T cell exhaustion

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Disclosures

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Founder and Scientific Advisor: TTMS, Inc.

Disclosures

I am thrilled to be invited to give a one-hour talk on T cell exhaustion.

However, exhaustion is still a controversial topic, as not everyone necessarily agrees what it means to be exhausted (or if it even *is* its own state/fate)

Thus, talking about it in its entirety can be quite.. political.

I will thus try to give a balanced talk (more PBS, less Fox News) regarding the importance of this functional fate in cancer immunology.

If you are trying to turn on T cells, it's critical that said T cells have the capacity to be functional

- However T cells have a wide variety of functional and differentiation states, and some of them are dysfunctional **even with proper stimulation**
  - **Anergy**: A functional state in which TCR triggering alone induces a transcriptional and post-translational program preventing future reactivity through the TCR (even if that future stimulation *includes* costimulation)
  - **Senescence**: A pathologic state in which time and extensive cell division result in T cells that are chronically secretory but respond poorly to antigen
    - Also lose costimulatory receptors preventing a full stimulation
  - **Exhaustion**: A terminal differentiation state driven by persistent antigen that results *in progressive loss of polyfunctionality*
- All of these dysfunctional states seem to be 'read out' the same way
  - Loss of cytokine production
  - Loss of proliferative capacity
  - Loss of cytotoxicity
- **All of these dysfunctional states are important in cancer!**

History of T cell exhaustion

TABLE 1

LCMV-specific CTL and Antibody Responses in Acutely and Persistently Infected BALB/c WEHI Mice

CTL response*

TABLE VIII

Suppression of CTL Response and Establishment of Persistent Infection by LCMV Clone 13, a Genetic Variant Isolated from Spleen of Carrier Mice

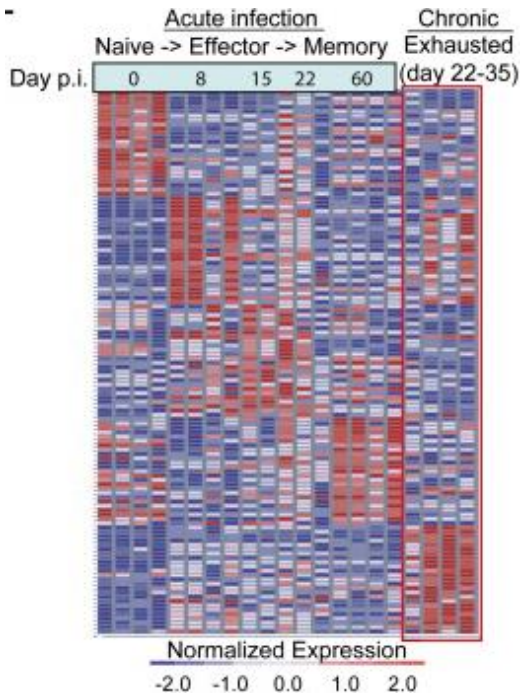
Source of spleen cells transferred*	Challenge virus	LCMV-specific CTL in spleen: Percent specific ^{51}Cr release from BALB CL-7 ($H\text{-}2^d$) targets (E/T, 50:1)			LCMV-specific antibody in serum	LCMV titer in serum (log_{10} PFU/ml)				
						Days postinfection				
		Uninfected	LCMV Armstrong infected	LCMV clone 13 infected	ELISA titer (log 2)	8	30	45	55	90
Normal	LCMV Armstrong	3	58	52	13.9	<1.6	<1.6	<1.6	NT‡	NT
		0	52	54	14.1	<1.6	<1.6	<1.6	NT	NT
		1	52	57	13.7	<1.6	<1.6	<1.6	NT	NT
Normal	LCMV Clone 13§	2	6	8	12.7	4.7	5.4	4.1	4.3	5.1
		0	0	0	14.6	4.3	5.3	4.2	4.6	4.3
		0	2	3	13.3	3.9	5.1	4.7	NT	NT
Normal	LCMV Armstrong + LCMV clone 13	3	7	12	13.3	4.0	5.4	4.8	5.3	4.7
		0	6	2	13.8	4.1	5.4	4.2	5.3	3.9
		1	8	7	12.9	3.8	5.1	5.0	5.3	5.1

* 5×10^7 spleen cells from adult BALB/c WEHI mice were transferred intravenously into normal adult BALB/c WEHI mice

rier mice)	2	0	4	5	2	2	6	<4.7
	3	2	3	3	2	2	4	<4.7
	4	4	2	3	4	5	7	<4.7
	5	5	2	4	2	2	5	9.8
	6	0	1	5	0	1	3	7.5

- The term ‘exhaustion’ was coined thirty years ago in chronic viral infection
- A major advance of the ability to study exhausted T cells was in lymphocytic choriomeningitis virus (LCMV) infection in mice
- LCMV has two commonly used strains that differ by two amino acids
- This amino acid results in antigen chronicity in vivo

Chronic infections helped identify targets present on exhausted T cells



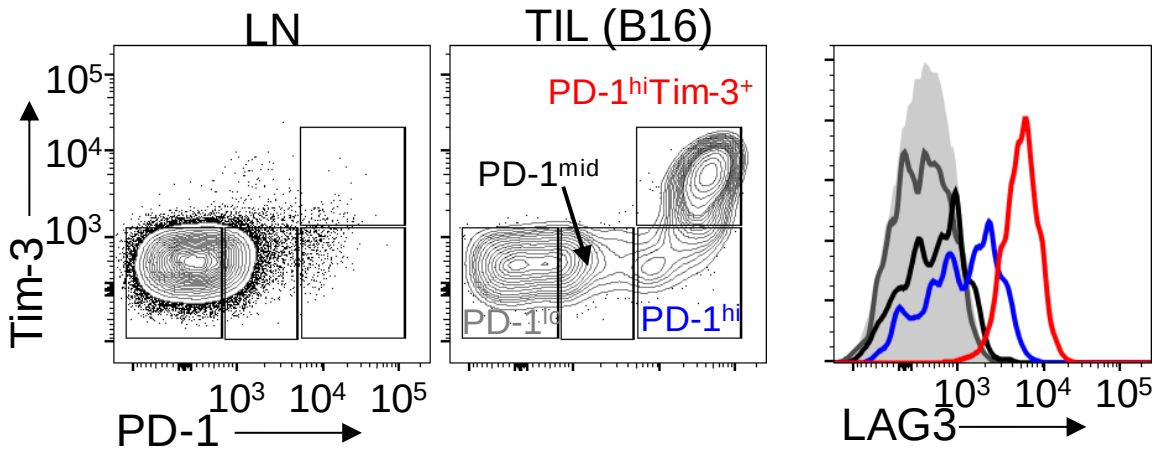
I. Inhibitory receptors				
Gp49b	12.24	4.29	4.48	
Cd244	11.02			
Ctla4	6.93	2.30	2.33	
Pdcd1	6.55			
Cd160	4.08			A
Klrc1		4.41	2.03	A
Ptger4	4.07			
Gp49b	4.04	2.56	2.49	
Lag3	3.25			
Klra9	2.88	2.04		
Klrg1	2.59	36.90	7.36	A
Klra3	2.20			
Ptger2	2.08			A

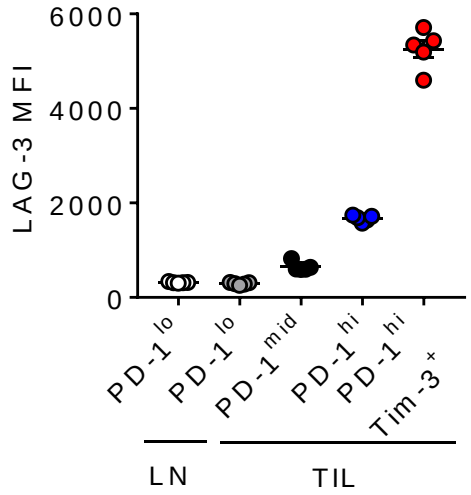
II. Cell surface receptors and ligan				
Tnfsf6	16.29	6.12	5.61	
Ctla2b	8.64	14.44	11.60	
Ctla2b	8.30	12.72	12.77	
Itgax		7.60	3.07	A
Nrp	5.65			
Tnfrsf9	5.42			A
Pglyrp1	5.01			A
Tmem49	4.62	2.79		A
Il18r1		4.21	4.93	
Klrk1		4.05	3.86	A
Adam19	3.80	2.37		A
Itga4		3.75		
Klrk1		3.58	2.56	A
Ly6c		3.56	2.79	
Itgb1	3.52	3.94	3.61	
Tnfrsf1b	3.48			
Ly6a	3.24	4.06	4.07	
Alcam	2.92			
Cd9	2.60			
Pglyrp1	2.50			A
Igsf10		2.48		A
Cd7	2.45			
Glycoprot	2.38			
Itga4	2.35	4.35		A
Tnfrsf1a	2.31			A
Cd48		2.26		
Itgb2		2.26		
Fcgr2b		2.23	2.45	
Il1rl1		2.19		
Itgav	2.18			A
Itgb1	2.17			
Gpr65	2.12			
Cdam			2.11	A

