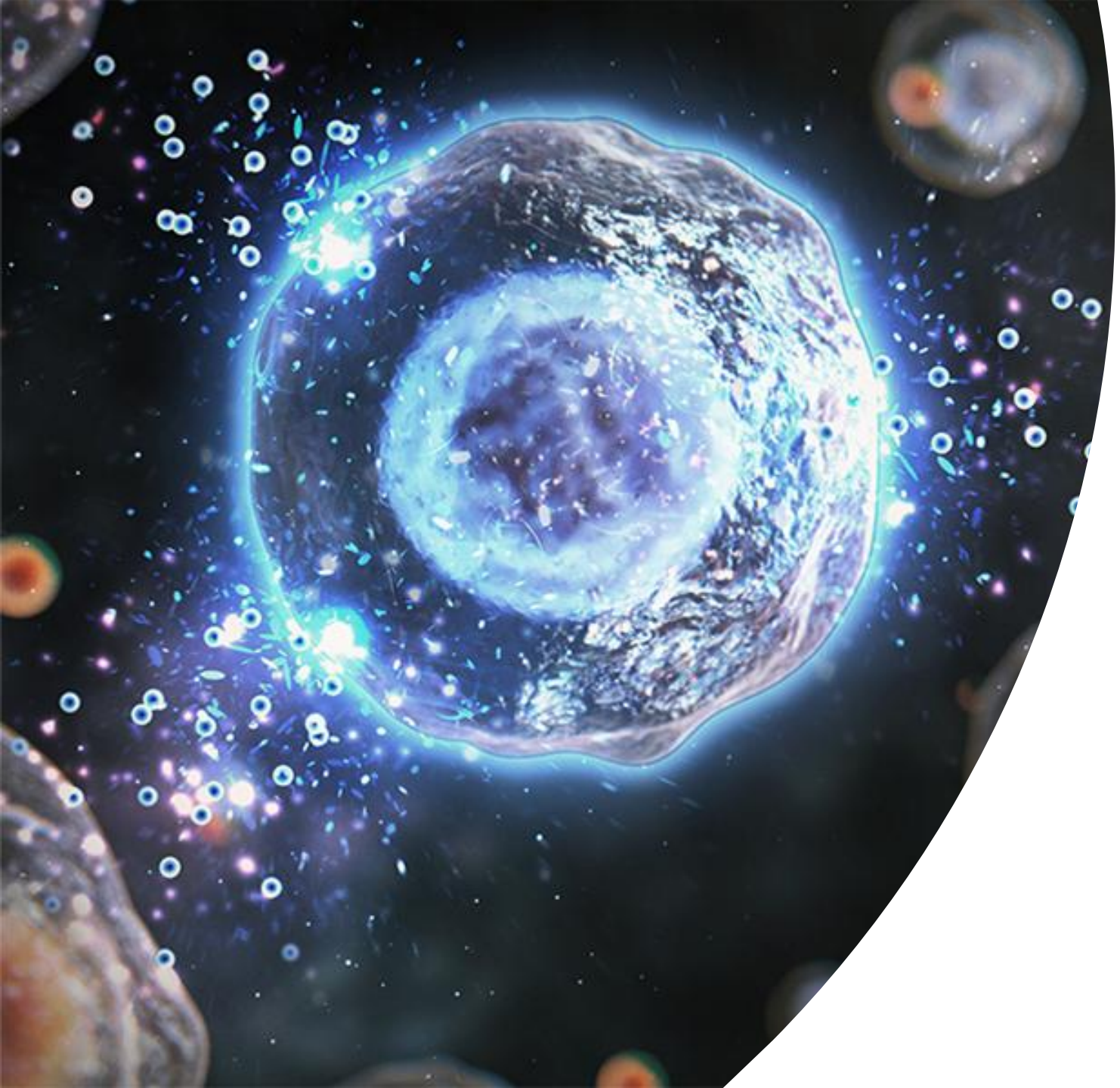


Exercise 12

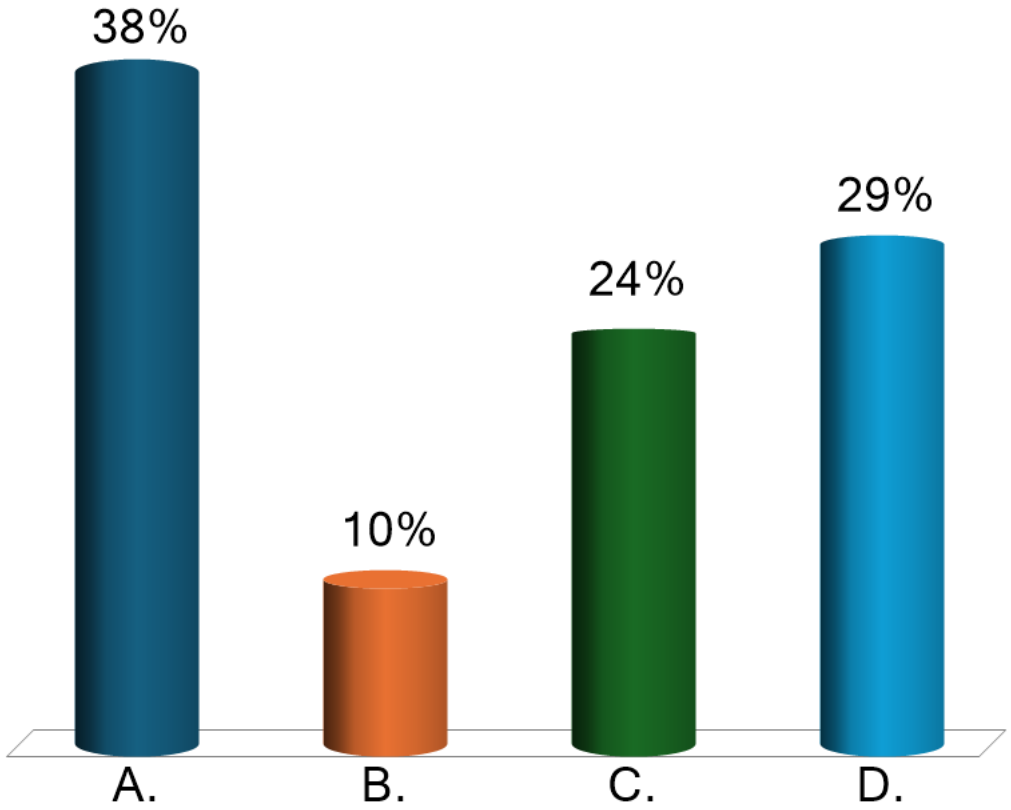
L13- Special Vaccines
L1-11- Review of the whole course

30/05/ 2025

TA:
Emile Dorchies
(emile.Dorchies@epfl.ch)

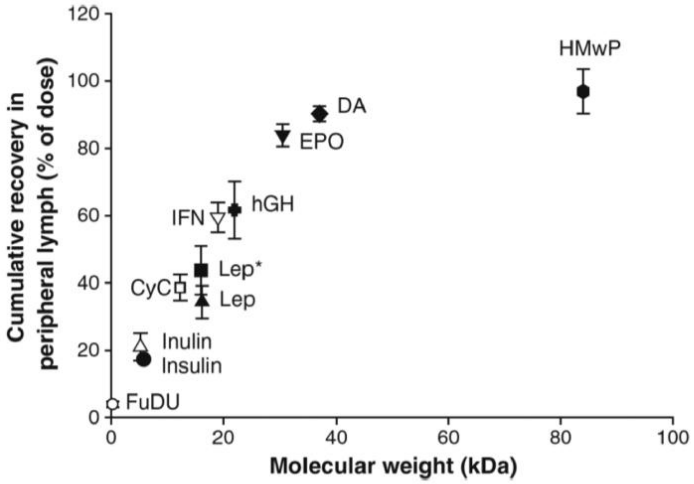
11. What are ways to preferentially target lymph nodes for vaccine? (multiple answers possible)

- ✓ A. Albumin hitchhiking
- B. Immunize through mucosa
- C. Use nanoparticles of more than 100nm of diameter
- ✓ D. Target dendritic cells with anti-CD40 antibodies

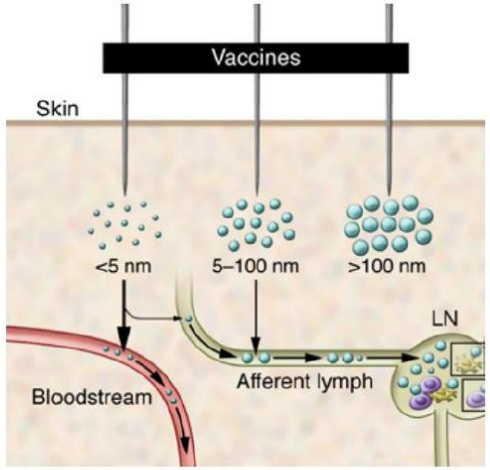


Size-Based LN Targeting

In general, materials larger than approximately 9 nm in diameter preferentially drain to lymphatics, whereas molecules/particles smaller than ~6 nm drain to the blood.



Adv. Drug Delivery Rev. 2011, 63, 890–900

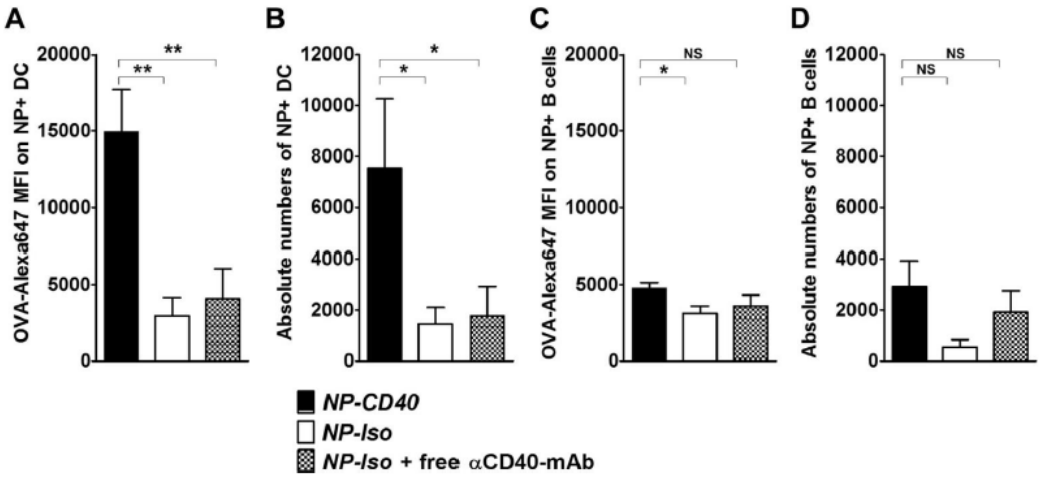


Moyer et al. *J. Clin. Invest.* **2016**
Melief et al. *J. Clin. Invest.* **2015**

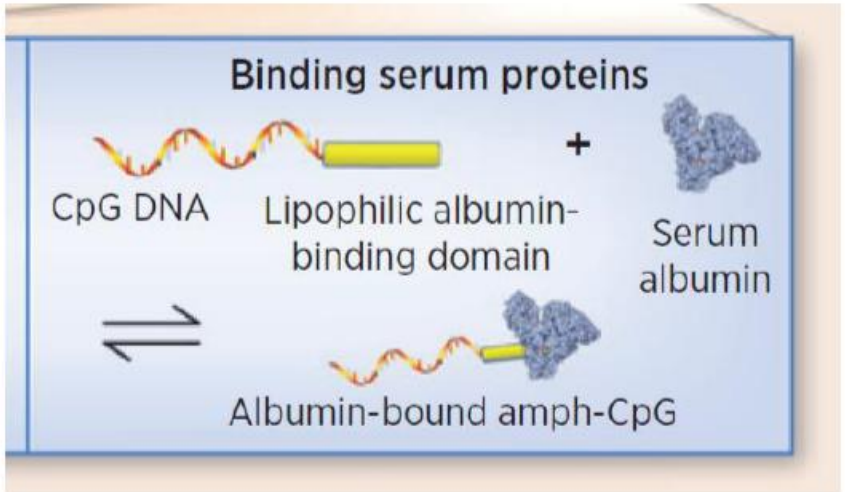
Promoting Vaccine Capture in Lymph Nodes

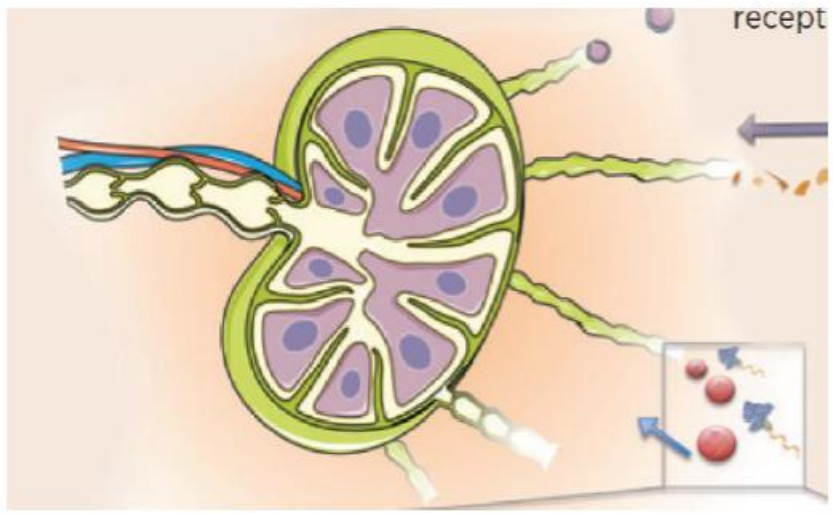
Incorporate targeting ligands for DCs, e.g.:

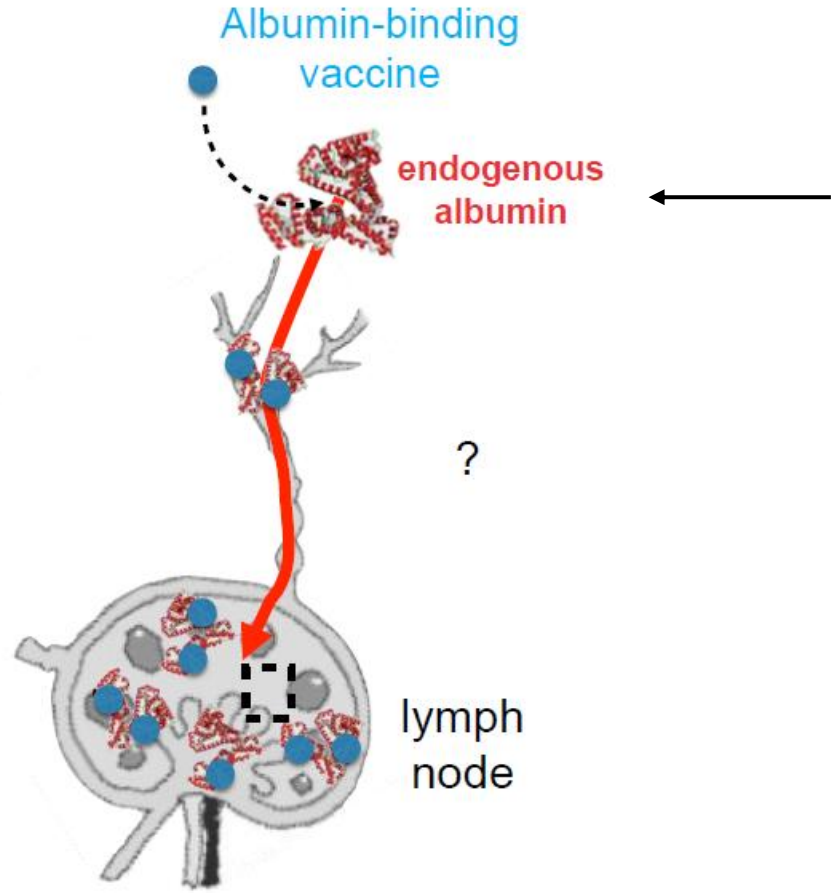
- Mannose
- Anti-CD40
- Anti-DEC-205
- Anti-CD11c



“albumin hitchhiking” vaccine target lymph nodes?







