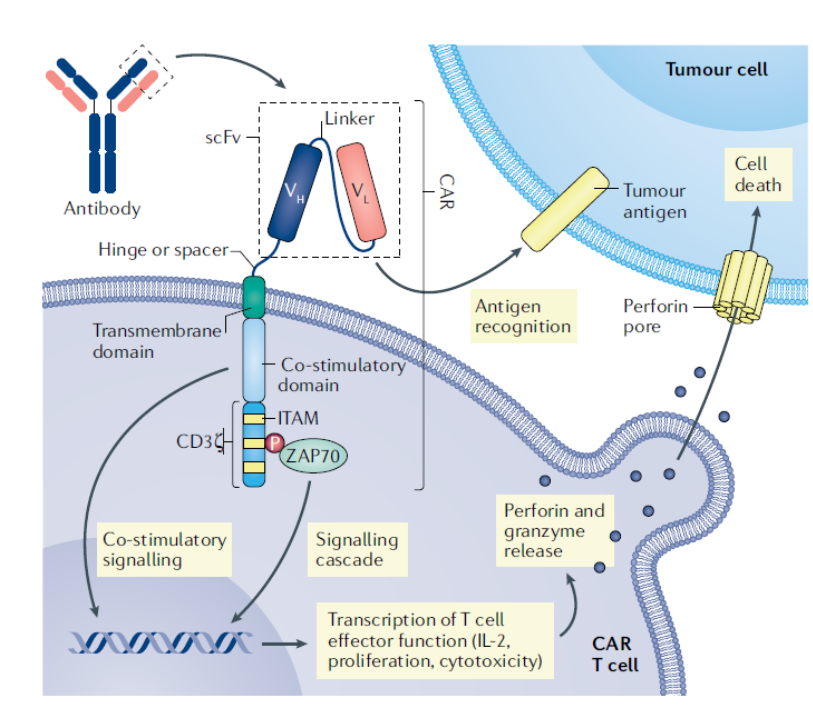
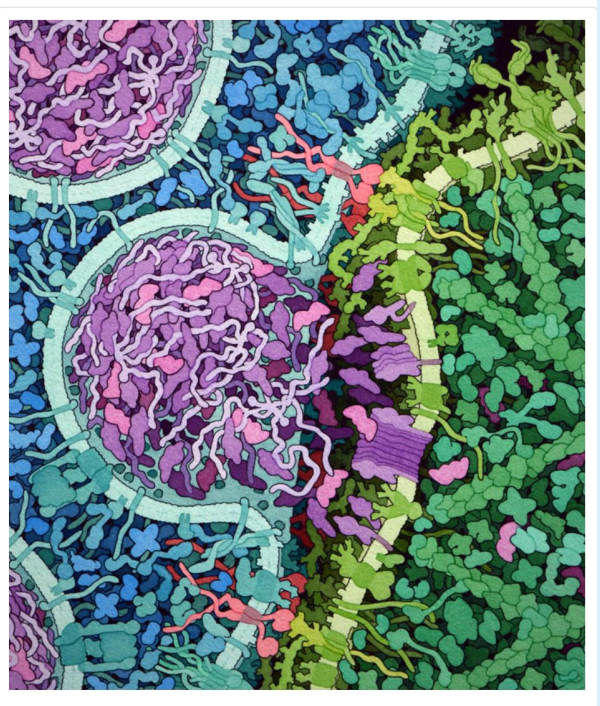
3d molecular designs
Transforming Science Learning

Living Drugs: *fighting cancer with CRISPR-engineered CAR-T cells.*





Tim Herman
3D Molecular Designs

Workshop Outline

- **Cancer Immunotherapy**

  Supercharging our own immune systems…

- **Connecting the dots…..**
  - ***CRISPR-Based Genome Editing…*** *just finished*
  - ***Tomorrow's Science Today…*** *coming soon*

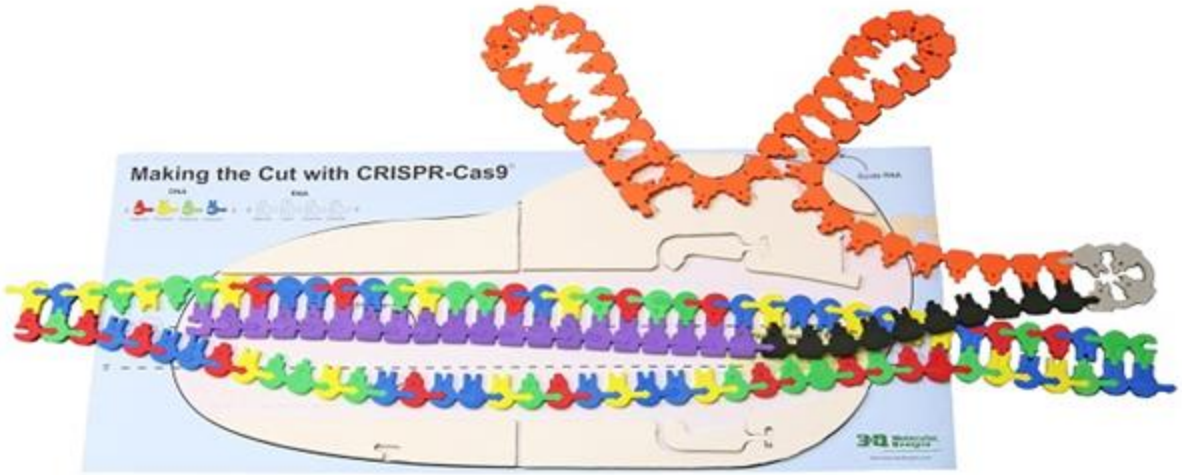
    An NIH SEPA project -- focused on infectious diseases

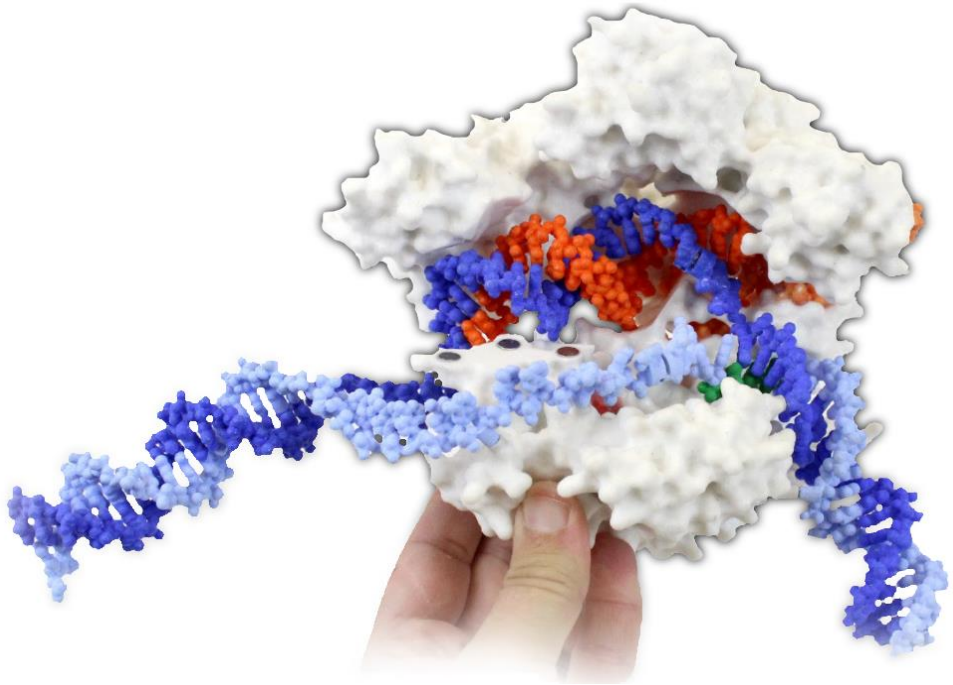
- **What is a CAR-T Cell,… and how do we make them?**

Just completed….a 5-year NIH-SEPA project focused on:

The Science and Ethics of CRISPR-based Genome Editing

New tools that will be used to engineer proteins…. and cells.





CRISPR II: Using Cas9 as a Genome Editing Tool ---- at 1:20pm on Saturday

Coming soon,... to a workshop near you.

