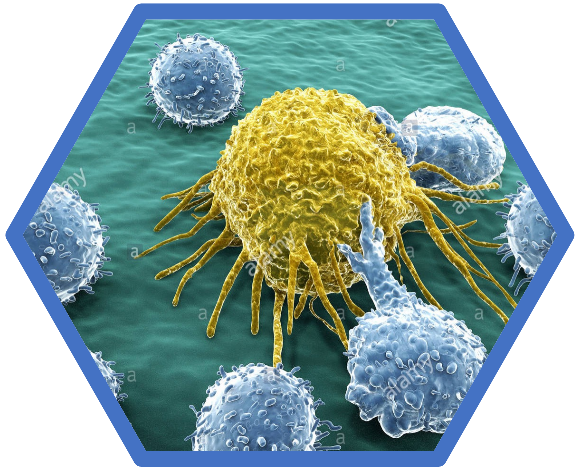
EPFL

Biomaterials for MX

MSE – 471

Maartje Bastings

Course 7: Materials for Immune Engineering



Course Content & Time Table

BLOCK 1: Introduction and materials overview

11-9	Lecture 1.	Intro to biomaterials and biology
18-9	Lecture 2.	Naturally derived biomaterials
25-9	Lecture 3.	Polymers and nanoparticles
2-10	Lecture 4.	Surfaces

BLOCK 2: Interactions and specific applications

9-10	Lecture 5.	Materials for drug delivery and targeting
16-10	Lecture 6.	Materials for cell adhesion
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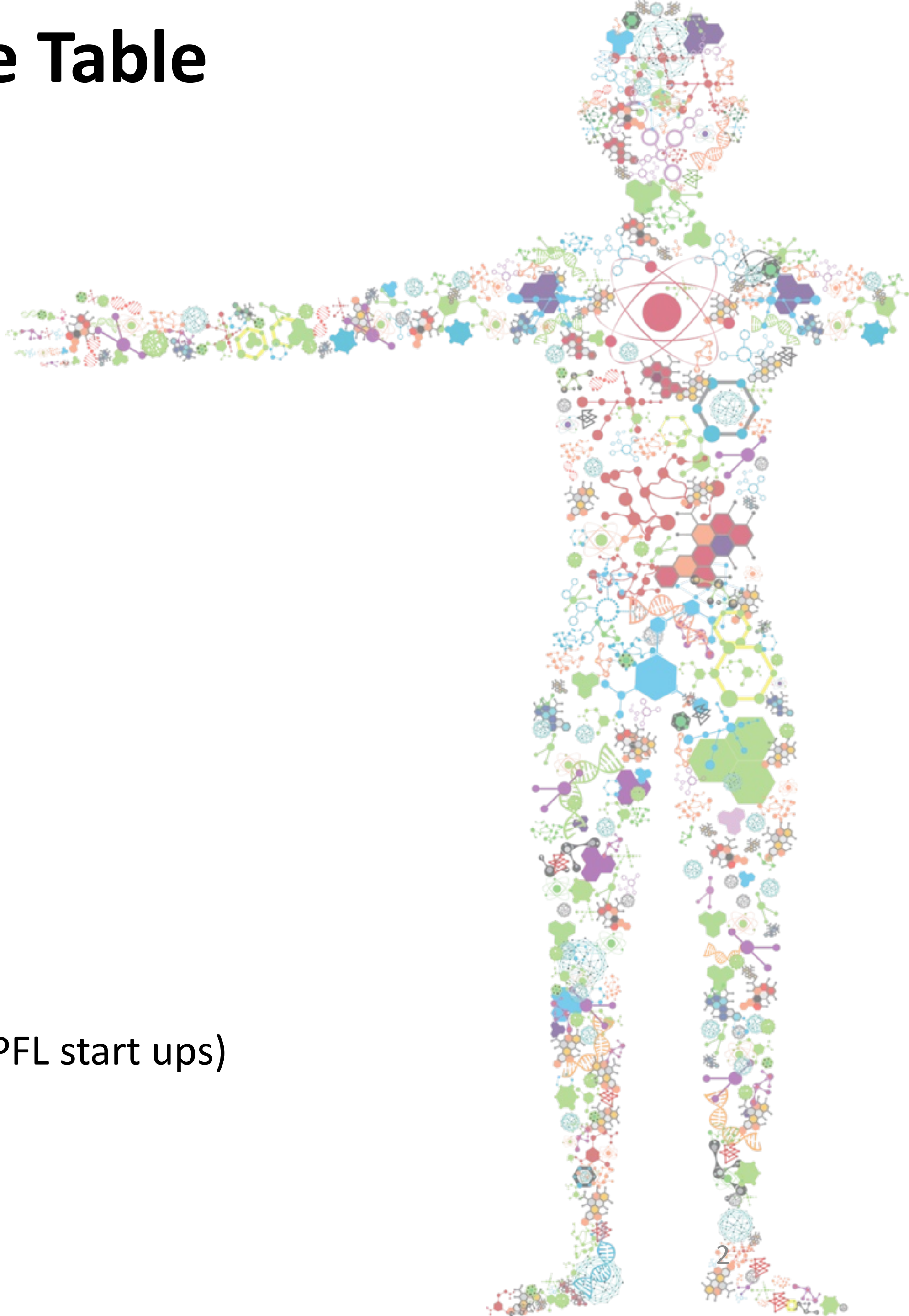
30-10	**Lecture 7.**	**Materials for immune engineering**
6-11	Lecture 8.	Materials for tissue engineering

BLOCK 3: Measurements, regulation and translation

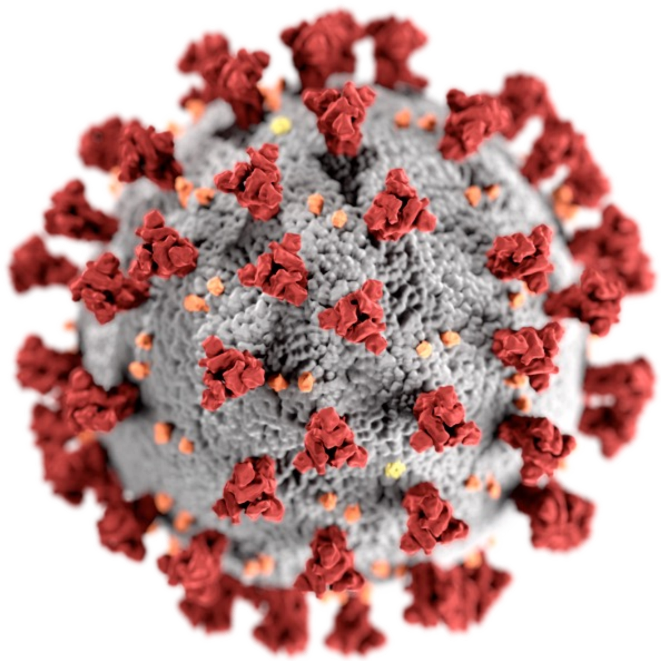
13-11	Lecture 9.	Characterization and performance
20-11	Lecture 10.	Sensors and diagnostic devices
27-11	Lecture 11.	Translation to industry, patents, spin-offs (EPFL start ups)
4-12	Lecture 12.	Regulatory aspects and trials (EPFL TTO)
11-12	Lecture 13.	Revision and conclusion
18-12	Open discussion and hand in of lab papers	



