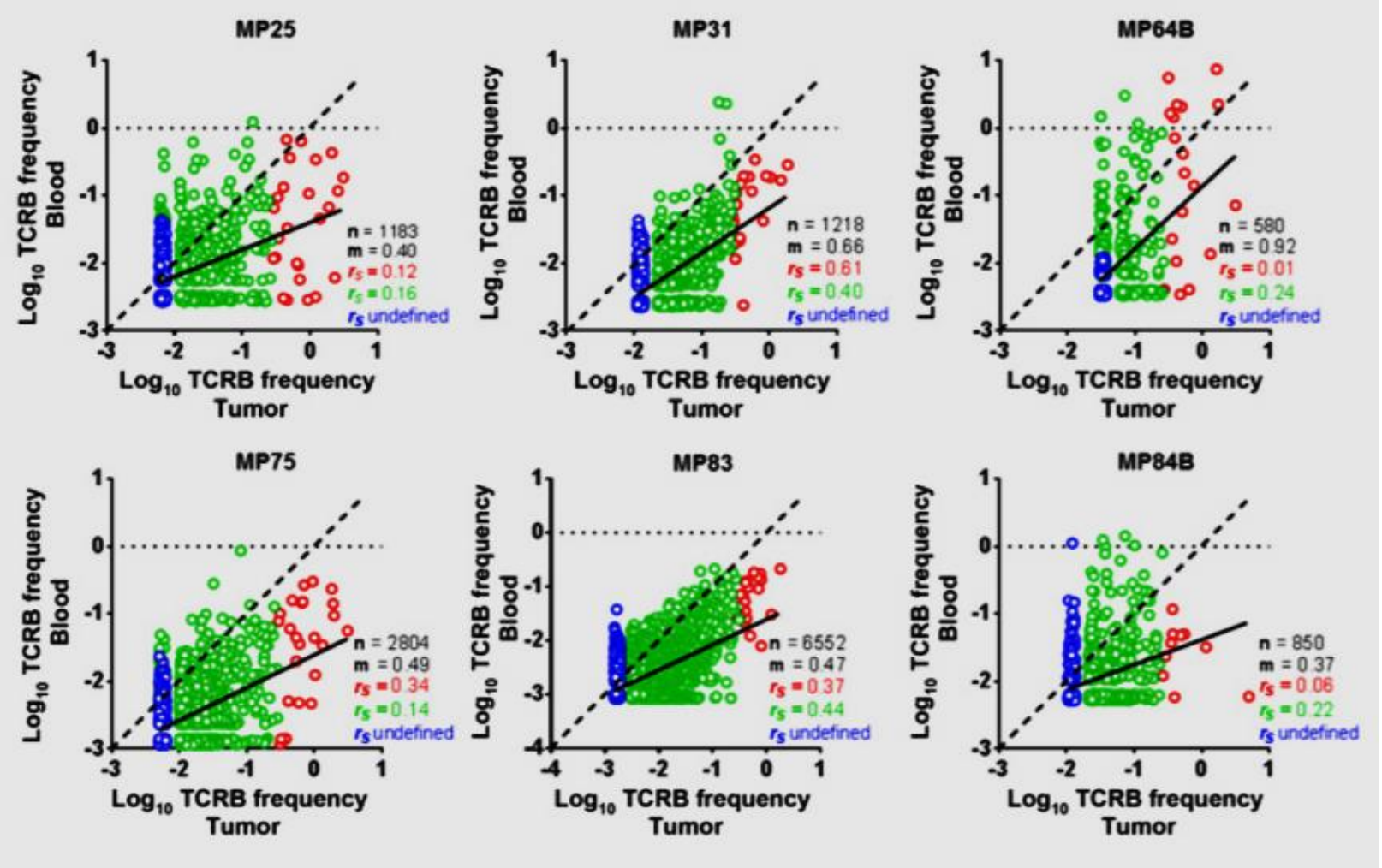
Deep TCR and BCR Sequencing as a Biomarker for Cancer



Bernatchez, Chantale Pancreatic Cancer

Clin Cancer Res; 23(23) December 1, 2017

3:15-4:15pm Small Group/Hands on Session

Michael T. Lotze, MD—
University of Pittsburgh

- The Adaptome
- TCR and BCR DAM-PCR, NGS
- Profiling and quantitating the B and T cell clonotypes

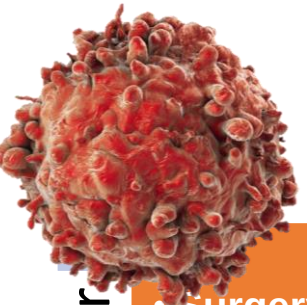
Disclosures-Consultant

- **Prometheus**
- **Celgene Cellular Therapeutics**
- **NeuMedicine**
- **Chairman of the Advisory Board, Immunocellular Therapeutics, Ltd.**
- **Intezyne**
- **VeraStem**
- **Checkmate, Inc.**
- **Pieris, Inc.**
- **Lion/Iovance CSO**
- **iRepertoire, Inc. (Hudson Alpha Institute)**
- **Torque, Inc.**
- **Adicet, Inc.**

Five Talks

Why Women Live Longer Than Men

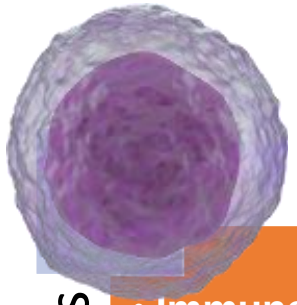
Foundations of Cancer Therapy (WuXing Again)



Tumor

- Surgery
- Chemotherapy
- Radiation

- Other Targets:
- Signal Transduction
- Autophagy
- Oncogenes
- Tumor Suppressor Genes

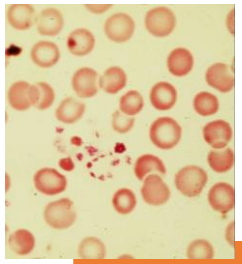


T Cells

- Immune Stimulants
- Checkpoint Inhibition
- Adoptive Cell Therapy (CARs, TIL)
- DC Vaccines
- Oncolytic Viruses

Endothelium

- Anti-VEGF
- Chloroquine
- Platelet Derived Growth Factor (PDGF)
- Fibroblast Growth Factor (FGF)
- TKI's (Sorafenib, Sunitinib, Axitinib, Pazopanib)