Tomorrow's Science Today: *preparing for the next pandemic.*

A new NIH SEPA project focused on infectious diseases

- **New Instructional Materials**
  - Models, models and more models....
    A Virus Collection,.. including CoV-2
    Antibodies/vaccines /and **your immune system**
    mRNA vaccines and multi-valent, ultra-potent vaccines…from synthetic biology.
  - Stories, stories and more stories....

- **A Professional Learning Experience**…beginning in the summer of 2024

But first,…enroll now in **Modeling the Molecular World**, 2023
Session 1 -- June 26–30, or
Session 2 -- July 17 – 21

STEM....

Engineering... *allows us to:*

* *design an effective mRNA vaccine for CoV-2*
* *design supercharged T-cells to fight cancer*

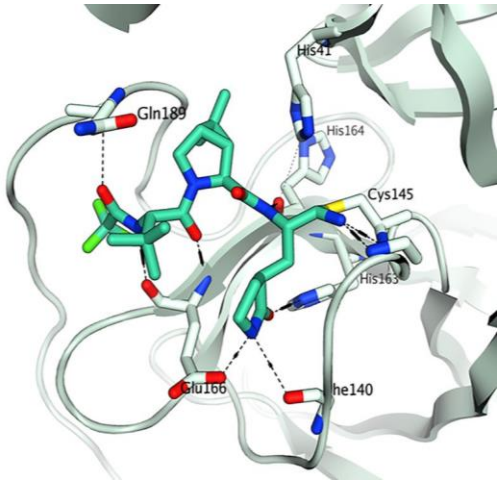
Science... *provided us with the nucleotide sequence of the CoV-2 spike protein.... and the field of molecular immunology*

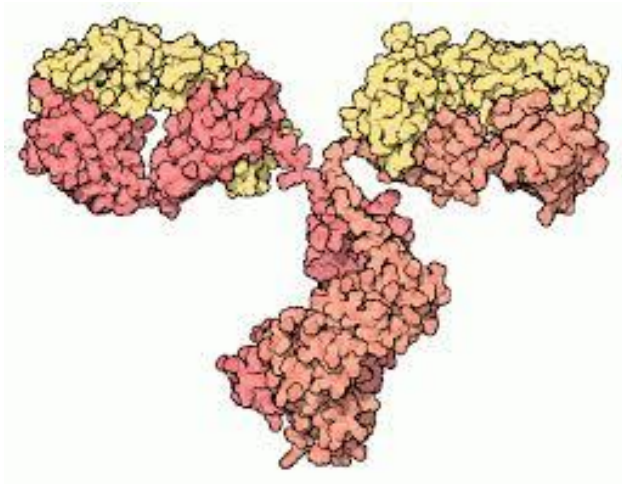
How can you incorporate principles of *engineering design* into your science classroom?

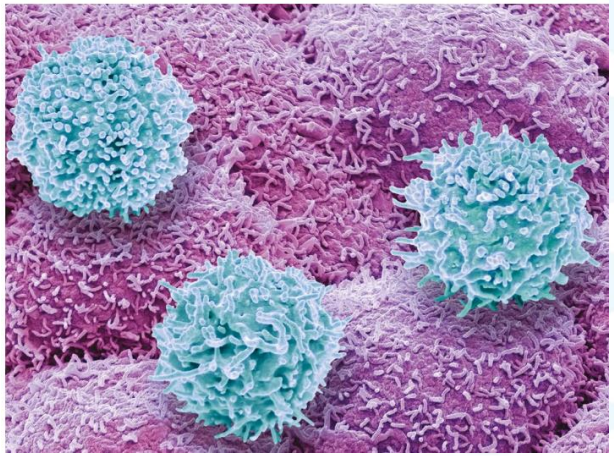
Teaching mRNA Vaccines – and the future of therapeutic RNAs – Friday at 10:40 am

Drugs,… *Yesterday,… Today,… and Tomorrow*

Small Molecules → **Biologics** → **Living Drugs**

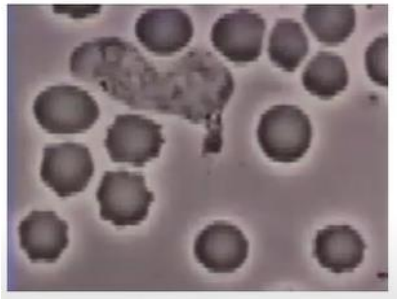






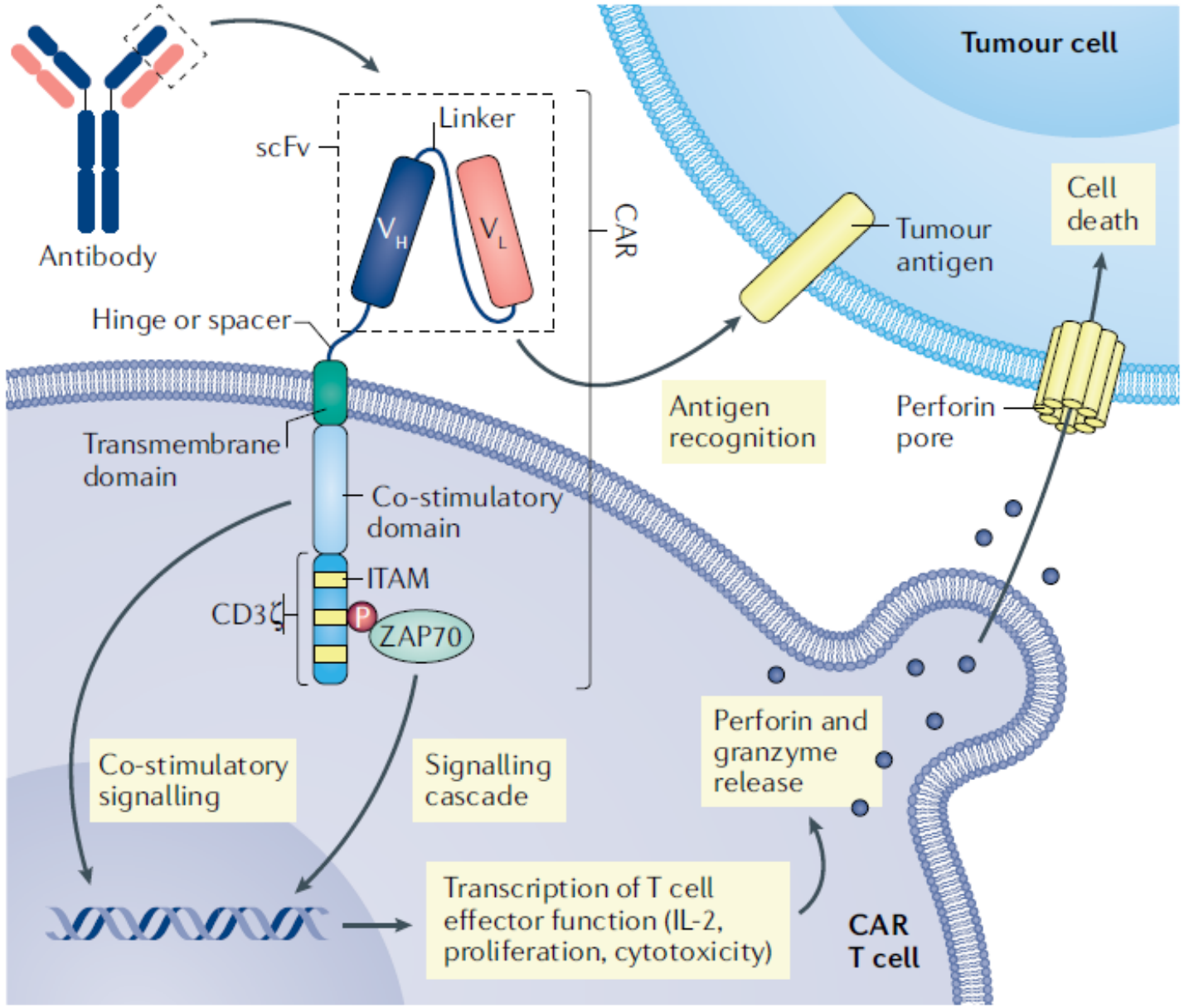
Deciphering CAR-T Cells: Exploring Functional Mechanisms to Drive Next Generation Immunotherapy

