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

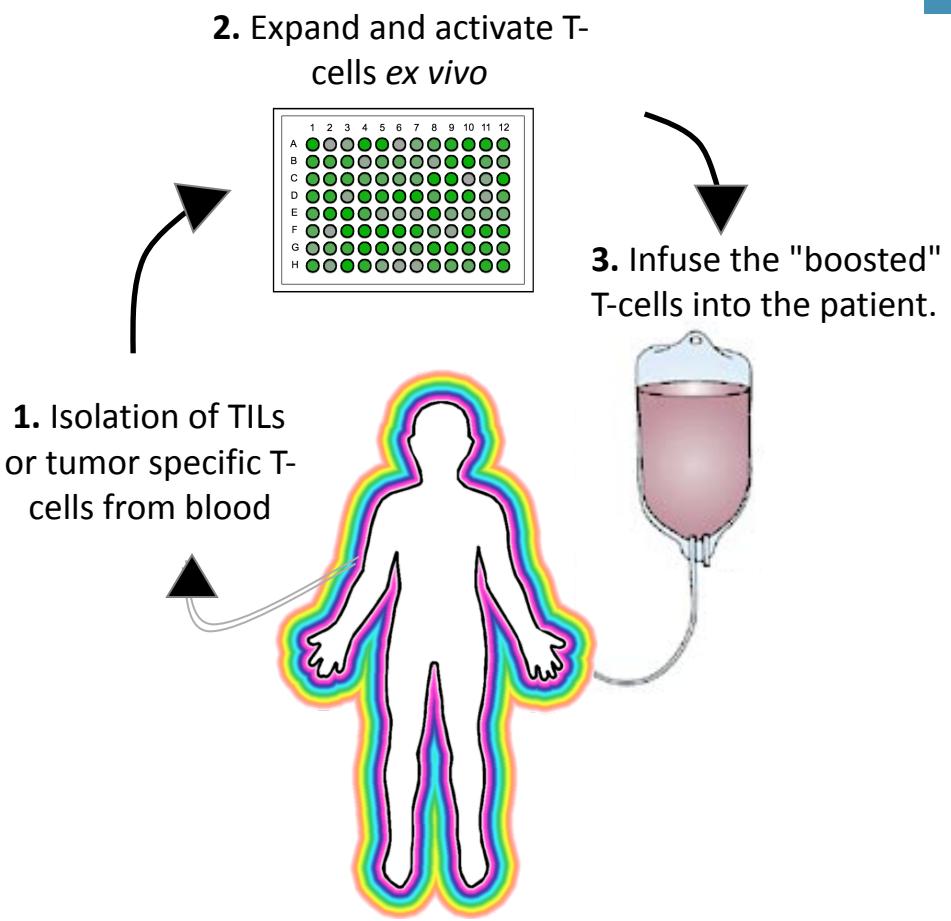
- **TIL** (Tumor infiltration T-lymphocytes therapy)

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).**

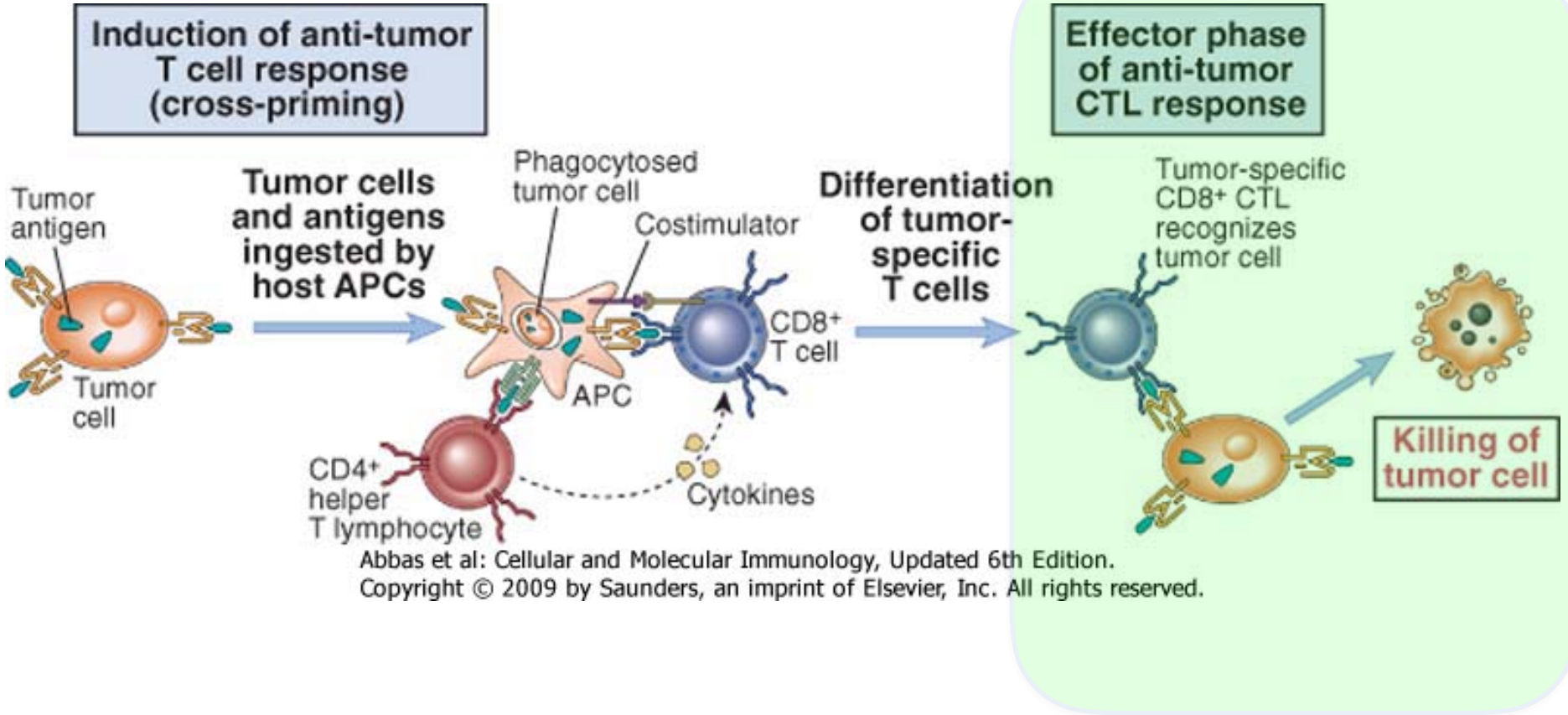
TIL

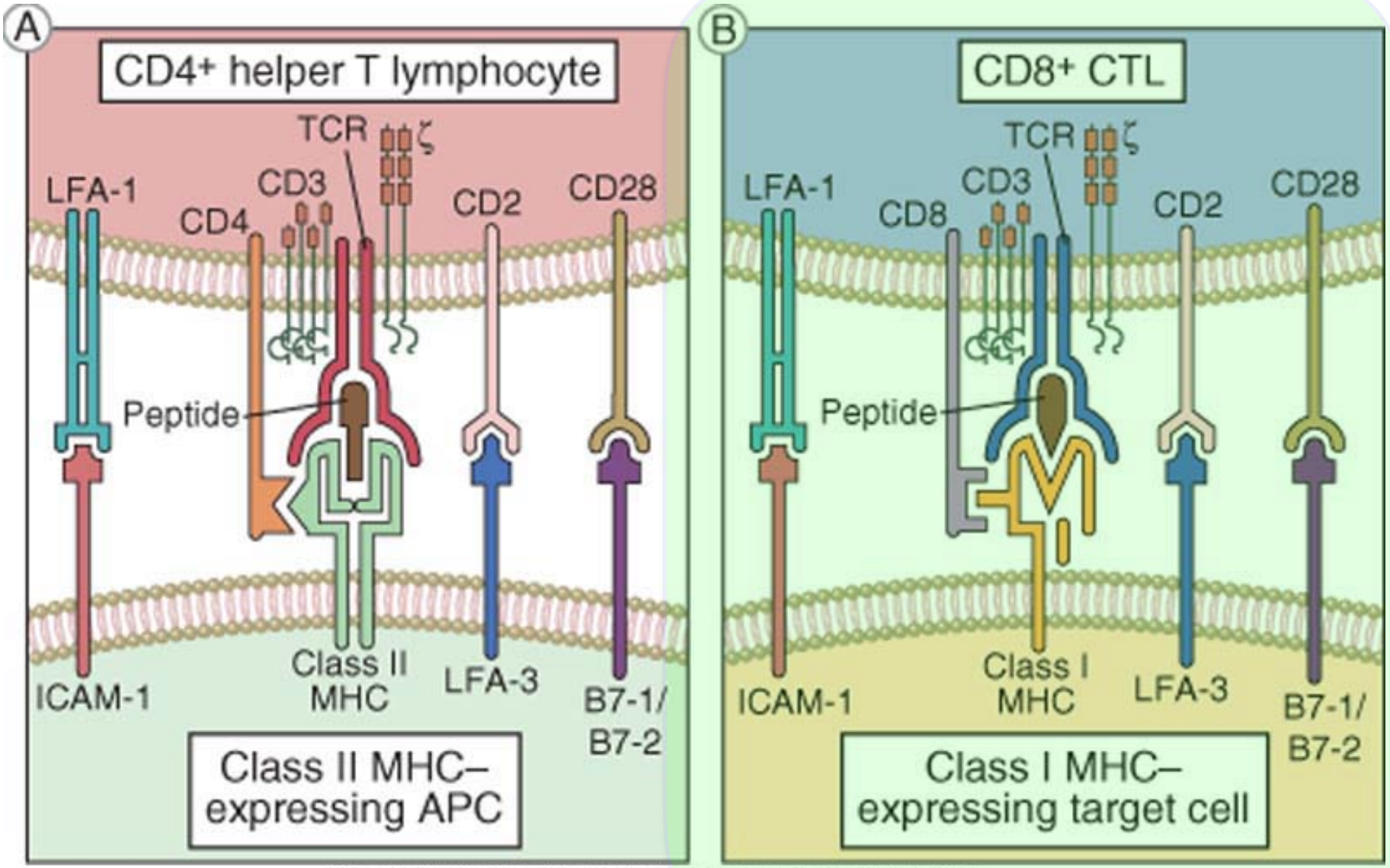


- **Target therapy with Tumor specific T cells**
  - Cancer: Melanoma
  - Autologous tumor infiltrating lymphocytes (TILs); “Live drug”
- **Advantages**
  - High response rate (>50%),
  - Long-term remission,
  - Less toxic & gentler to the patient
- **Limitation:**
  - Extraction of TILs,
  - Cell manufacturing

