SITC 2016

NATIONAL HARBOR, MD
NOVEMBER 9-13, 2016

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Society for Immunotherapy of Cancer

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T cell function and specificity in colorectal cancer

Arnold Han, MD,PhD

Columbia University

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Society for Immunotherapy of Cancer

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Presenter Disclosure Information

Arnold Han, MD, PhD

The following relationships exist related to this presentation:

Provisional U.S. patent related to TCR sequencing

Systemic Analysis of Human CRC

1.

Sample acquisition

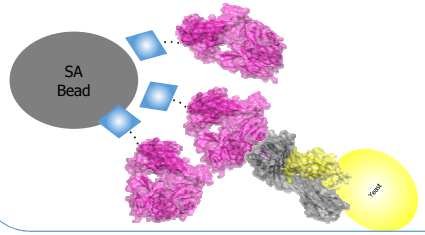
Tumor/Normal

T cell phenotyping

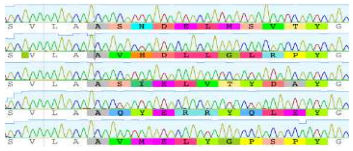
Understand phenotypes of tumor-specific T cell repertoire

2.

Screening



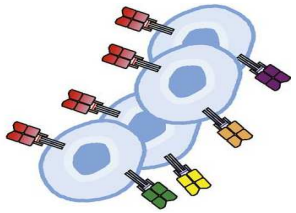
Deep-Sequencing & Bioinformatics Analysis



Determine T cell antigen specificities

3.

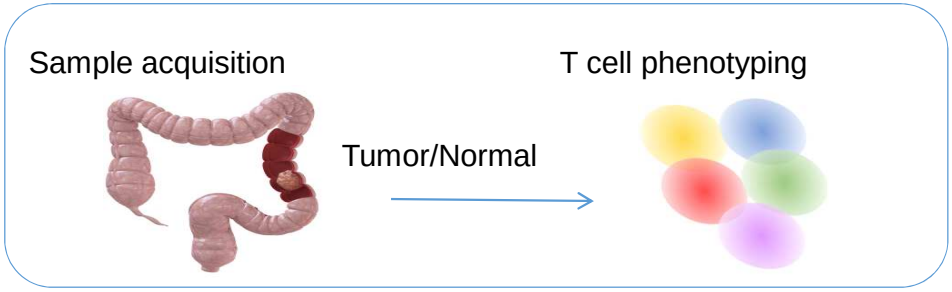
TCR-based therapies



Investigate therapeutic potential

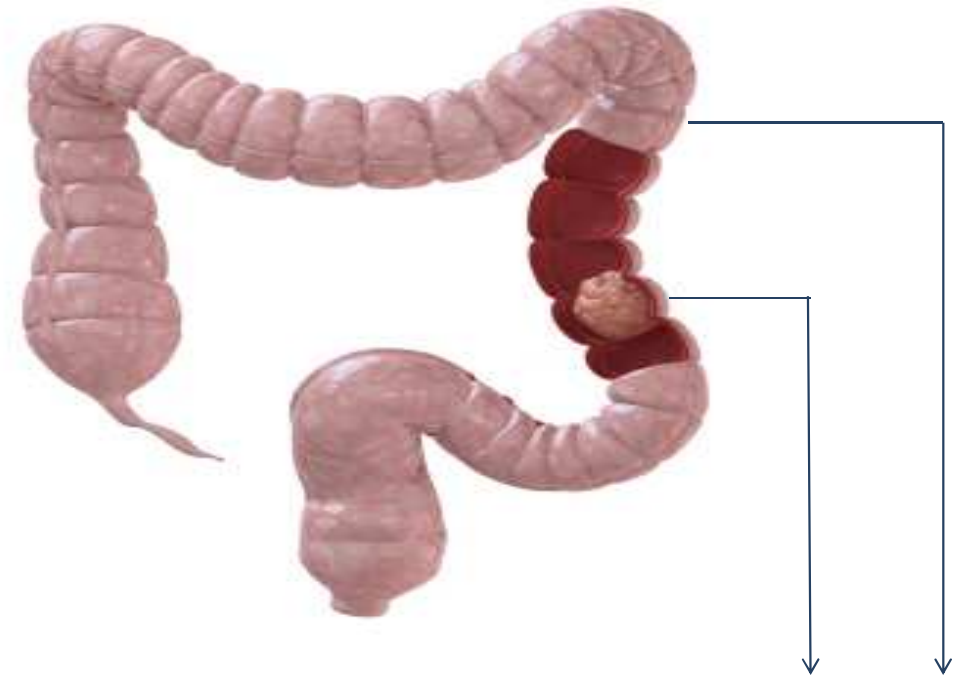
Arnold Han, MD, PhD

1.



