SCT				[107]
Obesity	[161]			
Neurological comorbidities		[66,67]		
Prior effusions	[162]			
LVEF		[32]		
High metabolic tumor burden	[163,164]			
Bilirubin levels	[32]			
Inflammatory parameters	[68,115,158,160,165–169]	[22,36,166,169–171,53,68,69,73,114,115,158,160]	[84,86]	
Cytopenias			[84,172]	
Leucopenia		[53]		[92,107,125,173,174]
Thrombopenia	[160,167]	[160]		
EASIX score	[160,175–177]	[160,175–177]		
HEMATOTOX score			[87]	[92]
Hypogammaglobulinemia				[91,107]
Fever	[167,169]	[53]		
Prior infections				[91,125,174]
No quinolone prophylaxis				[92]
Disease-related				
ALL	[112]	[67]		
IPI	[160]	[160]		
Aggressive subtype		[53]		
Disease burden	[11,30,61,68,98,99,167,178]	[32,67,99,113,170]		
Primary refractory	[158]	[158]		
Number of prior lines				[88,106,125]
> 2 lines of therapy		[116]		
Need of bridging therapy		[115]		[89]
SD or PD after bridging therapy	[37]			
CAR-*T*-related				
Lymphodepletion	[167]			[107]
CAR-T cell dose	[112,158,167,179]	[67,158]		[106]
CAR-T subpopulations	[98,167]			
CAR-T expansion	[68,98,178,180]	[98,170]		
CD28 construct	[111–113,115]	[22,113–116]	[84]	[116]
CRS		[1,53,67–69]	[84,85,172]	[107,118,174,181,182]
Tocilizumab		[53]		[89]
ICANS			[84]	[89,118]
Corticosteroids				[88,89,91,92,118,125,157,174]

Abbreviations: ALL, Acute lymphoblastic leukemia; CAR-T, chimeric antigen receptor *T*-cell; CIRS, Cumulative Illness Rating Scale; CRS, cytokine-release syndrome; EASIX, Endothelial Activation and Stress Index; ECOG, Eastern Cooperative Oncology Group; ICANS, immune effector cell-associated neurotoxicity syndrome; IPI, International Prognostic Index; LVEF, left ventricular ejection fraction; PD, progression of disease; SCT, stem cell transplantation; SD, stable disease.

Toxicities of CAR-T cells

Table 3
Strategies to CRS and/or ICANS prophylaxis.

Reference	Summary	Outcomes
Prophylactic tocilizumab		
Locke et al., 2017 [70]	Prophylactic tocilizumab on day + 2 in cohort 3 of ZUMA-1 trial (N = 31)	1/31 (3 %) patients with grade ≥ 3 CRS compared with 11 % in cohort 1 + 2. However, the incidence of grade ≥ 3[a] ICANS was 35 % (6 % grade 4, including 1 case of fatal brain edema), which was the cause of the closure of this cohort
Molostova et al., 2019 [61]	Tocilizumab before infusion in 37 pediatric patients with B-ALL treated with local manufactured antiCD19 CAR-T	CRS in 22 patients (59 %), 3 of them grade ≥ 3 (14 %) [a] ICANS in 15 patients (40 %): 10, grade 1–2; 1, grade 4; 2, grade 5 [a]
Caimi et al., 2021 [183]	Tocilizumab 1 h prior to infusion in 20 patients treated with anti-CD19 CAR-T cell for NHL	CRS in 10 patients (50 %) (all grade 1-2[b]). Grade 1–2 ICANS in 4 patients (20 %); grade 4 ICANS in 1 patient (5 %) [c]
Early use of tocilizumab		
Gardner et al., 2019 [184]	Phase 1/2 trial of the CD19 CAR *T*-cell product SCRI-CAR19v1 in 43 patients with B-ALL. The first 23 patients (DLT cohort) received tocilizumab and/or CS only if dose-limiting and/or life-threating toxicities associated with CRS or neurotoxicity. The next 20 treated patients (EI cohort), received tocilizumab and/or CS for persistent symptoms of mild CRS	Both cohorts (DLT and EI) had similar overall rates of CRS: 91 % (21/23) vs 95 % (19/20), respectively. There was a higher rate of severe CRS [a] in the DLT cohort (30 vs 15 %), although this finding lacked statistical significance (P = 0.29). The incidence of ICANS was similar between the 2 cohorts: 48 % (11/23) for DLT vs 50 % (10/20) for EI (P = 1). A similar rate of grade 3–4 ICANS [a] was seen (22 % [5/23] vs 25 % [5/20], respectively (P = 1)
Kadauke et al., 2021 [185]	Tocilizumab after CTL019 infusion in a cohort of patients with B-ALL and high-tumor burden (≥40 % bone marrow blasts), if two peaks of fever ≥ 38.5 °C in a 24-hour (N = 15). The primary endpoint was the incidence of grade 4 CRS [d]	Grade 4 CRS occurred in 4/15 (27 %) compared with 13/26 (50 %) of a historical cohort of high-tumor burden patients from the initial phase 1 CTL019 trial (P = 0.18)
Gaffney et al., 2022 [186]	Early use of tocilizumab and CS versus standard management based on risk factors (age, comorbidities, disease burden and per physician discretion) in 30 patients with hematologic malignancies	The incidence of CRS was 80 % with no cases of grade 3–4 CRS [a.] The incidence of ICANS was 37 % (13/30) with 10 % (3/10) of grade ≥ 3. Twelve patients (40 %) were stratified as early management, of whom 8 % (1/12) developed grade ≥ 3 ICANS vs 11 % (2/18) of patients stratified as standard management
Prophylactic CS		
Oluwole et al., 2021 [74,75]	Oral dexamethasone 10 mg/day on days 0 (before infusion), +1 and + 2 in cohort 6 of ZUMA-1 trial (N = 40). They also received earlier CS and tocilizumab for toxicity management	CRS was observed in 32 patients (80 %) with no cases of grade ≥ 3 CRS [b]. ICANS was observed in 23 patients (58 %): ≥ grade 3 (13 %) [a]. Shorter median CRS duration and delayed time to CRS onset was observed than in cohorts 1 + 2 and 4
Early use of CS		
Topp et al., 2019 & 2021 [41,42]	CS were initiated to manage all grade 1 CRS if no improvement after three days in cohort 4 of ZUMA-1 trial (N = 41)	The incidence of grade ≥ 3 CRS [b] and grade ≥ 3 ICANS [a] observed in cohort 4 (2 % and 17 %, respectively) was lower than in cohorts 1 + 2 (12 % and 29 %, respectively)
Liu et al., 2020 [187]	R/R B-ALL treated by CAR-T in three Chinese clinical trials [anti-CD19, sequential CART therapy after transplantation and anti-CD22] (N = 68)	CRS was observed in 64/68 patients (94.1 %): 79.4 % grades 1–2 and 14.7 % grade 3 [d]. CS were administrated in 42 patients (61.8 %) within 1 month after CAR-T cell infusion. The use of CS did not impact CAR-T cell expansion or outcome
Prophylactic anakinra		
Park et al., 2021 [38]	Anakinra 100 mg SC every 12 h for at least 10 days from day + 2 or after 2 documented fever episodes prior to day + 2 in R/R LBCL or MCL receiving CD19 CART cell (N = 31)	CRS was observed in 21 patients (68 %) with 6 % of grade 3–4 CRS [c.] ICANS was observed in 4 patients (13 %), with 2 grade 3 ICANS (6 %) [c].
Frigault et al., 2021 [188]	Anakinra 200 mg SC from 4 h prior to axi-cel infusion to a total of 7 days in R/R LBCL	CRS was observed in 5/6 patients with no cases of grade 3–4 CRS [b]. Grade 3 ICANS was observed in 2/6 patients [a] (preliminary analysis)
Other strategies		
Kenderian et al., 2020 [76]	Lenzilumab (GM-CSF inhibitor) on day 0 (6 h before CD19 CAR-T cell) for ICANS and CRS prevention in R/R LBCL. Phase 1/2 Zuma-19 study (NCT04314843)	No published results yet
Maakaron et al., 2021 [78]	Oral simvastatin 40 mg daily from 5 days before apheresis plus intrathecal dexamethasone on days + 1, +6, +13 of axi-cel infusion for ICANS prevention in R/R LBCL. Phase 1 study (NCT04514029)	The incidence of ICANS was 20 % (1/5 patients) with no cases of grade 3–4 ICANS (preliminary analysis)
Ortíz-Maldonado et al., 2021 [8]	Fractionated infusion of manufactured CD-19 CAR-T cell with full administration depending on the absence of CRS in cohort 3 of NCT03144583 trial (N = 28)	The incidence of grade ≥ 3 CRS in this cohort was 7 % (2/28). No cases of grade ≥ 3 ICANS were observed

Abbreviations: ALL, Acute lymphoblastic leukemia; Axi-cel; axicabtagene ciloleucel; CAR-T, chimeric antigen receptor *T*-cell; CARTOX, chimeric antigen receptor *T*-cell therapy-associated toxicity; CRS, cytokine-release syndrome; CS, corticosteroids; CTCAE, common terminology criteria for adverse events; DLT, dose-limiting toxicities; EI, early intervention, GM-CSF, granulocyte–macrophage colony-stimulating factor; ICANS, immune effector cell-associated neurotoxicity syndrome; LBCL, large B-cell lymphoma; MCL, mantle cell lymphoma; NHL, non-Hodgkin lymphoma; R/R, relapsed/refractory; SC, subcutaneous.
[a]Graded according to CTCAE.
[b]Graded according to Lee criteria.
[c]Graded according to CARTOX criteria.
[d]Graded according to Penn scale.

Toxicities of CAR-T cells

Table 4
Management of CRS and ICANS.