Rosenberg SA & Dudley ME 2009 Current Opinion of Immunology

Induction of a T-response against tumor



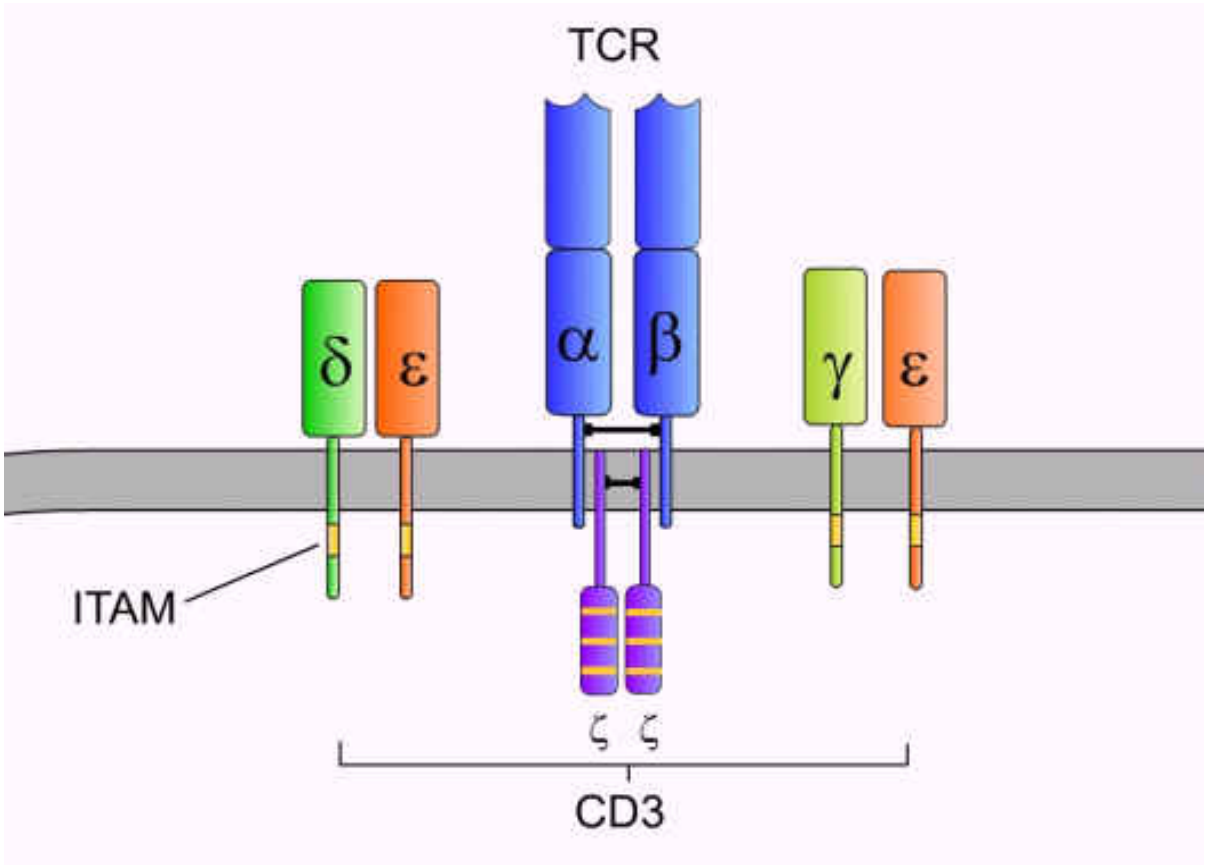


Low number of TIL

Adaptive Cell Transfer Therapy

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

TCR



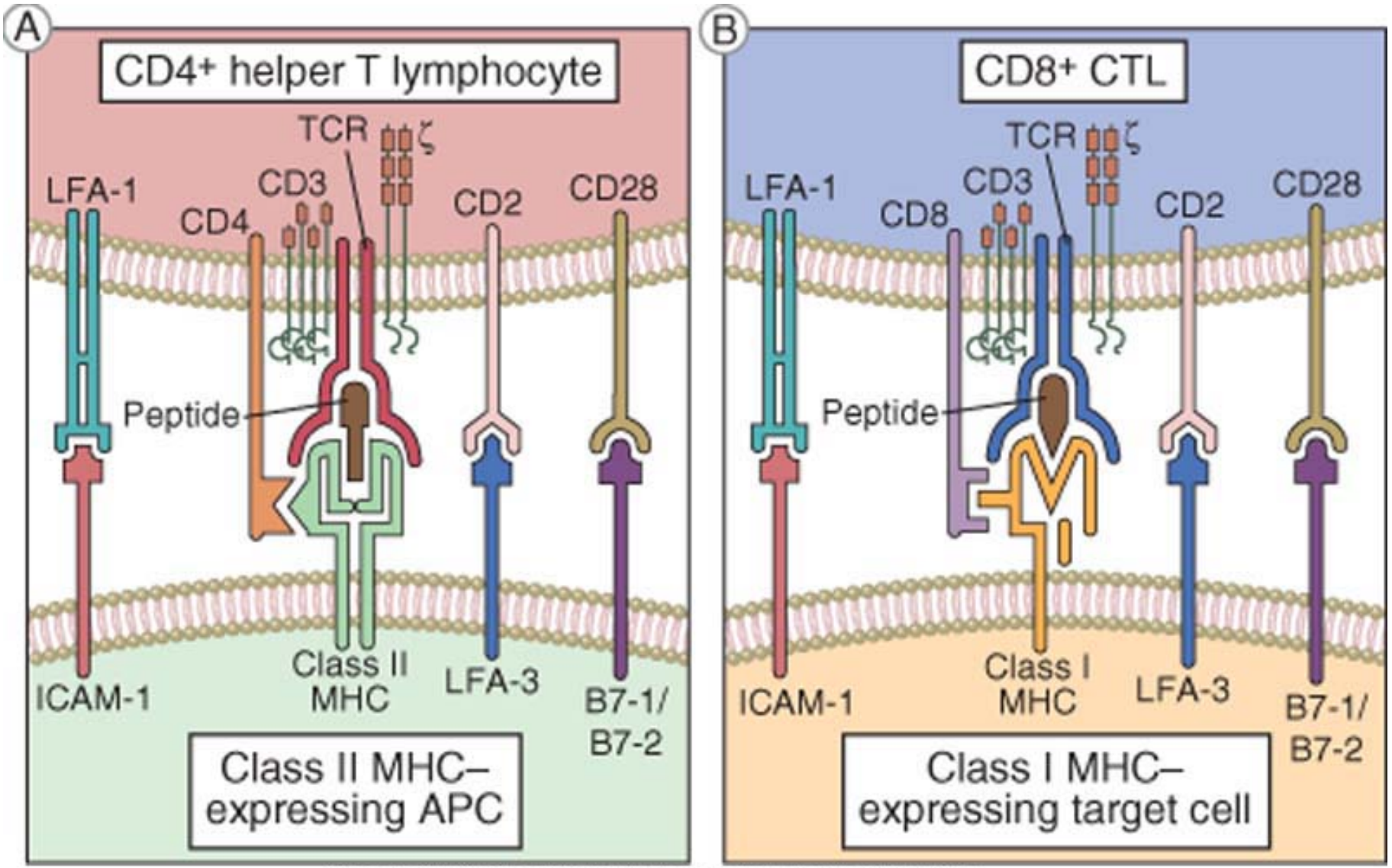
TCR complex :TCR, CD3, ζ

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.

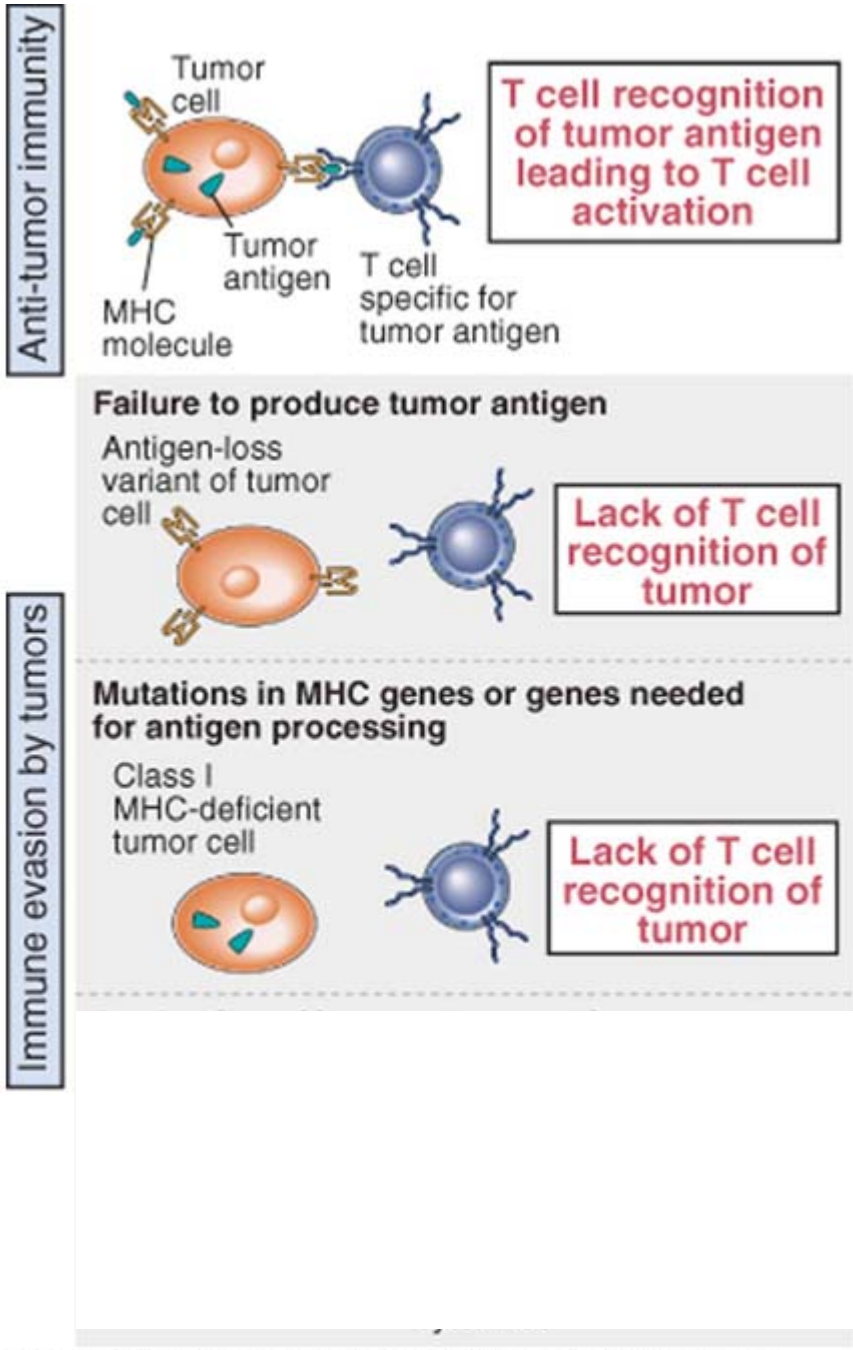
Morgan, R. A.et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. **Science 314, 126–129 (2006).**



HLA-restricted response – tumor escape

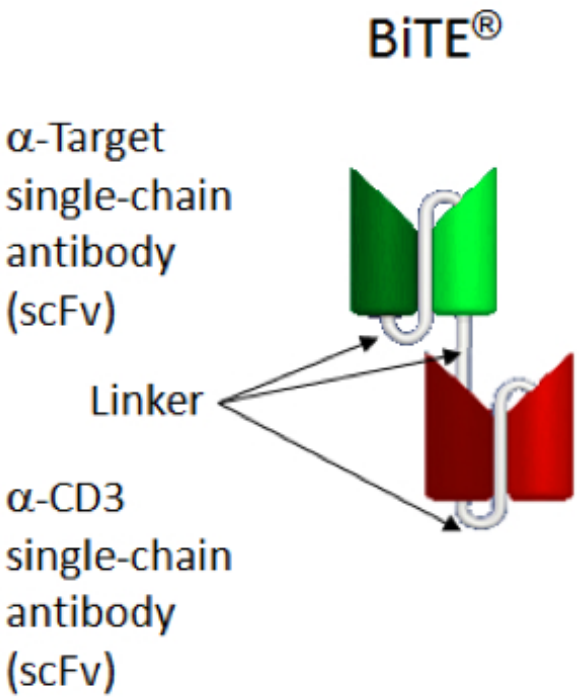
Tumor escape (4)

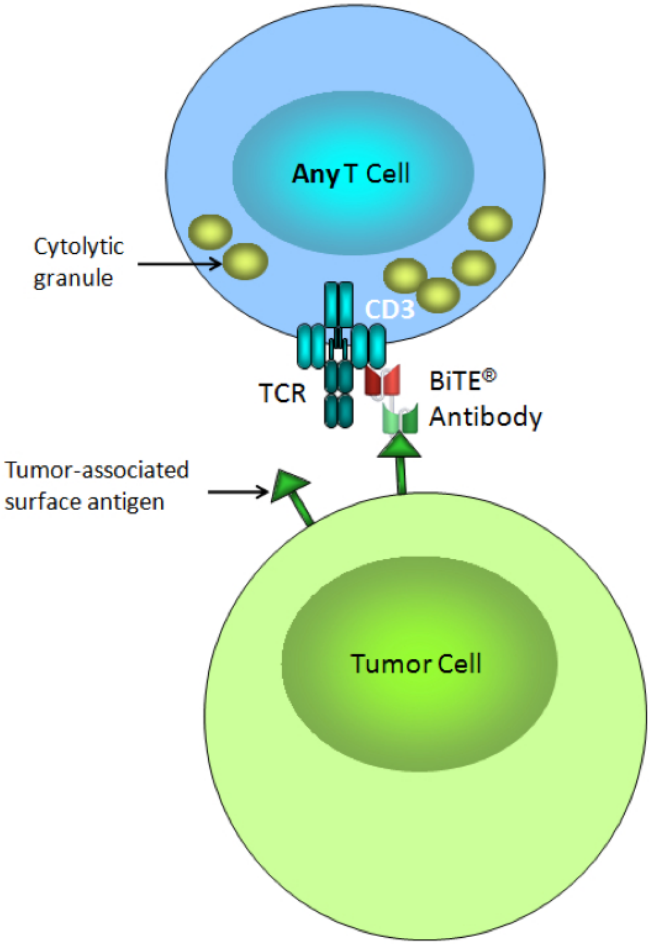
- Some tumor cells reduce the expression of MHC I