- Microarray analysis of T cells responding to chronic versus acute viral infection revealed several cell surface molecules highly upregulated on T cells as they progressed to exhaustion
  - PD-1
  - LAG3
  - TIM-3/HAVCR2
  - TIGIT
  - 2B4
  - CD160
  - And so on…
- However, **costimulatory** molecules are also overexpressed on terminally exhausted T cells (4-1BB, OX40, GITR)
- In other words: these cells are **hungry for signals** and thus become dependent on the presence of the ligands: a dangerous proposition in the tumor microenvironment

Observing similar phenotypes in cancer-responsive T cells

- As cancer represents a source of chronic activation, T cells in tumors also succumb to T cell exhaustion
- Consistent with chronic infections, tumor reactive T cells progressively upregulate co-inhibitory molecules and lose polyfunctionality
- However, this is still a hotly debated topic!



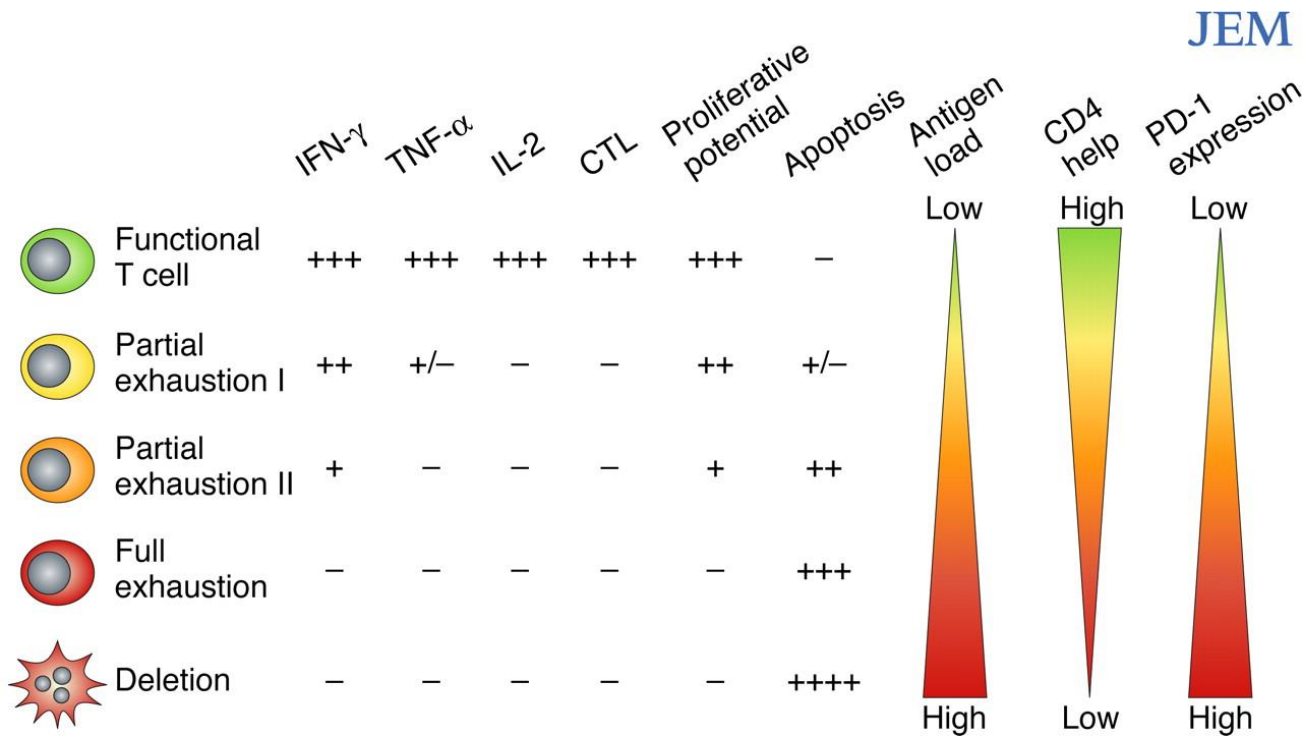


Defining T cell exhaustion as a differentiation state

- T cell differentiation fates like Th1, Th2, effector-memory, etc., all have several common features
  - A distinct functional program (cytokine production)
  - A transcriptional network that drives that program
  - An epigenetic landscape that supports those transcriptional networks
- While still under debate, many in the field believe that exhausted T cells are essentially a distinct subset of T cells rather than a functional state – we will discuss the underlying data for these suppositions

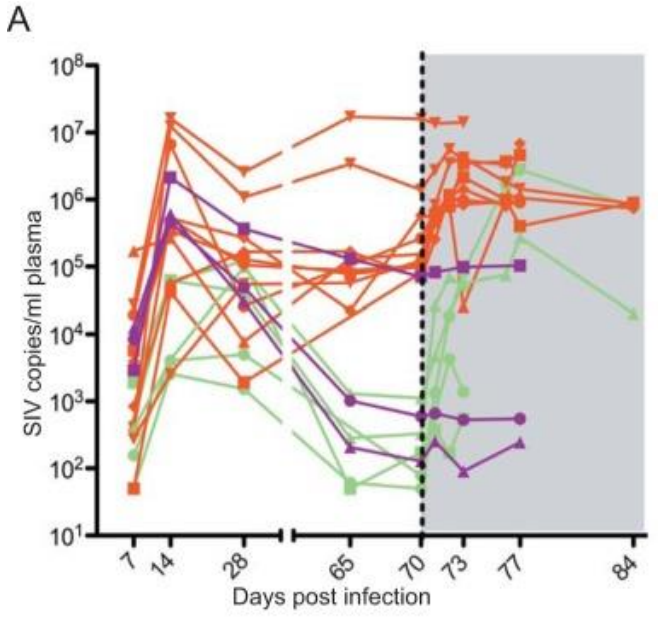
Progression and functional defects of exhausted T cells

- Exhaustion is not strictly hyporesponsive
- Indeed, exhausted T cells still maintain some degree of cytokine production
- But it is the polyfunctionality that is lost: multiple cytokines, killing ability, and proliferative potential that truly defines exhaustion