Understand phenotypes of tumor-specific T cell repertoire

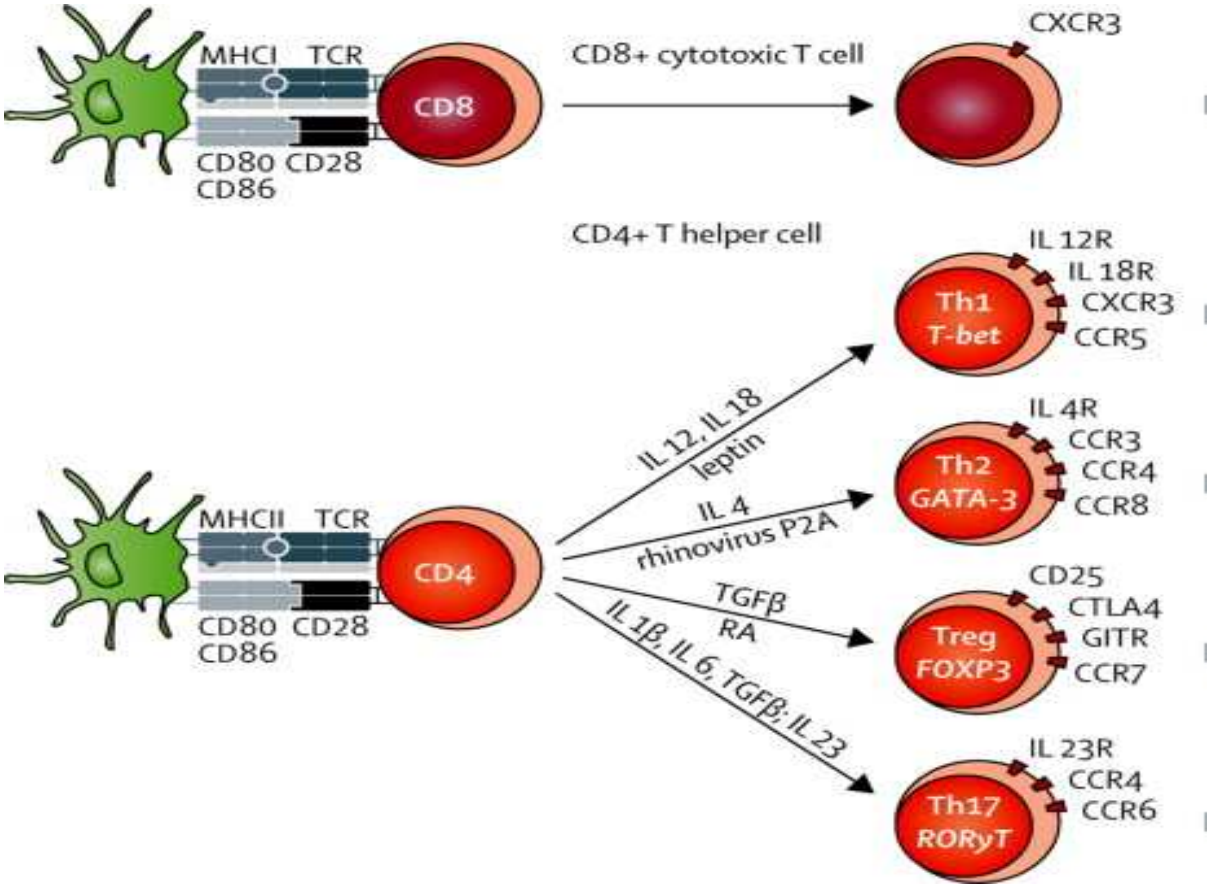
Experiment



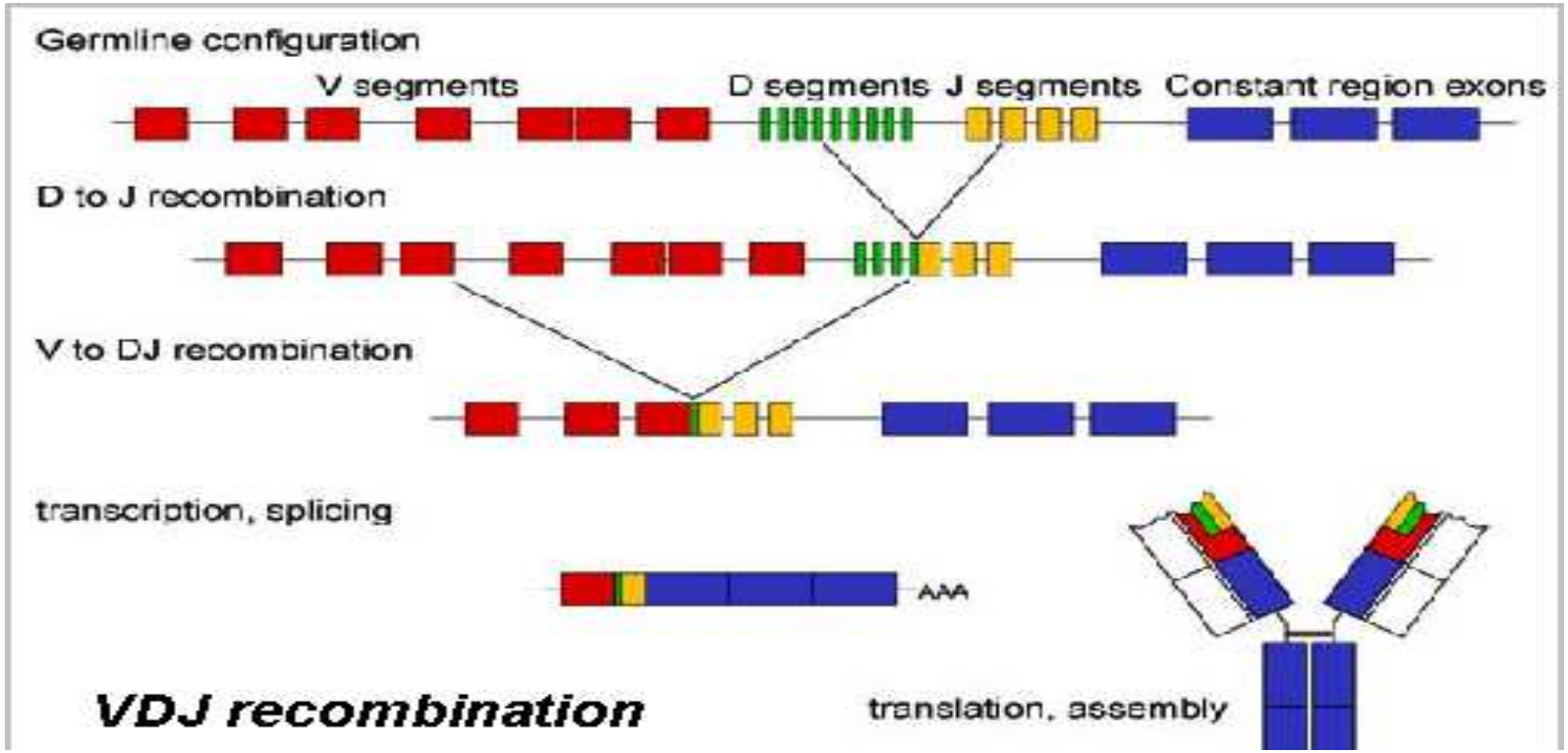
Extract and Analyze Single T cells

Single-Cell vs Bulk Analysis

The Complexity of T cell Function



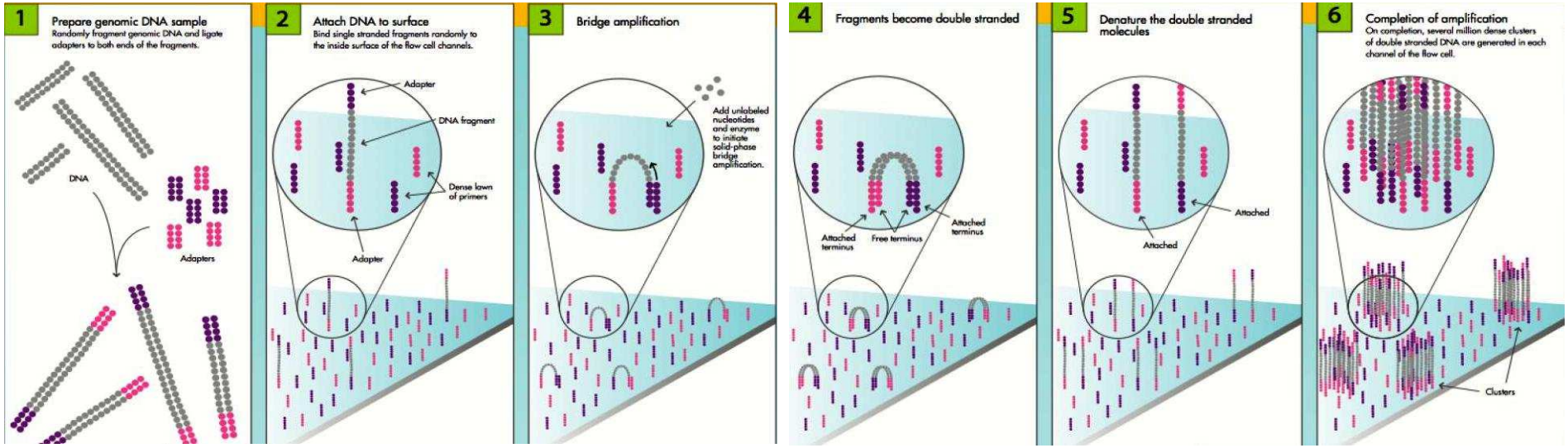
TCR diversity: V(D)J Recombination



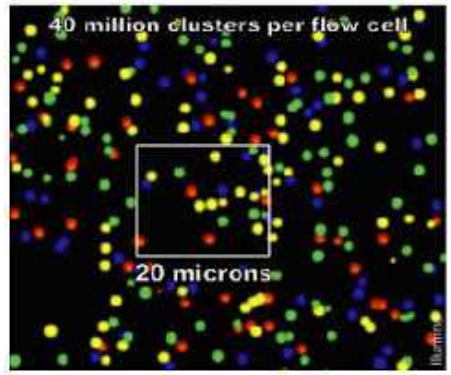
Diversity ~ 10^{15}

Janeway et al

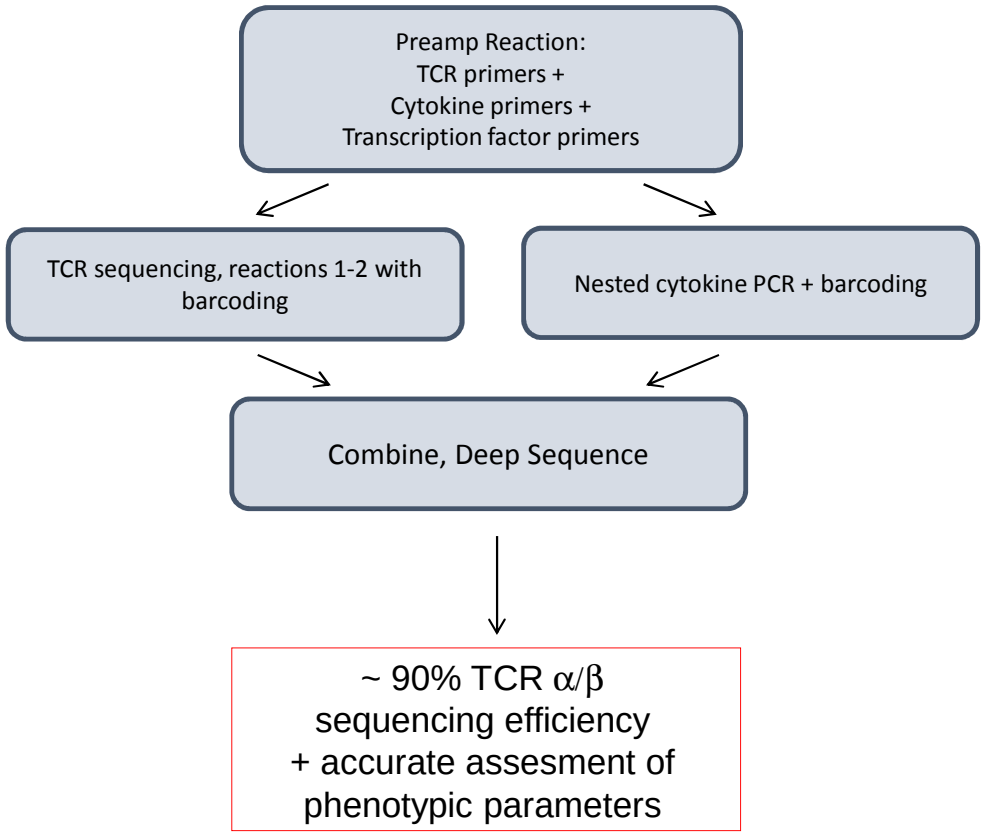
Deep Sequencing



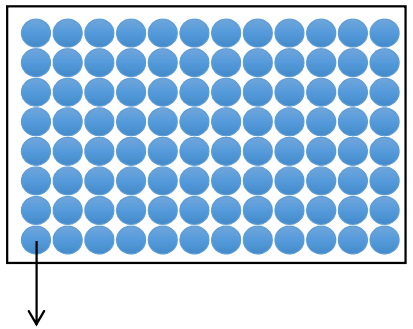
VS. Conventional (Sanger Based Sequencing):
Single Molecules are Sequenced
Throughput > 10^7



