ASCPT

FROM MOLECULE TO PATIENT

ASCPT 2019 ANNUAL MEETING

FROM MOLECULE TO PATIENT

Basic immunology for clinical pharmacologists: application to cancer therapy

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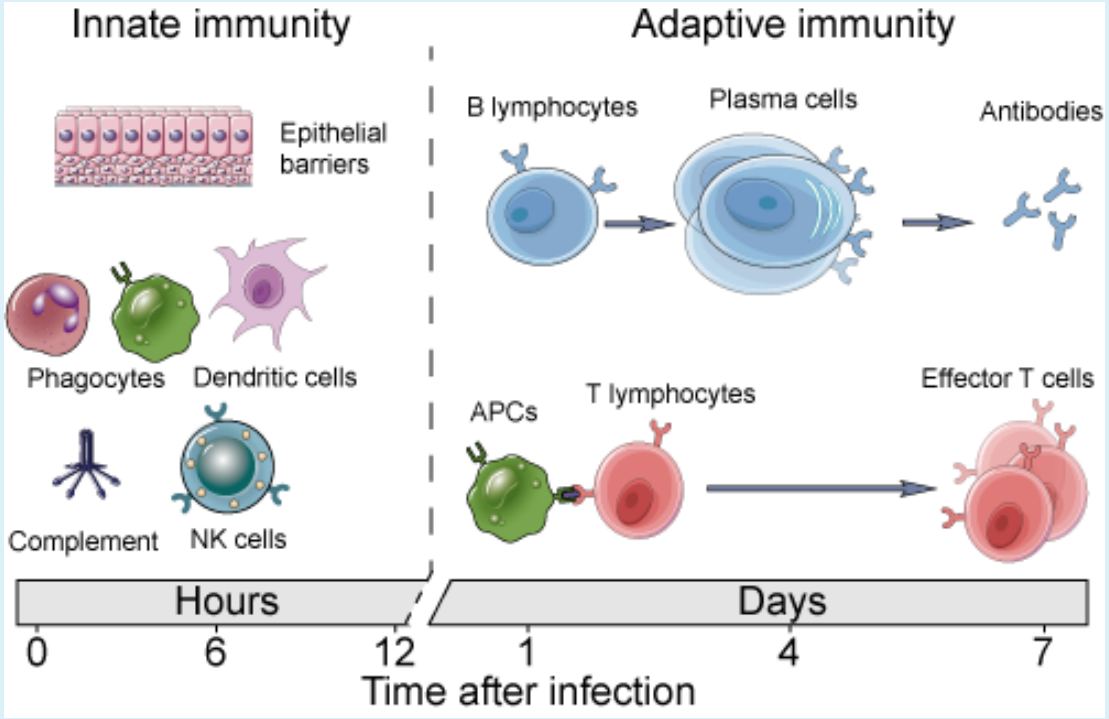
University of Virginia

What do you know? True or False

- Transgenic T cells only recognize intracellular antigens?
- Therapeutic mononclonal antibodies are only used to block checkpoint inhibitor molecules?
- Only dendritic cells are antigen presenting cells?
- Adaptive immunity only recognizes mutations in tumor cells?
- Immunohistochemistry is the most useful way to monitor anti-tumor immunity?

Fundamental components of immunity

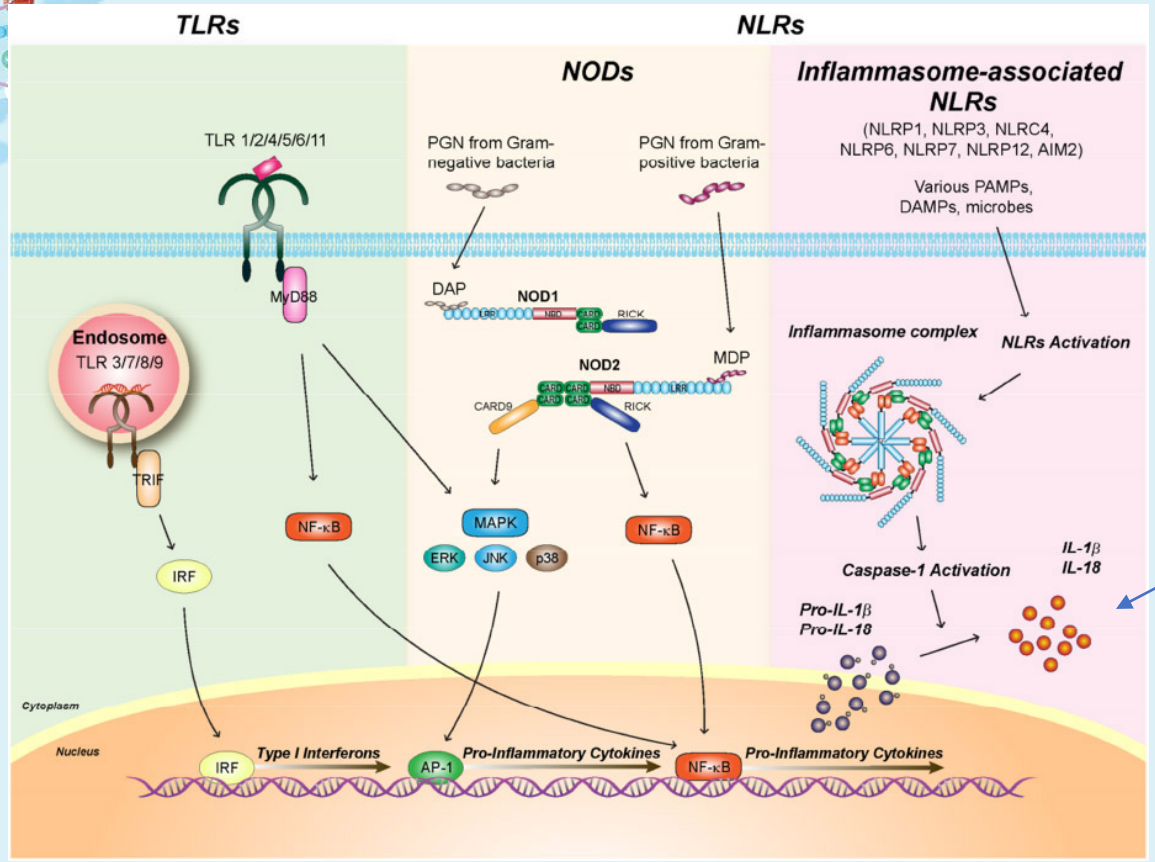
- Rapid response
- Pattern recognition
- Scavenger receptors
- Cytokines and other instructive molecules
- Direct response for host defense
- Phagocytosis
- Anti-microbial activity



- slow response
- Increasing affinity receptors
- Memory
  - Recirculation
  - Self-renewal
  - Qualitative changes

https://www.creative-diagnostics.com/innate-and-adaptive-immunity.htm

Pattern recognition molecules drive innate inflammation



Inflammation provides context for adaptive immune response

J Bacteriol Virol. 2014 Sep;44(3):215-225.

Adaptive immunity of primarily composed of T cells and B cells

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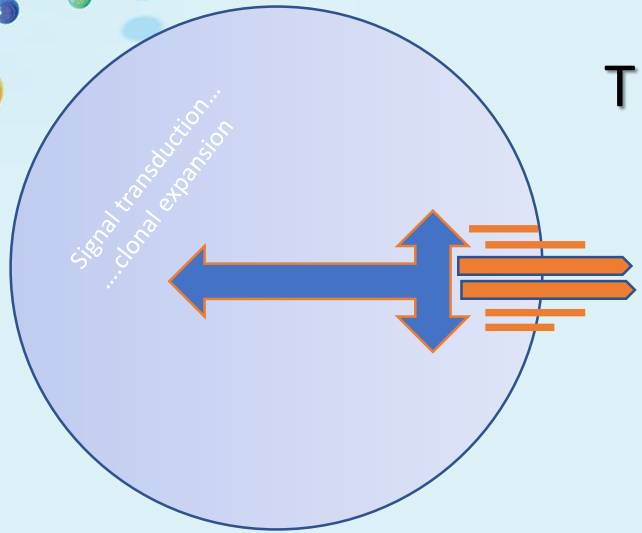
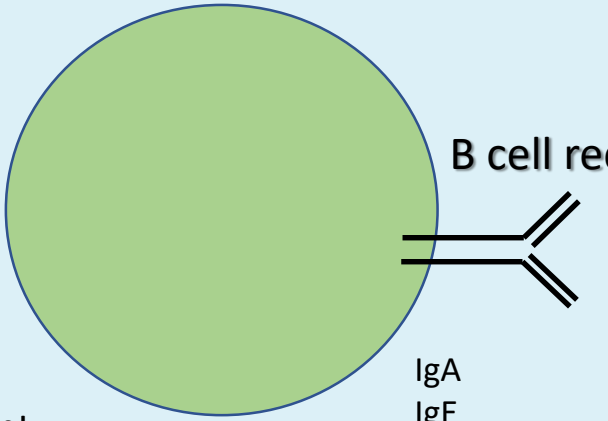


T cell receptor

T cell subtypes:
CD8= cytotoxic
CD4= helper

<u>Antigen receptors:</u>

- Highly diverse due to recombination during lymphocyte development
- 2.5e7 possible combinations
- Clonal selection drives specificity