A video…featuring Wendell Lim…
https://youtu.be/EAA-aSEZasw
start viewing at 24:20 … or at 31:30

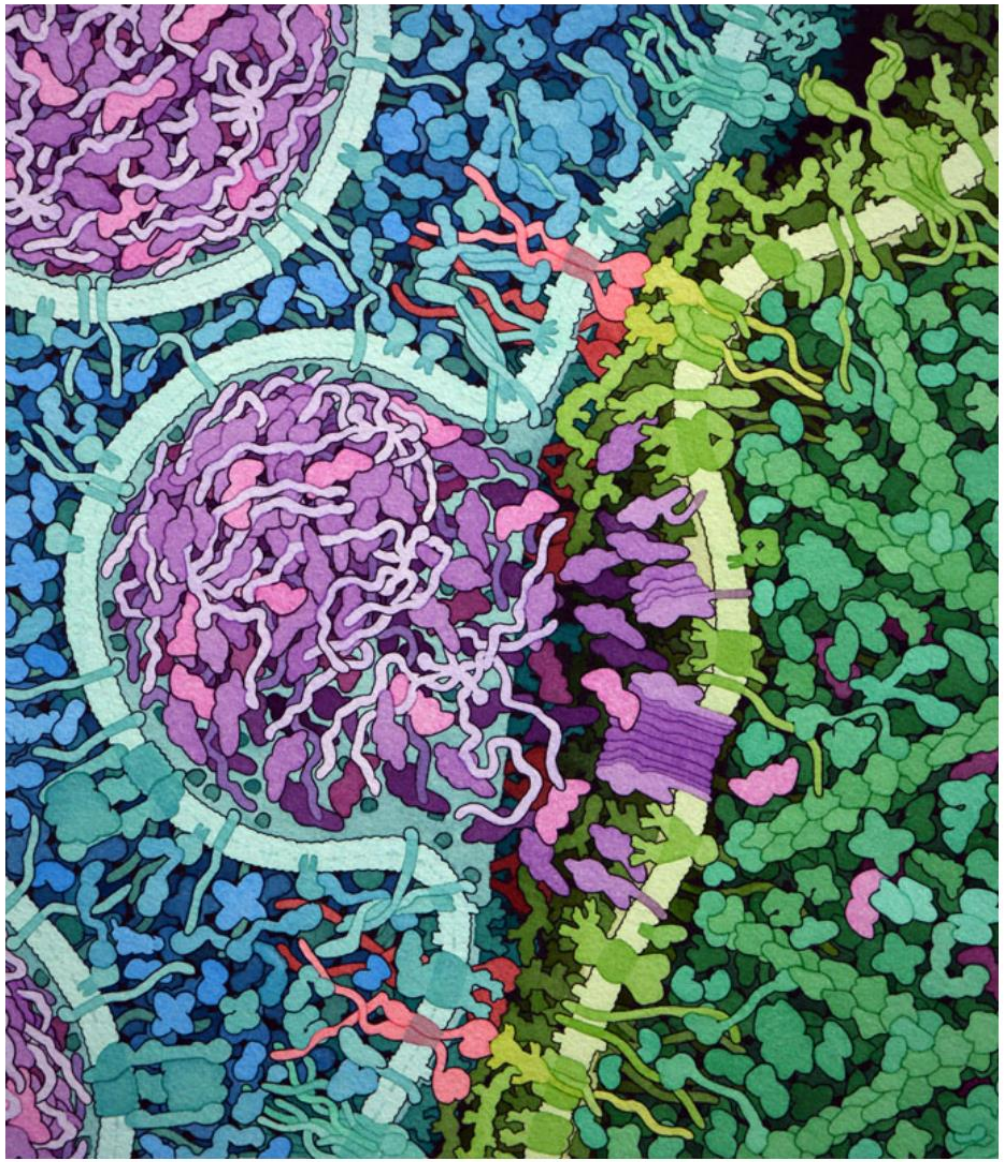


https://www.youtube.com/watch?v=EAA-aSEZasw

Fig. 1 | **Schematic of a basic second-generation CAR T cell.** The extracellular portion of the chimeric antigen receptor (CAR) molecule is typically generated from a monoclonal antibody against the target. The variable heavy (V_H) and variable light (V_L) chains, also known as the single-chain variable fragment (scFv), from the antibody sequence are connected by a linker to form the antigen-specific region of the CAR molecule. The hinge or spacer region anchors the scFv to the transmembrane region that traverses the cell membrane. Intracellularly, the co-stimulatory domain and CD3ζ chain signal once the scFv portion of the CAR recognizes and binds tumour antigen. Co-stimulatory signals are dependent on the co-stimulation domain used: CD28 is dependent on PI3K, whereas 4-1BB requires tumour necrosis factor (TNF) receptor-associated factors (TRAFs) and nuclear factor-κB (NF-κB). The CD3ζ chain contains three immunoreceptor tyrosine-based activation motif (ITAM) domains that, upon phosphorylation (P), signal through ζ-associated protein of 70 kDa (ZAP70). Downstream signalling leads to T cell effector functions including release of perforin and granzyme, leading to cell death of the target tumour cell. IL-2, interleukin 2.



Artistic illustration of an engineered T cell (on the left side in blue) recognizing and attacking a leukemia cell (on the right side in green). The CAR molecule is shown in red, bound to CD19 on the leukemia cell. This has led to activation of the T cell, which releases perforin (purple), forming pores in the cell surface. Granzymes (magenta) then enter through the pore and initiate apoptosis to kill the cancer cell.



Molecule of the Month: Chimeric Antigen Receptors

T cells may be engineered with chimeric antigen receptors to attack cancer cells.

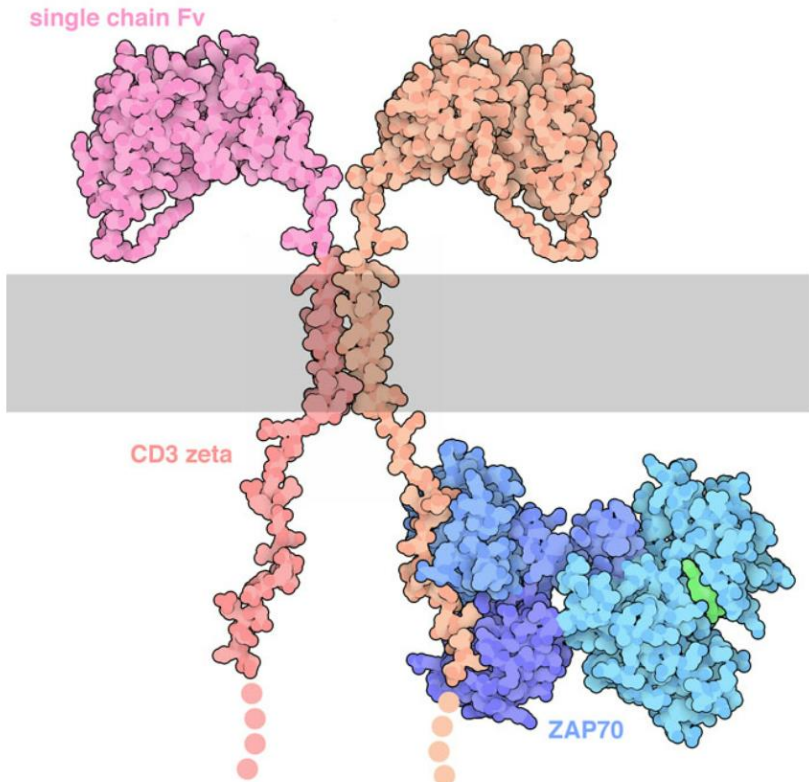
The immune system is expert at defending us from dangers, but when faced with cancer cells, the immune system often fails. Cancer cells are normal human cells that have been transformed into rogues. They usually have many genetic changes that allow them to grow without normal limitations. Unfortunately, most of these genetic changes mutate proteins that are inside the cancer cell, so the immune system may not detect them. Today, researchers are developing ways to retool the immune system to fight specific forms of cancer.

