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Chimeric Antigen Receptor T-Cells (CAR-T):

what are

and

how they work

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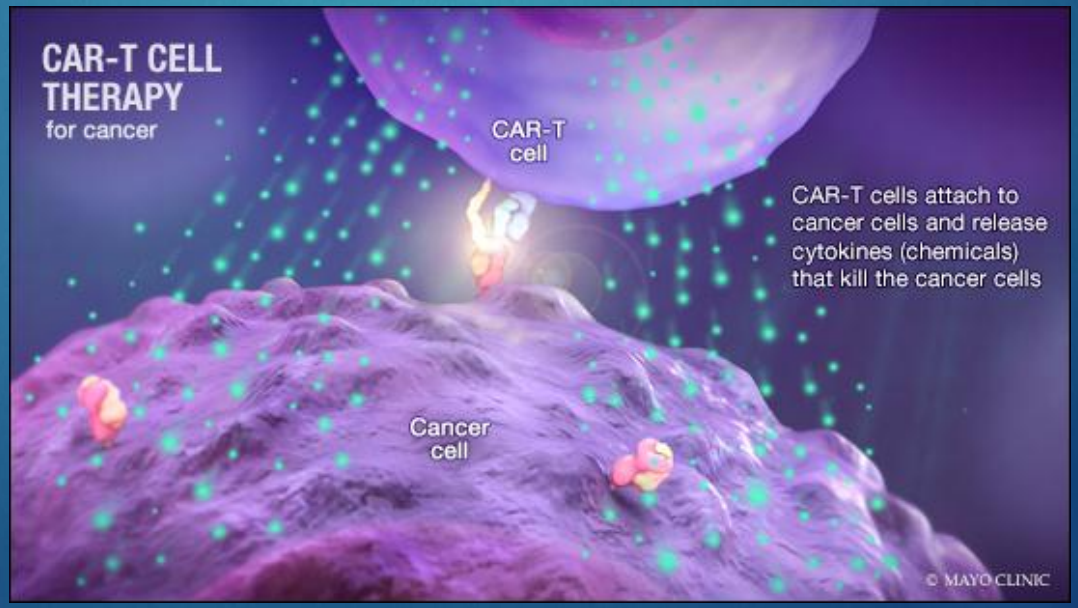
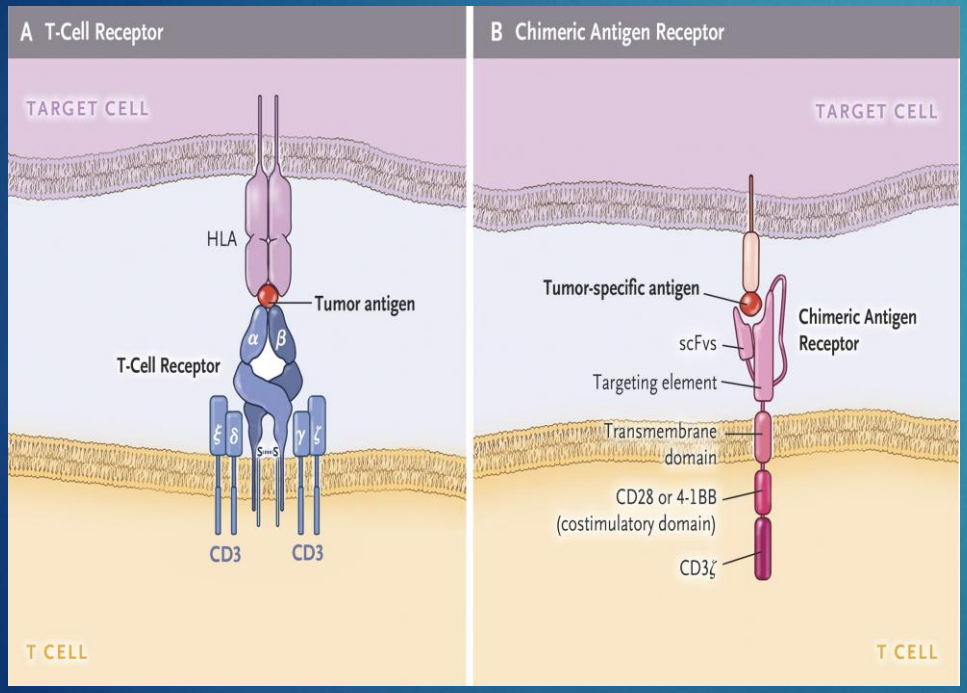
Background and introduction