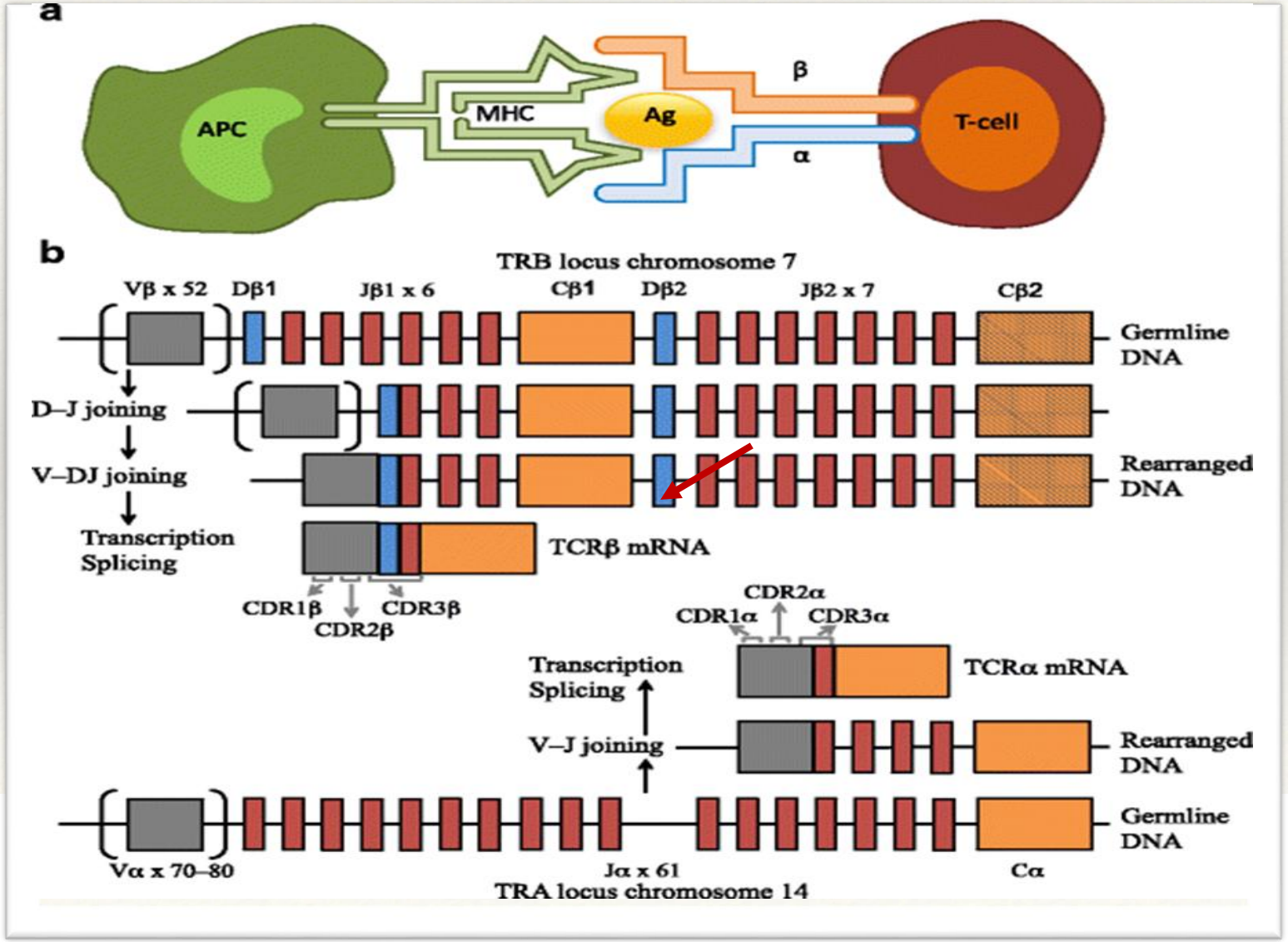


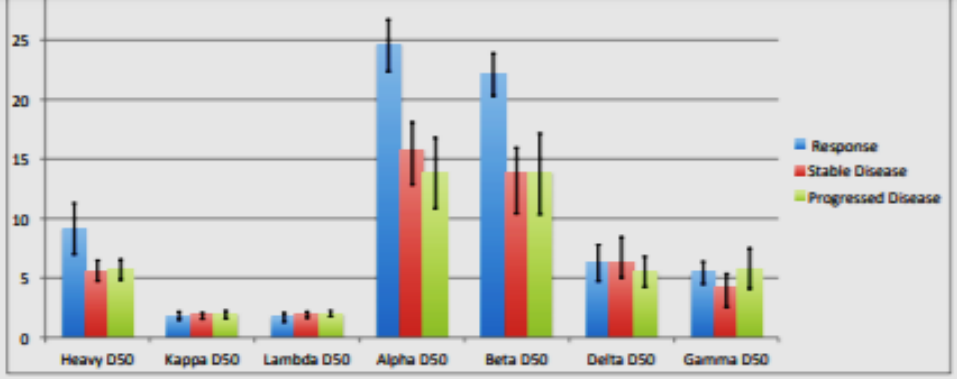
Platelets and RBC

- Erythropoietin
- Thrombopoietin
- Interleukin 11
- Red Cell Infusions

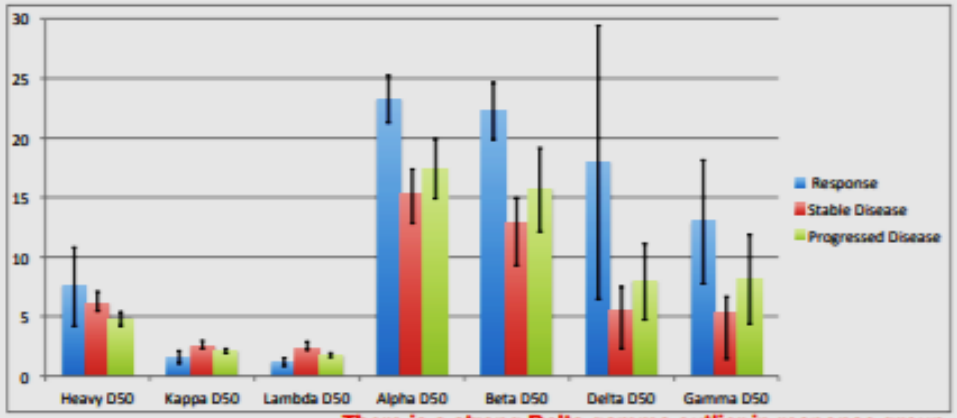
- Platelet Derived Growth Factor (PDGF)

TCR/BCR Diversity Enabling Recognition of Antigen Is Generated by V(D)JC Recombination



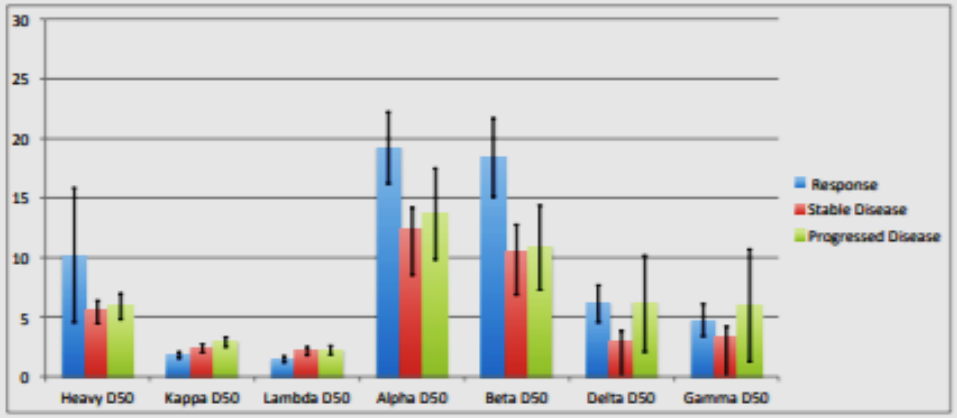


Time-Point-2



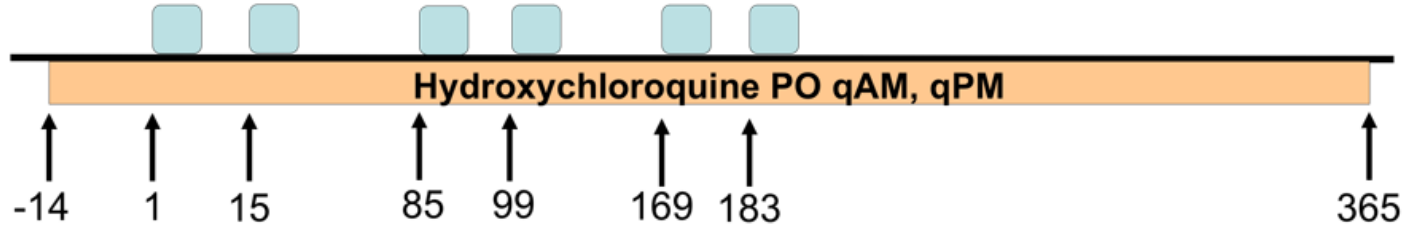
There is a strong Delta gamma outlier in response group