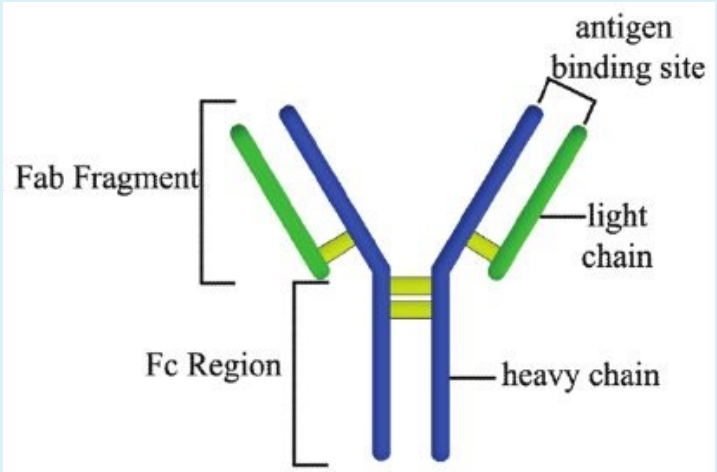
B cell receptor

IgA
IgE
IgG
-IgG1, IgG2, IgG3, IgG4
IgM
IgD

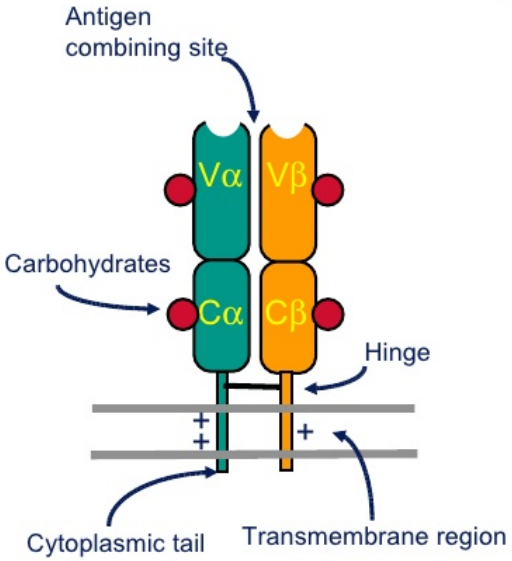
Fc
Fab

Antibody isotype influences activity

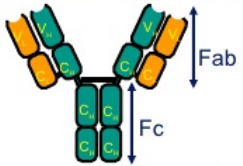
Antibody and TCR structure



The T cell antigen receptor



Resembles an Ig Fab fragment



Domain structure: Ig gene superfamily
Monovalent

No alternative constant regions

Never secreted

Heterodimeric, chains are disuphide-bonded

Very short intracytoplasmic tail

Positively charged amino acids in the TM region

Antigen combining site made of juxtaposed Vα and Vβ regions

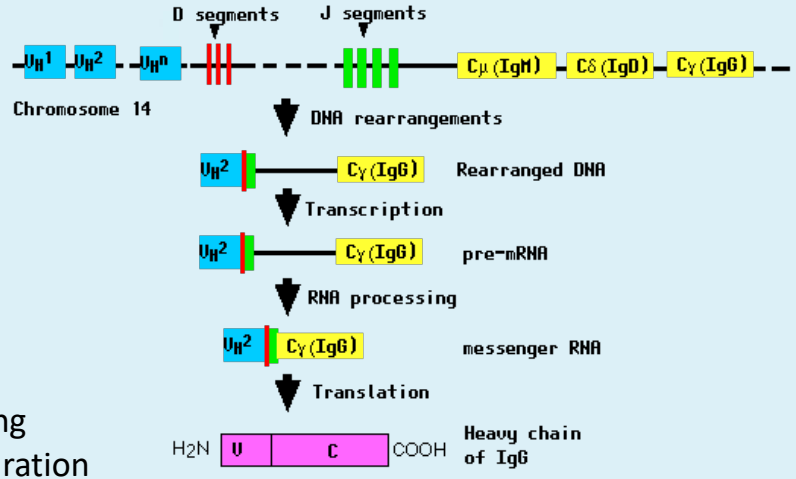
30,000 identical specificity TcR per cell

https://www.slideshare.net/rajud521/t-cell-antigen-receptor

Generation of diversity in lymphocyte antigen receptors

- 2.5e7 possible combinations!
- Can't be germline encoded (compare with PRR and NK-R)
- Allelic exclusion: 1 antigen receptor per cell
- Fitness of receptor is defined by positive and negative selection

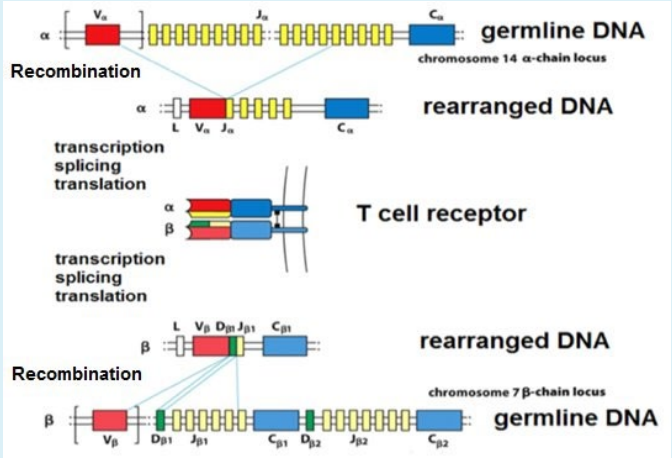
BCR:



-class switching
-Affinity maturation
-somatic hypermutation

http://www.biology-pages.info/A/AgReceptorDiversity.html

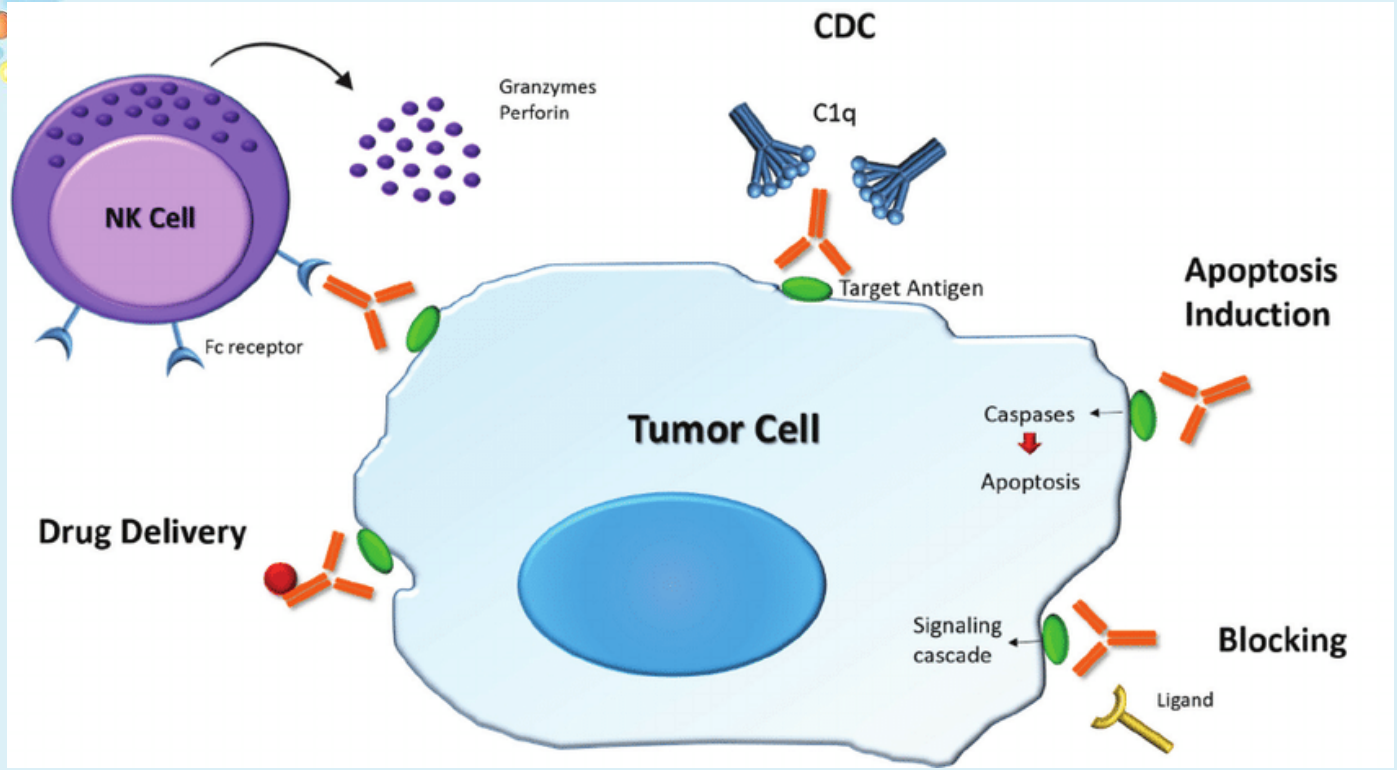
TCR:



- TCR sequence is unique to each T cell

adapted from Janeway CA et al. 2001 (26)

Functions of antibodies



Loureiro, et al. (2015). Challenges in Antibody Development against Tn and Sialyl-Tn Antigens. Biomolecules. 2015. 1783-1809. 10.3390/biom5031783.

Different Ab isotypes have different activities

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Functions and properties of immunoglobulin

	Immunoglobulin								
	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	IgD	IgE
Classical pathway of complement activation	++	+	+++	–	+++	–	–	–	–
Alternative pathway of complement activation	–	–	–	–	–	+	–	–	–
Placental transfer	+++	+	++	–/+	–	–	–	–	–
Binding to macrophage and phagocyte Fc receptors	+	–	+	–/+	–	+	+	–	+
High-affinity binding to mast cells and basophils	–	–	–	–	–	–	–	–	+++
Reactivity with staphylococcal Protein A	+	+	–/+	+	–	–	–	–	–

Figure 4-17 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

Agonist antibodies mimic ligands; role for cross-link via FcR binding

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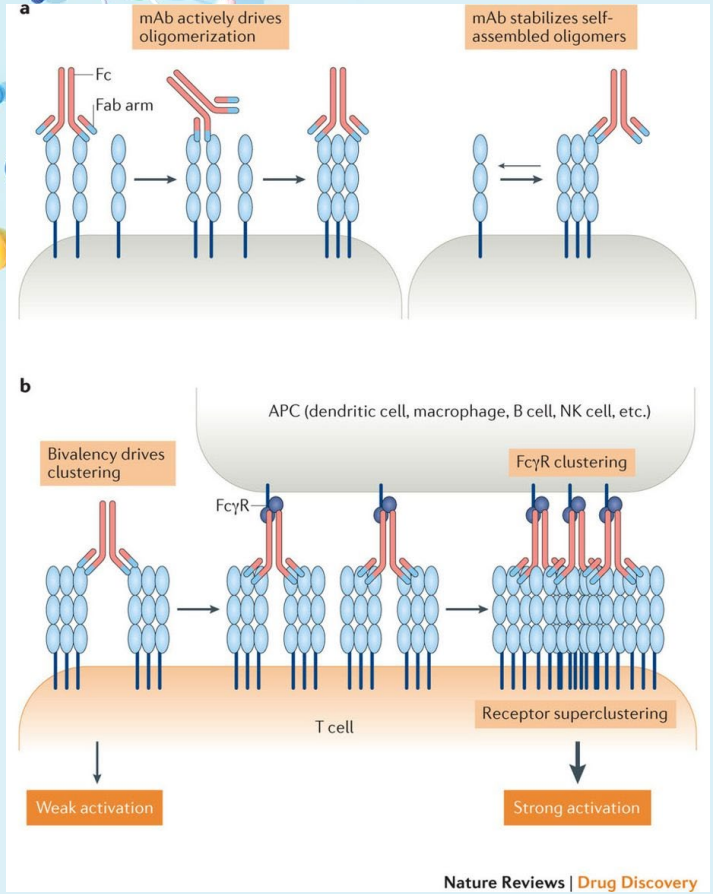
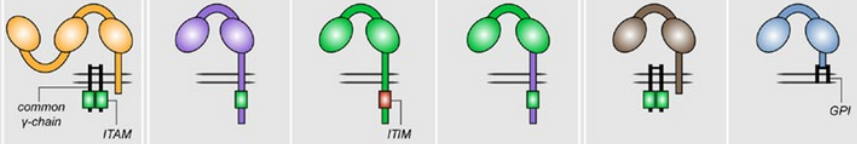
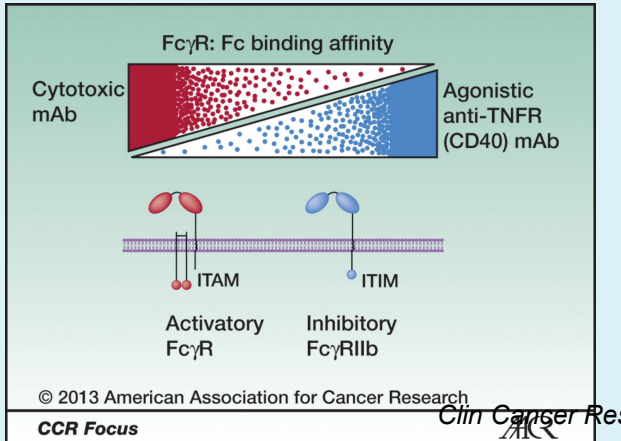