Albumin binds lipophilic molecules or proteins

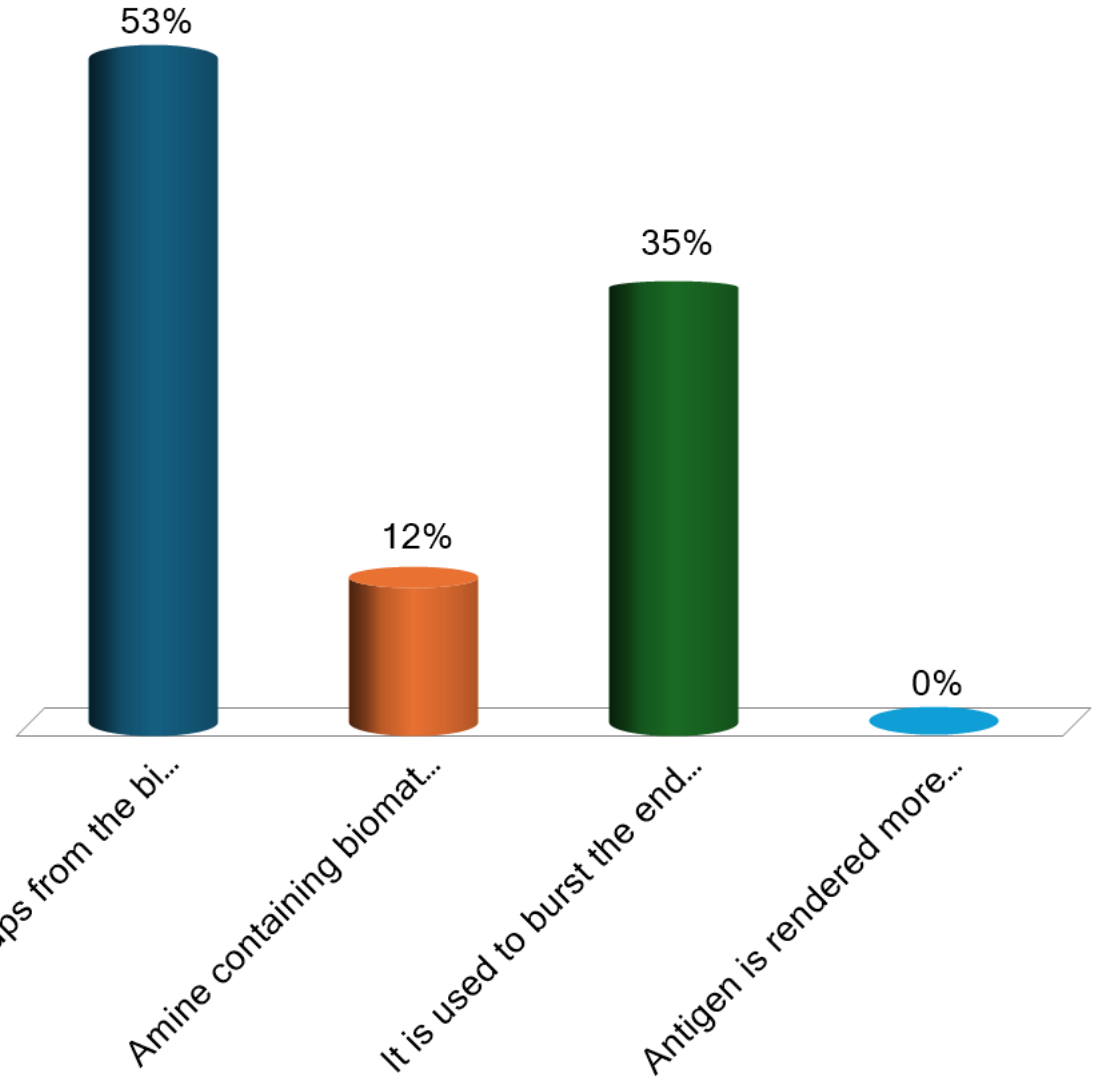
Tsopelas, C. & Sutton, R. *J. Nucl. Med.* **43**, 1377–1382 (2002);
Faries, M. B. *et al. Ann. Surg. Oncol.* **7**, 98–105 (2000).

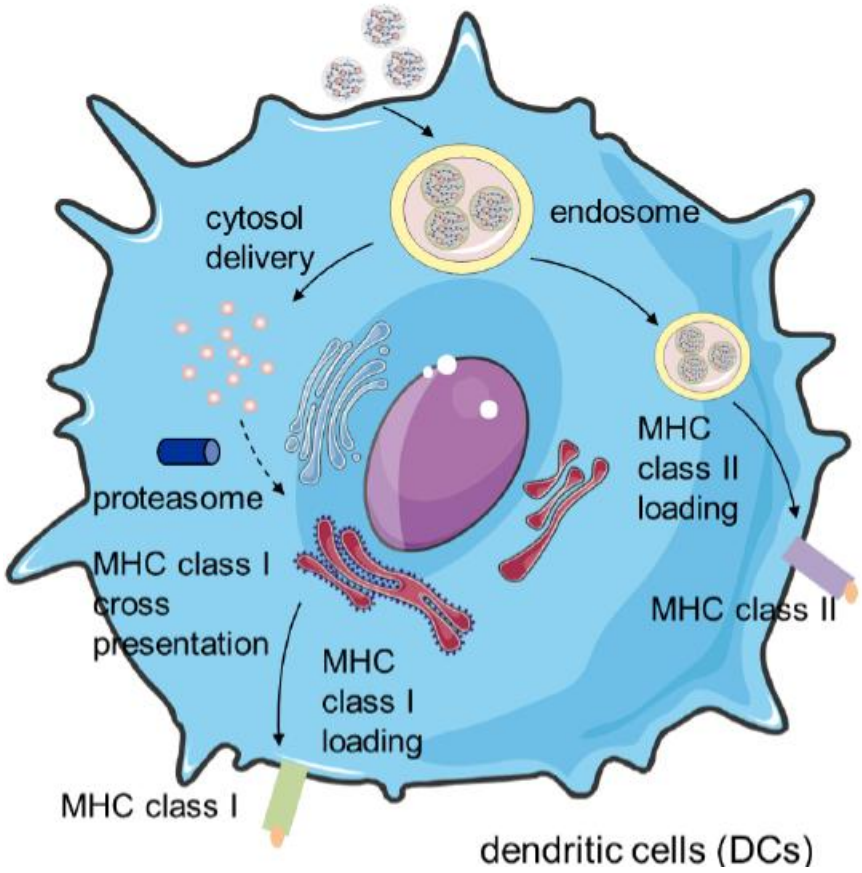
Saha, S. *et al. Am. J. Surg.* **191**, 305–310 (2006)

Cancer Immunology Research **2015,** *3*, 836-43.

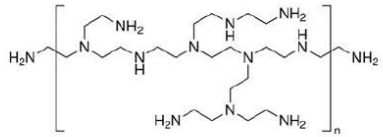
2. What is **TRUE** the « proton sponge » effect of biomaterials? (multiple answers possible)

- ✓ A. Amine groups from the biomaterial are protonated at lower pH, bringing H+ and counterions into endosome and increasing osmotic pressure
- B. Amine containing biomaterial are used as pH stabilizer by absorbing endosomal H+
- ✓ C. It is used to burst the endosome and foster antigen presentation on HMC-I on APC
- D. Antigen is rendered more immunogenic by protonation

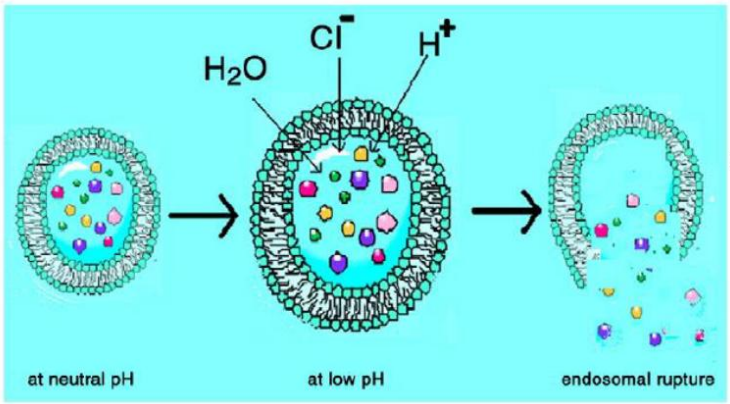




Endosomal escape facilitated by biomaterials



“proton sponge” effect



Osmotic pressure change

$-NH_2 \rightarrow -NH_3^+$

J. Controlled Release **151**, 220-228 (2011).

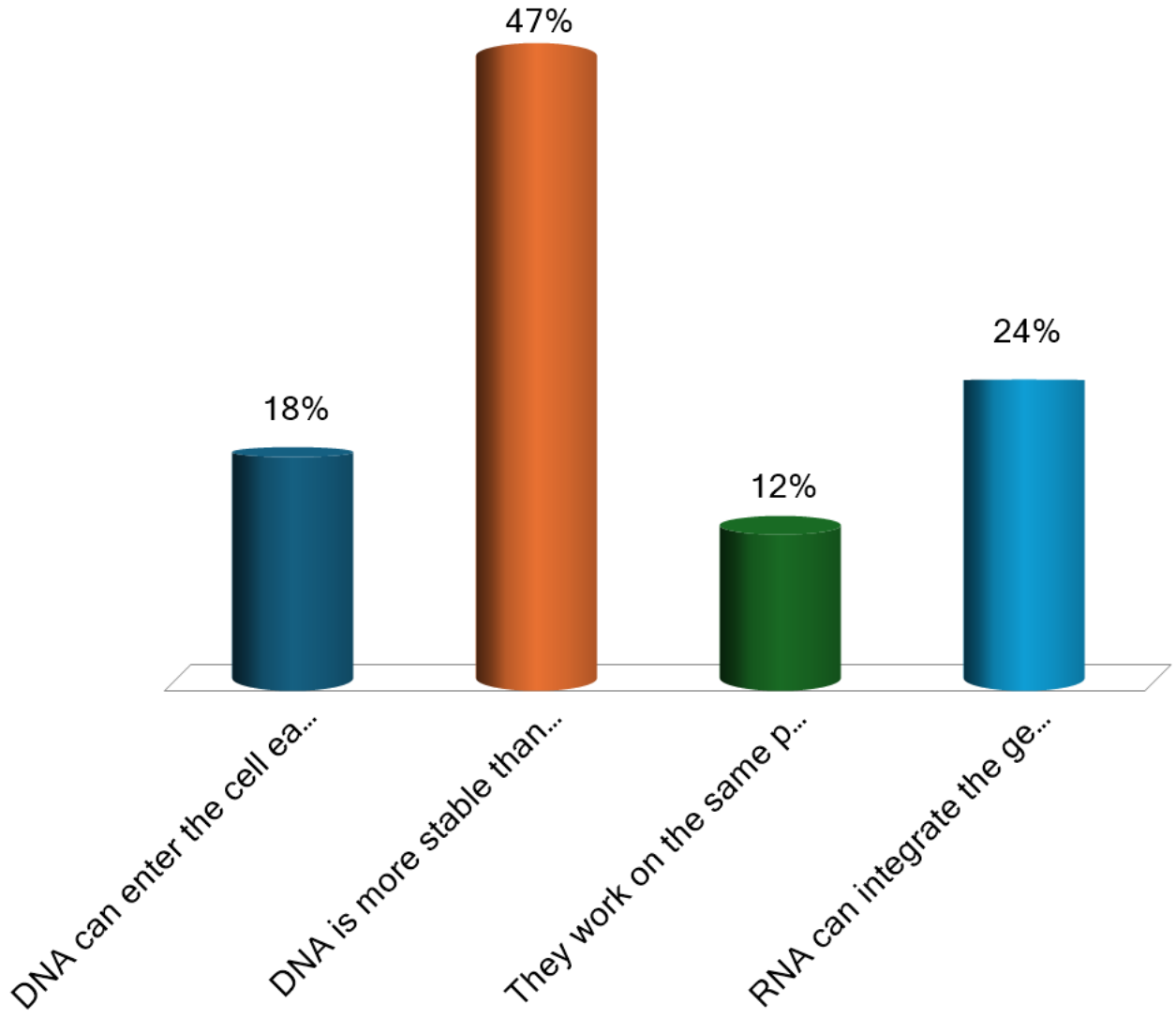
3. What are main differences between RNA and DNA vaccines? (multiple answers possible)

A. DNA can enter the cell easily

✓B. DNA is more stable than RNA

✓C. They work on the same principle

D. RNA can integrate the genome more easily



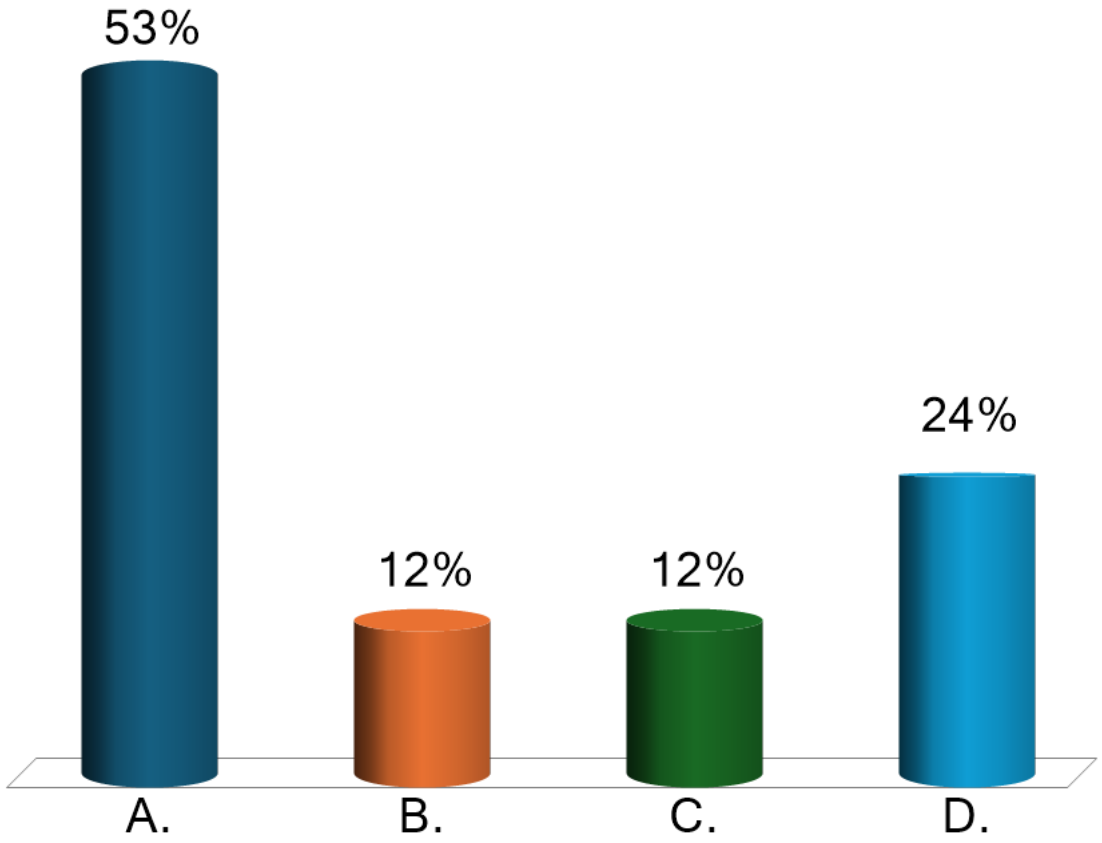
4. What are the advantages of mRNA-based vaccines? (Multiple answers possible)

✓A. Easy and fast to be manufactured

B. easy storage/transport

C. Simple design made only of raw RNA

✓D. Cheap



mRNA-Based Vaccines

The underlying principle is delivery of a transcript that encodes one or more immunogens into the host cell cytoplasm, where translation **generates immunogenic proteins** that are subsequently sequestered intracellularly, incorporated into the cell membrane, or secreted.

The mRNA is generated by transcribing a DNA template synthesized **once the genetic sequence encoding the immunogen is known** and disseminated globally.

Advantages: high safety, easy and fast manufacturing compared to more conventional approaches, less expensive easy to be modified, much less possible for the mRNA to integrate into the genome than a DNA-based vaccine.