Time-Point-3

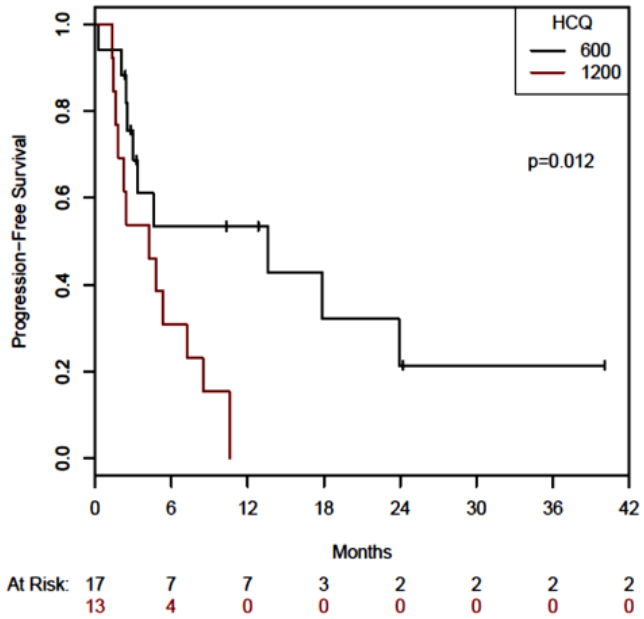


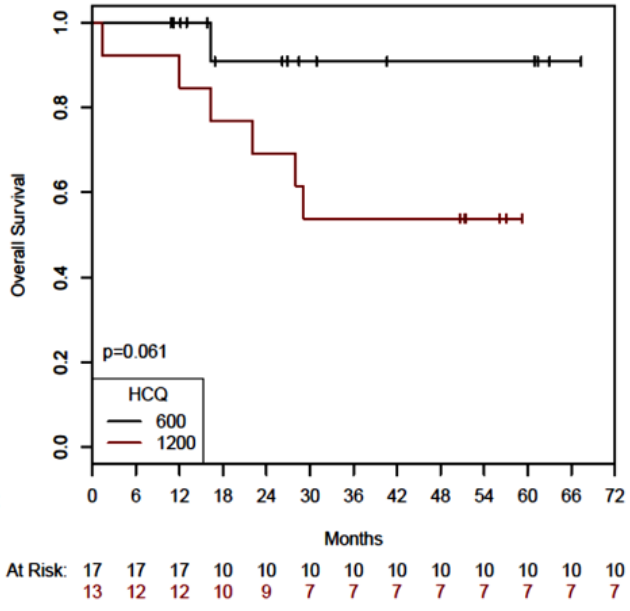
STUDY DESIGN

Aldesleukin
600,000 IU/kg IV q8hrs

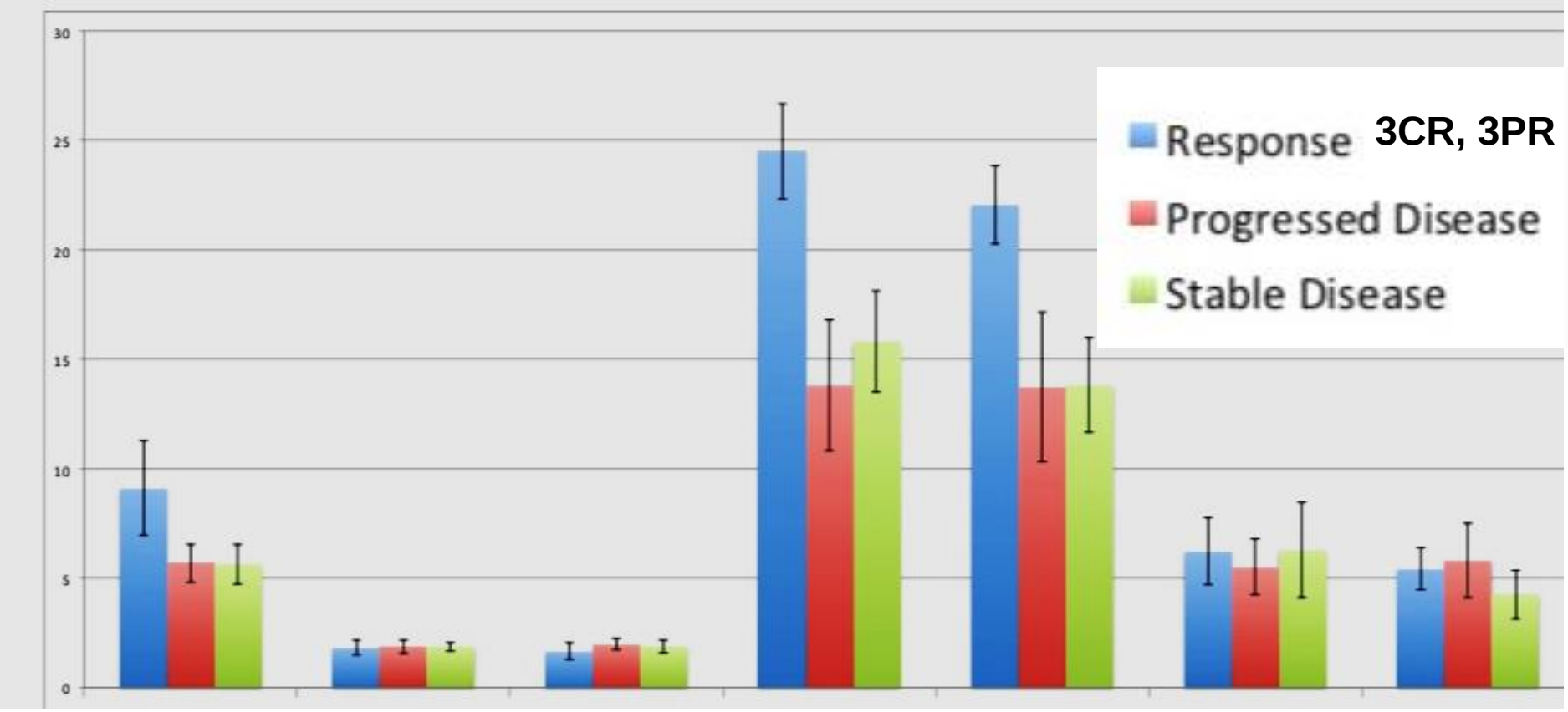


PFS AND OVERALL SURVIVAL





D50 in 29 Advanced Renal Cancer Patients Treated with High Dose Interleukin 2 (IL-2) and Hydroxychloroquine Associated with Clinical Response

