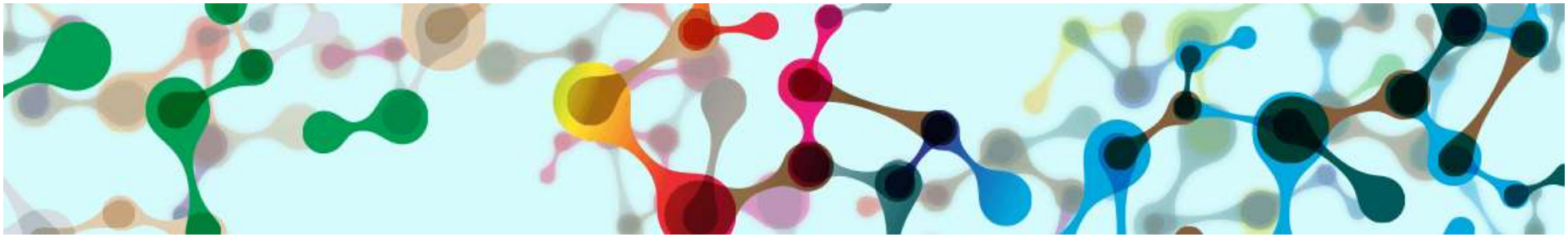


Peripheral T cell expansion predict tumor infiltration and clinical IO response

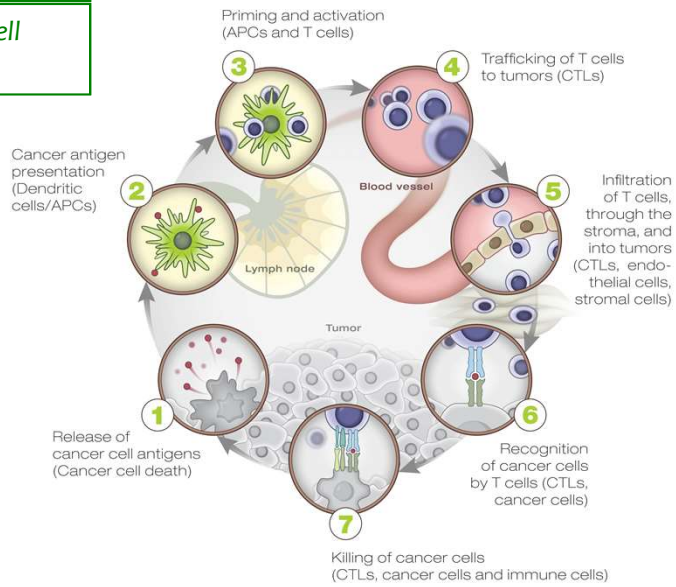
Jane Grogan
CSO, ArsenalBio

[Former employee Genentech – studies represented here]

Changing our understanding of how checkpoint inhibition: reversing T cell "exhaustion" is not the whole story

Anti-PD1/PD-L1

Expansion of T cell compartment



- **Assumed that PD-L1/PD-1 blockade re-invigorates "exhausted" T cells in tumors**
- **Drug discovery & development has been driven by this view**
- **It is likely incorrect: blockade may act to *expand* T cell responses**

Chen & Mellman (2013) Immunity

Anti-PD1/PD-L1

Immunosuppression in tumor bed

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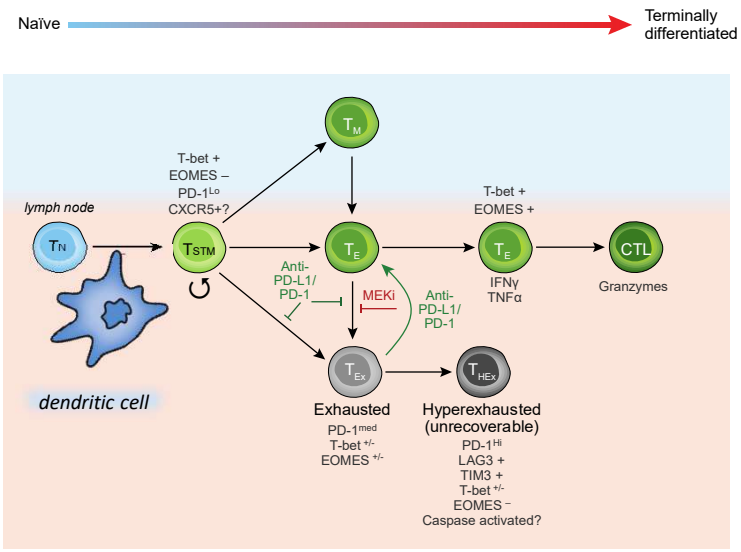
Evidence that checkpoint blockade acts to expand or activate T cells



Exhaustion is epigenetically locked by TOX and may be irreversible

- Wherry lab, Schietinger lab, Zhen lab, Wu Lab, Rao lab

Anti-PD-L1/PD-1 expands T stem cell memory cells in infection & tumors, little actual evidence for exhaustion reversal

- Ahmed lab
- **Tscm are PD1+/TIGIT+, but TIM3-/Lag3-**

CD28 signaling via B7.1 is required for anti-PD-L1 efficacy

- Ahmed lab, Mellman lab

PD-L1 on dendritic cells is key to regulating T cell function by PD-1 in anti-tumor immunity

- Mellman lab, Tang lab

PD-1 therapy elicits recruitment of new TILs, not expansion of pre-existing exhausted TILs

- Howard Chang et al; Wu/Grogan, et al

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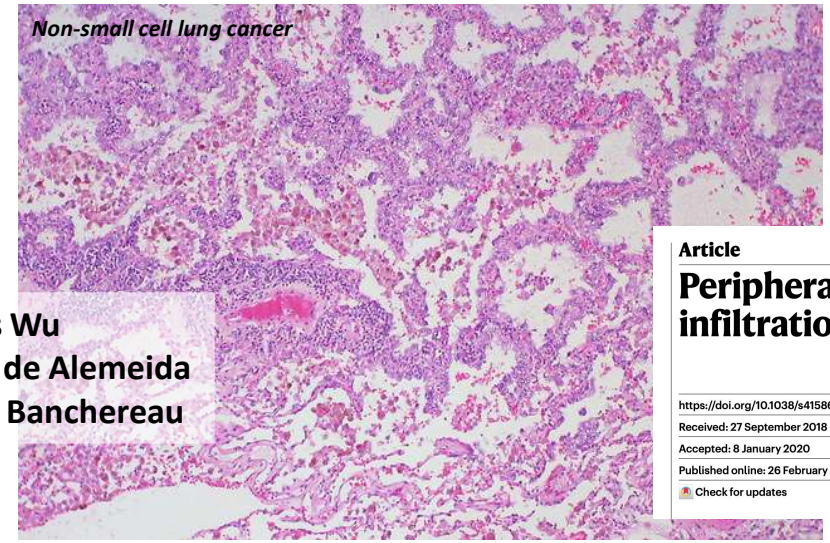
Establishing the origin of expanded TILs during PD-(L)1 therapy:
TCR clonotypes in tumor, normal adjacent tissue, and blood