CRS		ICANS	
General recommendations for all grades of CRS [a] - Blood test including coagulation and inflammation parameters - Screening for infection and initiation of empiric antimicrobial therapy following institutional protocol - Supportive care such as IV hydration, antipyretics and physical treatment if needed		General recommendations for all grades of ICANS [a] • Complete neurologic exam including the ICE score with frequent reassessments (i.e., every 6 hours or if clinical deterioration) • Neuroimaging MRI if available or CT. It may be repeated every 2-3 days if refractory grade 3-4 ICANS • EEG evaluation. It may be repeated every 2-3 days if refractory grade 3-4 ICANS • Lumbar puncture is recommended for grade 3-4 of neurotoxicity and may be considered for refractory grade 2 after neuroimaging. Consider also fundoscopic examination • Aspiration preventions such as avoiding oral treatment and CNS depressants	
Grade	**Management**	**Grade**	**Management**
Grade 1: Fever [b], with or without constitutional symptoms	• Apply general recommendations • Consider administering tocilizumab in patients with persistent fever (more than 3 days) despite the implementation of supportive care and without any clinically or microbiologically defined infection [c]	**Grade 1:**ICE score 7-9 without depressed level of consciousness	• Apply general recommendations • Initiate seizure prophylaxis if no prior administration (i.e., levetiracetam 500 mg every 12 hours)
Grade 2: Fever [b] plus hypotension responding to fluids and/ or hypoxia responding to $< 40\%$ FiO_2	• IV fluid resuscitation (20-30 mL/kg within the first 2 hours) and/or oxygen therapy • Initiate tocilizumab 8 mg/kg IV [c] over 1 hour (not to exceed 800 mg/dose). If no improvement the dose can be repeated every 8 hours with a maximum of 4 total doses • Consider DXM 10 mg IV every 6 hours if no improvement after two doses of tocilizumab	**Grade 2:**ICE score 3-6 and/or mild depressed level of consciousness (awakens to voice)	• Start DXM 10 mg IV every 6 hours • Tocilizumab may be considered only with concurrent CRS
Grade 3: Fever [b] plus hypotension managed with one vasopressor (with or without vasopressin) and/ or hypoxia requiring $\geq 40\%$ FiO_2	• Implement measures as per grade 2 • Include vasopressors as needed • Perform echocardiogram	**Grade 3:**ICE score 0-2 and/or depressed level of consciousness (awakens only to tactile stimulus) and/or any clinical seizure focal or generalized that resolves rapidly or nonconvulsive seizures on EEG that resolve with intervention and/or focal/local edema on neuroimaging	• Implement measures as per grade 2 • If clinical or non-convulsive seizures on EEG, optimize antiepileptic drug therapy after neurology consultation
Grade 4: Fever [b] plus hypotension requiring multiple vasopressor (excluding vasopressin) and/ or hypoxia requiring positive pressure	• If no improvement observed with all the above measures, consider administration of high-dose methylprednisolone at a dose of 1000 mg IV per day for 3 days with a progressive tapering of the dose until grade 1 CRS [d] • If refractory, alternate therapy such as anakinra or siltuximab may be used. In this scenario, basal IL-1 or IL-6 might be useful to choose therapy • Consider cyclophosphamide (1000 mg single dose) or antithymocyte globulin (i.e., rabbit thymoglobulin 2 mg/kg) to eliminated CAR-T lymphocytes	**Grade 4:**Stupor or coma, life-threatening prolonged seizure (> 5 min), repetitive clinical or electrical seizures without return to baseline in between, deep focal motor weakness and/or diffuse cerebral edema on neuroimaging; decerebrate or decorticate posturing; cranial nerve VI palsy; papilledema; or Cushing's triad	• If no improvement observed with all the above measures, consider administration of high-dose methylprednisolone at a dose of 1000 mg IV per day for 3 days with a progressive tapering of the dose until grade 1 ICANS [d] • If refractory, alternate therapy such as anakinra or siltuximab may be used. In this scenario, basal IL-1 or IL-6 might be useful to choose therapy • Consider administration of intrathecal hydrocortisone with or without methotrexate • Consider cyclophosphamide (1000 mg single dose) or antithymocyte globulin (i.e., rabbit thymoglobulin 2 mg/kg) to eliminate CAR-T lymphocytes

Abbreviations: ASTCT, American Society of Transplantation and Cellular Therapy; CBC, complete blood count; CMP, comprehensive metabolic panel; CMV, cytomegalovirus; CNS, central nervous system; CRP, C-reactive protein; CRS, cytokine-release syndrome; CS, corticosteroids; CT, computed tomography; DXM, dexamethasone; EBV, Epstein-Barr virus; EEG, electroencephalogram; ICANS, immune effector cell–associated neurotoxicity syndrome; ICE, immune effector cell–associated encephalopathy; IL, interleukin; ICU, intensive care unit; IV, intravenous; LDH, lactate dehydrogenase; MRI, magnetic resonance imaging; PCT, procalcitonin; PTT/PT, prothrombin time/ partial thromboplastin time.

a. CRS and ICANS are graded according to ASTCT consensus grading.

b. Fever is defined as temperature $\geq 38\ ^{\circ}C$ not attributable to any other cause.

c. Consider administration of concomitant DXM 10 mg every 12 h for 3 doses as ICANS prophylaxis if tocilizumab is used.

d. I.e., reducing the dose by 50% every 48 h.

Toxicities of CAR-T cells

Table 5
Infection prophylaxis after CAR-T therapy.

Antibacterial	Consider antibiotic prophylaxis (penicillin or fluoroquinolones) in patients with ANC < 0.5 x10^9/L
Anti-fungal	Consider fluconazole (200 mg/day) or micafungin (50 mg IV/day) in patients with ANC < 0.5 x10^9/L Consider posaconazole(300 mg/day), in patients treated with tocilizumab or corticosteroids [a] with persistent ANC < 0.5 x10^9/L (>4 weeks)
Anti-pneumocystis	Co-trimoxazole 80/400 mg once daily or 160/800 mg three times per week, from LD conditioning or after ANC > 0.5 x10^9/L, until 6 months post-CAR-T cell infusion, if CD4 + count $> 0.2 \times 10^9$/L In case of prolonged cytopenias or co-trimoxazole allergy consider pentamidine inhalation (300 mg once every month), dapsone 100 mg daily, or atovaquone 1500 mg once daily
Anti-viral	Acyclovir 800 mg every 12 h or valacyclovir 500 mg every 12 h to initiate from LD conditioning until 6 months post-CAR *T*-cell infusion, if CD4 + count $> 0.2 \times 10^9$/L For hepatitis B infection prophylaxis with entecavir/tenofovir is recommended the first year after CAR-T cell infusion. Consider monitoring viral load every 3–6 months during the first year after discontinuation

Abbreviations: ANC, absolute neutrophil count; CAR-T, chimeric antigen receptor *T*-cell; IV, intravenous; LD, lymphodepletion.
a. At least 30 days after the last dose of tocilizumab or corticosteroids.

Graft-versus-host disease

Few data are available on CAR-*T*-induced graft-versus-host disease [GVHD] [152,153], which can be triggered by cytokine storm following CAR-T infusion. Patients with prior allogeneic SCT have been widely included in pivotal trials [1,4,8] and there are also reports in a real-life settings [154–156]. GVHD does not seem to be a major concern after CAR-T therapy. However, a 6-month period between SCT and CAR-T infusion is suggested, and CAR-T infusion should be avoided if active GVHD is present.

Other complications

Hemophagocytic lymphohistiocytosis

Hemophagocytic lymphohistiocytosis (HLH) after CAR-T therapy is a rare but potentially life-threatening dysfunctional immune response [7,32,54,89,122] characterized by hyperactive macrophages and lymphocytes, proinflammatory cytokine hypersecretion, tissue infiltration, hemophagocytosis, and organ damage [136]. Delayed coagulopathy has

Secondary malignancies

Cardiovascular toxicities

Cardiovascular events occurred in up to a third of patients in CAR *T*-cell clinical trials [145–147]. Clinical manifestations are closely related to high-grade CRS, including tachycardia, hypotension, arrhythmia, systolic dysfunction, and cardiac arrest. Heart failure after CAR-T therapy is probably reversible [90], in a similar way to critical illness or stress-induced cardiomyopathy [146].

Cardiovascular events are thought to be driven by a pathway of widespread immune and inflammatory activation similar to CRS [146]. To our knowledge, no cardiac infiltration by CD19 CAR-T has been described. However, Linette et al. reported two fatal cardiogenic shocks

Tumoral lysis syndrome

The destruction of malignant cells after lymphodepleting chemotherapy and/or CAR-T infusion increases the risk of tumor lysis syndrome (TLS), particularly in patients with high tumor burden. This is characterized by metabolic abnormalities including hyperuricemia, hyperkalemia, hyperphosphatemia, and hypocalcemia, which leads to severe renal impairment [149,150]. TLS is currently infrequent after

Toxicities of CAR-T cells

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The pathogenesis, diagnosis, prevention, and treatment of CAR-T cell therapy-related adverse reactions

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Toxicities of CAR-T cells

TABLE 1 Approved CAR-T cell therapy.

Name (trade name)	Company	Target antigen	CAR construct (Crees and Ghobadi, 2021; Anderson, 2022)	Listing date	Indication
Tisagenlecleucel (Kymriah)	Novartis	CD19	Second generation, CD3ζ+4-1BB Lentiviral vector	FDA 2017.08.30 EMA 2018.08.27	Paediatric and young adult patients (age 3–25 years) with r/r B-ALL; adult (≥18 years) patients with r/r DLBCL (Braendstrup et al., 2020)
				FDA 2022.05.27	Adult patients with r/r FL (Fowler et al., 2022)
Axicabtagene ciloleucel (Yescarta)	Kite pharma	CD19	Second generation, CD3ζ+CD28 Retroviral vector	FDA 2017.10.18 EMA 2018.08.27	Adult patients with LBCL failing at least two other kinds of treatment (including r/r DLBCL, r/r PMBCL, high-grade BCL and DLBCL arising from FL) (Jacobson et al., 2020)
Brexucabtagene autoleucel (Tecartus)	Kite pharma	CD19	Second generation, CD3ζ+CD28 Retroviral vector	FDA 2020.07.24 EMA 2020.12.17	Adult patients with r/r MCL Adults with r/r B-ALL (Tbakhi and Reagan, 2022)
Lisocabtagene maraleucel (Breyanzi)	Juno Therapeutics/ Bristol Myers Squibb	CD19	Second generation, CD3ζ+4-1BB Lentiviral vector	FDA 2021.02.05	Adult patients with r/r LBCL failing at least two other kinds of treatment (including r/r DLBCL, r/r PMBCL, high-grade BCL, Grade 3B FL) (Crees and Ghobadi, 2021)
Idecabtagene Vicleucel (Abecma)	Bristol Myers Squibb	BCMA	Second generation, CD3ζ+4-1BB Lentiviral vector	FDA 2021.03.26 EMA 2021.08.19	Adult patients with r/r MM (Sharma et al., 2022)
Relmacabtagene autoleucel (relma-cel)	JW Therapeutics	CD19	Second generation, CD3ζ+4-1BB Lentiviral vector	NMPA 2021.09.03	Adult patients with r/r DLBCL (Ying et al., 2021)
Ciltacabtagene autoleucel (Carvykti)	Legend Biotech/ Janssen Biotech	BCMA (consisting of two BCMA-binding domains)	Second generation, CD3ζ+4-1BB Lentiviral vector	FDA 2022.02.28	Adult patients with r/r MM (Berdeja et al., 2021)

FDA, Food and Drug Administration; EMA, European Medicines Agency; NMPA, National Medical Products Administration; r/r B-ALL, relapsed or refractory B-cell acute lymphoblastic leukaemia; r/r DLBCL, relapsed or refractory diffuse large B-cell lymphoma; LBCL, large B-cell lymphoma; PMBCL, primary mediastinal B-cell lymphoma; BCL, B-cell lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; MM, multiple myeloma.

Toxicities of CAR-T cells

TABLE 2 Adverse reactions related to CAR-T cell therapy.