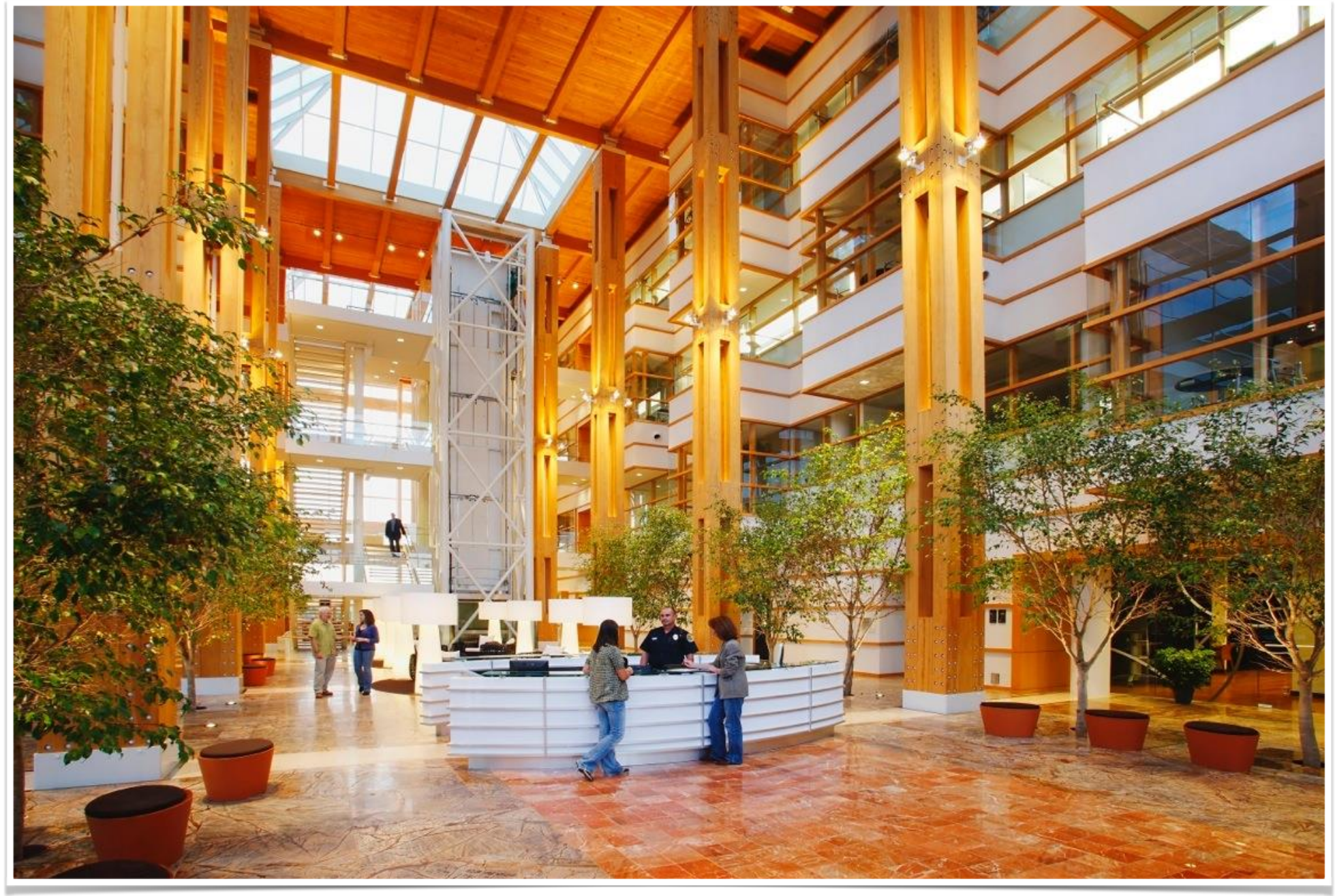


IgH κ λ α β δ γ

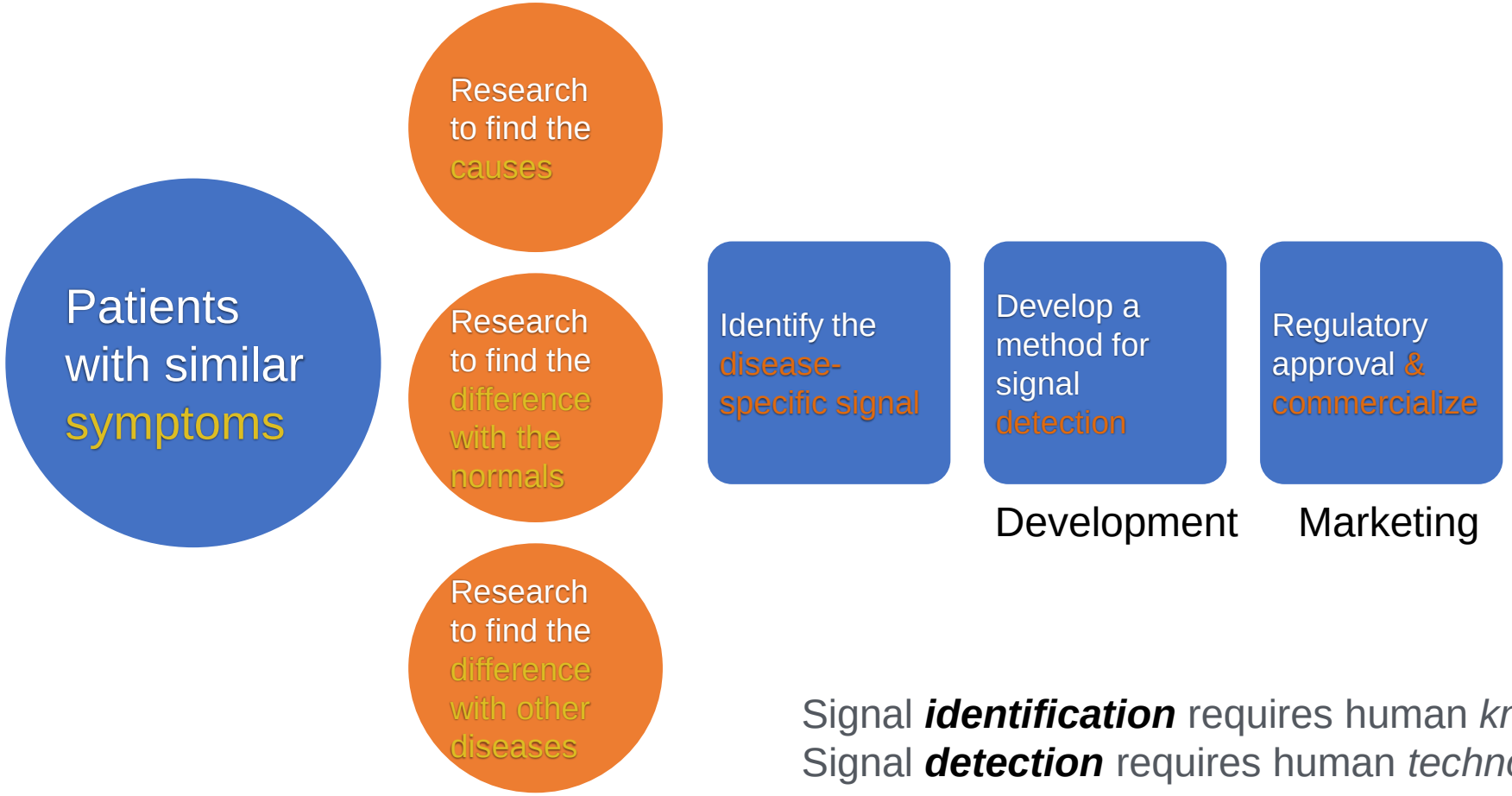
Evaluating Cancer Immunity: Robust NextGen Sequencing of Immune Repertoires in Blood, Tumor, and Single Cells

Jian Han, MD., Ph.D.

Faculty Investigator,
HudsonAlpha Institute for Biotechnology
Director, The R10K Project
CEO, iRepertoire

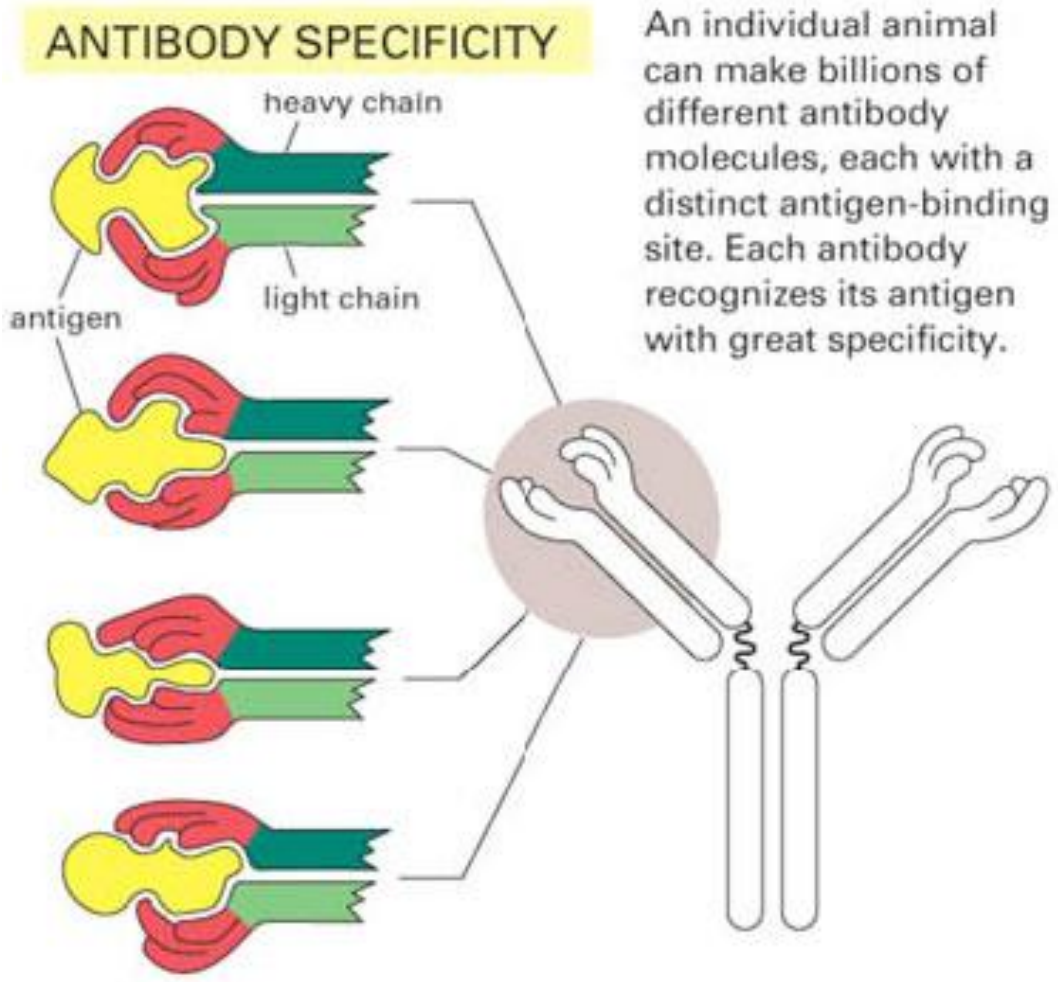


Re-thinking diagnostics



Signal ***identification*** requires human *knowledge*;
Signal ***detection*** requires human *technology*.