Thomas Wu
Patricia de Alemeida
Romain Banchereau

Article

Peripheral T cell expansion predicts tumour infiltration and clinical response

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Thomas D. Wu[1,5], Shravan Madireddi[2], Patricia E. de Almeida[2], Romain Banchereau[3], Ying-Jiun J. Chen[4], Avantika S. Chitre[2], Eugene Y. Chiang[2], Hina Iftikhar[2], William E. O'Gorman[3], Amelia Au-Yeung[3], Chikara Takahashi[3], Leonard D. Goldstein[1], Chungkee Poon[6], Shilpa Keerthivasan[2], Denise E. de Almeida Nagata[2], Xiangnan Du[2], Hyang-Mi Lee[2], Karl L. Banta[2], Sanjeev Mariathasan[3], Meghna Das Thakur[7], Mahrukh A. Huseni[7], Marcus Ballinger[7], Ivette Estay[7], Patrick Caplazi[8], Zora Modrusan[4], Lélia Delamarre[2], Ira Mellman[2], Richard Bourgon[1] & Jane L. Grogan[2,9]✉

Wu et al, Nature, 2020

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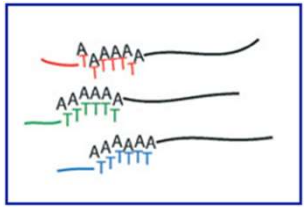
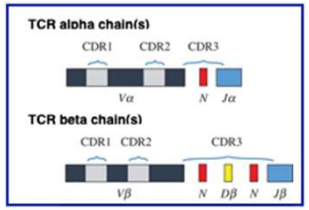
Details can be revealed by studying single T cells

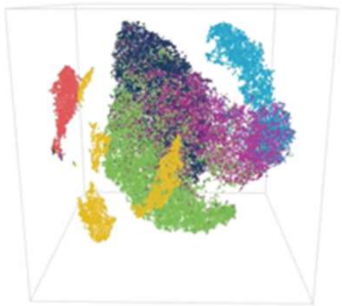
10X Genomics Gel Bead technology

scRNA-seq

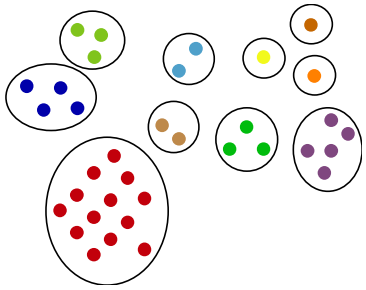


scTCR-seq





Analyze cells by transcript profiles



Group cells by clonotype

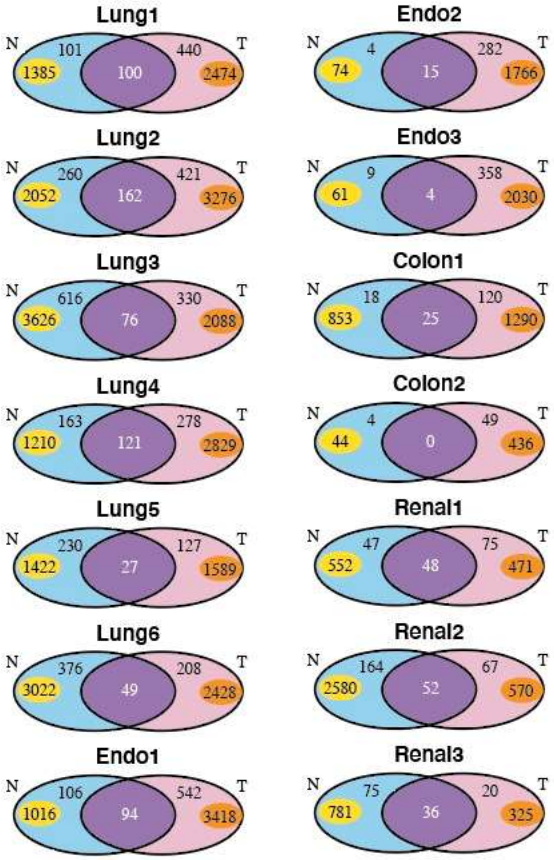
Patient	Src	Single-cell RNA-seq T cells Transcripts	T cells Cells	Non-T cells Transcripts	Non-T cells Cells	scTCR-seq Typed	scTCR-seq Clones
Lung1 (CD3)	T	27,930,187	7,335	509,263	127	6,105	4,684
	N	17,403,400	5,944	615,976	225	4,376	
Lung2 (CD3)	T	41,594,246	8,486	1,403,112	297	7,905	6,482
	N	29,248,258	7,105	1,340,805	298	6,312	
Lung3 (CD3)	T	10,005,925	6,698	476,462	264	4,688	7,011
	N	19,132,841	11,594	680,102	412	7,903	
Lung4 (CD3)	T	5,258,284	8,992	101,957	189	4,857	4,833
	N	3,254,282	6,120	90,328	168	2,875	
Lung5 (CD45)	T	8,453,692	2,901	10,993,570	2,383	2,377	3,487
	N	8,781,381	3,765	9,056,514	1,959	2,938	
Endo1 (CD3)	T	12,131,599	10,670	621,543	404	7,050	5,387
	N	4,498,595	7,278	161,051	290	2,615	
Endo2 (CD3)	T	5,966,732	7,425	384,890	384	3,732	2,190
	N	399,141	1,718	28,921	108	202	
Endo3 (CD3)	T	9,239,225	5,228	941,197	295	3,929	2,485
	N	203,092	355	9,638	30	131	
Colon1 (CD45)	T	7,278,551	2,422	6,006,689	1,456	1,969	2,364
	N	4,583,949	1,651	4,499,591	2,053	1,150	
Colon2 (CD45)	T	1,987,154	642	9,596,073	2,632	594	535
	N	725,454	153	3,729,254	1,486	66	
Lung6 (CD45)	T	22,118,576	3,809	14,685,726	1,663	3,734	9,948
	N	24,326,797	7,211	7,663,565	1,296	5,847	
	B	18,385,925	4,154	18,526,827	4,081	3,838	
Renal1 (CD45)	T	4,429,006	2,362	12,984,393	5,003	1,878	1,599
	N	2,046,228	2,698	3,657,947	3,235	1,112	
	B	2,714,048	1,216	7,960,749	3,600	813	
Renal2 (CD45)	T	9,320,594	2,799	16,150,376	2,730	2,455	3,960
	N	11,104,386	4,270	12,383,975	3,706	3,755	
	B	4,213,162	1,653	20,425,570	6,918	1,388	
Renal3 (CD45)	T	3,095,674	1,308	19,445,899	5,416	707	2,009
	N	5,148,240	1,610	20,697,490	3,993	1,355	
	B	5,508,115	2,051	5,546,902	1,902	1,132	
Total		330,486,739	141,623	211,376,355	59,003	99,788	56,974