Immune System & Materials

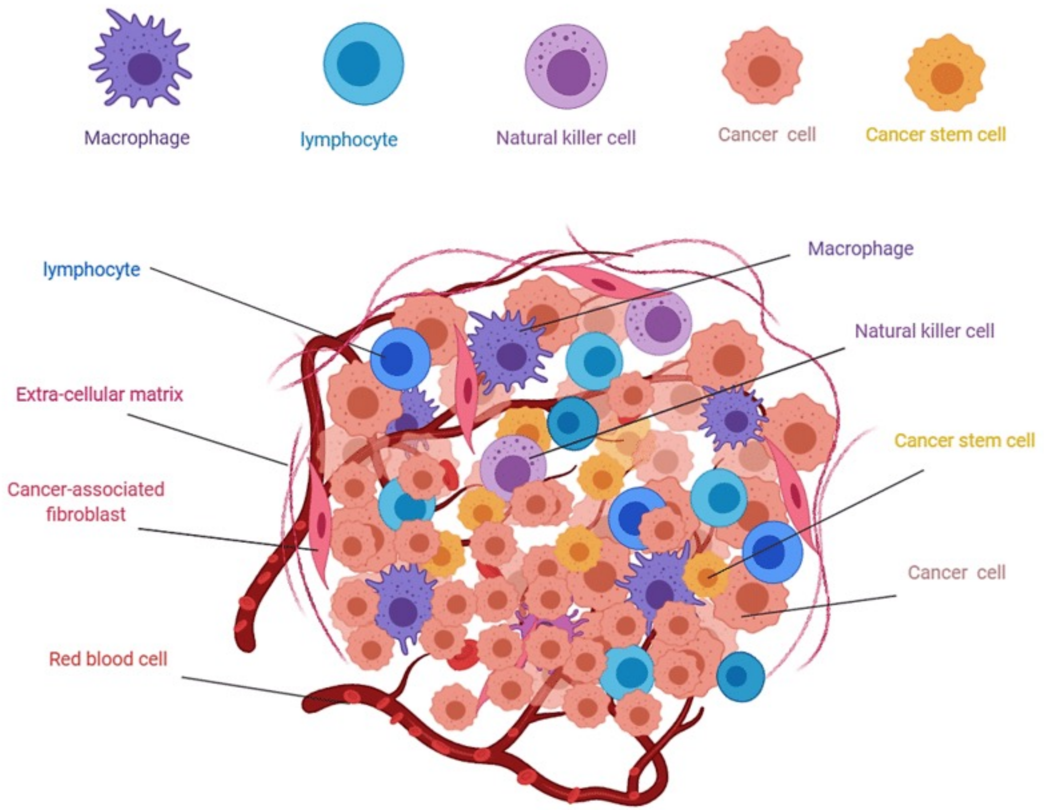
VACCINES

Pathogens

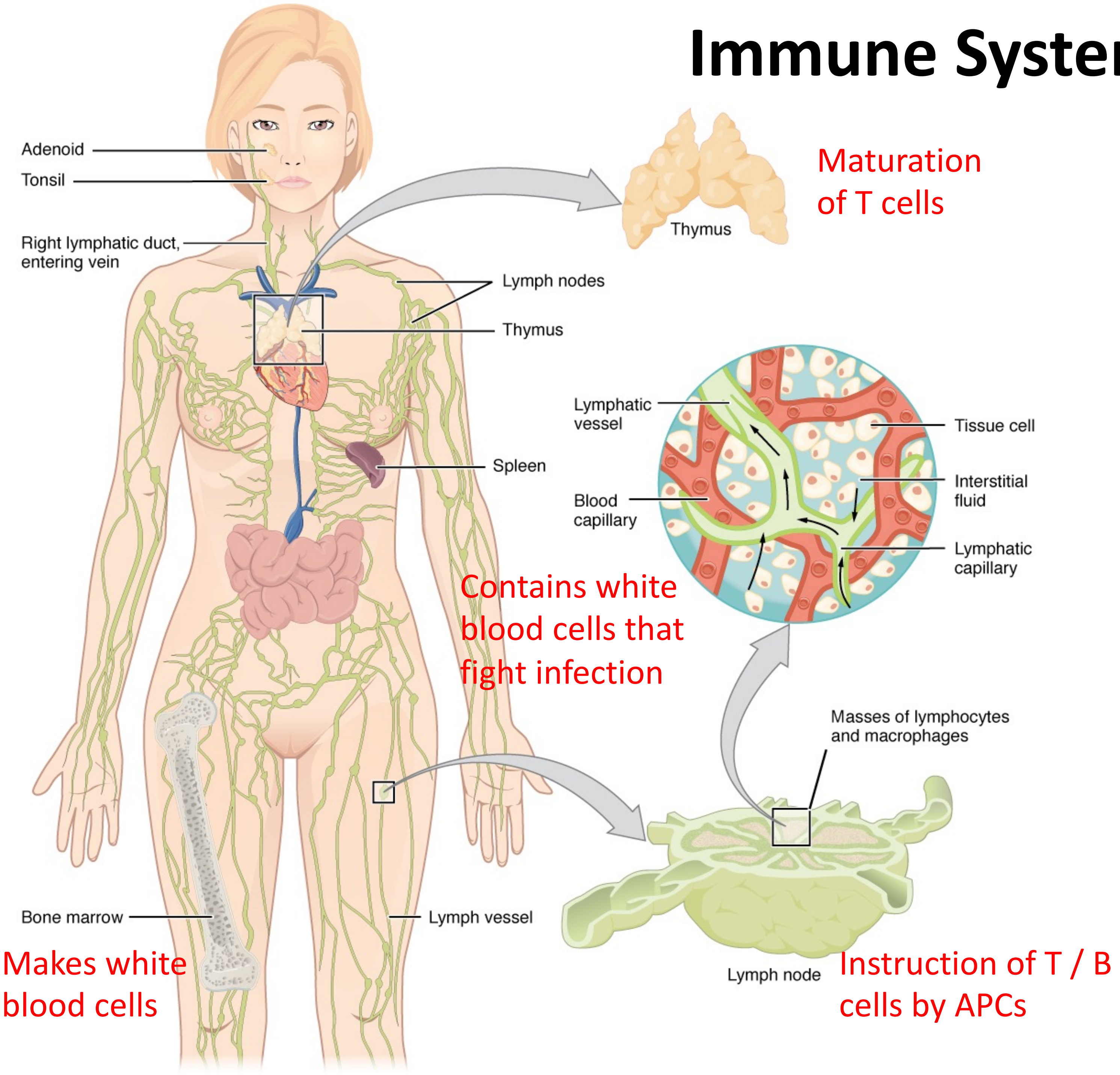


CAR-T / immune therapy

Cancer



Immune System

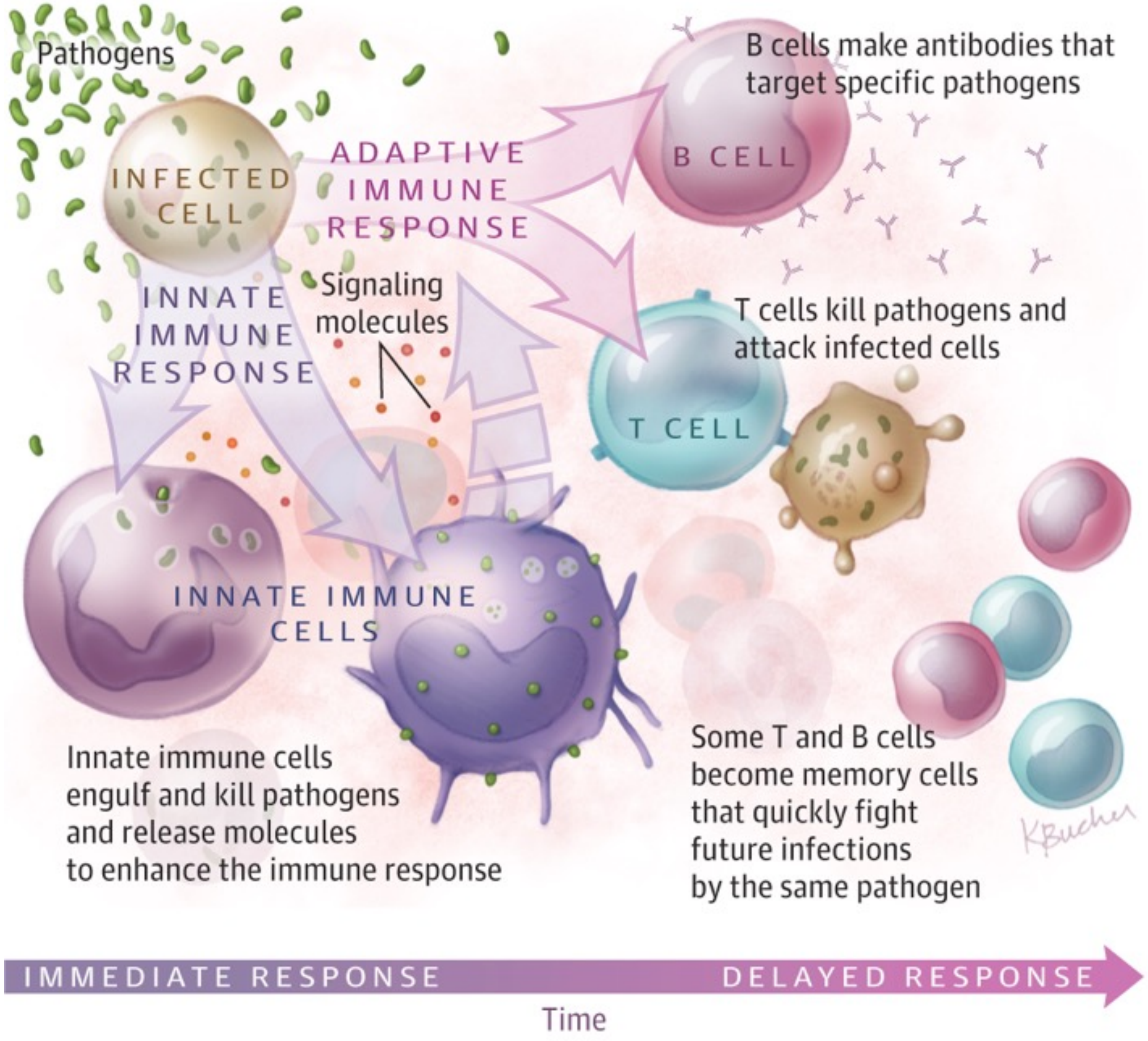


3 levels to protect us from invaders:

1) **Natural barriers** to keep bacteria / viruses out
2) **Innate system**: circulating cells that stop invaders from spreading
3) **Adaptive system**: active attack when invaders are there, triggered by 2

https://med.libretexts.org/Bookshelves/Ancillary_Materials/Laboratories/SC%3A_BIOL_250_-_HUMAN_ANATOMY_(Aquino)/18%3A_The_Lymphatic_System

Innate and Adaptive immune system



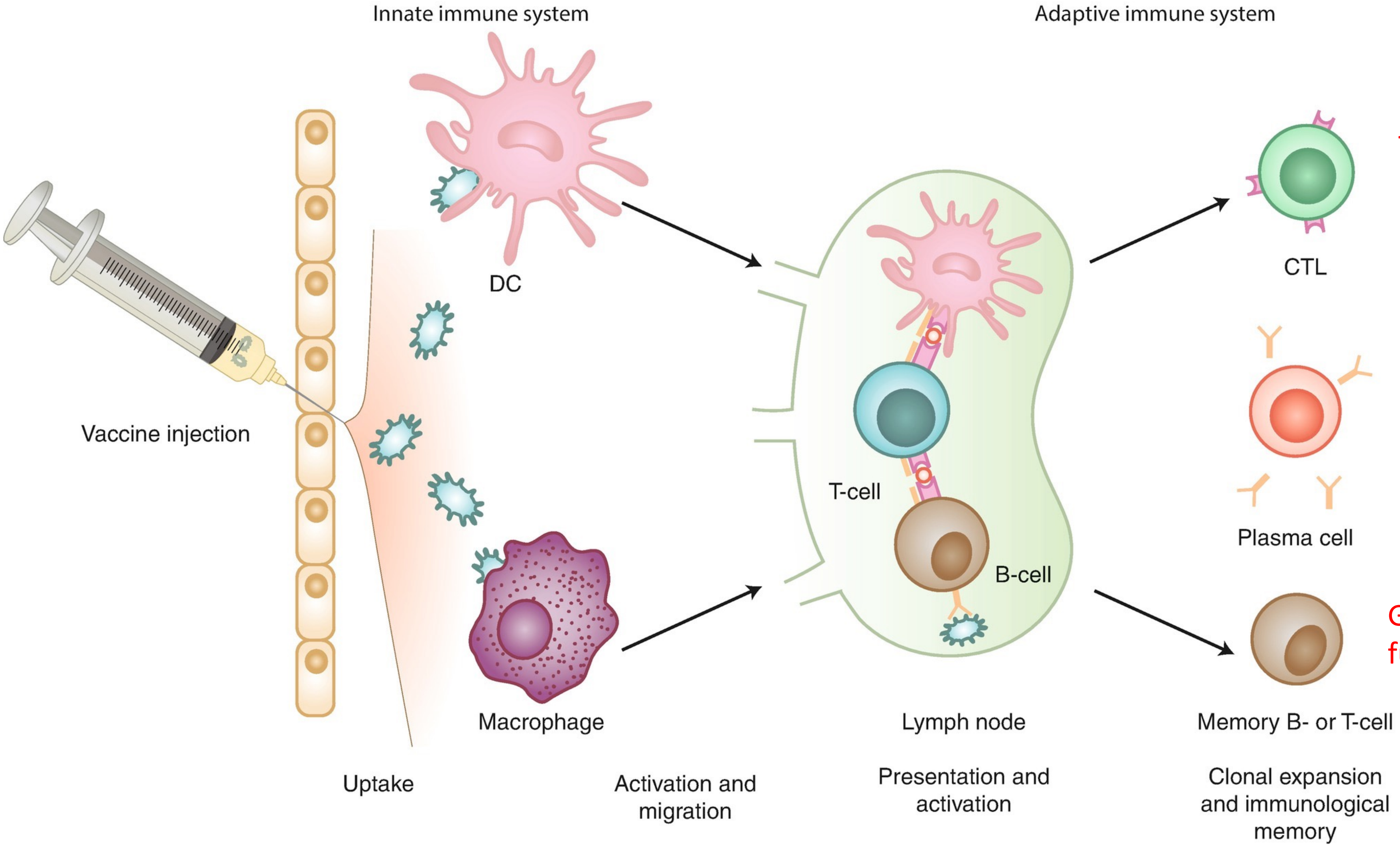
Innate Immune Response

- Immediate & (mostly) broad
- Macrophages
- Dendritic cells
- Release factors to activate adaptive response

Adaptive Immune Response

- B cells
- T cells
- Highly specific
- Are activated in lymph nodes by antigen presenting cells (APCs)
- Direct fight & memory
- Delayed response

Steps in Vaccination



T cells:
Kill infected cells

B cells:
Make antibodies

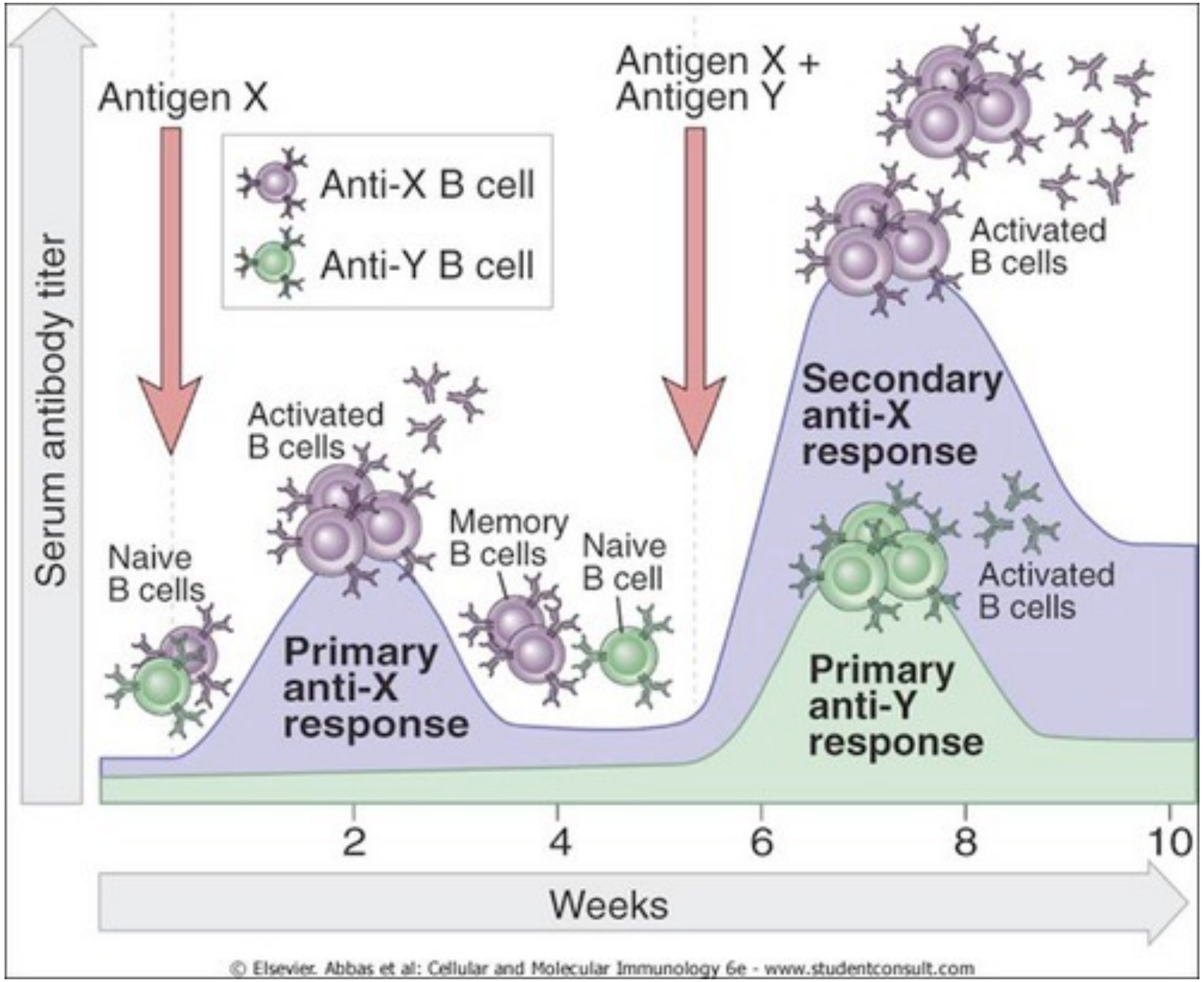
Generate memory for future challenge

How does vaccination prevent disease?

The **memory B cells** keep a **memory** of the organism that you were vaccinated against. If you are ever exposed to that organism, memory cells will recognize it straight away, and **rapidly start multiplying**. Because they have already been trained to produce antibodies against the organism, they are able to produce a large number of antibodies very quickly (within hours).

The **antibodies attach** to the invading organisms and prevent them from attacking your healthy cells. Because the antibodies are produced so quickly, they are able to **fight the disease before you even get sick**.

This accelerated and more intense immune response generated by the memory B cells is known as the **secondary response**. It is faster and more effective because all the preparations for the attack were made when you were vaccinated.