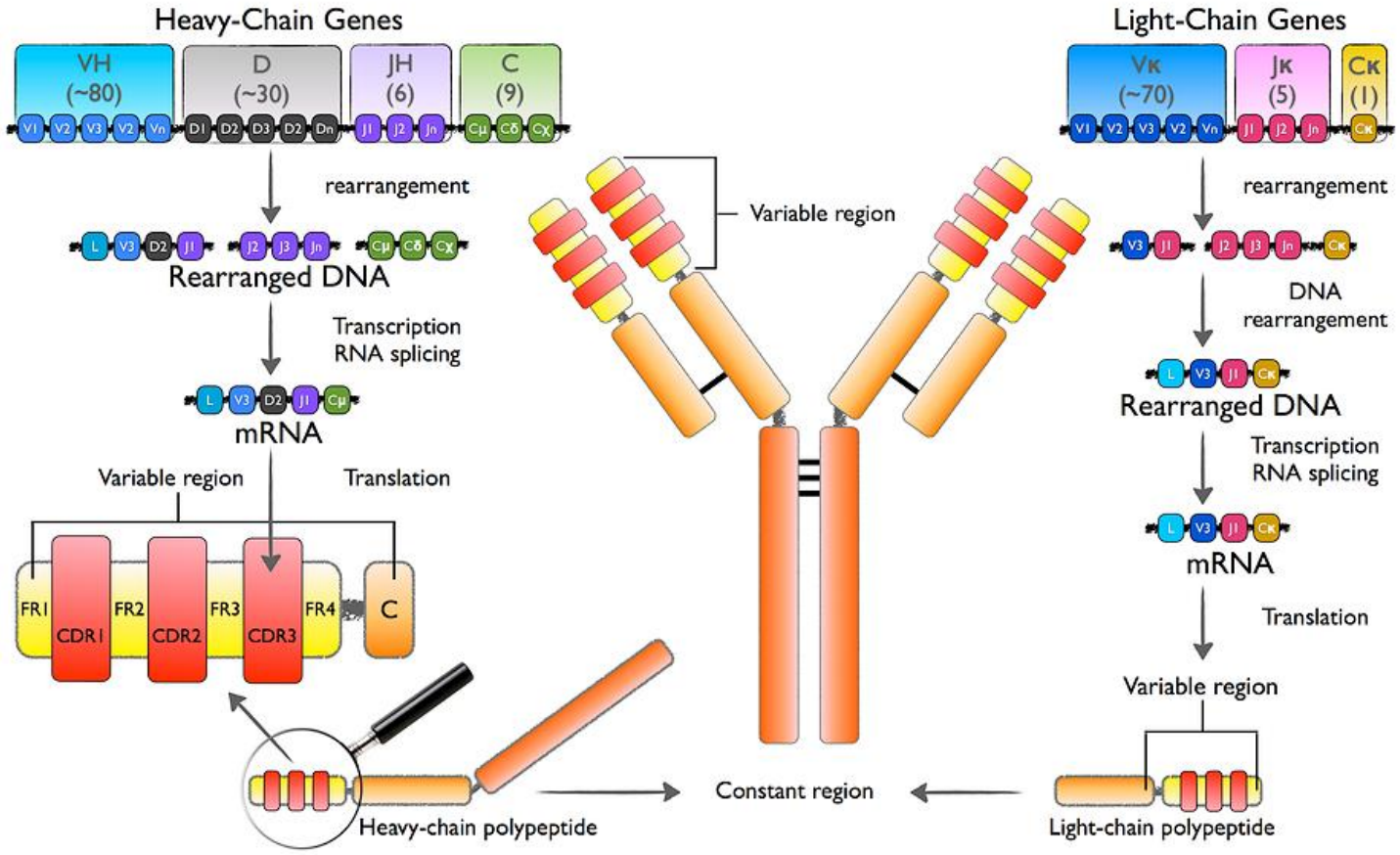
Immune receptors are disease sensors



They are very specific, and sensitive and it is non-invasive.

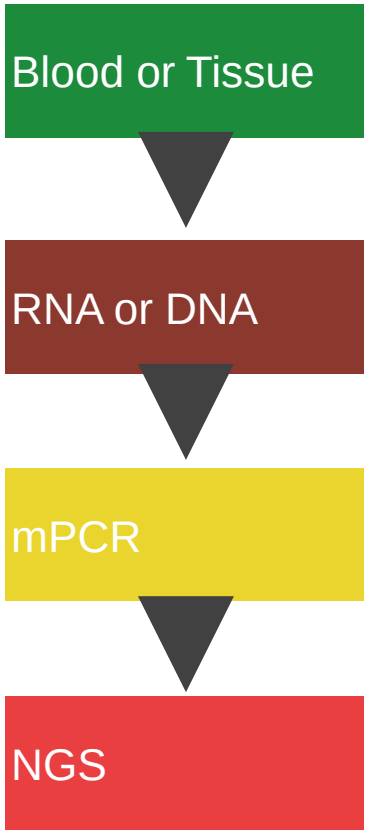
The immune system is the best doctor

Repertoire Diversity



Human: 10^{15} TCRs!!! Each cell has its own barcode.

Methods



PNAS

High throughput sequencing reveals a complex pattern of dynamic interrelationships among human T cell subsets

Chunlin Wang[a], Catherine M. Sanders[b], Qunying Yang[b], Harry W. Schroeder, Jr.[c], Elijah Wang[b], Farbod Babrzadeh[a], Baback Gharizadeh[a], Richard M. Myers[b], James R. Hudson, Jr.[b], Ronald W. Davis[a,1], and Jian Han[b,1]

[a]Stanford Genome Technology Center, Palo Alto, CA 94304; [b]HudsonAlpha Institute for Biotechnology, Huntsville, AL 35806; and [c]Departments of Medicine and Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294

Contributed by Ronald W. Davis, December 8, 2009 (sent for review October 9, 2009)

Developing T cells face a series of cell fate choices in the thymus and in the periphery. The role of the individual T cell receptor (TCR) in determining decisions of cell fate remains unresolved. The stochastic/selection model postulates that the initial fate of the cell is independent of TCR specificity, with survival dependent on additional TCR/coreceptor "rescue" signals. The "instructive" model holds that cell fate is initiated by the interaction of the TCR with a cognate peptide-MHC complex. T cells are then segregated on the basis of TCR specificity with the aid of critical coreceptors and signal modulators [Chan S, Correia-Neves M, Benoist C, Mathis (1998) *Immunol Rev* 165: 195–207]. The former would predict a random representation of individual TCR across divergent T cell lineages whereas the latter would predict minimal overlap between divergent T cell subsets. To address this issue, we have used high-throughput sequencing to evaluate the TCR distribution among key T cell developmental and effector subsets from a single donor. We found numerous examples of individual subsets sharing identical TCR sequence, supporting a model of a stochastic process of cell fate determination coupled with dynamic patterns of clonal expansion of T cells bearing the same TCR sequence among both CD4+ and CD8+ populations.