Adverse reaction	Main symptoms	Relationship with CRS	Characteristic
CRS	Fever; Hypotension; Hypoxia; DIC; Multi organ system toxicities	—	• Systemic inflammatory reaction caused by a large number of inflammatory factors
ICANS	Aphasia; Headache; Mild encephalopathy; Focal neurological Deficit; Tremor; Seizures; brain edema	CRS is one of the main inducers of ICANS, ICANS and CRS may occur simultaneously or not	• The breakdown of the BBB and capillary leakage lead to the entry of pro-inflammatory cytokines and CAR-T cells into the CSF to damage the CNS.
Cardiovascular toxicity	Hypotension; Sinus tachycardia; Increased serum troponin levels; Arrhythmia; Reduced LVEF; Cardiogenic shock; QT prolongation; Heart failure	CRS is one of the main inducers of cardiovascular toxicity, which can lead to serious direct and indirect cardiovascular complications	• Abnormal elevation of inflammatory cytokines IL-6, VWF, Ang-2, TNF-α and off-target cross-reaction of CAR-T cells to actin can lead to cardiovascular toxicity
Hematologic toxicity	Neutropenia; Thrombocytopenia; Leucopenia; Anemia; B-cell aplasia; Coagulopathy	Patients with severe CRS were more likely to develop late hematologic toxicity	• Neutropenia is closely related to infectious complications • B-cell aplasia is a common toxicity of anti-CD19 CAR-T therapy
HLH/MAS	Ferritin is extremely elevated; High fever; Hepatosplenomegaly; Hemocytopenia; Coagulopathy	HLH/MAS is a severe manifestation of CRS, so it is difficult to distinguish diagnosis of them	• The incidence of HLH/MAS is low, but its mortality is high and prognosis is poor
Skin toxicity	Rash; Dry skin; Purpura; Papules; Maculopapular; Urticarial rash; Bullous eruptions; Oral mucositis	CRS is one of the inducers of skin toxicity, and the reduced immune function induced by CRS may lead to skin infections in patients	• The clinical manifestations and mechanisms of skin toxicities are still poorly understood • Currently, there are no guidelines to diagnose and treat skin toxicity
Pulmonary toxicity	Respiratory failure	CRS is one of the main inducers of pulmonary toxicity	• The incidence of pulmonary toxicity is lower than that of CRS and ICANS. • There are definite clinical diagnostic indicators about pulmonary toxicity
Renal toxicity	Adrenal insufficiency; Electrolyte disorders; Kidney failure; Acidosis	CRS is one of the main inducers of renal toxicity	• The incidence of renal toxicity is lower than that of CRS and ICANS. • There are definite clinical diagnostic indicators about renal toxicity • Usually symptomatic treatment
Hepatotoxicity	Liver injury	CRS is one of the main inducers of hepatotoxicity	• The incidence of hepatotoxicity is lower than that of CRS and ICANS. • There are definite clinical diagnostic indicators about hepatotoxicity
Gastrointestinal toxicity	Diarrhea; Vomiting; Bleeding; Nausea	CRS is one of the main inducers of gastrointestinal toxicity	• The incidence of gastrointestinal toxicity is lower than that of CRS and ICANS. • There are definite clinical diagnostic indicators about gastrointestinal toxicity • Usually symptomatic treatment

CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; DIC, disseminated intravascular coagulation; BBB, blood brain barrier; CSF, cerebrospinal fluid; CNS, central nervous system; LVEF, left ventricular ejection fraction; IL, interleukin; Ang-2, angiopoietin-2; VWF, von willebrand factor; TNF-α, tumor necrosis factor alpha; HLH/MAS, Hemophagocytic Lymphohistiocytosis/Macrophage Activation Syndrome.

Toxicities of CAR-T cells

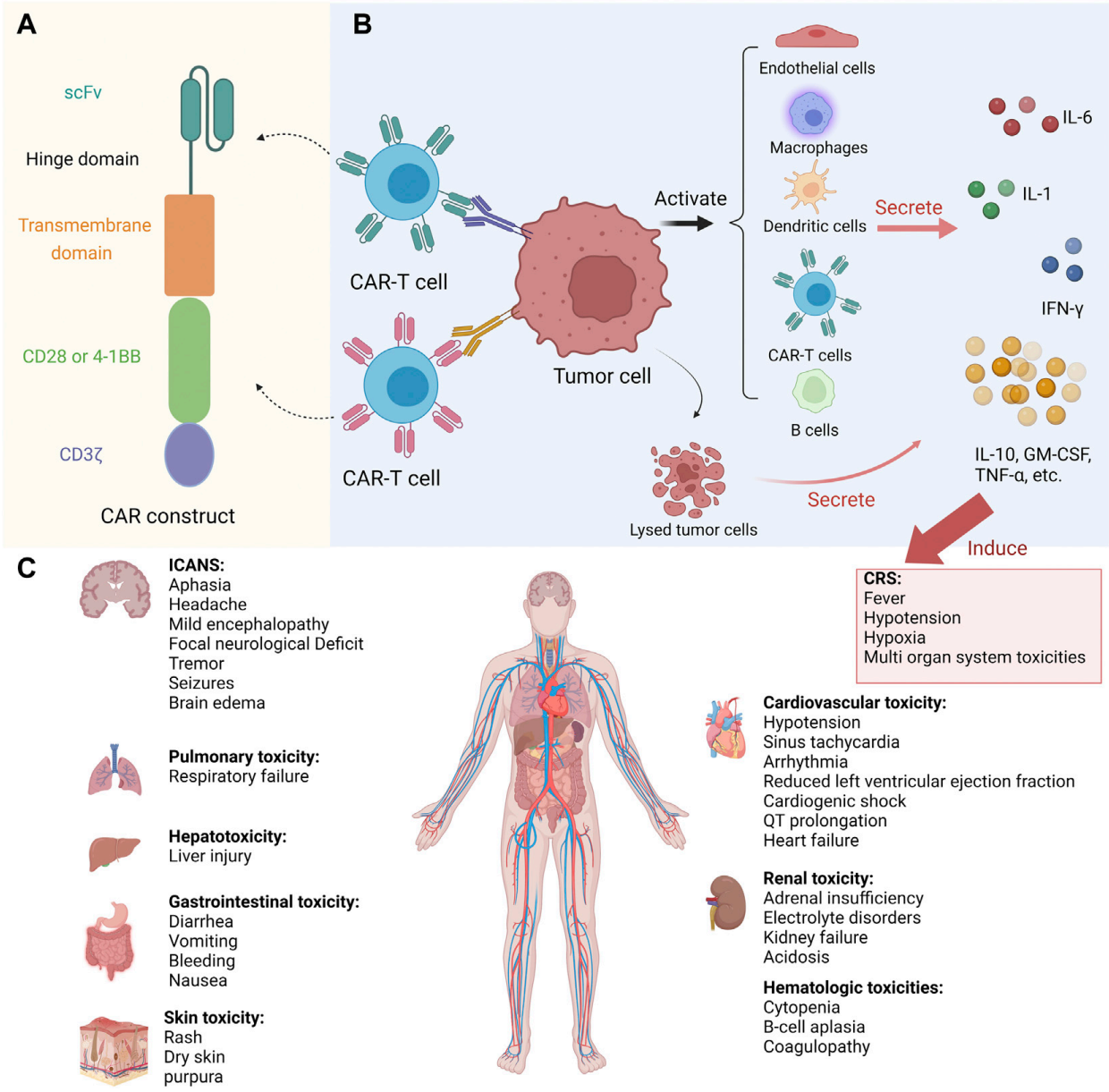


FIGURE 1
Toxicities during CAR-T therapy. **(A)** The structure of CAR. **(B)** Pathogenesis of CRS. **(C)** Organ systemic toxicities induced by CRS. Abbreviations: CAR, chimeric antigen receptor; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; scFv, single-chain variable fragment; IL, interleukin; IFN-γ, interferon gamma; GM-CSF, granulocyte macrophage colony-stimulating factor; TNF-α, tumor necrosis factor alpha. This figure created with BioRender.com.

Toxicities of CAR-T cells

TABLE 4 Management of CRS.

CRS management system	Lee criteria (Lee et al., 2014)	CARTOX criteria (Neelapu et al., 2018a)	ASTCT consensus criteria (Neelapu, 2019)	ASCO guideline (Santomasso et al., 2021)
Grade 1	• Vigilant supportive care (treat fever and neutropenia if present, antipyretics, analgesics as needed)	• Fever: Acetaminophen and hypothermia blanket; Consider tocilizumab 8 mg/kg IV or siltuximab 11 mg/kg IV for persistent (lasting >3 days) and refractory fever	•Antipyretics and IV hydration	• Supportive care with antipyretics, IV hydration, and symptomatic management of organ toxicities and constitutional symptoms
	• Assess for infection	• Organ toxicity: Symptomatic management	• Diagnostic work-up to rule out infection	• Consider empiric broad-spectrum antibiotics if neutropenic
	• Monitor fluid balance	• Empiric broad-spectrum antibiotics and filgrastim if neutropenic	• Consider growth factors and antibiotics if neutropenic	• If neutropenia, consider empiric broad-spectrum antibiotics and G-CSF (GM-CSF is not recommended)
		• Maintenance IV fluids for hydration		• In patients with persistent (>3 days) or refractory fever, consider managing as per grade 2
Grade 2	• Maintenance of adequate hydration and blood pressure	• Fever: manage fever as in grade 1 CRS	• Supportive care as in grade 1	• Supportive care as per grade 1
	• Vigilant supportive care (monitor cardiac and other organ function closely), if the patient doesn't have extensive co-morbidities or older age	• Hypotension: IV fluid bolus of 500–1,000 ml of normal saline; Second IV fluid bolus if pressure remains <90 mmHg; Tocilizumab or siltuximab for the hypotension refractory to fluid boluses (tocilizumab can be repeated after 6 h); If hypotension persists, start vasopressors, consider transfer to ICU), dexamethasone (10 mg q6h, IV)	• IV fluid boluses and/or supplemental oxygen	• Administer tocilizumab (8 mg/kg, IV); Repeat q8h if no improvement in signs and symptoms of CRS; Limit to a maximum of three doses in a 24 h period, with a maximum of four doses total
	• Tocilizumab (adults 4 mg/kg, children 8 mg/kg) ± corticosteroids (methylprednisolone 2 mg/kg/day, dexamethasone 0.5 mg/kg maximum 10 mg/dose), if the patient has extensive co-morbidities or older age	• Hypoxia: supplemental oxygen; Tocilizumab or siltuximab ± corticosteroids and supportive care	• Tocilizumab ± dexamethasone or its equivalent of methylprednisolone	• In patients with hypotension that persists after two fluid boluses and after one to two doses of tocilizumab, may consider dexamethasone (10 mg q12h, IV) for one to two doses and then reassess
		• Organ toxicity: symptomatic management of organ toxicities, as per standard guidelines; Tocilizumab or siltuximab ± corticosteroids and supportive care		• Manage per grade 3 if no improvement within 24 h of starting tocilizumab
Grade 3	• Maintenance of adequate hydration and blood pressure	• Fever: manage fever as in grade 1 CRS	• Supportive care as in grade 1	• Supportive care as per grade 2 and include vasopressors as needed
	• Vigilant supportive care	• Hypotension: IV fluid bolus, tocilizumab and siltuximab as recommended for grade 2 CRS; Increase dexamethasone to 20 mg q6h IV, if refractory; Transfer to ICU, obtain echocardiogram, and perform haemodynamic monitoring	• Consider monitoring in intensive care unit	• Tocilizumab as per grade 2 if maximum dose is not reached within 24 h period plus dexamethasone (10 mg q6h, IV) and taper once symptoms improve
	• Tocilizumab (adults 4 mg/kg, children 8 mg/kg) ± corticosteroids	• Hypoxia: supplemental oxygen including high-flow oxygen delivery and non-invasive positive	• Vasopressor support and/or supplemental oxygen	• If echocardiogram was not already performed, obtain ECHO to assess cardiac function and

(Continued on following page)

Toxicities of CAR-T cells

TABLE 5 Current interventional clinical trials aiming to reduce CAR-T specific toxicities.