Disadvantages: a delivery system is necessary difficulty in storage and transportation, others to be determined

Do not enter the cells by itself

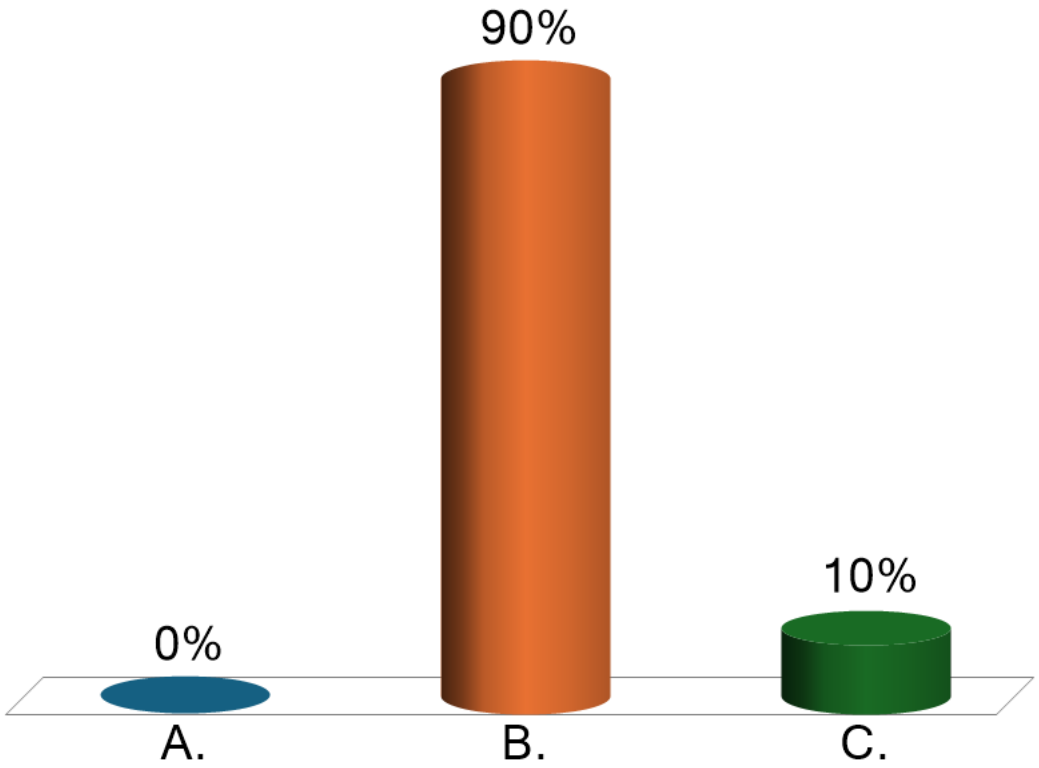
RNA is unstable compared to DNA (need to refrigerated and stabilized)

5. What are the principle behind mRNA vaccines?

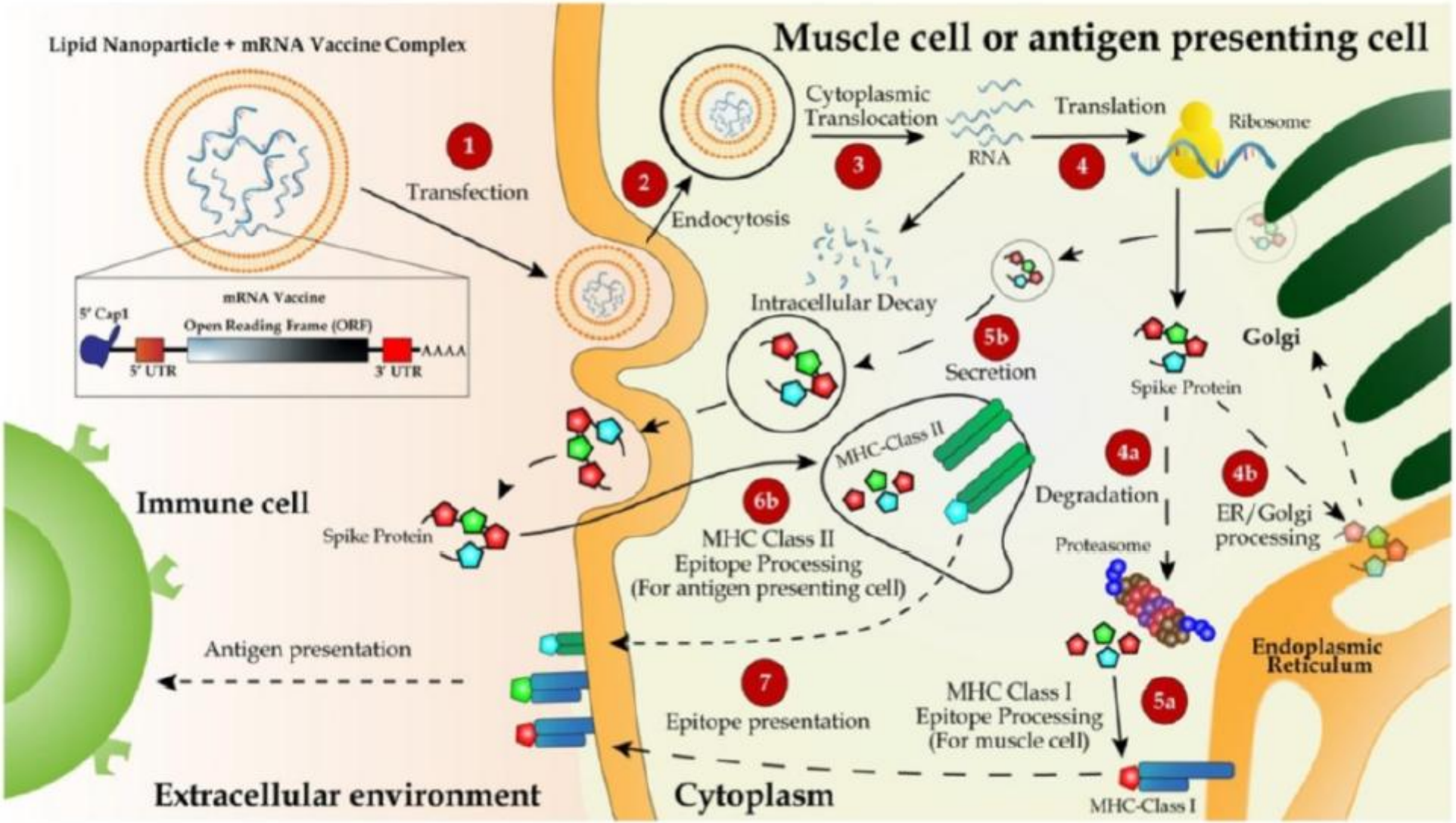
A. mRNA coding an antibody targeting a pathogen enters plasma cells and make them produce neutralizing antibodies

✓ B. mRNA coding a pathogen's protein makes the patients cell express this antigen transiently

C. mRNA is retrotranscribed into DNA, incorporated into the genome and make cells express an antigen indefinitly



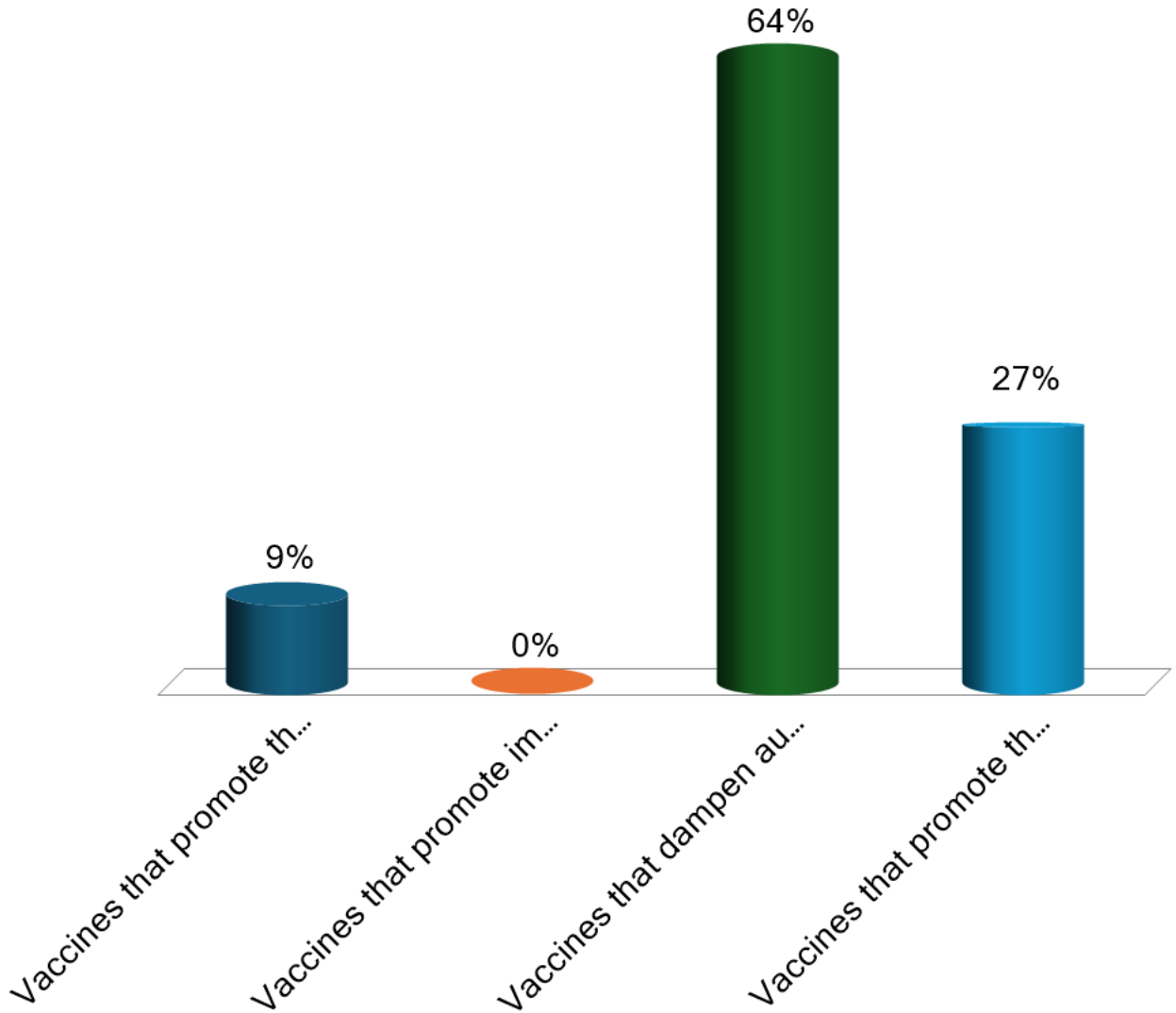
Cellular fates of mRNA vaccines



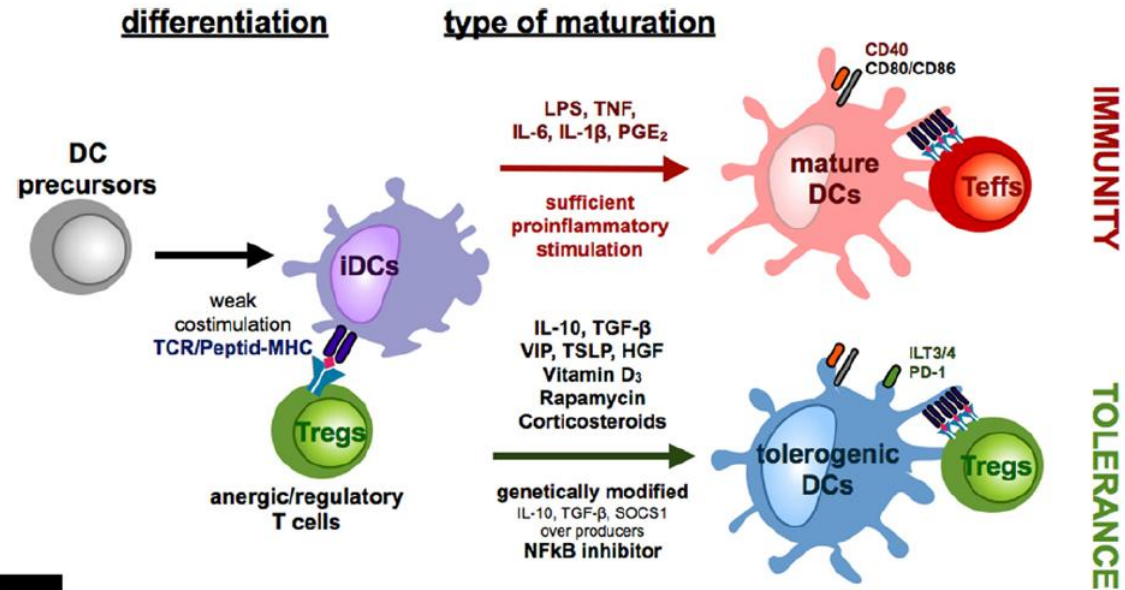
Int. J. Biol. Sci. 2021, Vol. 17, 2021: 17(6): 1446-1460. doi: 10.7150/ijbs.59233

6. Tolerogenic vaccines are…

A. Vaccines that promote the formation of effector T cells
B. Vaccines that promote immune reaction toward pathological self-antigens
✓ C. Vaccines that dampen autoimmune diseases
D. Vaccines that promote the formation of anergic and regulatory T cells by strong TCR/peptide-MHC stimulation



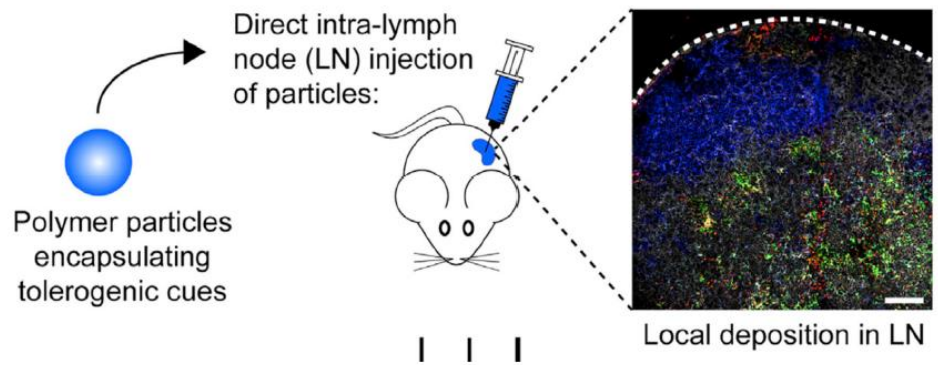
Tolerogenic vaccination for antigen-specific modulation



Raker et al Front Immunol 2015

Antigen-specific tolerogenic vaccines

multiple sclerosis (MS)



Microparticles containing:

- a peptide fragment of myelin oligodendrocyte glycoprotein (MOG)
- rapamycin (Rapa): a regulatory signal

Cell Reports **16**, 2940-2952 (2016).

Review of the whole course

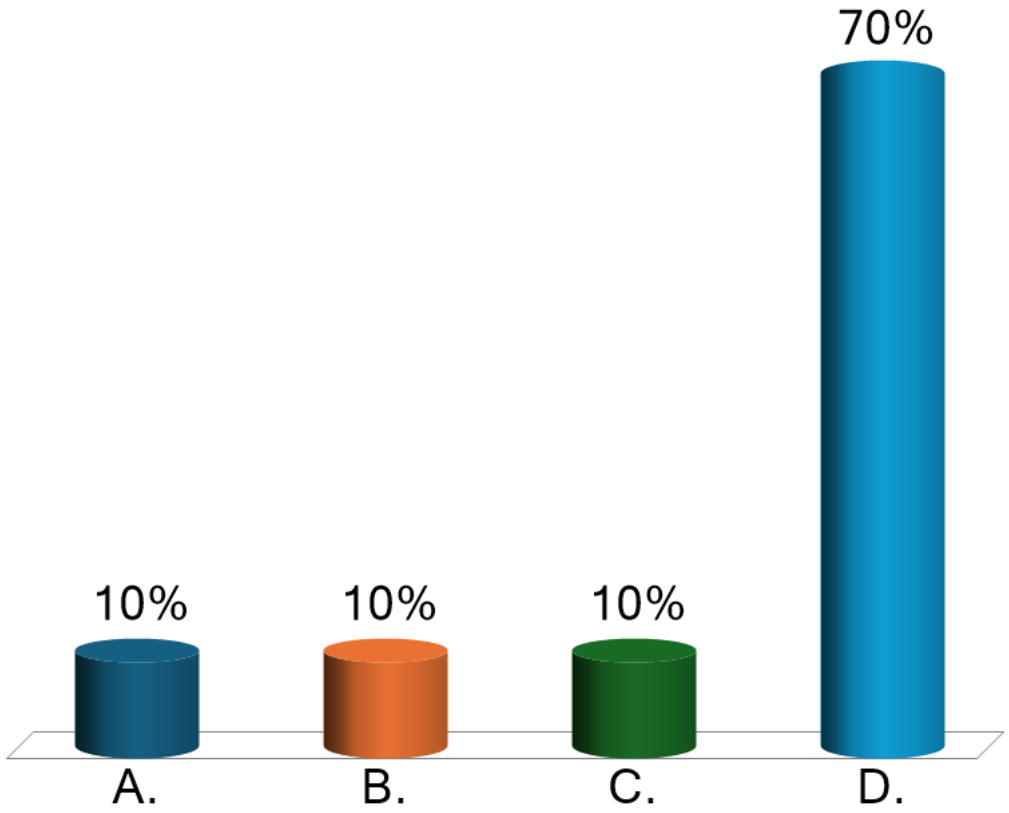
7. Which antibodies should we use to measure OVA-reactive CD8+ T cells in a tumor which express OVA?