Name	FcγRI CD64	FcγRIIa CD32a	FcγRIIb C32b	FcγRIIc CD32c	FcγRIIIa CD16a	FcγRIIIb CD16b
Structure						
Function	Activating	Activating	Inhibitory	Activating	Activating	Activating
Affinity	High	Low	Low	Low	Low	Low
SNP		131H/R R: reduced affinity to IgG2	232I/T T: decreased inhibitory activity	57Q/X X: stop codon (non-functional protein)	158F/V V: increased affinity to IgG1/3/4	NA1/2 NA2: reduced affinity to IgG1/3

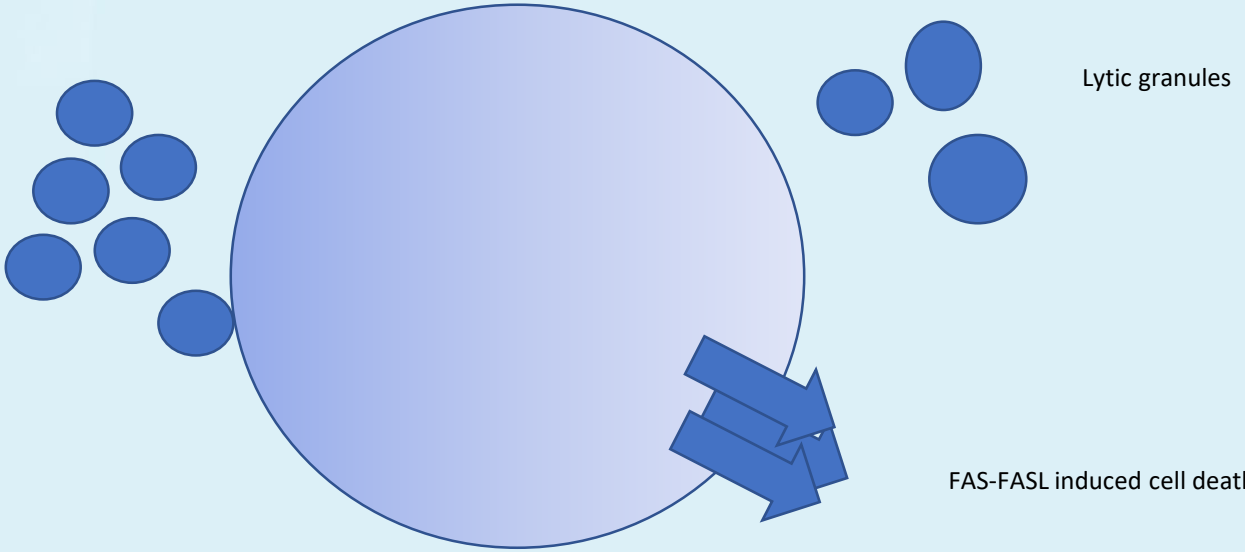
Vogelpoelet al). Frontiers in immunology. 6. 79. 10.3389/fimmu.2015.00079.



FcR available?

Clin Cancer Res; 19(5); 1035–43

T cells are the major mediators of tumor immunity due to their effector activities



- The production of these molecules and cytolytic activity are commonly monitored to examine “functional” immunity

Why Immunotherapy for Cancer?

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- Immune system is exquisitely specific: chemo- and radiation therapy are not; even TKIs can be off-tumor.
- Immune system spreads to many areas of the body and is quick to respond upon re-exposure to antigen.
- Immune system remembers. Responses are durable.
- Immunity can be engineered and personalized with synthetic biology.

T cells in tumor are a good prognostic indicator

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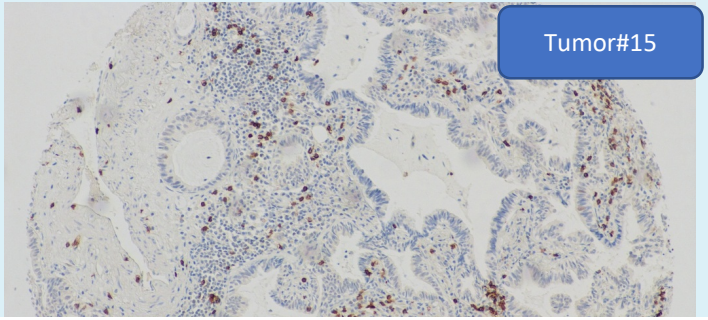
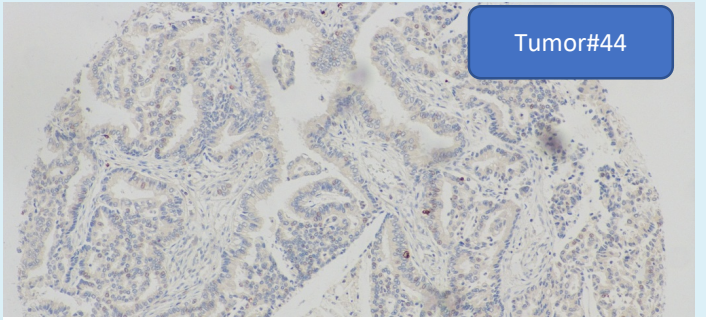
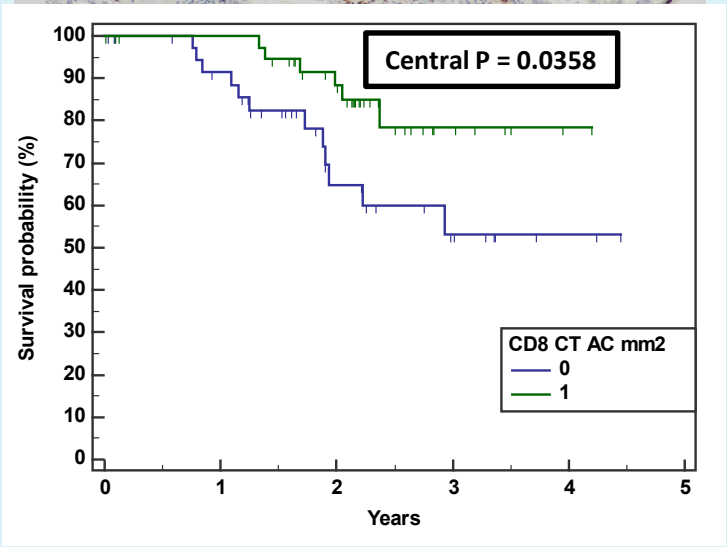
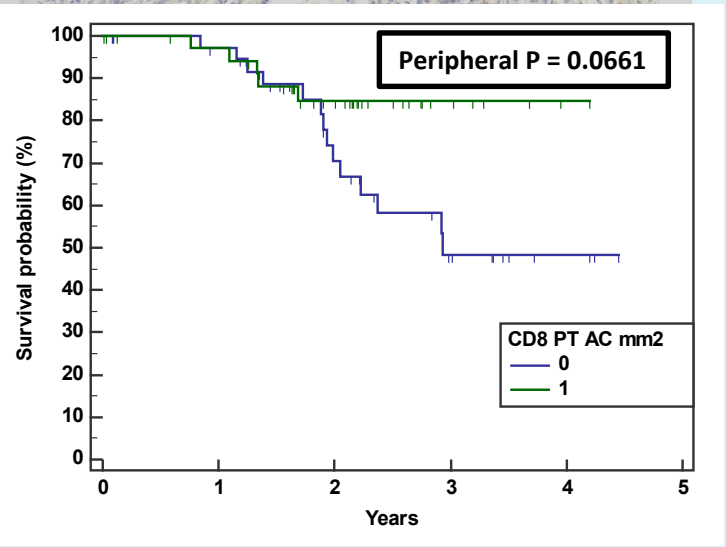
CD8









What are these immune cells; how did they get here and why are they relevant?