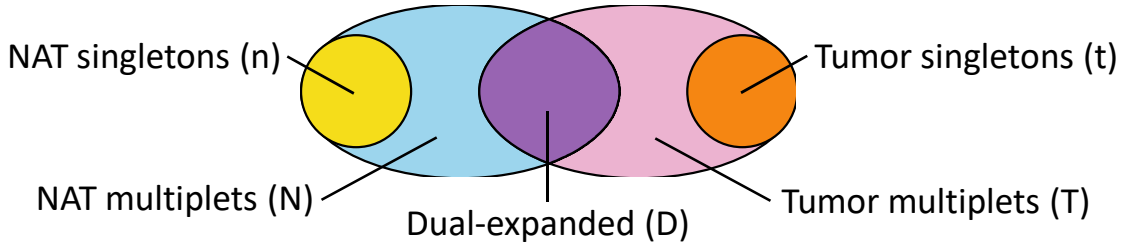
Applied to tumor, Normal Adjacent Tissue (NAT), blood from 14 patients before treatment

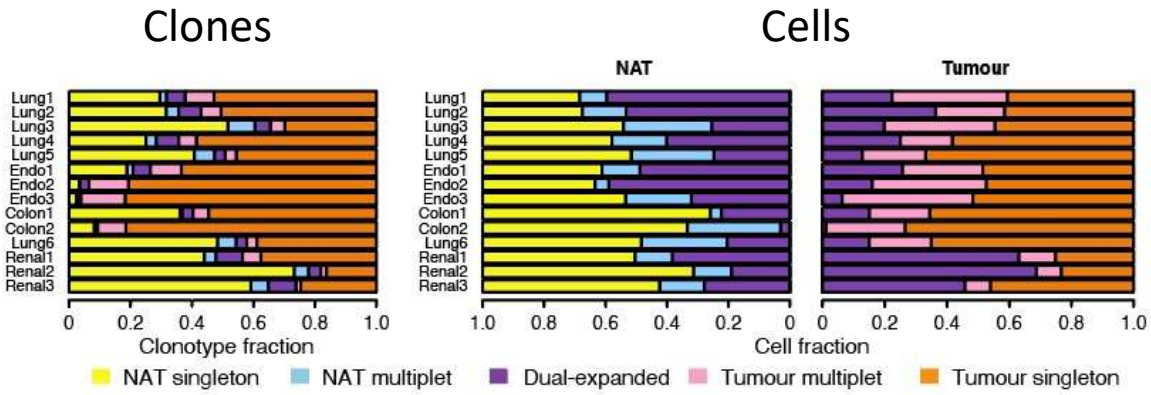
↓

141,623 T cells with 56,974 distinct clonotypes

Extensive sharing of clones in tumor and NAT

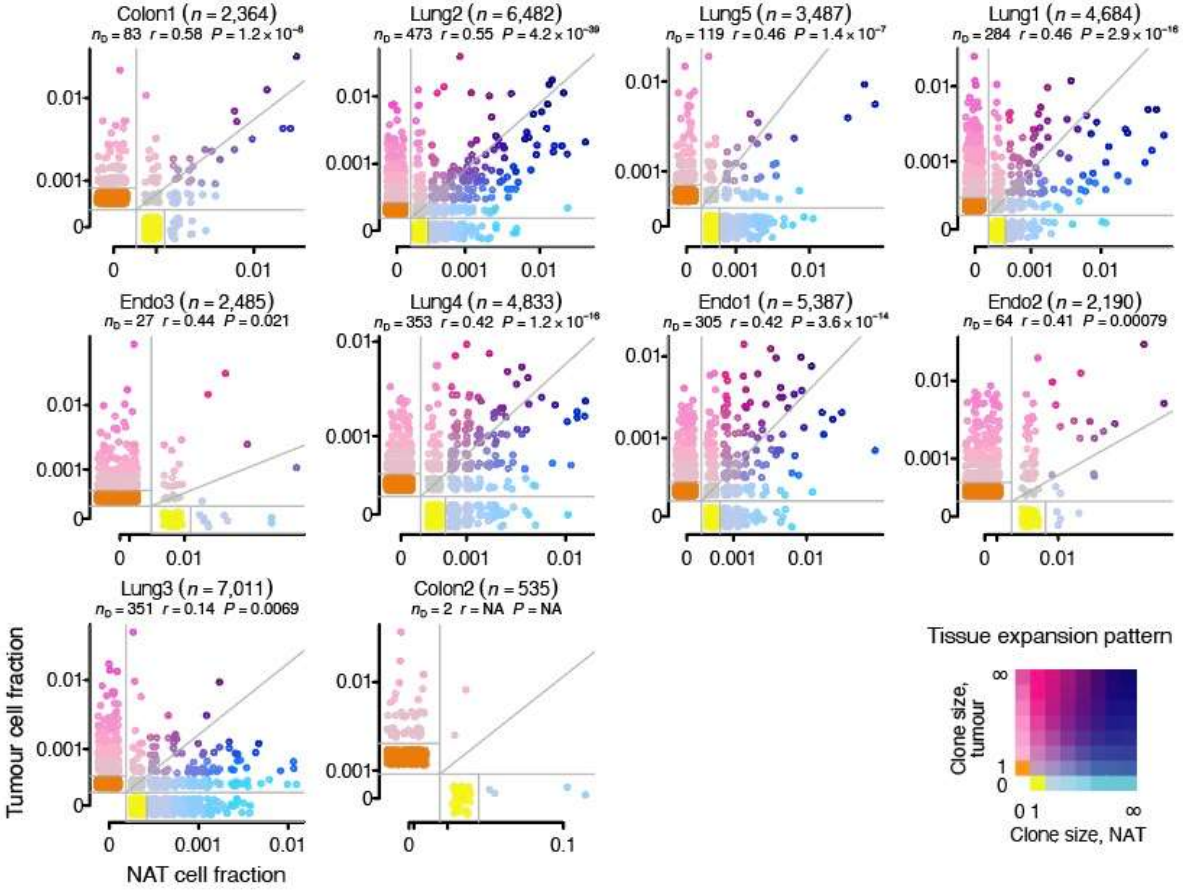


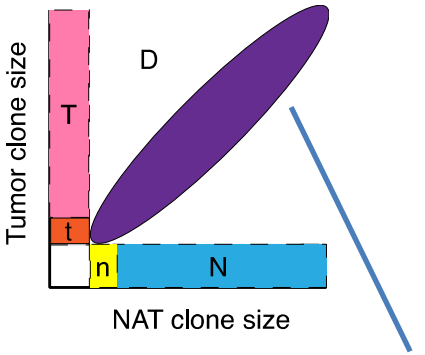




Observation: Expanded T cells in tumor and NAT often had sibling clones in the other compartment