A. CD19 , peptides from OVA bound to tetramer

B. CD3, CD8

C. CD3, CD4, CD8, CD45

✓ D. CD3, CD8, peptides from OVA bound to tetramer



Identifying antigen-specific T-cells

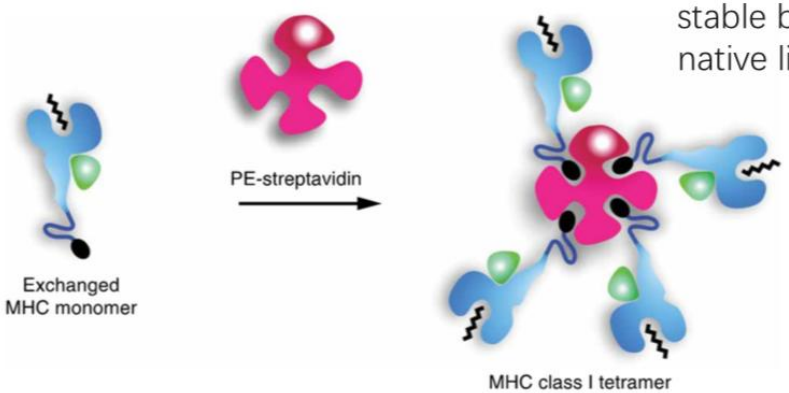
Peptide-MHC interaction

KD of TCR binding to pMHC:

- CD8 TCRs: 10 nM – 1 µM
- CD4 TCRs: 1-10 µM

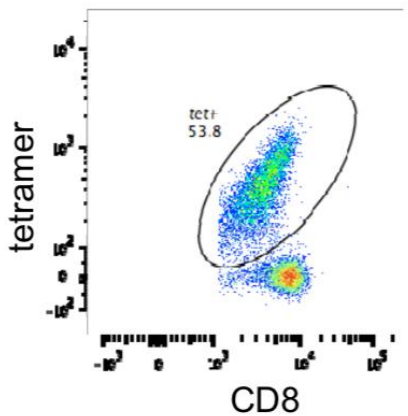
Original paper:

John D. Altman; Paul A. H. Moss; Philip J. R. Goulder; Dan H. Barouch; Michael G. McHeyzer-Williams; John I. Bell; Andrew J. McMichael; Mark M. Davis. (1996) "Phenotypic Analysis of Antigen-Specific T Lymphocytes". Science. 274 (5284): 94–96.



Using multivalency to achieve stable binding of TCRs to their native ligands

Nature Protocols **1**, 1120 - 1132 (2006)



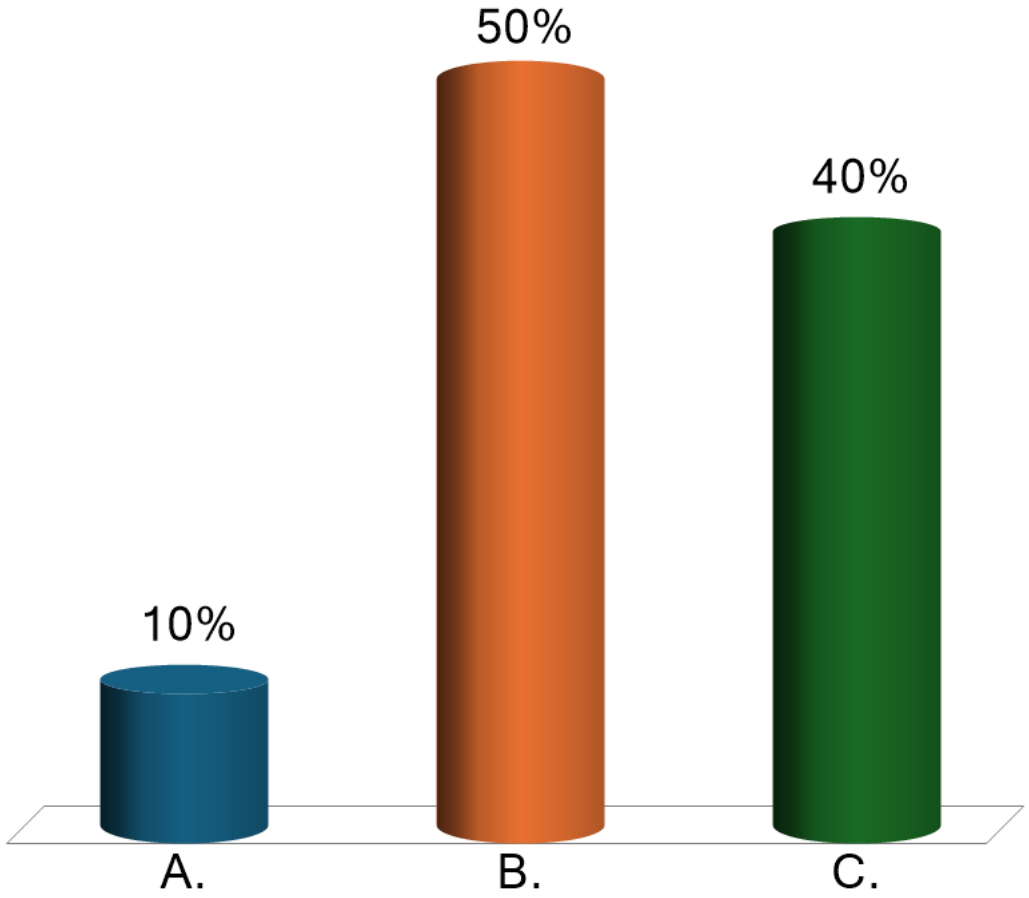
Limit of detection:
~0.1-0.2% among
T cells
(1 in 1,000)

8. For the cancer immunity cycle, which one is correct?

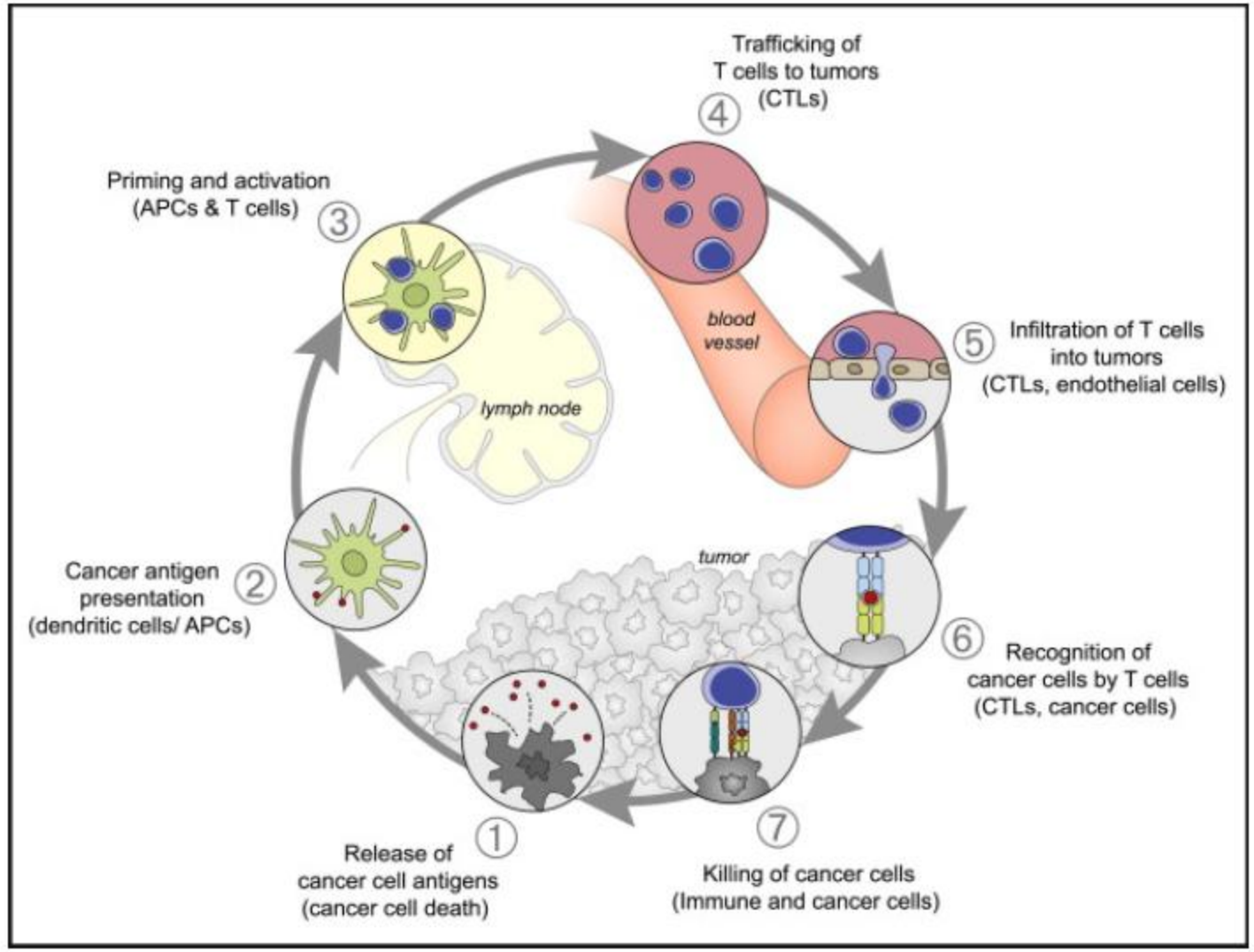
A. Cancer cell death →priming and activation →trafficking of T cells to tumors → cancer antigen presentation → infiltration of T cells into tumors →recognition of cancer cells by T cells → killing of cancer cells

✓ B. Cancer cell death→cancer antigen presentation→priming and activation→trafficking of T cells to tumors→infiltration of T cells into tumors→recognition of cancer cells by T cells → killing of cancer cells

C. Cancer cell death →cancer antigen presentation → trafficking of T cells to tumors →infiltration of T cells into tumors→priming and activation → recognition of cancer cells by T cells → killing of cancer cells



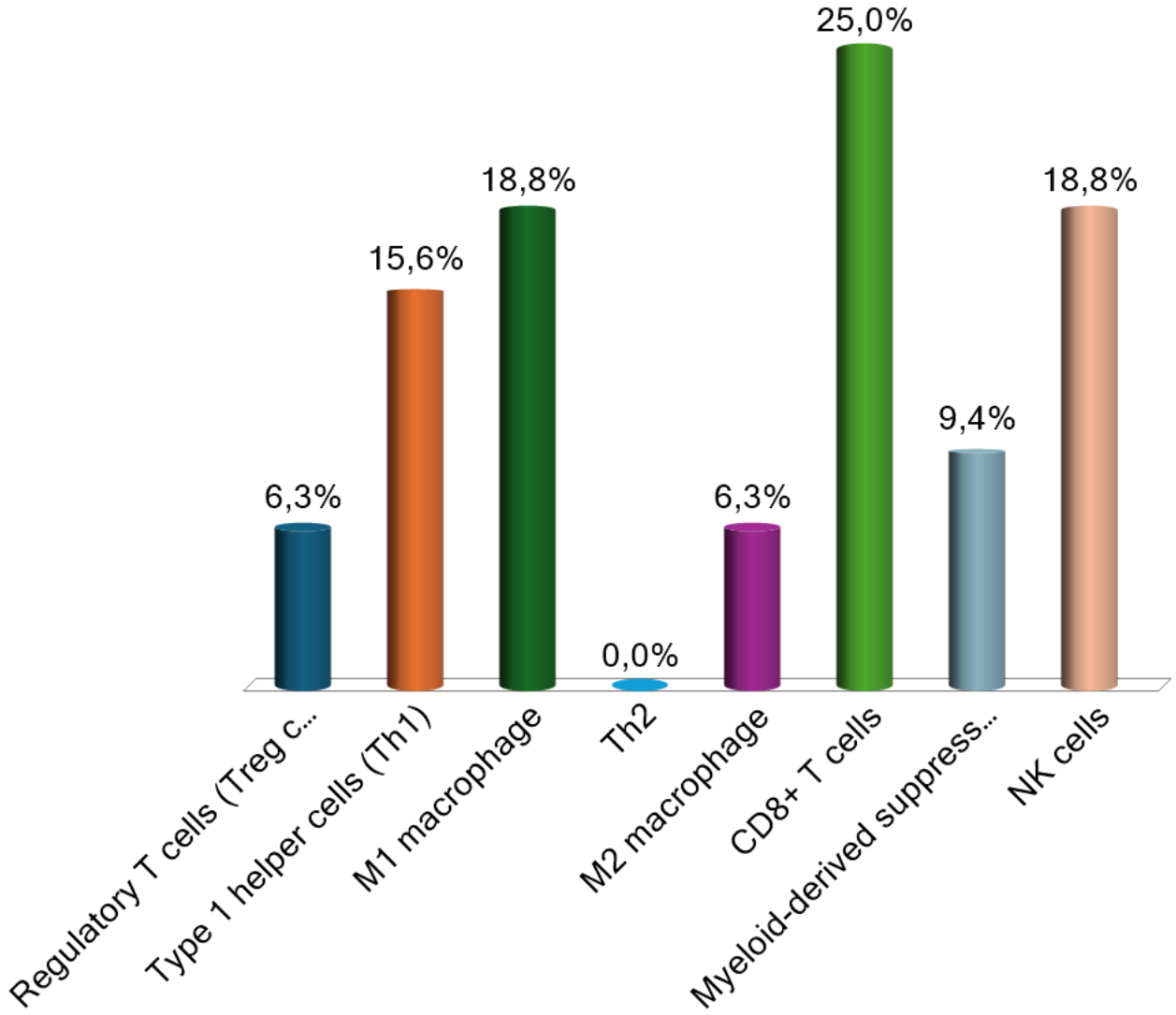
Cancer-immunity cycle

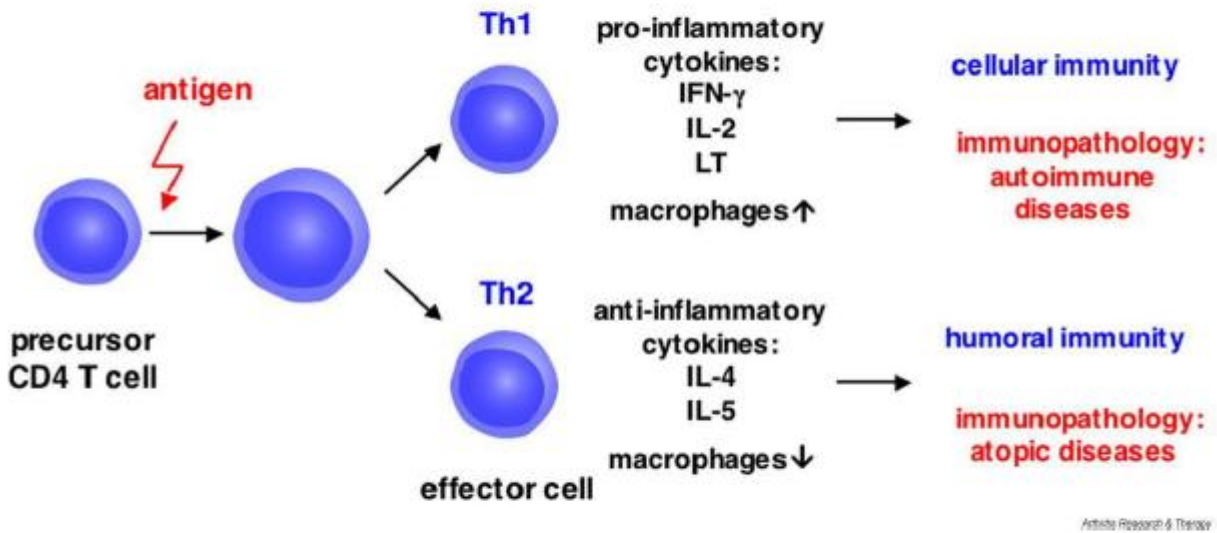


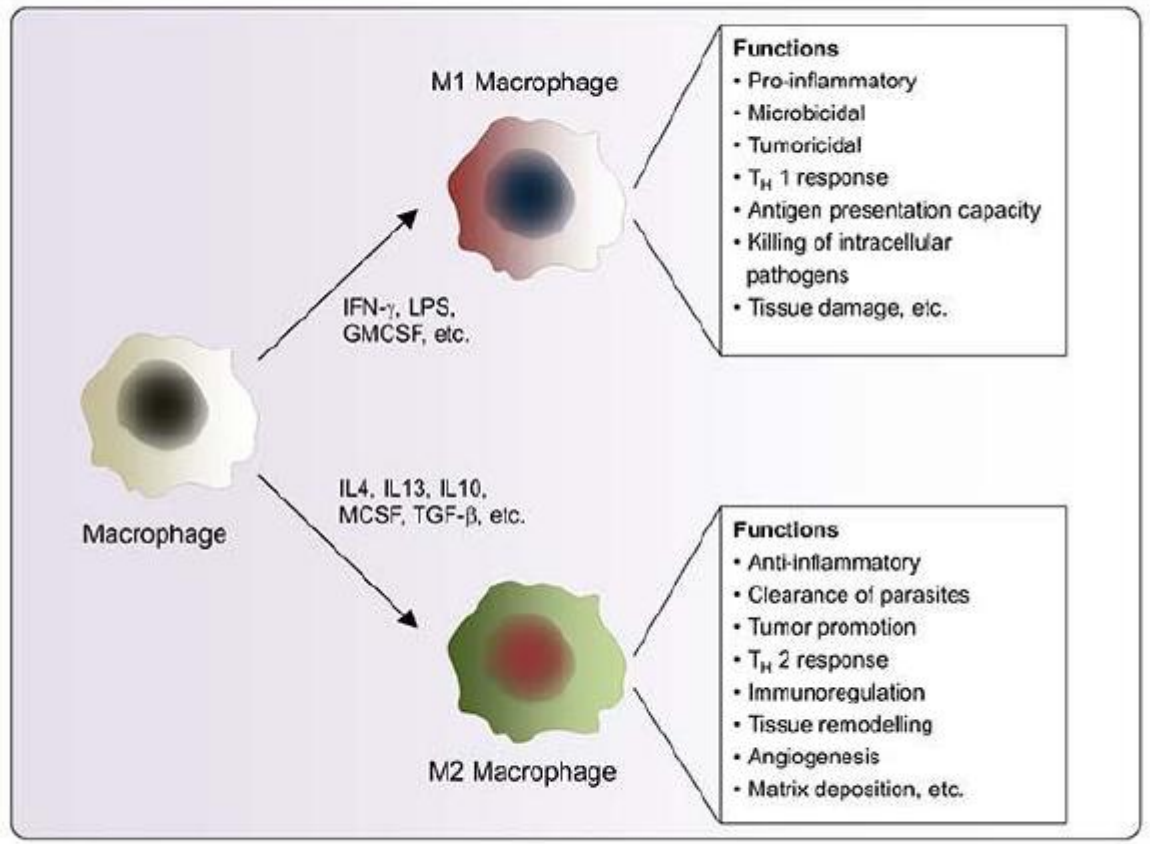
Chen, D. S. and I. Mellman (2013). Immunity **39**(1): 1-10.