CDR3 | clonal expansion | immune repertoire | T cell receptor

the V(D)J combination, form the center of the antigen binding site where they often play a critical role in defining the affinity and specificity of the receptor for individual peptide-MHC complexes (5) of both the TCRα and TCRβ chains. Our goal was to produce comprehensive, unrestricted profiles of TCR diversity for key subsets of T cells isolated from the blood of a healthy individual at sequence-level resolution.

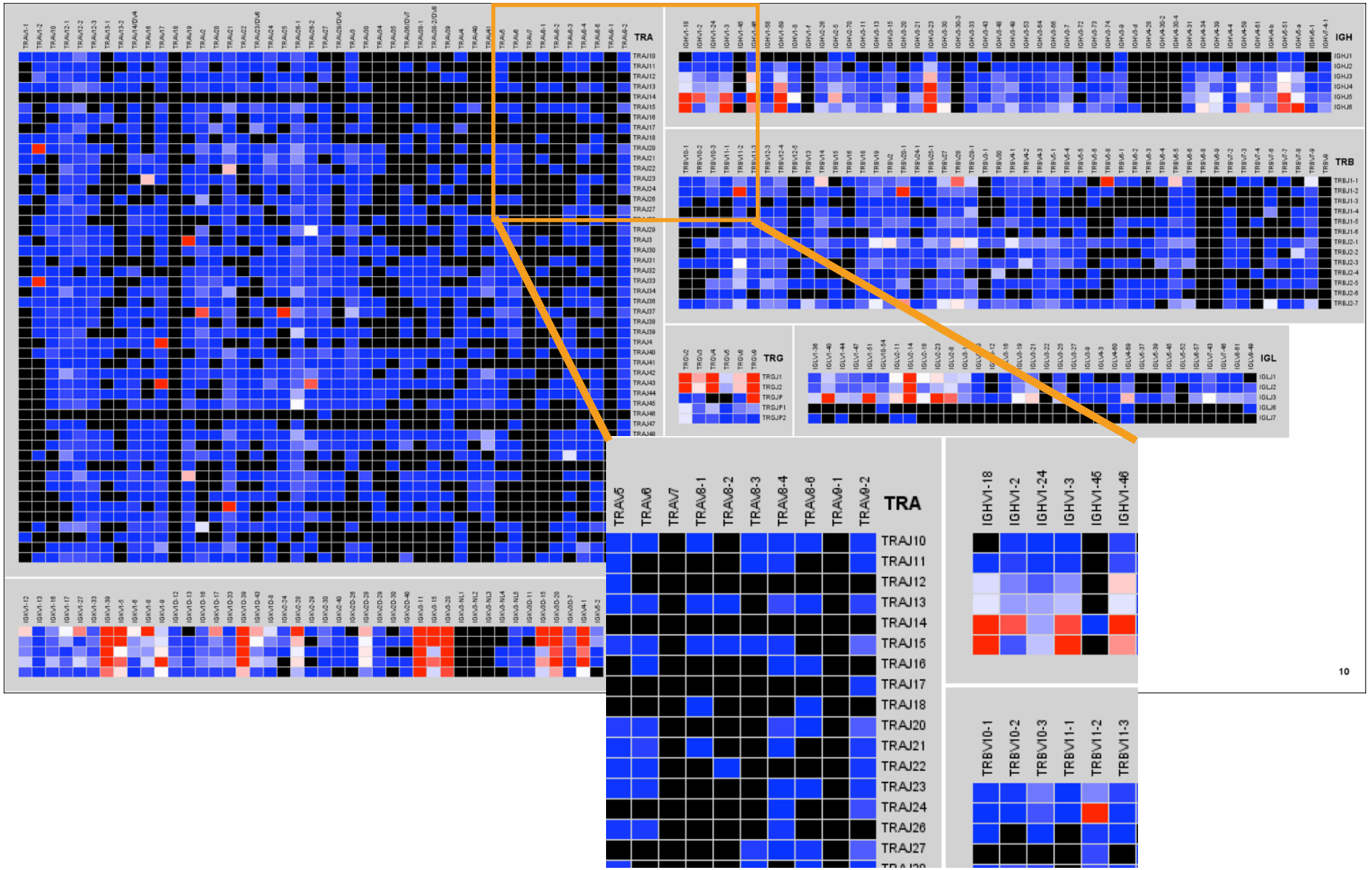
Results

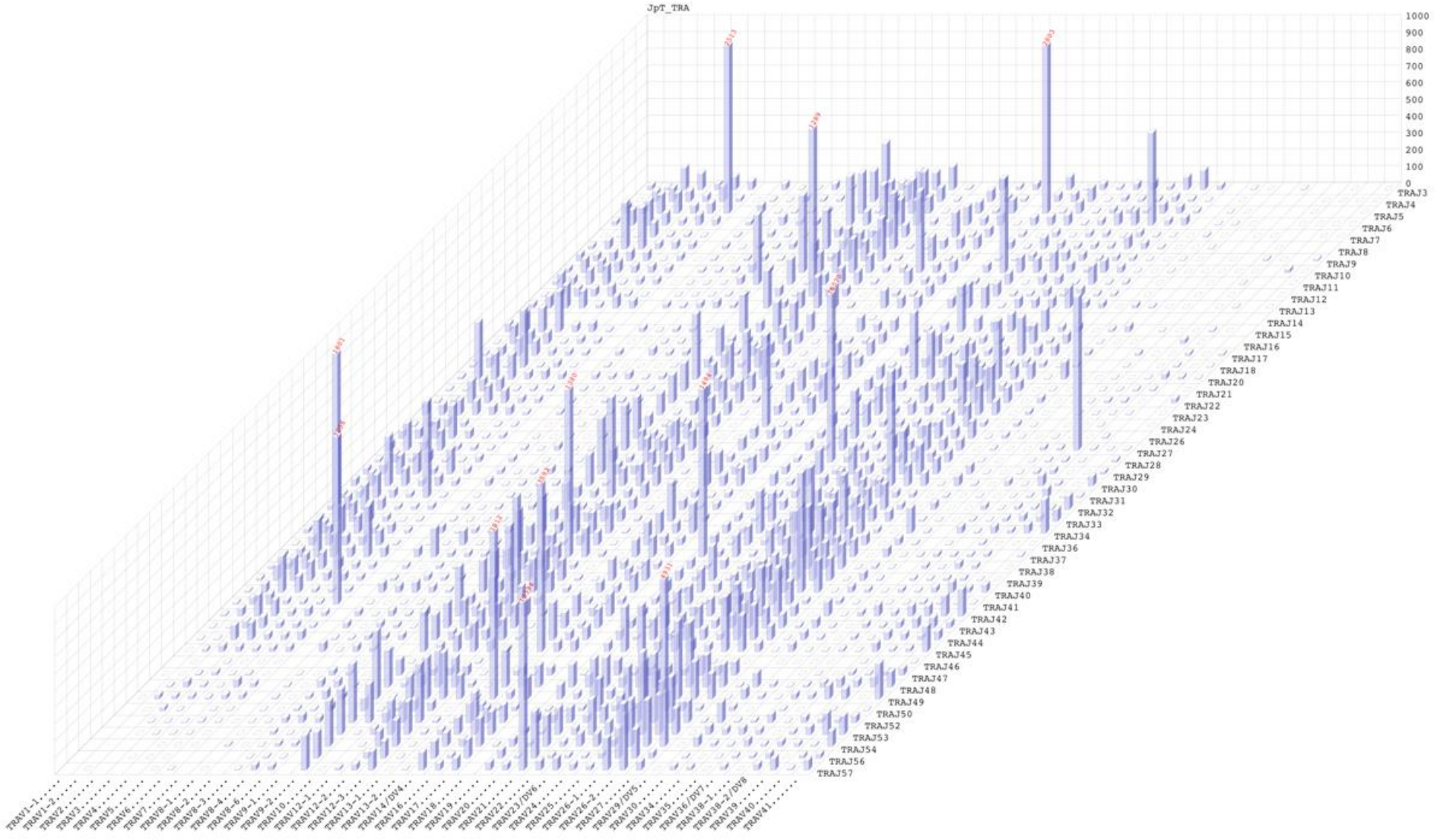
In total, approximately 1.67 million effective sequence reads, which correspond to sequenced cDNA molecules, were generated for eight distinct T cell populations isolated from peripheral blood from a healthy, east Asian male, age 48, who had no known illnesses at the time of blood donation and reported feeling normal and well during the month before the sampling of his blood (Table 1). The first amplification sampled CD3+ T cells in general (pan T) (Figs. S1 and S2). Four additional amplifications (Tc, Tr, Th1, and Th2) sampled T cell subsets with divergent effector functions; the final three amplifications (Tn+t, Ta, and Tm) sampled T cells at different stages of T cell development (*SI Text*, Figs. S1 and S3, and Tables S1–S3). From these sequence reads, about 1.48 million CDR3 intervals were identified, totaling 169,977 and 113,290 unique CDR3 intervals for TCRα and TCRβ chains, respectively. With a few exceptions, a highly random