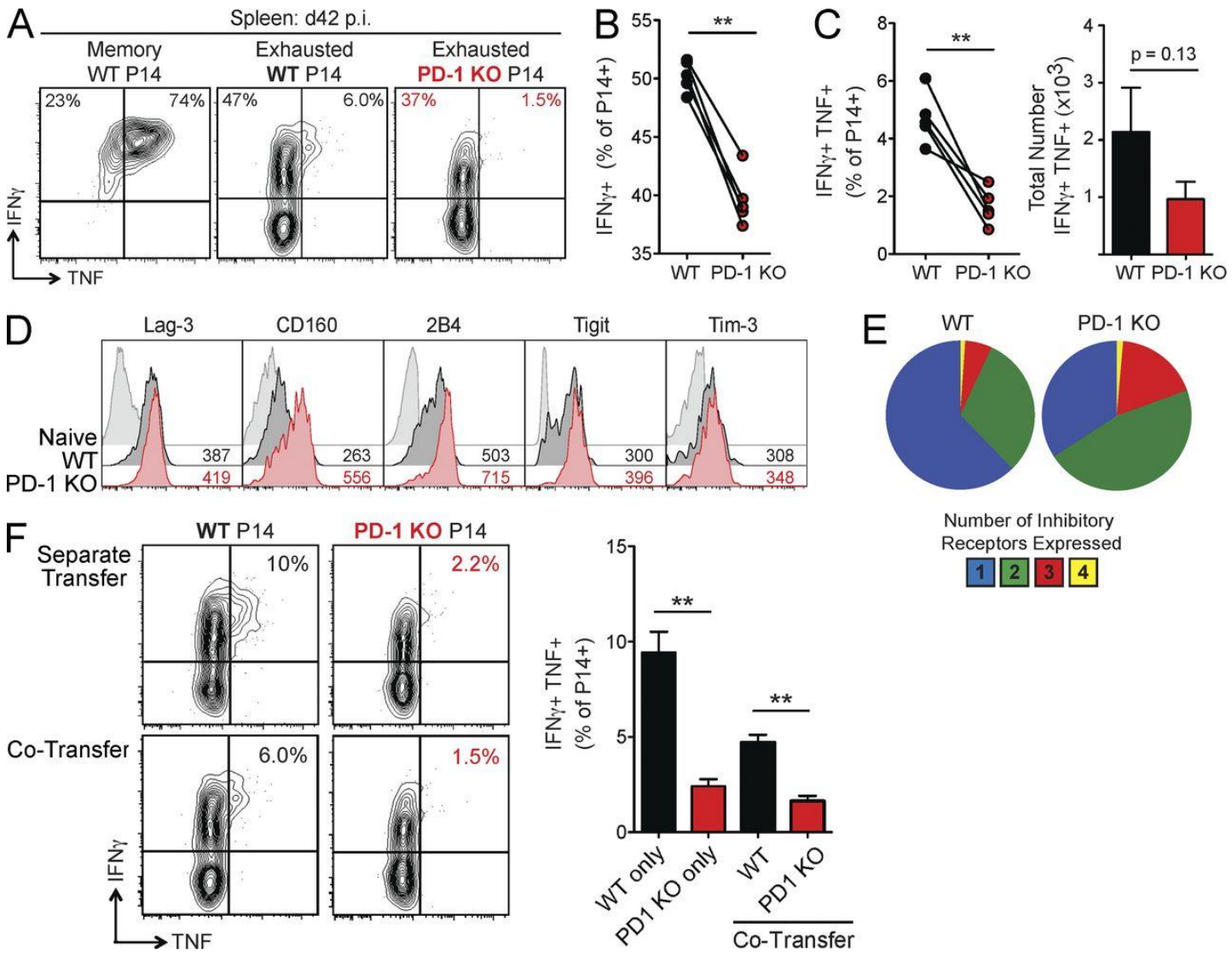
Are exhausted T cells dysfunctional?

- While this seems like an obvious question, it is not a clear answer
- Depletion of exhausted T cells in chronic infections, despite their ‘dysfunctional state’ leads to rampant and lethal viremia
- While similar experiments have not been necessarily done in cancer, these data suggest exhausted T cells retain *some* function
- Indeed, exhausted T cells can still transcribe *Ifng* as well as cytotoxicity genes



So what DRIVES T cell exhaustion?

PD-1 signaling does not *cause* T cell exhaustion



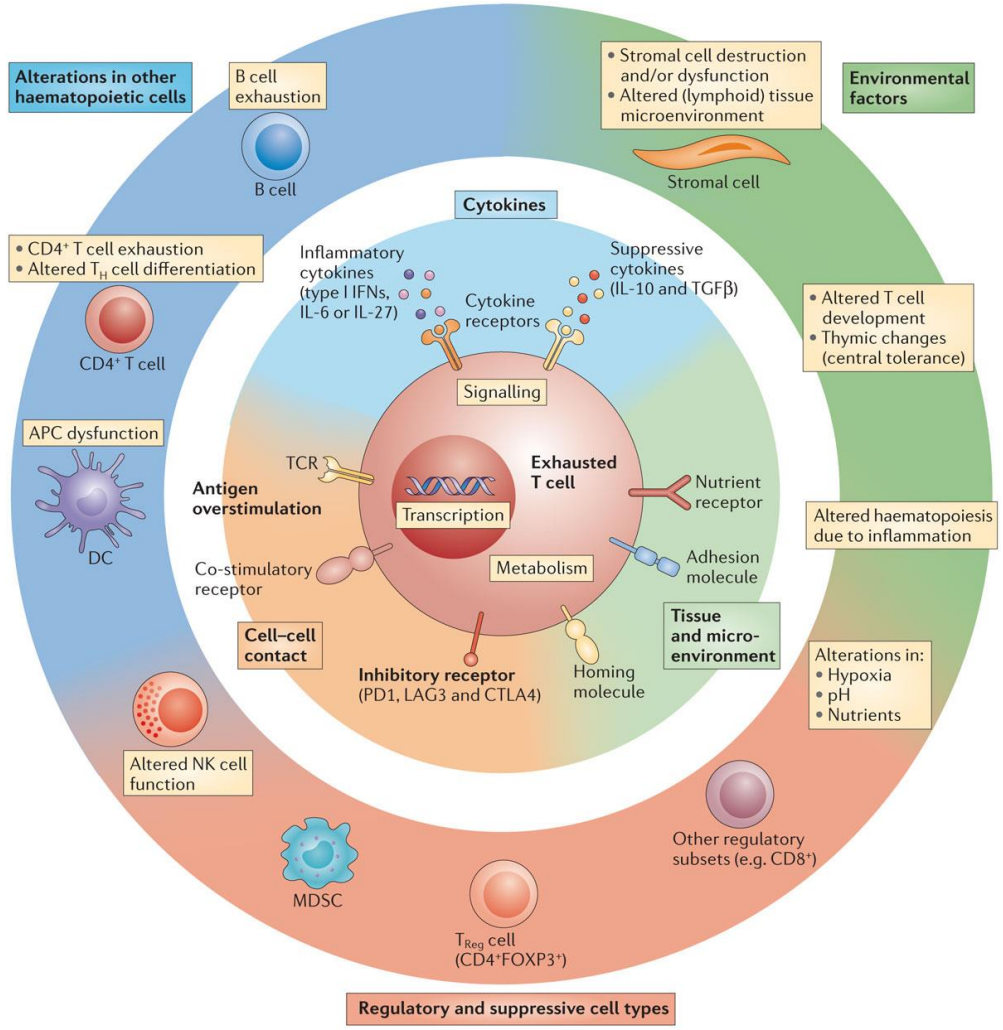
PD-1 deficient T cells still become exhausted in chronic viral infection

In fact, they are even more severely exhausted.

So what does?

Drivers of the exhausted T cell fate

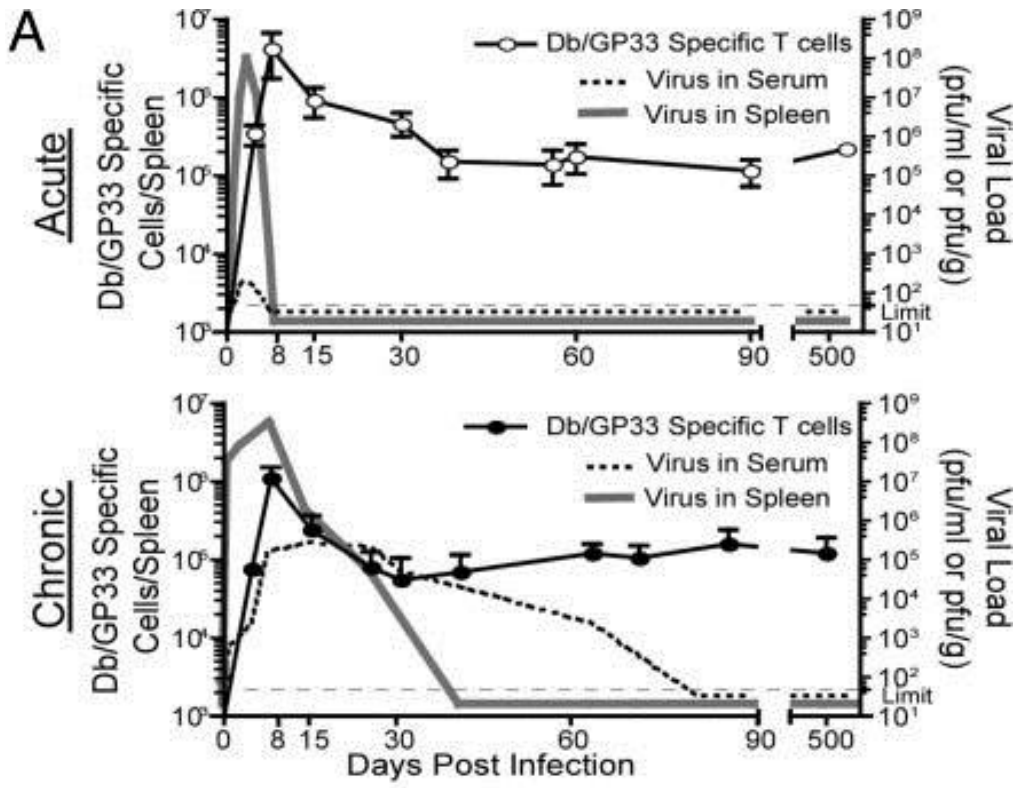
- Antigen – Persistent antigen is associated with T cell exhaustion
- Inflammation – cytokines can play roles in driving certain components of the exhausted cell phenotype
- Microenvironment – Hypoxia, metabolic stress