9. According to the understanding of cancer immunology, which of the following cells is antagonist of growing tumors? (multiple answers possible)

A. Regulatory T cells (Treg cells)
✓B. Type 1 helper cells (Th1)
✓C. M1 macrophage
D. Th2
E. M2 macrophage
✓F. CD8+ T cells
G. Myeloid-derived suppressor cells (MDSC)
✓H. NK cells




antigen
precursor
CD4 T cell
Th1
pro-inflammatory
cytokines:
IFN-γ
IL-2
LT
macrophages↑
cellular immunity
immunopathology:
autoimmune
diseases
Th2
anti-inflammatory
cytokines:
IL-4
IL-5
macrophages↓
effector cell
humoral immunity
immunopathology:
atopic diseases
Arthritis Research & Therapy


M1 Macrophage
IFN-γ, LPS,
GMCSF, etc.
Macrophage
IL4, IL13, IL10,
MCSF, TGF-β, etc.
M2 Macrophage
Functions
• Pro-inflammatory
• Microbicidal
• Tumoricidal
• TH 1 response
• Antigen presentation capacity
• Killing of intracellular pathogens
• Tissue damage, etc.
Functions
• Anti-inflammatory
• Clearance of parasites
• Tumor promotion
• TH 2 response
• Immunoregulation
• Tissue remodelling
• Angiogenesis
• Matrix deposition, etc.

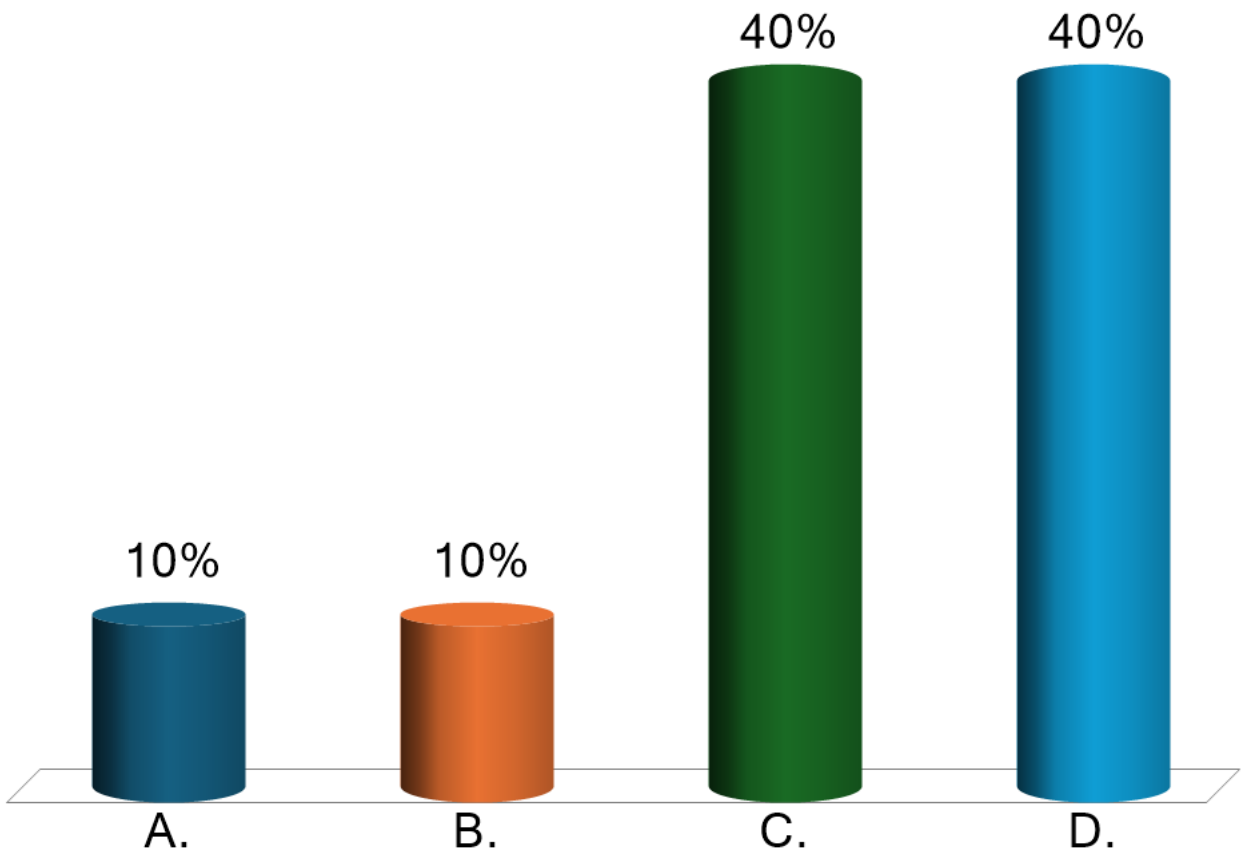
10.What is the reason behind Enhanced Permeation and Retention (EPR) in tumors?

A. Narrow fenestrations in the blood vessels of tumors

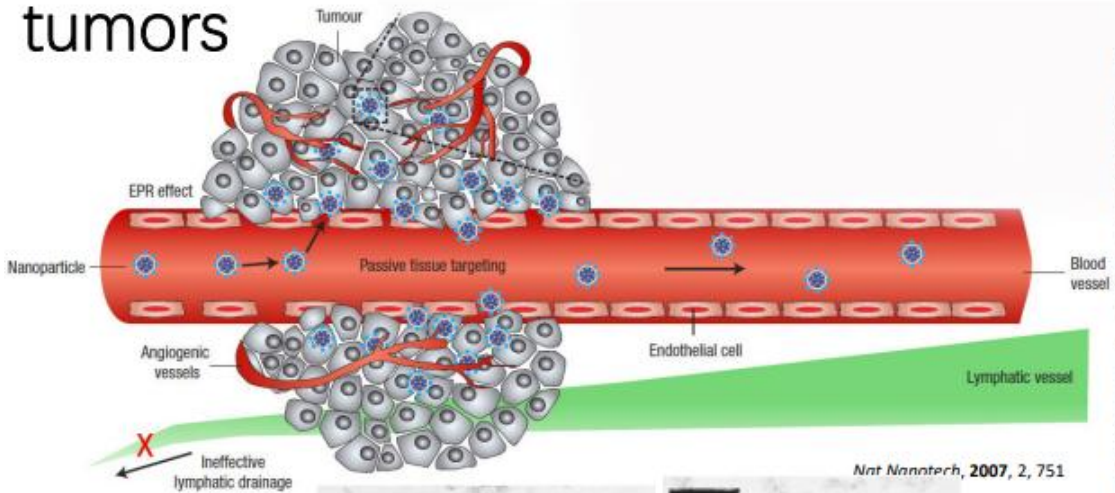
B. The large amount of lymphatic drainage

C. Wide fenestrations in the blood vessels of tumors

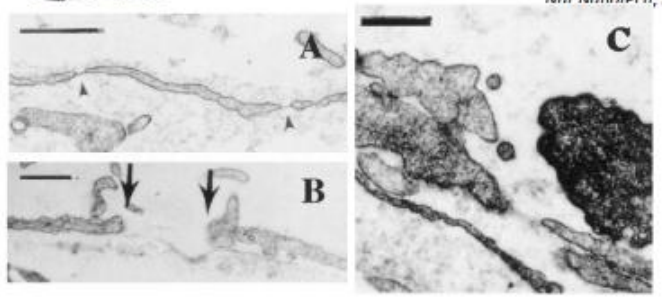
D. Both A and B



Enhanced permeation and retention effect in tumors



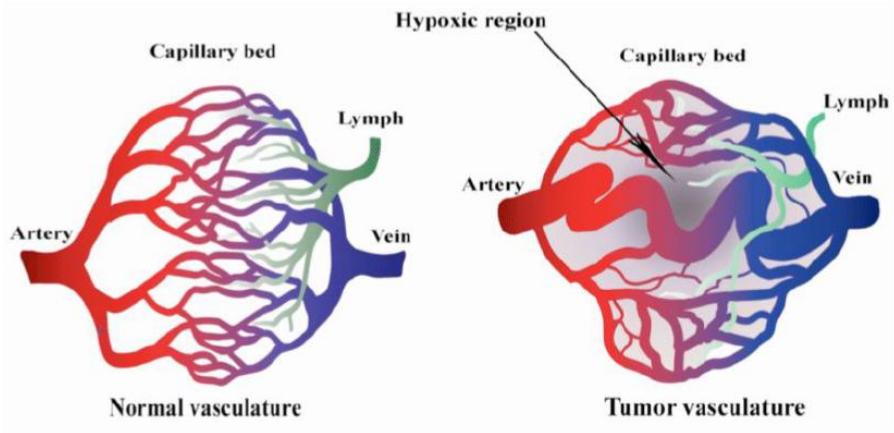
Nat Nanotech, **2007**, 2, 751

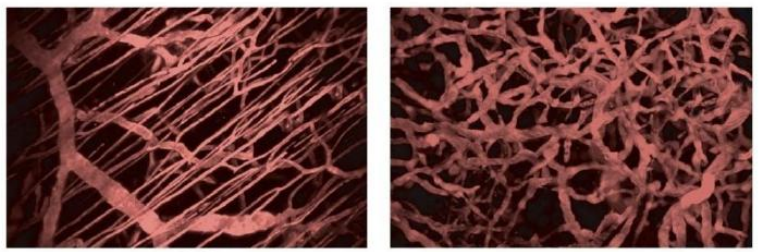


Pore size: 380-780 nm

PNAS, ***1998***, *95, 4607*

EPR relies on specific pathophysiological characteristics of tumors vs. healthy tissues. In healthy tissues, low-molecular-weight drugs easily extravasate out of blood vessels, while nanomedicines are unable to do so, because of their size. Conversely, in tumors, the abnormally wide fenestrations in the blood vessels allow for the extravasation of materials with sizes up to several hundreds of nanometers. This, together with the absence of lymphatic drainage, leads to a relatively effective and selective accumulation of nanomedicines in tumors.

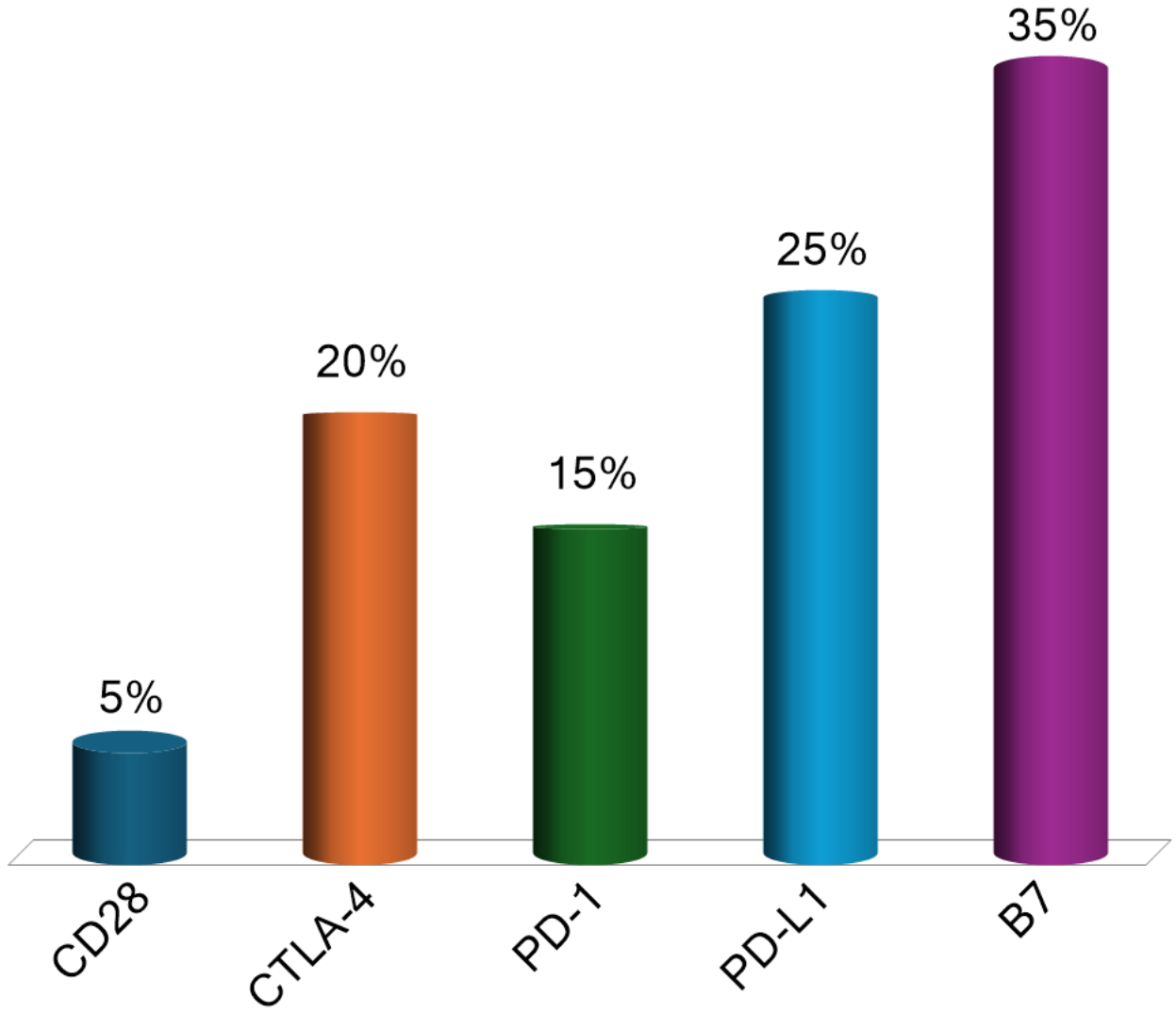




normal tissue **tumor**

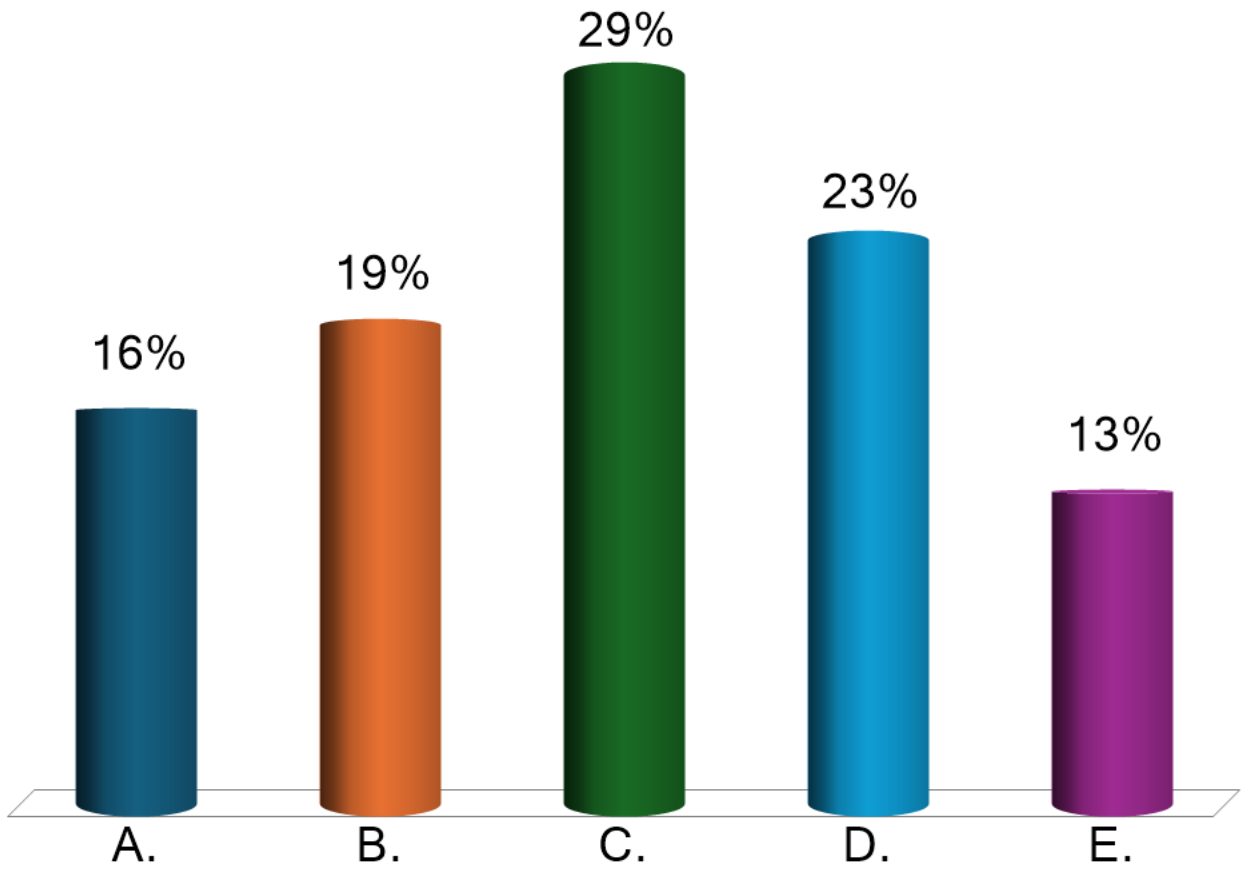
11. Which of these receptors are NOT on the T cell surface (multiple answers possible)