Single-cell gene expression and TCR sequencing by deep sequencing

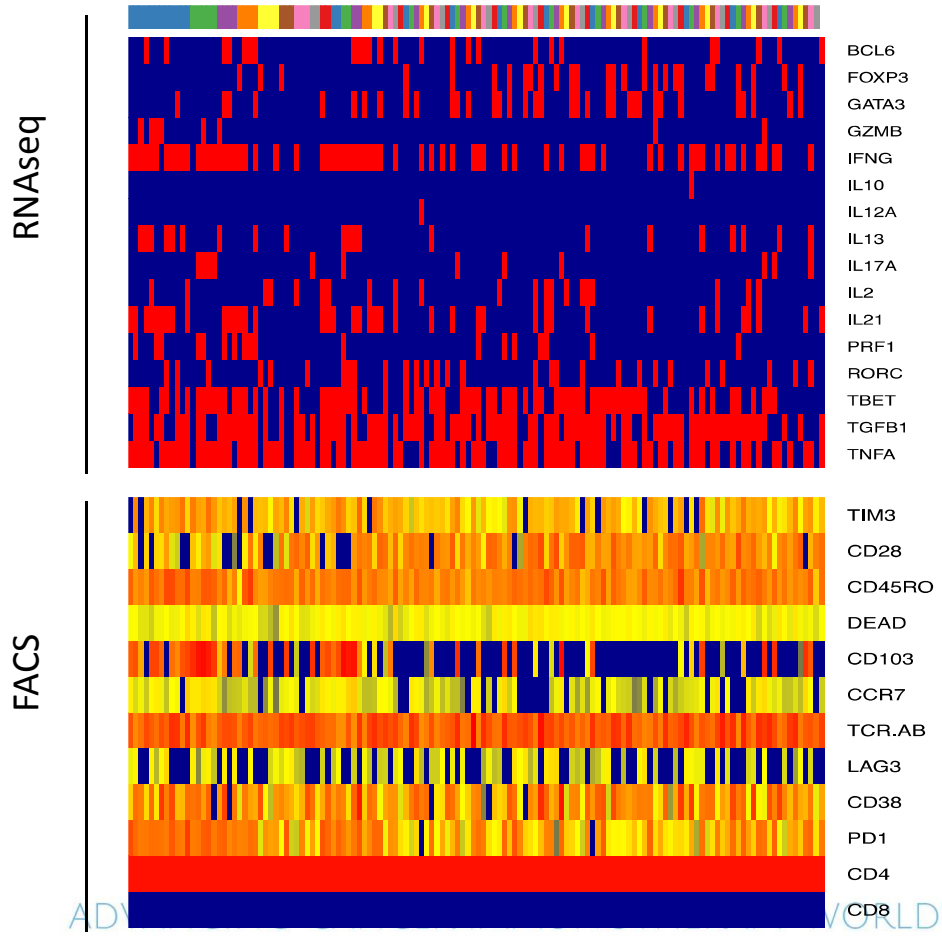


Han et al, *Nature Biotechnology, 2014*



Unique DNA sequence barcode (Cell-Specific)
TCR sequences
Cytokine/Transcription Factor Sequences

Paired TCR and phenotypic analysis: 35+ parameters/cell



Visualizing high dimensional T cell data: T-SNE

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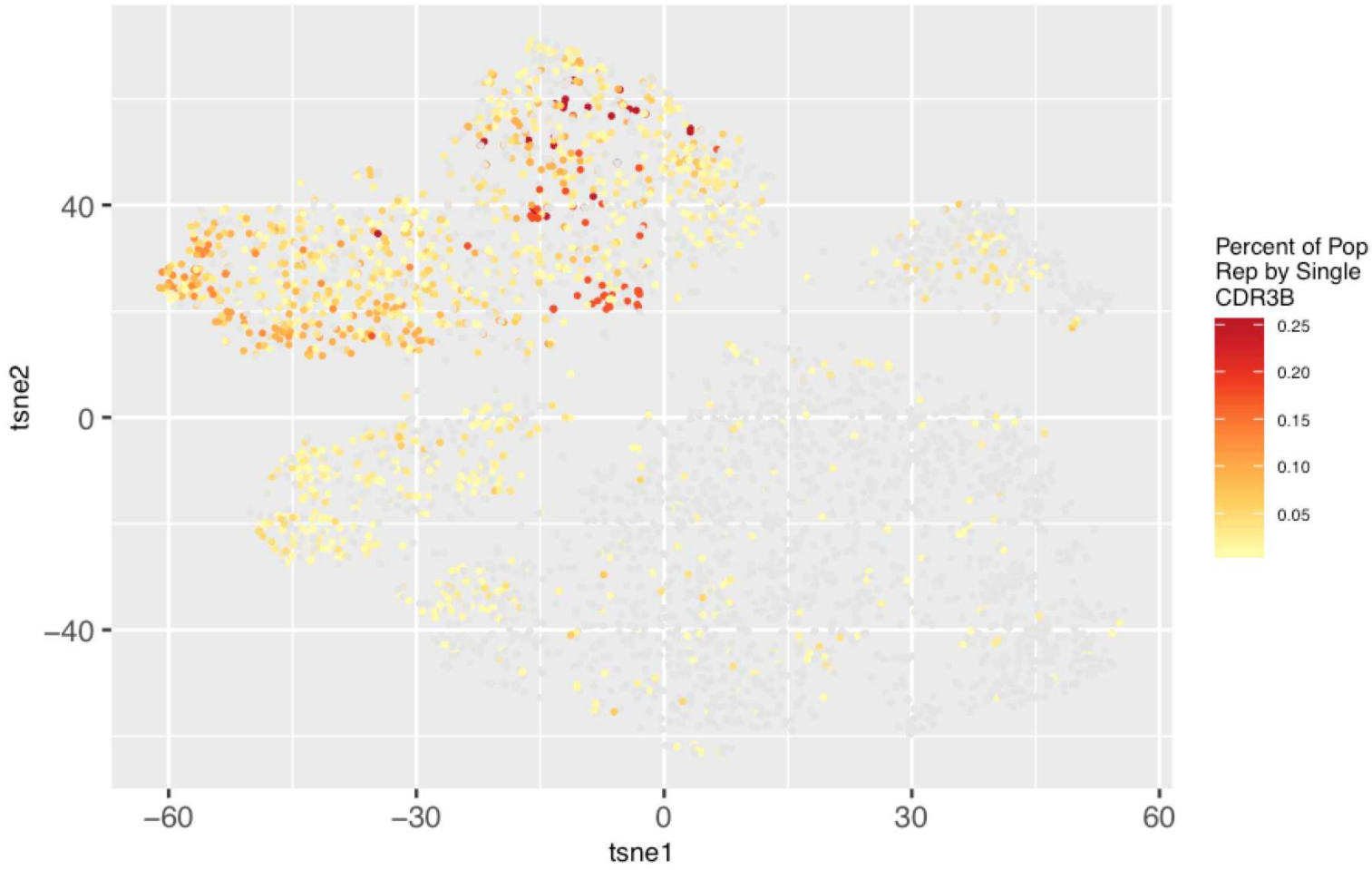
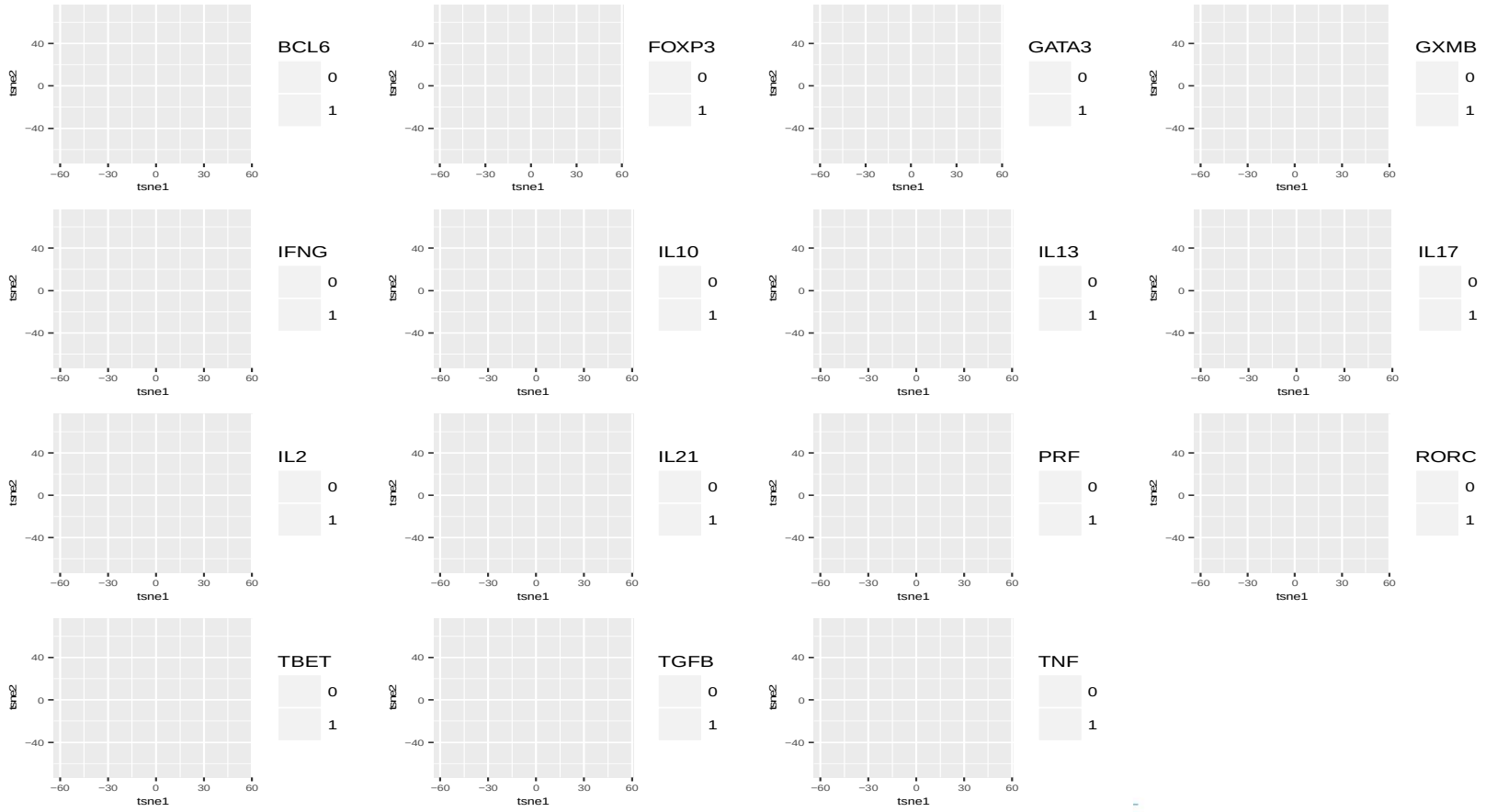
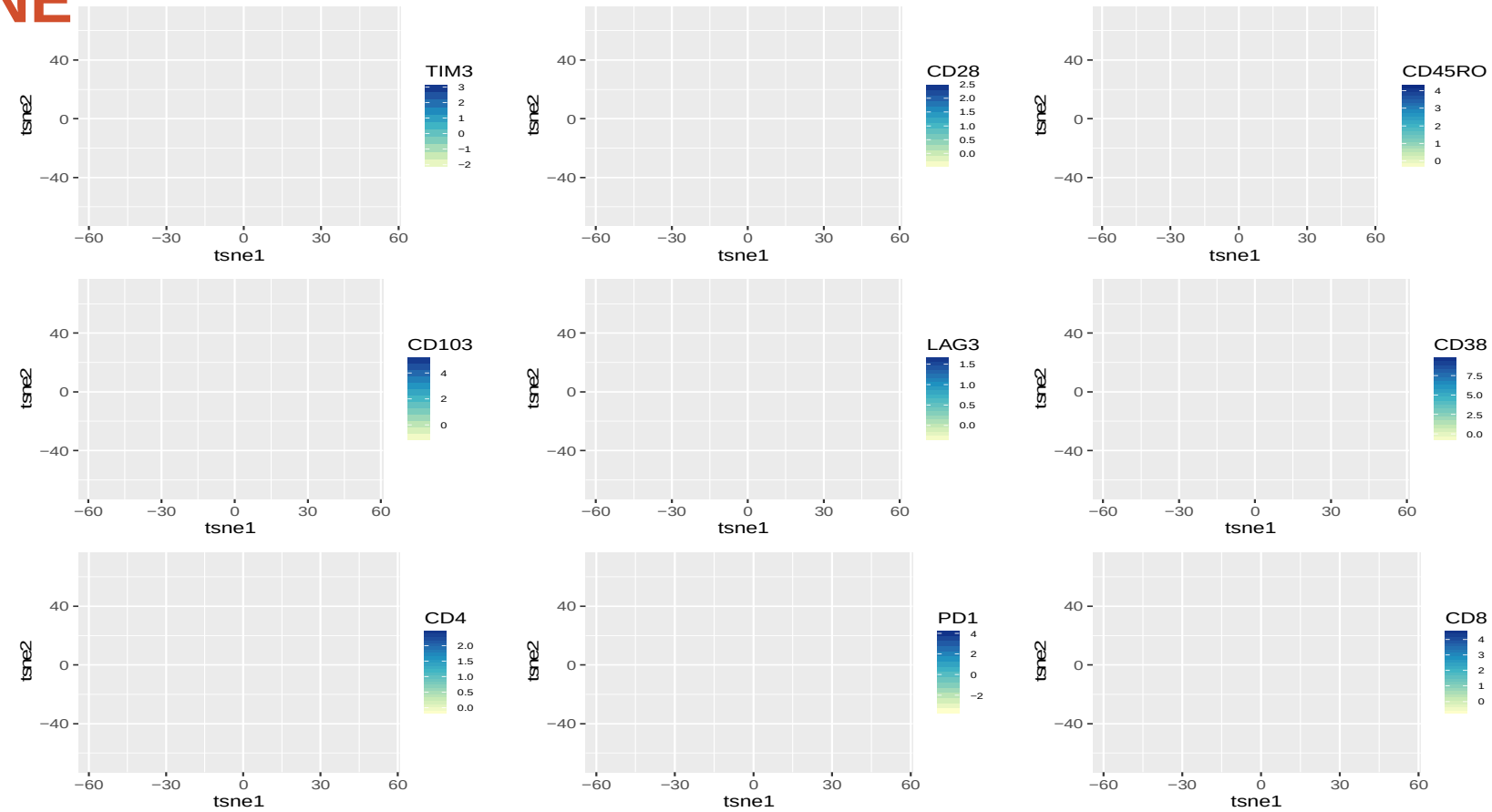