Patterns of dual expansion differed across patients



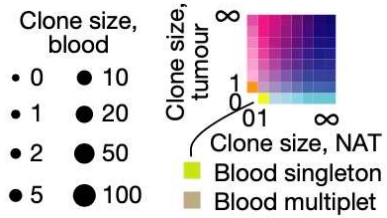


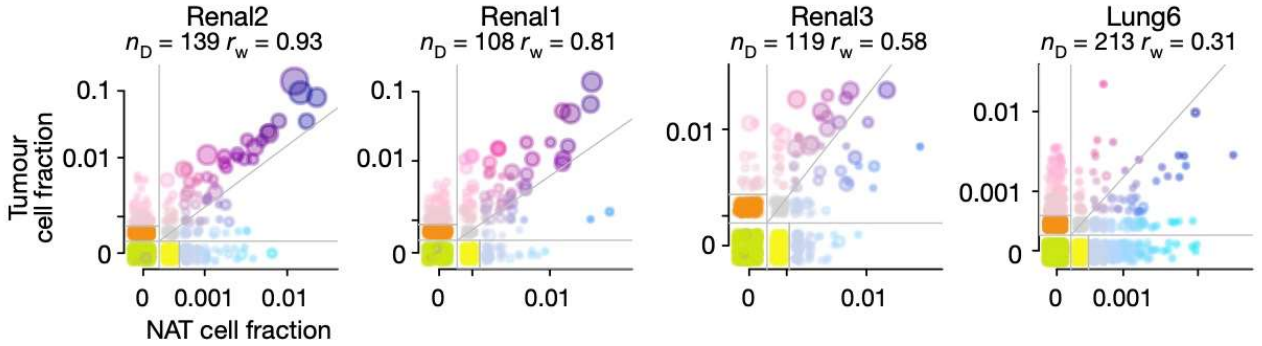
Approximately equal clone sizes in tumor and NAT: Parallel dual expansion

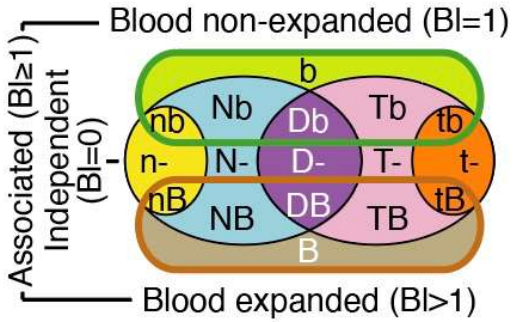
Hypothesis: Could parallel dual expansion be coming from a common source, such as peripheral blood?

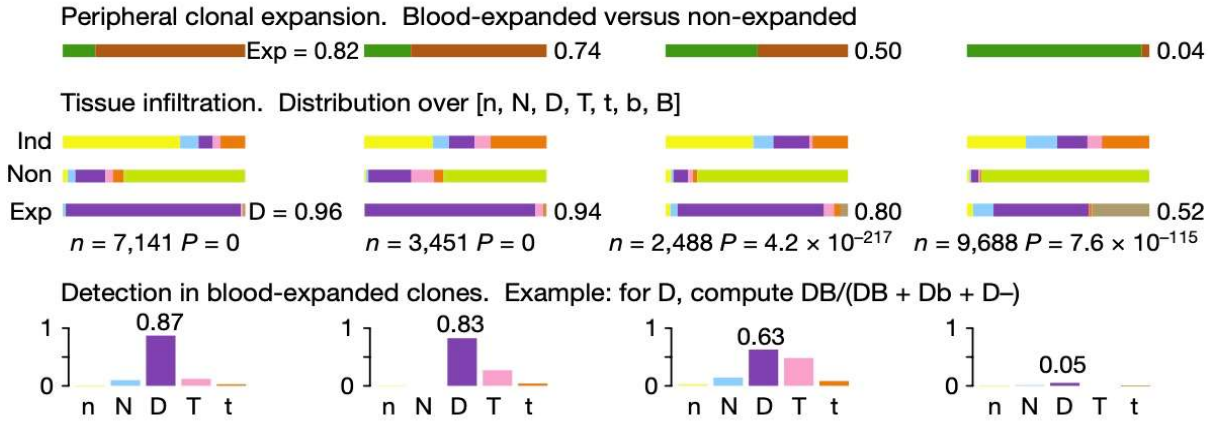
Support from our four patients with peripheral blood samples

clone size in blood by the size of the dot



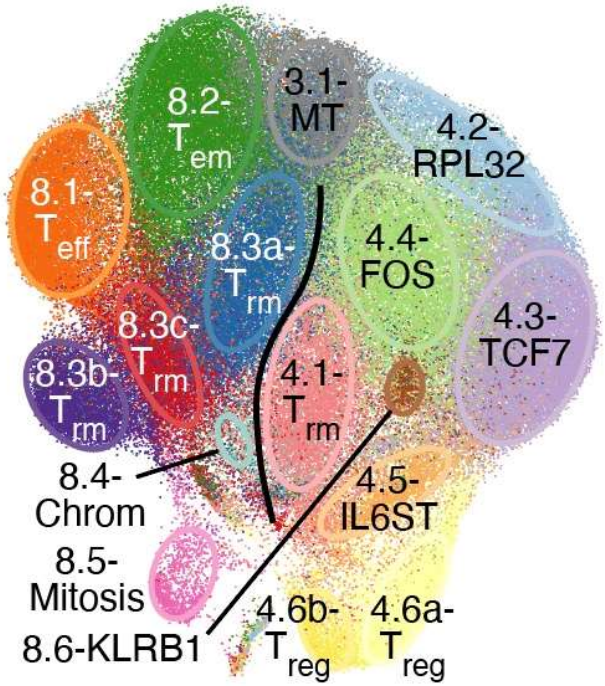






Integrating scRNA-seq and scTCR-seq data shows different expansion behaviors by T cell subset