Engineered T cells

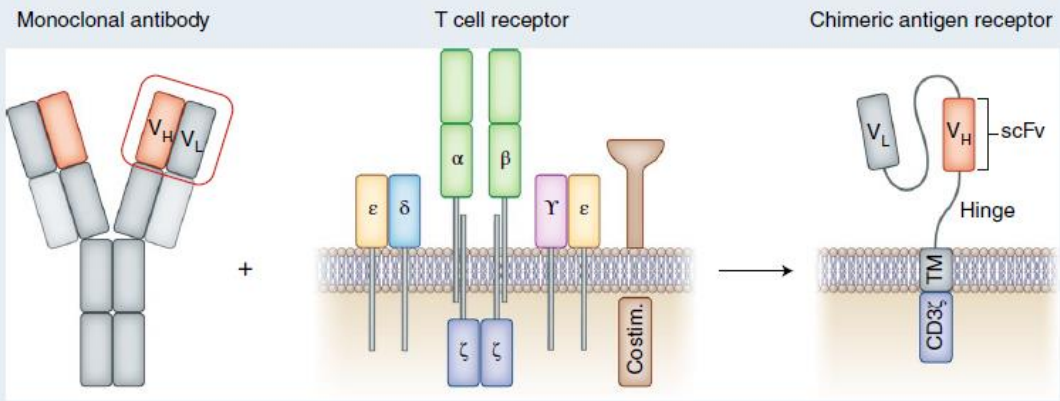
T cells have the ability to recognize virus-infected cells using T-cell receptors, and then destroy them. Unfortunately T cells are not as effective against cancer cells, because the molecules on the surface of cancer cells often look much like the molecules on normal cells. Chimeric antigen receptors (CAR) are a way for doctors to arm T cells with the ability to recognize cancer cells. Researchers remove T cells from a cancer patient and add a gene for a CAR that recognizes their form of cancer. When these “CART” cells are returned to the patient, they search for cancer cells and destroy them.

Building a CAR

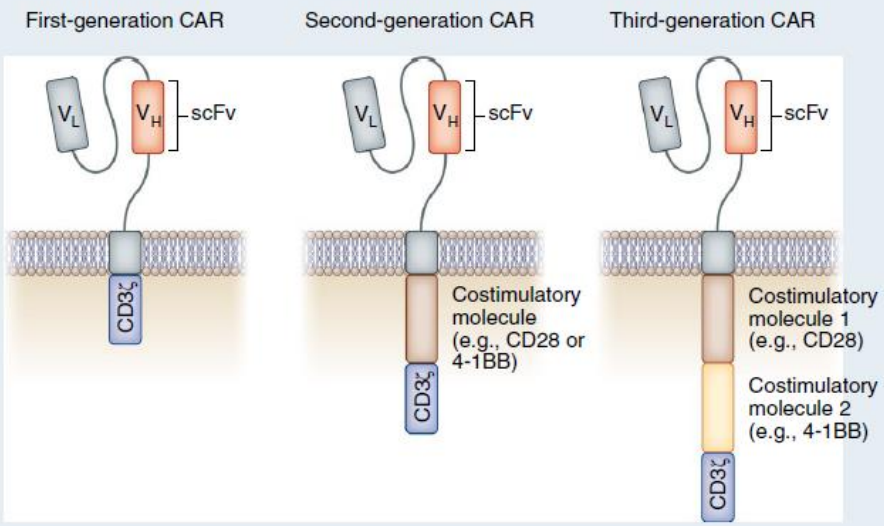
Chimeric antigen receptors are built by connecting several functional parts from different proteins, each with a specific job. A single-chain antibody variable fragment (Fv) recognizes a known cancer cell protein, targeting the tumor. These are generally engineered from monoclonal antibodies by using only the domains at the tip of the antibody, and connecting the two chains with a flexible linker. The antibody portion is connected to a transmembrane segment with another flexible linker. Inside the cell, one or more domains are taken from signaling proteins, which will activate the T cell once it finds a tumor cell.



This model of a chimeric antigen receptor is composed of a single-chain antibody Fv connected to a portion of CD3 zeta. It is shown in complex with the signaling molecule ZAP70. Four PDB entries were used to create the illustration: 2kh2, 2hac, 2oq1, and 2ozo.



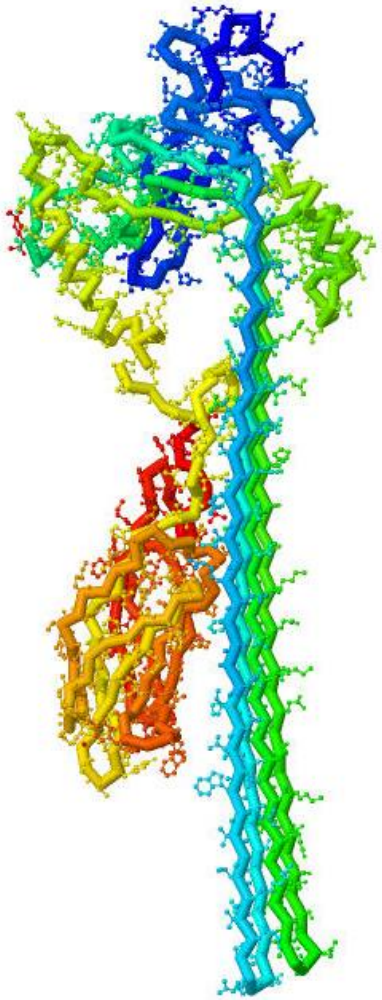
Structure of CAR T cells. CAR T cells are engineered by fusing the scFv of a monoclonal antibody (or another antigen-recognition domain) to a transmembrane domain and intracellular signaling domains capable of eliciting a T cell response. V_H, heavy chain; V_L, light chain; costim, costimulation; TM, transmembrane. Credit: Debbie Maizels/Springer Nature



CARs that contain only the CD3-ζ endodomain are known as first-generation CARs; those that contain one costimulatory domain (such as CD28 or 4-1BB) are known as second-generation CARs; and those that contain two or more costimulatory domains are known as third-generation CARs. Credit: Debbie Maizels/Springer Nature

Perforin

How T-cells kill….