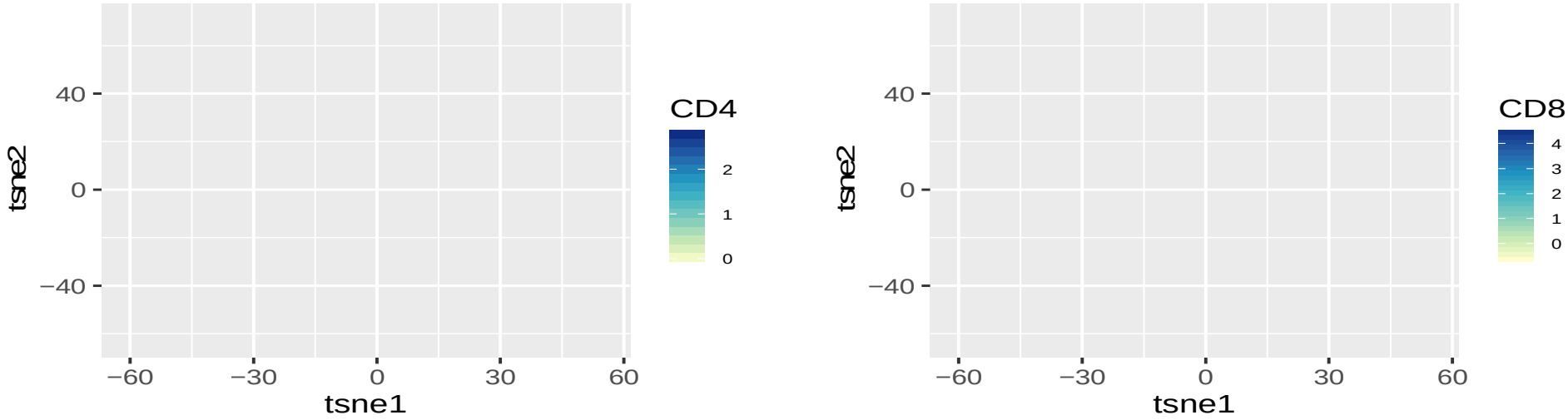
Visualizing high dimensional T cell data: T-SNE



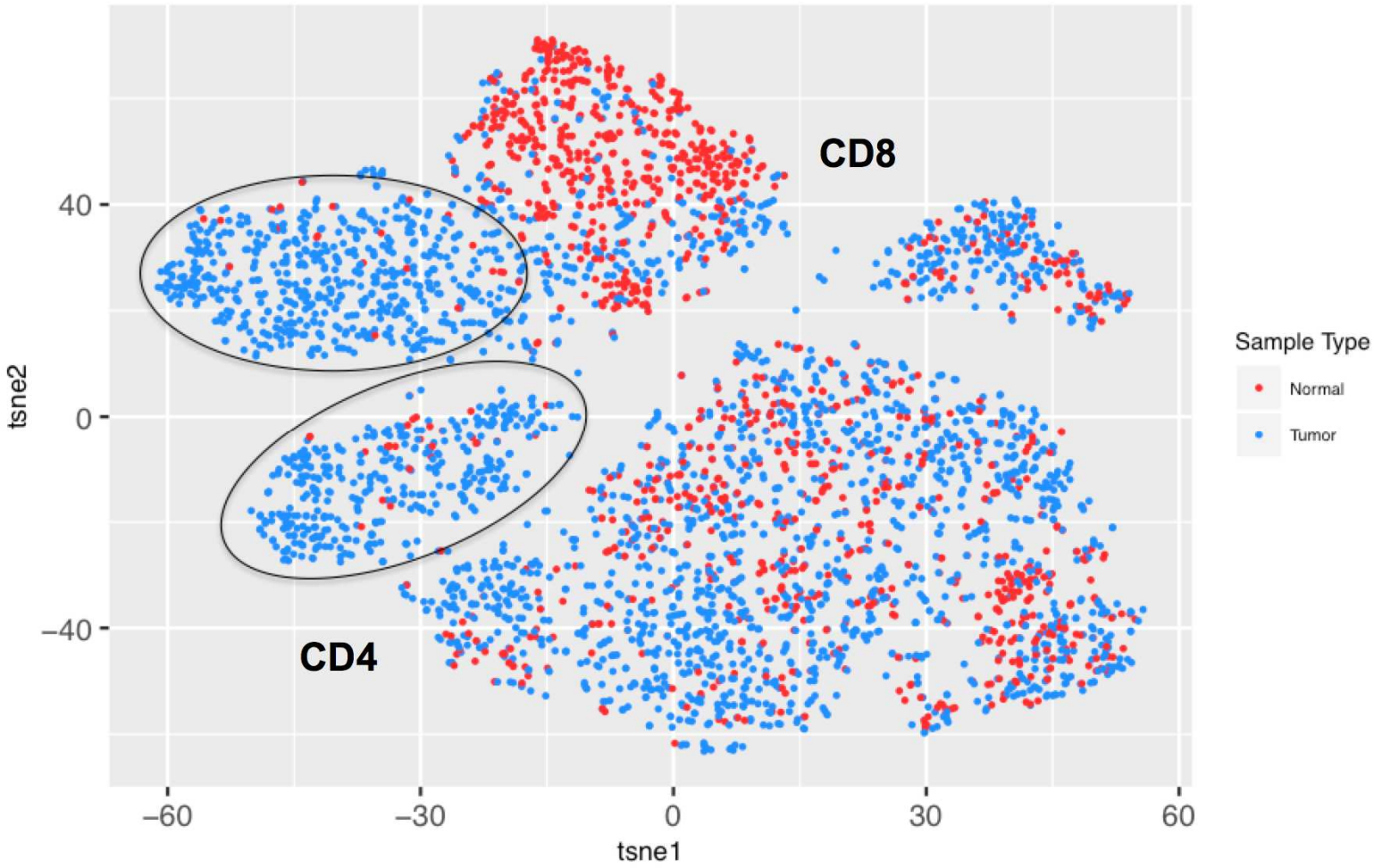
Visualizing high dimensional T cell data: T-SNE