Cancer-Immunity Cycle

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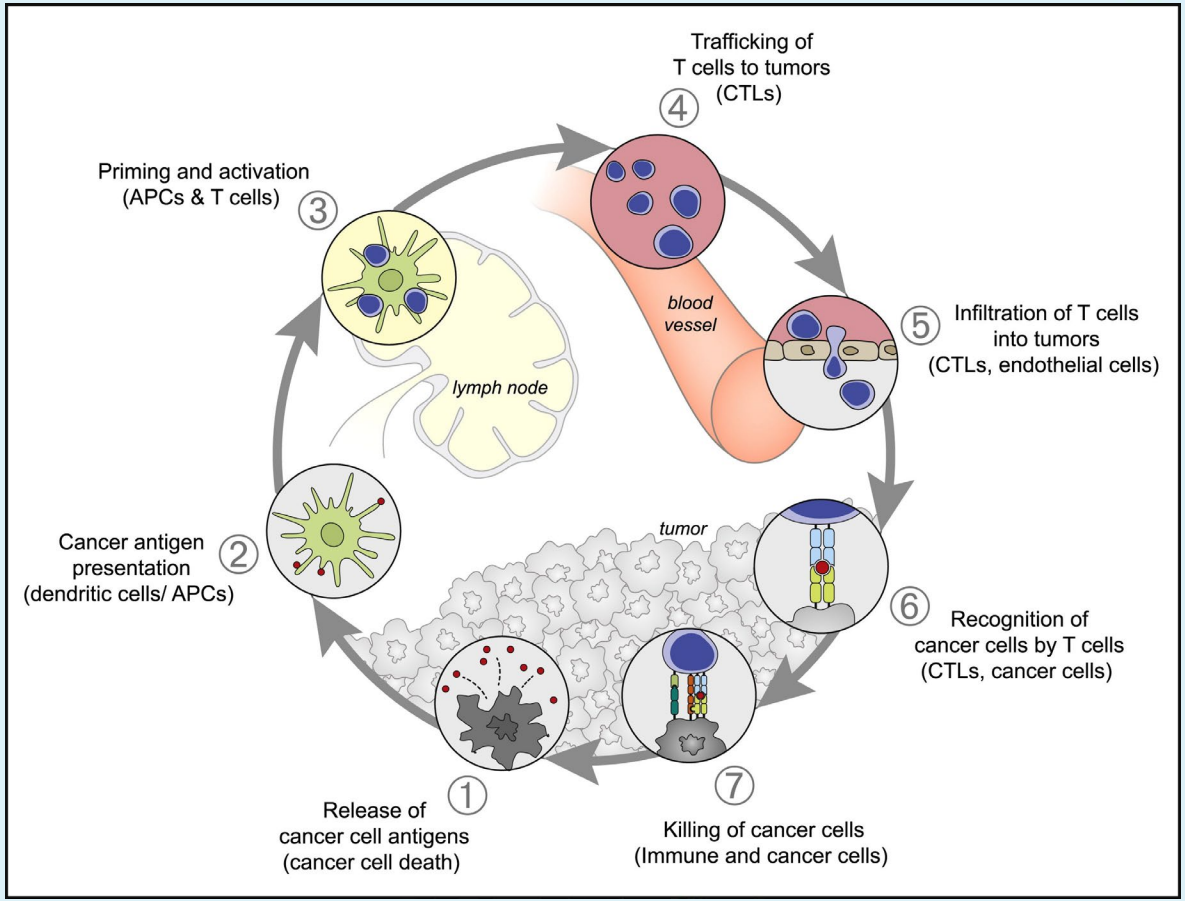

Today's goal is to help you understand this!

Chen and Mellman Immunity Volume 39, Issue 1, 25 July 2013, Pages 1-10

T cells recognize degraded proteins presented at the surface of cells: they peer inside cells (MHC restriction)

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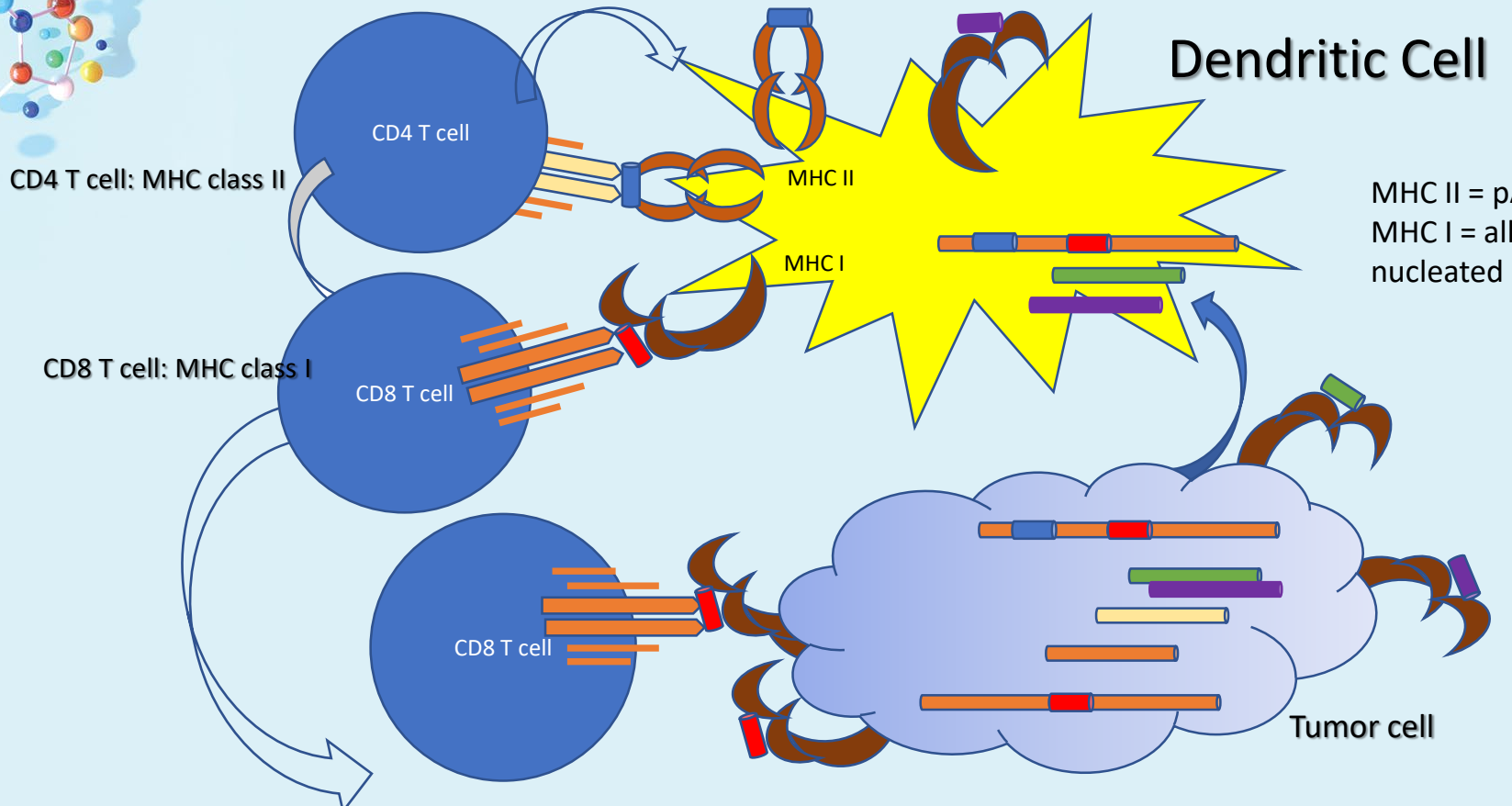
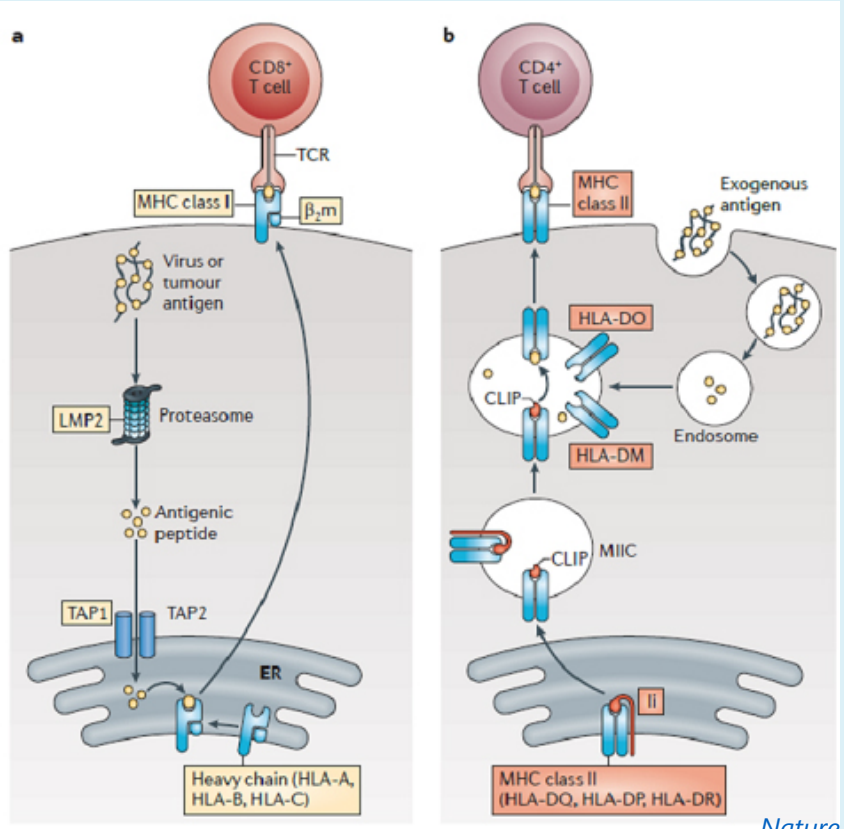
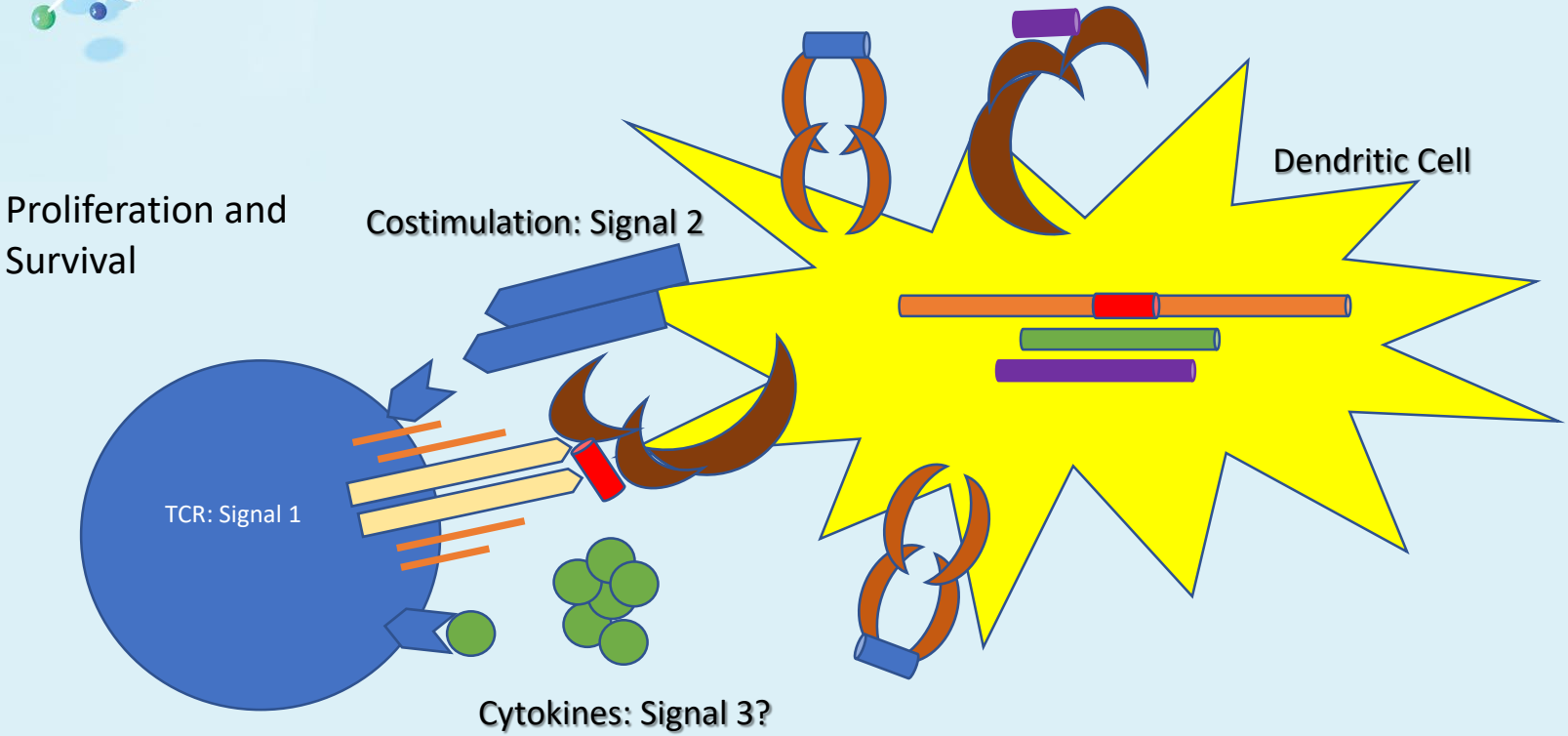