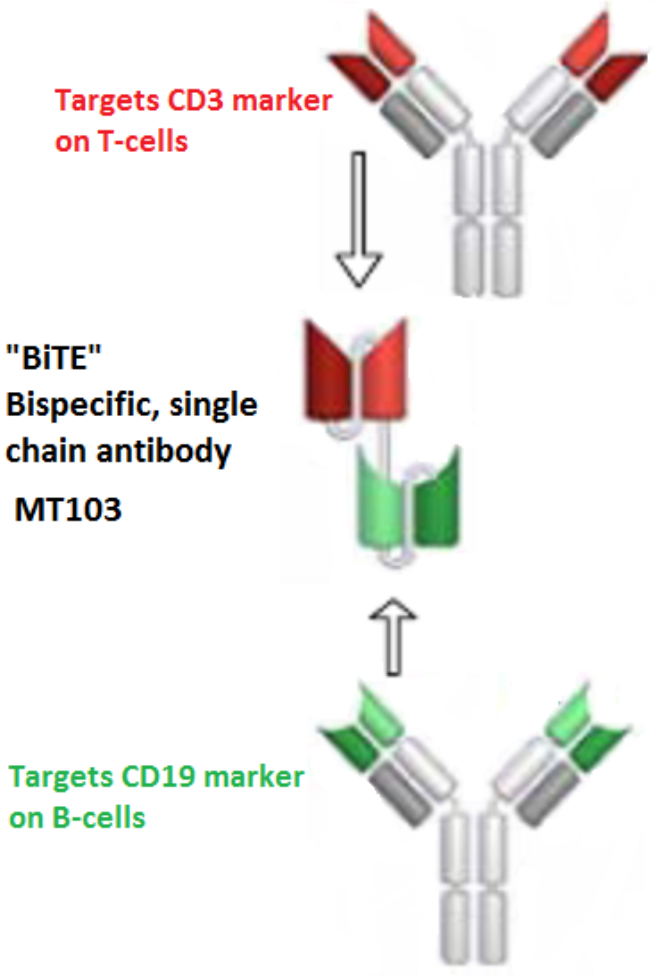
Any possibility to design an immunotherapeutic approach able to work independently from class I MHC?

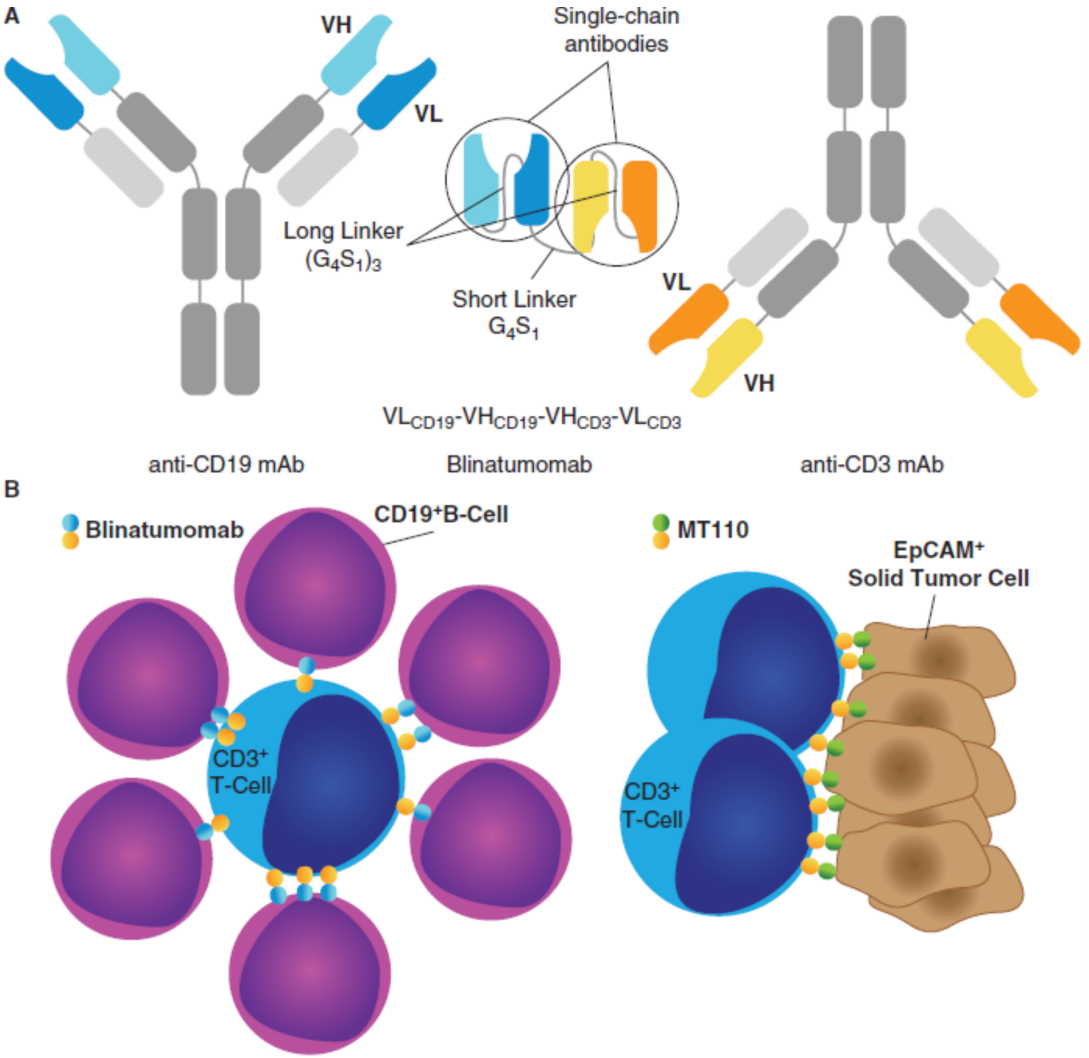
Bispecific T-Cell Engaging (BiTE) Antibody





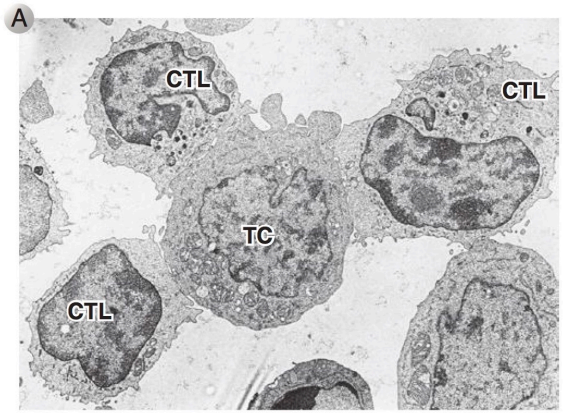
Blinatumumab

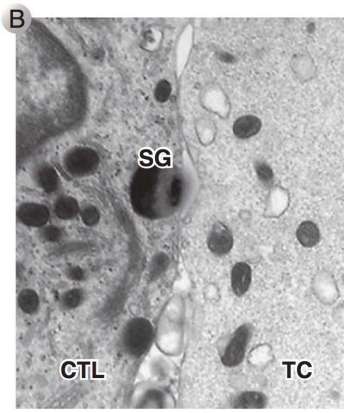


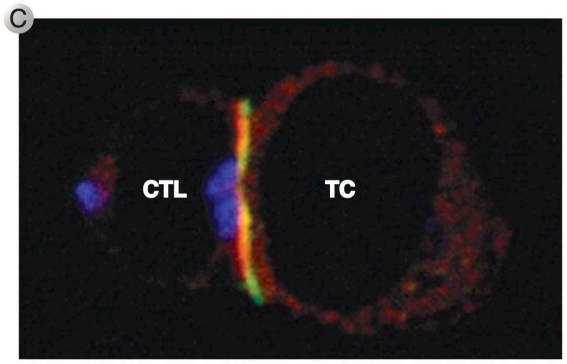


Structure and function of blinatumomab. A. The structural features of blinatumomab (MT103, AMG103) arise from monoclonal antibodies (mAbs) directed against CD19 and CD3. Single-chain antibodies are constructed from the light and heavy variable immunoglobulin domains (VL and VH) for each protein and connected 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]).

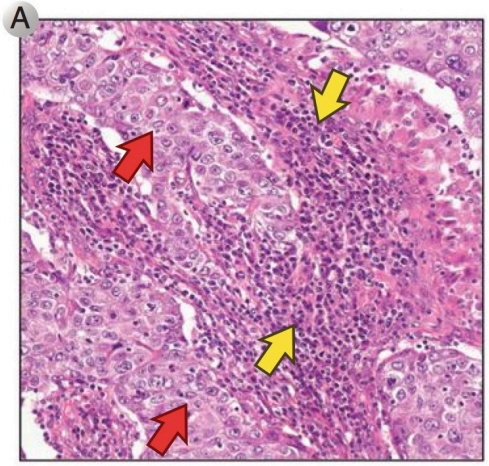
Induction of a T-response against tumor

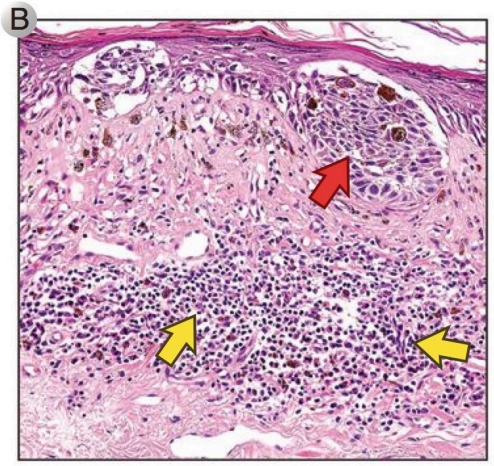


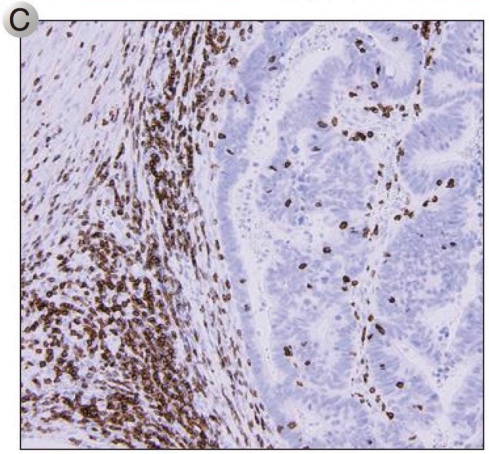


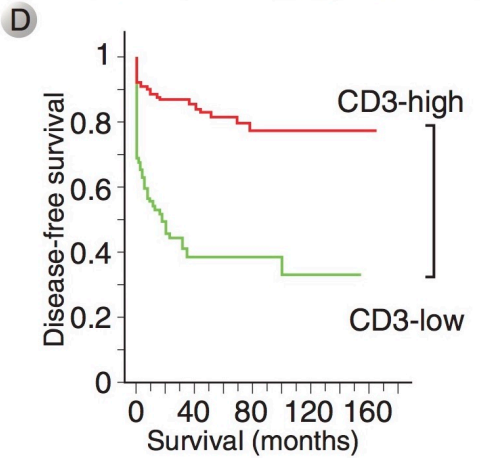


Cathepsins (blue) LFA-1 (green) Talin (red)









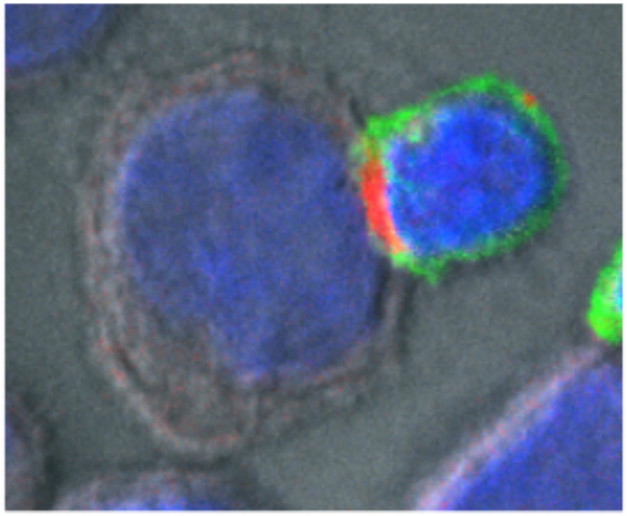


Figure 1C. Confocal microscope image displaying a cytolytic synapse between a tumor cell (with large Hoechst stained nucleus) and T cell (with small Hoechst stained nucleus and green signal recognizing CD45) in the presence of BiTE. Cytolytic synapse represented by activated PKCθ labeling (red) on T 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**