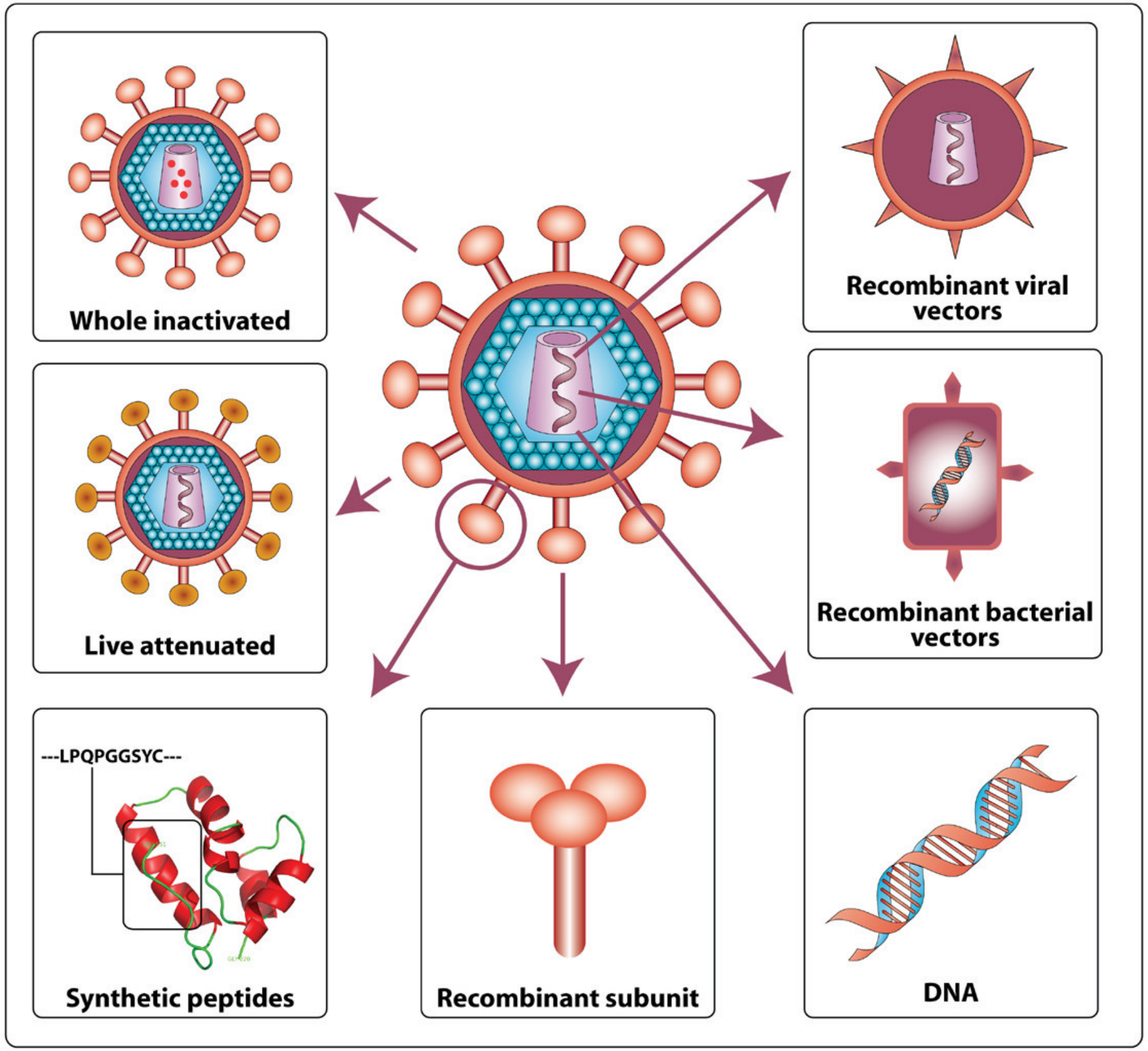


Different Types of Vaccines

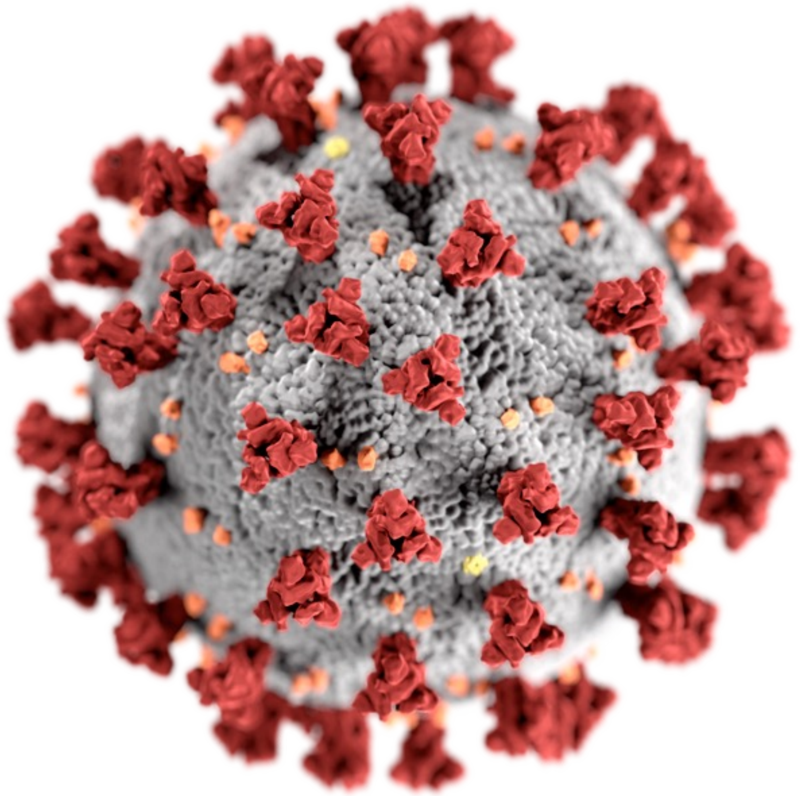
There are 5 main types of vaccines:

1. **live attenuated vaccines**, which contain a living, but weakened, form of the germ (organism);
2. **inactivated vaccines**, which contain a killed form of the organism;
3. **subunit vaccines**, which contain just the protein/lipid part of the organism that stimulates an immune response (the antigen);
4. **toxoid vaccines**, which contain an inactivated bacterial toxin (toxoid) – target immune response to the toxin instead of the whole germ
5. **RNA vaccines,** which delivery the mRNA that codes for pathogen specific protein receptors

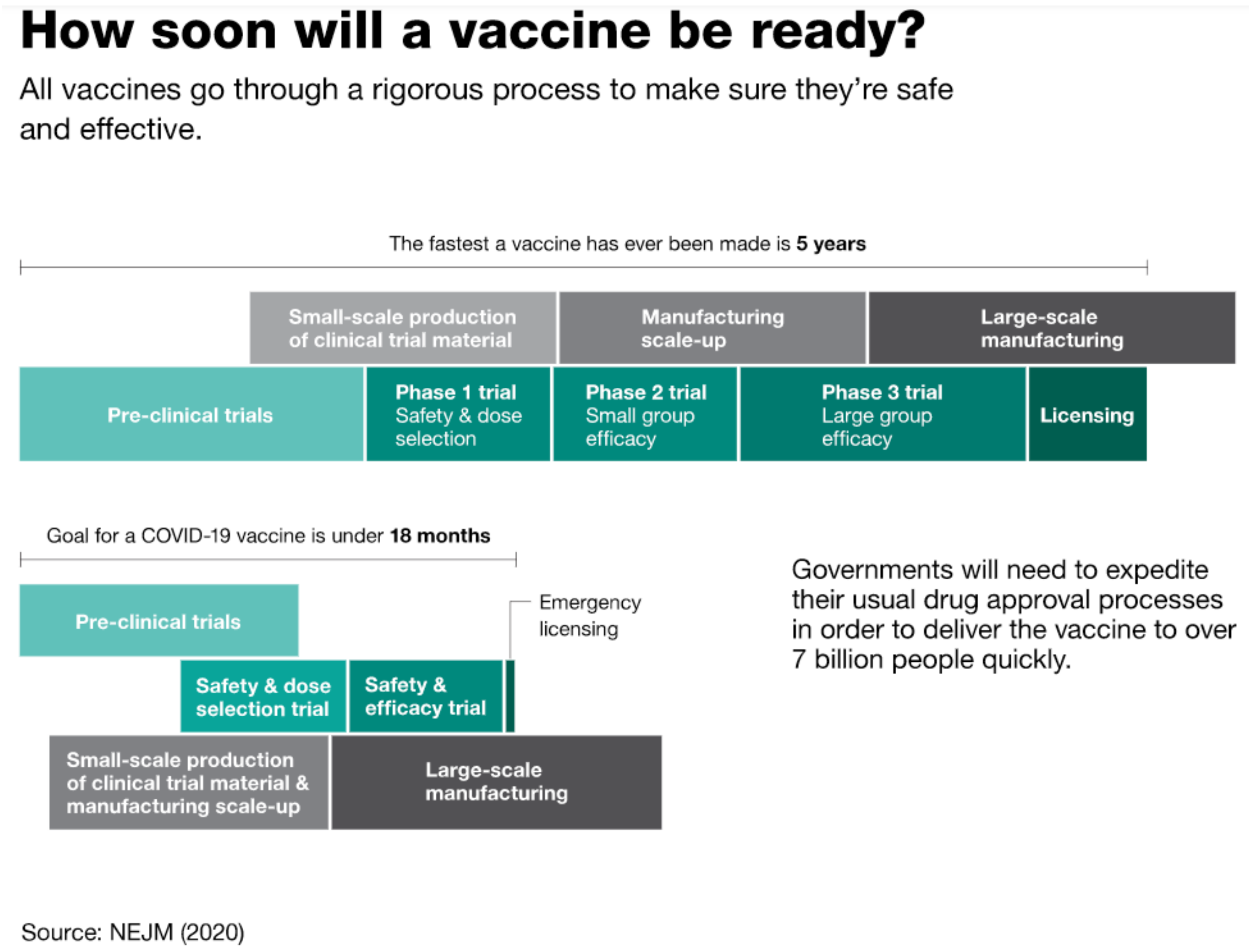
While the live vaccines can provide lifelong immunity after only one or 2 doses, **periodic booster doses** are needed to maintain immunity with some of the other types of vaccines.



The Vaccine against SARS-CoV-2 / COVID-19



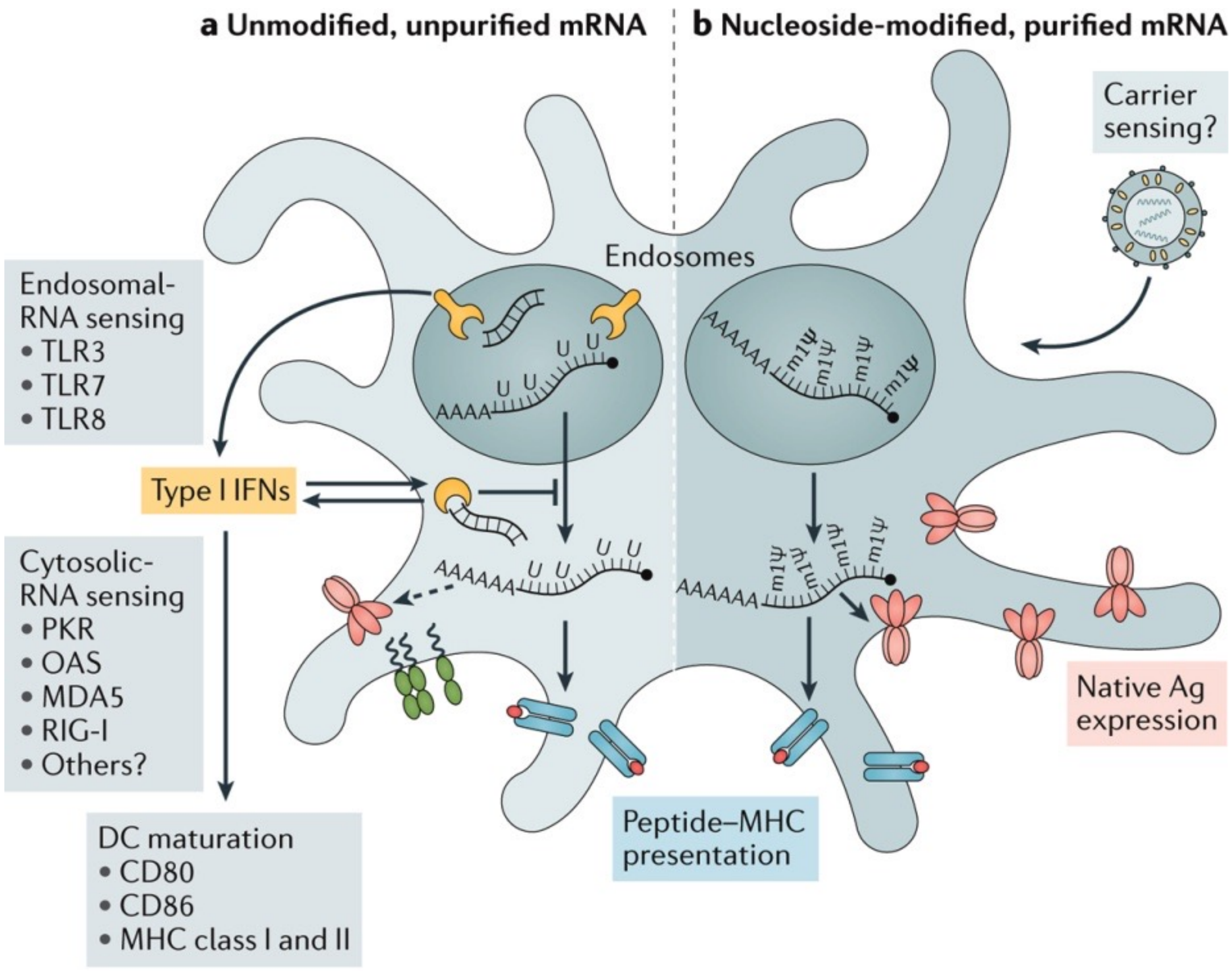
Problem with respiratory viruses:
Infective particles are extracellular
Cannot kill outside the body
Infection needs to happen first
to build immunity