Nature Reviews | Immunology

Antigen

- The persistence of antigen plays a key role in driving cells to become exhausted
- LCMV is a great system to study this as the differences between the virus strain are so minute
- T cell transfer studies confirmed a role for antigen in this process
  - T cells transferred from acutely infected mice into chronically infected mice become exhausted
  - T cells transferred from chronically infected mice early (1 week post infection) into acutely infected mice become memory
  - T cells transferred from chronically infected mice late (30 days post infection) into acutely infected mice remain exhausted
- TCR transgenic mice specific for model, viral, or tumor antigens have helped find similar types of scenarios (albeit with different kinetics) in tumor systems

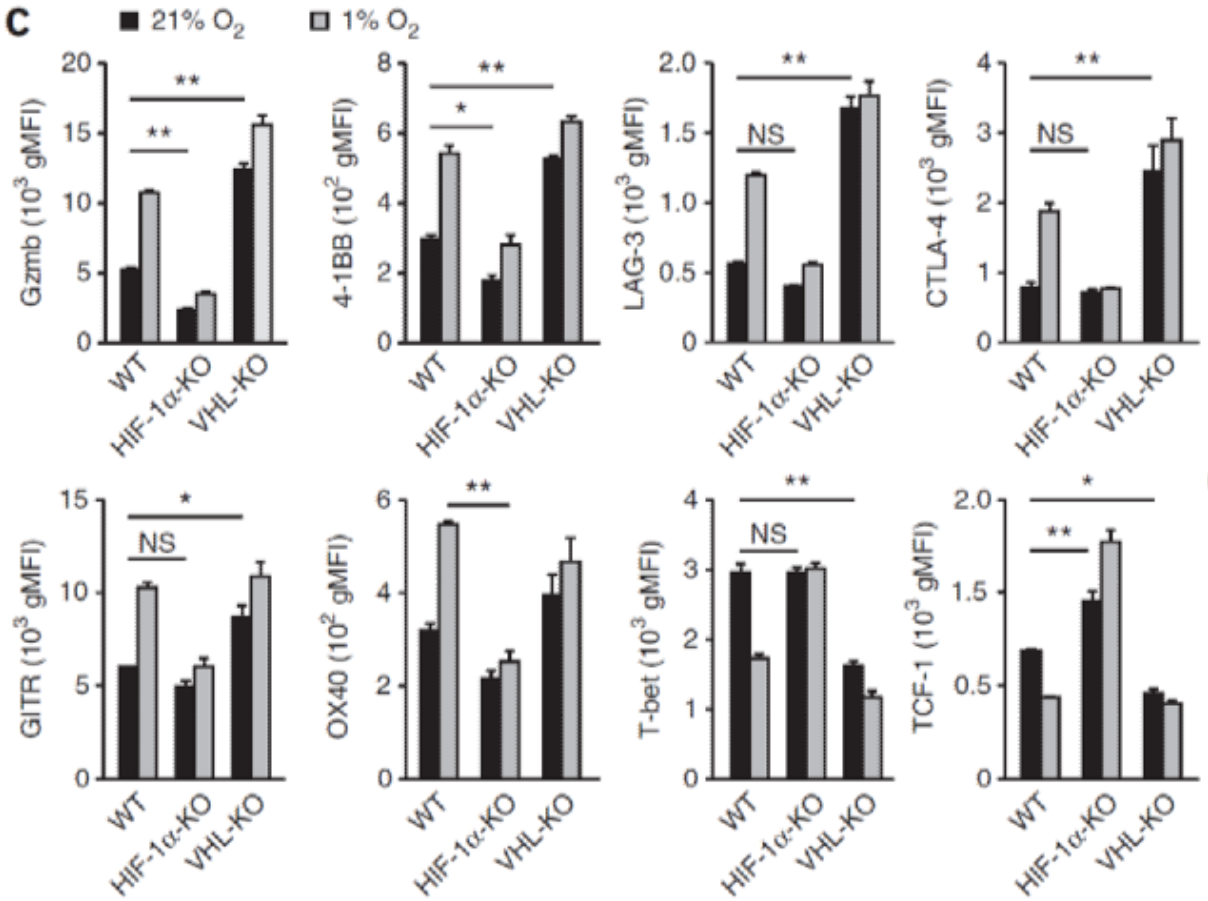


Inflammation

- Use of genetically modified mice in the LCMV system has allowed for direct interrogation of the role of inflammation in exhaustion
- Chronic antigen supposes chronic inflammation, and indeed in chronic infections type I interferon signaling can be overstimulated
- IFNAR-deficient T cells (or anti-IFNAR blockade) result in less chronic infection induced exhaustion
- Tumor necrosis factor (TNF) can also drive exhaustion (TNF blockade results in increased T cell function)
- A host of other factors can drive exhausted like programs, including
  - IL-6, IL-27
  - Treg derived factors like TGFb, IL-10
  - Lack of proper T cell help
  - Exposure to MDSC

Metabolic stress

- Exposure to metabolically stressful conditions has been shown to induce an exhausted-like state
  - Hypoxia: HIF1a drives co-inhibitory molecule expression, but also effector function
  - Low glucose, high lactate
  - ER stress/ ROS production
- How causative these states are in isolation still remains to be determined