Name	Clinical trials	Specific toxicities	Prophylactic drug	Recruitment status
Axicabtagene ciloleucel	NCT05459571	CRS ICANS	Dexamethasone: dexamethasone (10 mg, orally or IV) before CAR-T cell infusion	Recruiting
Axicabtagene ciloleucel	NCT04314843	ICANS	Lenzilumab: sequenced therapy of lenzilumab and axicabtagene ciloleucel on Day 0	Terminated (Development program terminated.)
Axicabtagene ciloleucel	NCT04150913	CRS ICANS	Anakinra: anakinra (dosage per protocol, SC) on days 0–6	Recruiting
Axicabtagene ciloleucel	NCT04514029	ICANS	Simvastatin: simvastatin (40 mg/day, orally) will be started at least 5 days prior to apheresis and will be continued until day +30 after infusion. Dexamethasone: intrathecal dexamethasone 8 mg on days −1, +6, +13 (± 2 days)	Recruiting
Axicabtagene ciloleucel	NCT04432506	CRS ICANS	Anakinra: anakinra SC on days 0–6	Active, not recruiting
Axicabtagene ciloleucel	NCT03954106	ICANS	Defibrotide: defibrotide 6.25 mg/kg/dose once daily as a single dose on CAR-T Day −5, −4, and −3 before lymphodepletion, then every 6 h daily for 8 days (CAR-T Day 0 to Day 7)	Terminated (Primary endpoint would unlikely to be met based on the unplanned interim assessment on the first 20 efficacy evaluable patients.)
Axicabtagene ciloleucel	NCT04205838	ICANS	Anakinra: anakinra SC every 6–12 h for 12–36 doses over 9 days	Suspended (funding)
Axicabtagene ciloleucel	NCT04071366	CRS	Itacitinib: itacitinib (200 mg/day, orally) for 30 days or itacitinib (200 mg bid, orally) for 30 days	Recruiting
Axicabtagene ciloleucel	NCT02348216	CRS ICANS	Cohort 3 Levetiracetam: levetiracetam (750 mg orally or IV, BID) starting on Day 0 Tocilizumab: tocilizumab (8 mg/kg IV over 1 h [not to exceed 800 mg]) on Day 2 Cohort 4 Corticosteroids: dexamethasone or methylprednisolone. Tocilizumab: tocilizumab (8 mg/kg IV over 1 h [not to exceed 800 mg] at lower grades of toxicity) Levetiracetam: levetiracetam (750 mg orally or IV, BID) starting on Day 0 Cohort 5 Levetiracetam: levetiracetam (750 mg orally or IV, BID) starting on Day 0 Cohort 6 Corticosteroids: dexamethasone prior to axicabtagene ciloleucel infusion on Day 0, Day 1 and Day 2 Tocilizumab: tocilizumab at lower grades of toxicity Levetiracetam: levetiracetam (750 mg orally or IV, BID) starting on Day 0	Active, not recruiting
Lisocabtagene maraleucel	NCT04359784	CRS ICANS	Anakinra: anakinra SC daily on days 0–13	Recruiting

CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; IV, intravenous; SC, Subcutaneous Injections.

Toxicities of CAR-T cells

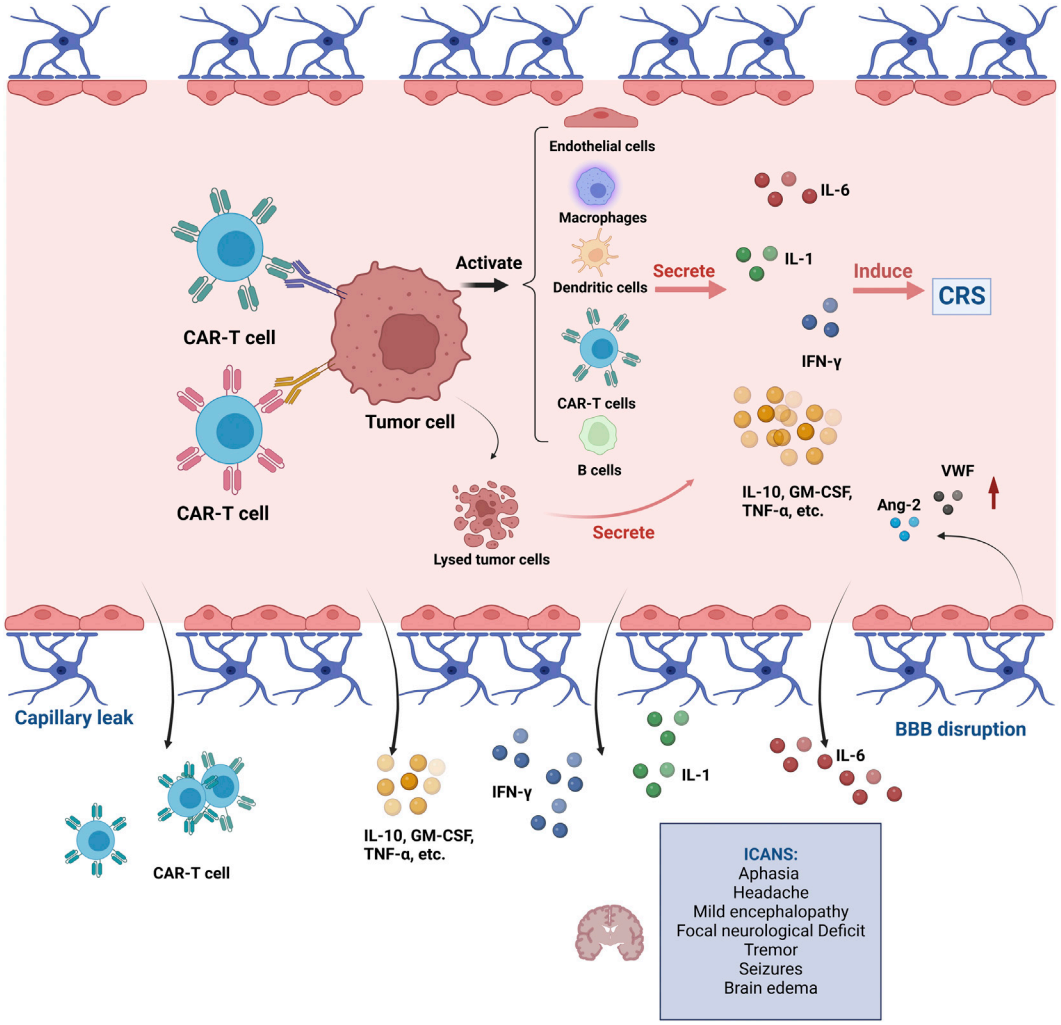


FIGURE 2
Pathogenesis of ICANS during CAR-T therapy. Abbreviations: CAR, chimeric antigen receptor; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; IL, interleukin; IFN-γ, interferon gamma; GM-CSF, granulocyte macrophage colony-stimulating factor; TNF-α, tumor necrosis factor alpha; BBB, blood brain barrier. This figure created with BioRender.com.

Toxicities of CAR-T cells

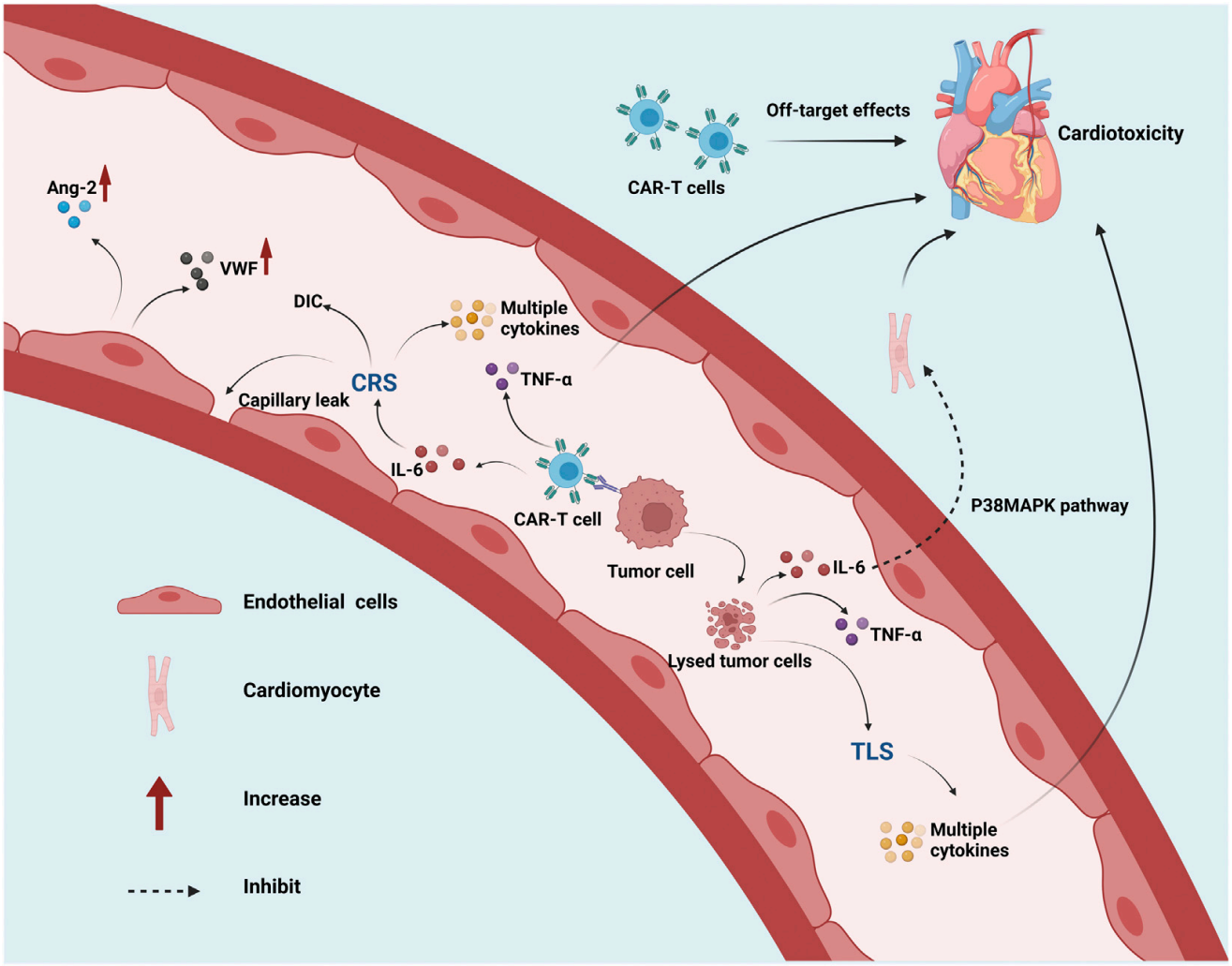
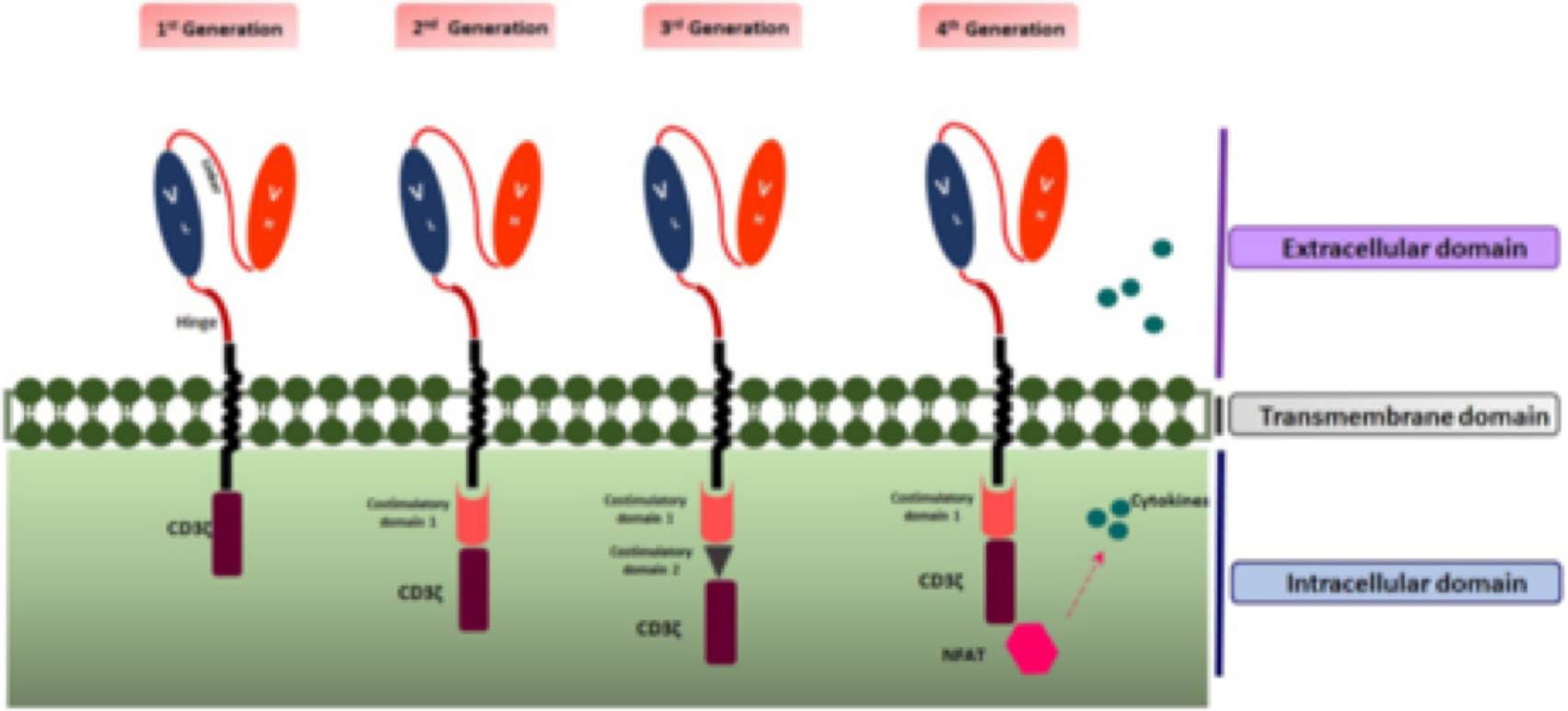