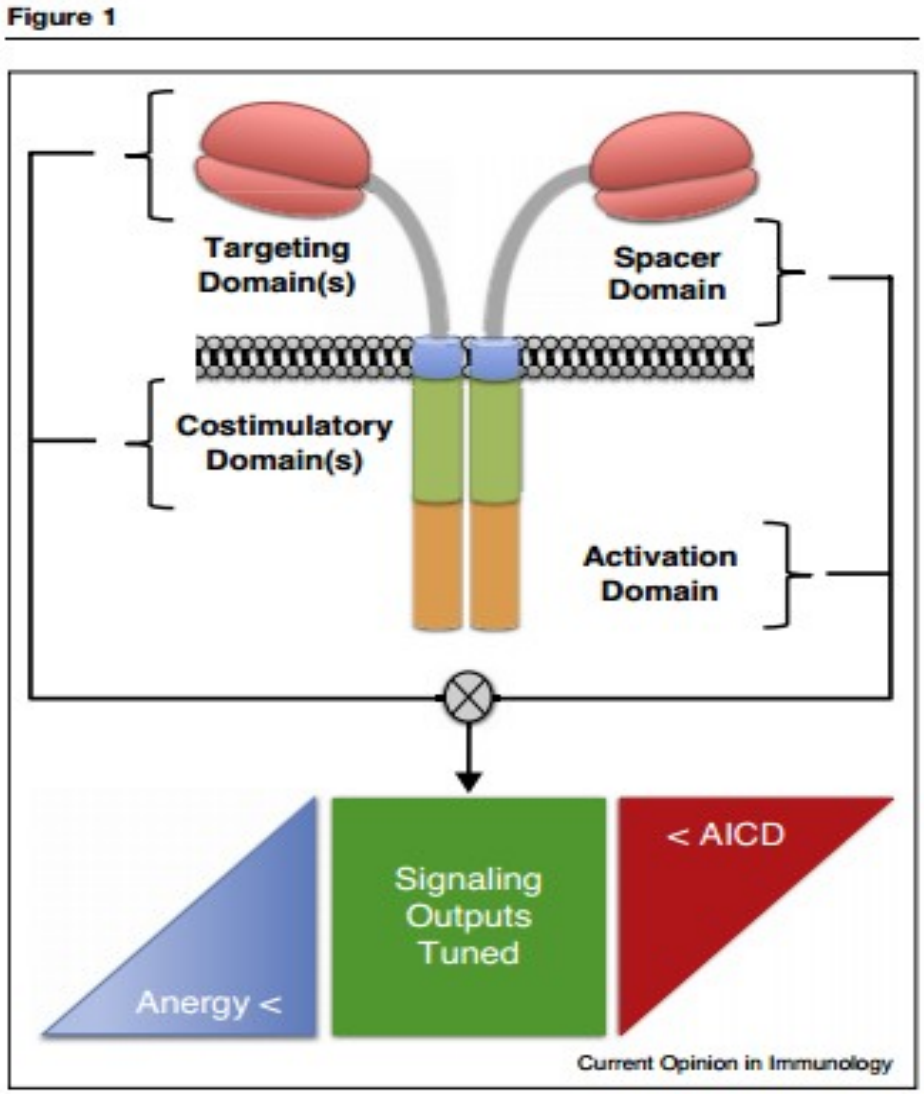
Adaptive Cell Transfer Therapy

- **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



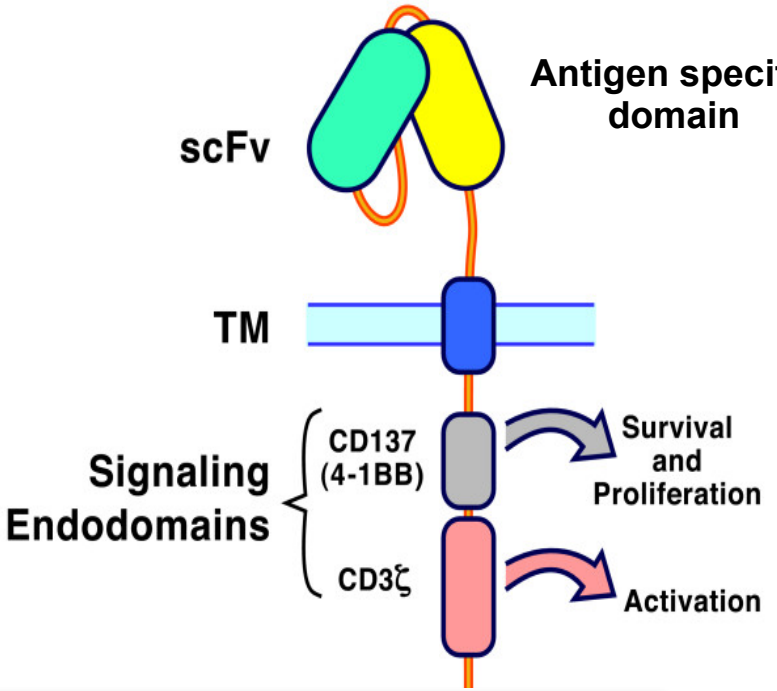
CARs consist of fusion molecules and are typically comprised of:

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



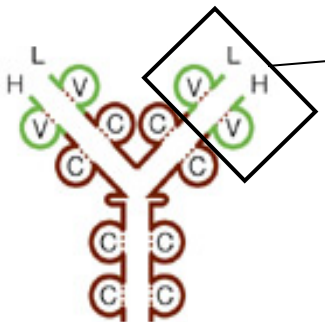
Advantages of CAR T cells

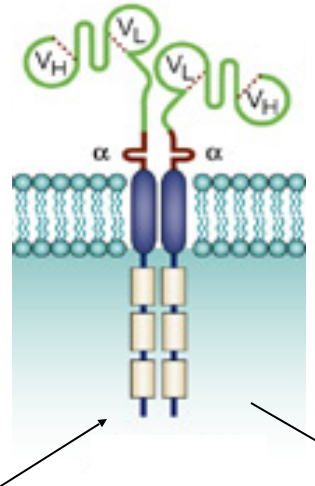
"Live drug"	Tumor recognition independent of HLA (no HLA typing needed)	Multiple anti-tumor immuno-modulators can be engineered	Target variety of antigens (self-protein, carbohydrate, glycolipid)

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

Generation of a tumor targeted chimeric antigen receptor (CAR)

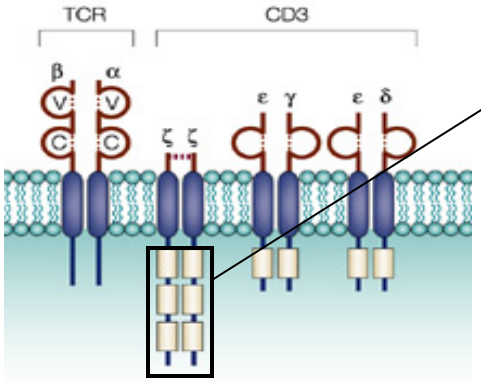
α-TAA mAb

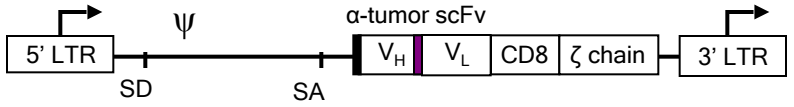




α-TAA scFv—CD8-ζ

TCR complex



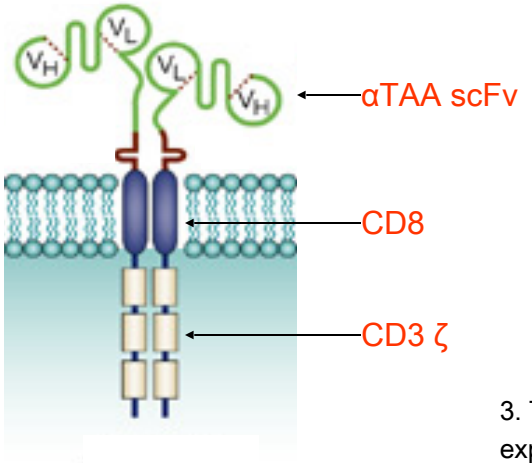


CAR retroviral vector

(??why a retrovirus??)