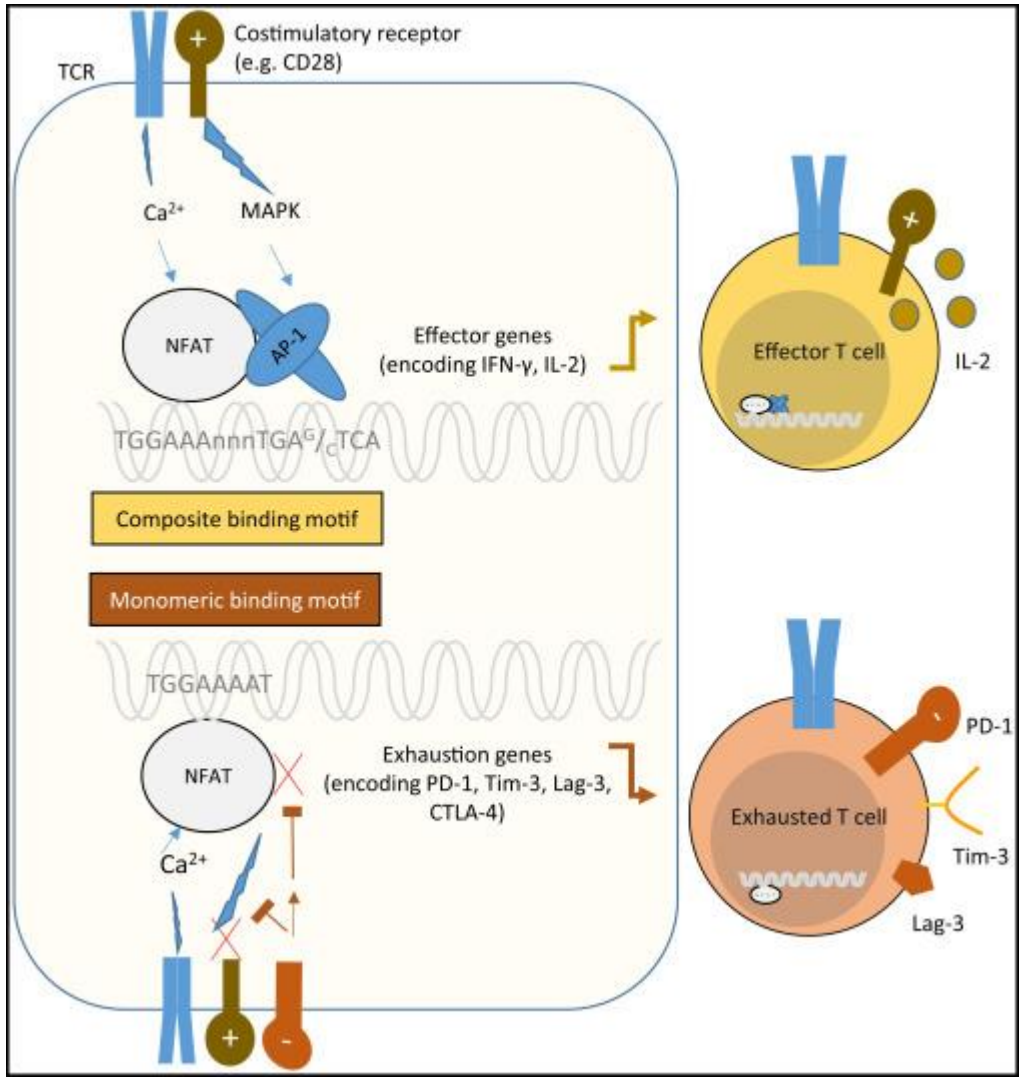


Maintenance of the exhausted T cell fate

- As a differentiation state rather than a functional one, exhausted T cells (especially terminally exhausted ones) are inclined to stay this way
- The biology of T cell exhaustion has been an important scientific endeavor as those targets that arise may be therapeutic targets to modify cellular function for modulation of the immune response

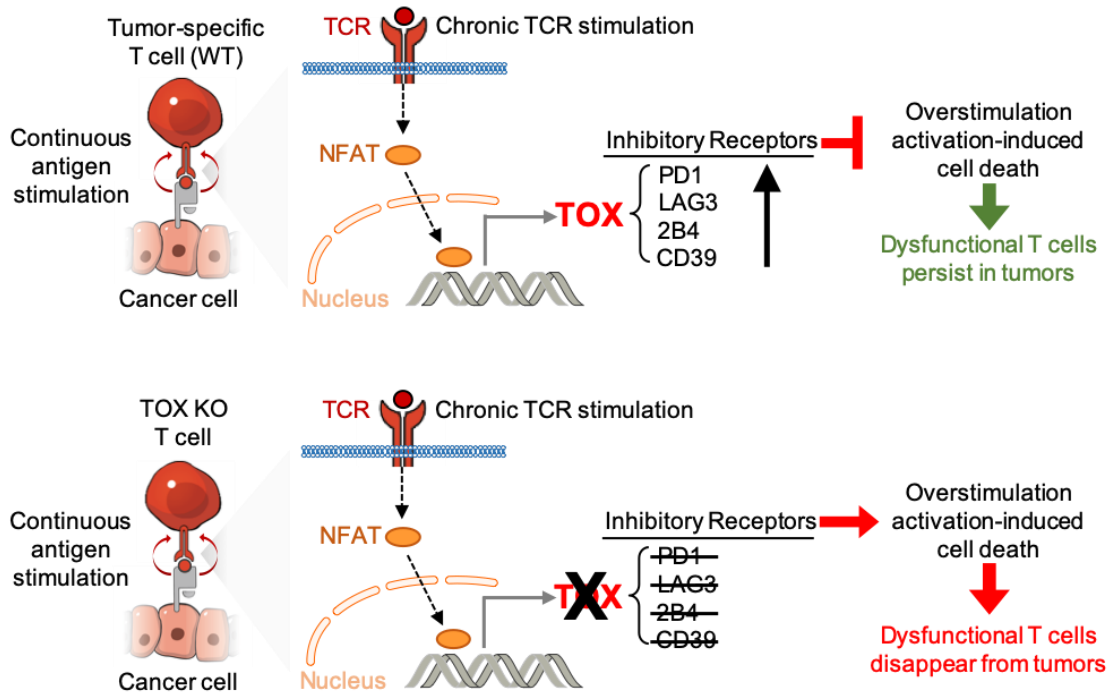
Transcriptional rewiring

- As chronic stimulation is a driver of exhaustion, NFAT, which is downstream of the TCR, is a major culprit in transcriptional changes induced
- NFAT typically partners with AP-1 (induced by costimulation) to program effector gene lineages
- However, chronic stimulation or co-inhibition promotes constitutive NFAT activity which is sufficient for an exhausted like state
- NFAT is not the whole story, however
- Maintenance of the phenotype likely requires transcription factors like
  - Blimp-1
  - T-bet
  - Eomesodermin
  - HIF1a
  - **TOX**
- However, what has been clear is that the transcriptional program of exhausted T cells does not necessarily explain their dysfunction



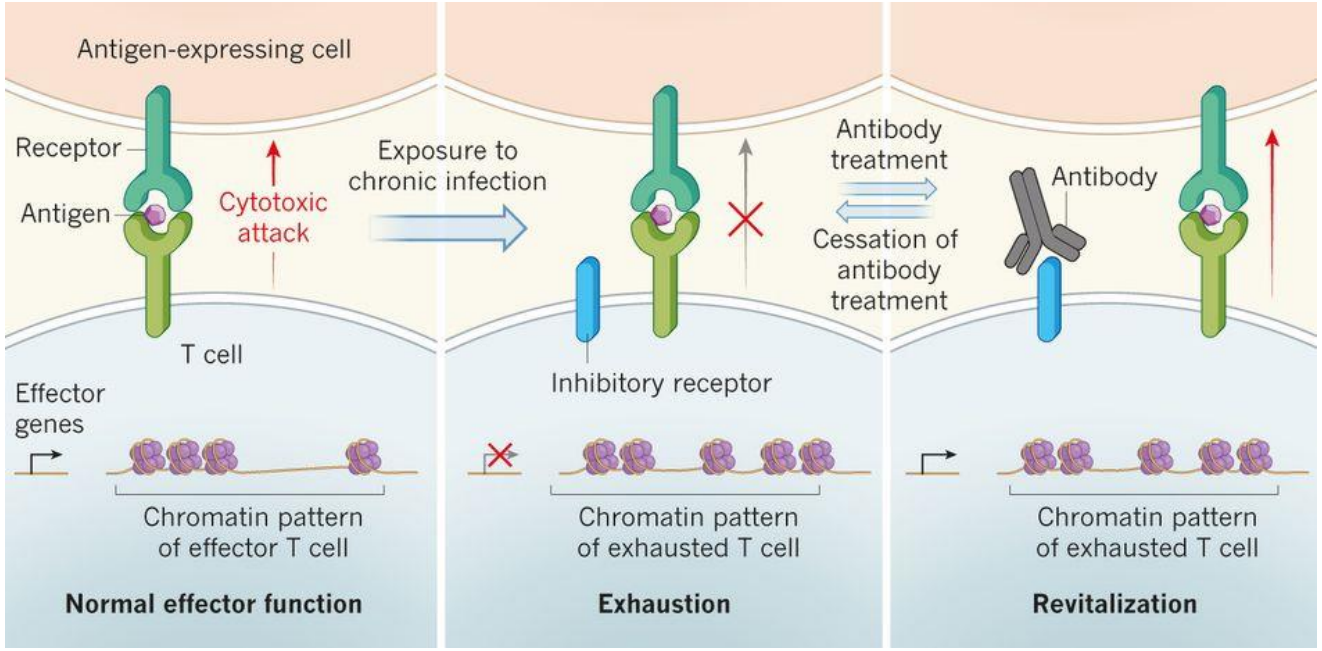
TOX in control of (some of) the exhaustion program

- A flurry of papers this year detailed a transcription factor called TOX that could set up the exhaustion program through the expression of a number of inhibitory receptors
- However, while TOX-deficient T cells in cancer *looked* less exhausted, they were still 'dysfunctional' meaning it's not the whole story (and my personal bias is that there will not be a single smoking gun behind exhausted T cells)