Perforin

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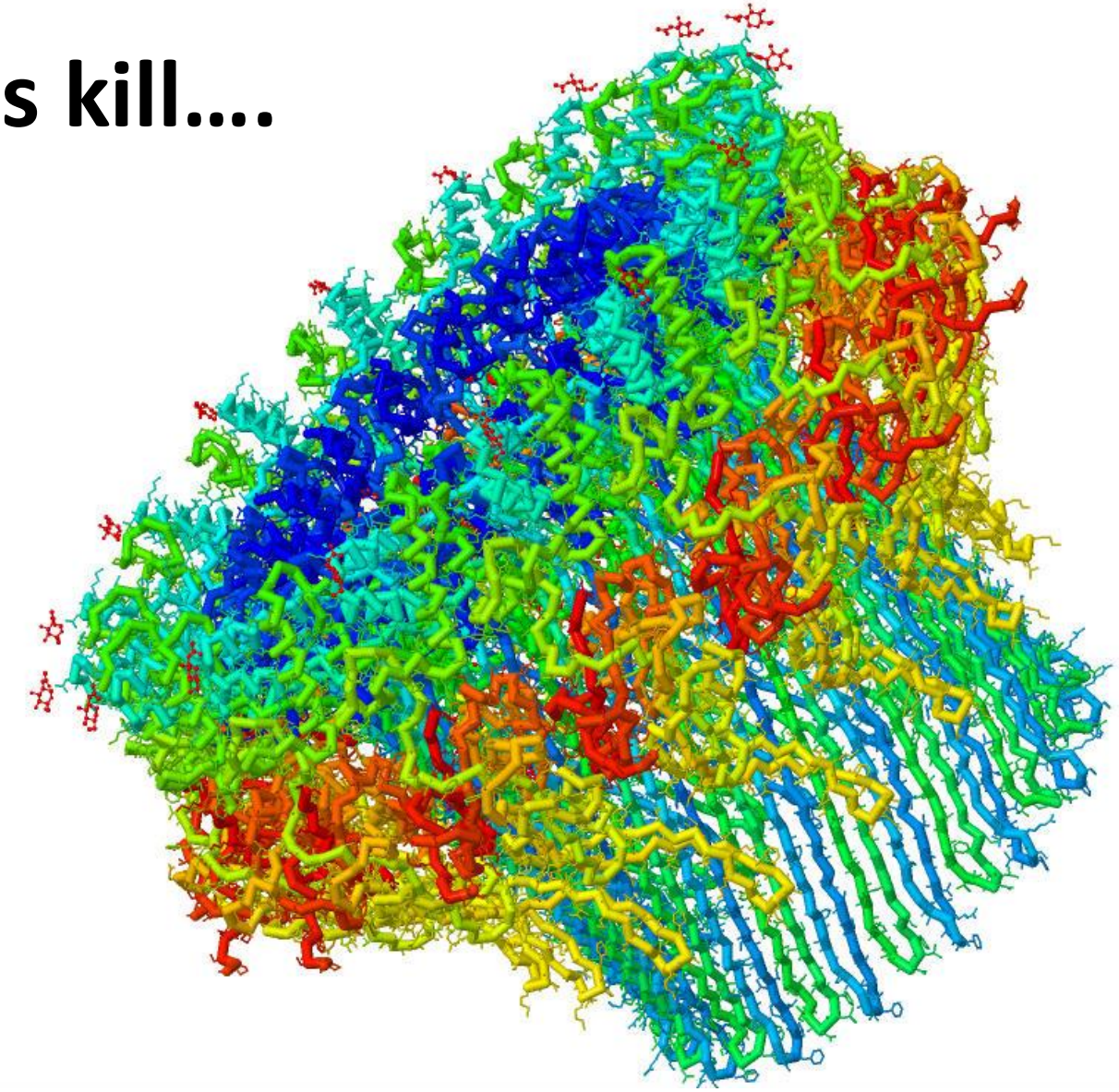
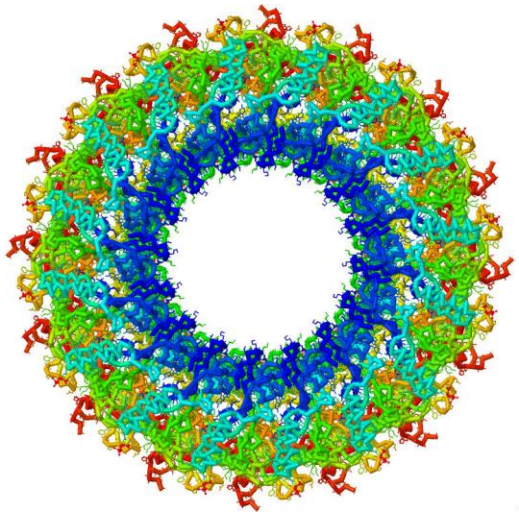
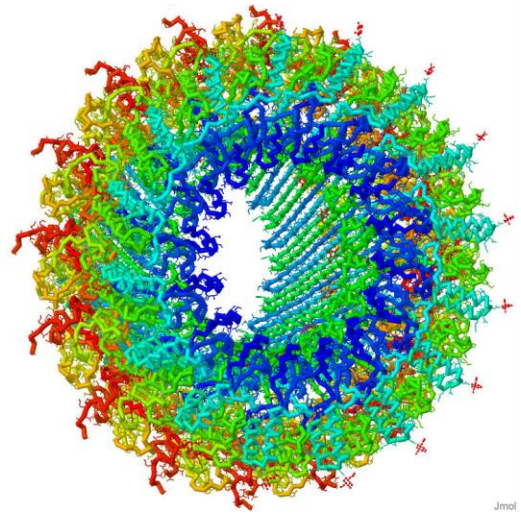