A. CD28
B. CTLA-4
C. PD-1
✓ D. PD-L1
✓ E. B7



11.Which of the following are ways to prevent systemic toxicity? (multiple answers possible)

A. Local injection of the drug
✓B. Hydrogel-mediated delivery
✓C. CpG DNA Nano-cocoon (DNC)
✓D. Microneedles
E. Cytokine fused to a tumor-targeted antibody

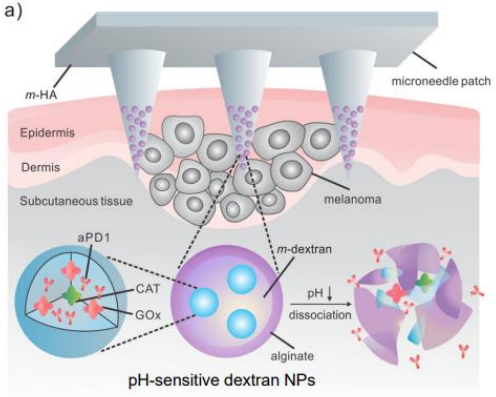


Microneedles enhance checkpoint blockade antibody therapy

- Each MN is composed of biocompatible hyaluronic acid (HA)
- MN is integrated with pH-sensitive dextran nanoparticles (NPs)
- NP encapsulate aPD1 and glucose oxidase (GOx).
- GOx is applied to convert blood glucose to gluconic acid in the presence of oxygen (O_2).
- Catalase (CAT) assists glucose oxidation by generation of O_2 and helps consume undesired hydrogen peroxide (H_2O_2)

glucose oxidase (GOx) converts blood glucose to gluconic acid

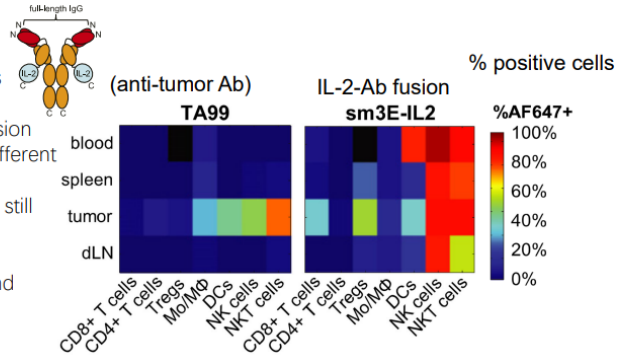
$$\text{glucose} + O_2 + H_2O \xrightarrow{GOx} \text{gluconic acid} + H_2O_2$$



Nano Lett. 2016, 16, 2334−2340

How about using tumor-targeted antibody?

- Anti-TA99 Ab targets tumor well as expected
- However, IL-2-Ab fusion protein behaved very different from the Ab alone
- Likely, the fusion IL-2 still target IL-2 receptors (dominates!)
- Systemic cytokines find systemic leukocytes



Tzeng, A., Kwan, B. H., Opel, C. F., Navaratna, T., & Wittrup, K. D. (2015). Proceedings of the National Academy of Sciences, 112(11), 3320–3325.

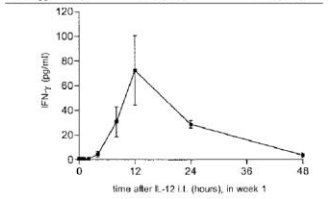
Does intra-tumoral injection help?

local injections ≠ local retention

https://theodora.com/rodent_laboratory/injections.html

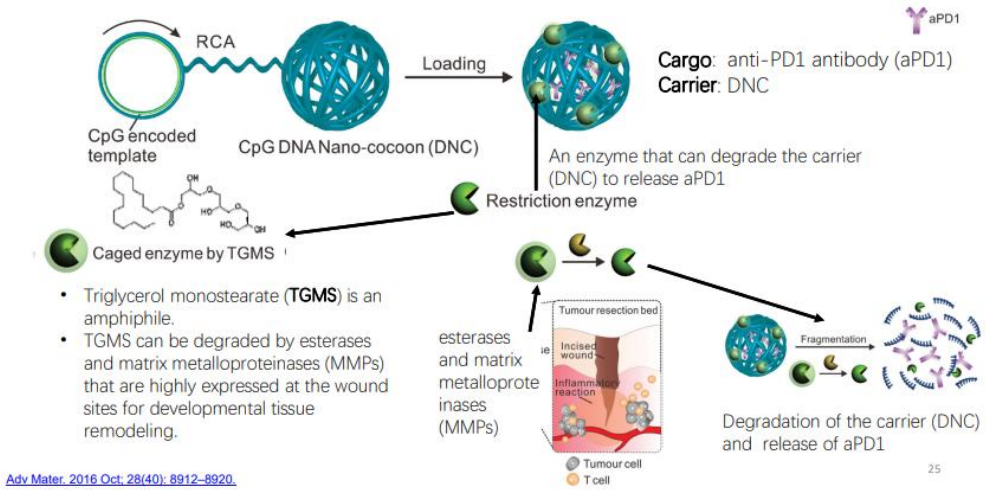
In humans with head and neck cancer: PK parameters for intratumoral IL-12:

	100 ng/kg	
	Week 1 (n = 3)	Week 6 (n = 2)
C_{max} (pg/ml)	362 (74.7)	763 (62.2)
T_{max} (h)	3 (1.4)	0.5 (0)
$t_{1/2}$ (h)	6.8 (0.1)	5.1 (0.4)
AUC (pg × h/ml)	4361 (1199)	6045 (2068)



van Herpen, Huijbens, R., Looman, M., & de Vries, J. (2003). Clin. Cancer Res.

How to better control the drug release?



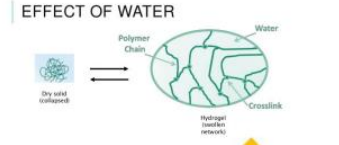
- Triglycerol monostearate (**TGMS**) is an amphiphile.
- TGMS can be degraded by esterases and matrix metalloproteinases (MMPs) that are highly expressed at the wound sites for developmental tissue remodeling.

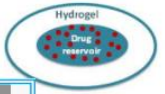
Adv Mater. 2016 Oct; 28(40): 8912–8920.

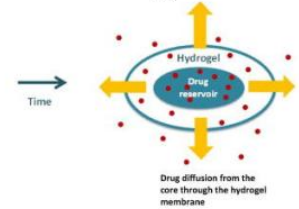
25

Hydrogel for deliverer of checkpoint blockade antibodies

A **hydrogel** is a network of polymer chains that are hydrophilic, sometimes found as a colloidal gel in which water is the dispersion medium. **Hydrogels** are highly absorbent (they can contain over 90% water) natural or synthetic polyme



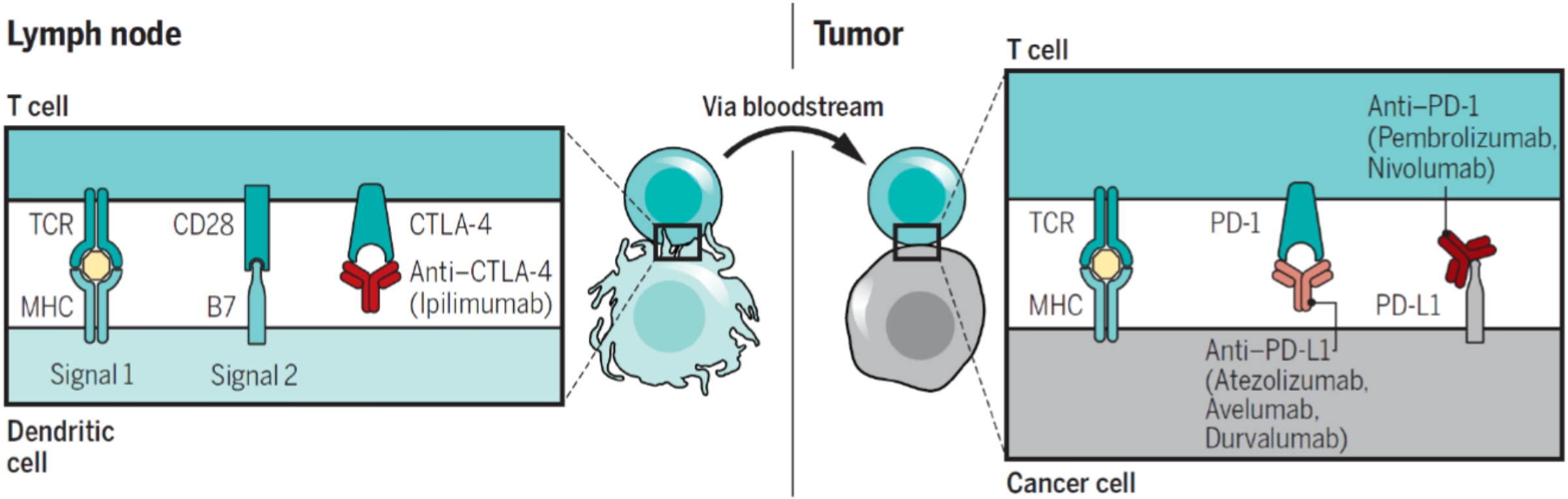




ONCOIMMUNOLOGY
2016, VOL. 5, NO. 2, e1074374 (12 pages)
http://dx.doi.org/10.1080/2162402X.2015.1074374

24

Checkpoint Inhibitors



Science 23 Mar 2018:
Vol. 359, Issue 6382, pp. 1350-1355
DOI: 10.1126/science.aar4060

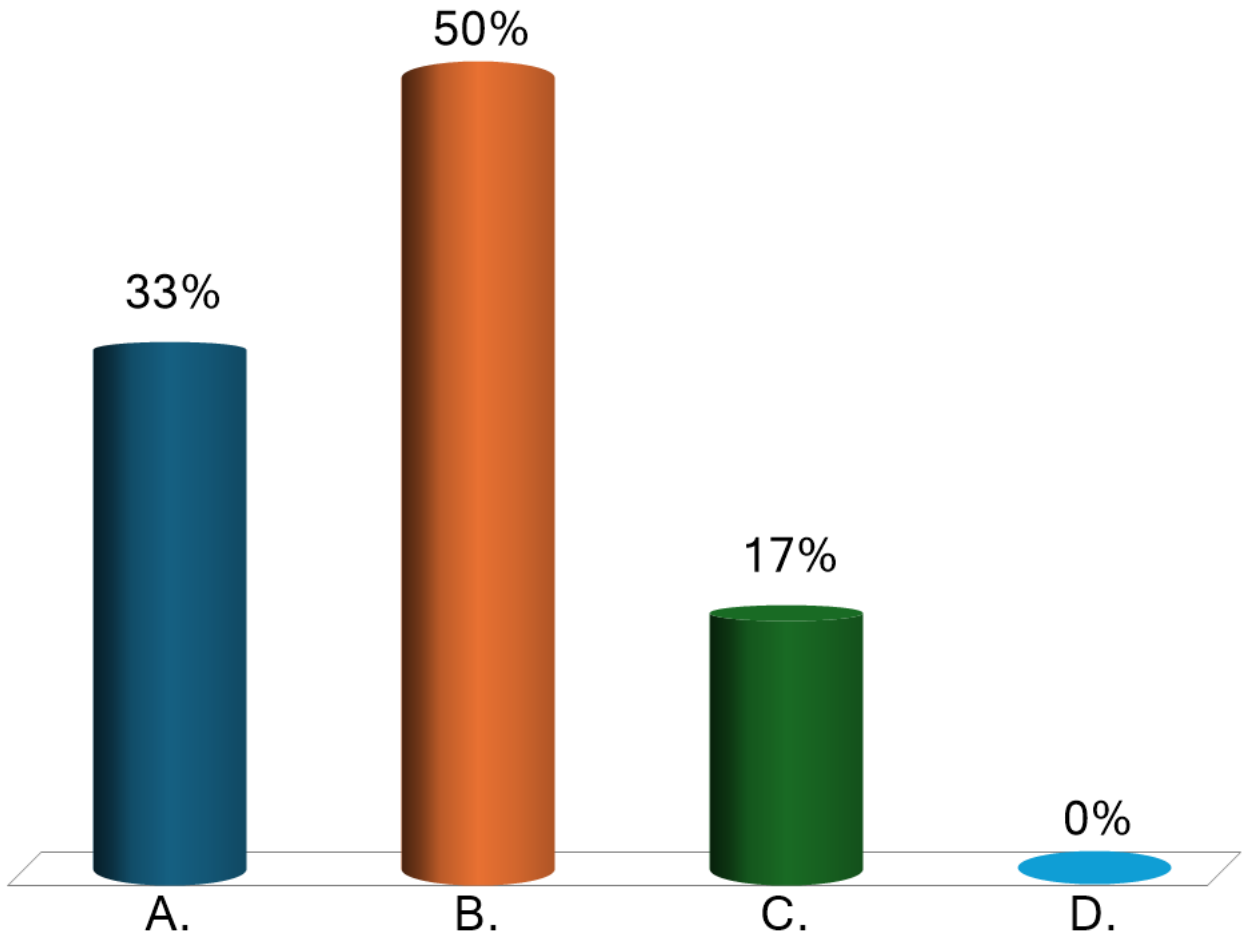
12.What is **TRUE** about CAR-T cells compared to TCR-T cells?