Antigen processing

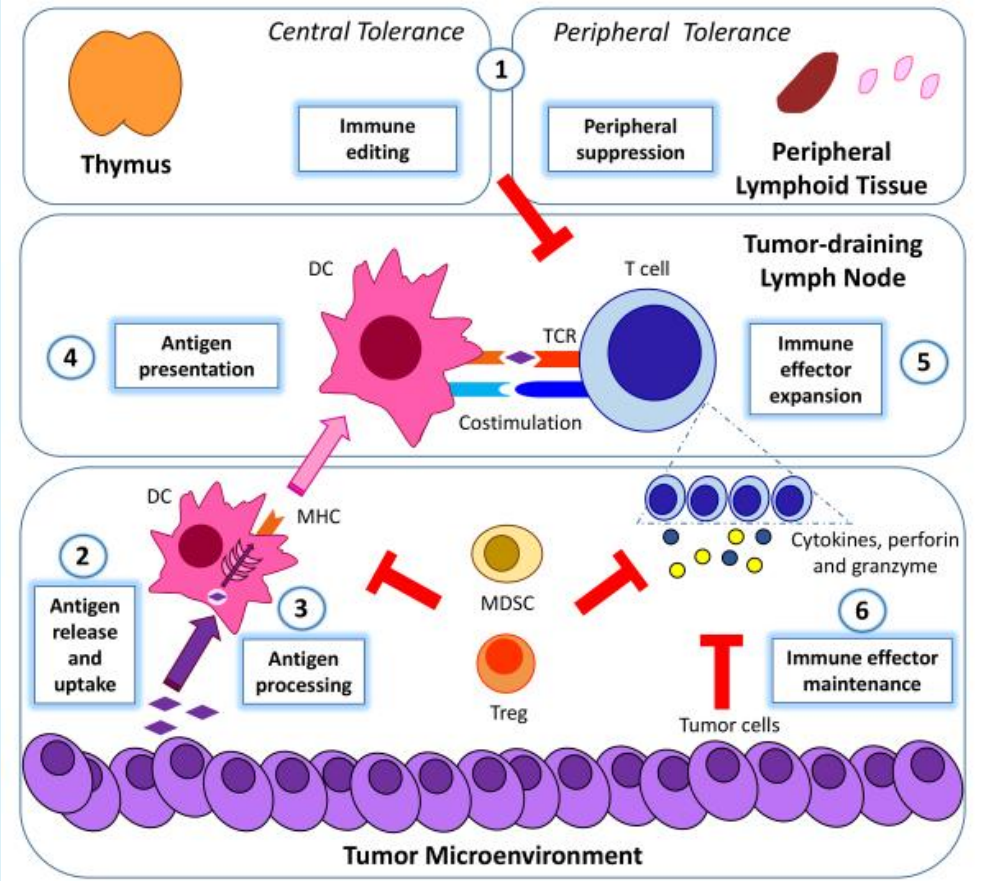


-Antigen can be acquired by multiple different receptor systems
-Macrophages and B cells can presented antigen acquired exogenously on MHC class II molecules
-Only DC can present exogenously acquired antigen on MHC class I molecules.

T cells require multiple “signals” for support….



Tolerance



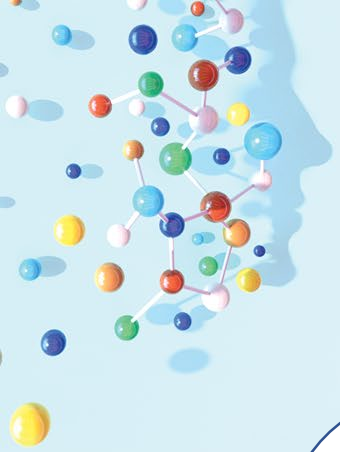
Mechanism to prevent the diversity of receptors generated during recombination from attacking the “self” proteins.

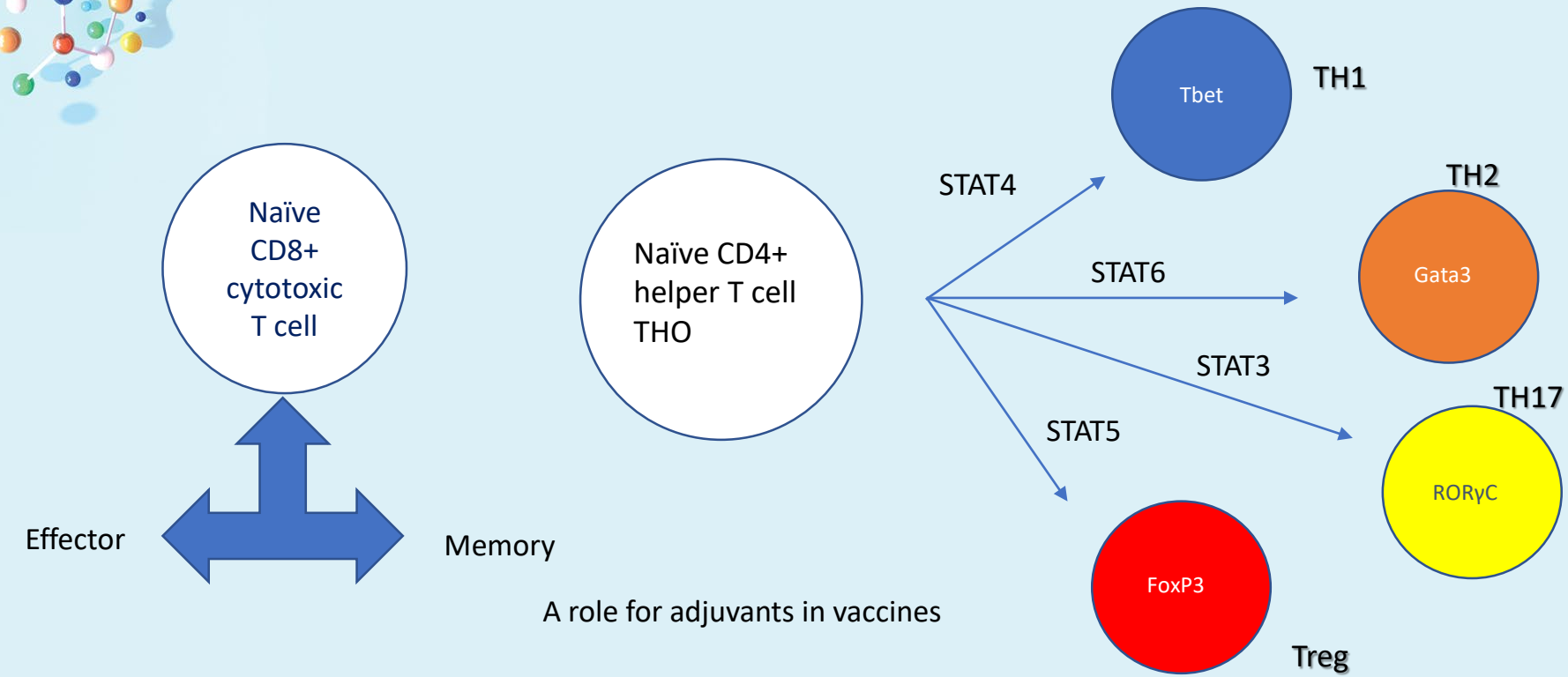
Peripheral tolerance is context dependent- inflammation (PRR…)

Resting DC shut-off T cells.

How is tumor antigen presented?

Environmental conditions dictate T cell differentiation and functions with canonical transcription factors





A role for adjuvants in vaccines

Clonal selection

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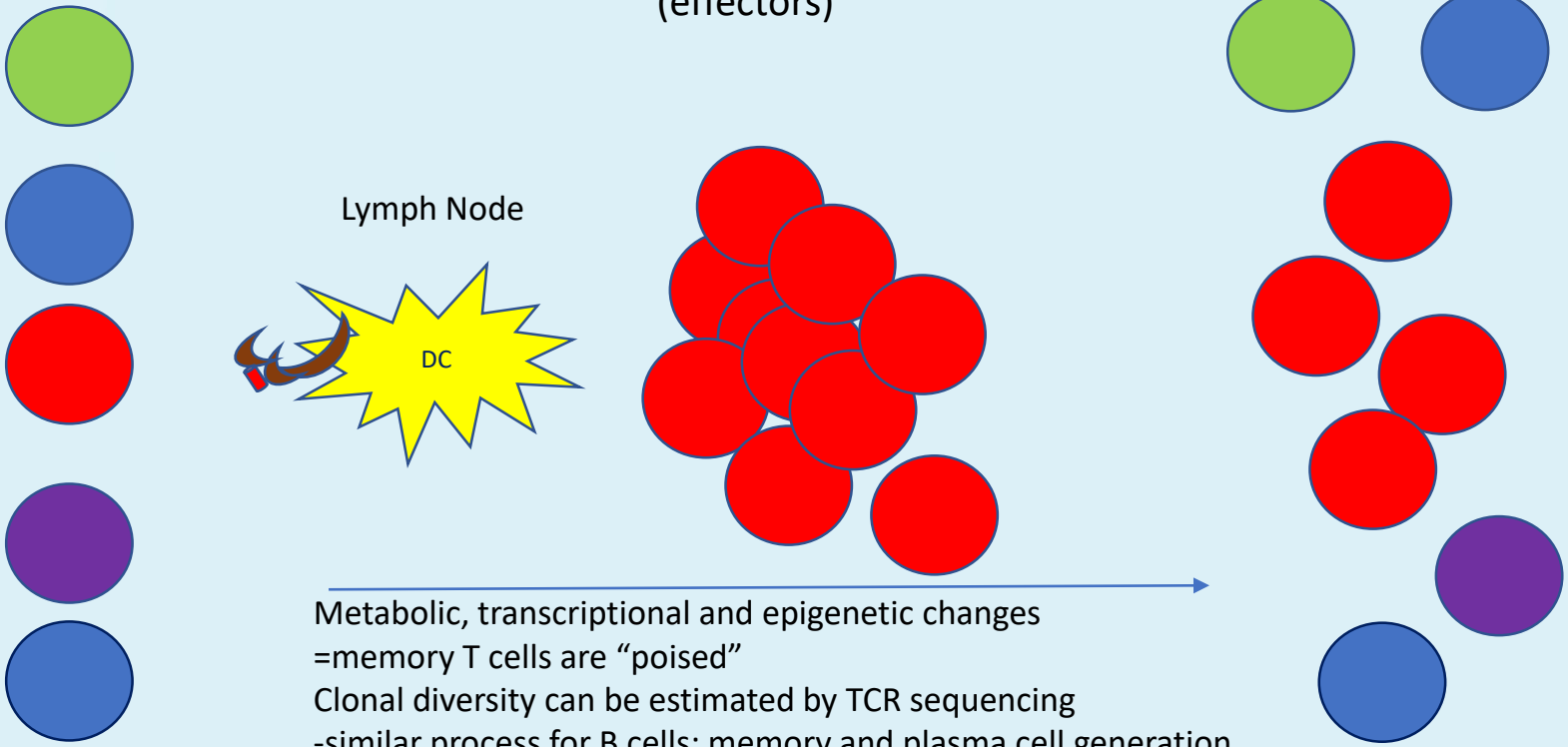
Naïve population