Generation of TAA-targeted T cells for treatment of Cancer

1. Construct a chimeric antigen receptor (CAR)

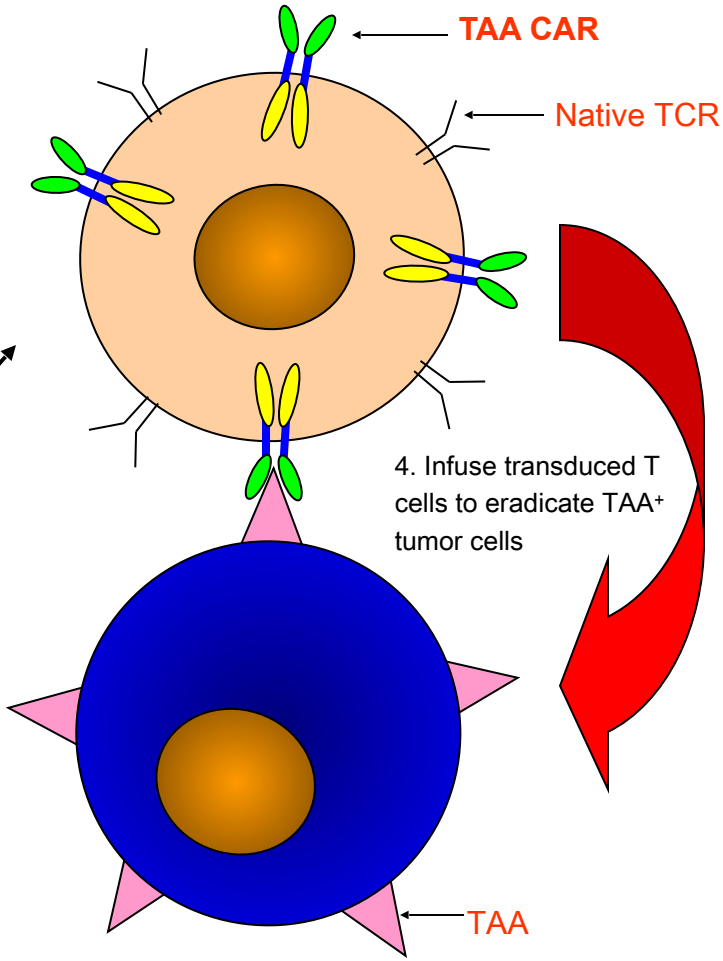


TAA CAR

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

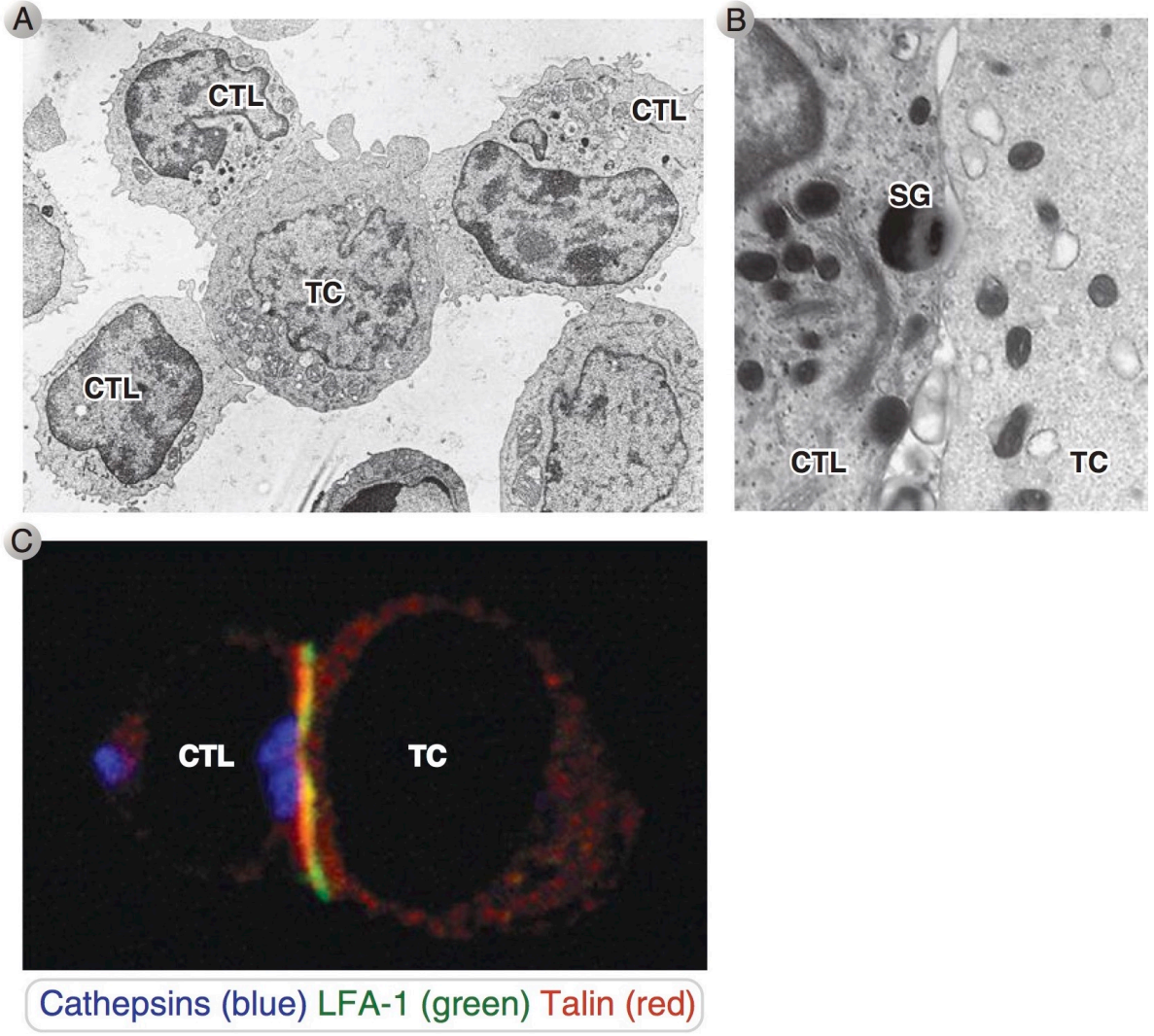
SFG-CAR

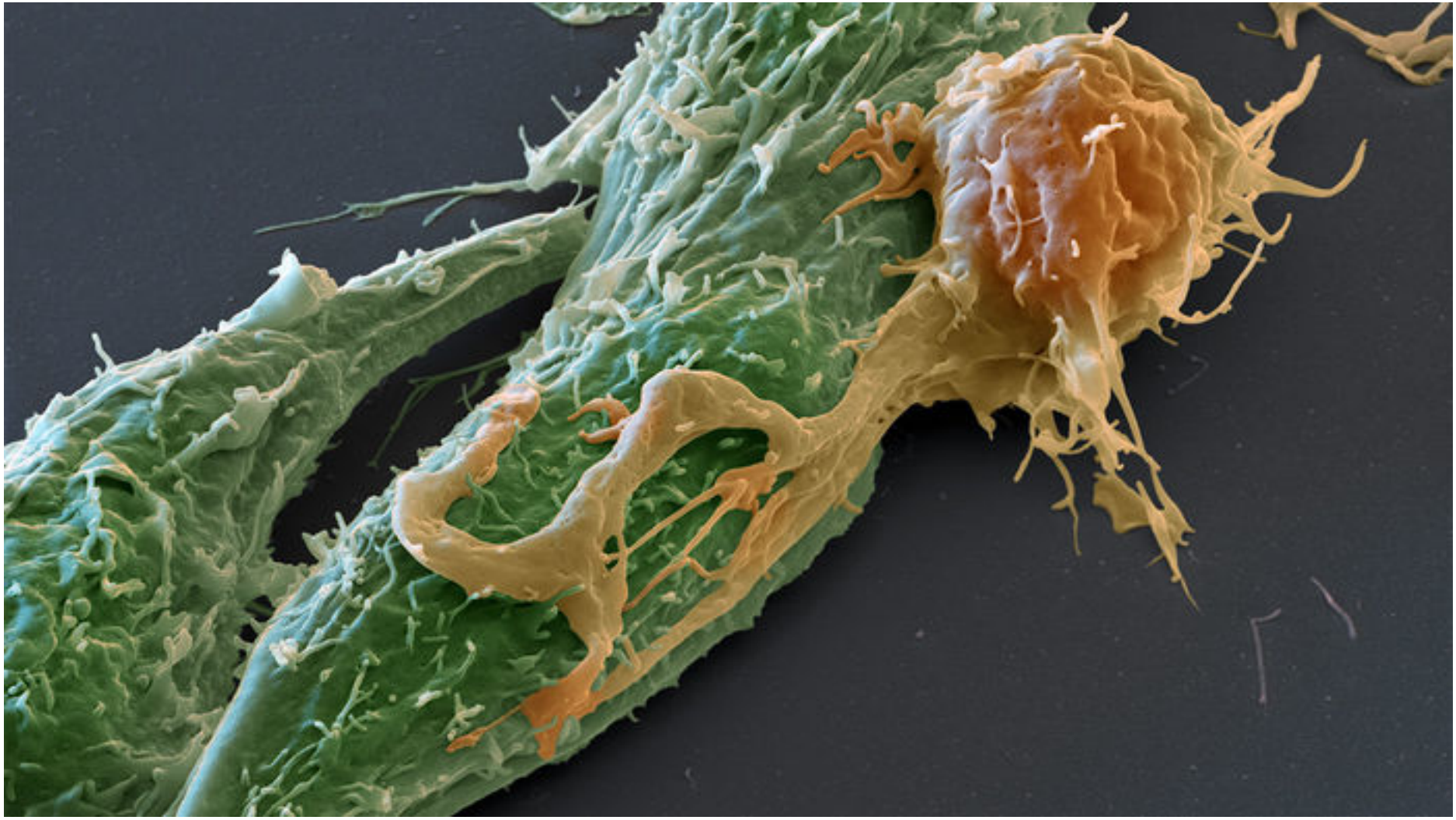
3. Transduce and expand patient T cells ex vivo

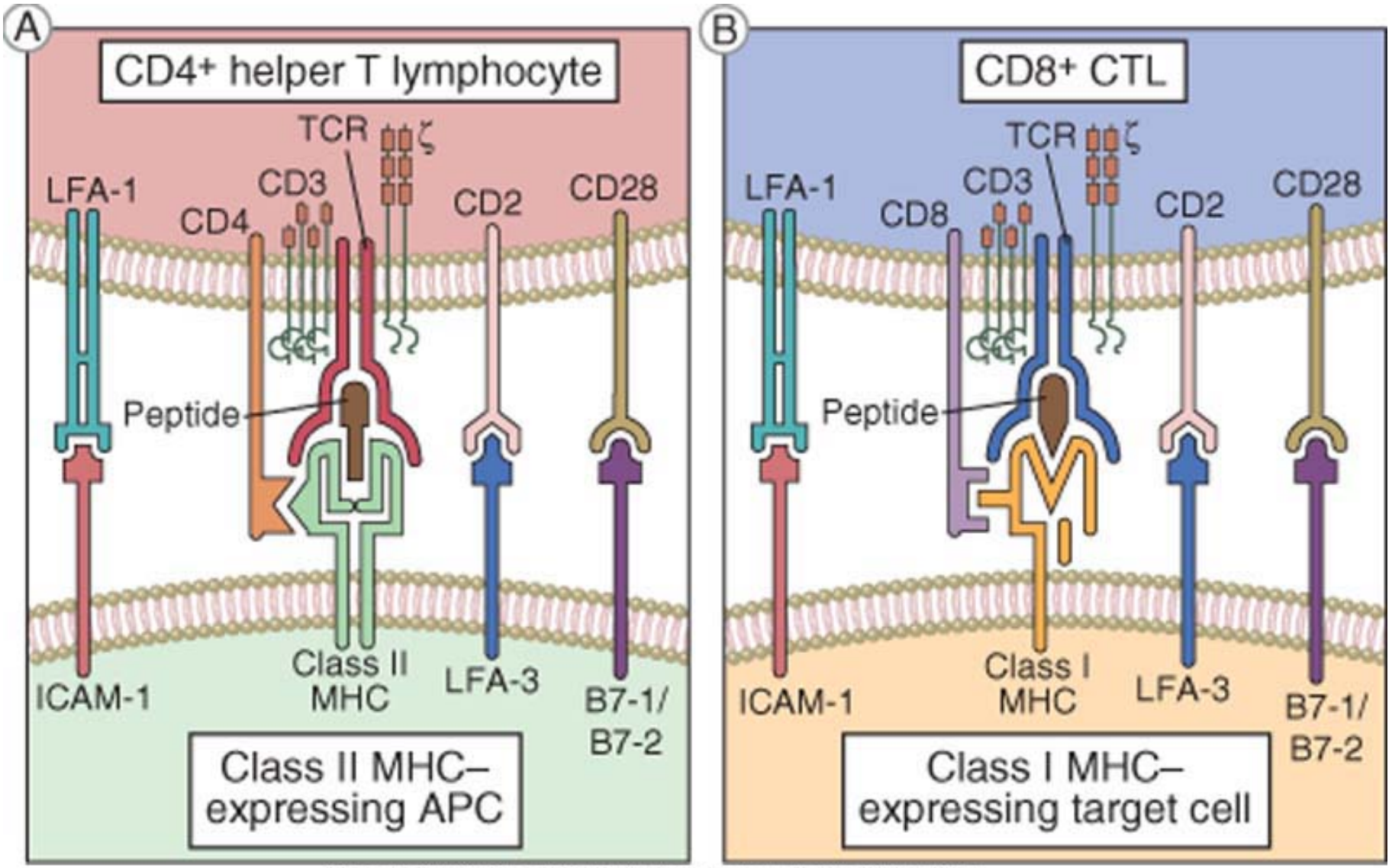


4. Infuse transduced T cells to eradicate TAA$^+$ tumor cells

Induction of a T-response against tumor







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

Challenges of CAR-T

* **Target selection**

Clinical significance of CAR-T cells

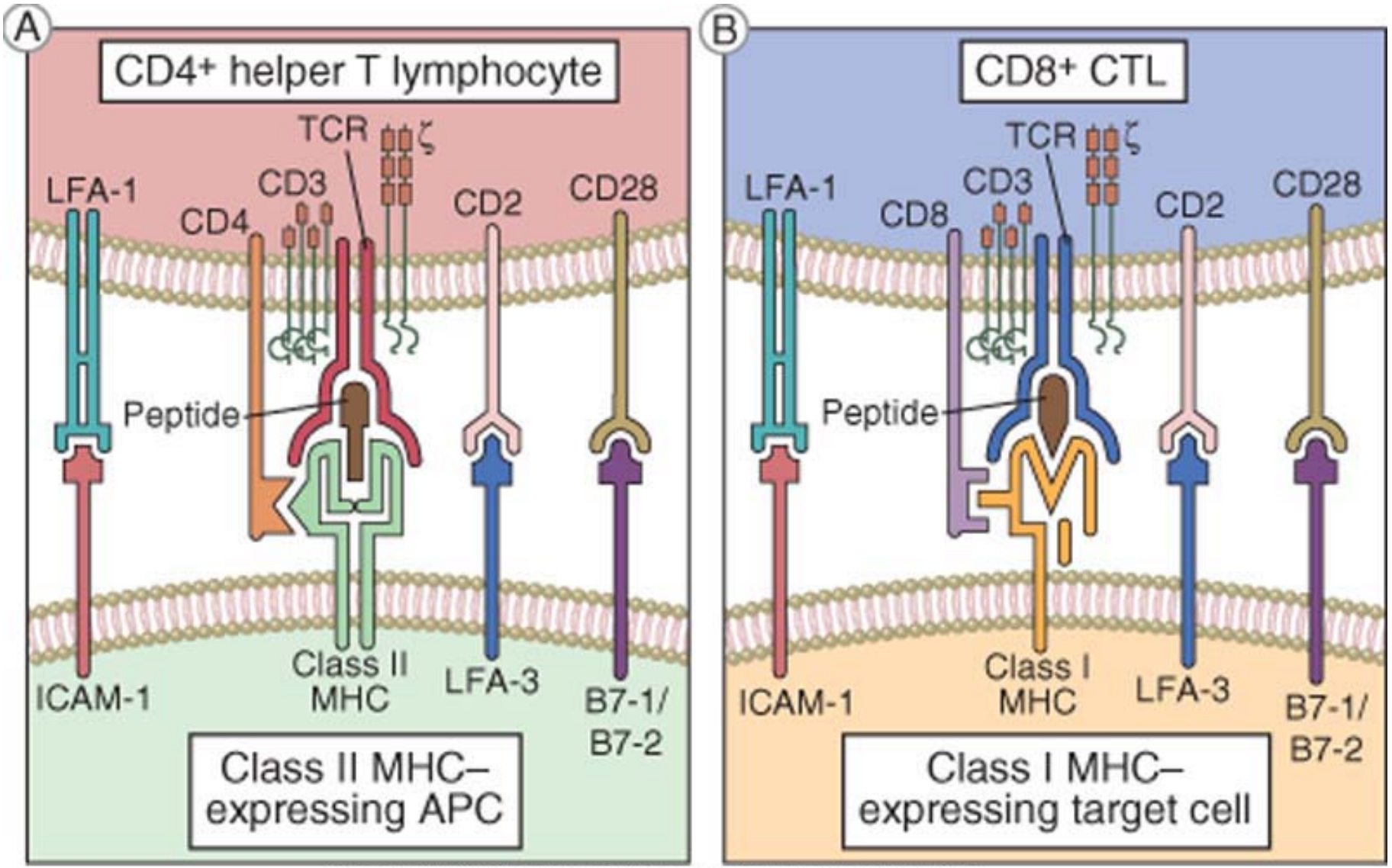
Target	CAR	Cancer	Objective response
CD19	CAR:CD28-CD3ζ	Lymphoma and CLL	N=7: 1CR, 5 PR & 1SD
	CAR:CD137-CD3ζ	ALL	2CR
	CAR:CD28-CD3ζ	ALL	5CR
CD20	CAR:CD137-CD28-CD3ζ	NHL	N=3: 1PR, 2NED
CEA	CAR-CD3ζ (1st gen)	Colorectal & breast	N=7: minor responses in two patients
GD2	CAR-CD3ζ (1st gen)	Neuroblastoma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