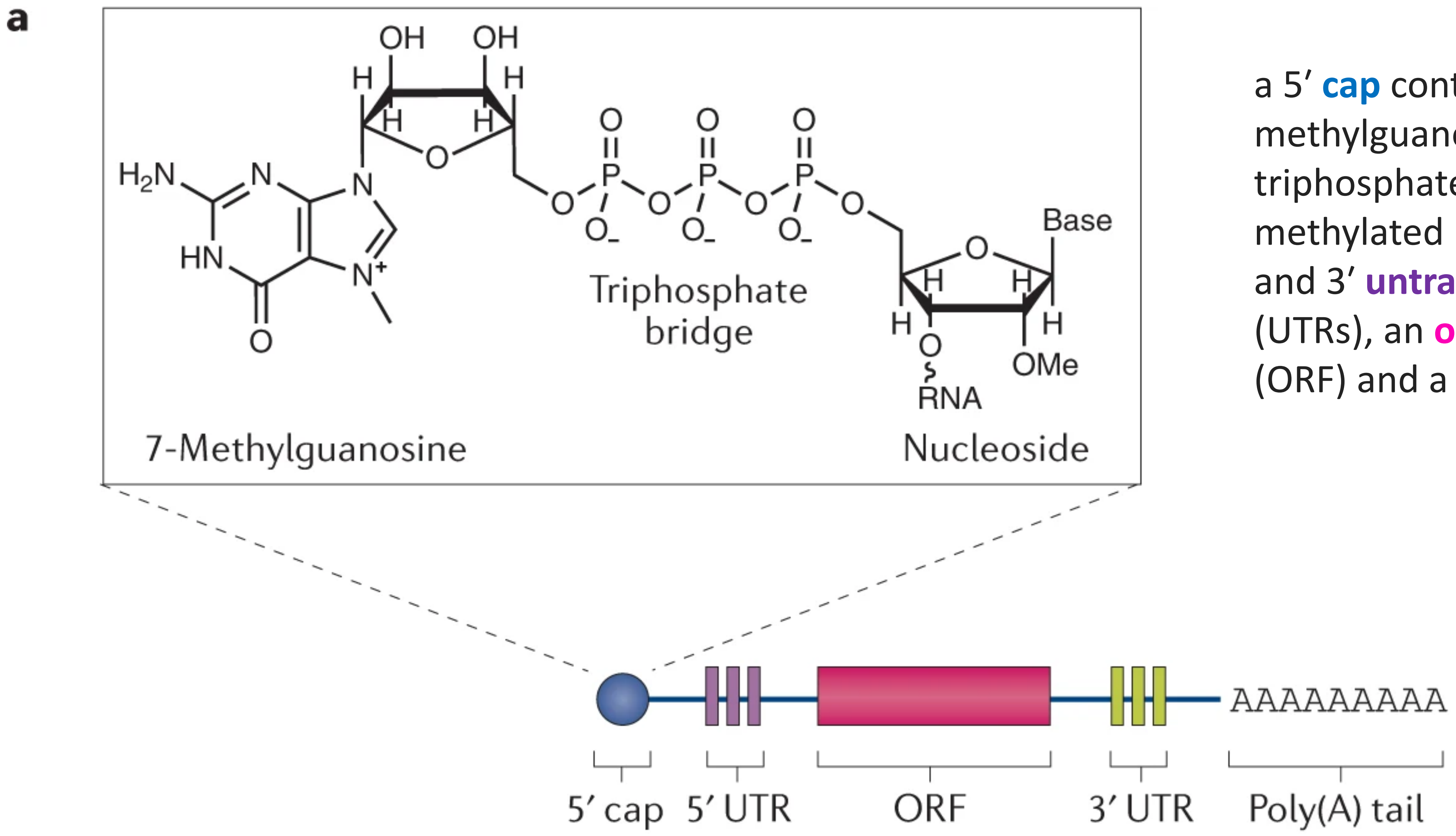
mRNA vaccines

PROBLEM (?): Exogenous mRNA is inherently immunostimulatory, as it is recognized by a variety of cell surface, endosomal and cytosolic innate immune receptors

→ innate immune sensing of mRNA has been associated with the inhibition of antigen expression and may negatively affect the immune response

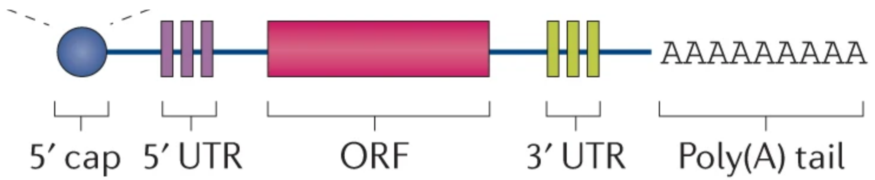


Designing the mRNA



a 5′ **cap** containing 7-methylguanosine linked through a triphosphate bridge to a 2′-*O*-methylated nucleoside, flanking 5′ and 3′ **untranslated regions** (UTRs), an **open reading frame** (ORF) and a **poly(A) tail.**

Designing the mRNA



CAP function
[1] the first or second nucleotide from the 5′ end is methylated on the 2′ hydroxyl of the ribose (2′-*O*-methylation), which **prevents recognition by cytosolic sensors of viral RNA**, and **prevents unintended immune responses**.
[2] **protects the mRNA sterically from degradation** by exonucleases,
[3] works **synergistically with the poly(A) tail** at the 3′ end, poly(A) binding proteins and translation initiation factor proteins to **circularize mRNA and recruit ribosomes for initiating translation**

5′ and 3′ **untranslated regions function**
[1] regulate mRNA translation, half-life and subcellular localization
[2] minimize mRNA degradation by excluding miRNA-binding sites
[3] minimize regions that prevent ribosomes from scanning the mRNA transcript (hairpins)

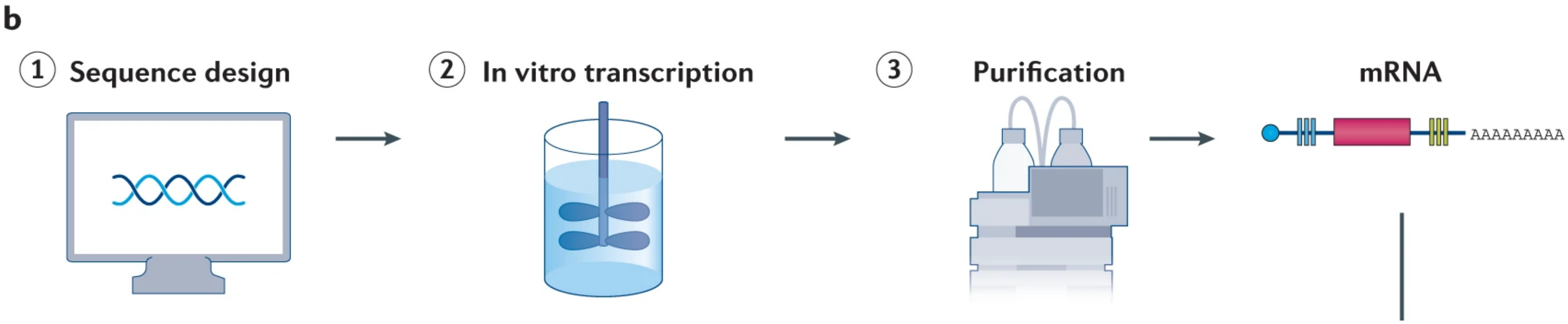
open reading frame of the mRNA vaccine is the most crucial component because it contains the coding sequence that is translated into protein.

contains modified nucleosides, such as pseudouridine, N1-methylpseudouridine or other analogues to reduce innate immune recoginition

Both the Moderna and Pfizer–BioNTech SARS-CoV-2 vaccines contain modified mRNA

polyA (~100nt) tail: protects

Producing the mRNA



(1) Once the genome of a pathogen has been sequenced, a sequence for the target antigen is designed and inserted into a plasmid DNA construct.

(2) Plasmid DNA is transcribed into mRNA by bacteriophage polymerases in vitro

(3) mRNA transcripts are purified by high performance liquid chromatography (HPLC) to remove contaminants and reactants.

mRNA is not stable → MATERIALS

Lipid-based nanoparticles (LNPs) are the most clinically advanced of the mRNA delivery vehicles.

All SARS-CoV-2 mRNA vaccines in development or approved for clinical use as of June 2021 employ LNPs.

Benefits for mRNA delivery: ease of formulation, modularity, biocompatibility and large mRNA payload capacity.