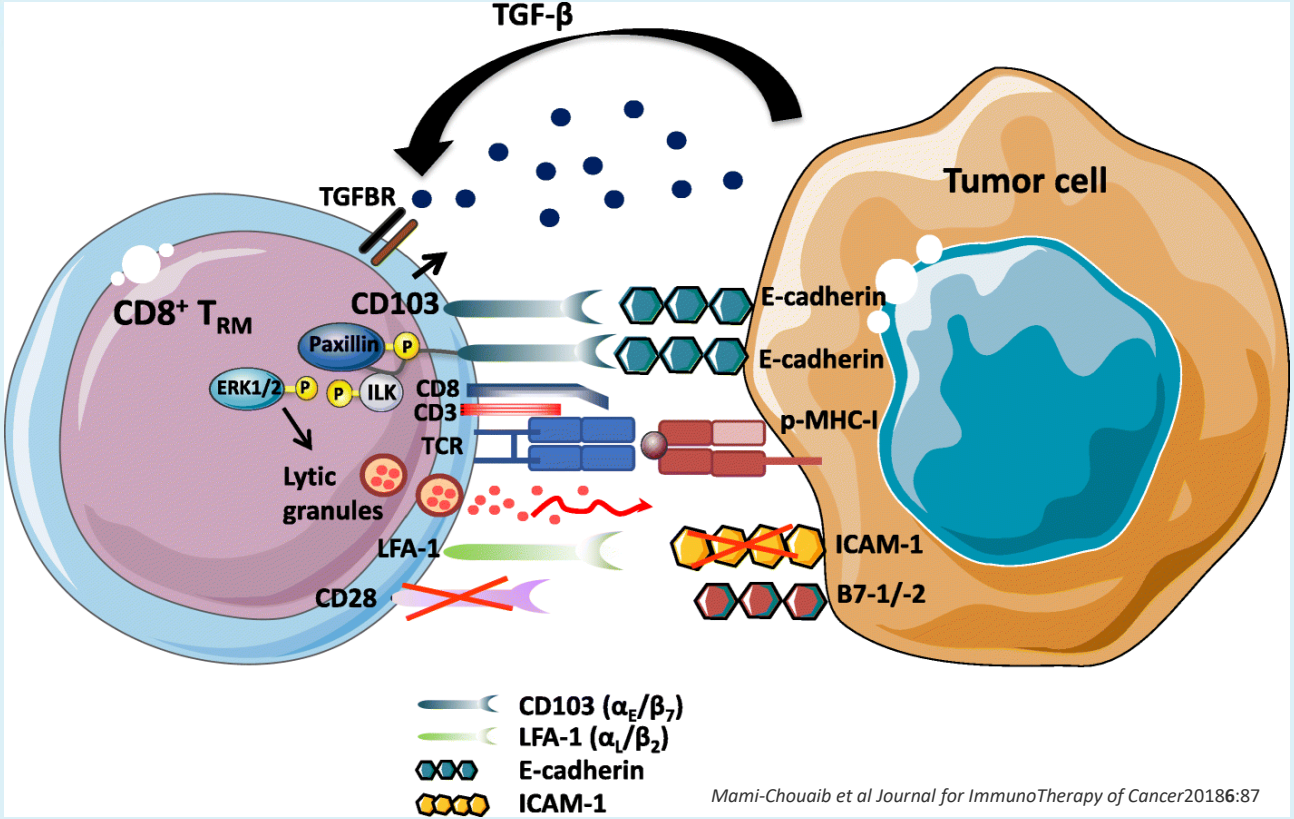
Primary response (effectors)

Memory



Metabolic, transcriptional and epigenetic changes
=memory T cells are “poised”
Clonal diversity can be estimated by TCR sequencing
-similar process for B cells; memory and plasma cell generation

Trafficking: how to get off the Beltway! Zipcodes and parking permits.



*Mami-Chouaib et al Journal for ImmunoTherapy of Cancer*2018**6**:87

Resistance Mechanisms: Darwinian or Newtonian?

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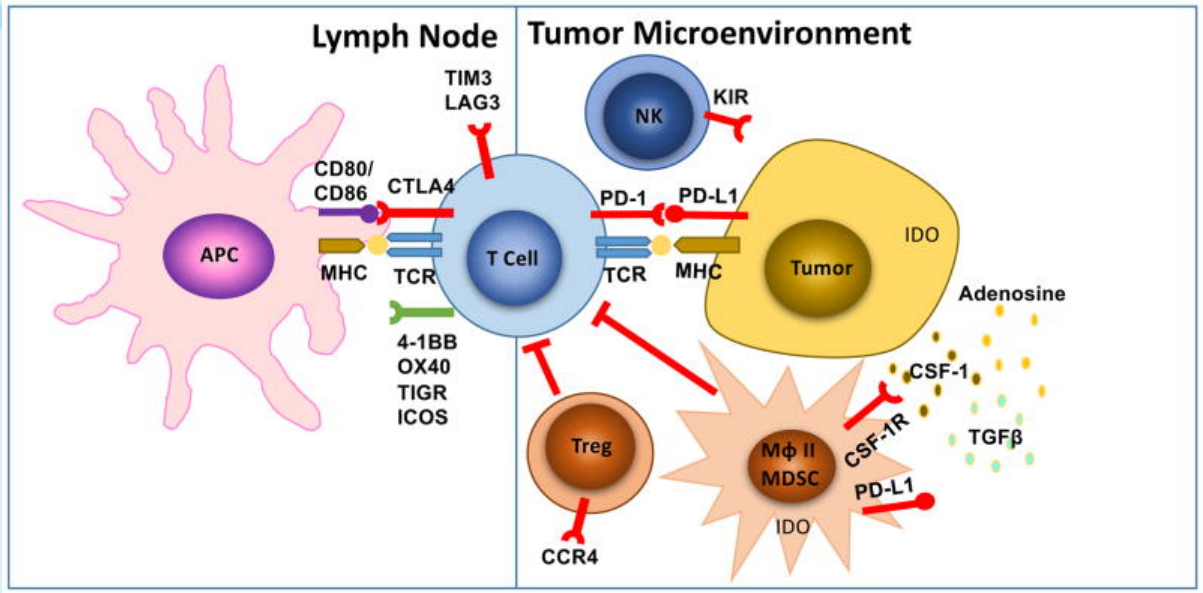
- Inflammation in the TME, commonly type I or type II IFN, drive responses designed to limit immunopathology



Sharma et al; Cell 168(4) 707-723 (2017)

Primary vs Adaptive Resistance

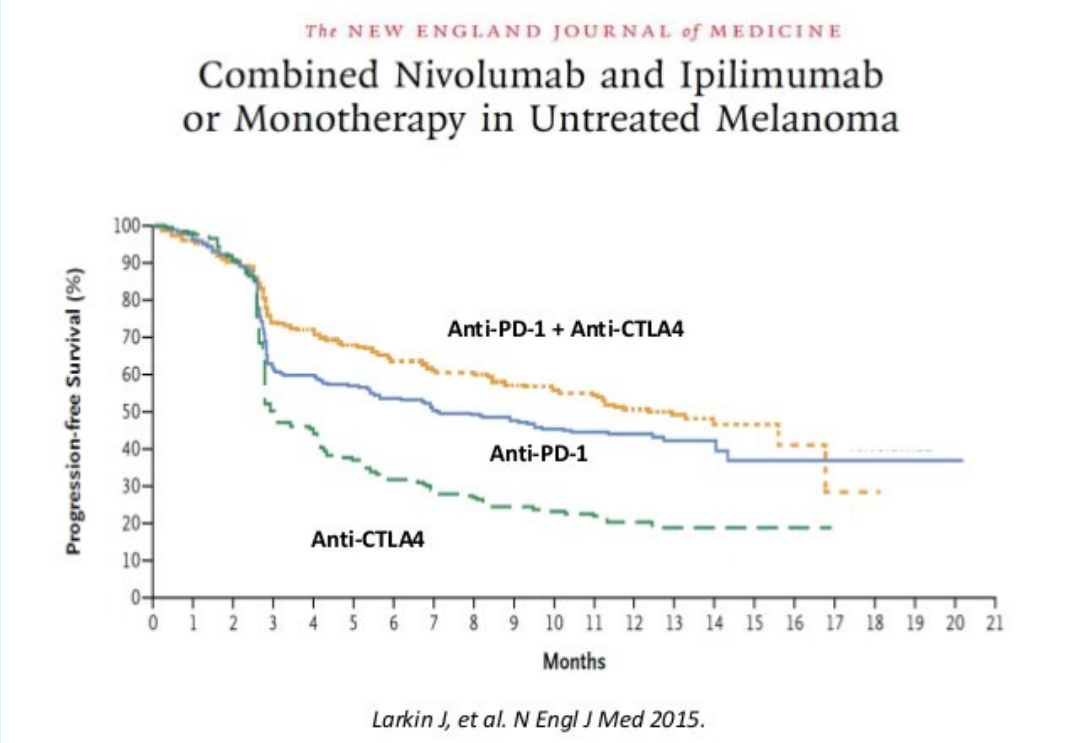
Target rich environment for immunologists