- intratumor clone and subclones heterogeneity and immunosuppressive microenvironment in cancer ecosystem contribute to inherent difficulties for tumor treatment;
- immuno-oncology is a successful strategy of adoptive cell transfer for treating metastatic cancer and breaking immunological tolerance to tumors («self» antigens);
- T-cell engineering and synthetic immunity for producing durable remissions in patients with treatment-refractory tumors.

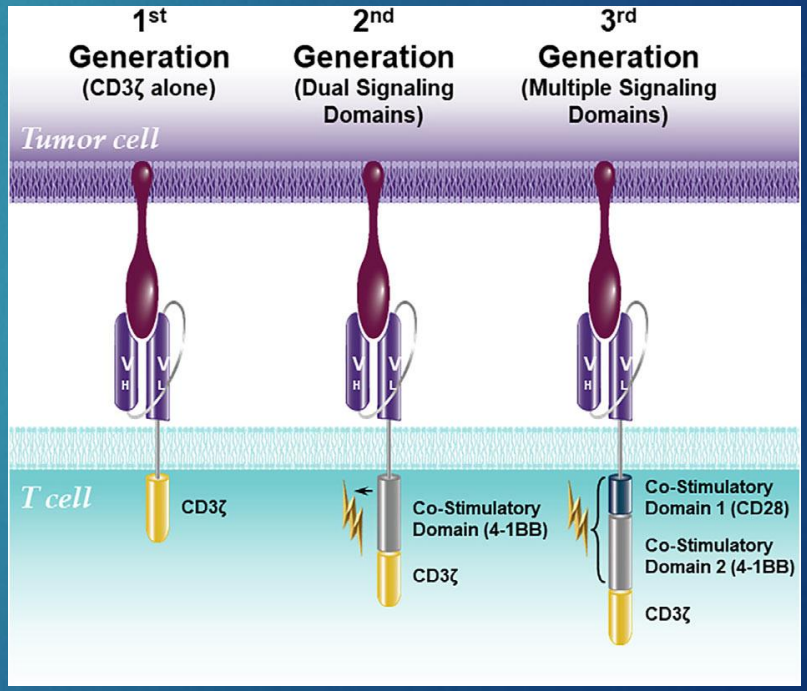


What are CAR-T Cells

- genetically redirect and reprogram T cells to overcome tolerance in cancer;
- patient's own T cells genetically engineered with gene encoding chimeric antigen receptor (CAR) that binds a tumor antigen;
- CARs consist of:
  - extracellular immunoglobulin-derived variable heavy and light chains to direct specificity;
  - intracellular signaling molecule comprised of TCR CD3 zeta chain;
  - co-stimulation signaling, such as CD28 and 4-1BB, that increases expansion and potency of engineered T cells.