A. The CAR is similar to a TCR, however the intracellular domain is switched to the intracellular domain of the IL2 receptor

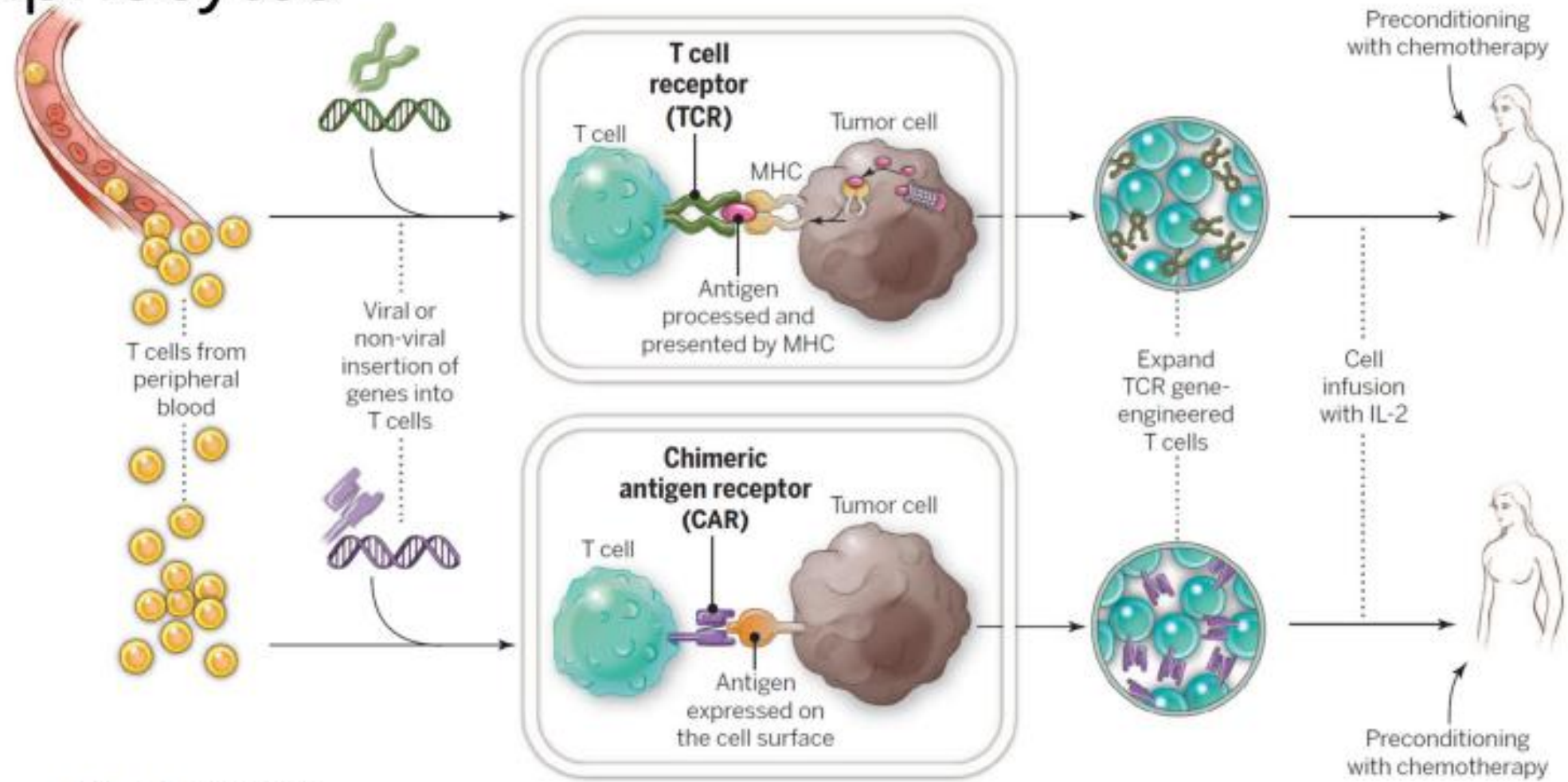
B. CAR-T cells are dependent on the recognition of target peptide in the MHC of target cells

C. CAR-T cells do not target the intracellular proteome ✓

D. The CAR possesses several adjacent extracellular CD3 domains

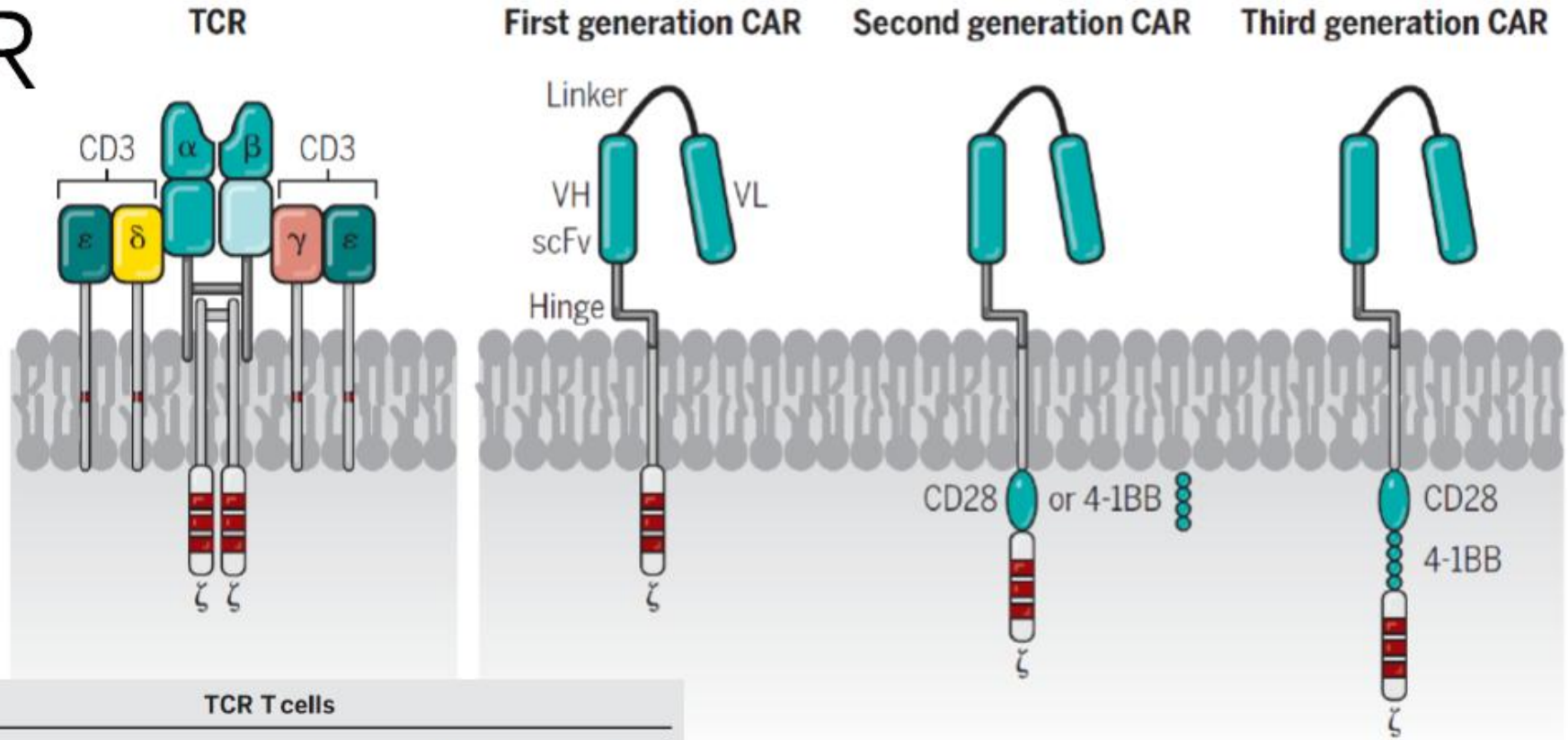


Gene-modification of peripheral blood lymphocytes



Science **348**, 62-68 (2015).

TCR vs. CAR



CAR T cells	TCR T cells
Signal amplification from synthetic biology: 200 targets can trigger CAR T cells (*57*)	Sensitive signal amplification derived by evolution of the TCR
Avidity-controllable	Low-avidity, unless engineered (*58*)
CAR targets surface structures: proteins, glycans	TCR targets intracellular proteome
MHC-independent recognition of tumor targets	Requires MHC class I expression and HLA matching on tumor
At least decade-long persistence (*59*)	Lifelong persistence
Serial killers of tumor cells (*60*)	Serial killers of tumor cells (*60*)
Cytokine release syndrome more severe than with TCR-based therapy	Off-tumor toxicity difficult to predict (*7*)

A CAR combines antigen-binding domains—most commonly, a single-chain variable fragment (scFv) derived from the variable domains of antibodies with the signaling domains of the TCR“ chain and additional costimulatory domains from receptors such as CD28, OX40, and CD137

Science **359**, 1361-1365 (2018).

13. Considering T cell metabolism, which of the following are **TRUE**? (multiple answers possible)

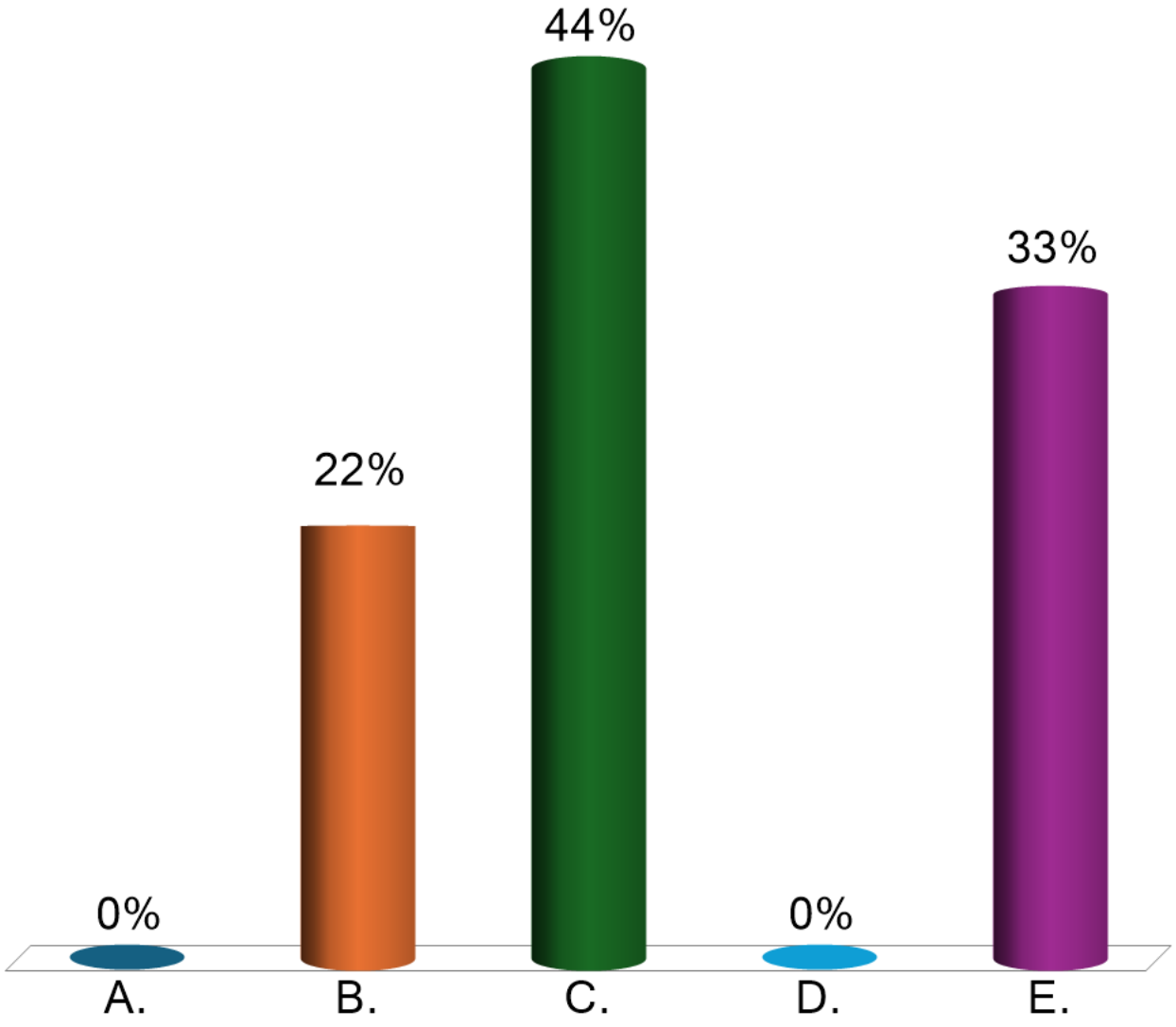
A. T cell diffrenciation state has no relation to metabolism

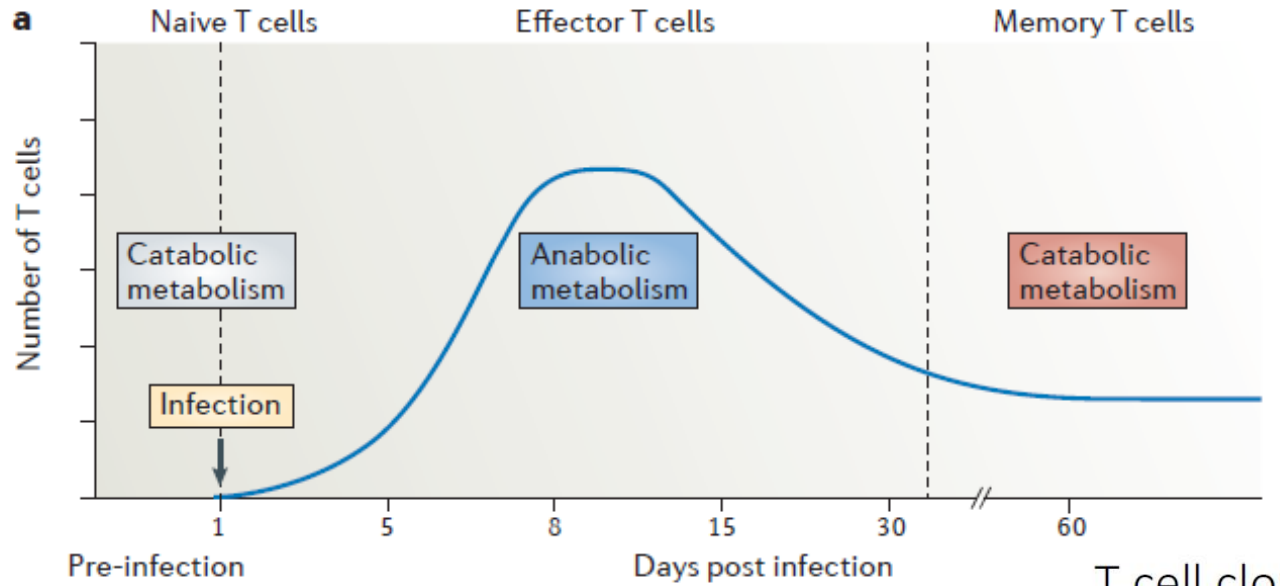
✓ B. Naive and memory have a catabolic metabolism

✓ C. Effector T cell have an anabolic metabolism

D. Arginin and tryptophan are not important in T cell metabolism

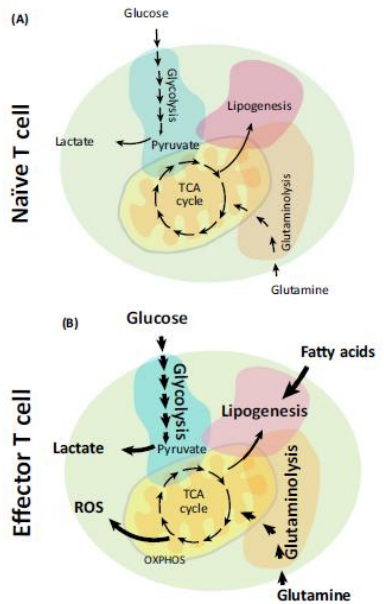
E. Enhancing reductive carboxylation fosters différenciation to memory T cells



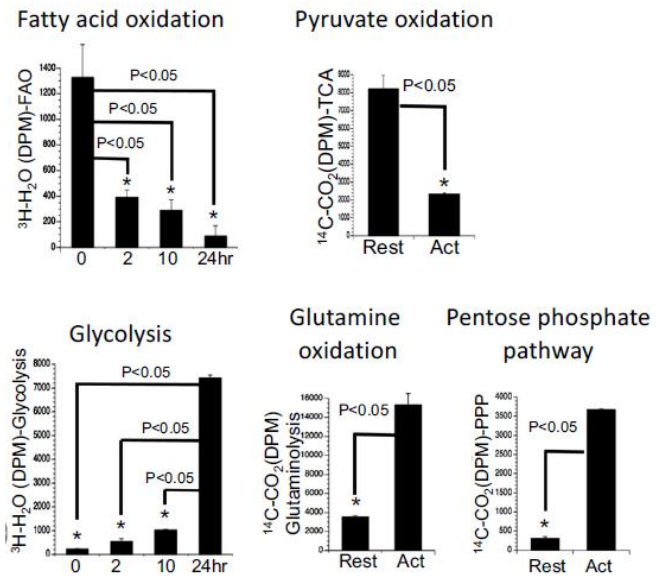


Kaech *et al.*, Nat. Rev. Imm. 2012

T cell clonal expansion requires a massive activation of specific metabolic pathways

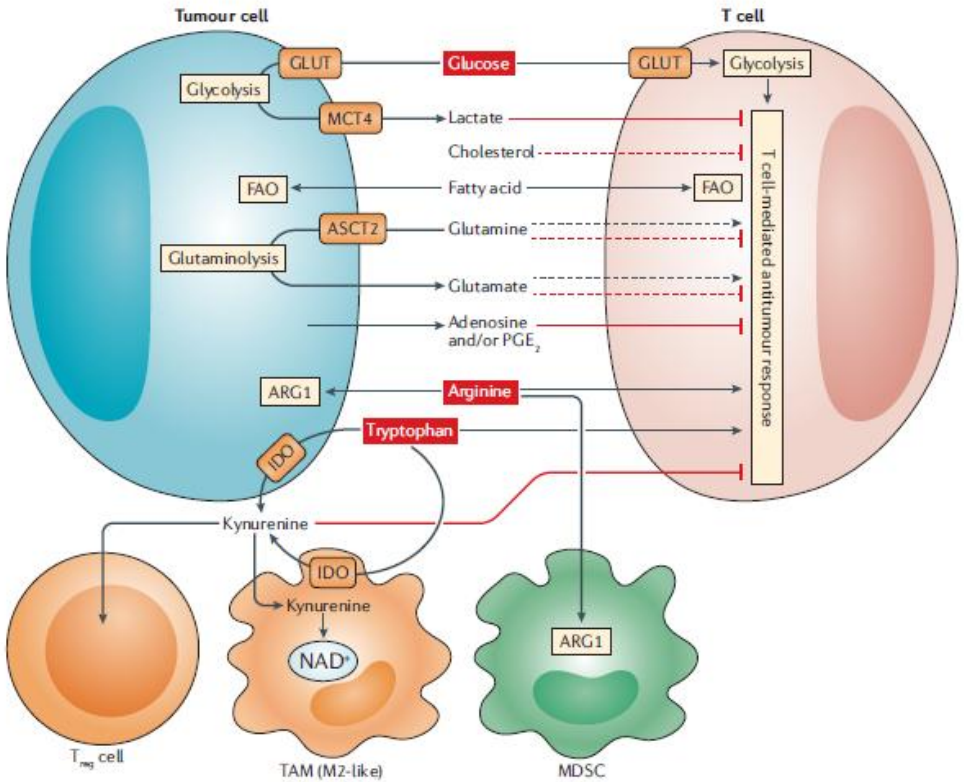


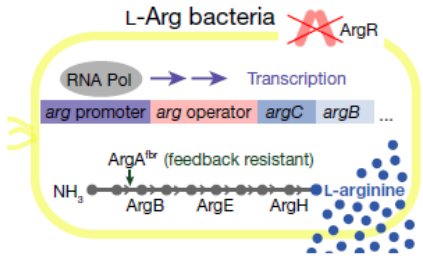
Zhang, Romero *et al.*, Trends Mol.Med. 2018