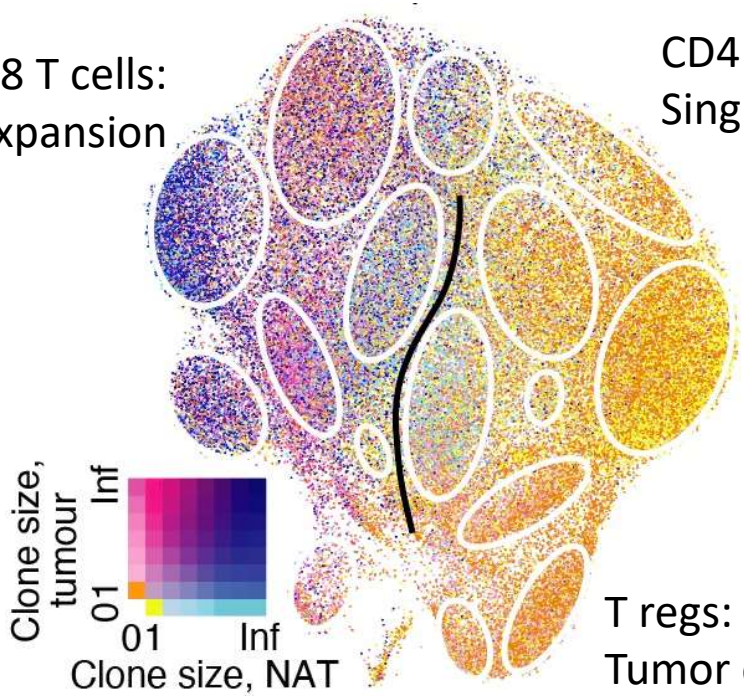
scRNA-seq data



scTCR-seq data

CD8 T cells:
Dual expansion

CD4 T cells:
Singletons

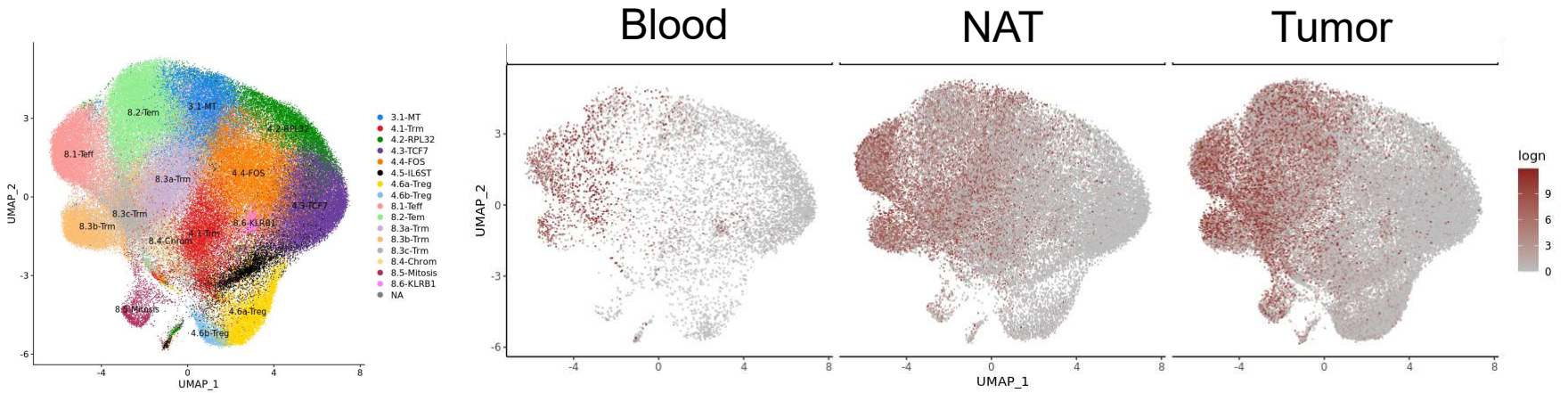


T regs:
Tumor expansion

Distribution of Clonal Freq Across Cell Types and Sample Sources

Expanded clones were detected mostly in Teff, Tem and Trm clusters

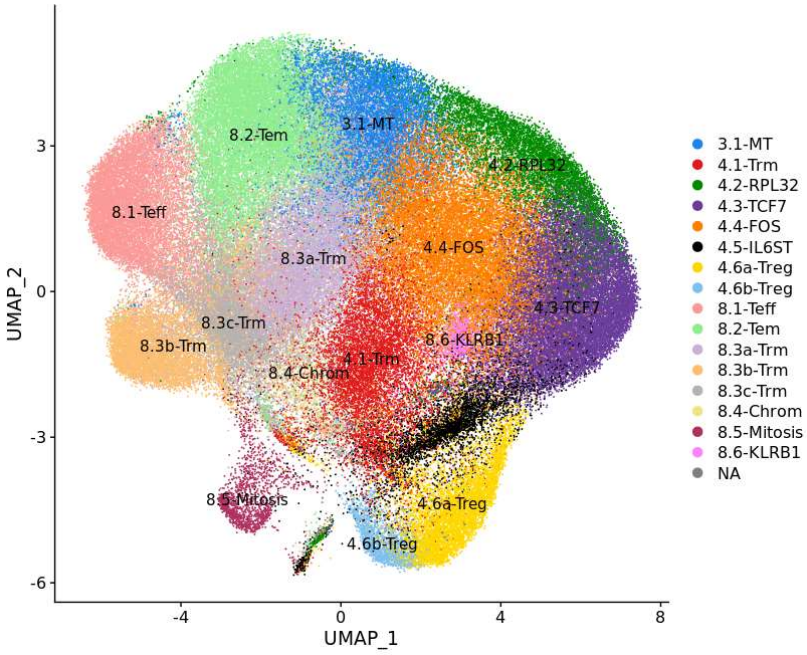
Coloring of Cells by Clonal Frequency, Separated by Sample Sources



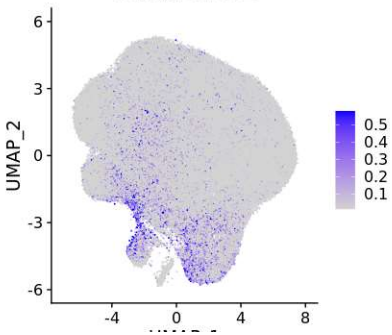
scRNA-seq data yielded several clusters:

Distribution of Exhaustion, Act and Cytolytic Signature Score

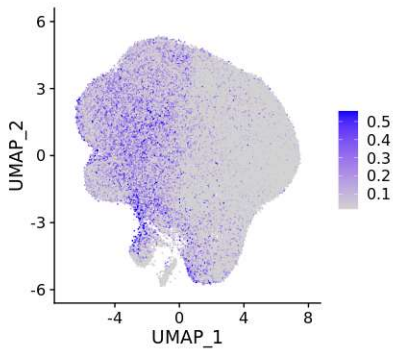
Coloring of Cells by Clusters



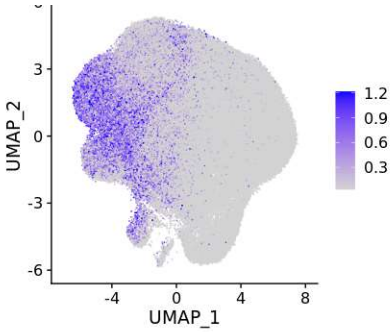
Exhaustion Scores



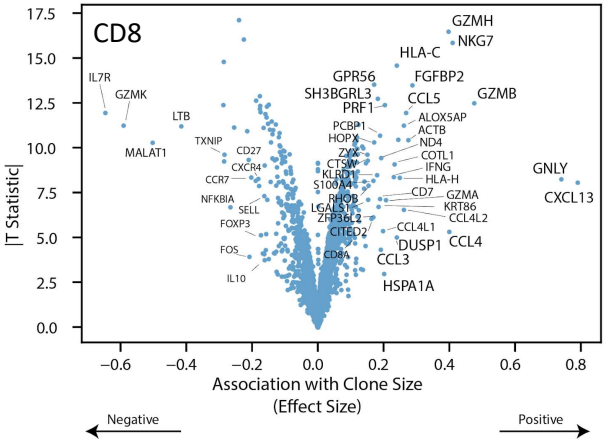
CD8



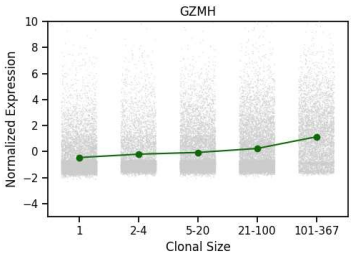
Cytolytic Scores



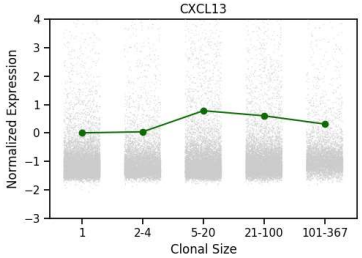
Genes encoding T cell effector genes positively correlated with T cell Clonal Expansion