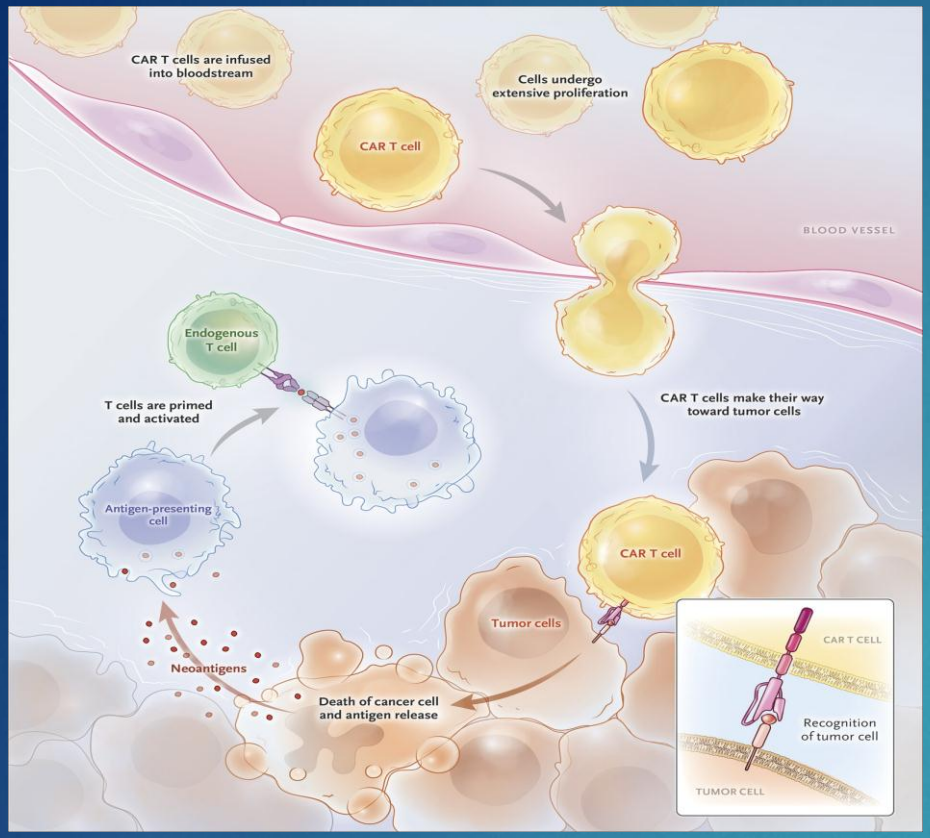


Structure of CARs and T-Cell Receptors.
June CH, Sadelain M.
N Engl J Med 2018;379:64-73



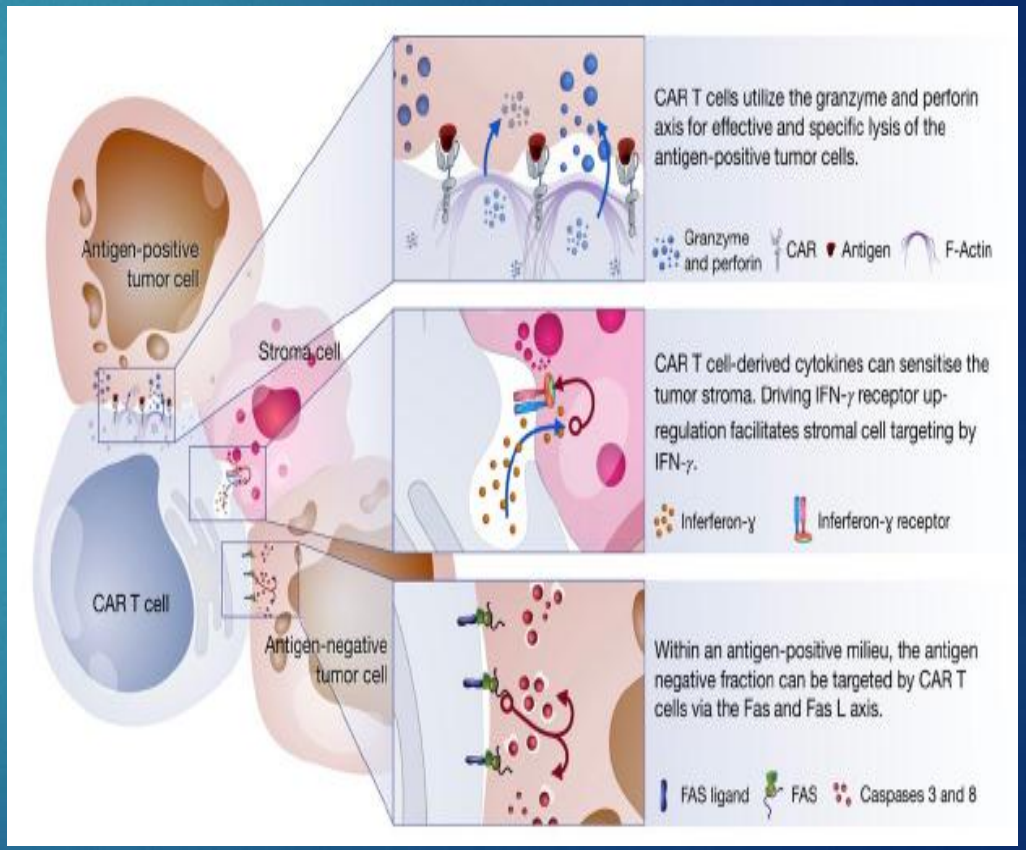
An introduction to chimeric antigen receptor (CAR) T-cell immunotherapy for human cancer
Feins S. et al.
Am J Hematol. 2019;94:S3–S9