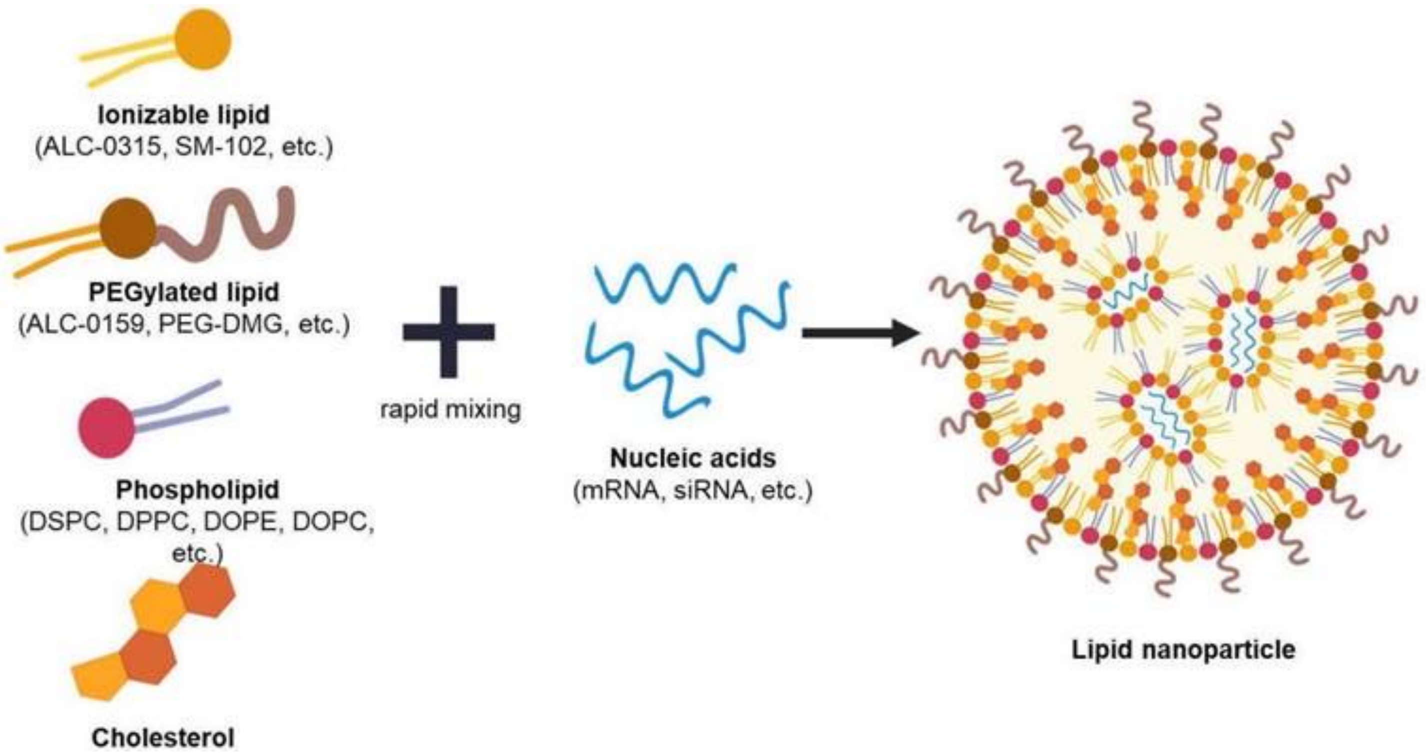
[1] the RNA drug

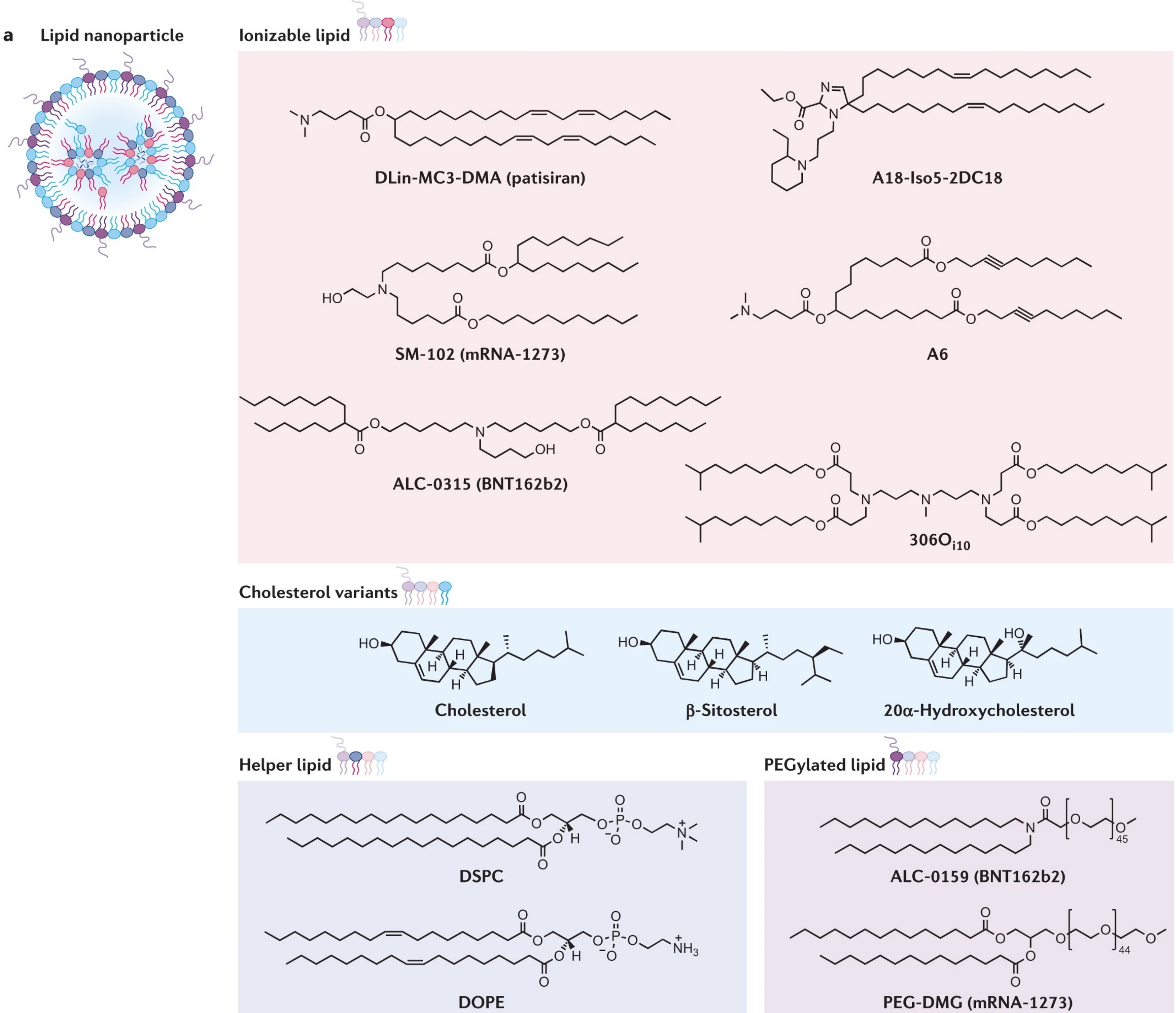
[2] an ionizable lipid

[3] cholesterol

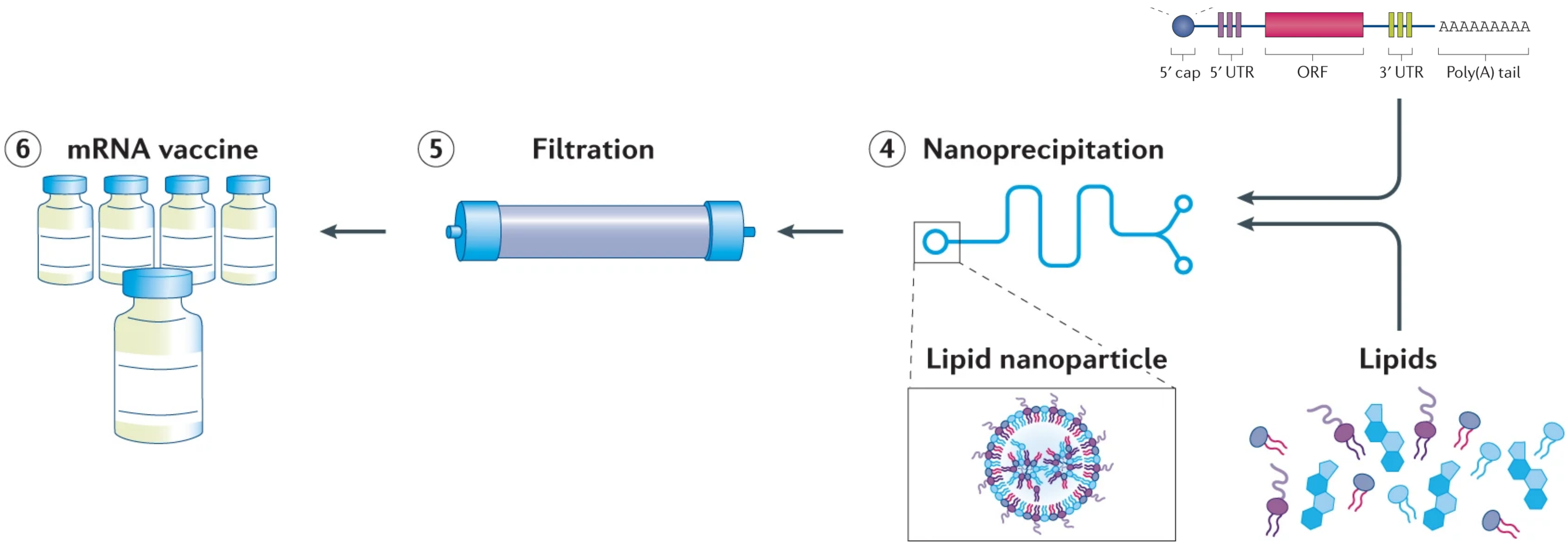
[4] a helper phospholipid

[5] a PEGylated lipid




a
Lipid nanoparticle
Ionizable lipid
DLin-MC3-DMA (patisiran)
A18-Iso5-2DC18
SM-102 (mRNA-1273)
A6
ALC-0315 (BNT162b2)
$306O_{i10}$
Cholesterol variants
Cholesterol
β-Sitosterol
20α-Hydroxycholesterol
Helper lipid
DSPC
DOPE
PEGylated lipid
ALC-0159 (BNT162b2)
PEG-DMG (mRNA-1273)

Vaccine formulation



(6) the filtered mRNA vaccine solution is stored in sterilized vials.

(5) The nanoparticle solution is dialysed or filtered to remove non-aqueous solvents and any unencapsulated mRNA and

(4) Purified mRNA is mixed with lipids in a microfluidic mixer to form lipid nanoparticles. Rapid mixing causes the lipids to encapsulate mRNA instantaneously and precipitate as self-assembled nanoparticles.

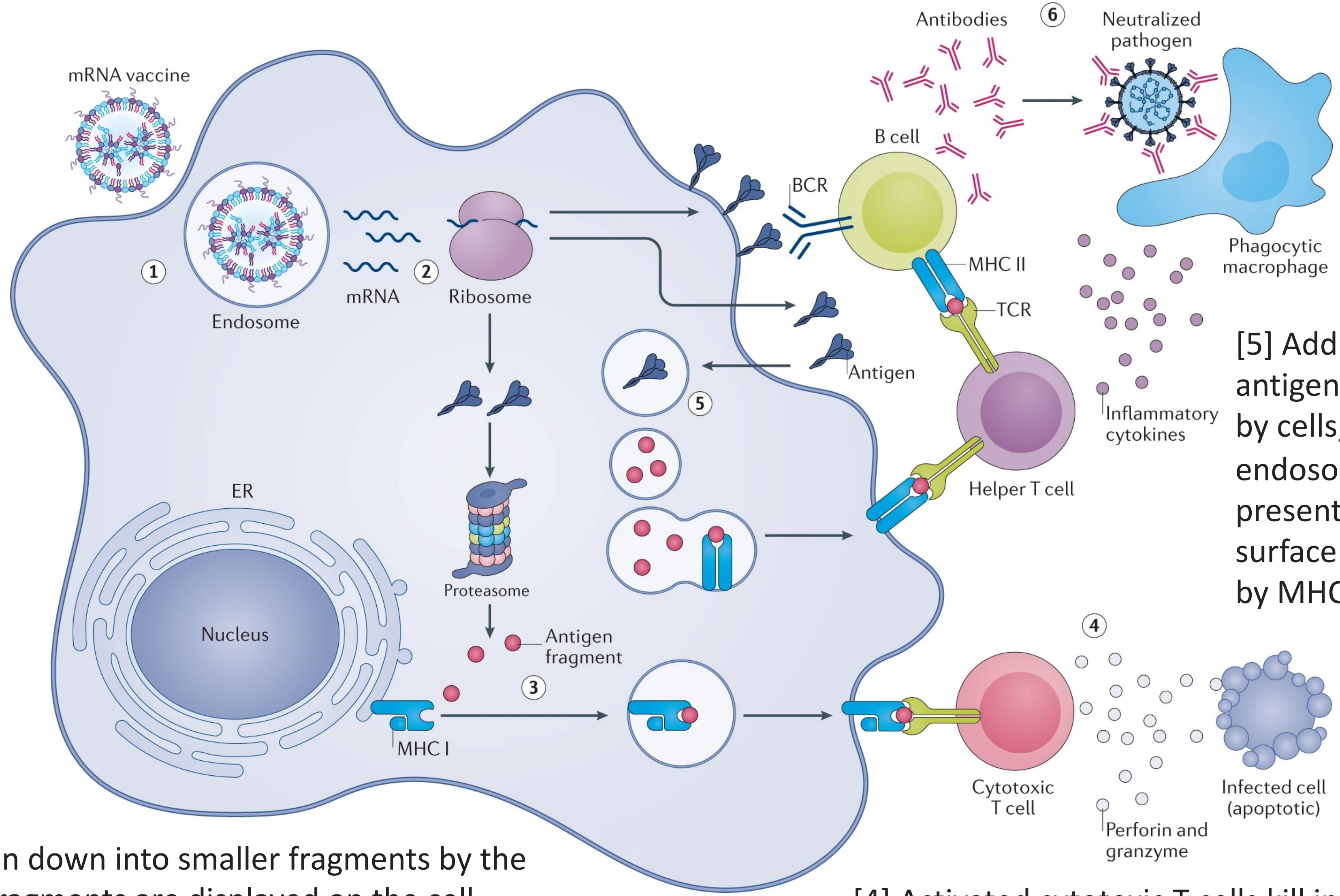

VACCINE
COVID-19

Vaccination

[6] Helper T cells facilitate the clearance of circulating pathogens by stimulating B cells to produce neutralizing antibodies, and by activating phagocytes, such as macrophages, through inflammatory cytokines.

[1] Injected mRNA vaccines are endocytosed by APCs

[2] After escaping the endosome, mRNA is translated into protein by the ribosome. The translated antigenic protein can stimulate the immune system in several ways.



[5] Additionally, secreted antigens can be taken up by cells, degraded inside endosomes and presented on the cell surface to helper T cells by MHC class II proteins.

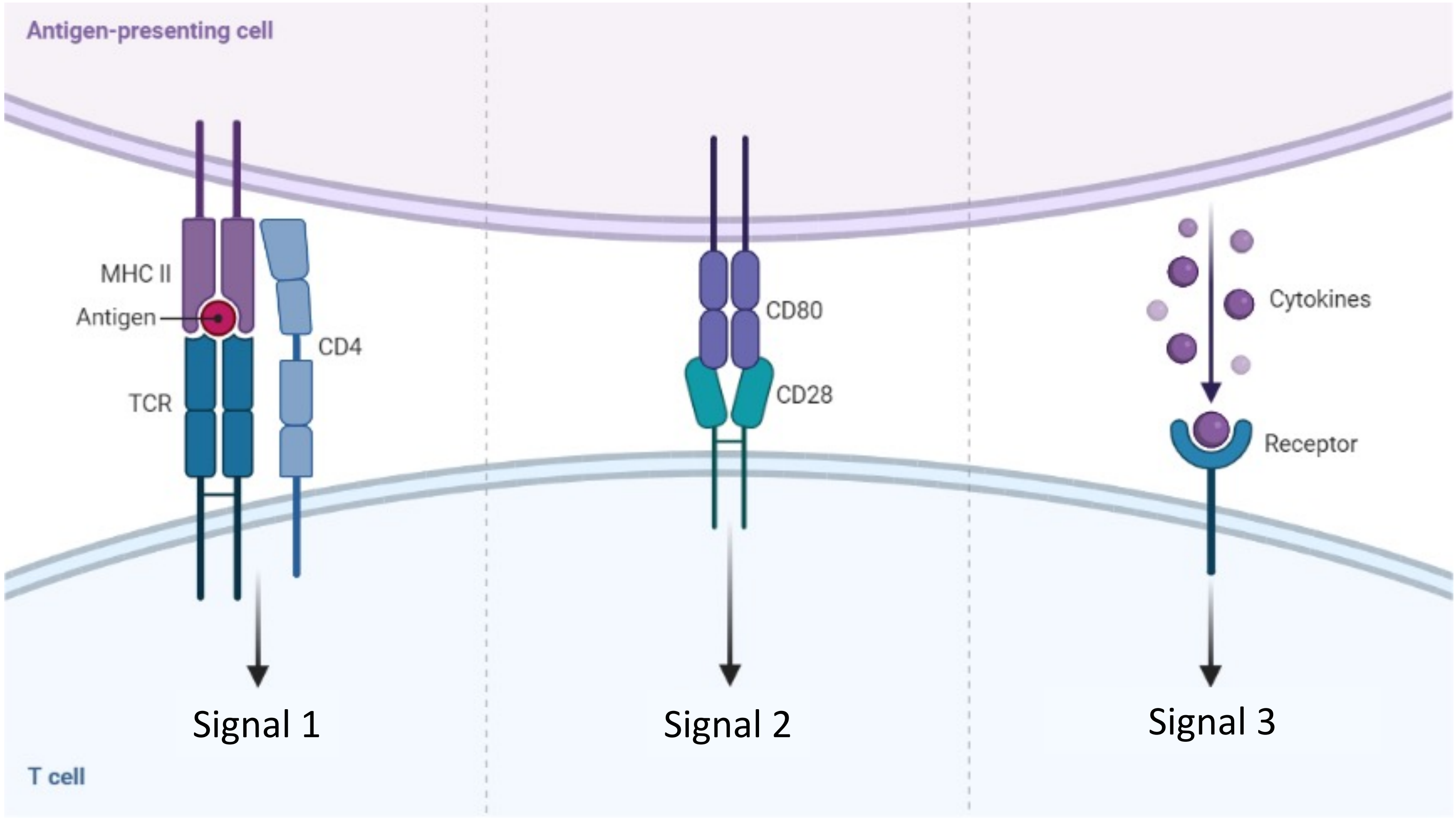
[3] Intracellular antigen is broken down into smaller fragments by the proteasome complex, and the fragments are displayed on the cell surface to cytotoxic T cells by major histocompatibility complex (MHC) class I proteins.

[4] Activated cytotoxic T cells kill infected cells by secreting cytolytic molecules, such as perforin and granzyme.