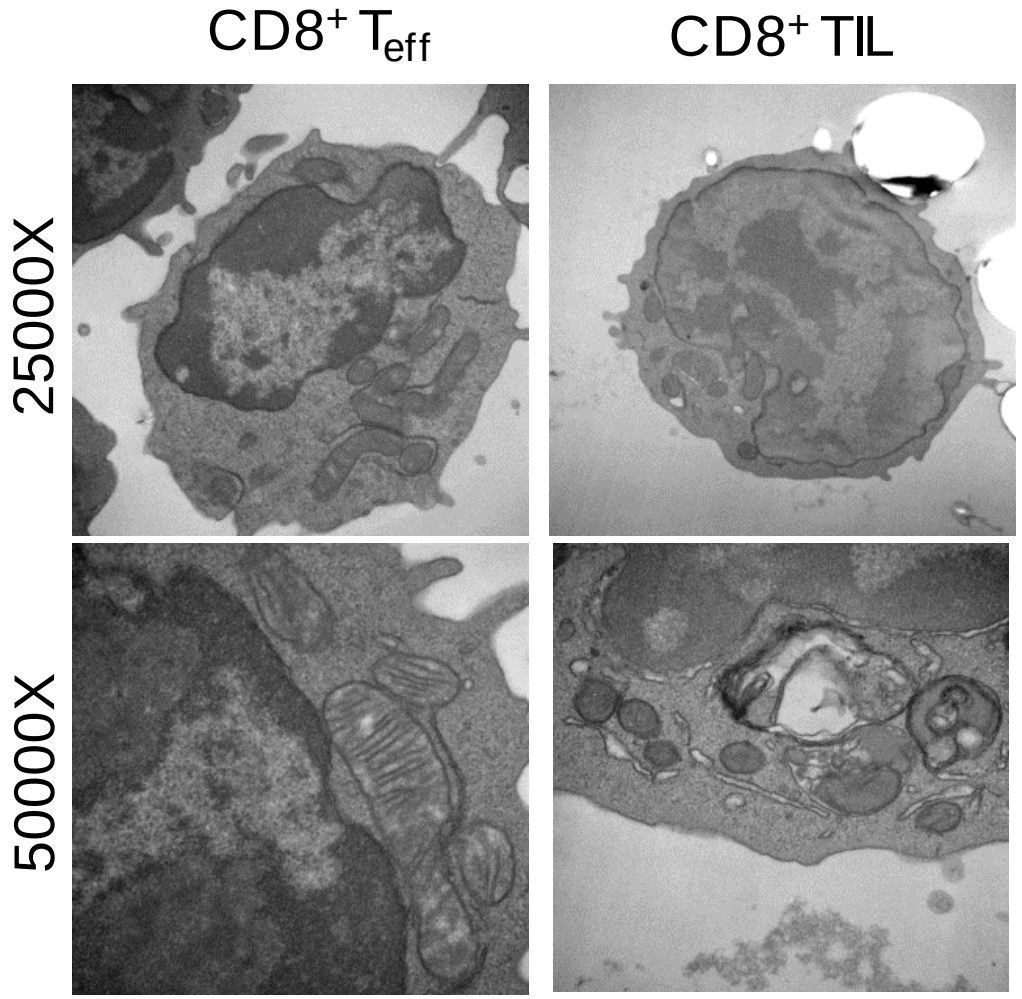


Epigenetics

- Differentiated cells (like Th1 and Th2, CTL and memory, regulatory versus conventional) have epigenetic programs which guide transcriptional machinery
- Several groups using ATAC-sequencing have shown that exhausted T cells have a distinct epigenetic signature
- This signature **is not** changed by PD-1 blockade, which means, inevitably, T cells will ‘slide’ back into their dysfunctional state



Metabolic insufficiency

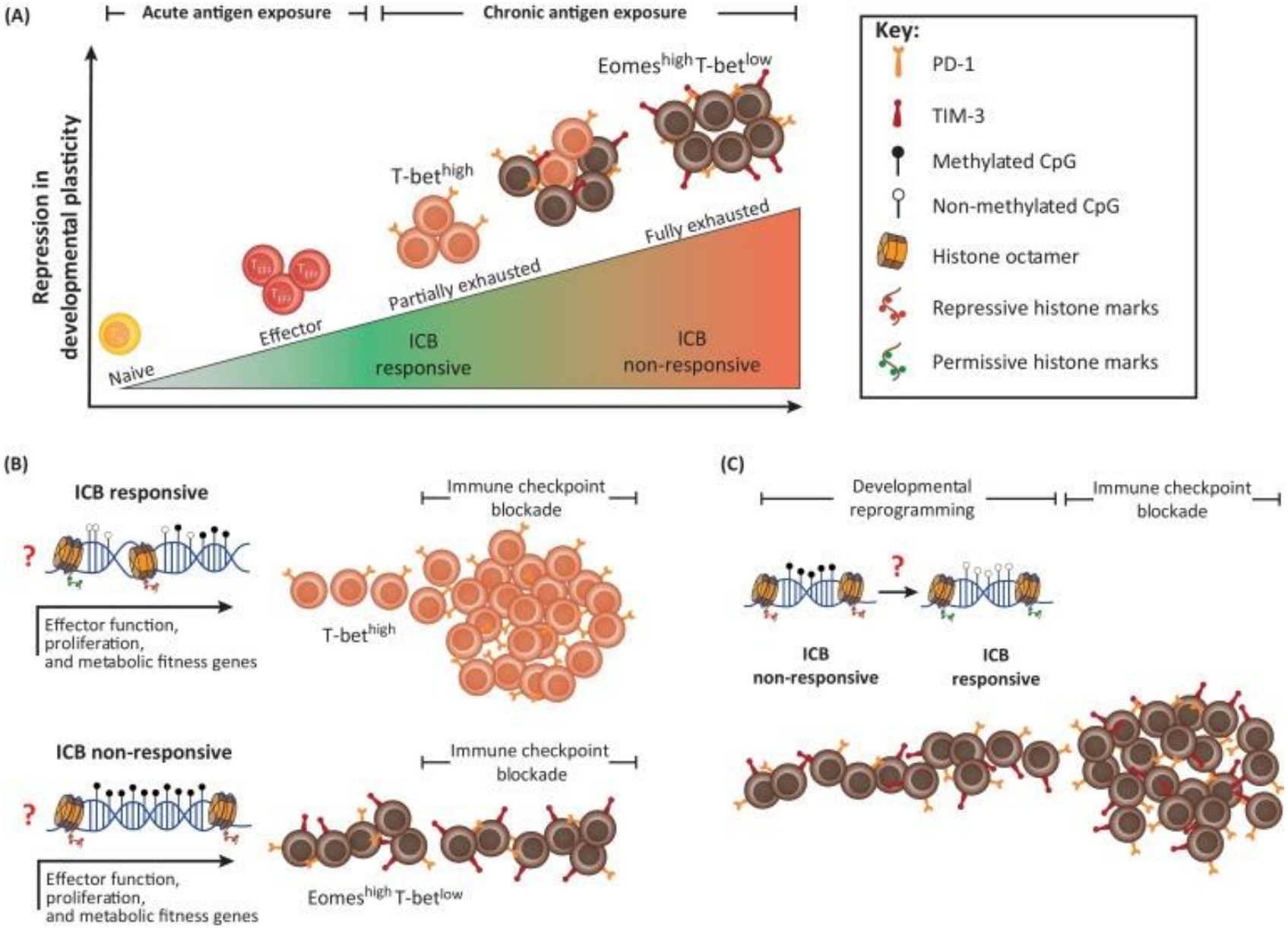


- Exhausted T cells are also characterized by severe metabolic deficiencies
- Exhausted T cells isolated from tumors (and in chronic infection) show repressed glucose uptake and accumulation of dysfunctional mitochondria
- Thus, exhausted T cells are energetically disadvantaged in a nutrient poor environment!

So, if PD-1 doesn't *cause* exhaustion and anti-PD1 doesn't rescue exhausted T cells….