Table 1. Sequence reads and CDR3 for different subsets of T cells

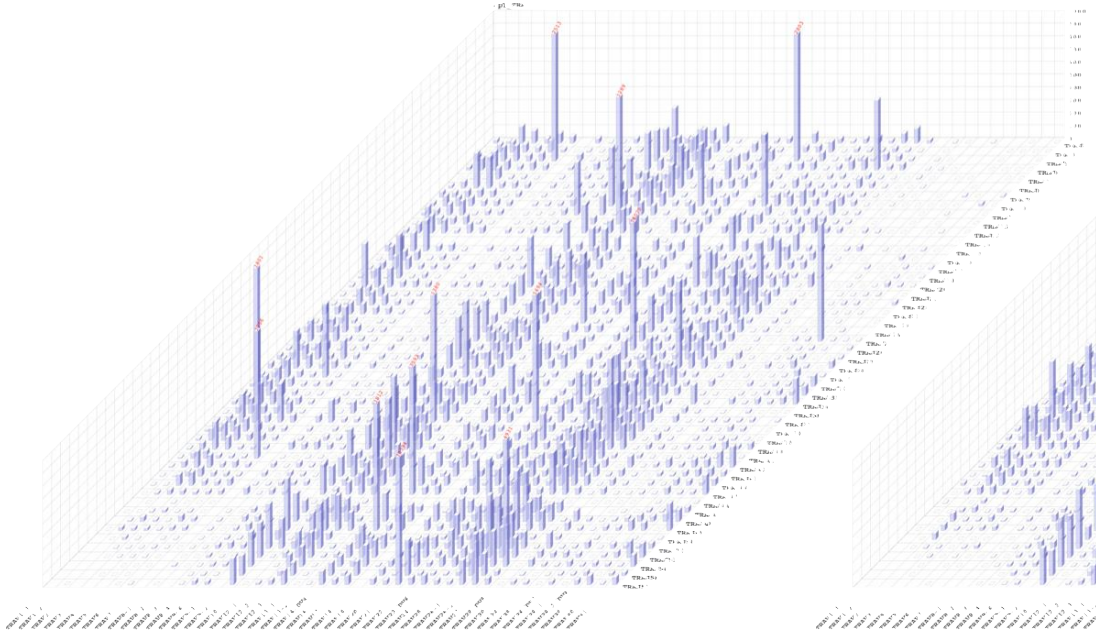
				Unique CDR3[†]			
				TCRα		TCRβ	
Subset	Cell count	Effective read*	Total CDR3	aa	na	aa	na
Tr	6.30×10^7	206,087	179,354	34,804	38,773	22,906	23,654
Th1	1.84×10^8	174,046	150,122	29,471	32,518	19,644	20,061
Th2	1.94×10^7	105,567	91,369	14,038	15,301	6,250	6,447
Tc	1.69×10^8	221,832	200,412	16,654	18,214	9,310	9,735
Tn+t	9.52×10^7	213,054	191,121	22,728	24,652	13,947	14,373
Ta	8.89×10^6	187,494	167,727	9,052	10,084	3,873	4,129
Tm	1.45×10^7	168,301	146,762	16,302	18,049	15,081	15,536
pan T	3.77×10^7	283,241	251,665	37,857	42,045	26,981	27,960
pan T[‡]	—	80,246	71,765	15,638	16,622	10,308	10,483
pan T[§]	—	30,579	27,263	7,794	8,130	5,334	5,416
Total	—	1,670,447	1,477,560	137,751	169,977	106,903	113,290
Public[¶]	—	1,311	1,222	203	210	916	938

2D Map

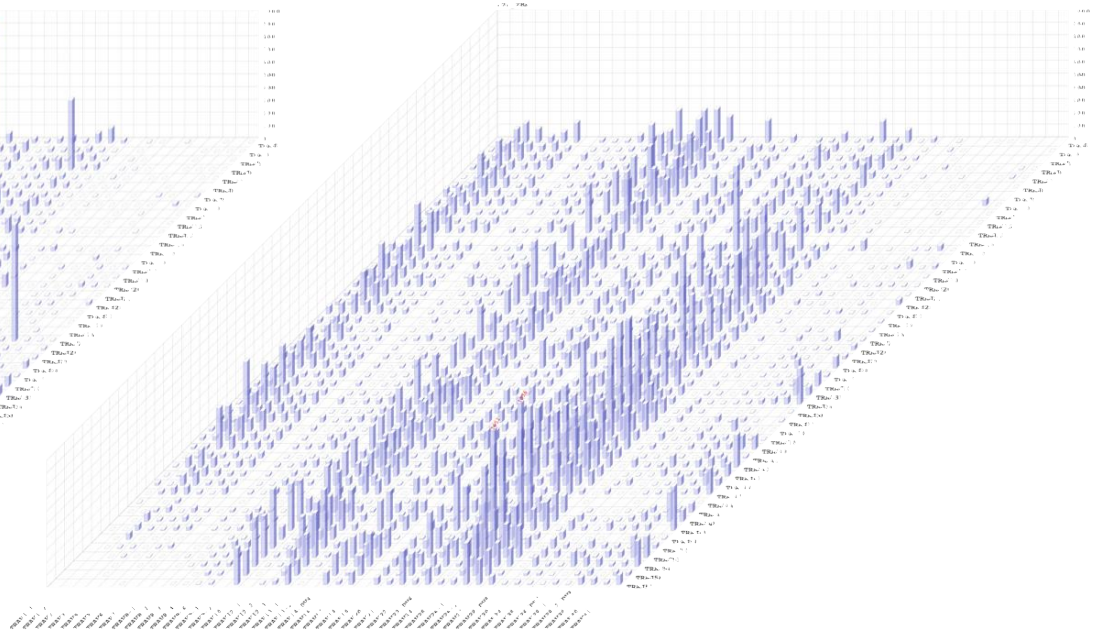

TRA
TRAJ10
TRAJ11
TRAJ12
TRAJ13
TRAJ14
TRAJ15
TRAJ16
TRAJ17
TRAJ18
TRAJ20
TRAJ21
TRAJ22
TRAJ23
TRAJ24
TRAJ26
TRAJ27
IGH
IGHJ1
IGHJ2
IGHJ3
IGHJ4
IGHJ5
IGHJ6
TRB
TRBJ1-1
TRBJ1-2
TRBJ1-3
TRBJ1-4
TRBJ1-5
TRBJ1-6
TRBJ2-1
TRBJ2-2
TRBJ2-3
TRBJ2-4
TRBJ2-5
TRBJ2-6
TRBJ2-7
TRG
TRGJ1
TRGJ2
TRGJP
TRGJP1
TRGJP2
IGL
IGLJ1
IGLJ2
IGLJ3
IGLJ6
IGLJ7
TRAV5
TRAV6
TRAV7
TRAV8-1
TRAV8-2
TRAV8-3
TRAV8-4
TRAV8-6
TRAV9-1
TRAV9-2
IGHV1-18
IGHV1-2
IGHV1-24
IGHV1-3
IGHV1-45
IGHV1-46
TRBV10-1
TRBV10-2
TRBV10-3
TRBV11-1
TRBV11-2
TRBV11-3
10