Tumor cell intrinsic:
-PDL1
-MHC

Extrinsic:
-Treg
-MDSC
-M2 macrophages
-granulocytes
-CAFs

Environmental:
-nutrient availability
-hypoxia
-acidification

Targeting resistance mechanisms works



Primary vs Adaptive Resistance
-JAK
-STK11/LKB1
-neoantigen loss

Need on-trial analysis to understand resistance

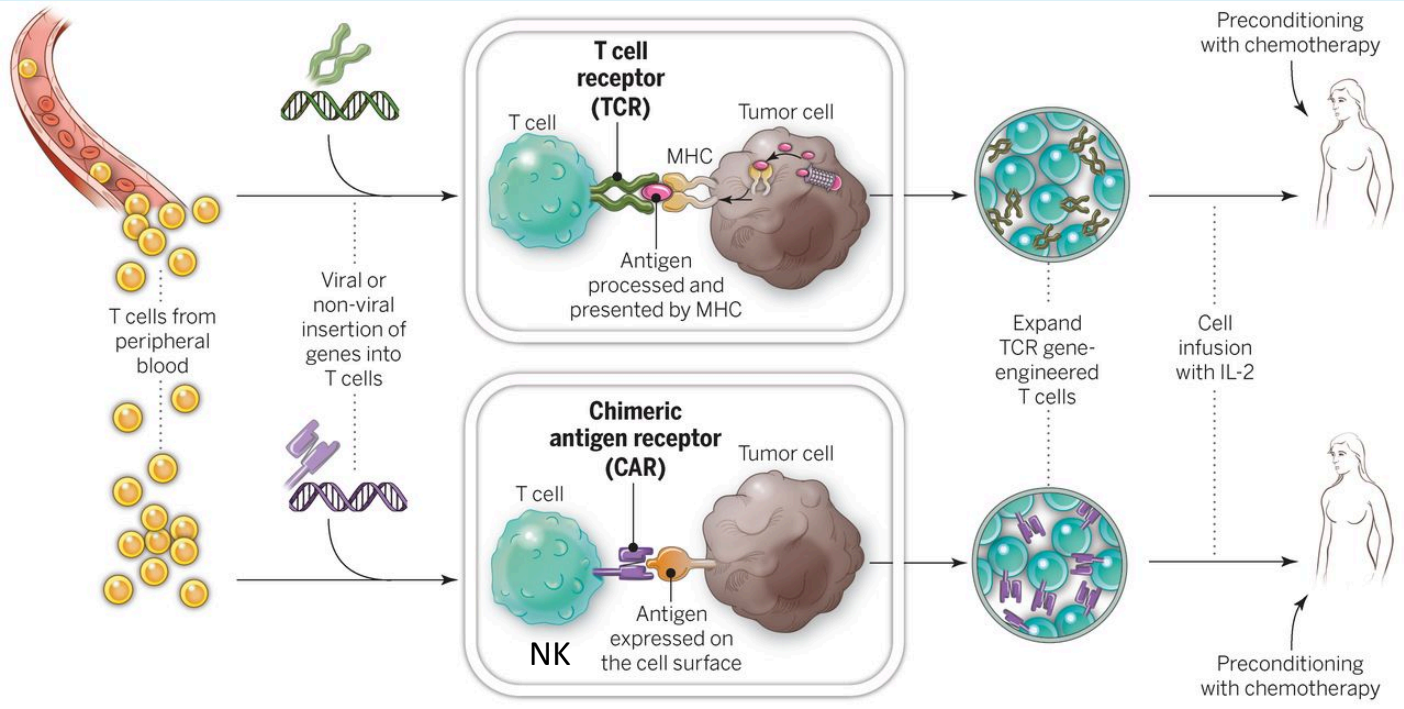
If the tumor contains no T cells…

A “cold” “desert” environment

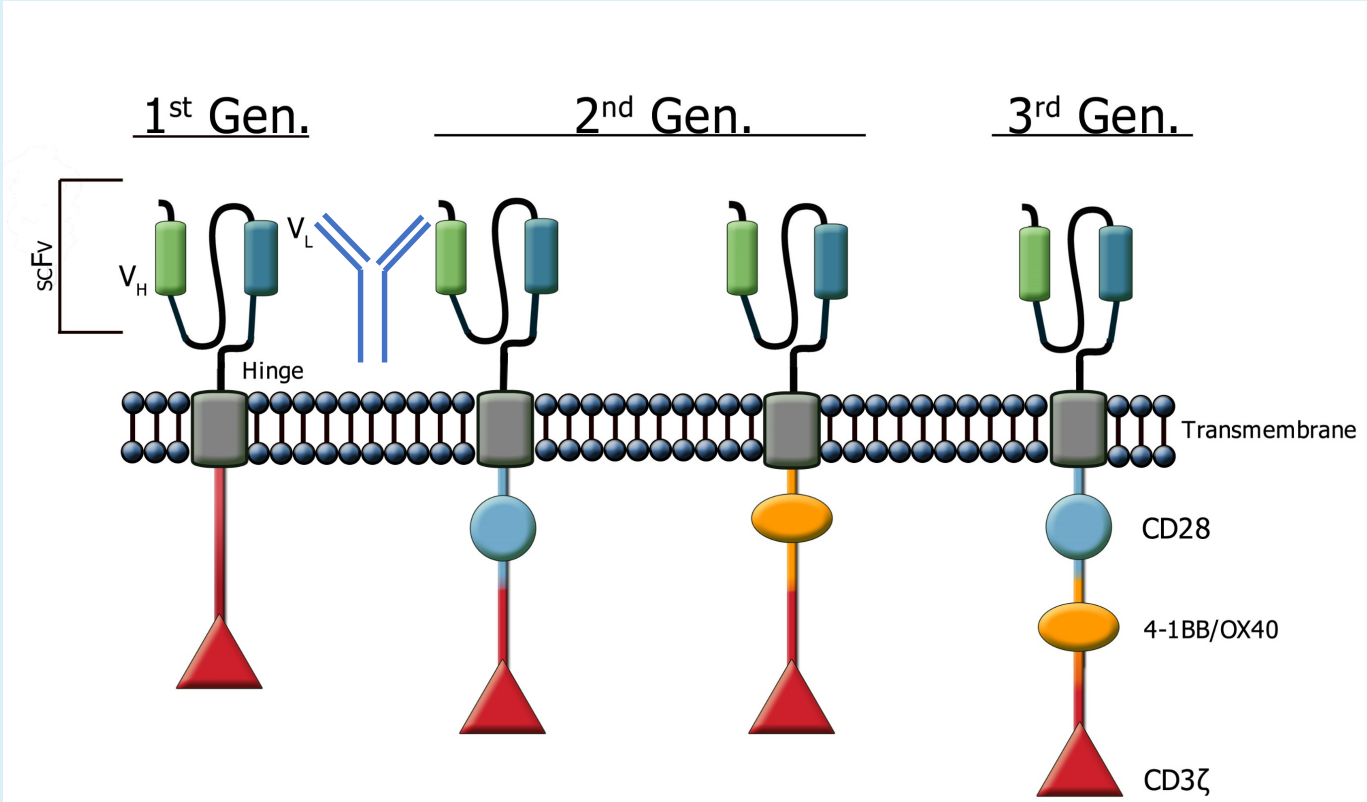
http://polarsoils.blogspot.com/2016/08/what-biome-is-antarctica.html

If the tumor contains no T cells…

- Side step host immunity by providing T cells.



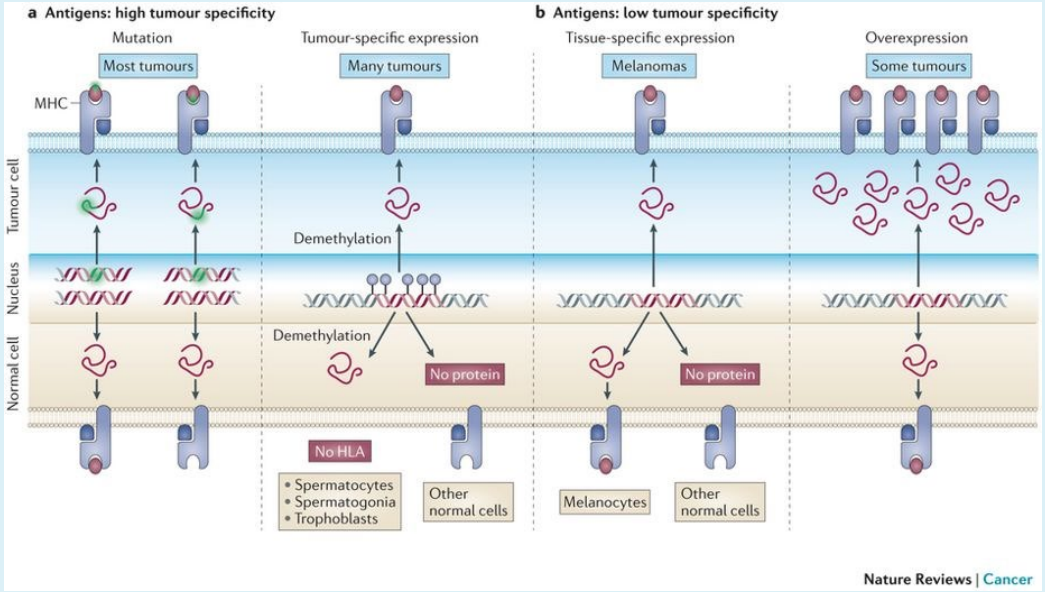
CAR-T and synthetic biology

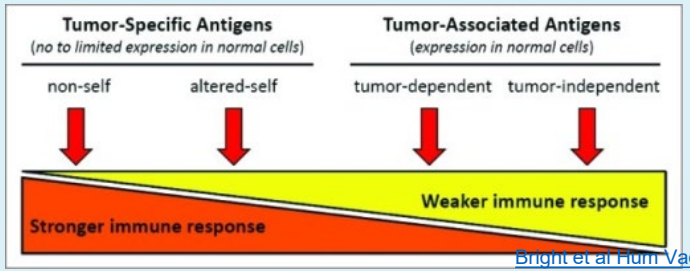


Cancer vaccines

- Prophylactic (preventative) or therapeutic
- Seeking clonal expansion and tumor infiltration
- Happy hunting ground of materials engineers!
- Generation of durable memory

Coulie et al Nature Reviews Cancer **volume14**, pages135–146 (2014)