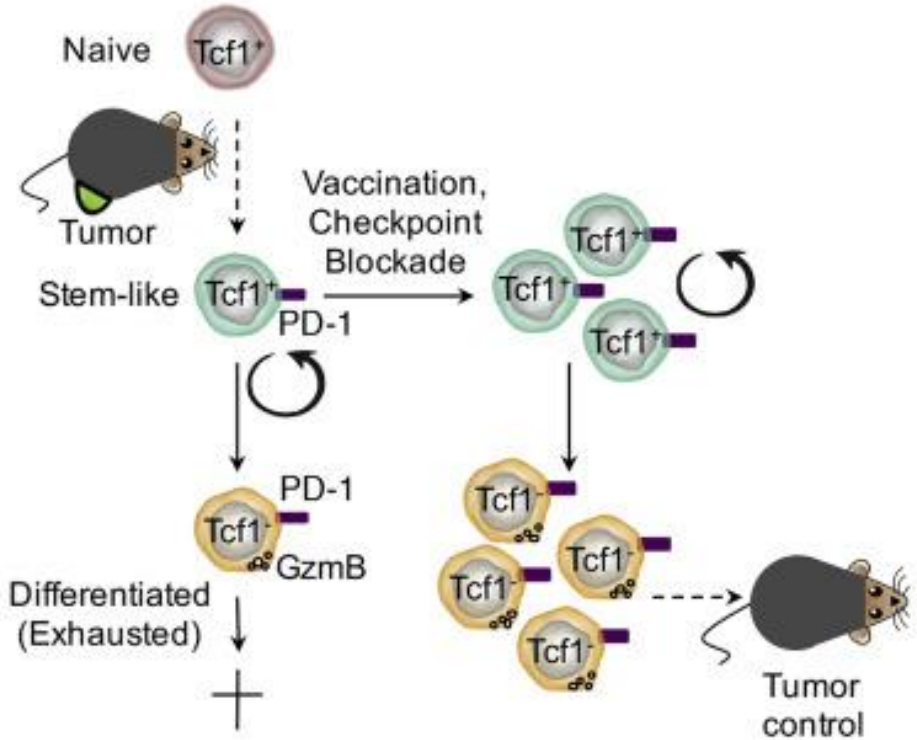
why does anti-PD1 work? Why are some people experiencing such dramatic responses on therapy?

Who does PD-1 blockade affect?



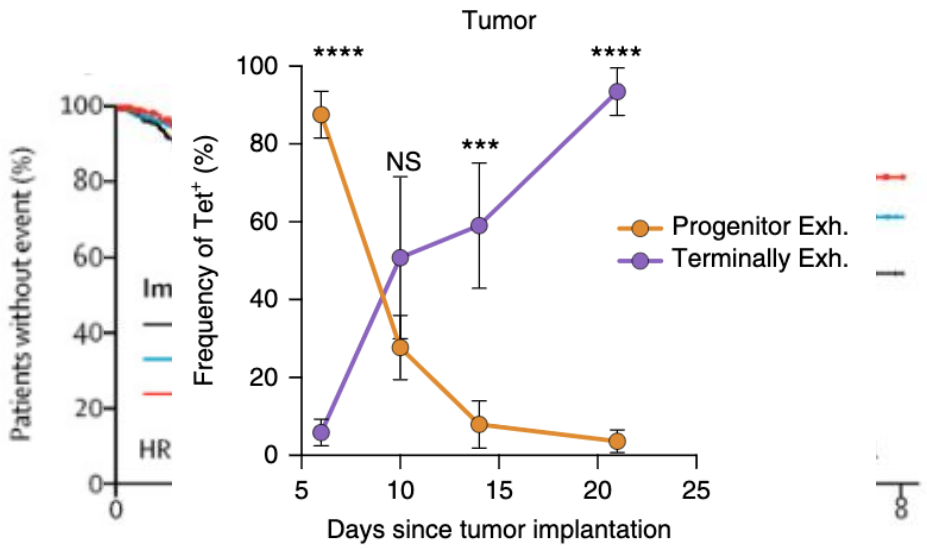
Trends in Molecular Medicine

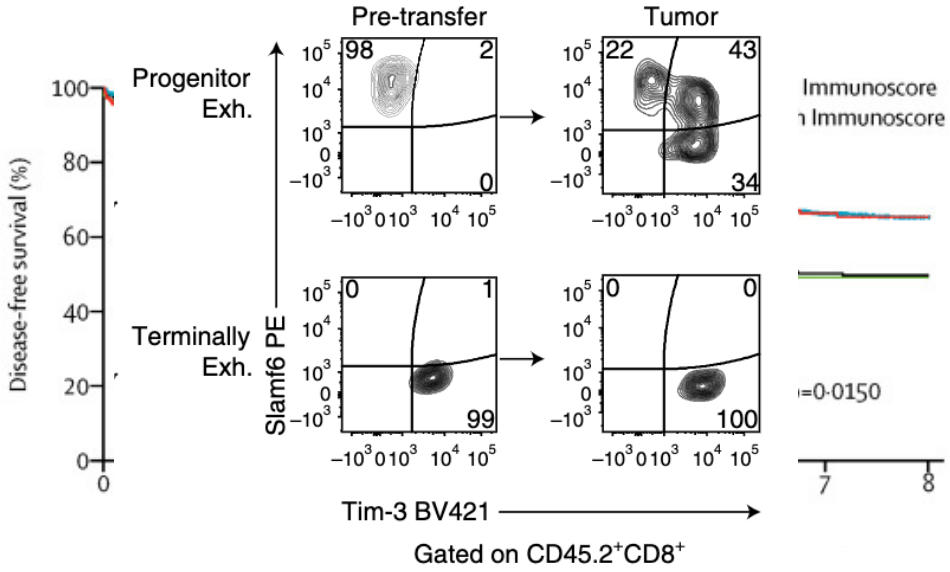
Who does PD-1 blockade affect?



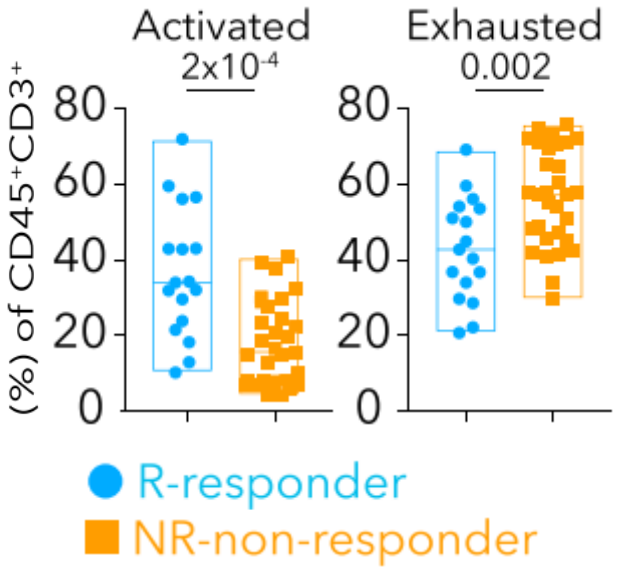
- Single cell sequencing of murine and human tumors indeed confirms this supposition: anti-PD1 likely causes changes to more stem-like PD-1 expressers rather than the terminally exhausted cells
- Thus, PD-1 blockade actually induces changes in cellular fate rather than the immediate function of the cell

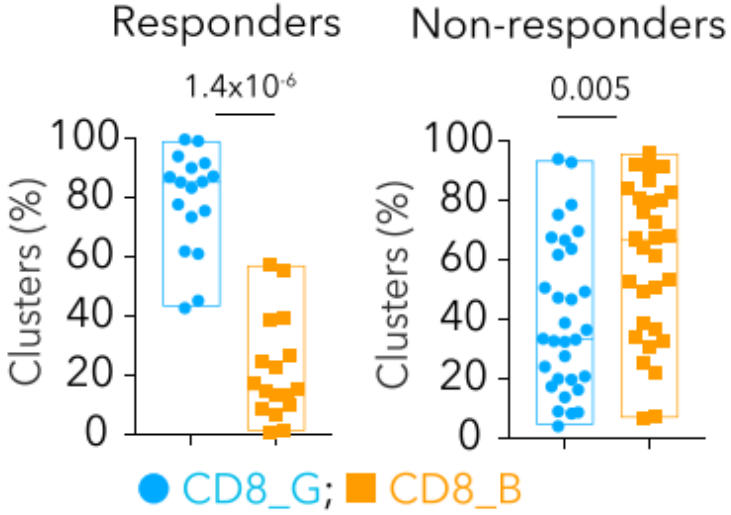
Terminally exhausted cells are not targets waiting to be reinvigorated, and may be bad for you





- ↑ T cell infiltration = ↑ EFS/DFS
- ↑ %Exhausted T cells (Texh) = ↓ Response to immTx
- ↑ Exhaustion signature (↑ IRs; ↓ TCF1/7) = ↓ Response
- αPD-1 therapy targets progenitor Texh (PD-1$^+$CXCR5$^+$ TIM-3$^-$), not terminally differentiation Texh (TIM-3$^+$)