Kershaw et. al. Nature Reviews Cancer, 2013

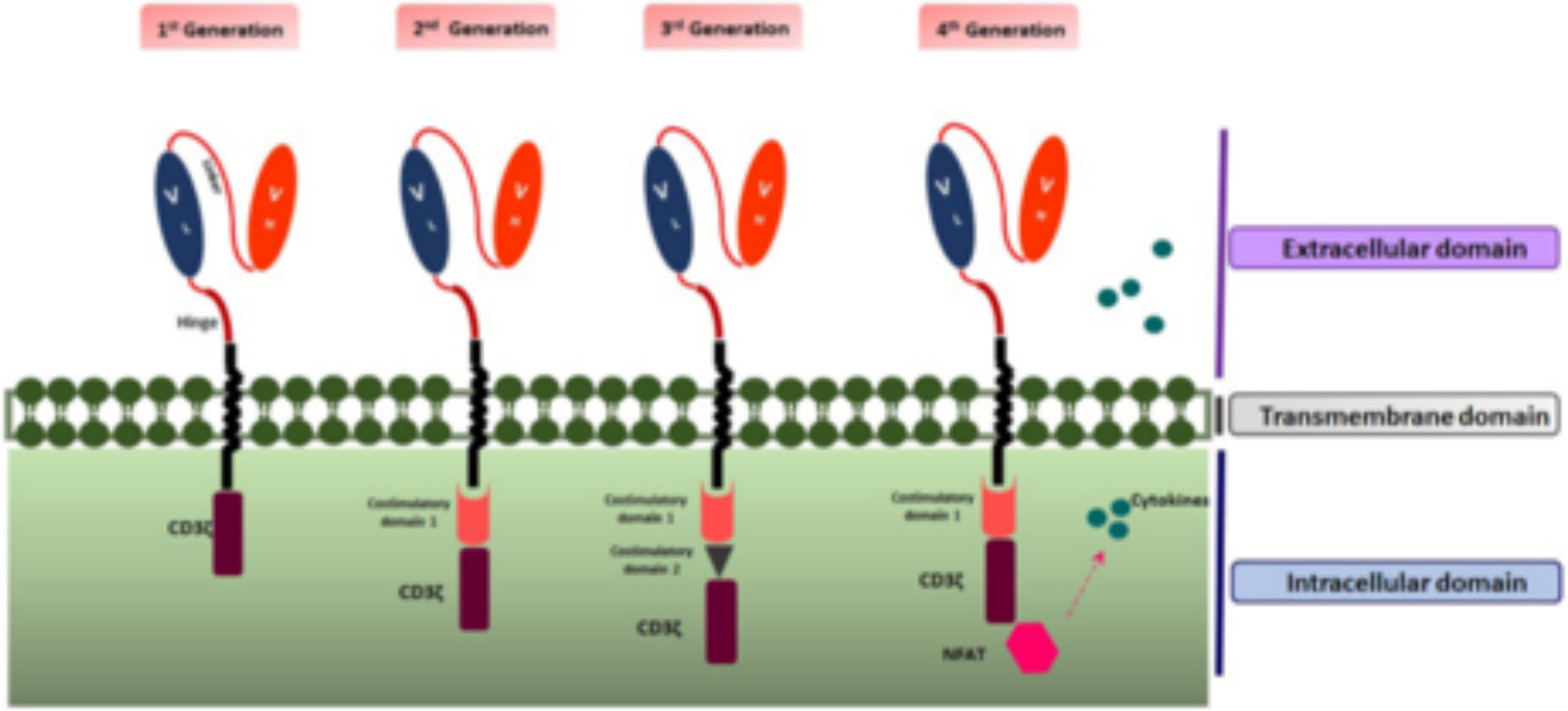
Challenges of CAR-T

* Target selection

* **Optimize co-stimulatory signaling of T cell effector functions**

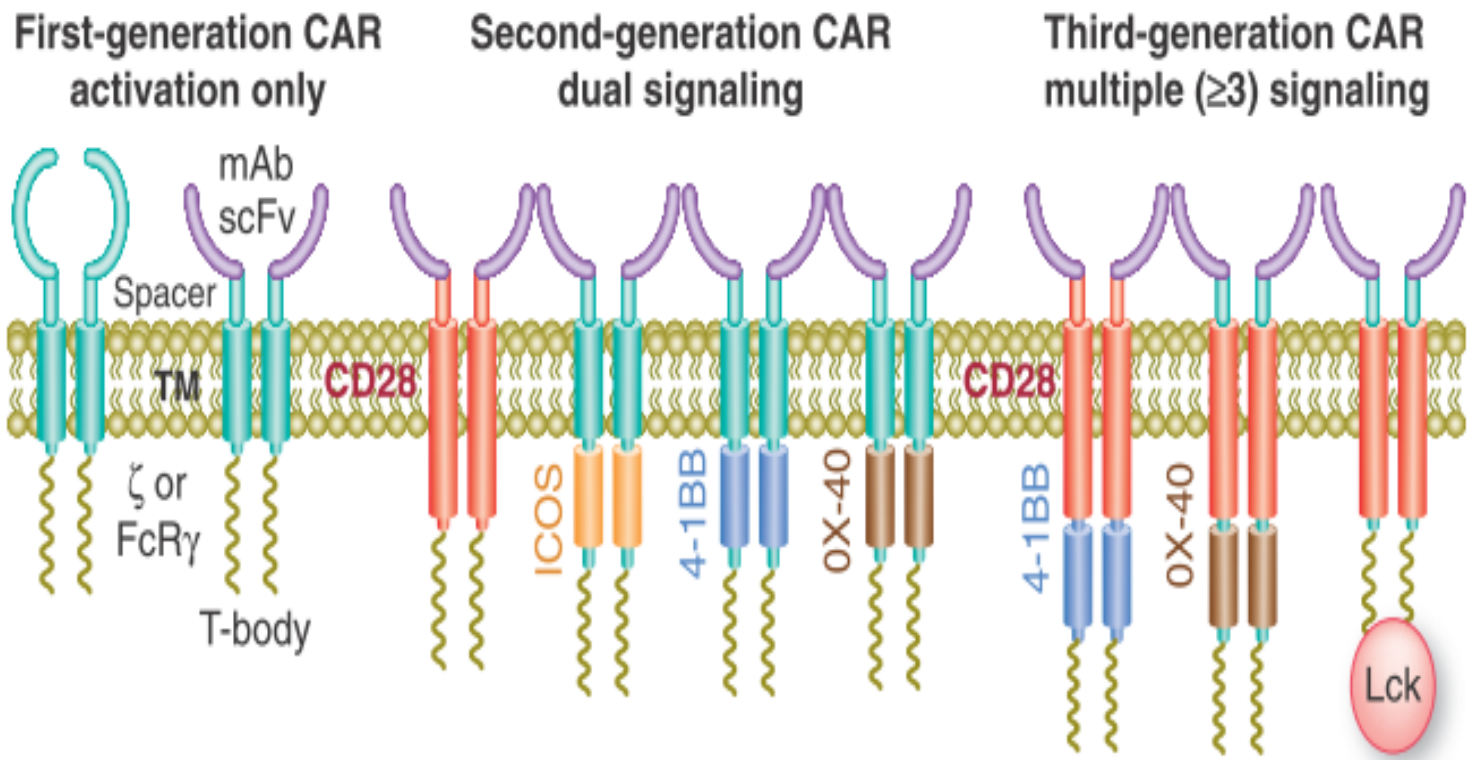


Evolution in CAR design



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, is under development
- Third-generation CARs seem to have improved proliferation, cytokine secretion and a better persistence in circulation

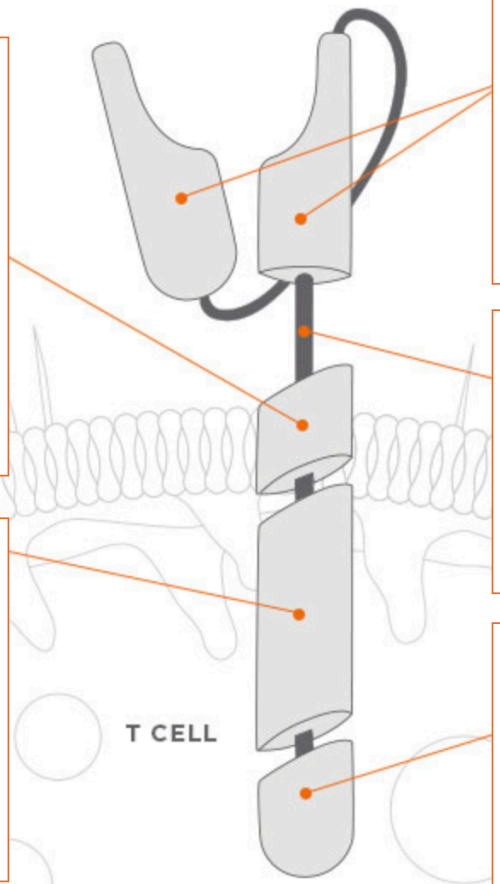
- **Unfortunately, this last generation of CARs may also be dangerous and the activation can be too strong leading to cytokine storm and eventually to death**

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) +

Challenges of CAR-T

* Target selection

* Optimize co-stimulatory signaling of T cell effector functions

* **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

- **Cytokine syndrome**
  - Elevated levels of pro-inflammatory cytokines
    - Treatable by anti-IL-6mAb and steroid