models give meaning to words…


Jmol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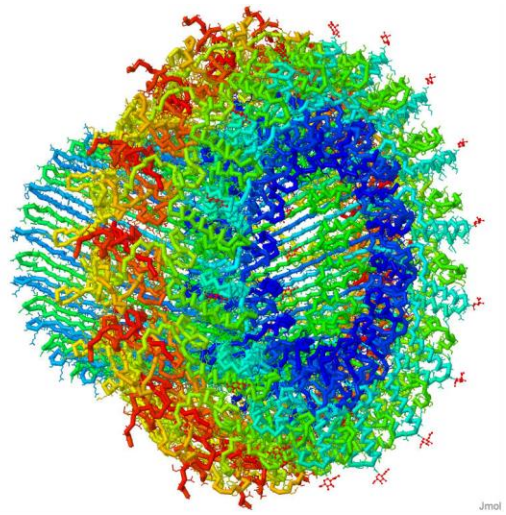
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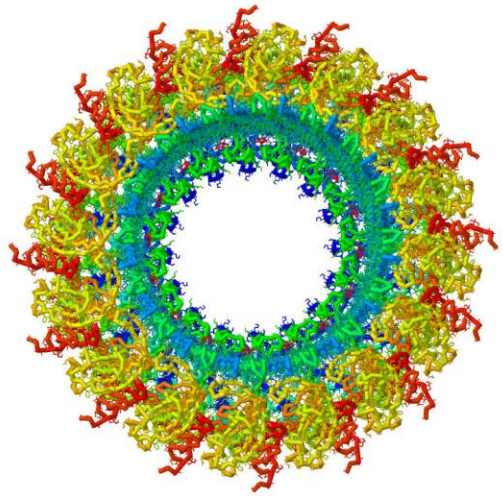
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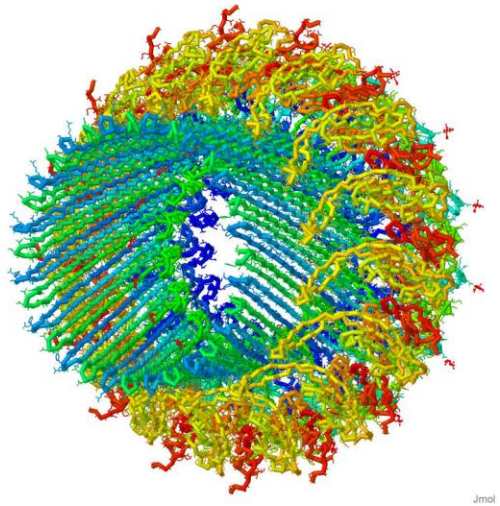
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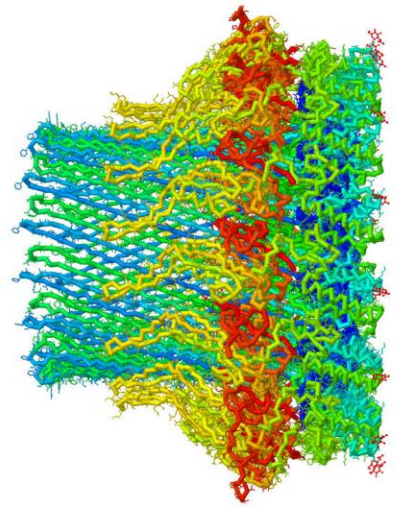
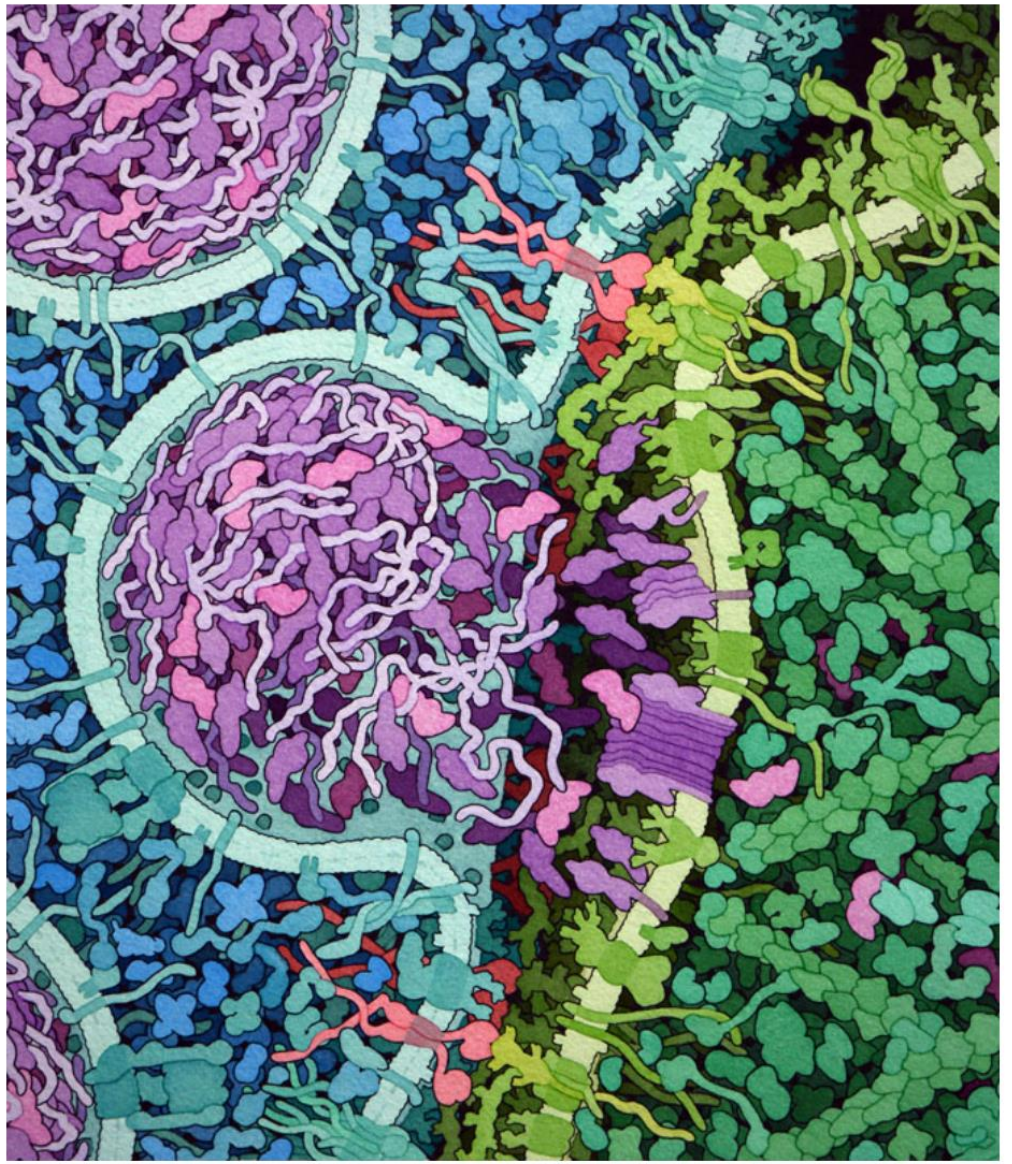
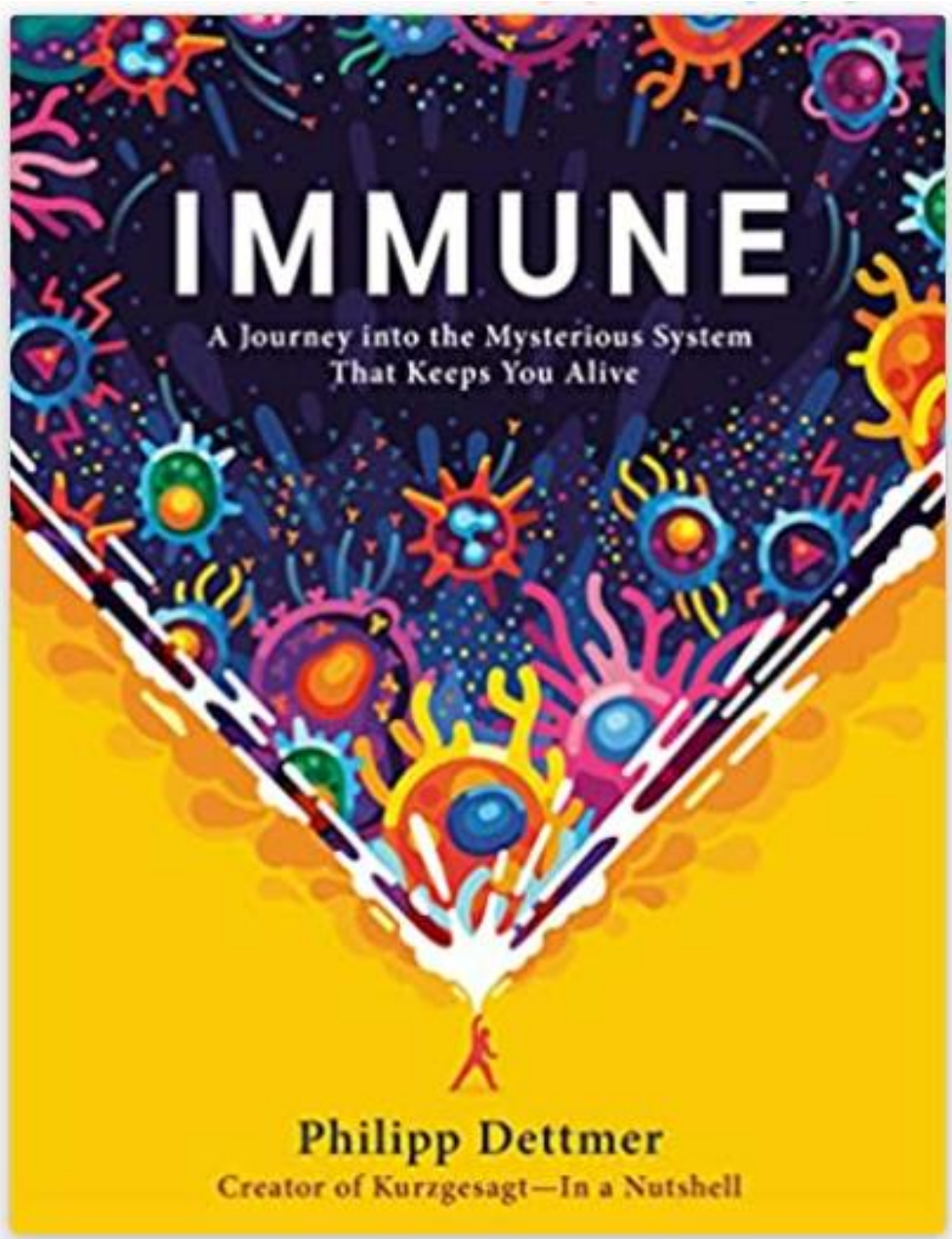

Jmol

Models make it real….

T-cells deploy perforin … which spontaneously assembles into a tube that punches a hole in the cancer cell … through which granzymes enter to trigger apoptosis of the cancer cell.

Is science cool,… or what?

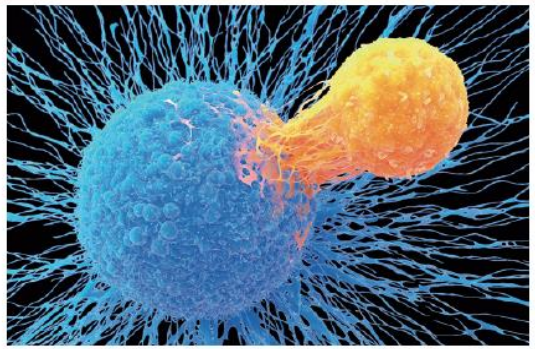



IMMUNE
A Journey into the Mysterious System That Keeps You Alive
Philipp Dettmer
Creator of Kurzgesagt—In a Nutshell

T cells

milestones

Milestones advisors



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Visit the interactive timeline online
The *Nature Milestones: T cells* collection contains all of the articles included herein along with details of other important events and multimedia components that are presented in an interactive timeline. Visit the content online using the QR code or URL provided on page S2.

Contributing journals
Communications Biology, Nature, Nature Communications, Nature Immunology, Nature Methods, Nature Reviews Drug Discovery, Nature Reviews Endocrinology, Nature Reviews Immunology, Nature Reviews Rheumatology

Citing the Milestones
Nature Milestones: T cells includes Milestone articles written by our editors and an online collection of previously published material. To cite the full project, please use *Nature Milestones: T cells* https://www.nature.com/collections/t-cells-milestones (2022). Should you wish to cite any of the individual Milestones, please list Author, A. Title. *Nature Milestones: T cells* <Article URL> (2022). To cite articles from the Collection, please use the original citation, which can be found online.