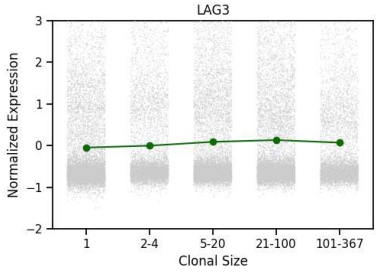
Cytokines & Effector molecules



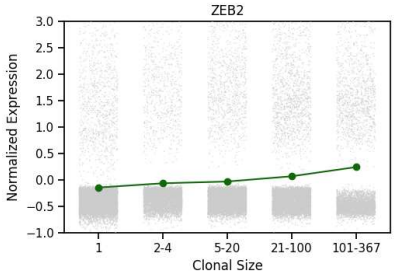
Cell trafficking & Adhesion



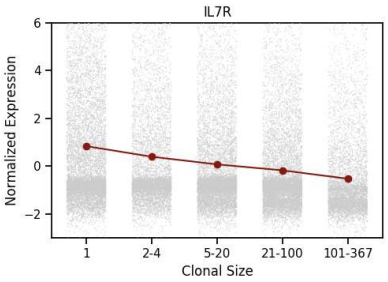
Exhaustion markers



Transcription factors

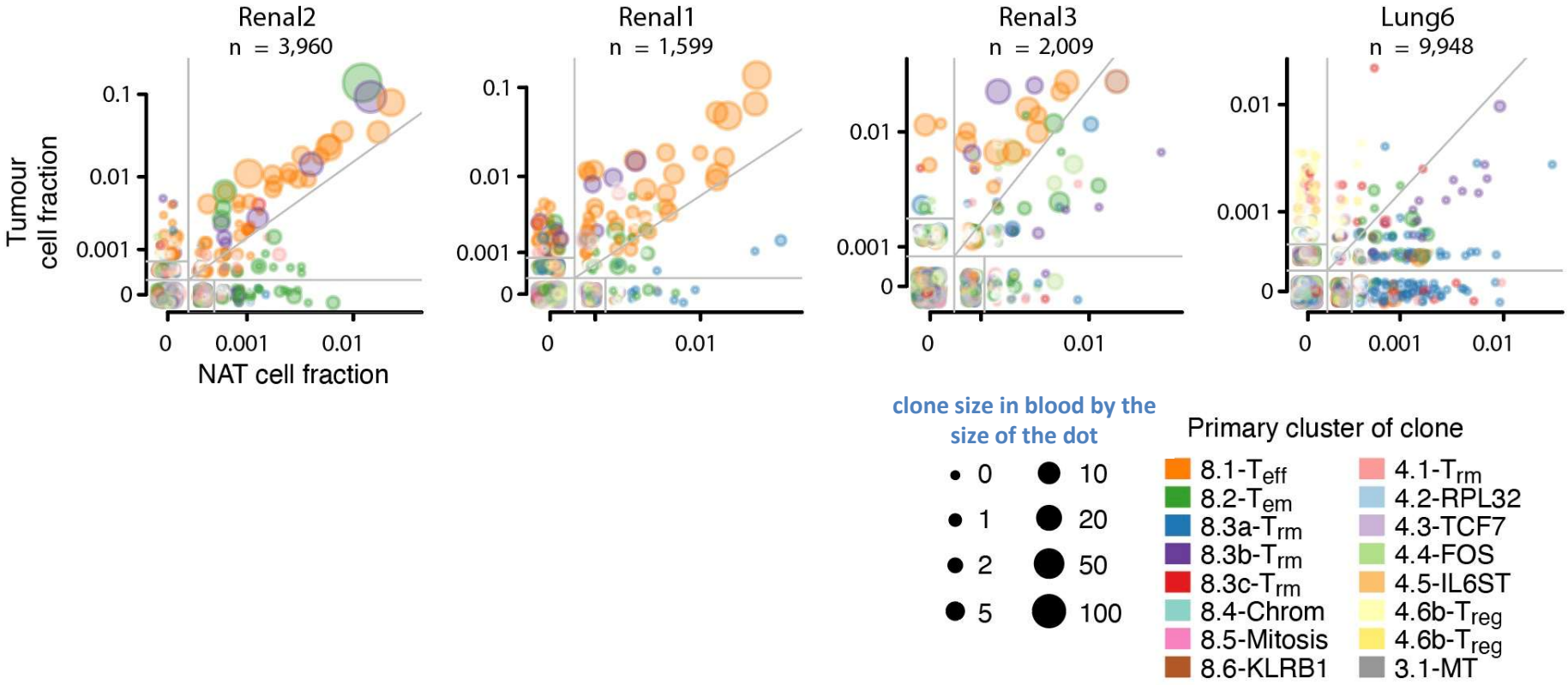


Survival Signals

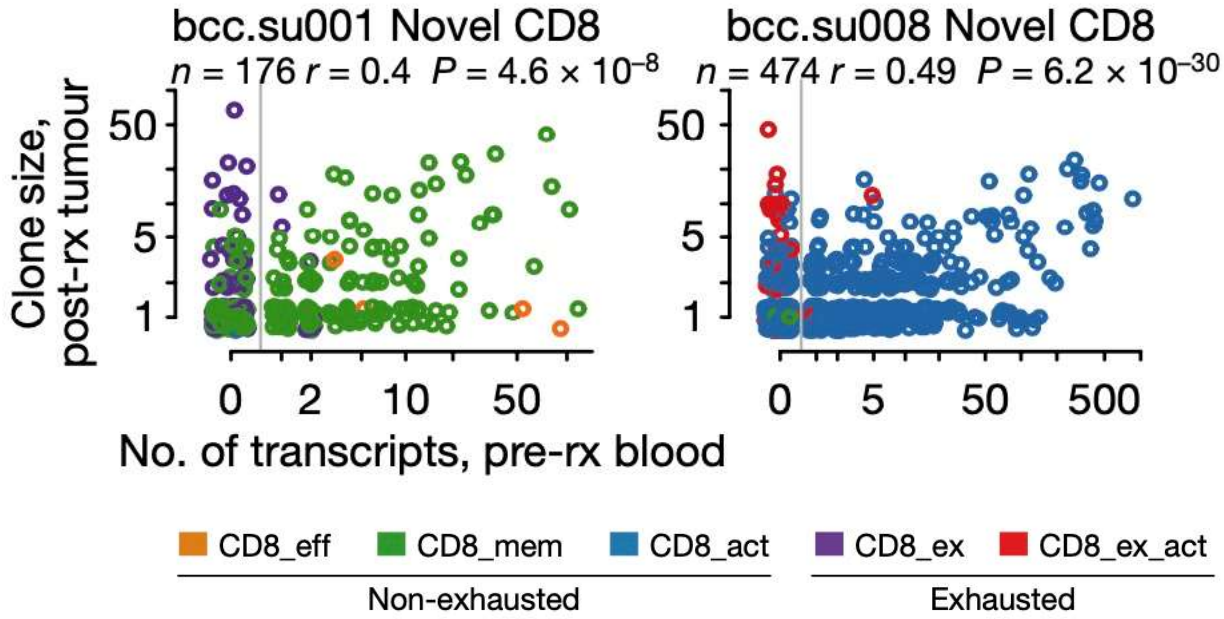


David DeTomaso, Grace Zheng

T-effector cells (8.1) account for most infiltration from peripheral blood into tumor



Clone sizes correlate between pre-treatment blood and post-treatment tumor



Suggests that peripheral blood may be the source of at least some of the novel clones

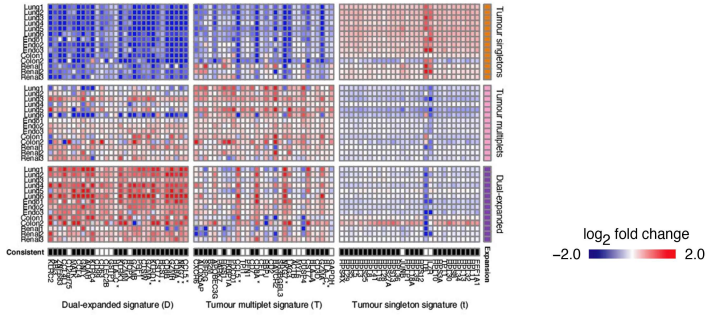
Chang, Nat Med, 2019

Could patient variability in peripheral or dual expansion explain clinical response to IO?