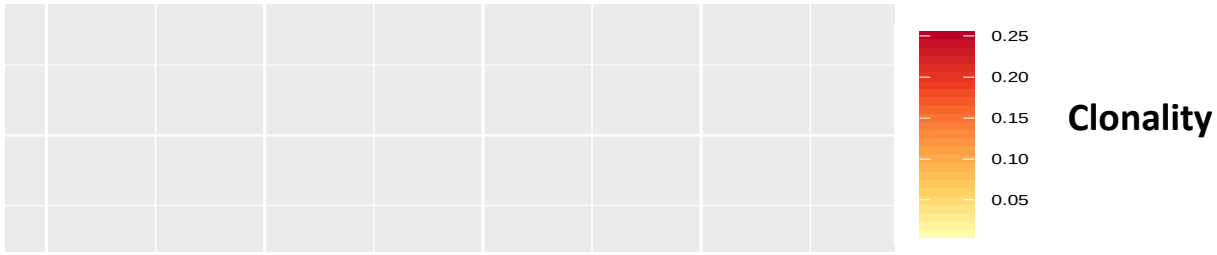
Visualizing high dimensional T cell data: T-SNE

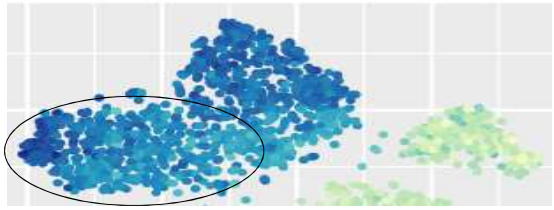


Phenotypes of TILs

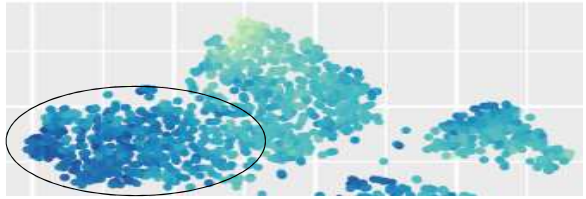


Distinguishing features of CD8 TILs

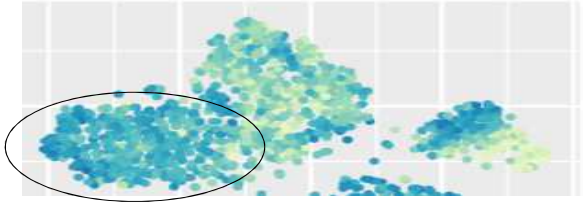




CD103

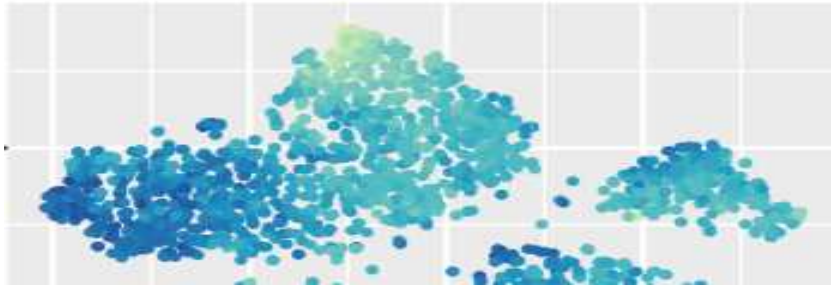


PD1

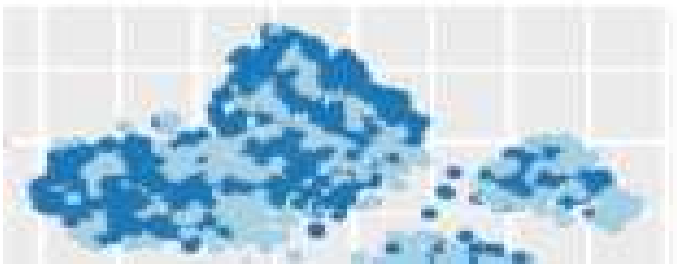


CD45RO

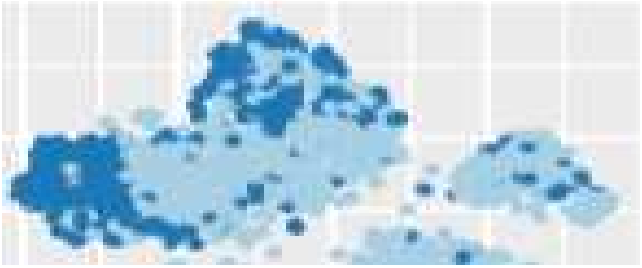
PD1 and exhaustion? : CD8



PD1



Perforin



Granzyme

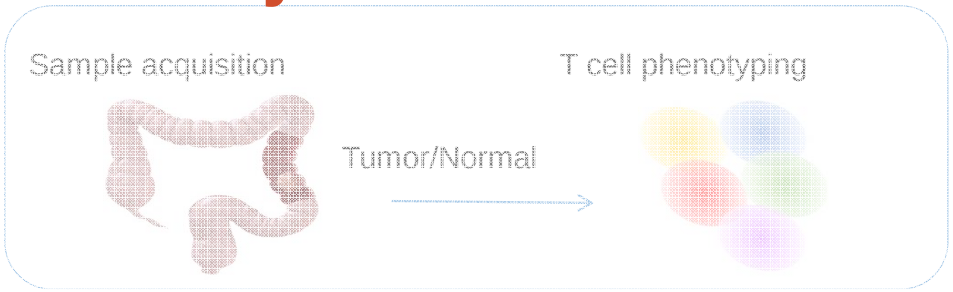
Ongoing: T cells in cancer
All cells/cancers are NOT equal

- Compare T cell profiles between tumors
  - correlate with disease stage, prognosis, and degree of mutation (MSS, MSI)
- Identify functionally significant cell types
  - Identify targets for therapy
  - Adoptive Transfer

Single-Cell RNAseq is coming

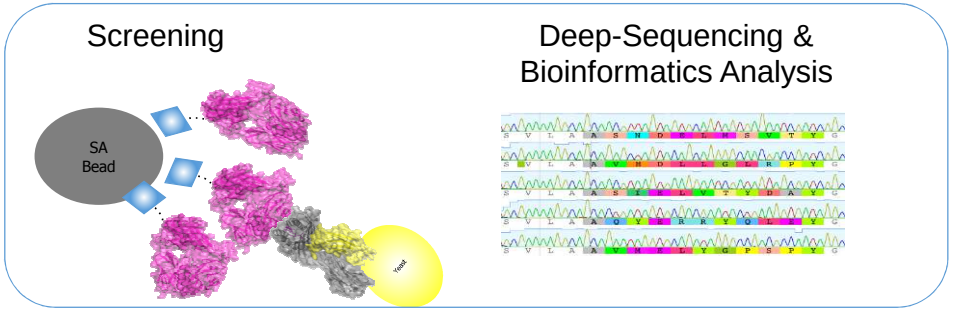
Systemic Analysis of Human CRC

1.



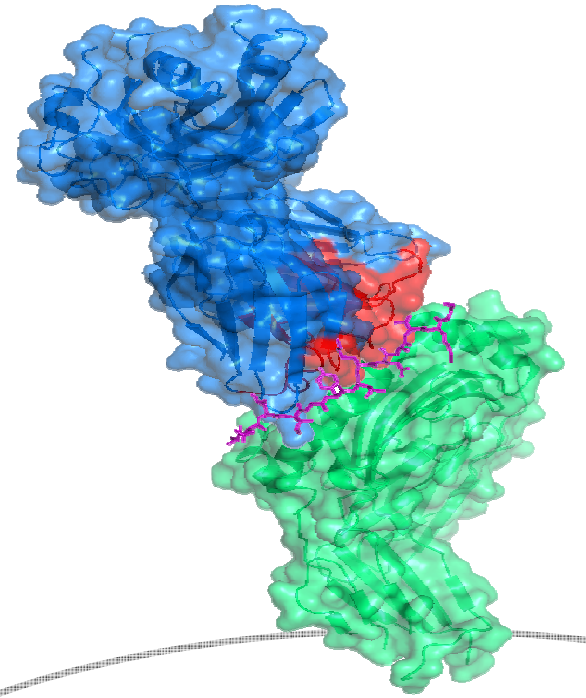
Understand phenotypes of tumor-specific T cell repertoire