How CAR-T Cells work



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

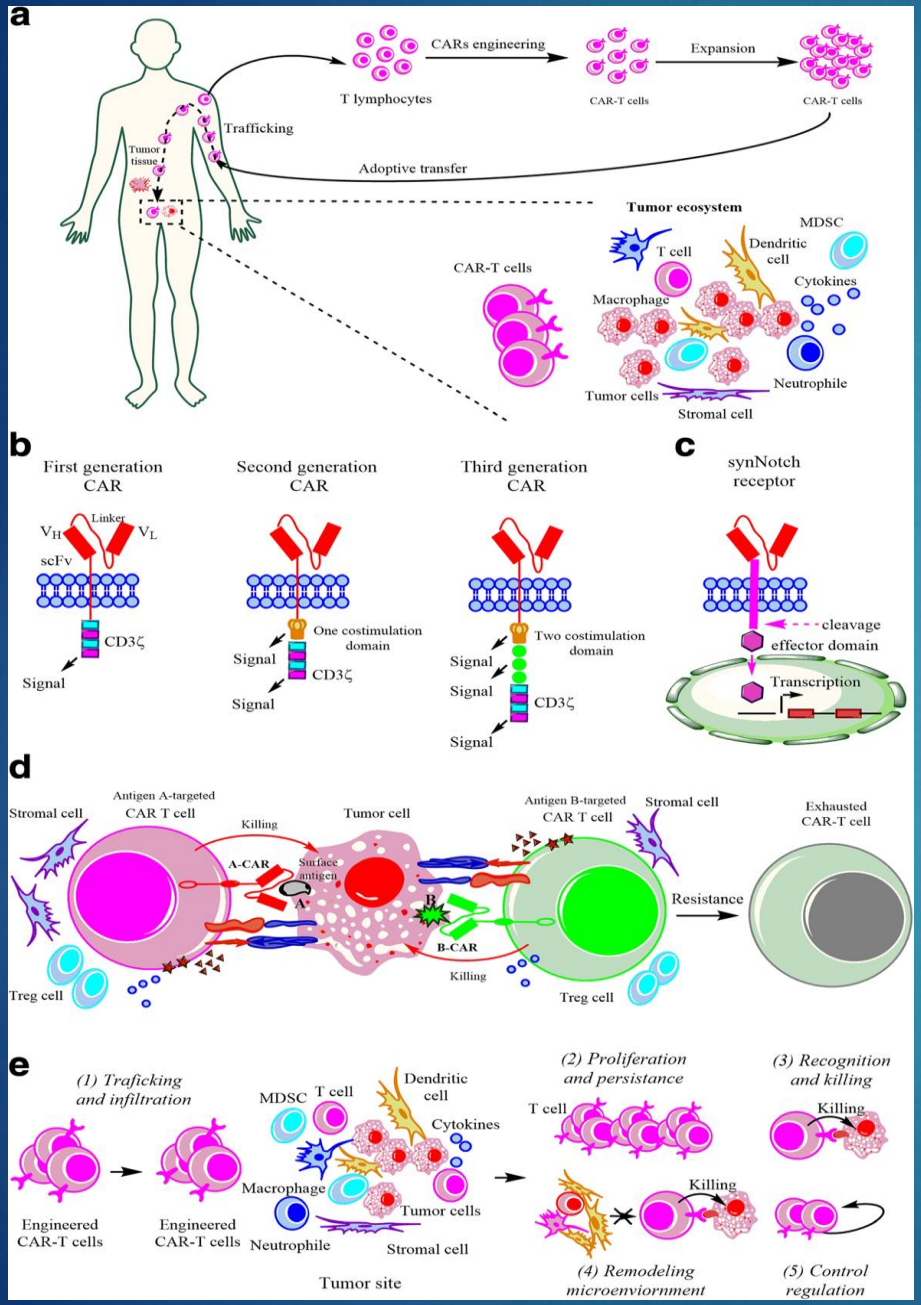
- **Engrafting, trafficking to tumor, and proliferating extensively after infusion.**