Clinical trials of atezolizumab (anti-PDL1) with bulk RNA-seq from pre-treatment tumor (but unfortunately no TCR measurements in tumor or blood)

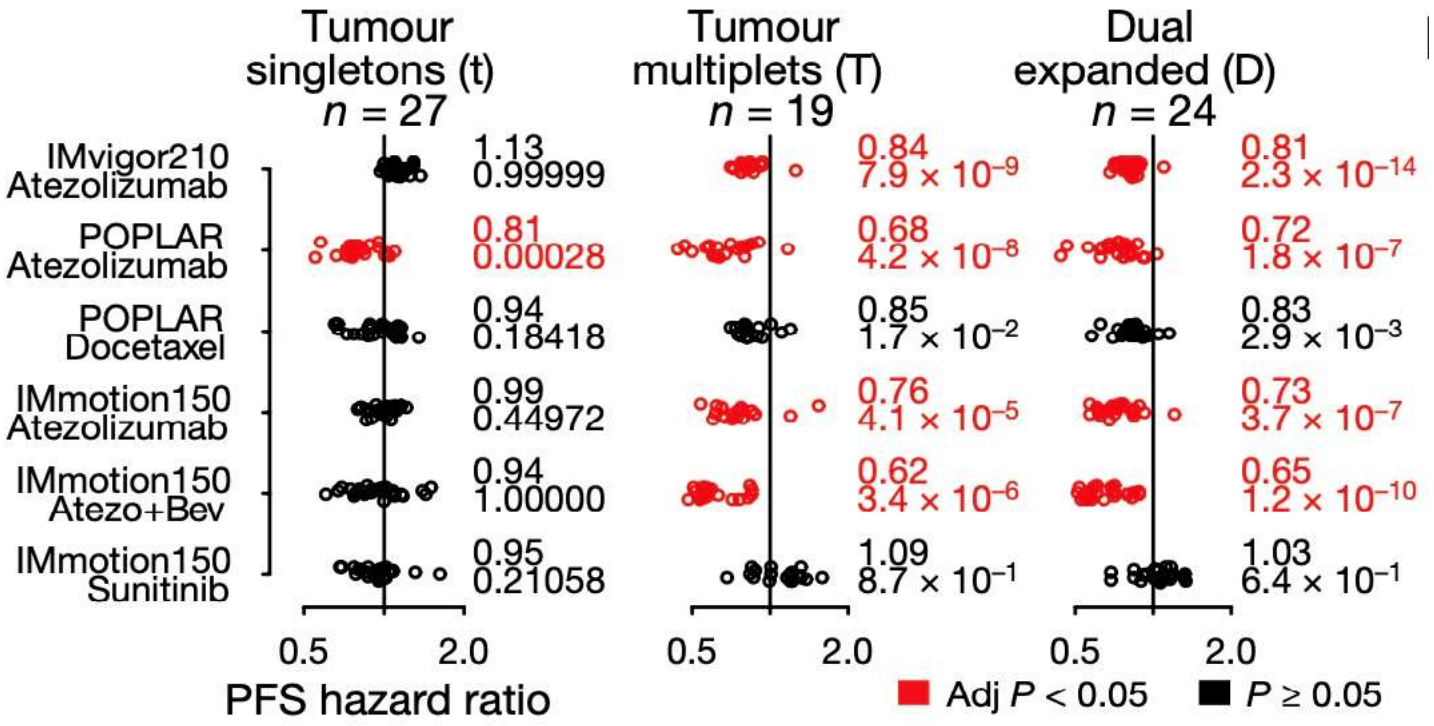
- IMvigor210: A single-arm trial studying locally advanced or metastatic urothelial carcinoma. 354 patients with bulk RNA-seq samples.
- POPLAR: A dual-arm trial of non-small-cell lung carcinoma comparing 93 patients with bulk RNA-seq treated with atezolizumab and 100 patients with docetaxel chemotherapy
- IMmotion150: A triple-arm trial of renal cell carcinoma treated with atezolizumab (86 patients), atezolizumab plus the VEGF inhibitor bevacizumab (88 patients), or the tyrosine kinase inhibitor sunitinib (89 patients)

Developed a gene signature of clonal expansion in tumors



Wu et al, Nature, 2020

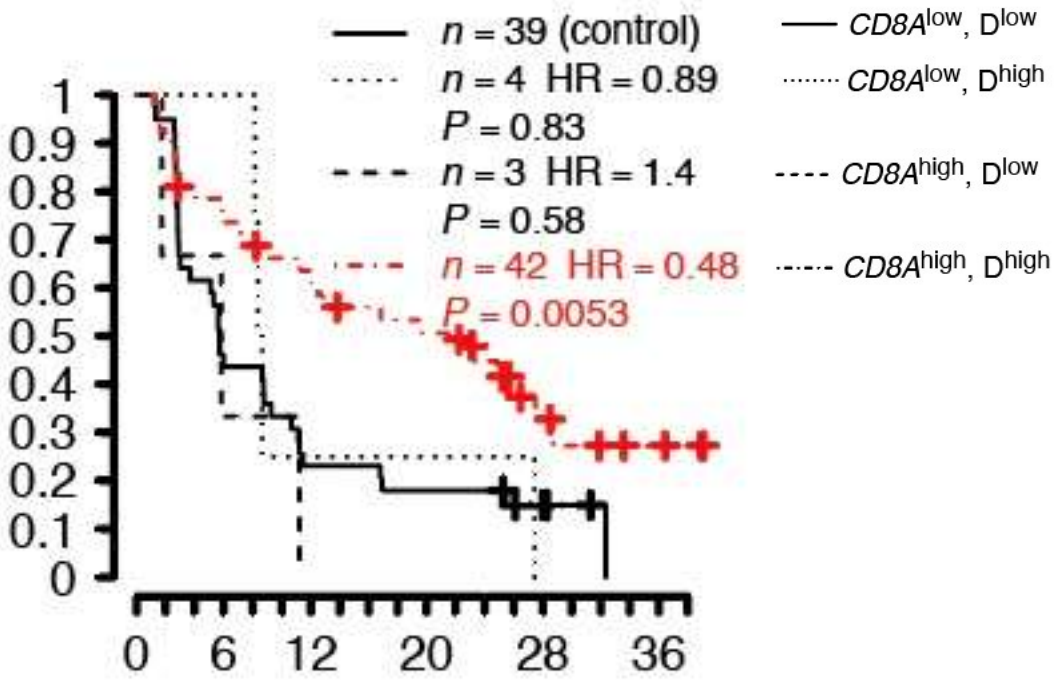
Gene set analysis shows expansion signatures associate with clinical response