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    Antibodies/vaccines /and your immune system
    mRNA vaccines and multi-valent, ultra-potent vaccines…from synthetic biology.
  - Stories, stories and more stories….
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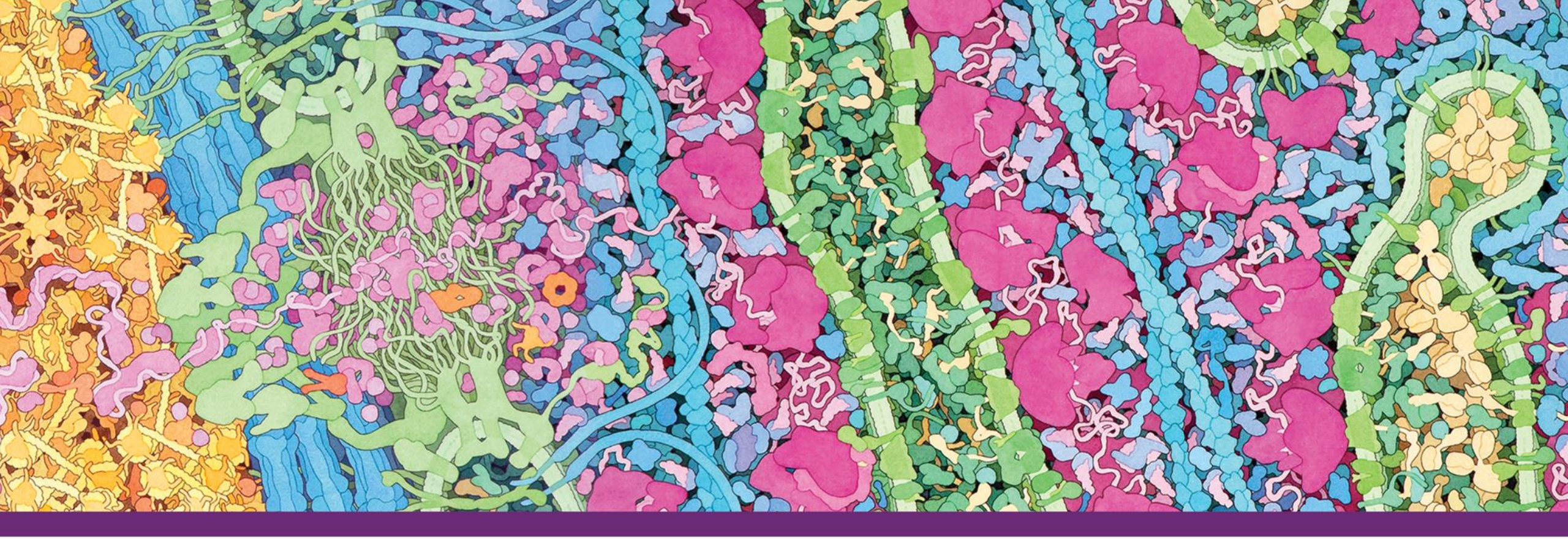


3d molecular designs

Transforming Science Learning

RESEARCH ARTICLE SUMMARY

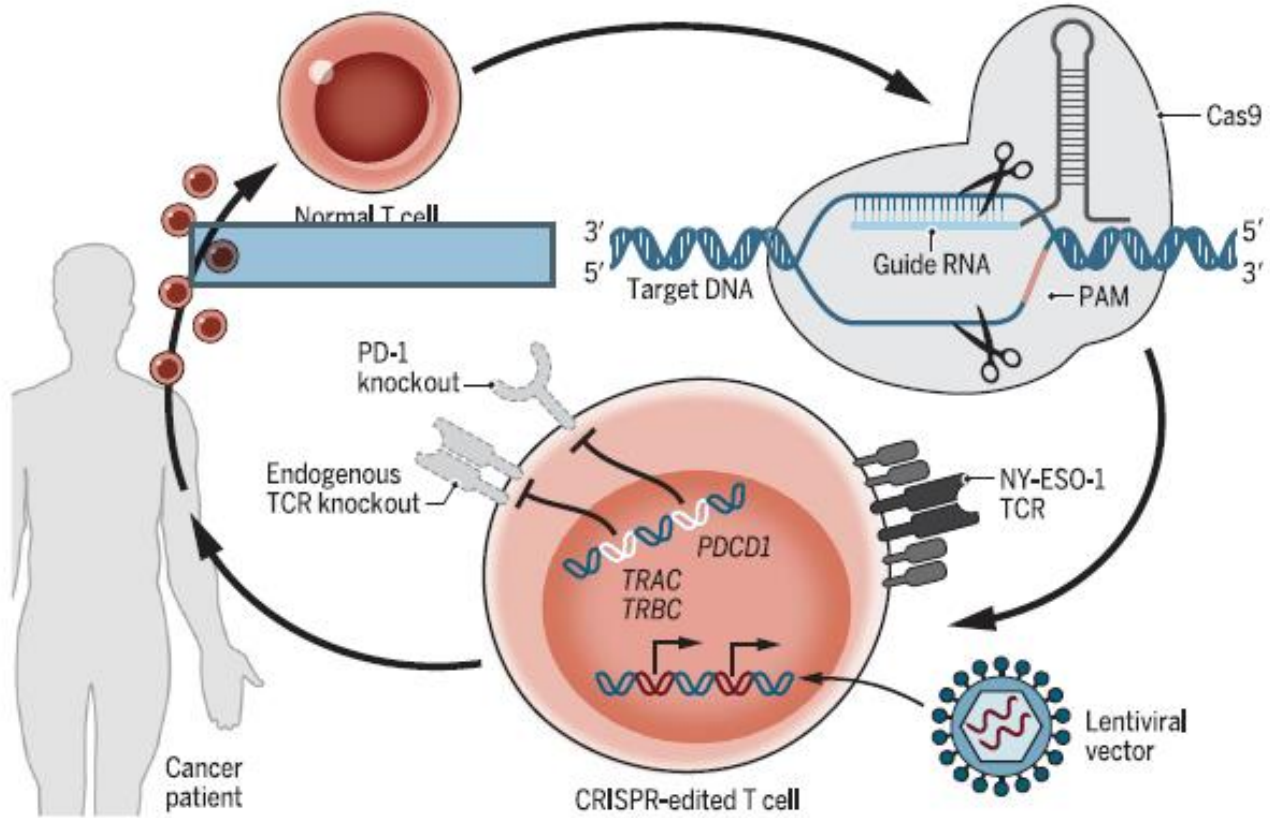
CLINICAL TRIALS

CRISPR-engineered T cells in patients with refractory cancer

Edward A. Stadtmauer*†, Joseph A. Fraietta*, Megan M. Davis, Adam D. Cohen, Kristy L. W
Eric Lancaster, Patricia A. Mangan, Irina Kulikovskaya, Minnal Gupta, Fang Chen, Lifeng Tia
Vanessa E. Gonzalez, Jun Xu, In-young Jung, J. Joseph Melenhorst, Gabriela Plesa, Joanne
Tina Matlawski, Amanda Cervini, Avery L. Gaymon, Stephanie Desjardins, Anne Lamontagn
January Salas-Mckee, Andrew Fesnak, Donald L. Siegel, Bruce L. Levine, Julie K. Jadlowsky
Regina M. Young, Anne Chew, Wei-Ting Hwang, Elizabeth O. Hexner, Beatriz M. Carreno,
Christopher L. Nobles, Frederic D. Bushman, Kevin R. Parker, Yanyan Qi, Ansuman T. Satpat
Howard Y. Chang, Yangbing Zhao, Simon F. Lacey*, Carl H. June*†

INTRODUCTION: Most cancers are recognized and attacked by the immune system but can progress owing to tumor-mediated immunosuppression and immune evasion mechanisms. The infusion of ex vivo engineered T cells, termed adoptive T cell therapy, can increase the natural antitumor immune response of the patient. Gene therapy to redirect immune specificity combined with genome editing has the potential to improve the efficacy and increase the safety of engineered T cells. CRISPR coupled with CRISPR-associated protein 9 (Cas9) endonuclease is a powerful gene-editing technology that potentially allows the ability to target multiple genes in T cells to improve cancer immunotherapy.