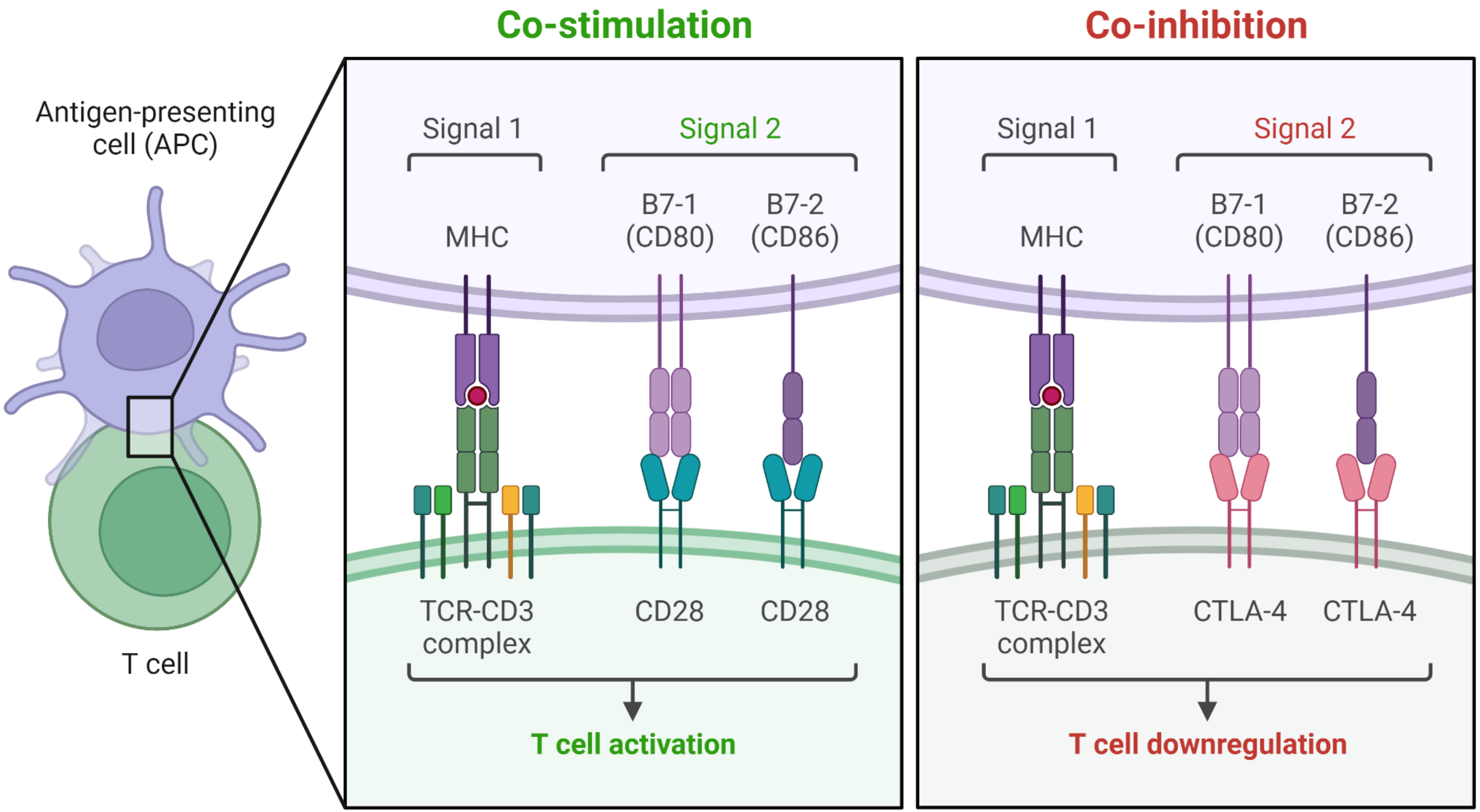
Vaccination side effects

BREAK

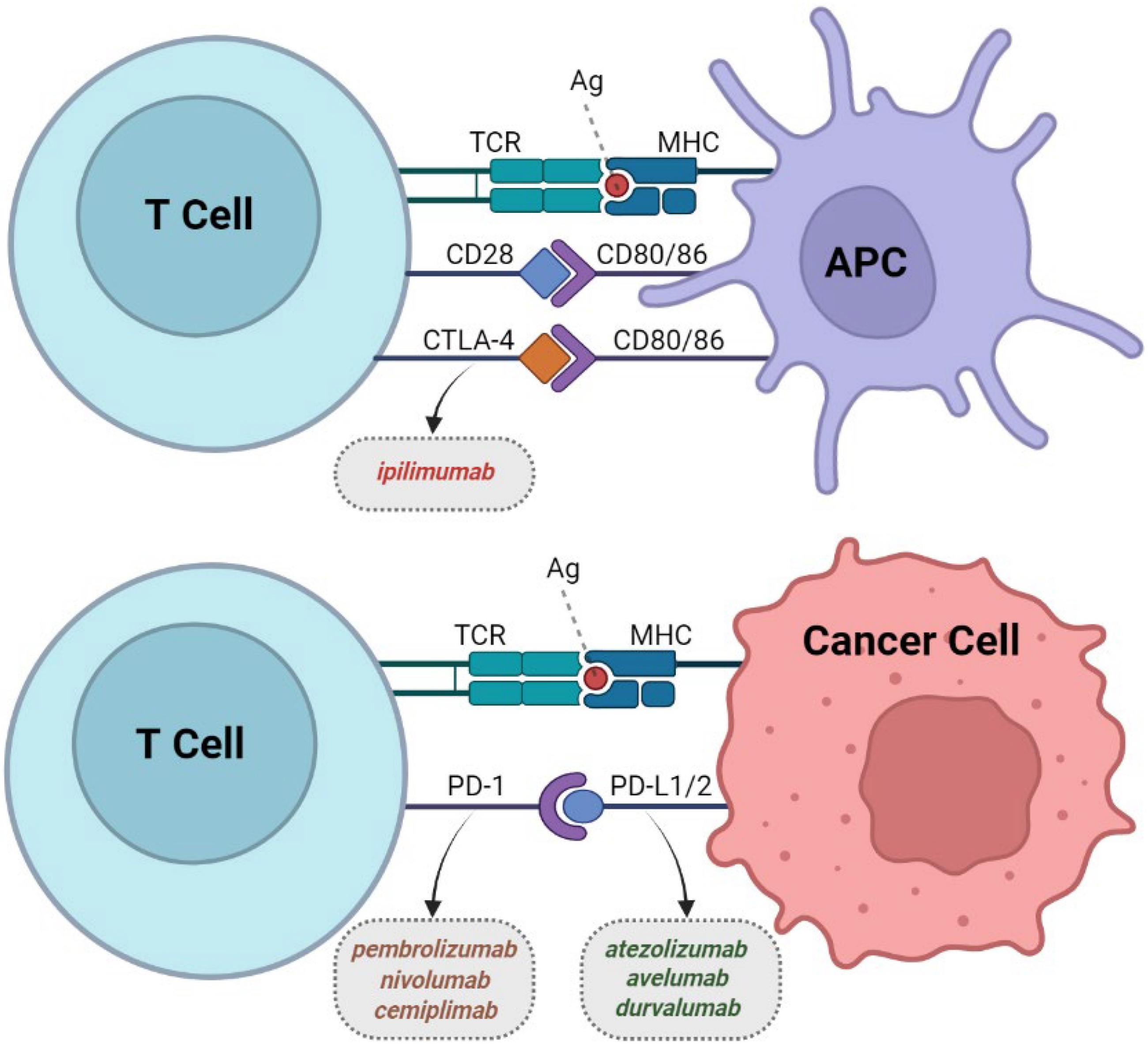
Immune Communication



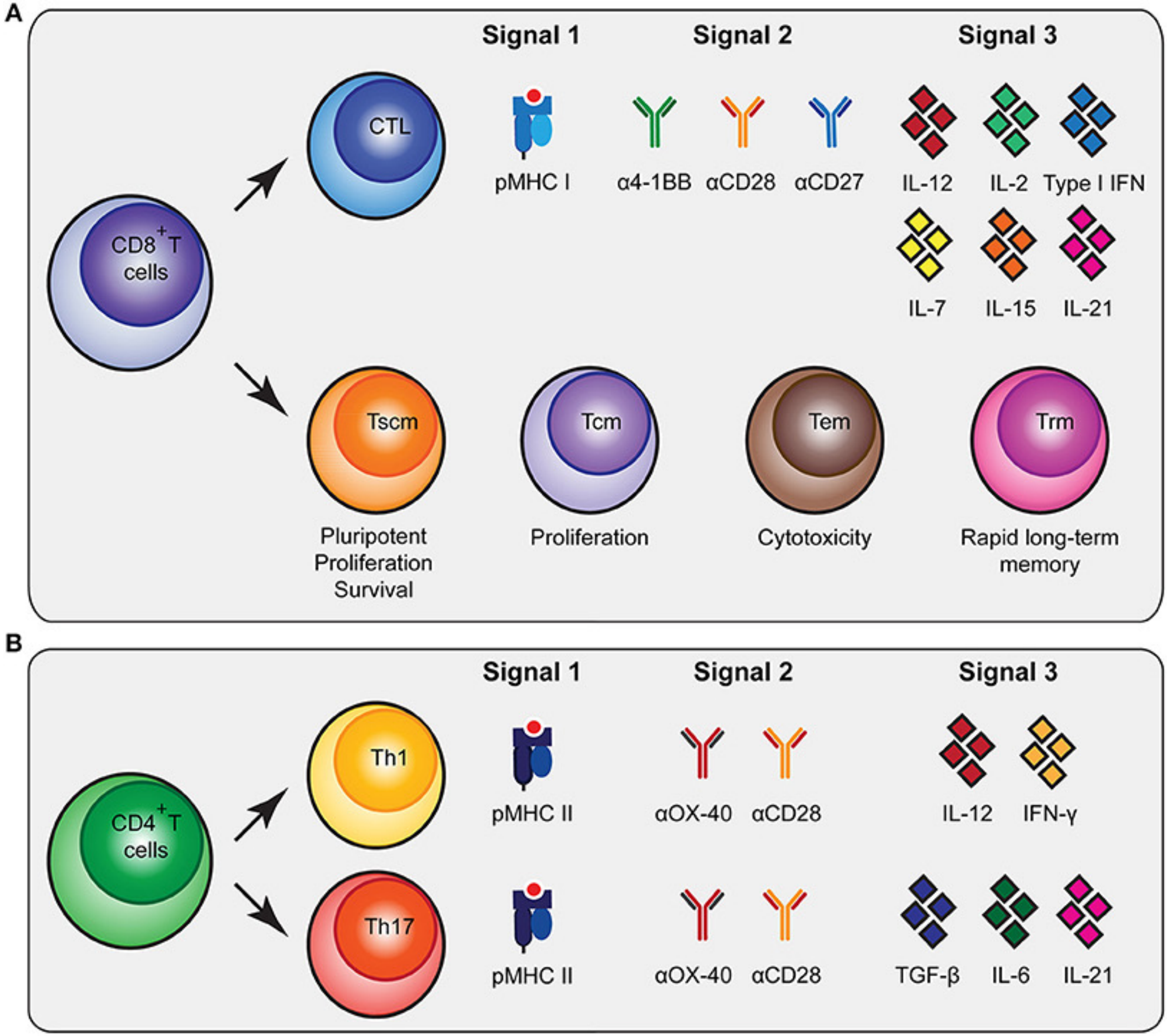
Immune Communication



Cancer checkpoint therapy



Synthetic cues for T cell stimulation → Combine in Materials!

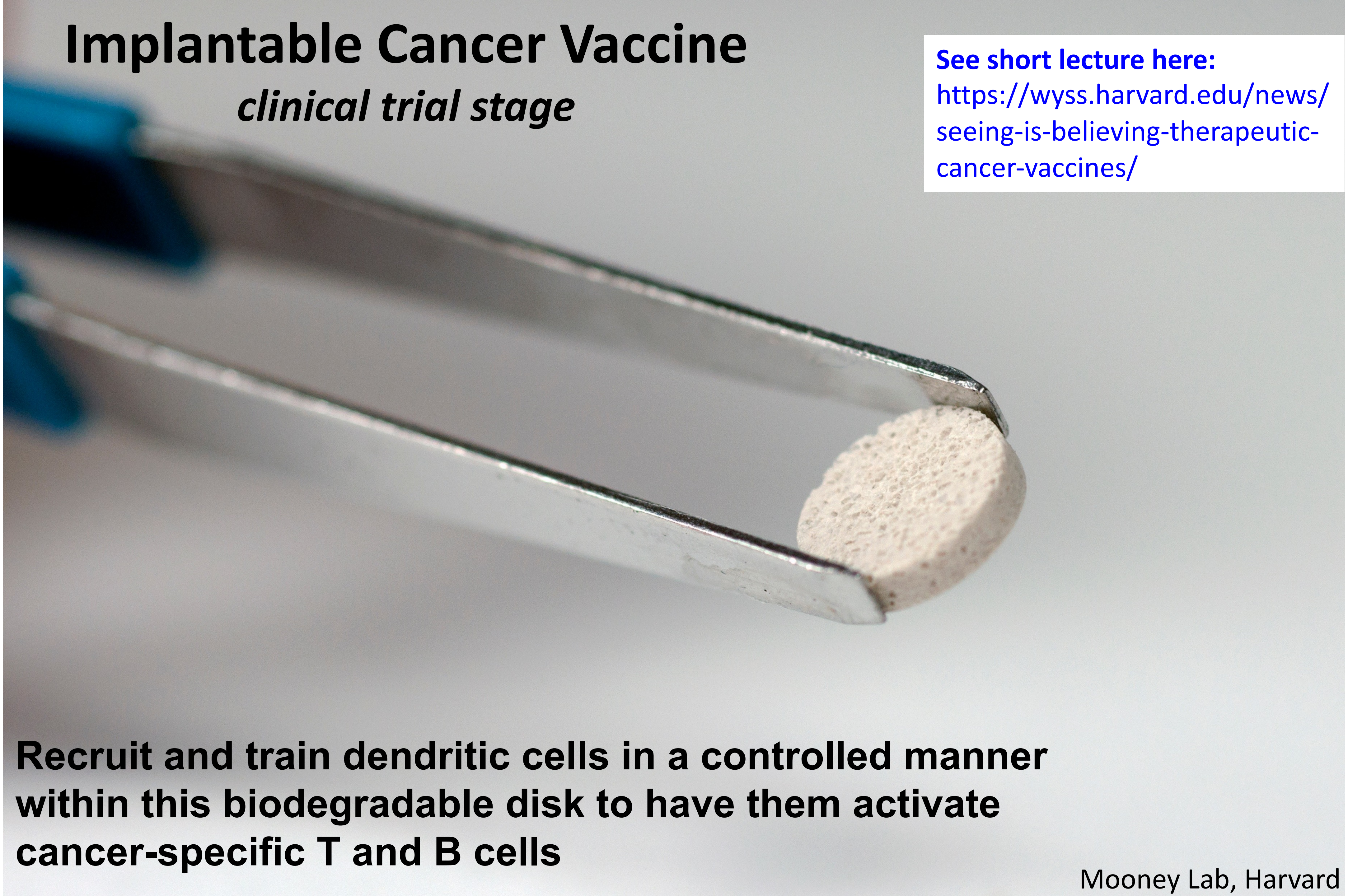


Schluck et al. Frontiers in Immunology 2019

Implantable Cancer Vaccine

clinical trial stage

See short lecture here:
https://wyss.harvard.edu/news/seeing-is-believing-therapeutic-cancer-vaccines/