Benmebarek M. et al. International Journal of Molecular Sciences 2019, 20, 1283

- **Killing Mechanisms of CAR-T Cells:**
  - perforin and granzyme axis: targeting antigen positive fraction;
  - cytokine secretion: stromal cell sensitization;
  - Fas and FasL axis: targeting antigen-negative fraction.

How CAR-T Cells work



Engineering chimeric antigen receptor-T cells for cancer treatment
Baixin Ye et al. Molecular Cancer (2018) 17:32

- engage surface antigens of tumors in an HLA-independent manner, not limited by tumor antigens presentation;
- insensitive to tumor escape mechanism related to MHC loss;
- synergize with endogenous immune response;
- target the tumor ecosystem:
  - cells interaction and niche cells: regulate interaction between heterogeneous tumor clones and immune cells;
  - immunosoppressive tumor microenvironment: remodel microenvironment through release of soluble factors that regulate the function of stromal and immune cells;
- persist long term in vivo: permanent anti-tumor effects

Responses to CAR-T Cells therapy

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

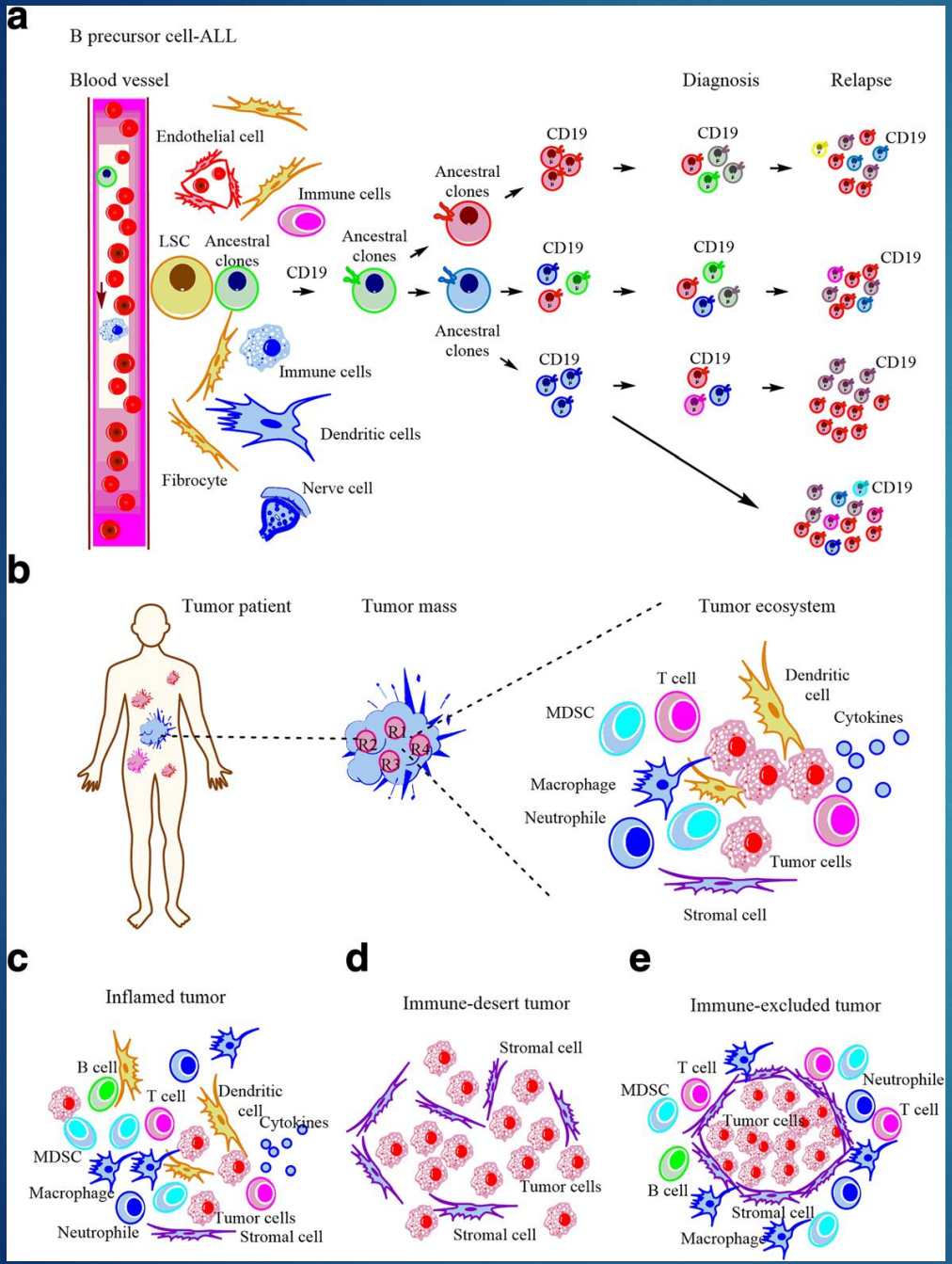
Disease	Response Rate	Comments	Reference
	percent		
Leukemia			
B-cell acute lymphoblastic leukemia (in adults)	83–93	High initial remission rates; unresolved issue is whether CAR T-cell therapy is definitive therapy or should be followed by allogeneic hematopoietic stem-cell therapy	Park et al.,[35]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