- **Tumor lysis syndrome**

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

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

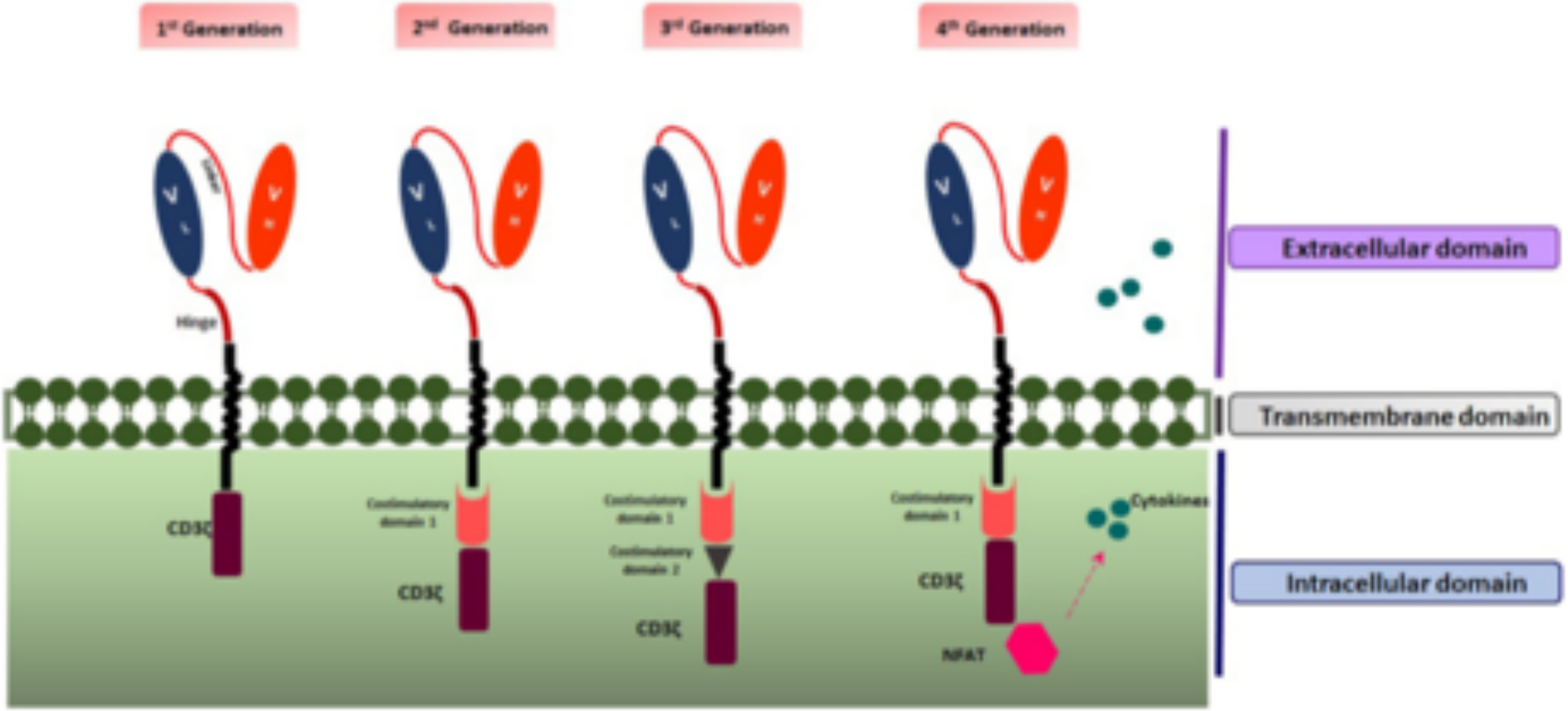
- Toxicities
  - On target/off tumor toxicities
  - Cytokine syndrome
- Tumor microenvironment
  - Presence of MDSCs & Treg in tumor
  - Immunosuppressive agents

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)

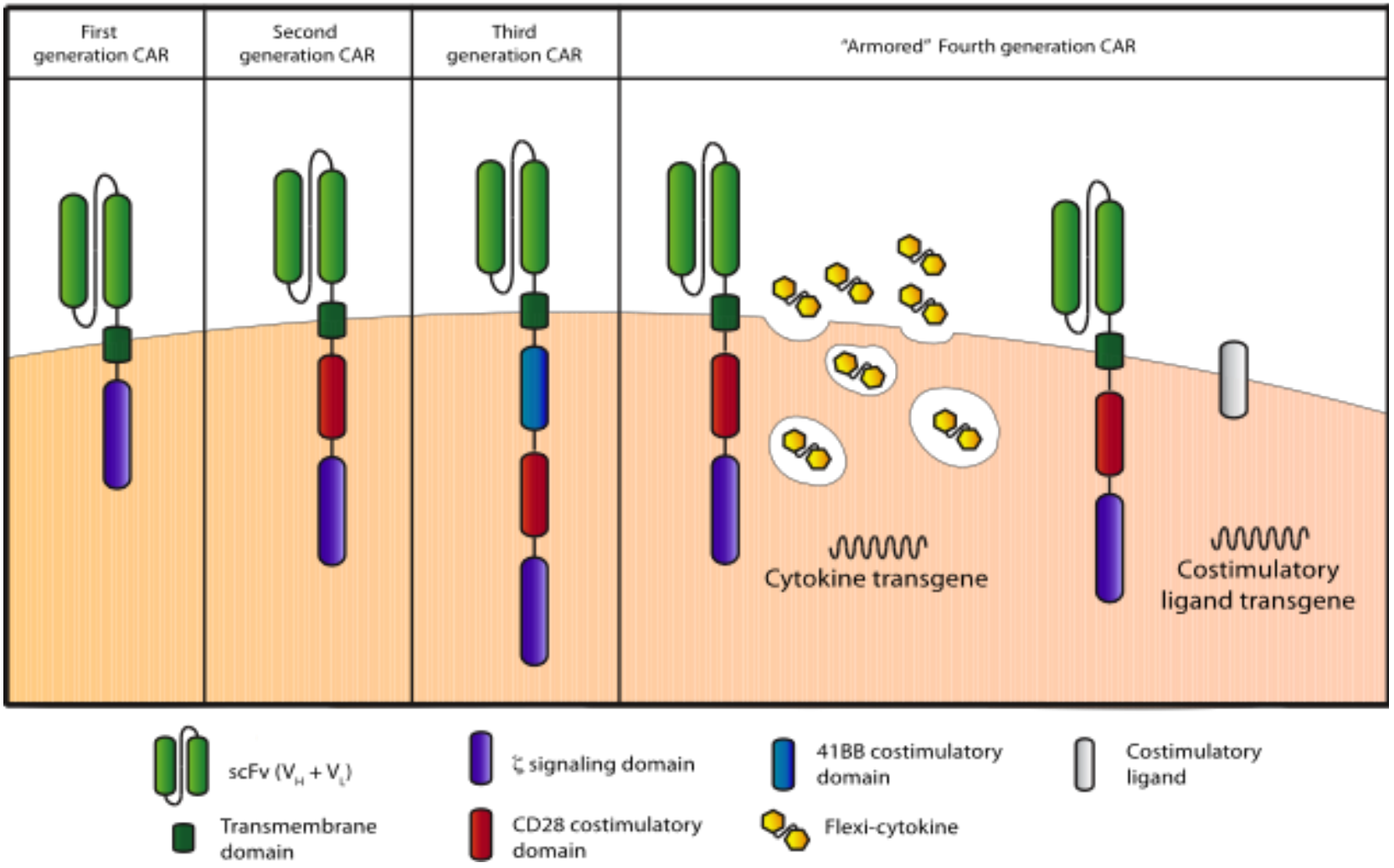
Evolution in CAR design



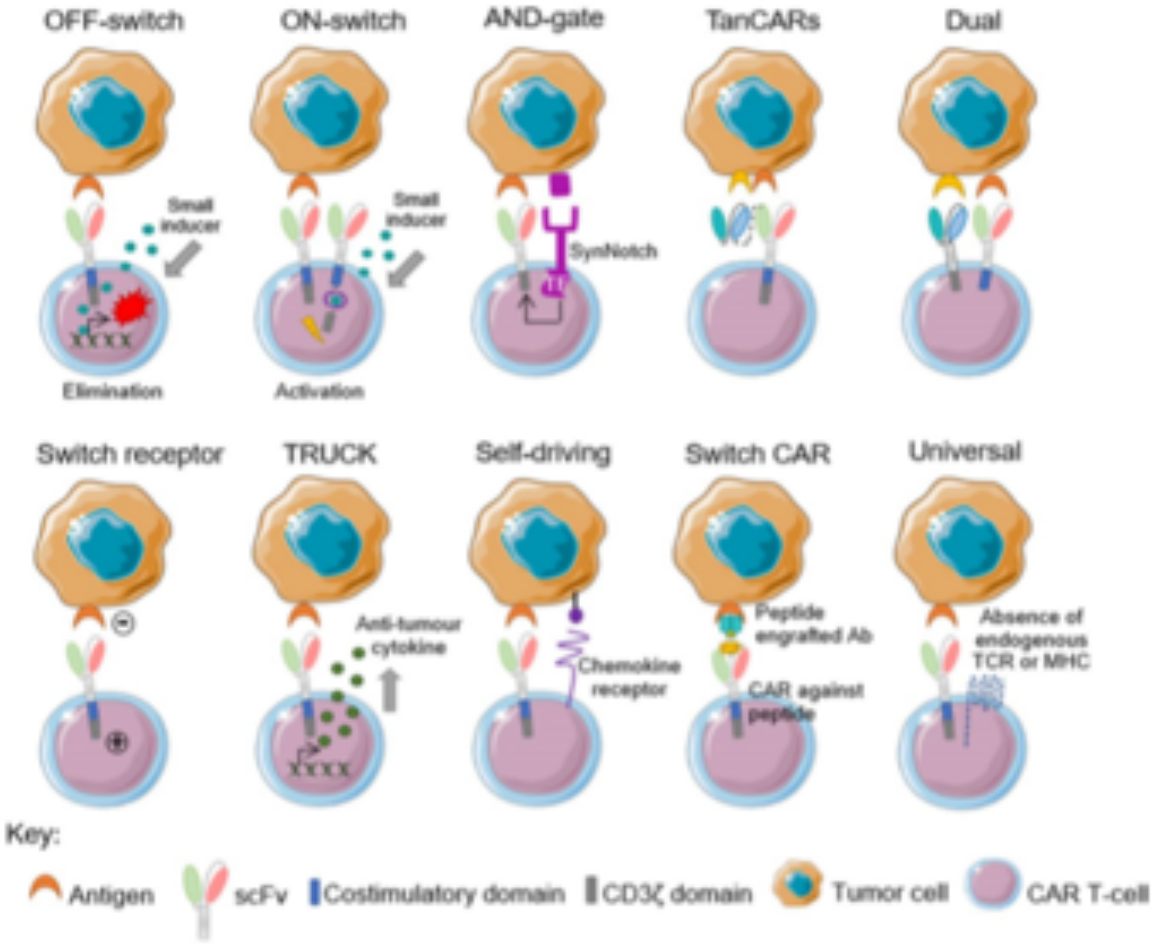
(Elahi, Khosh, Tahmasebi, & Esmaeilzadeh, 2018)

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



Evolution in CAR design



Next generation CARs (Gowrishankar et al., 2018)

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

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

Persistence of long-lived plasma cells and humoral immunity in individuals responding to CD19-directed CAR T-cell therapy

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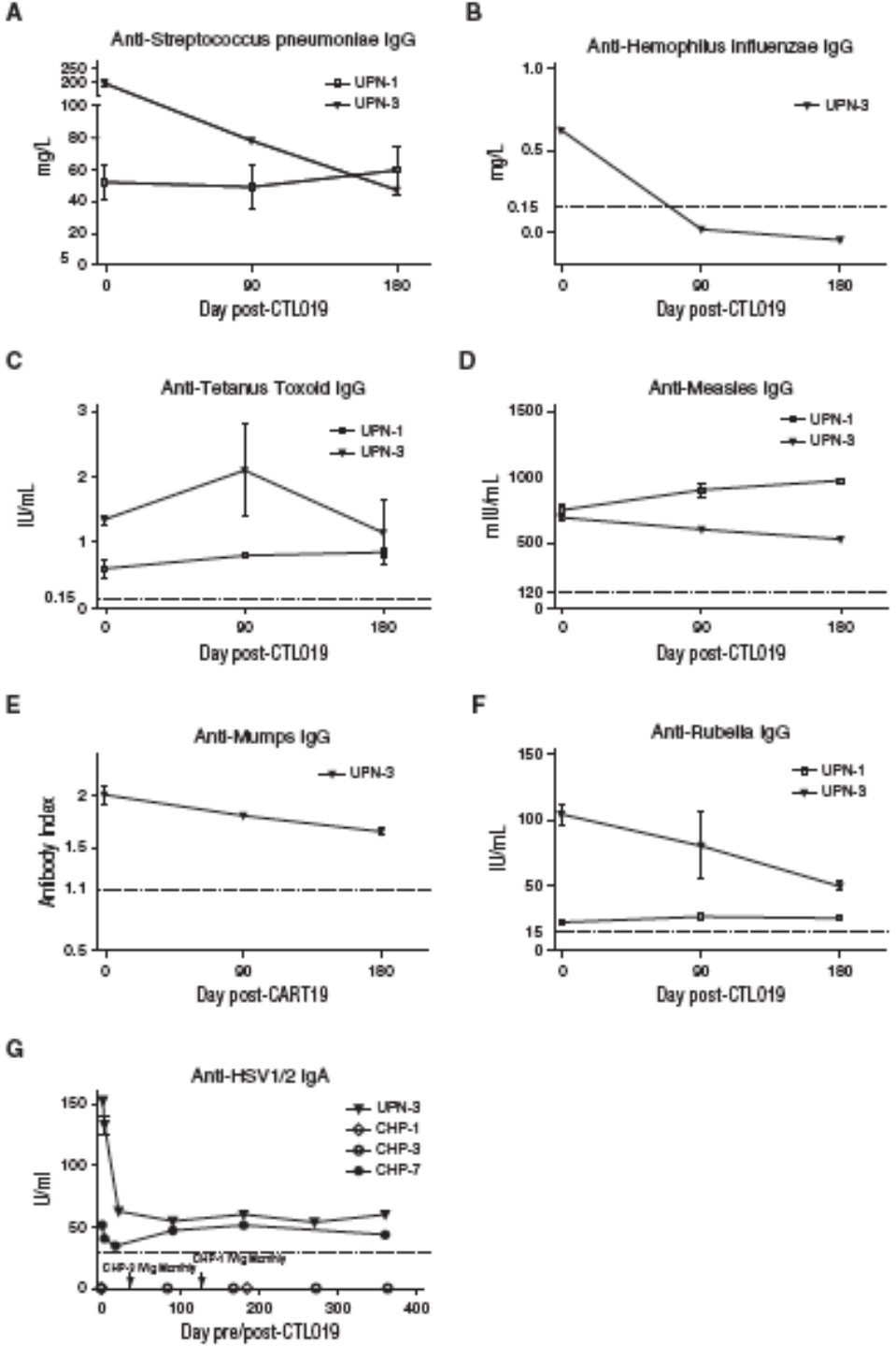
Key Points