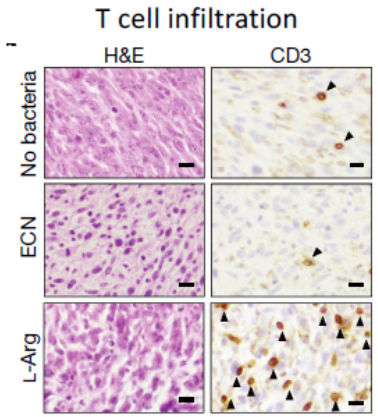


Wang *et al.*, Immunity 2011

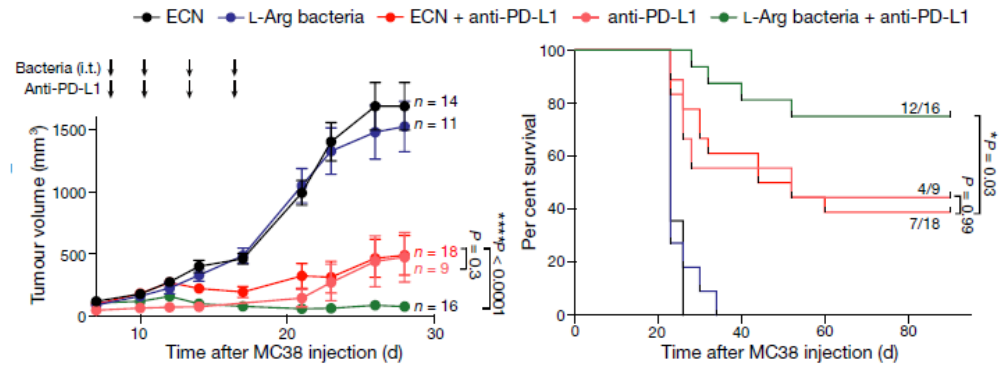
Immunotherapy is enhanced by nutrient supplementation through metabolically engineered bacteria







Arginine supply synergizes with checkpoint blockade immunotherapy



Canale *et al.*, 2021 Nature

Li, Wenes, Romero *et al.*, Nat.Rev.Clin.Onc. 2019

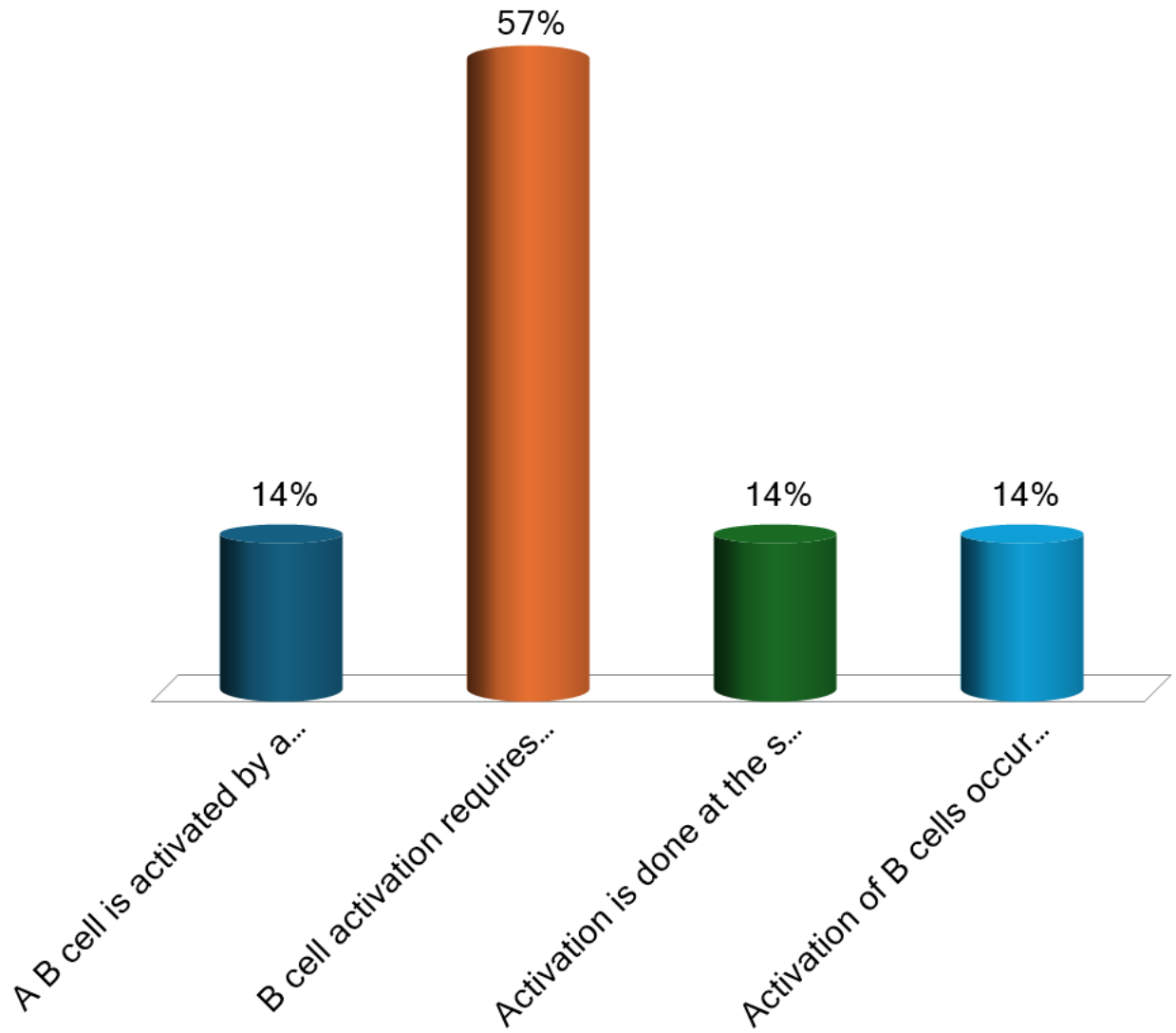
14. Which of the following statement regarding B cell activation is **TRUE**?

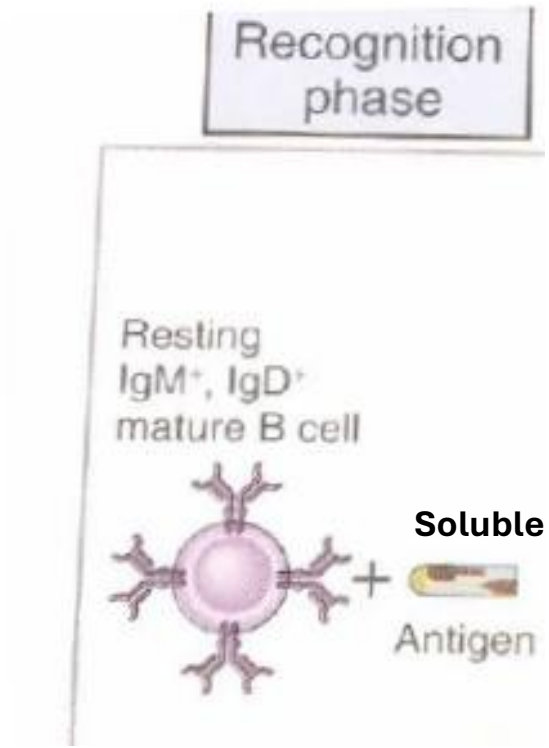
A. A B cell is activated by a APC presenting a MHC-peptide

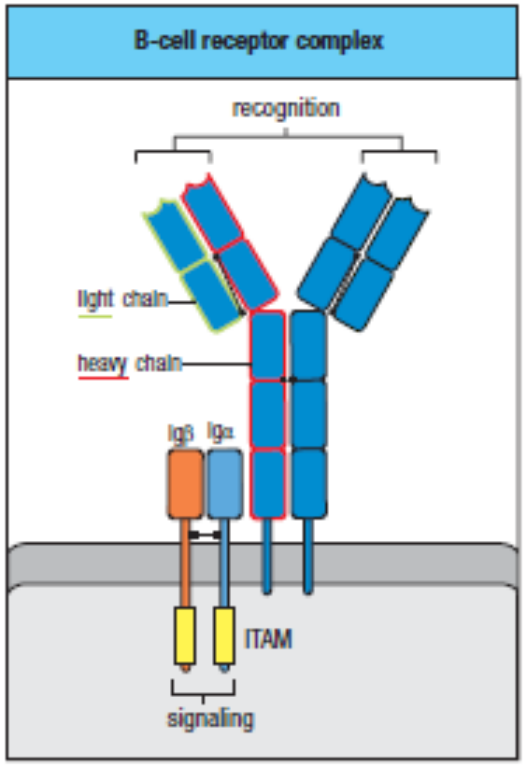
✓ B. B cell activation requires CD40L presentation on CD4 T cells

C. Activation is done at the site of injection

D. Activation of B cells occurs before T cell activation







No MHC required

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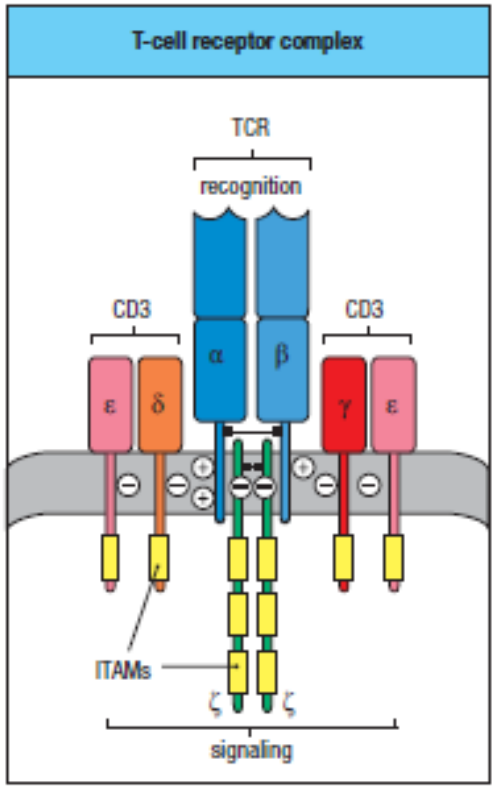
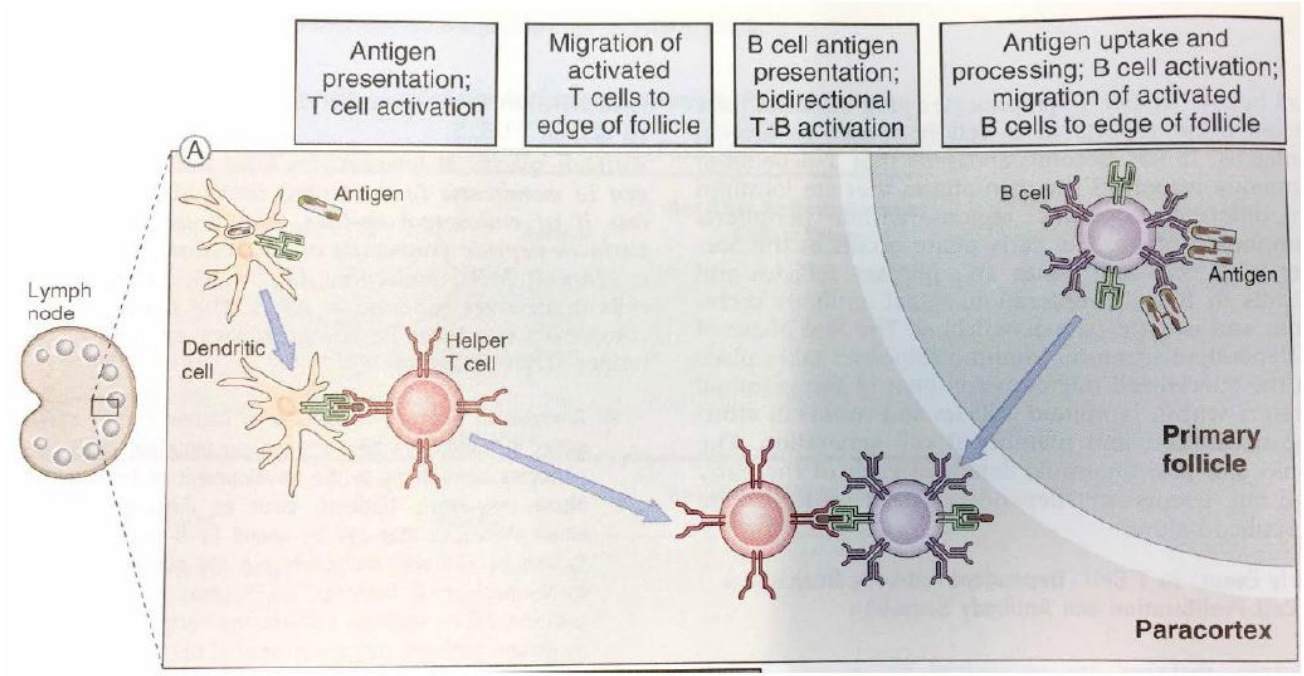


Janeway's Immunobiology, 9th ed.



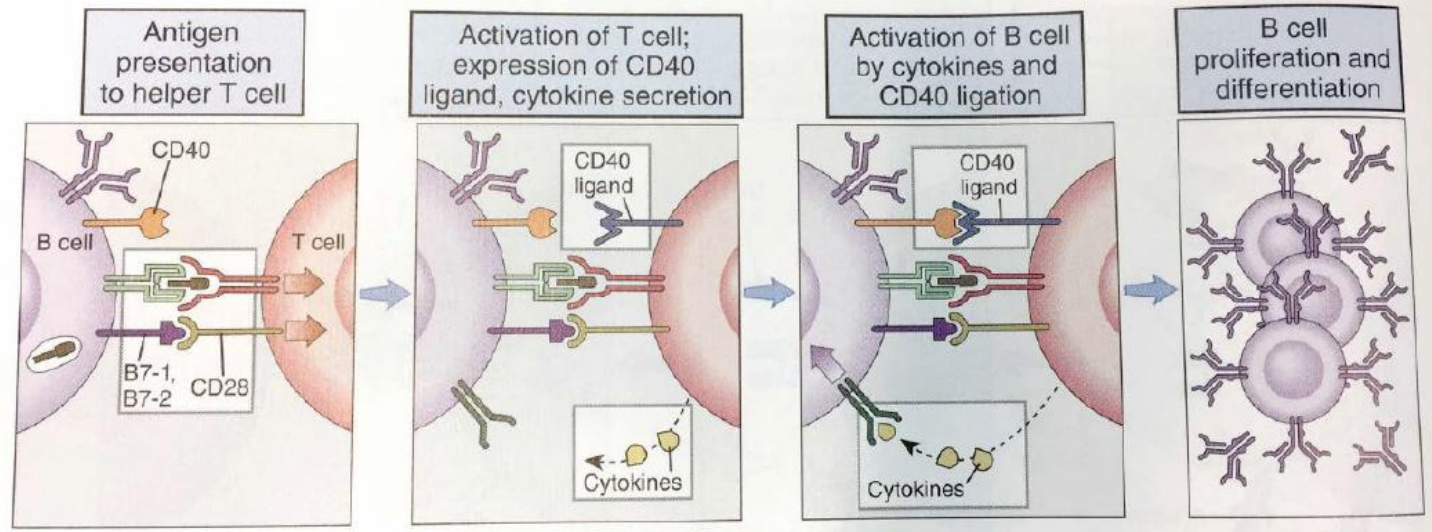


Figure 9–9 Bidirectional molecular interactions between B and T lymphocytes.
In this model for the role of multiple ligand-receptor pairs in T cell–dependent B cell activation, antigen is presented by B cells and induces the expression of costimulators B7-1 and B7-2. Helper T cells recognize the antigen (in the form of peptide-MHC complexes) and the costimulators and are stimulated to express CD40 ligand. CD40 ligand then binds to CD40 on the B cells and initiates B cell proliferation and differentiation. MHC, major histocompatibility complex.

Final exam: Wednesday 25.06.2025 - 9:15 to 12:15
room CM 1 1, CM 1 121 and CM 1 4

Example on moodle

Essay: deadline 30/05/2025 at 23h59 (tonight)

Make sure to submit it on moodle
File name: “Last name_first name”

If you have any questions:

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jiayi.tan@epfl.ch