* ND denotes not determined.

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

- dramatic clinical responses
- high rates of complete remission for B-cell malignancies

Resistance to CAR-T Cells therapy



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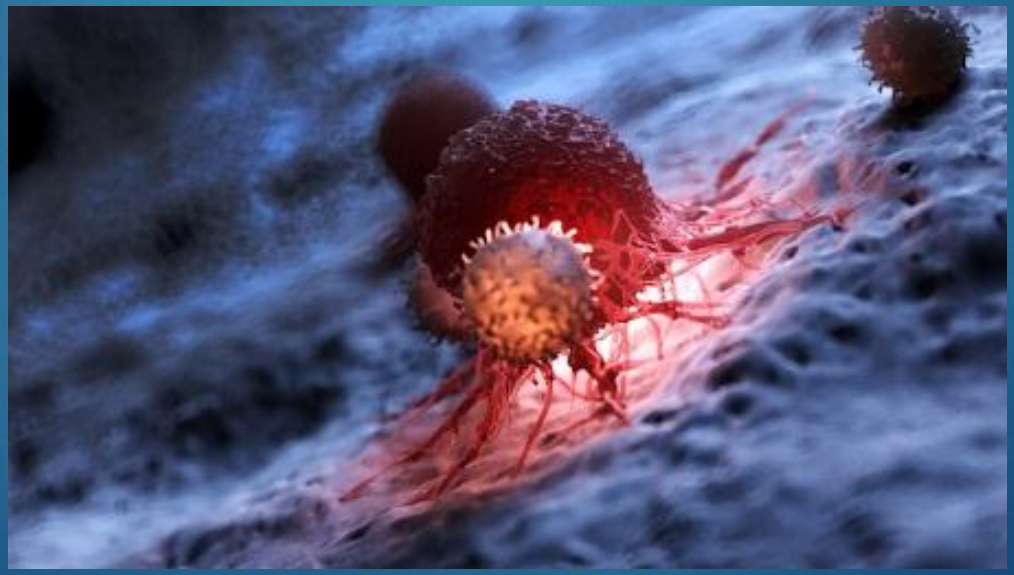
- expression of tumor CD19 variant:
  - loss CD19 antigen or epitope;
  - alternative splicing, homozygous or biallelic frameshift mutations in CD19 : non-membrane bound form of CD19 or poorly express variant that lacks an epitope recognized by CAR;
- tumor ecosystem:
  - interaction between malignant, immune, stromal, endothelial cells and cytokines;
- cancer immune phenotypes:
  - different response of immuno-inflammed, immuno-desert and immuno-excluded phenotype.