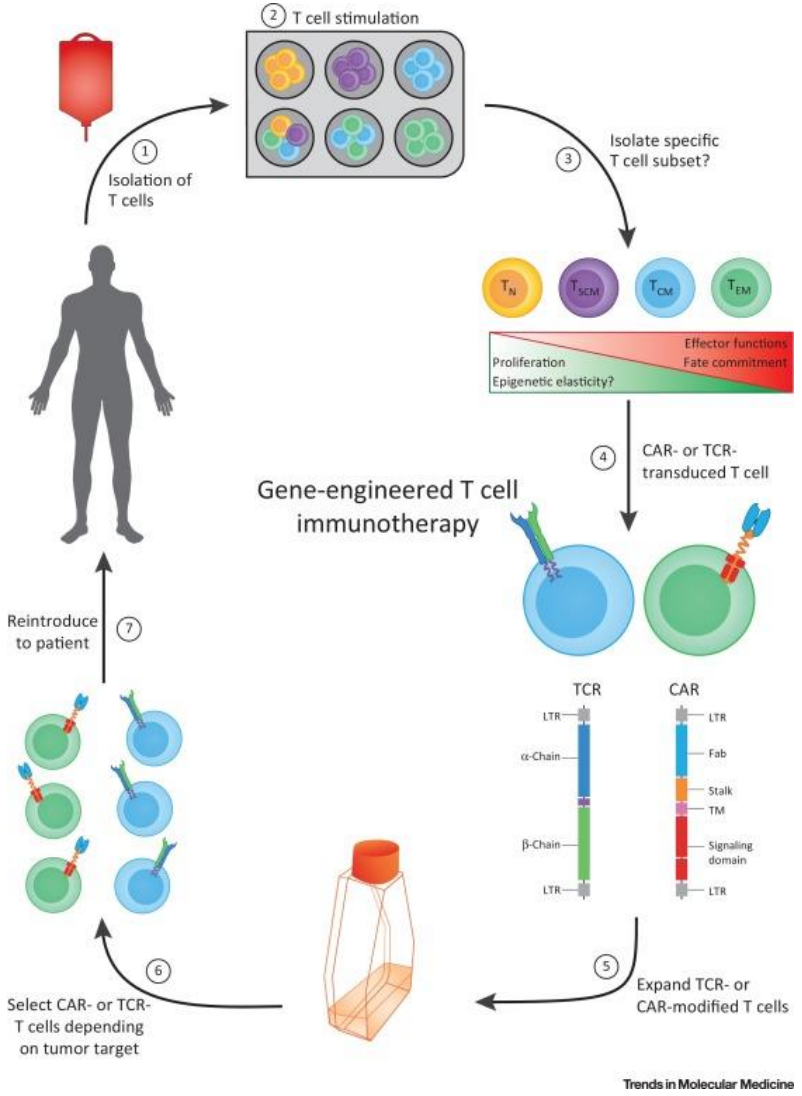




What about combinatorial therapies?

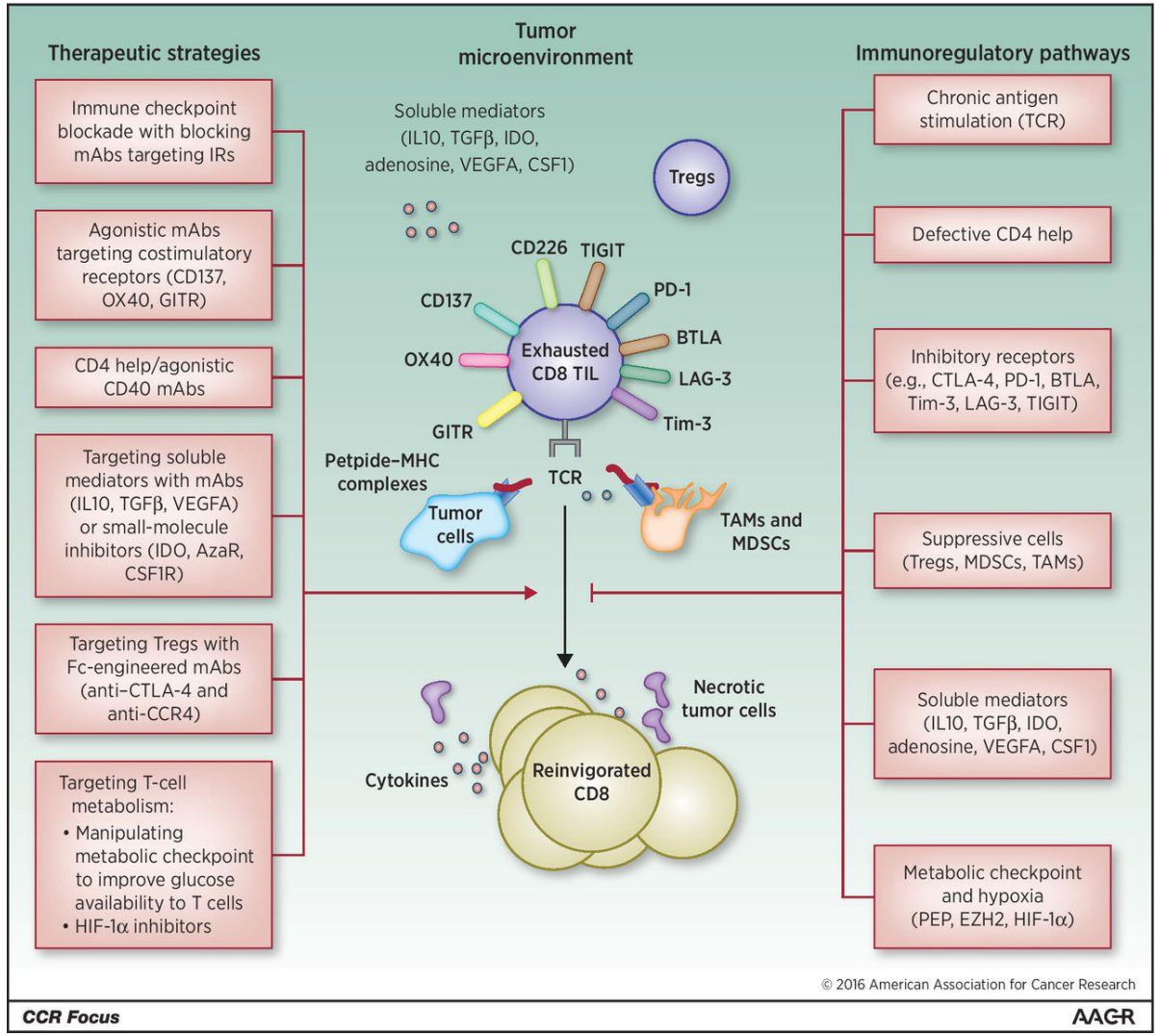
- In ipi/nivo combinations, supposedly, aCTLA-4 may act to prime new T cells, while aPD-1 sustains them/prevents their altered differentiation
- Unfortunately, the newest modalities target molecules whose signaling and downstream biology remain unclear
  - LAG3
  - TIM-3
  - TIGIT
- Understanding the underlying biology to these targets will undoubtedly reveal to us whether these terminally exhausted T cells can be rejuvenated or not

Exhaustion likely affects adoptive cell therapies



- CAR-T cells see a single antigen, and indeed chronic/tonic signaling of these receptors has been shown to lead to metabolic insufficiency and exhaustion
- TIL derive from tumor-reactive, partially or terminally exhausted cells that are cultured in vitro: if their epigenetic identity is maintained even after *ex vivo* culture, it is likely expanded and reinfused TIL will still succumb to the same phenotypes

What is the role of the microenvironment in causing exhaustion?



- Unlike chronic infections, solid tumors have a distinct microenvironment
- Once the tumor microenvironment is established, **the drivers of T cell exhaustion are all concentrated at the tissue site**
- Tumor cells present chronic antigen
- Stromal cells and immature myeloid cells generate persistent inflammation
- Treg cells and MDSC can generate IL-10, TGFb, inhibitory metabolites
- Tortuous angiogenesis combined with high tumor cell metabolism creates hypoxia and nutrient deprivation
- Physical barriers prevent new T cell infiltration

Concluding points for discussion

- T cell exhaustion in cancer arises from a number of tumor- and host-derived factors, including antigen, inflammation, and metabolic stress
- Are tumor-infiltrating T cells exhausted in the same way that those in chronic infections are?
- If exhaustion really is a state of terminal differentiation, are they salvageable?
- Given that these cells are often constitute the majority of tumor infiltrating T cells, what type of measures (artificial, heroic, crazy) could be deployed to reverse these inhibitory states?