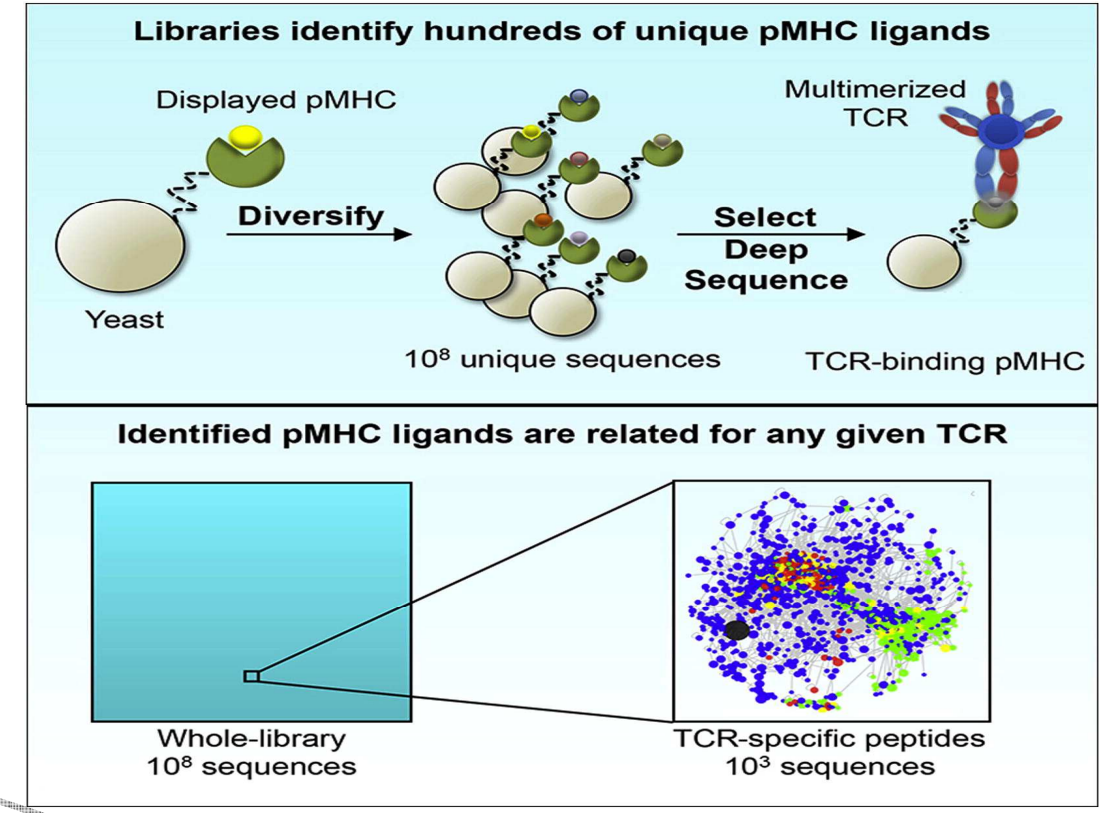
2.



Determine T cell antigen specificities

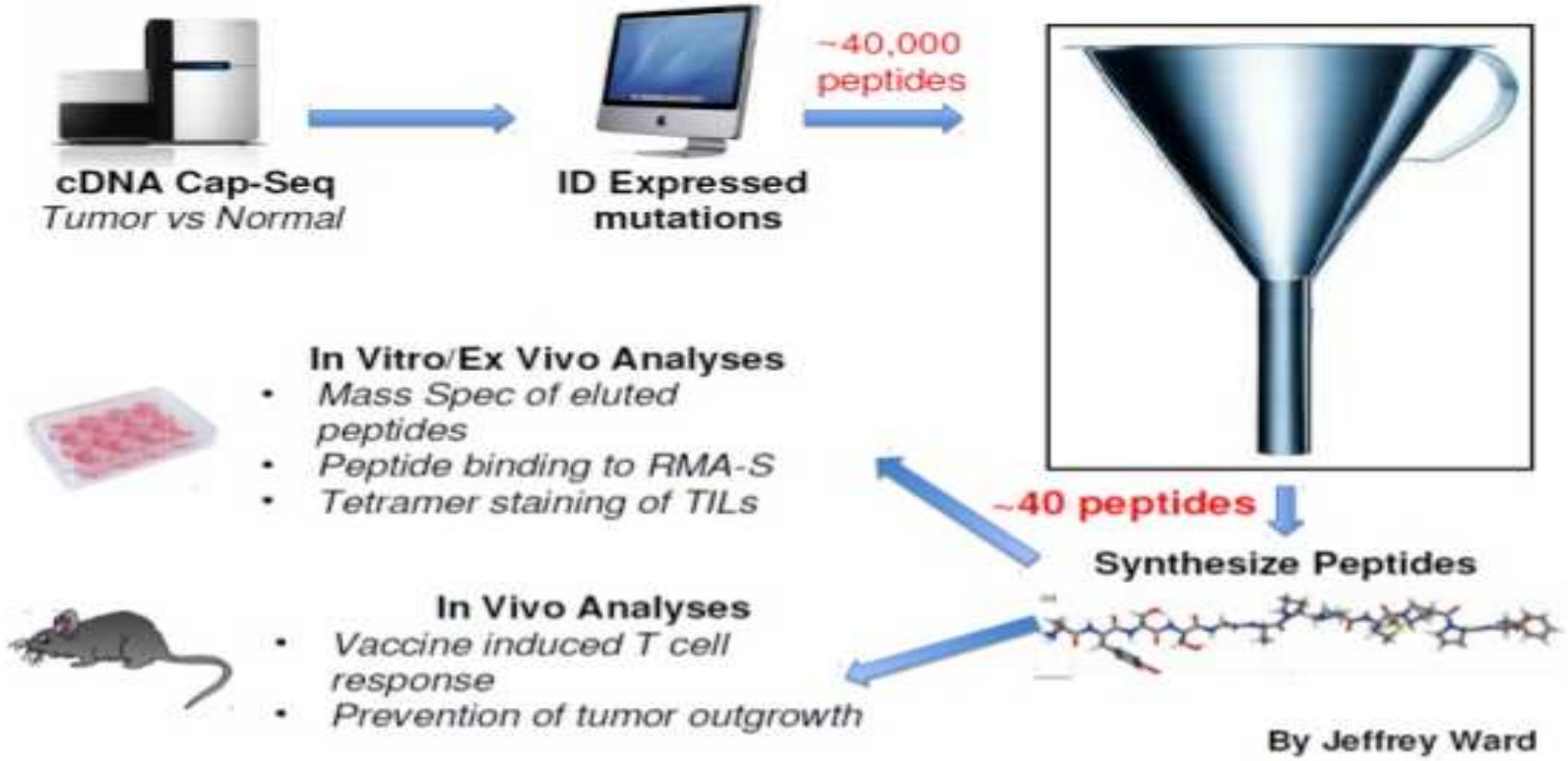
T cell antigen discovery





ME Birnbaum et al. Cell. 2014

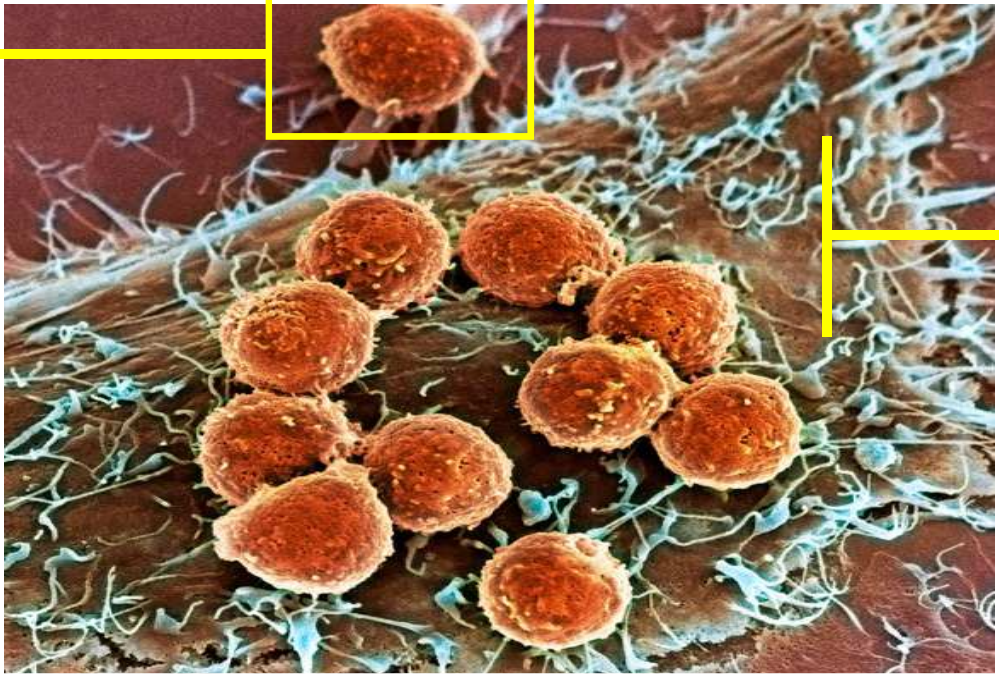
Neo-antigens: Personalized Tumor Vaccines



Nature Medicine, 2013

TCR specificity: Different approaches to the same question

Begin with T cell
Sequence TCR
Screen Random Libraries



Begin with tumor
Sequence tumor
Predict Neo-Epitopes
Test Epitopes

Pros:
Unbiased method
Let tumor tell us what antigens are relevant
Self-Antigens, CT antigens, Exogenous Antigens

Cons:
Peptide Length
MHC restriction
Undercoverage of peptide space
CANNOT DETERMINE A SINGLE ANTIGEN

TCR sequences isolated from single clones

Patient A (HLA A:02:01/02:01)

CDR3α	CDR3β	Pair Frequency
CAGGGGADGLTF	CASSLGLEQFF	22
CVVTETNAGKSTF	CASSADTGVNQPQHF	4
CALSEAEAAGNKLTF	CASSLGGGHTEAFF	3
CALSEAGMDSNYQLIW	CASSLVNGLGYTF	3
CAMREGRYSGAGSYQLTF	CATSRDRGQDEKLFF	3
CAVNSGNTGKLIF	CSARDYQGSQPQHF	1
CAVPFLYNQGGKLIF	CSARDYQGSQPQHF	1
CAVGEIVGTASKLTF	CASSYYIKFEQYF	1
CAVNDFNKFYF	CASSADTGVNQPQHF	1

Patient B (HLA A:02:01/02:06)

CDR3α	CDR3β	Pair Frequency
CALMNYGGATNKLIF	CASMGRSYGYTF	9
CAVETSNTGKLIF	CASSQGVGQFKNTQYF	4
CALSAGASGAGSYQLTF	CASSSSGGLVDTQYF	3
CAASSTGNQFYF	CASSLSGRQGGSYEQYF	2
CAVDSGGYNKLIF	CASSIPRGSSQPQHF	1