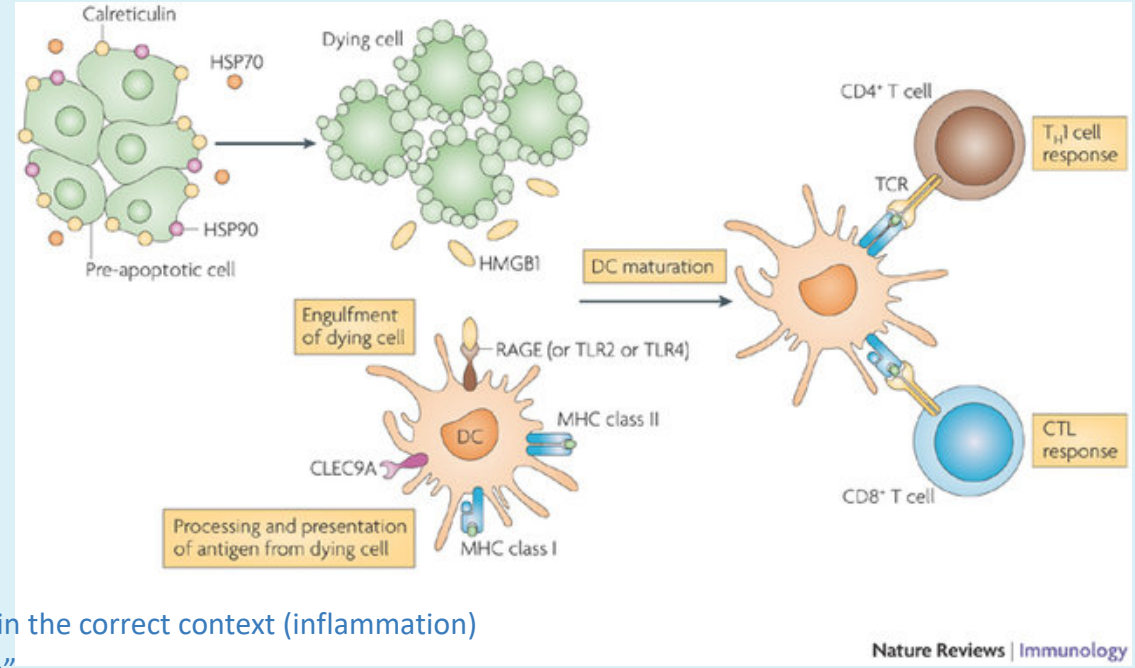
Cell-based vaccines
Protein based
Vector based
Adjuvants!

Bright et al Hum Vaccin Immunother. 2014 Nov; 10(11): 3297–3305.

Rationale for combination therapies

- Chemotherapies
- Radiation therapy
- Oncolytic viruses
- Targeted therapies
- Immunotherapies
- Epigenetics
  - Immunogenic cell death
    - Release of antigen in the correct context (inflammation)
    - ATP/NAD…"find me"
    - Cell surface calreticulin "eat me"
    - HMBG1/HSP/mitoDNA…"get an upset stomach from me"

Green DR,Ferguson T, Zitvogel L,Kroemer G Immunogenic and tolerogenic cell death. Nat Rev Immunol 9 353-363



Also, disrupt TME immunosuppression "wipe and reset"?

What are prognostic and diagnostic biomarkers with immunotherapy?

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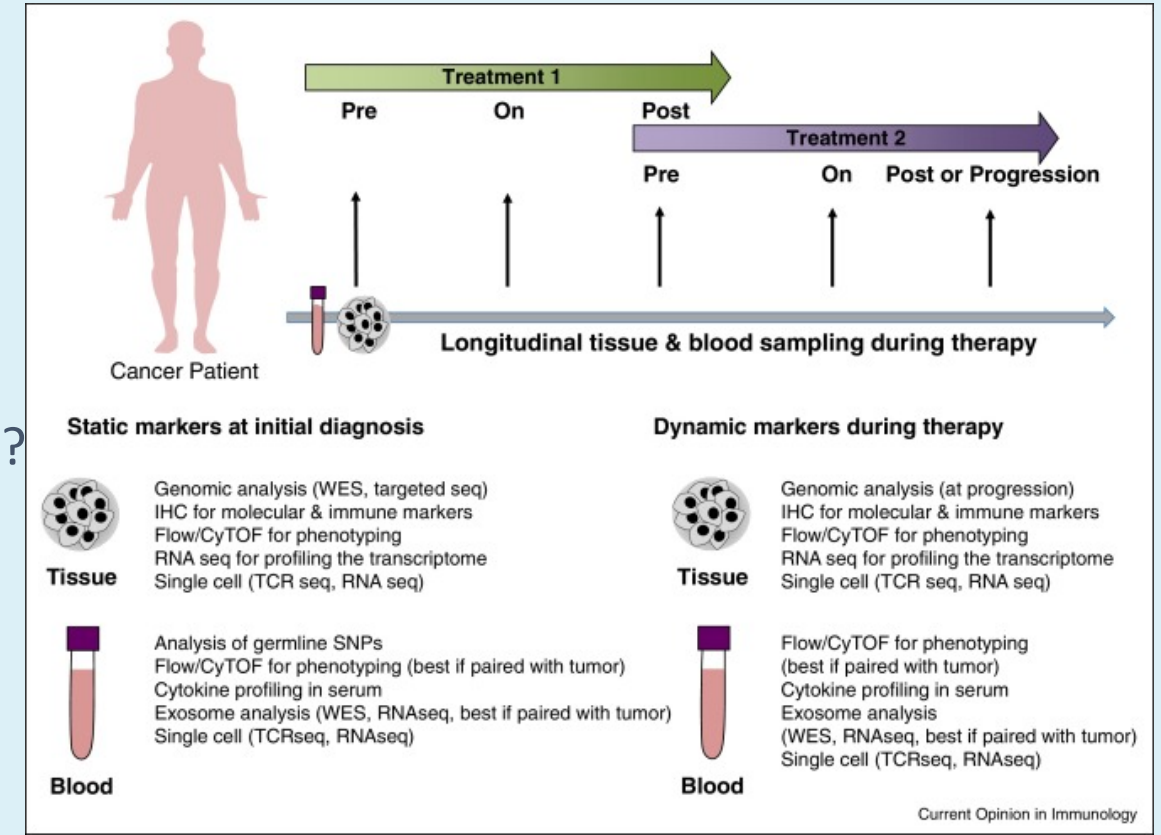
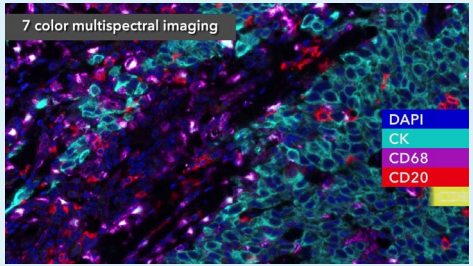
- How to monitor immunity?
- How to monitor resistance?
- Is enumerating CD8s sufficient?
  - Functional state
    - Cytokine production (Elispots)
    - Proliferative capacity



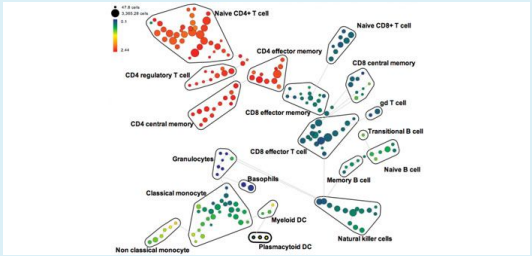
Emerging Technologies used for biomarkers



Beyond H&E/IHC

- Multispectral
- MIBI
- Flow cytometry/Cytof (low numbers?)
- Sequencing (sc?) with algorithms such as CIBERSORT
- Nanostring
- Epigenetic analysis (ATACseq)

- Intratumoral vs systemic?
- Liquid biopsies.



- Immunoscore
- CD8:Treg
- Inflammatory gene signatures
- TCR profiling

specificity

How are we intervening in tumor immunology?

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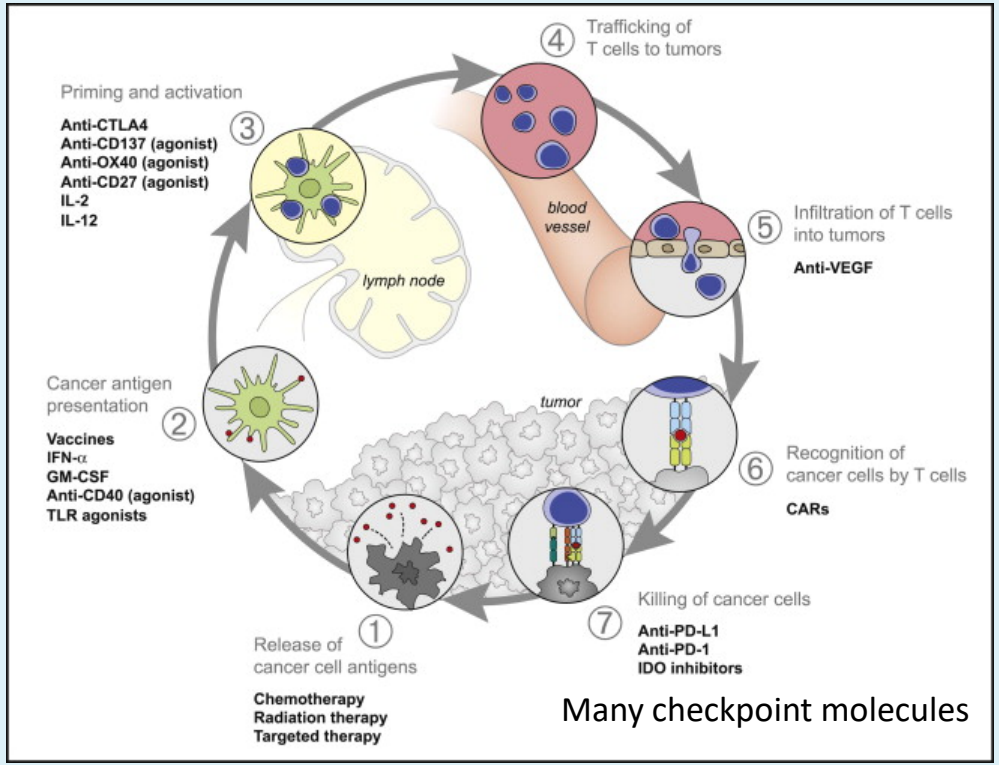
- Blocking Ab:
  - Checkpoint molecules
  - Chemokines/receptors
  - Cytokines
- Stimulatory Ab
  - Receptors
- Targeting/delivery
  - ADC
- Immunomodulatory SMI
  - Metabolic enzymes
  - Transcription factors
  - Epigenetic modification
- Generally not targeting tumor directly, but the IS

Access to tissue and on-target activity



Many checkpoint molecules

Chen and Mellman Immunity Volume 39, Issue 1, 25 July 2013, Pages 1-10

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