FIGURE 3
Pathogenesis of cardiovascular toxicity during CAR-T therapy. Abbreviations: CAR, chimeric antigen receptor; CRS, cytokine release syndrome; IL, interleukin; TNF-α, tumor necrosis factor alpha; MAPK, mitogen-activated protein kinase; TLS, tumor lysis syndrome; DIC, disseminated intravascular coagulation; Ang-2, angiopoietin-2; VWF, von willebrand factor. This figure created with BioRender.com.

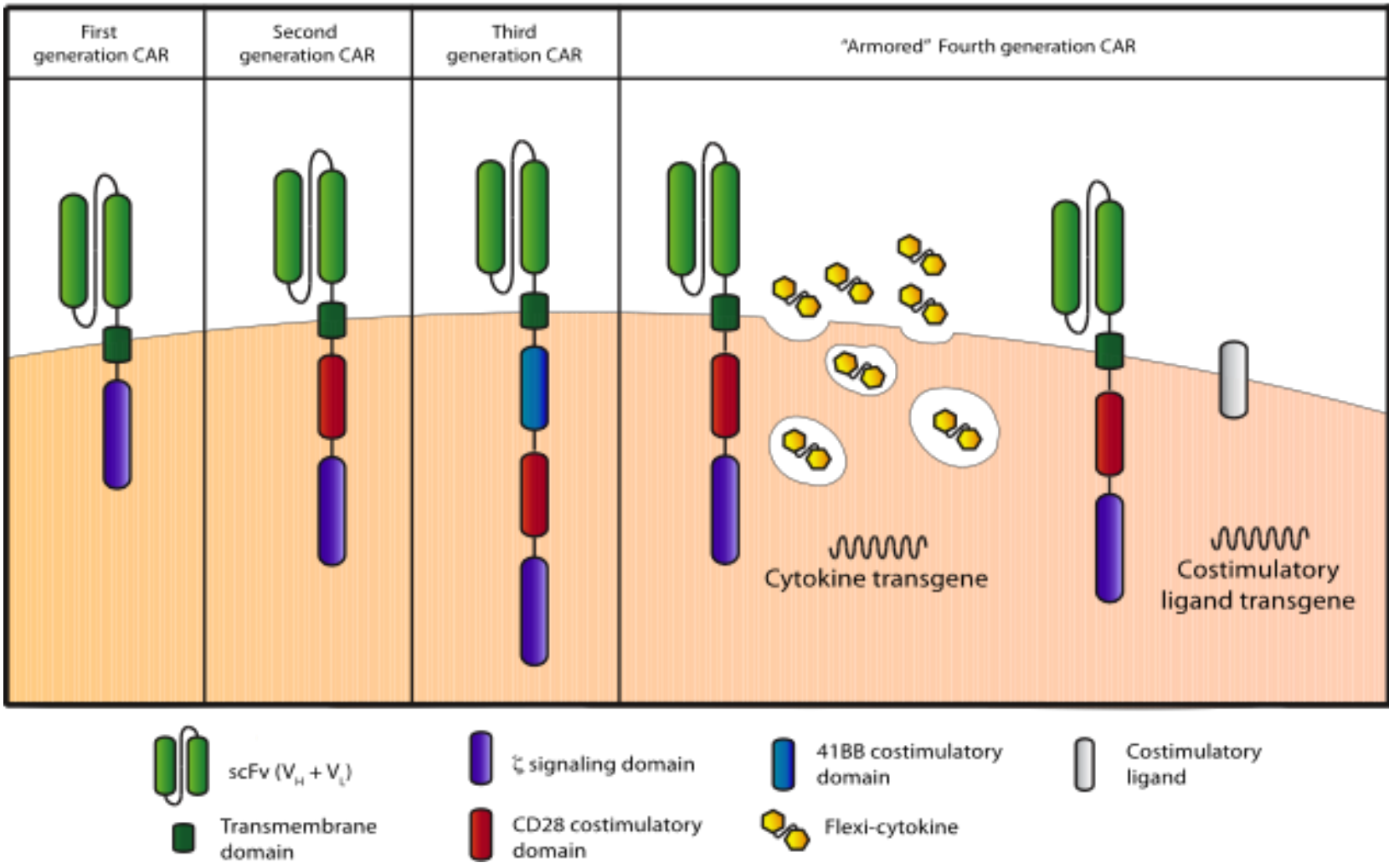
Evolution in CAR design



(Elahi, Khosh, Tahmasebi, & Esmaeilzadeh, 2018)

First-generation CARs: including activating receptors such as CD8/CD3-ζ fusion receptors;
Second-generation CARs: providing dual signaling to direct combined activating and co-stimulatory signals;
Third-generation CARs: comprising more complex structures with 3 or more signaling domains.
Fourth-generation: ….

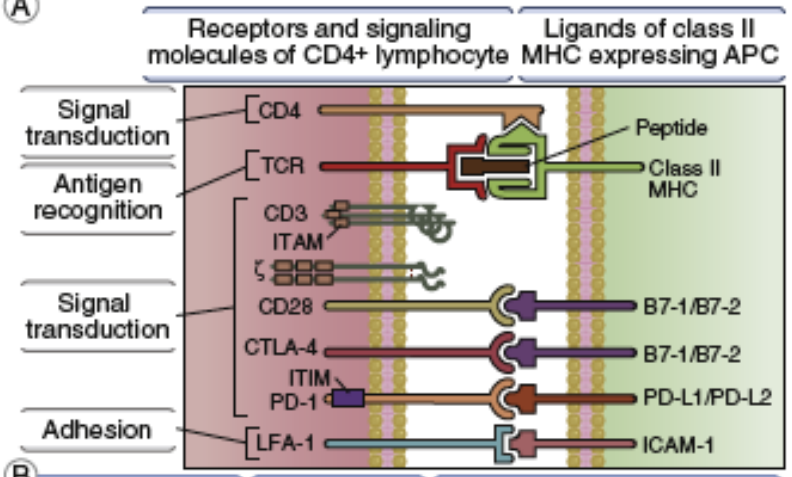
Moving Forward: Armored CARs



IL-12

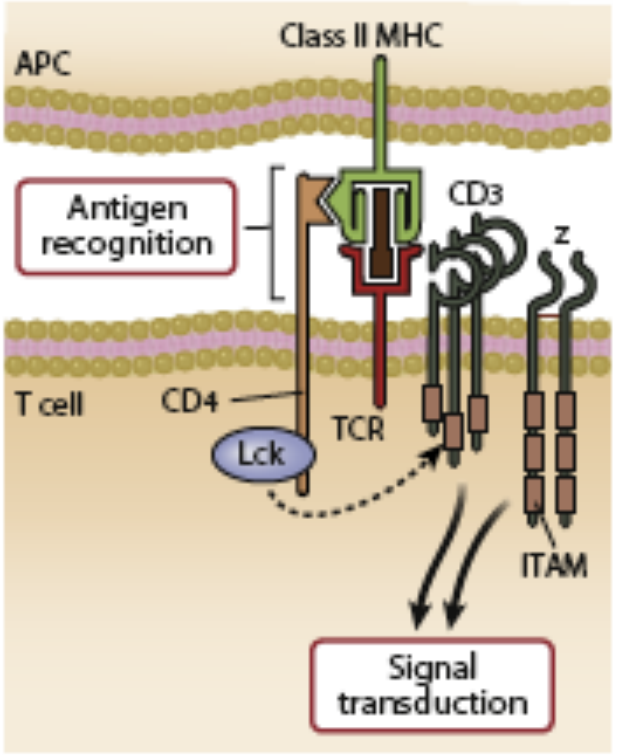
- A heterodimeric cytokine secreted by activated APCs, neutrophils and macrophages.
- Induces Th1 $CD4^+$ T cell response enhancing IL-2 and IFN-γ secretion
- Enhances T cell clonal expansion and effector function in concert with TCR signaling (signal 1) and CD28 co-stimulation (signal 2), serving as a signal 3.
- Avoids/reverses T cell anergy
- May overcome Treg mediated effector T cell inhibition
- Recruits and activates NK cells
- Clinical trials in cancer using systemic IL-12 therapy has been limited by severe inflammatory side effects

A



B

Surface molecules of T lymphocytes	Function	Ligand	
		Name	Expressed on
CD3	Signal transduction by TCR complex	None	
ζ	Signal transduction by TCR complex	None	
CD4	Signal transduction	Class II MHC	Antigen presenting cells
CD8	Signal transduction	Class I MHC	All nucleated cells
CD28	Signal transduction (costimulation)	B7-1/B7-2	Antigen presenting cells
CTLA-4	Inhibition	B7-1/B7-2	Antigen presenting cells
PD-1	Inhibition	PD-L1/PD-L2	Antigen presenting cells, tissue cells, tumor cells
LFA-1	Adhesion, signal transduction	ICAM-1	Antigen presenting cells, endothelium