Greater clinical response with above-median expression of genes in expansion signatures

Dual-variate analysis of survival using both CD8A and expansion signature scores

Example of dual-variate analysis: IMmotion150, Atezo+Bev arm



Discordant patients are informative

Conclusion

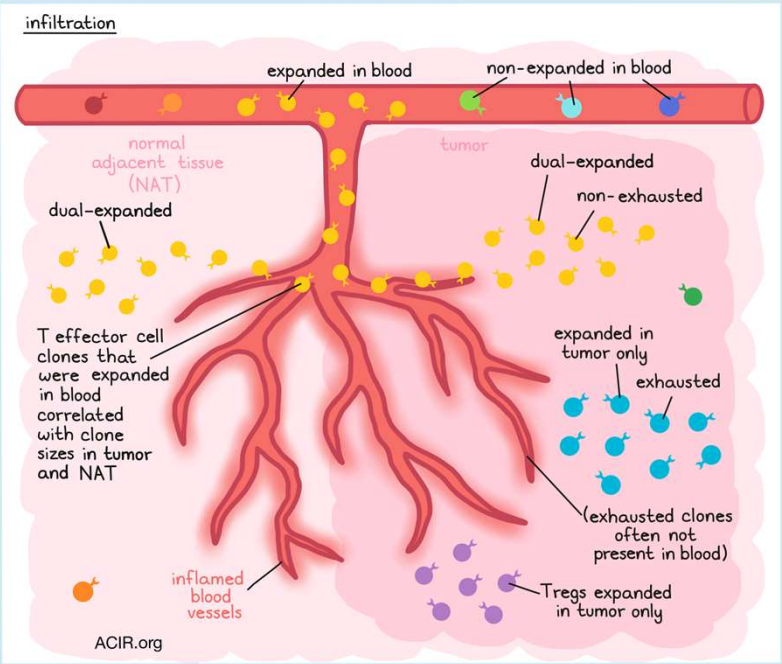
- Clonally expanded T cell population found in abundance in both tumor and adjacent normal tissue (NAT)

- A large fraction of T cells shared T cell receptors (TCRs) present in both tumor and NAT that these T cells had an effector phenotype - mostly Teff, Tem, Trm clusters

- We identify the source of these T cells in the peripheral blood.
  - the fraction of expanded clones in blood is a proxy for the extent of expansion within the tumor, with up to 87% of those TCRs captured in blood
  - that the extent of peripheral clonal expansion explains many other phenomena, including the appearance of novel T cell clones in tumors after immunotherapy (reported by Yost in 2019) and the presence of virally reactive T cells (reported by Simoni et al. in 2018 and Scheper et al. in 2019)

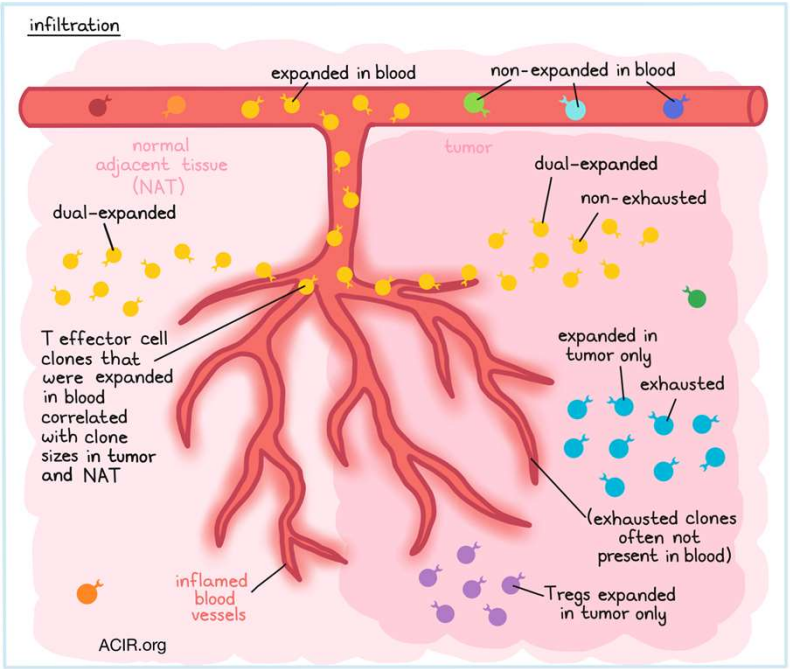
- These expanded T cell clones predicted response to cancer immunotherapy

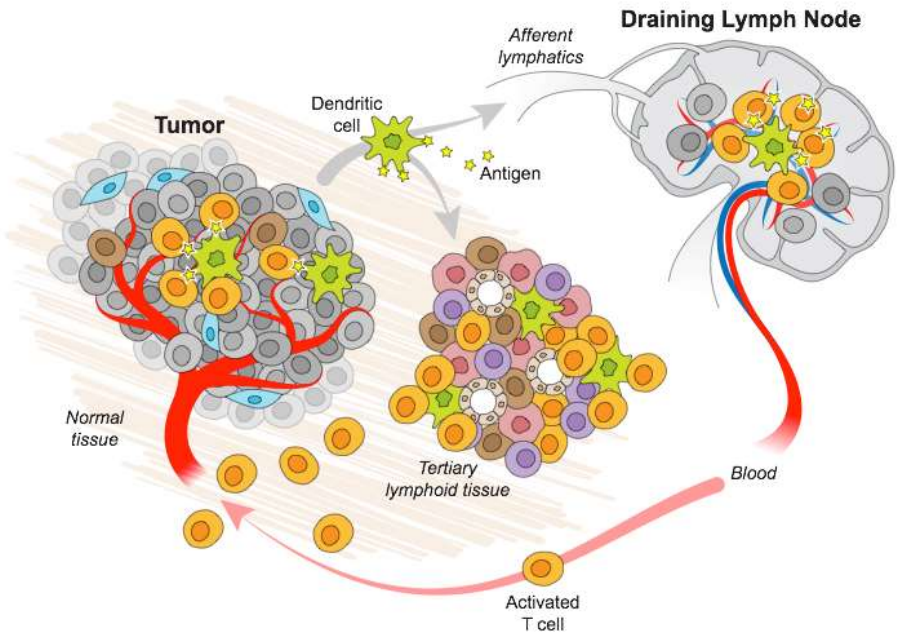
A model for clonal expansion of T cells in tumors



- A local, blood-independent expansion that leads to exhausted T cells
- A peripheral, blood-associated expansion that supplies fresh, non-exhausted T cells
- This model causes us to reconsider whether cancer immunotherapy acts by “reversing” exhaustion

A model for clonal expansion of T cells in tumors





Beyond the tumor: What are the peripheral and tissue-specific mechanisms that promote endogenous as well as exogenous TCR- and CAR-T responses

Acknowledgements

Genentech Team

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