Ineffective CAR-T Cells

- immunosenescence and exhaustion: CAR-T proliferation and persistence in vivo;
- failure of engraftment;
- contamination of autologous peripheral blood mononuclear cells with monocytes



Reported Toxic Effects of CAR-T Cells

Table 2. Reported Toxic Effects of CAR T Cells.

CAR Specificity and Adverse Effect	Reference
CD19 CAR	
B-cell aplasia and hypogammaglobulinemia	Kochenderfer et al.,[52] Kalos et al.[53]
Cytokine release syndrome	Davila et al.,[36] Lee et al.,[54] Teachey et al.[55]
Dermatitis	Rubin et al.[56]
Hematophagocytic lymphohistiocytosis and macrophage activation syndrome	Grupp et al.,[32] Porter et al.,[41] Teachey et al.[55]
Neurologic effects such as ataxia and aphasia	Brudno and Kochenderfer[57]
Cerebral edema	Gust et al.[58]
B-cell maturation antigen CAR: the cytokine release syndrome	Riches et al.[59]
Mesothelin CAR: anaphylaxis (antibody to murine single-chain variable fragments)	Maus et al.[60]
Carbonic anhydrase IX CAR: cholangitis (on-target)	Lamers et al.[61]
HER2/neu CAR: lethal cytokine release syndrome	Morgan et al.[62]
Carcinoembryonic antigen–related cell-adhesion molecule 5 (CEACAM5) CAR: hemorrhagic colitis (on-target)	Thistlethwaite et al.[63]

- targeting non specific surface antigens and cross-reactivity;
- on-target and off-target effects:
  - cytokine release syndrome;
  - neurologic dysfunction;
  - B-cell aplasia.

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Conclusions and perspectives

- ***CAR-T Cells as regenerative medicine application;***

- CAR-T Cells as personalized cancer treatment;

- CAR-T Cells as new type of medicine integrated with chemotherapy, immune checkpoint inhibitors (such as anti PD-1, PD-L1 and CTLA-4), antibody-based therapy, target therapy, surgery and radiation therapy;

- ***specific training for equipe unit is required;***

- ***several hurdles remain for therapeutic optimization;***

- identify the critical functional challenges to address the target disease from multiple different dimensions (synNotch receptor to induce customized immune responses);

- ***CAR-T cells with desirable characteristics and functions in:***
  - trafficking, proliferation and persistence;
  - recognition tumor cells: targeting dual or more surface antigens;
  - killing tumor cells: identification of specific tumor antigen targets;
  - remodeling the tumor ecosystem: shape immunosuppressive microenvironment;
  - feedback-control regulatory system: reduce adverse side effects.