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Review

Engineering Next-Generation CAR-T Cells for Better Toxicity Management

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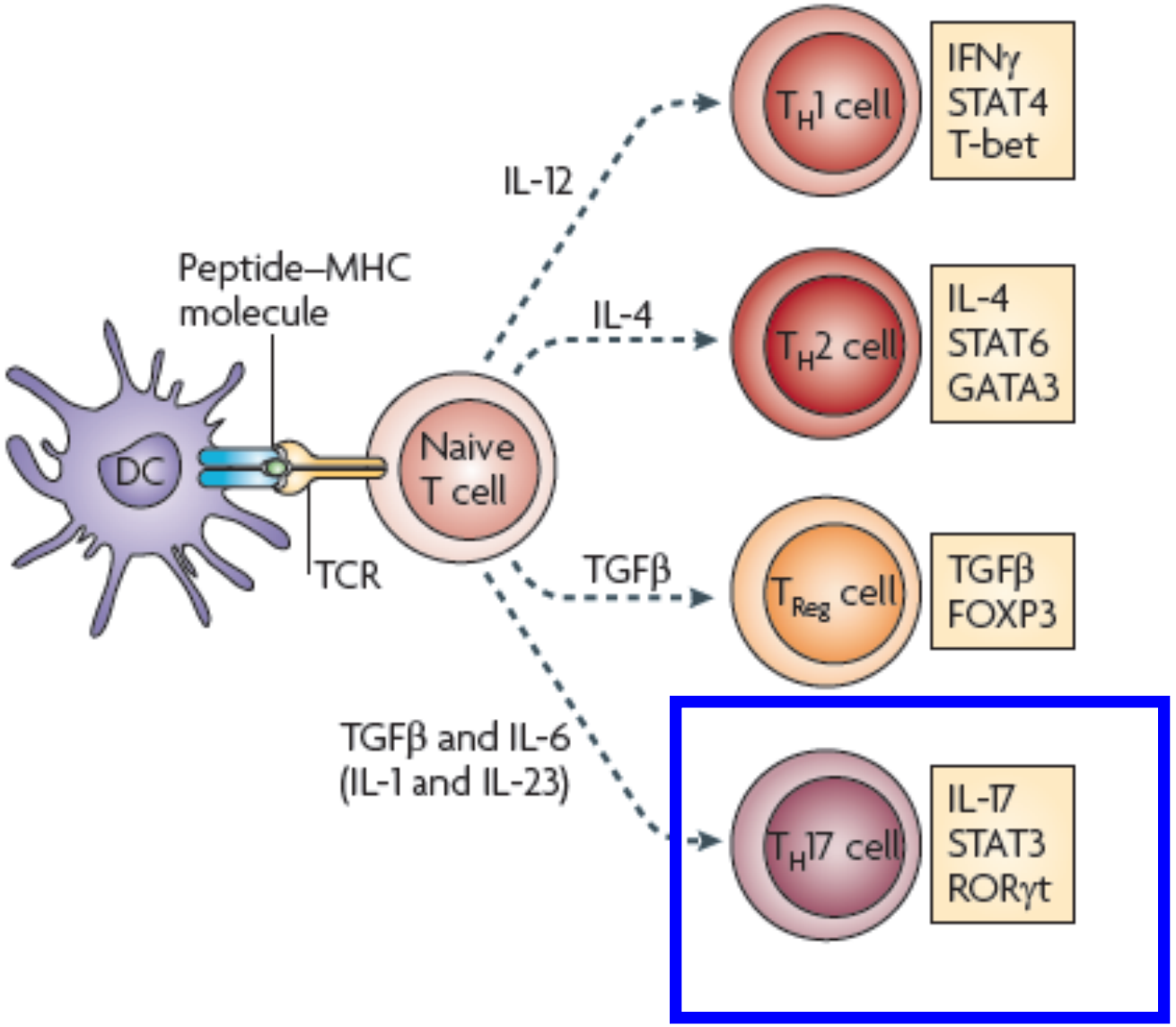
Determinants of successful CAR-T cells

- **Tumor target**
  - Target antigen is critical determinant for efficacy & safety
  - Ideal target uniquely express on tumor cells or on cells which are not essential for survival
- **Efficacy & Long-term persistence**
  - Subtypes of CD4+T cells (Th1, Th2, Th17, Th9 cells),
  - CD8+T cells
    - naïve, central memory; long-term
    - effector; active but short lived
- **Trafficking of CAR T cells to tumor**
  - Expression of addressins
  - Route of CAR-T cell infusion
    - Intra-tumoral/intravenous
  - Optimal co-stimulation of T cells

Classification of T-cell

- Cytotoxic T-cell (CD8)
- Helper T-cell (CD4)
- Regulatory/suppressor T-cell (T-Reg)
- Memory T-cell

Adoptive T cell therapy: Right T cell population?



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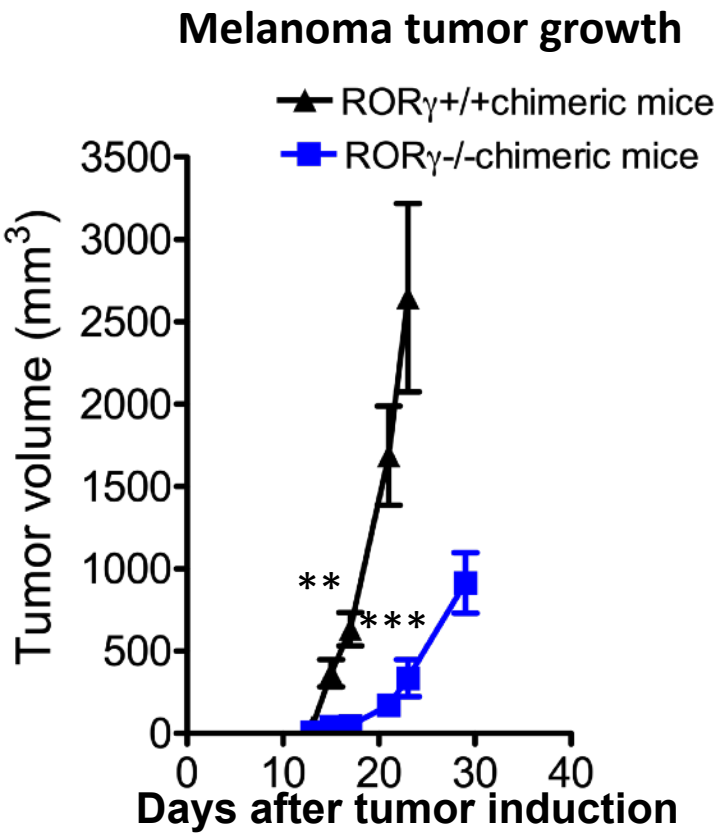
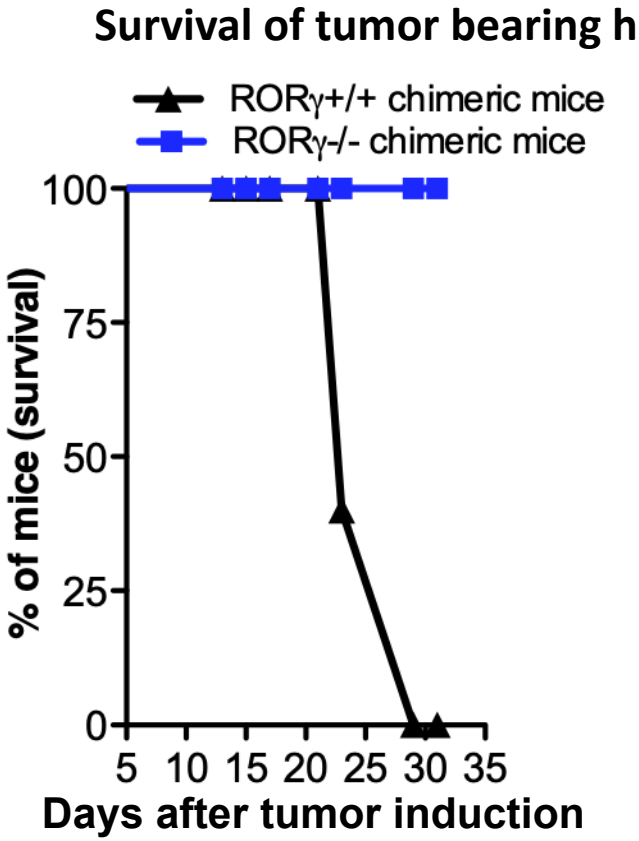
Tumor growth suppression in RORγ-/- mice (Th17 cell deficient)





Abrogation of Th17 pathways promotes anti-tumor immune responses

CANCER

Antitumor activity without on-target off-tumor toxicity of GD2–chimeric antigen receptor T cells in patients with neuroblastoma

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The reprogramming of a patient's immune system through genetic modification of the T cell compartment with chimeric antigen receptors (CARs) has led to durable remissions in chemotherapy-refractory B cell cancers. Targeting of solid cancers by CAR-T cells is dependent on their infiltration and expansion within the tumor microenvironment, and thus far, fewer clinical responses have been reported. Here, we report a phase 1 study (NCT02761915) in which we treated 12 children with relapsed/refractory neuroblastoma with escalating doses of second-generation GD2-directed CAR-T cells and increasing intensity of preparative lymphodepletion. Overall, no patients had objective clinical response at the evaluation point +28 days after CAR-T cell infusion using standard radiological response criteria. However, of the six patients receiving $\geq 10^8$/meter^2 CAR-T cells after fludarabine/cyclophosphamide conditioning, two experienced grade 2 to 3 cytokine release syndrome, and three demonstrated regression of soft tissue and bone marrow disease. This clinical activity was achieved without on-target off-tumor toxicity. Targeting neuroblastoma with GD2 CAR-T cells appears to be a valid and safe strategy but requires further modification to promote CAR-T cell longevity.