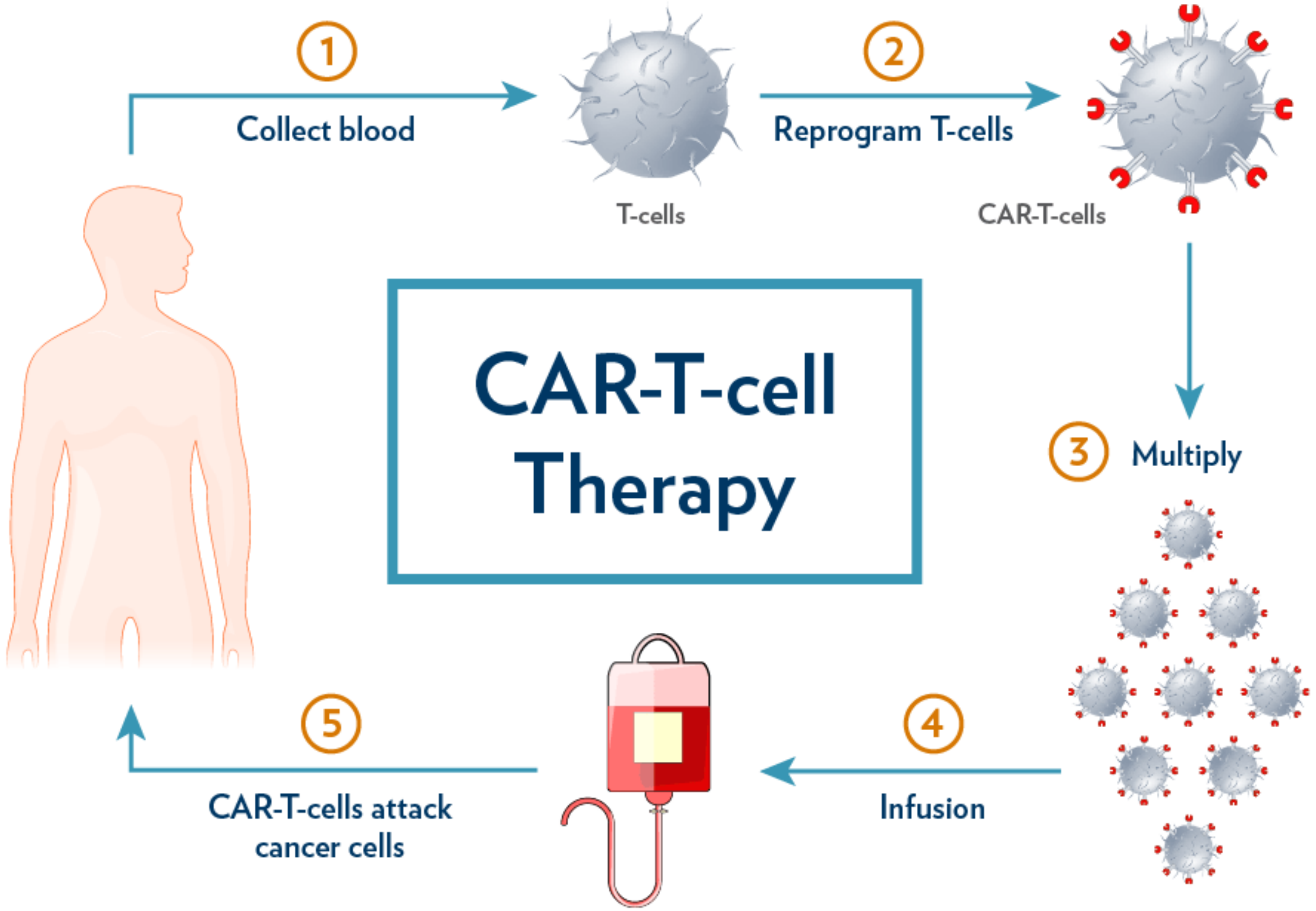


Recruit and train dendritic cells in a controlled manner within this biodegradable disk to have them activate cancer-specific T and B cells

Mooney Lab, Harvard

https://www.youtube.com/watch?v=y2-aIH2LhHI

CAR T-cell therapy: A "living drug"

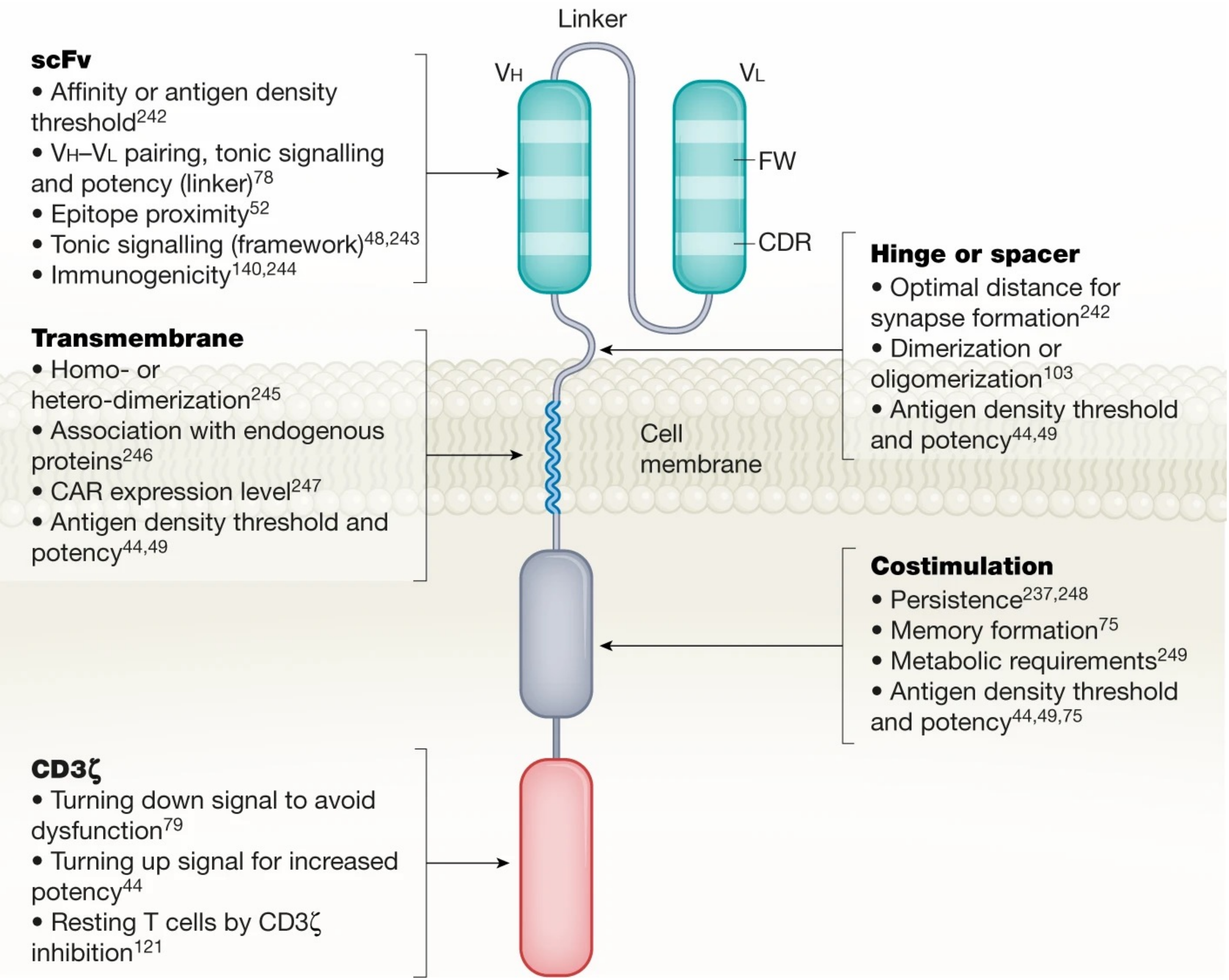


CARs

Chimeric antigen receptor

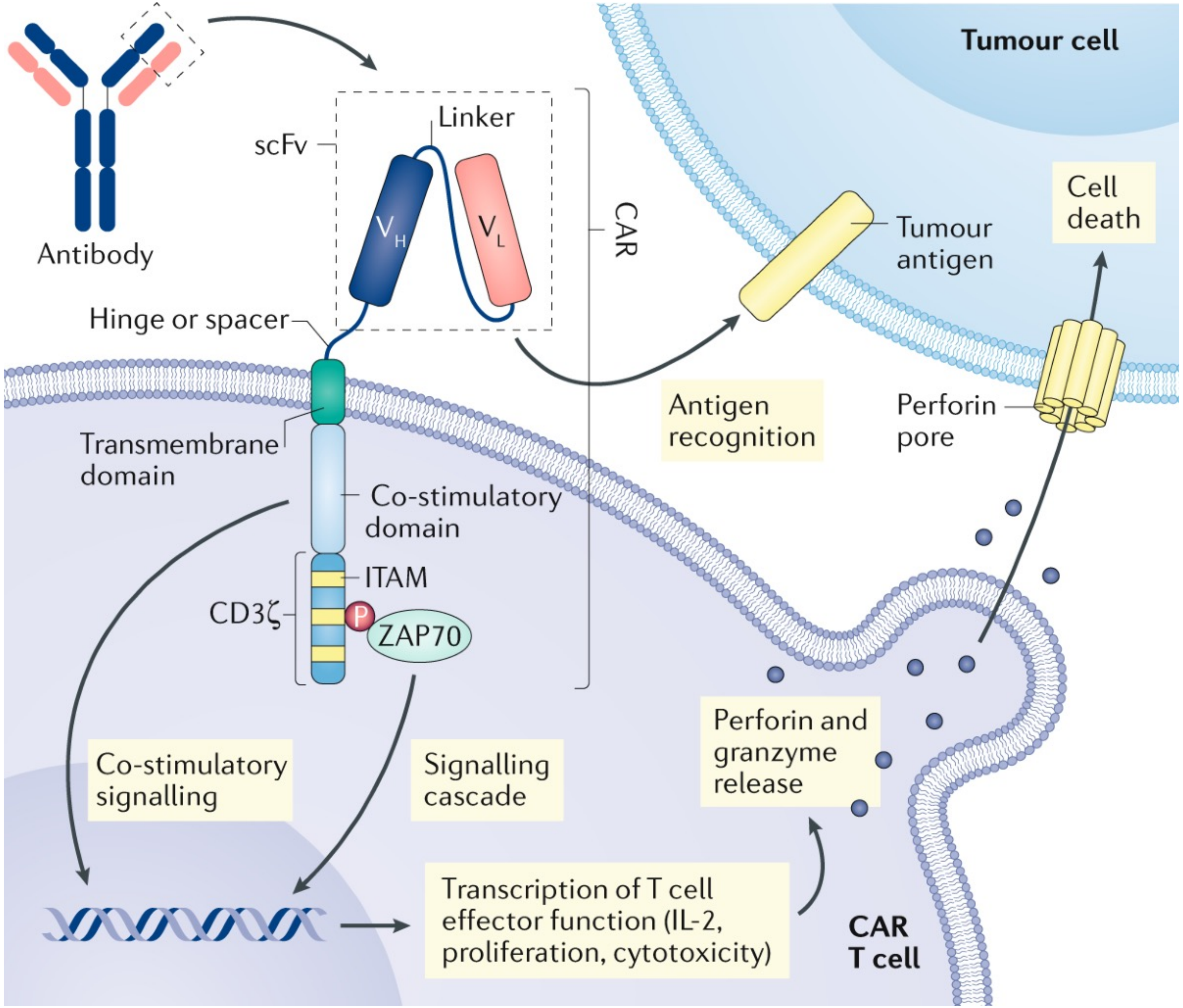
Approved for CD19 and BCMA
(B cell maturation antigen)

**Q: Why not more targets?
can you think of risks?**



Labanieh, L., Mackall, C.L. CAR immune cells: design principles, resistance and the next generation. *Nature* **614**, 635–648 (2023).