Thanks

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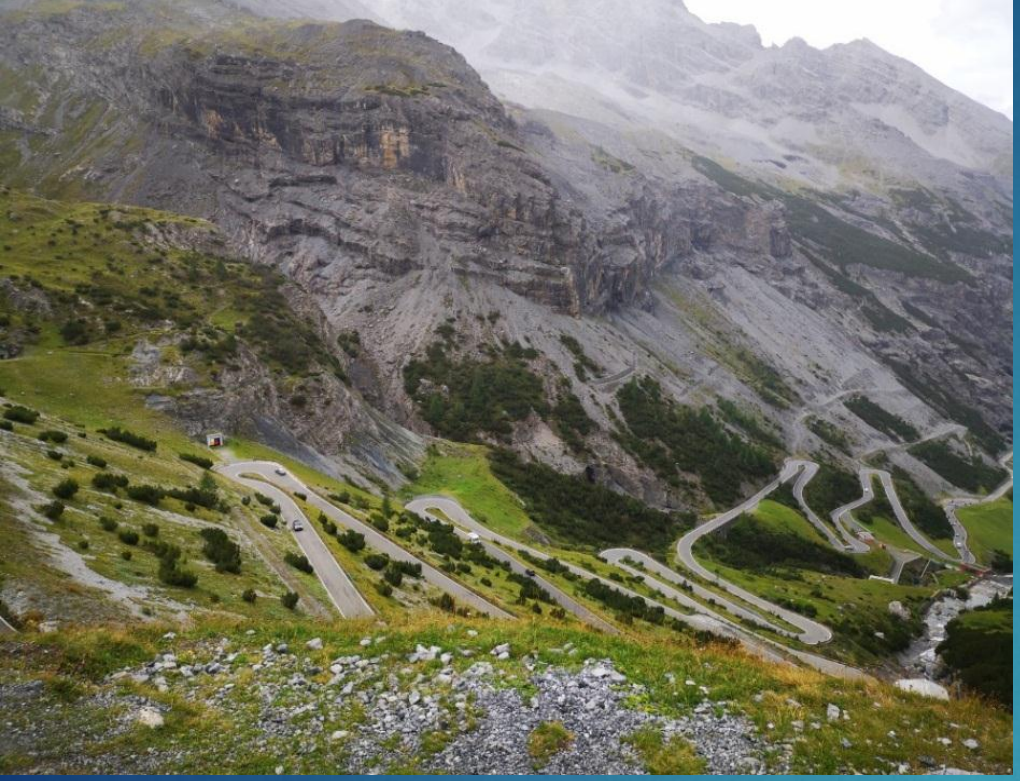

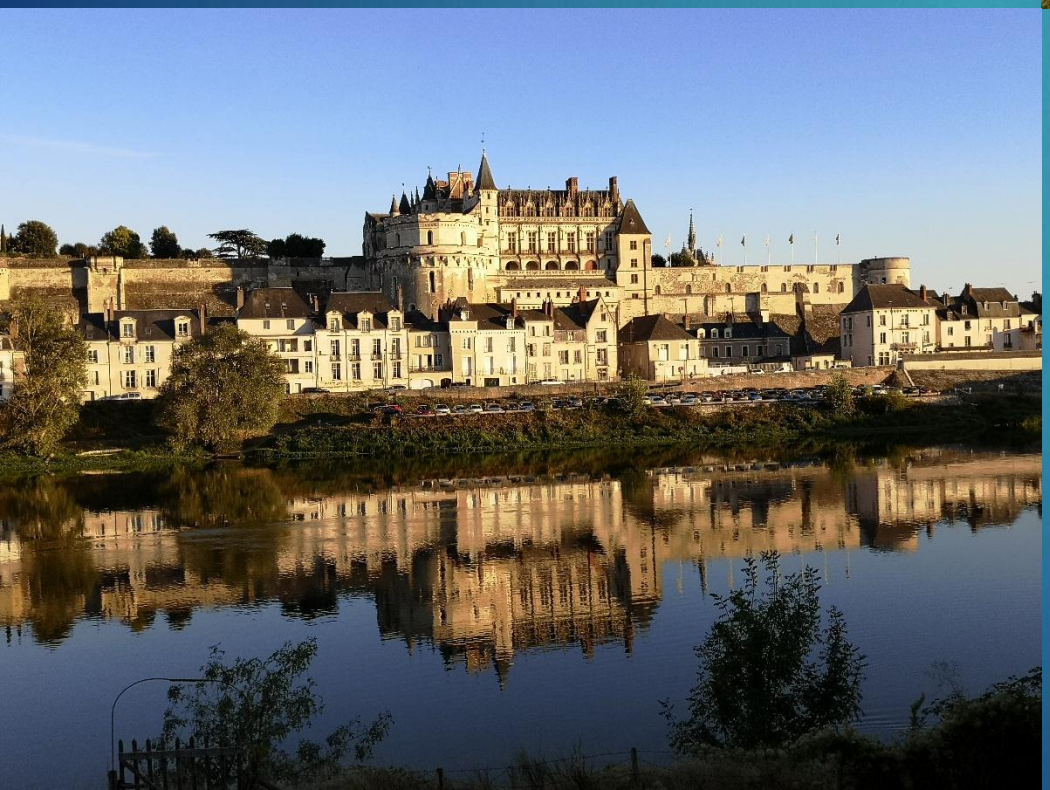


Thank you very much for your attention