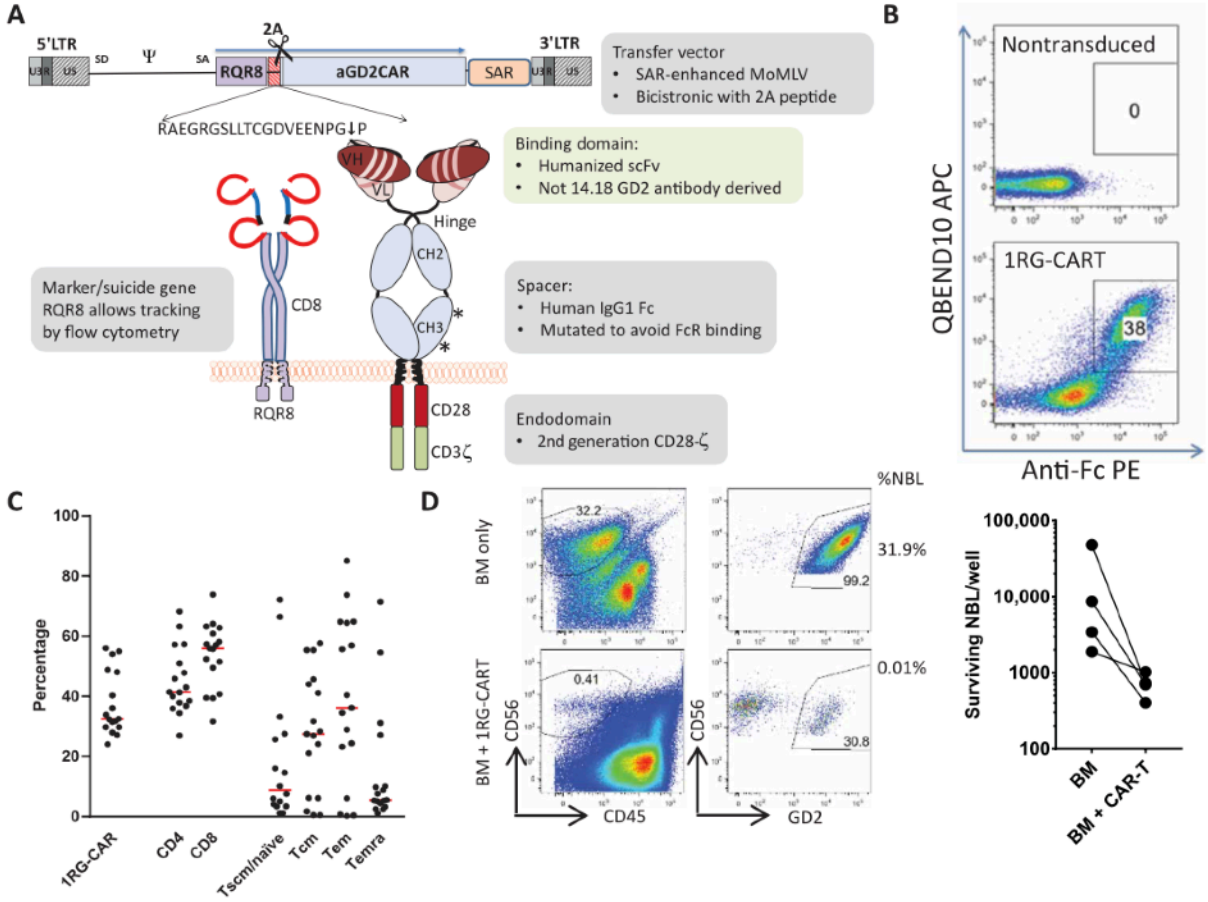


Fig. 1. 1RG-CART design, expression, and function. (**A**) The GD2-CAR (chimeric antigen receptor) consists of the single-chain variable fragment (scFv) of humanized anti-GD2 antibody K666 (*14*), as previously described (*13*). This is connected to the human immunoglobulin IgG1 hinge and Fc regions. To avoid unintended binding of innate immune cells expressing IgG Fc receptors (FcγRs) to CH2CH3, essential binding motifs needed for IgG1-FcγR interaction were mutated (*15*). The extracellular part of the CAR is fused to the transmembrane and the intracellular components of CD28 linked in cis to CD3ζ. RQR8 depicts the sort/suicide component and is coexpressed with GD2-CAR using a foot-and-mouth virus–derived 2A cleavage site. LTR, long terminal repeat; MoMLV, moloney murine leukemia virus-based vector; SAR, scaffold attachment region. (**B**) Coexpression of GD2-CAR and RQR8 is demonstrated in transduced T cells by staining for GD2-CAR using anti-human Fc binding the CH2CH3 spacer and by staining for RQR8 using QBEnd10. (**C**) Shown is the percentage of viable $CD3^+$/1RG-$CART^+$, $CD4^+$, and $CD8^+$ cells as well as the proportion of viable $CD3^+$/1RG-$CART^+$ lymphocytes in different T memory subsets [here defined as $CD45RA^+CCR7^+$, T stem cell memory (Tscm)/naïve; $CD45RA^-CCR7^+$, T central memory (Tcm); $CD45RA^-CCR7^-$, T effector memory (Tem); and $CD45RA^+CCR7^-$, T effector memory reexpressing CD45RA (Temra)]. Shown are data points for each of the 17 1RG-CART cell products manufactured and the median as a horizontal red line. (**D**) Depletion of autologous neuroblastoma (NBL) cells in infiltrated bone marrow (BM) by 1RG-CART cells (example on the left and degree of depletion for each sample on the right).

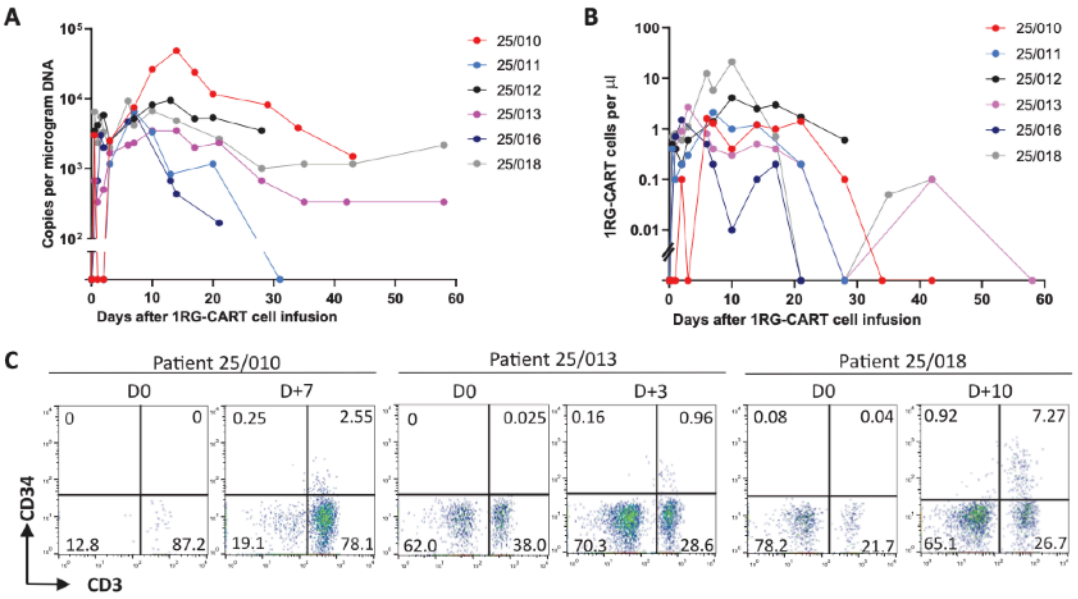


Fig. 2. 1RG-CART in vivo expansion. (**A**) Shown is the detection of 1RG-CART cells by qPCR expressed as copies/µg of DNA at indicated time points after CAR-T cell infusion for the six patients treated on DL4 and DL5. Marking quantification was below the limit of detection for the six patients treated on DL1 to DL3. The value at day +28 for patient 25/011 is 0. (**B**) Shown is the detection of 1RG-CART in peripheral blood by flow cytometry after staining for RQR8 at indicated time points for the six patients treated on DL4 and DL5. (**C**) Shown is the percentage of $CD3^+$/QBEnd10$^+$ cells of $CD45^+$/7-AAD$^-$ cells in peripheral blood at day 0 (D0) and at indicated time points after 1RG-CART administration for patients 25/010, 25/013, and 25/018.

secondary cytopenia was likely, at least in part, due to bone marrow infiltration. In summary, using cell doses of up to $10^9/m^2$, no dose-limiting toxicity, including neurotoxicity, was seen.

Tumor responses are associated with immune activation but are incomplete

Using Response Evaluation Criteria in Solid Tumors (RECIST) (*18*) and immune RECIST (*19*) for soft tissue target lesions and Curie and International Society of Pediatric Oncology European Neuroblastoma Group (SIOPEN) scoring for metastatic deposits identified by metaiodobenzylguanidine (^{123}I-mIBG) scans (*20*), no objective clinical responses were recorded at the day +28 reassessment (table S5, A and B). Three of the six patients treated with CAR-T cell doses of $\geq 10^8/m^2$ in DL4 and DL5 had clinical signs of progressive disease confirmed with MRI and/^{123}I-mIBG scintigraphy at 3 to 4 weeks

1RG-CART activity are described in detail below.

Patient 25/010 was an 8-year-old girl with relapsed metastatic neuroblastoma after four lines of previous treatment. At trial entry, she had widespread bone metastases and extensive bone marrow infiltration. She required opioid analgesia for pain, and her performance score was 60%. She received $1 \times 10^8/m^2$ 1RG-CART cells. On day +5, she developed fever and hypotension requiring fluid boluses and was supported with nasal cannula oxygen. At that time, she had raised C-reactive protein (CRP) with serum interleukin-6 (IL-6) and IL-10 concentrations in keeping with grade 3 CRS. These symptoms resolved after a single dose of tocilizumab (Fig. 3A). From day +7, she developed weight gain, tender hepatomegaly, and ascites. She had a low serum albumin, coagulopathy, and raised soluble CD25 [16,100 pg/ml (normal, <2500)], raised triglycerides (1.75 mM), and raised ferritin (3724 µg/ml; peak, 17,208 µg/ml) (Fig. 3B). These symptoms of sustained immune activation resolved by day +22 with supportive care including strict fluid management, 20% albumin infusion, and spironolactone. On day +21, she had an episode of acute back pain. Biochemical analysis of blood showed changes consistent with tumor lysis: raised potassium and phosphate serum concentrations and a peak lactate dehydrogenase (LDH) of 4017 IU/liter (Fig. 3C). Supportive care was provided, including hydration and salbutamol to treat hyperkalemia. Her symptoms resolved by day +24, and all analgesia was stopped. As per standard management of febrile neutropenia, regular blood cultures were taken; with the exception of a single blood culture taken on day +10, these showed no growth throughout the period to day +28. At disease reassessment on day +28, her performance status had markedly improved to 90%. Histological assessment of the bone marrow, which, at baseline, was heavily infiltrated with neuroblastoma, showed extensive tumor

Summary

CAR-T cells

- T cells transduced with tumor-specific Chimeric Antigen Receptor (CAR)
- Tumor recognition independent of HLA (no HLA typing needed)
- Target: variety of tumor antigens (protein, carbohydrate, glycolipid)
- High response rate (up to 88%): pre-clinical and clinical findings