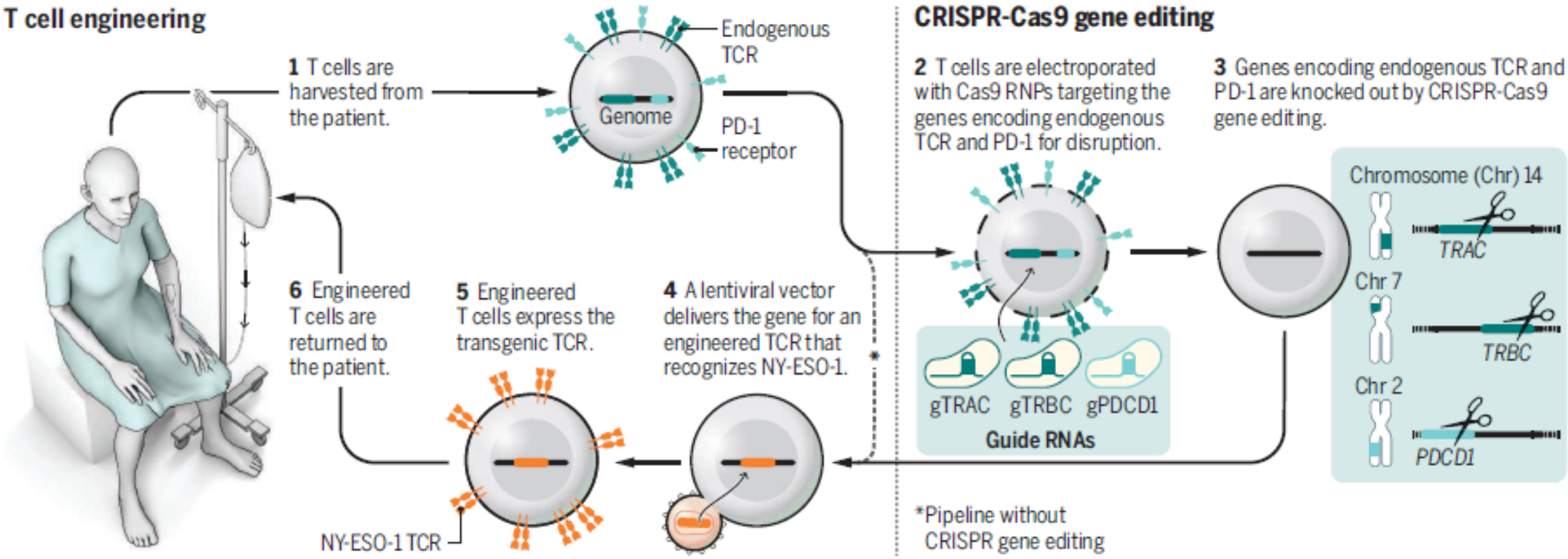
RATIONALE: Our first-in-human, phas
trial (clinicaltrials.gov; trial NCT0339
designed to test the safety and fea
multiplex CRISPR-Cas9 gene editing
from patients with advanced, refracto
A limitation of adoptively transferre
ficacy has been the induction of T ce
tion or exhaustion. We hypothes
removing the endogenous T cell recep
and the immune checkpoint mole
grammed cell death protein 1 (PD
improve the function and persisten
neered T cells. In addition, the remov
has the potential to improve safety a
toxicity that can be caused by autoi



CRISPR-Cas9 engineering of T cells in cancer patients. T cells (center) were isolated from the blood of a patient with cancer. CRISPR-Cas9 ribonuclear protein complexes loaded with three sgRNAs were electroporated into the normal T cells, resulting in gene editing of the *TRAC*, *TRBC1*, *TRBC2*, and *PDCD1* (encoding PD-1) loci. The cells were then transduced with a lentiviral vector to express a TCR specific for the cancer-testis antigens NY-ESO-1 and LAGE-1 (right). The engineered T cells were then returned to the patient by intravenous infusion, and patients were monitored to determine safety and feasibility. PAM, protospacer adjacent motif.

Modifying engineered T cells with CRISPR-Cas9 gene editing

Engineered T cells with improved anticancer activity can be generated through the targeted disruption of immunomodulatory genes, such as programmed cell death protein 1 (*PDCD1*, which encodes PD-1), and T cell receptor (TCR) genes (*TRAC* and *TRBC*), using CRISPR-Cas9 delivered as preformed ribonucleoproteins (RNPs). These cells are then modified to express an engineered TCR that recognizes cancer-testis antigen 1 (NY-ESO-1) expressed by cancer cells.



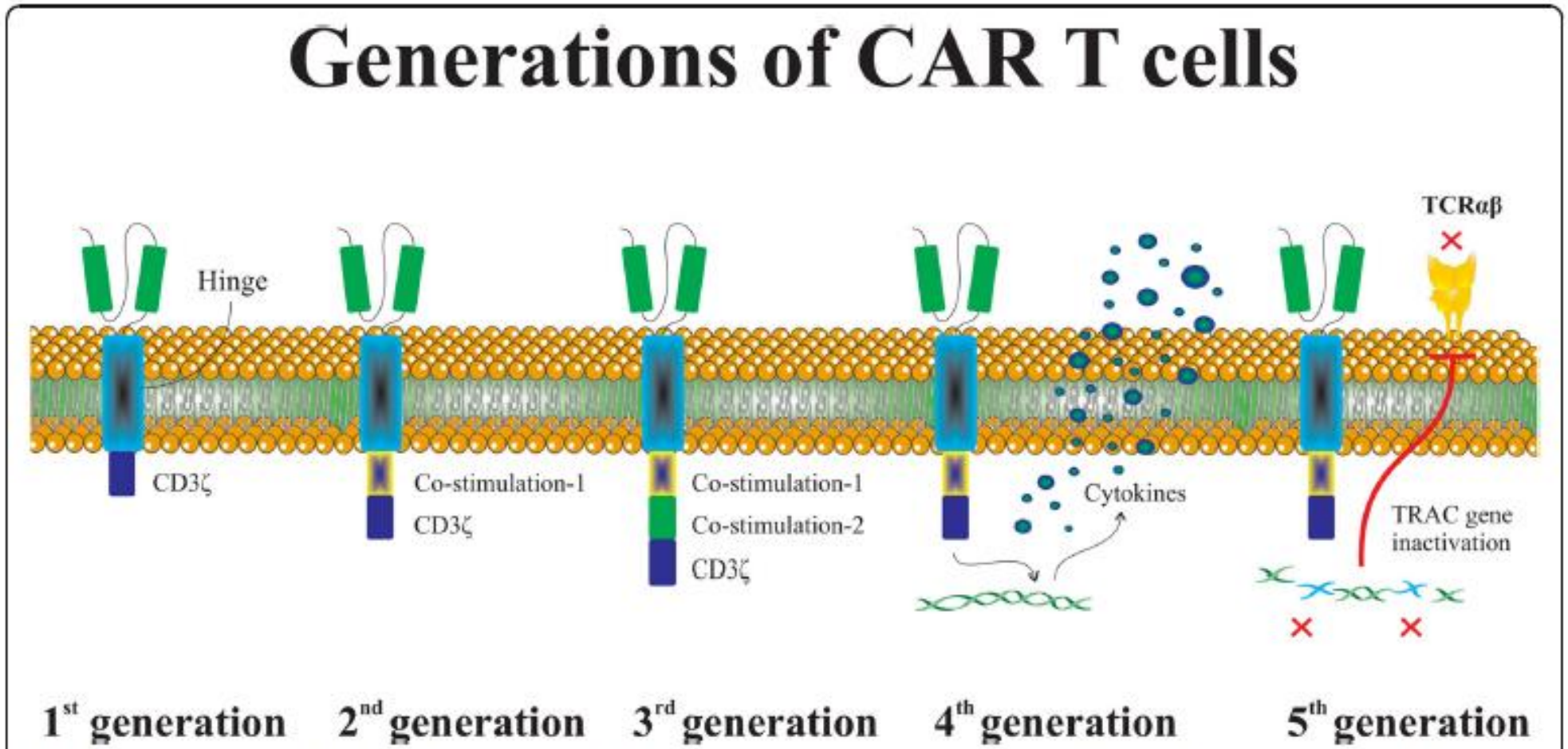


Fig. 1 The basic structure of CAR-T cell generations. The first generation of CARs contains only a CD3ζ as a well-documented intracellular signal domain. The second and third generations of CARs involve one or two costimulatory molecules in addition to CD3ζ, respectively. As well, the fourth generation of CAR-T cells strongly motivates the downstream transcription factor to prompt cytokine generation following interrelation between CAR and target antigen. Prominently, the genome edition technologies, such as CRISPR-Cas9, have been widely used to construct TRAC (TCR)-deficient CAR-T cells, establishing fifth generation of CAR-T cells