Mechanism



Larson, R.C., Maus, M.V. Recent advances and discoveries in the mechanisms and functions of CAR T cells. *Nat Rev Cancer* **21**, 145–161 (2021). https://doi.org/10.1038/s41568-020-00323-z

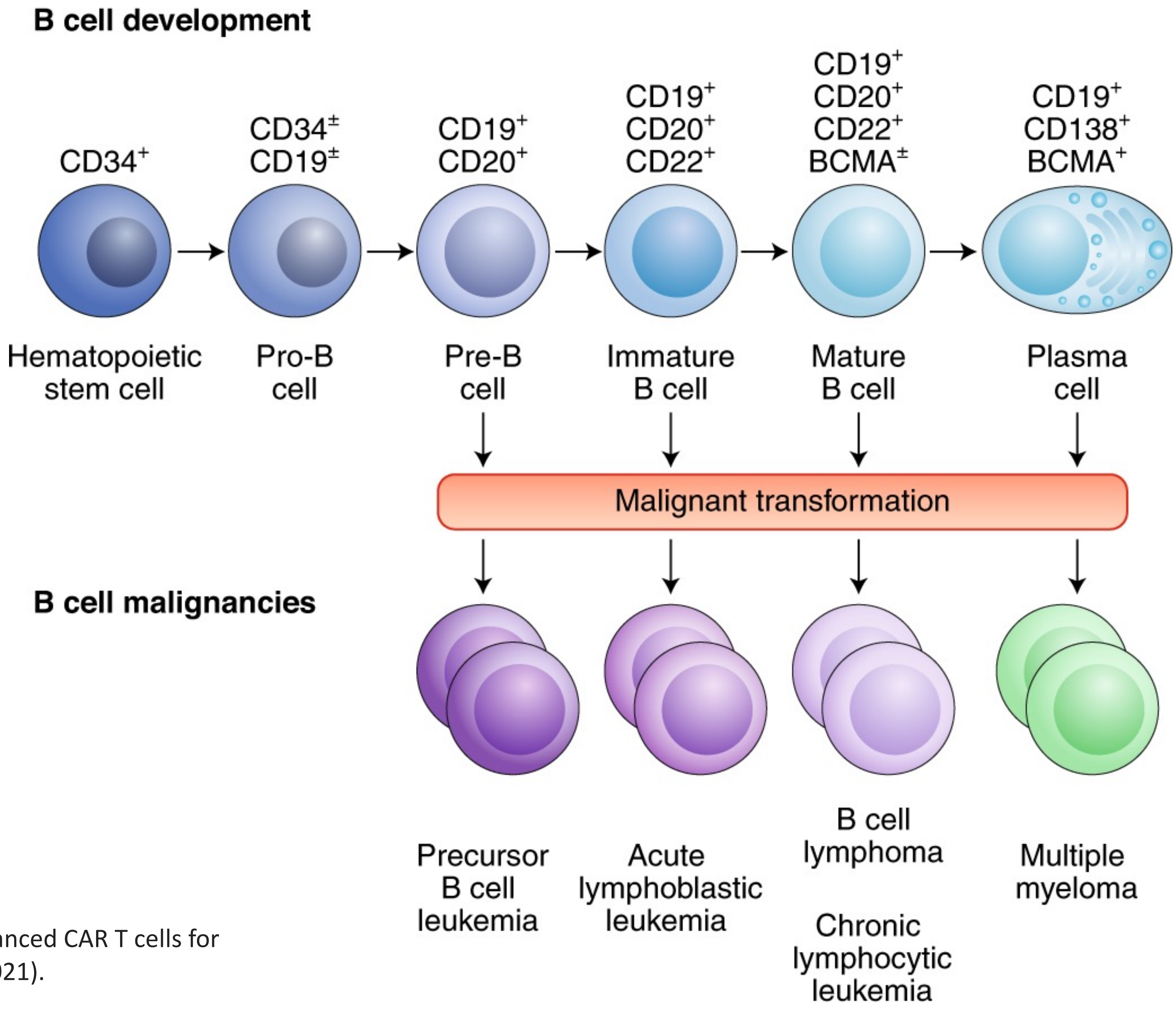
CAR T-cell therapy: A "living drug"

FDA-Approved CAR T-Cell Therapies

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

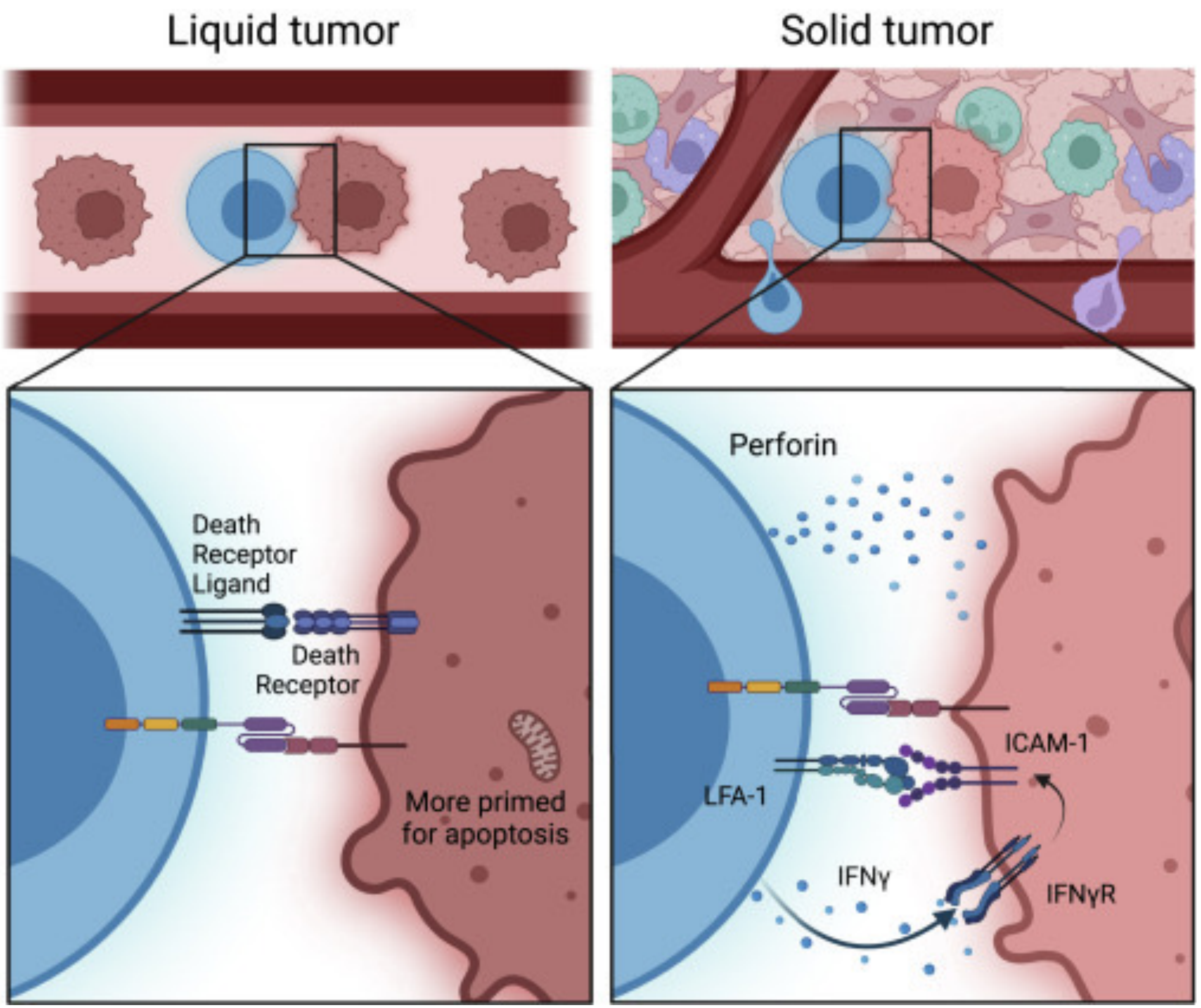
CD19 as target

CD19 serves as an ideal target for CAR-directed therapies because it is expressed on most B cell malignancies (including CLL, B-ALL, and many NHL), it is not expressed on hematopoietic stem cells, and elimination of all CD19+ B cells in the body is a manageable on-target treatment effect



Milone, M.C., Xu, J., Chen, SJ. *et al.* Engineering-enhanced CAR T cells for improved cancer therapy. *Nat Cancer* **2**, 780–793 (2021). https://doi.org/10.1038/s43018-021-00241-5

Differences in CAR T cell killing between liquid and solid tumors



While **liquid tumors are easy** for CAR T cells to access, either directly in the blood or via the lymph system, CAR T cells must be recruited into solid tumors, where they will **encounter many other cells within the tumor microenvironment that can influence their function**.

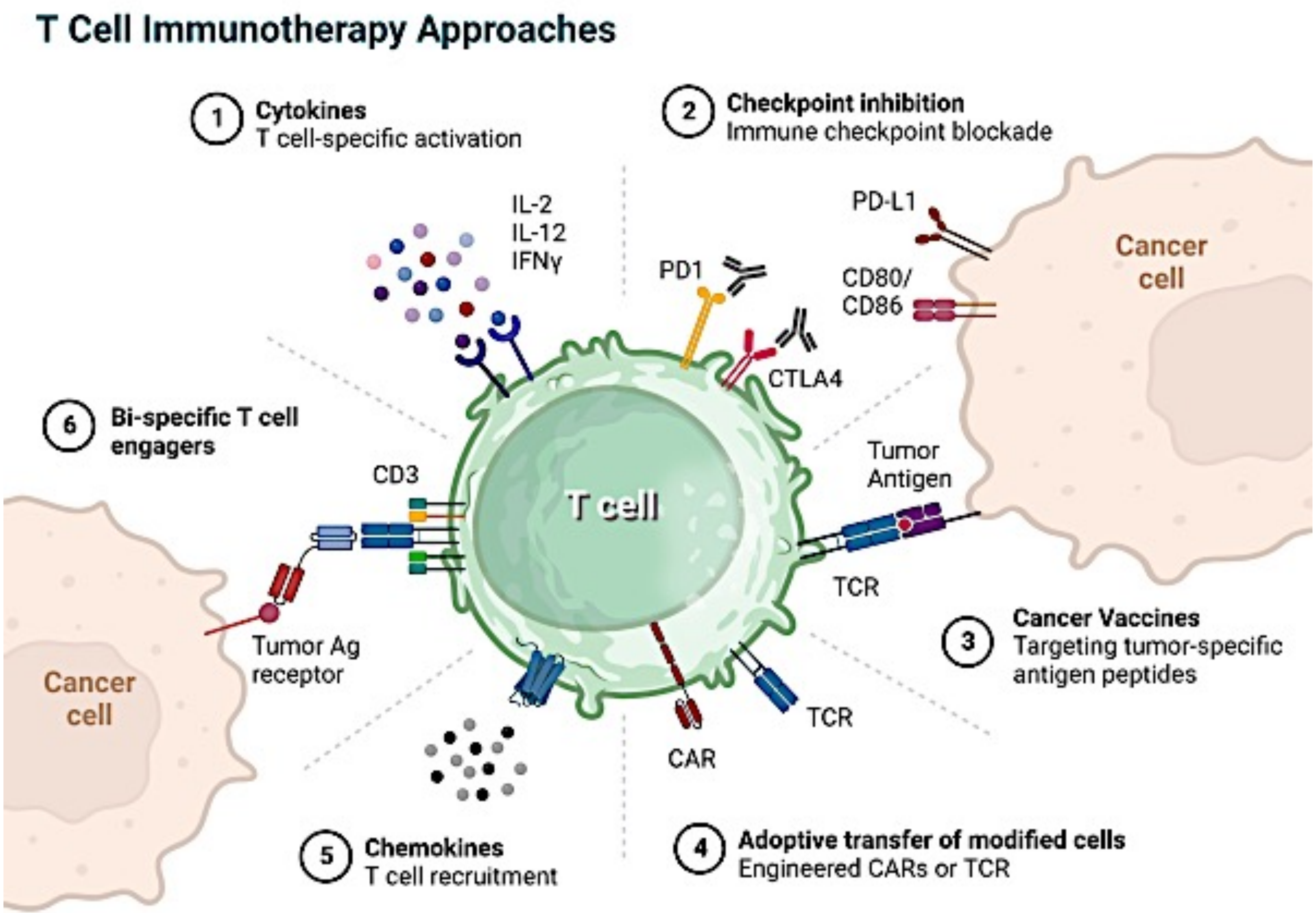
During CAR T cell killing, liquid tumors may not require perforin or INF-γ but do require death receptor expression on the tumor cell. Liquid tumors are also more primed for apoptosis compared with solid tumors.

Solid tumors require perforin for CAR T cell killing, but do not require death receptor expression/signaling in the tumor cell. Solid tumors also require IFN-γ receptor expression by the tumor cell for efficient CAR T cell killing. IFN-γ signaling increases ICAM-1 on the solid tumor cell surface, which interacts with LFA-1 on the CAR T cell to increase the cell binding strength

Volume 3, Issue 8, 12 August 2022, Pages 538-564
https://www.sciencedirect.com/science/article/pii/S2666634022001830#fig3

https://www.youtube.com/watch?v=Sz11e0r2L_s

https://www.youtube.com/watch?v=CxxuqIDyF_I



https://bpsbioscience.com/cancer-and-immunotherapy

Conclusion

1. Materials hold great promise in improving the efficacy and reducing toxicity of current cancer immunotherapeutic strategies

2. Many different approaches are being investigated
   - Synthetic expansion of T cells ex vivo
   - In vivo delivery of T cells
   - Delivery of antigens/adjuvants to dendritic cells
   - Scaffolds for in vivo stimulation

3. Careful selection, formulation, design and characterization of biomaterials is essential for controlled and efficient immunomodulation