- CD19-targeted T-cell immunotherapy reveals that a population of PCs lacking CD19 expression survives long-term, independent of B cells.
- Preexisting humoral immunity to vaccine-related antigens can persist in patients despite marked B-cell aplasia after CTL019 immunotherapy.

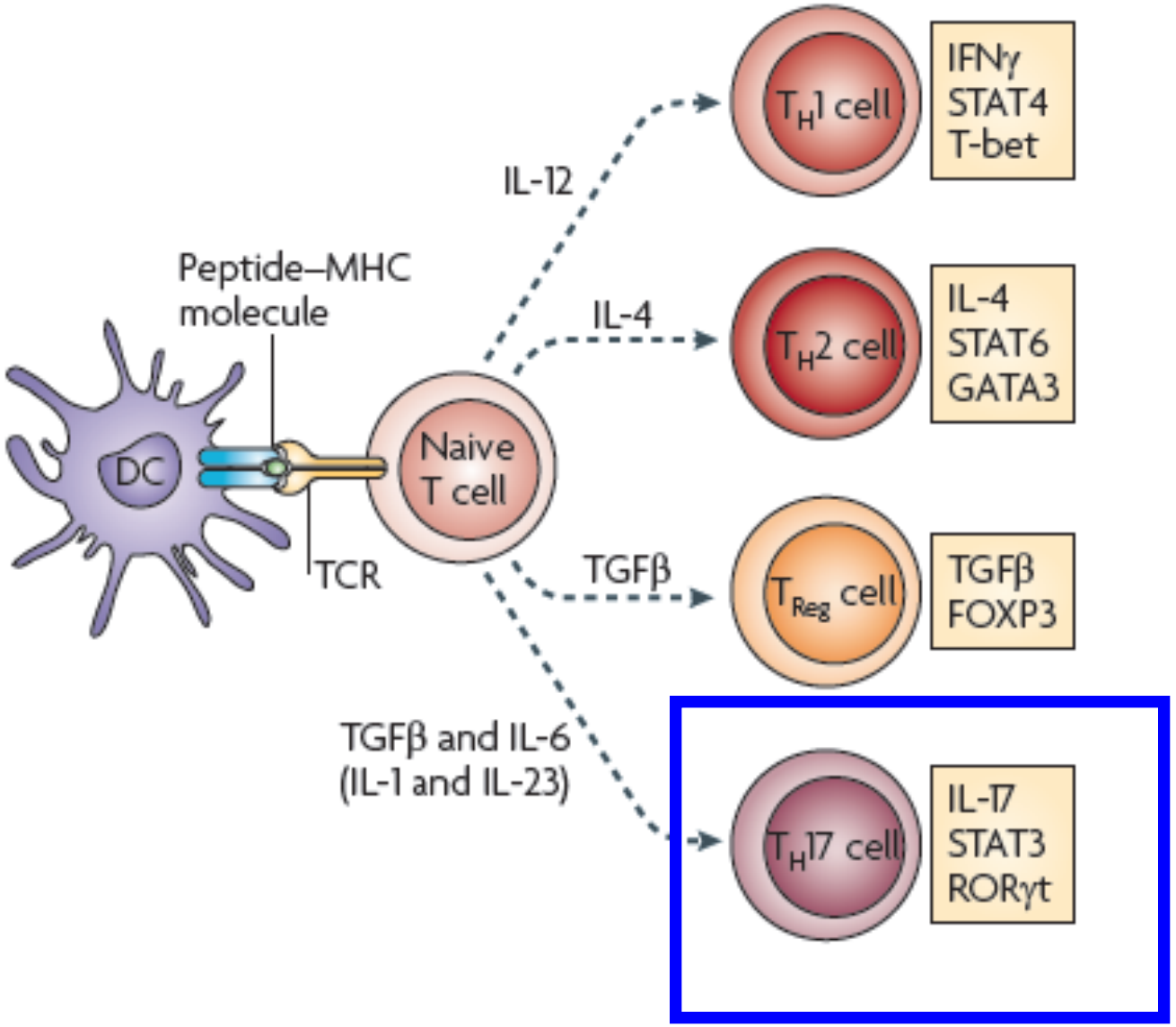
The mechanisms underlying the maintenance of long-lasting humoral immunity are not well understood. Studies in mice indicate that plasma cells (PCs) can survive up to a lifetime, even in the absence of regeneration by B cells, implying the presence of long-lived PCs as a mechanism for long-lasting immunity. Evidence from humans treated with anti-CD20, which depletes circulating B cells, also suggests B-cell–independent long-term survival of some PCs. On the other hand, antibody responses may be sustained solely by short-lived PCs with repopulation from clonally related memory B cells. To explore PC longevity and humoral immunity in humans, we investigated the fate of PCs and their antibodies in adult and pediatric patients who received chimeric antigen receptor–based adoptive T-cell immunotherapy targeting CD19 to treat B-cell lineage malignancies (CTL019). Treatment with CTL019 is frequently associated with B-cell aplasia that can persist for years. Serum antibody titers to vaccine-related antigens were measured, and quantitative assessment of B cells and PCs in blood and bone marrow was performed at various time points before and after CTL019 therapy. While total serum immunoglobulin concentrations decline following CTL019-induced B-cell aplasia, several vaccine/pathogen-specific serum immunoglobulin G and A (IgG and IgA) titers remain relatively stable for at least 6 and 12 months posttreatment, respectively. Analysis of bone marrow biopsies after CTL019 revealed 8 patients with persistence of antibody-secreting PCs at least 25 months post-CTL019 infusion despite absence of $CD19^{+}CD20^{+}$ B cells. These results provide strong evidence for the existence of memory B-cell–independent, long-lived PCs in humans that contribute to long-lasting humoral immunity. **(*Blood*. 2016;128(3):360-370)**


A
Anti-Streptococcus pneumoniae IgG
UPN-1
UPN-3
mg/L
Day post-CTL019
B
Anti-Hemophilus influenzae IgG
UPN-3
mg/L
Day post-CTL019
C
Anti-Tetanus Toxoid IgG
UPN-1
UPN-3
IU/mL
Day post-CTL019
D
Anti-Measles IgG
UPN-1
UPN-3
mIU/mL
Day post-CTL019
E
Anti-Mumps IgG
UPN-3
Antibody Index
Day post-CART19
F
Anti-Rubella IgG
UPN-1
UPN-3
IU/mL
Day post-CTL019
G
Anti-HSV1/2 IgA
UPN-3
CHP-1
CHP-3
CHP-7
U/ml
CHP-2 Mg Monthly
CHP-1 Mg Monthly
Day pre/post-CTL019

Classification of T-cell

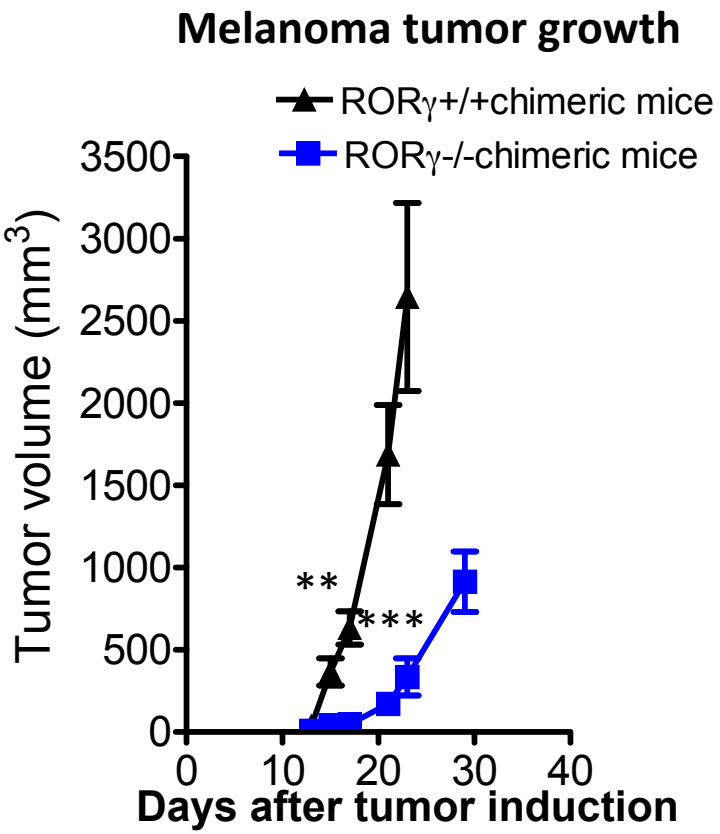
- Cytotoxic T-cell (CD8)
- Helper T-cell (CD4)
- Regulatory/suppressor T-cell (T-Reg)
- Memory T-cell

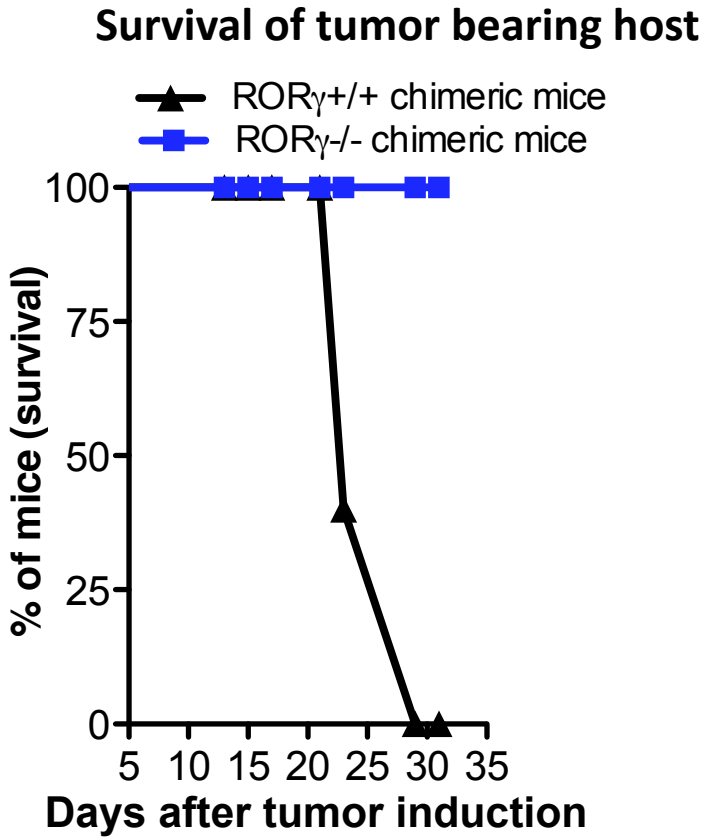
Adoptive T cell therapy: Right T cell population?



Zou W & Restifo NP Nature Reviews Immunology 2010

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





Abrogation of Th17 pathways promotes anti-tumor immune responses