= $CD8^+$ $PD1^-$

= $CD8^+$ $PD1^+$

CALSEARGGATNKLIF	CASSRDTVNTEAFF	4	CALSEARGGATNKLIF	CASSRDFVSNEQYF	2

3 TCRs converge the HLA*A2:01 library

Patient A

5	TRBV7-9	CASSLVNGLGYTF	TRAV19	CALSEAGMDSNYQLIW		TCR 9
4	TRBV10-1	CASSRDTVNTEAFF	TRAV19	CALSEARGGATNKLIF		CD8-1s

Patient B

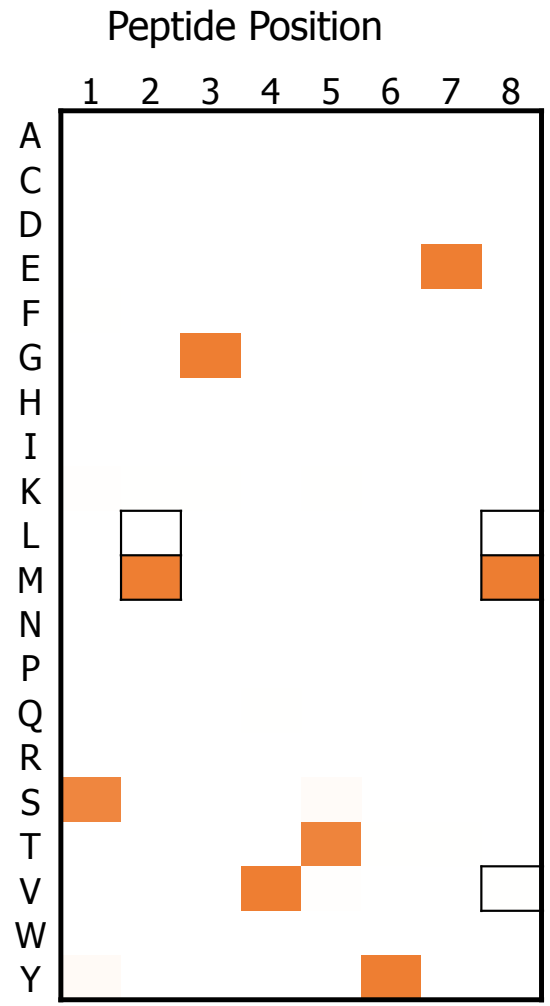
1	TRBV10-1	CASSRDFVSNEQYF	TRAV19	CALSEARGGATNKLIF	*	CD8-2s

Patient A	C	A	S	S	R	D	T	V	N	T	E	A	F	F
Patient B	C	A	S	S	R	D	F	V	S	N	E	Q	Y	F

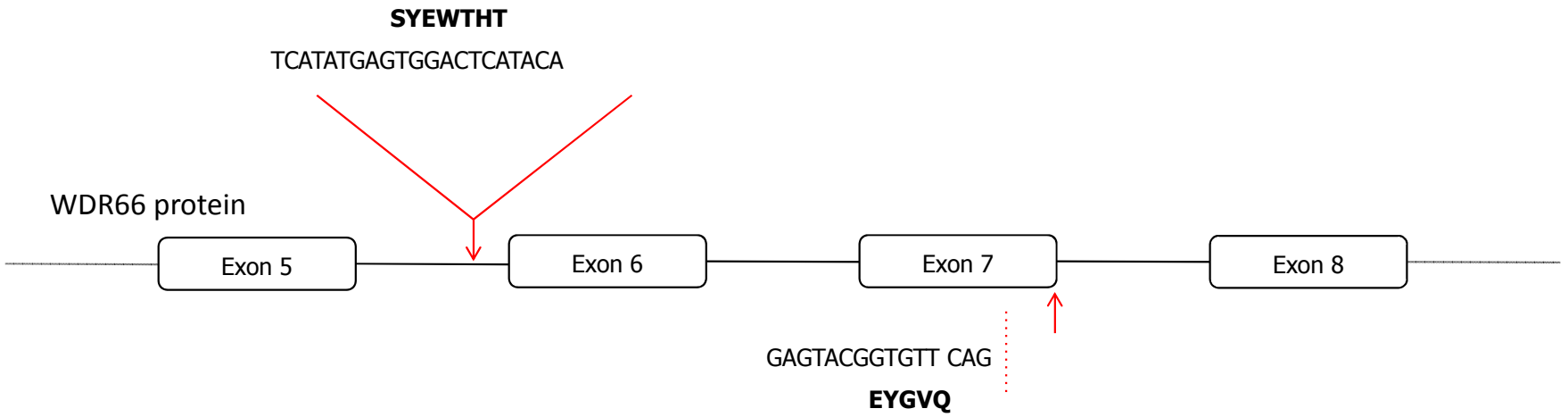
* = Shared with Normal Tissue

TCR 9

Peptide								Naïve	RD 1	RD 2	RD 3	RD 4
S	M	G	V	T	Y	E	M	0	3	6744	71690	141732
Y	M	G	V	S	Y	E	M	0	0	55	2430	1781
Y	M	G	V	V	Y	E	M	0	1	158	1070	142
K	M	G	V	T	Y	E	M	0	0	9	511	183
K	K	K	Q	K	T	T	V	0	1	100	430	98
F	M	G	V	T	Y	E	M	0	0	18	275	181
F	M	G	V	S	Y	E	M	0	0	5	165	60
G	L	G	V	S	Y	E	M	0	0	4	152	62
N	L	G	V	S	Y	E	M	0	0	4	93	13
T	L	G	V	T	Y	E	M	0	0	3	74	0
K	M	G	V	L	Y	E	M	0	0	3	61	88
Q	L	R	R	C	V	I	L	0	3	225	60	89
L	K	L	D	Y	G	Q	M	0	2	160	34	43
F	M	G	V	T	Y	E	V	0	0	0	19	176
S	M	G	V	T	F	E	V	0	0	8	14	0



Exome sequencing results reveal putative epitope for TCR 9



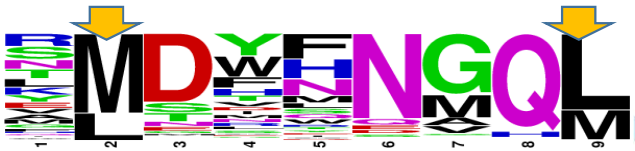
WT: EYGV.....QNYVTF
Mut: EYGVSYEWTHTQNYVTF

Putative Epitope: EYGVSYEW

TCRs isolated from Patient A and Patient B share peptide motif

CD8-1s TCR

Peptide	Naïve	RD 1	RD 2	RD 3	RD 4
L M D M H N G Q L	0	16	10775	20608	39700
R L D A M N G Q L	0	7	6484	11464	807
R M D Y N N M Q M	0	3	3656	8193	5229
S M D T F Q G Q M	0	7	4053	6926	1074
G M D Y H N G H L	0	3	3518	6103	217
Y L D F H N G Q L	0	7	3341	6022	19091
L M D Y T N M Q L	0	5	2536	4742	186
N L D W A N V Q L	0	4	2359	4702	150
M M D L H N G Q L	0	3	2271	4439	21190
K M D Y H E G Q L	0	1	2256	4434	410
T L D G F N G Q M	0	1	2177	3982	359
V M S H F E G Q L	0	1	2388	3830	376
A M D Y L N A Q L	0	4	1911	3440	215
Q L D W N N M Q M	0	8	1726	3433	102
R M G Y H N G Q L	0	2	2010	3261	367

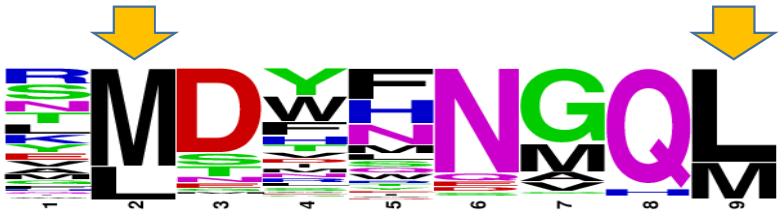


CD8-2s TCR

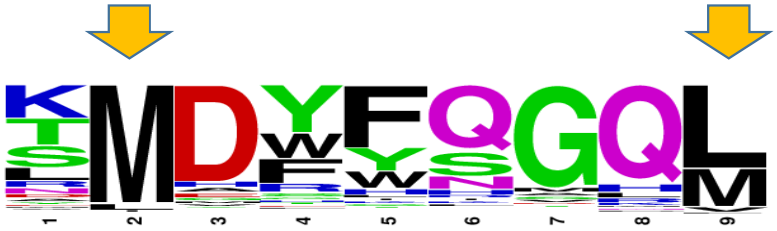
Peptide	Naïve	RD 1	RD 2	RD 3	RD 4
T M D F Y Q G Q L	0	0	2185	105899	170631
K M D Y F S G Q L	0	0	2214	83644	22803
S M D W F Q G Q M	0	0	894	50234	137230
L M D Y W Q G Q L	0	0	1104	31733	14304
N M M W F Q G Q L	0	0	352	8382	1456
K M H W F N G Q L	0	0	397	7366	451
T M D Y W Q G H L	0	0	332	6050	309
R M D R F N G Q L	0	0	591	5962	199
S M D T F Q G Q M	0	0	604	5601	197
V M S H F E G Q L	0	0	376	4065	83
L M D Y T N M Q L	0	0	322	2750	68
K M D Y H I G Q M	0	0	226	2403	83
V M D H F Q A Q L	0	0	170	1975	69
N M G F E N M Q L	0	0	132	1144	22
Y L D H K T L R L	0	15	866	881	285