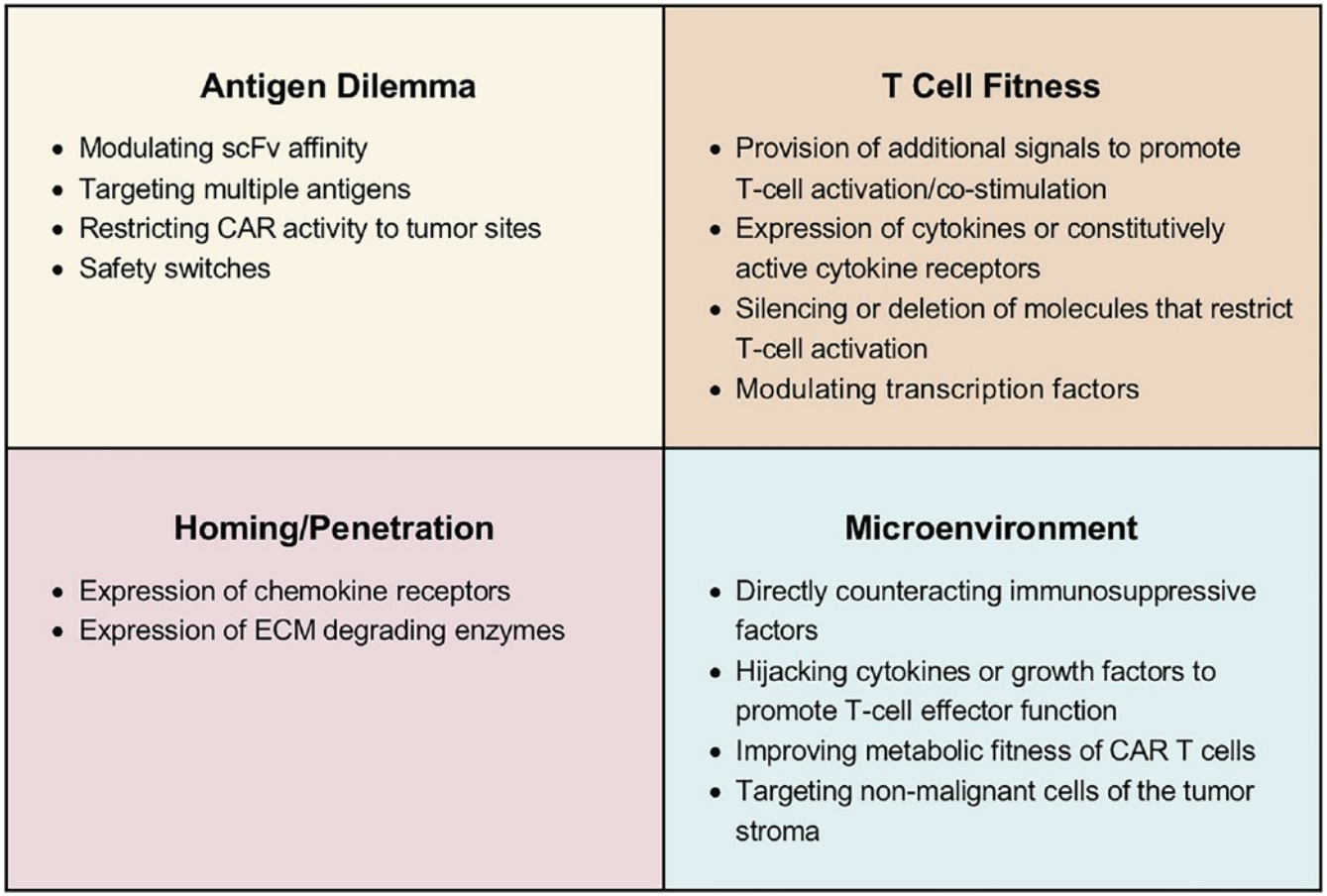
Limitation of CAR-T cells

- Toxicities
  - On target/off tumor toxicities
  - Cytokine syndrome
- Tumor microenvironment
  - Presence of MDSCs & Treg in tumor
  - Immunosuppressive agents

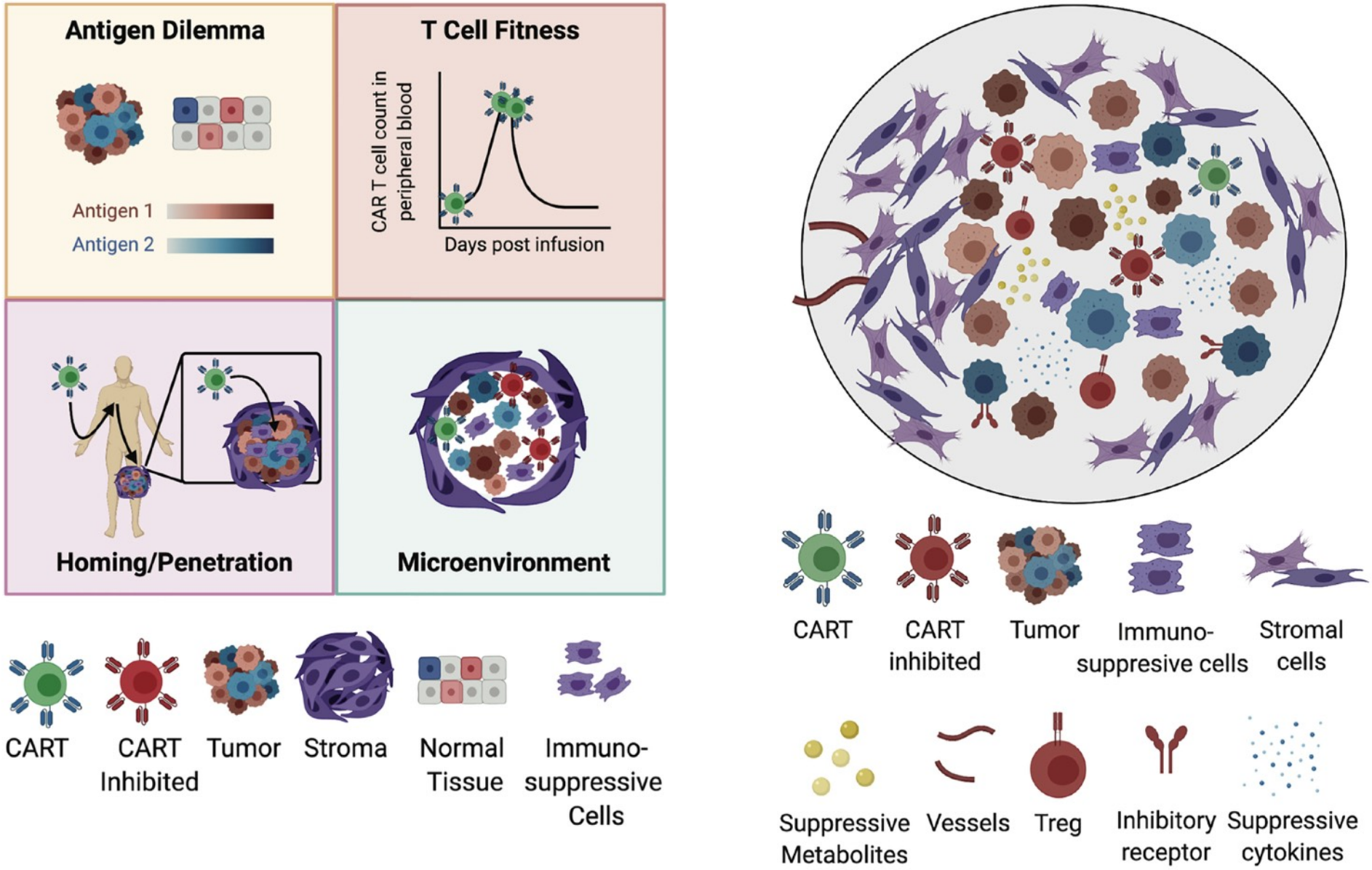
The hostile tumor microenvironment

The tumor microenvironment contains multiple inhibitory factors designed to potentially suppress effector T cells.

- $CD4^{+}$ $CD25^{hi}$ $FoxP3^{+}$ regulatory T cells (Tregs)
- MDSCs
- TAMs
- Expression of inhibitory ligands by tumor (PD-L1)
- Tumor secretion of T cell suppressive cytokines (TGF-β and IL-10)



Molecular Therapy 2020 282320-2339; DOI: (10.1016/j.ymthe.2020.09.015)



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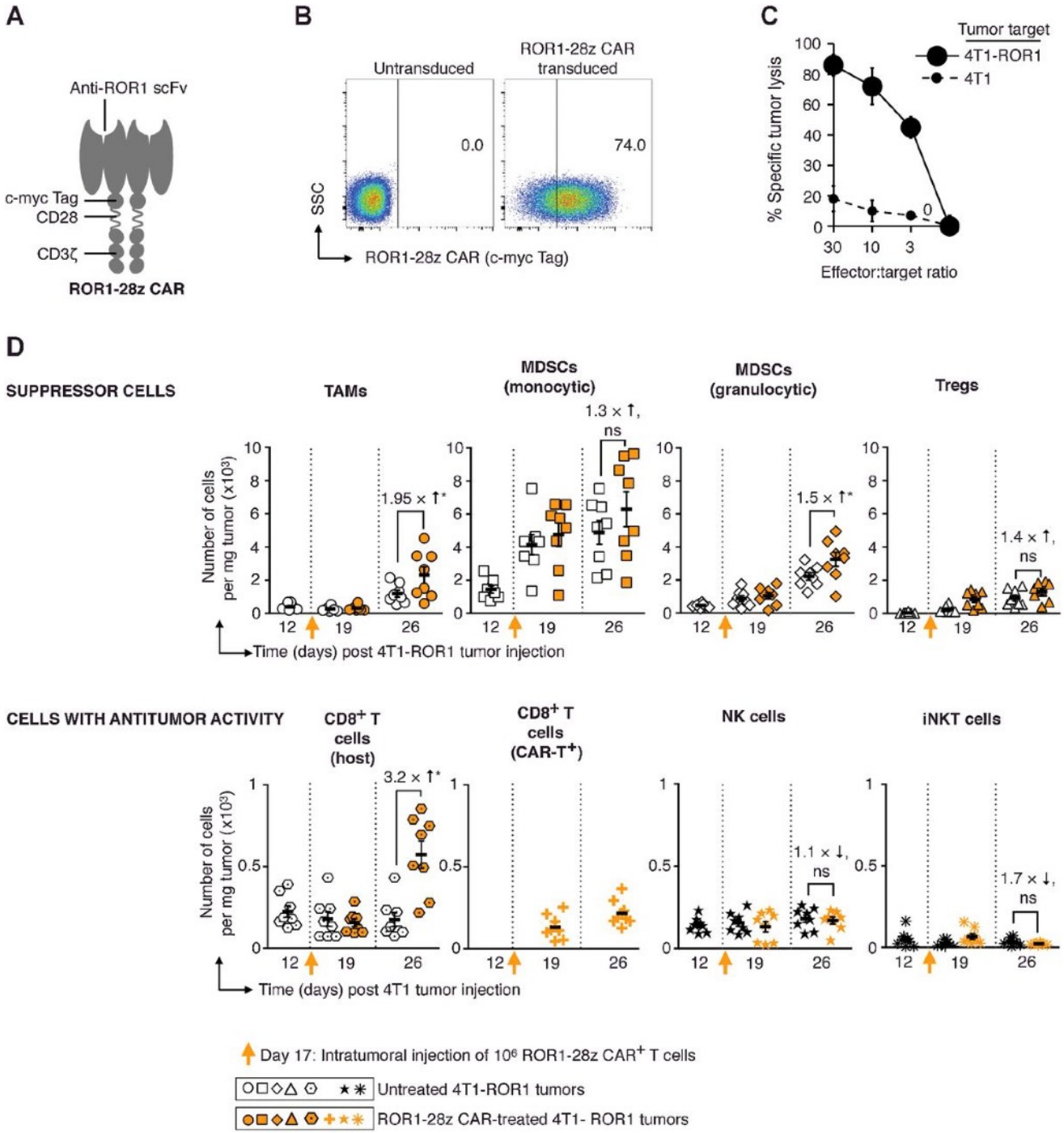

Fan Zhang et al.
Cancer Res 2018;78:3718-3730

REVIEW ARTICLE

blood advances

Next-generation cell therapies: the emerging role of CAR-NK cells

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T cells engineered with chimeric antigen receptors (CARs) have revolutionized the field of cell therapy and changed the paradigm of treatment for many patients with relapsed or refractory B-cell malignancies. Despite this progress, there are limitations to CAR-T cell therapy in both the autologous and allogeneic settings, including practical, logistical, and toxicity issues. Given these concerns, there is a rapidly growing interest in natural killer cells as alternative vehicles for CAR engineering, given their unique biological features and their established safety profile in the allogeneic setting. Other immune effector cells, such as invariant natural killer T cells, γδ T cells, and macrophages, are attracting interest as well and eventually may be added to the repertoire of engineered cell therapies against cancer. The pace of these developments will undoubtedly benefit from multiple innovative technologies, such as the CRISPR-Cas gene editing system, which offers great potential to enhance the natural ability of immune effector cells to eliminate refractory cancers.