CD8-1s and CD8-2s share a common prediction: wild-type MED23

CD8-1s TCR



CD8-2s TCR



MED23 = Mediator of RNA polymerase II transcription subunit 23

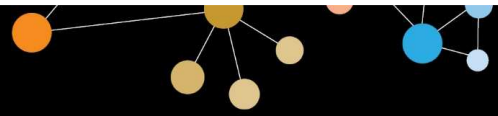
TLHYYEMHL

Role in Ras-active lung cancer
Yang X et al. PNAS. (2012)

Role in tumorigenesis for hepatocellular carcinoma.
Guo Y et al. J Gastroenterol Hepatol. (2015)

Role in esophageal squamous cell carcinoma.
Shi J et al. Mol Carcinogenesis. (2014)

Putative role in colorectal cancer
Jo YS et al. Pathol. Oncol. Res. (2015)



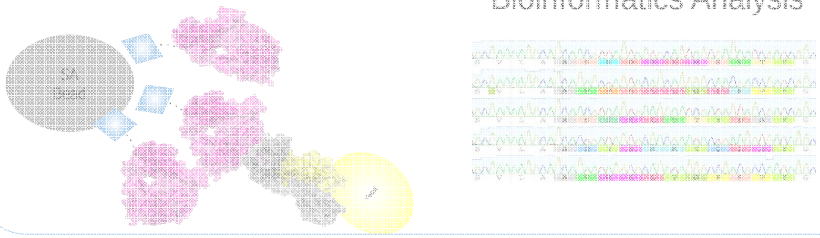
Future Directions:
Antigens driving T cells in colorectal cancer

- Link antigen specificities to T cell phenotypic profiles
- Can we find common TCR motifs or specificities across different patients?
- Systematic identification of tumor antigens in mouse models

1. Sample acquisition → Tumor/Normal → T cell phenotyping

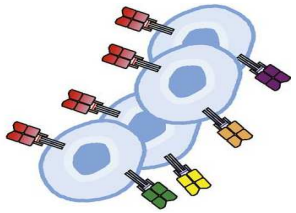
Understand phenotypes of tumor-specific T cell repertoire

2. Screening → Deep-Sequencing & Bioinformatics Analysis



Determine T cell antigen specificities

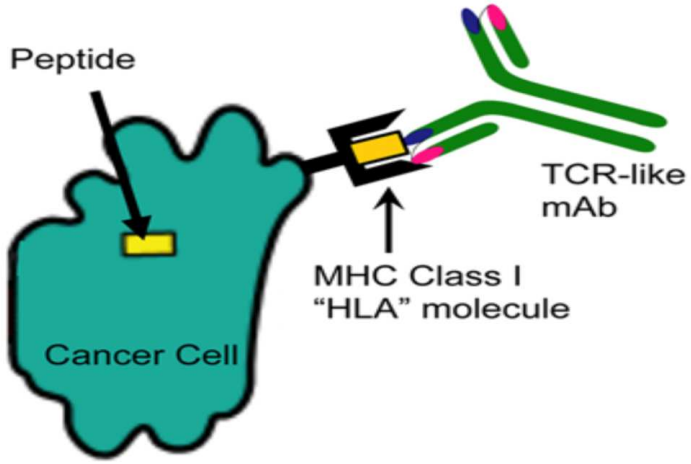
3. TCR-based therapies



Investigate therapeutic potential

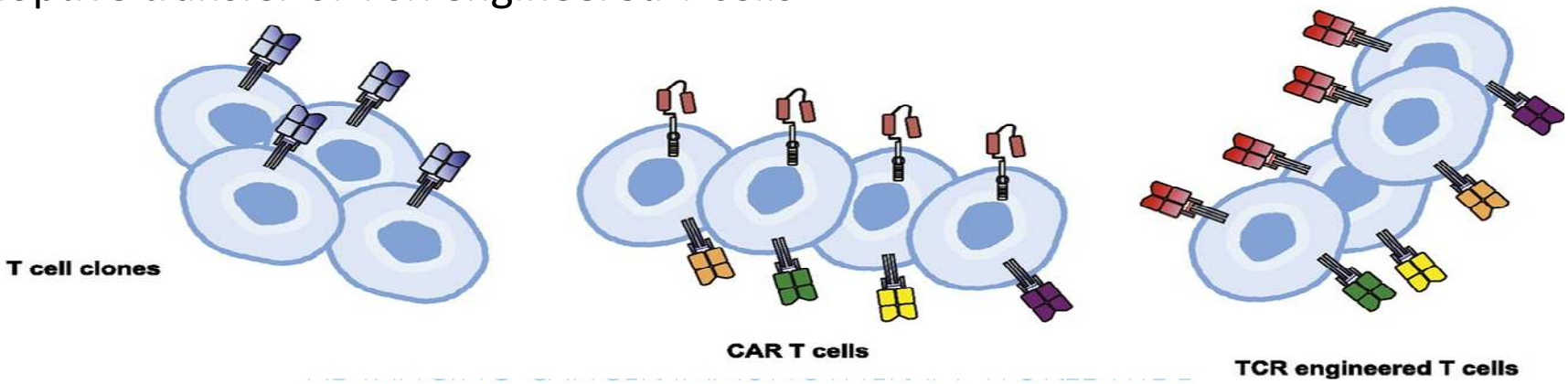
Future Directions: Therapeutic Implications

1. TCR mimic antibodies



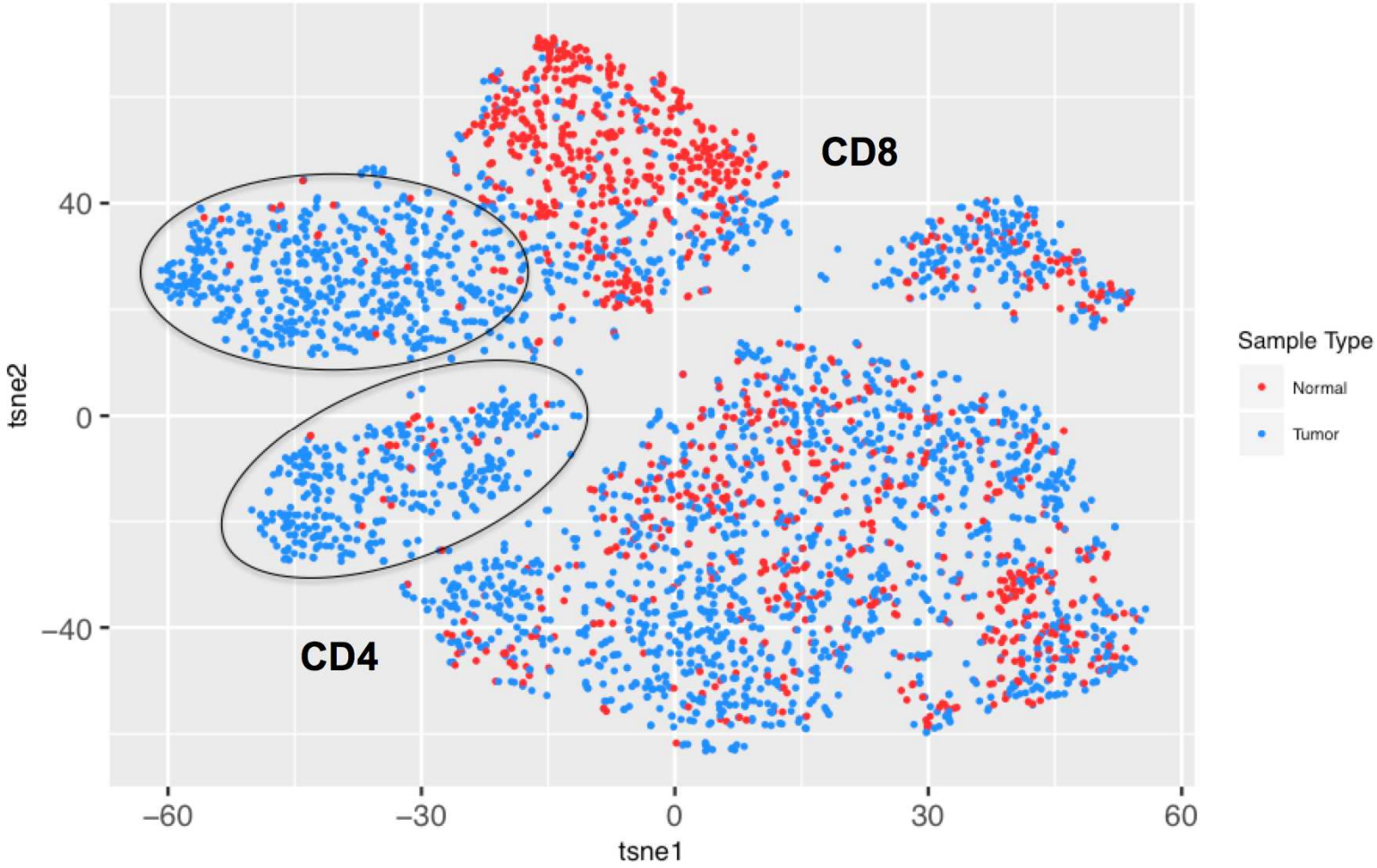
Scheinberg, 2013

2. Adoptive transfer of TCR engineered T cells



Maus, 2014

Therapeutically Relevant TIL TCRs



Davis Lab
Mark Davis
Trevor Hinshaw
Leo Hansmann
Jake Glanville

Garcia Lab
K. Christopher Garcia
Marvin Gee
Michael Birnbaum
Suzanne Fisher
Juan Mendoza

Quake Lab
Stephen Quake
John Beausang

Khatri Lab
Purvesh Khatri
Shane Lofgren