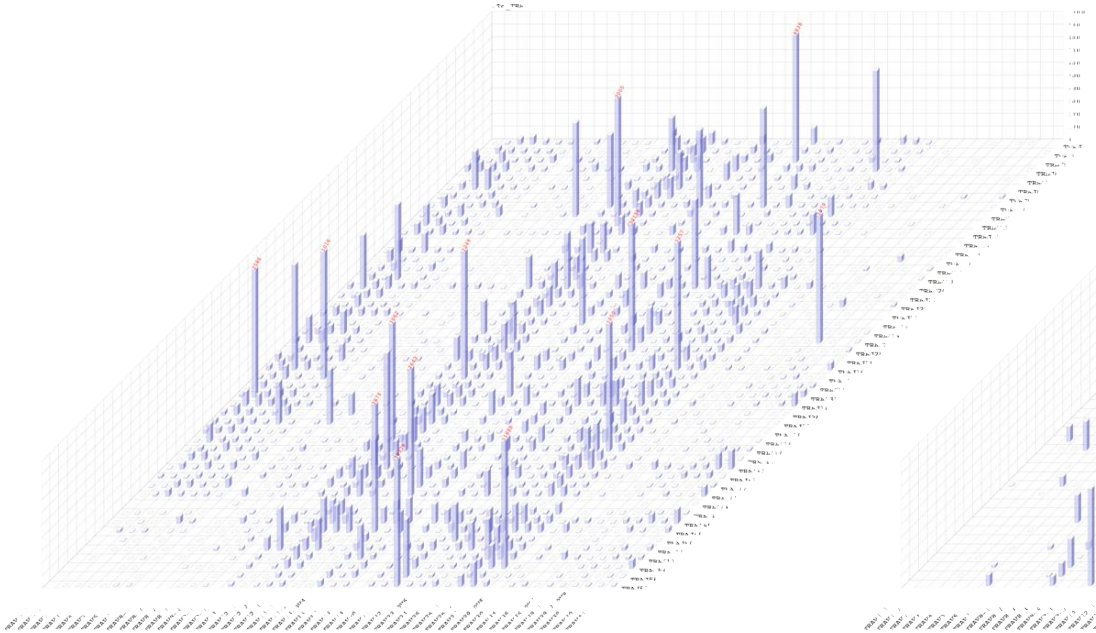
Normal Asian Male Pan T cell TCRA



PanT

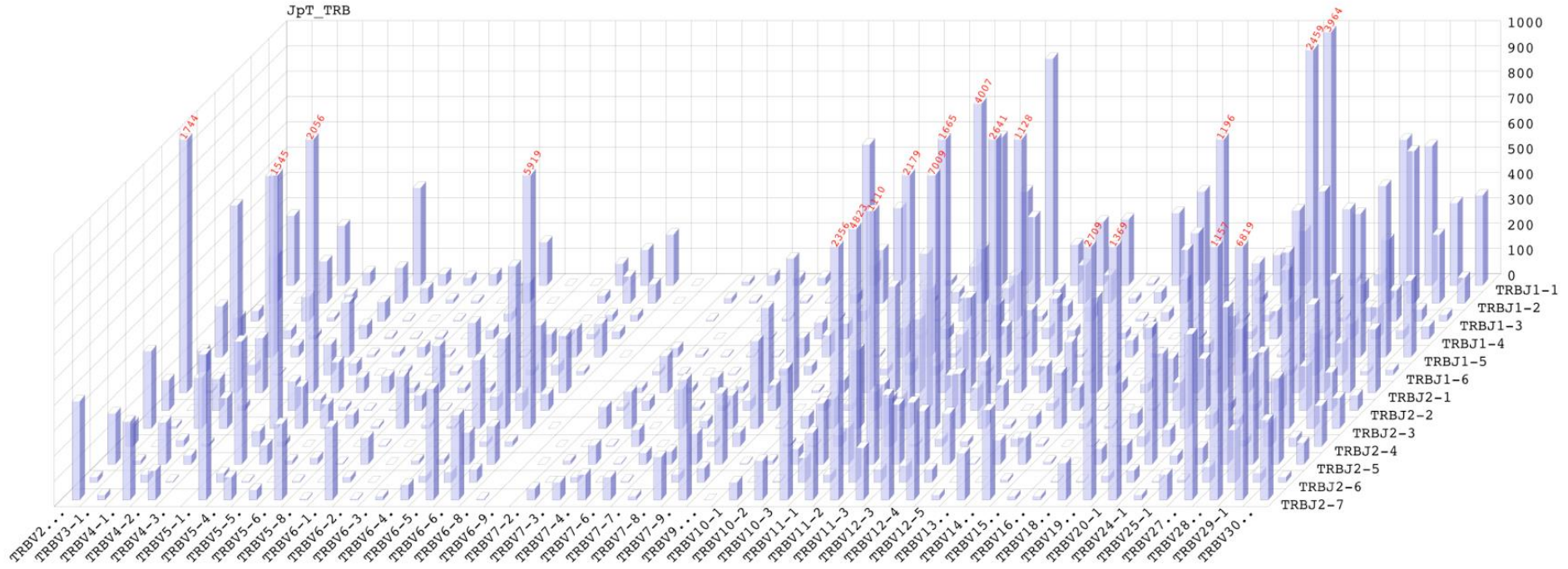


Treg

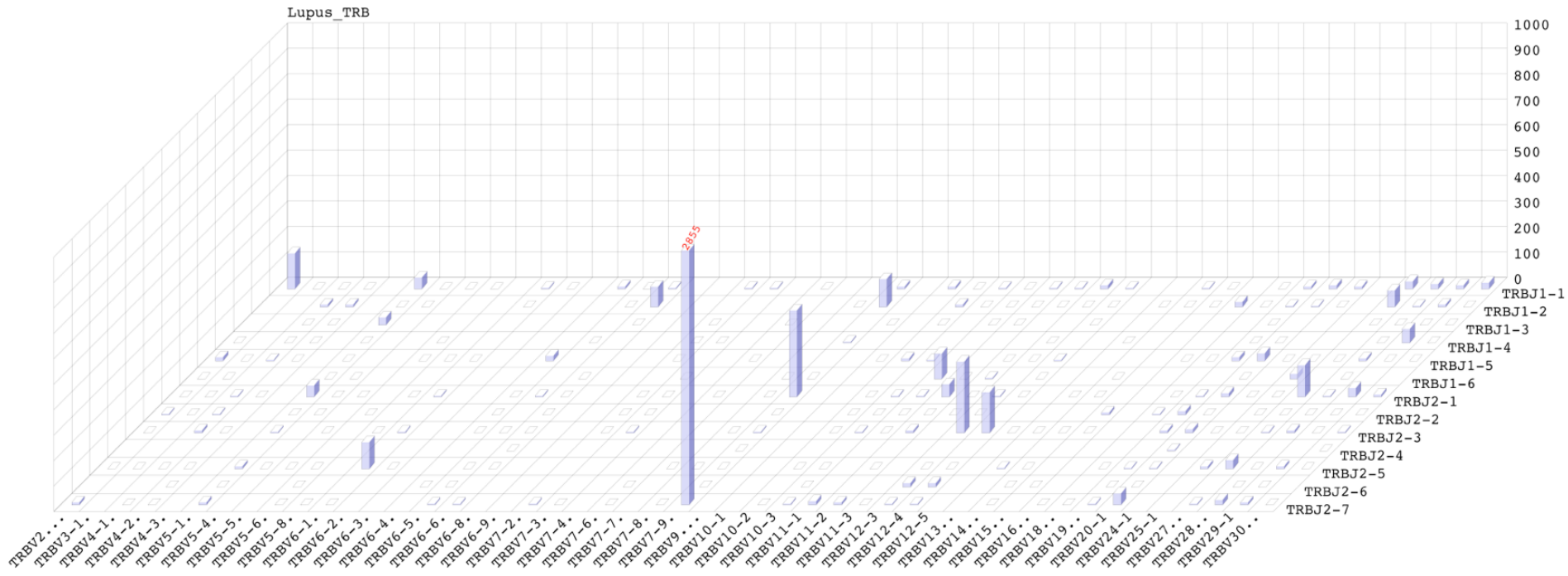


Tc

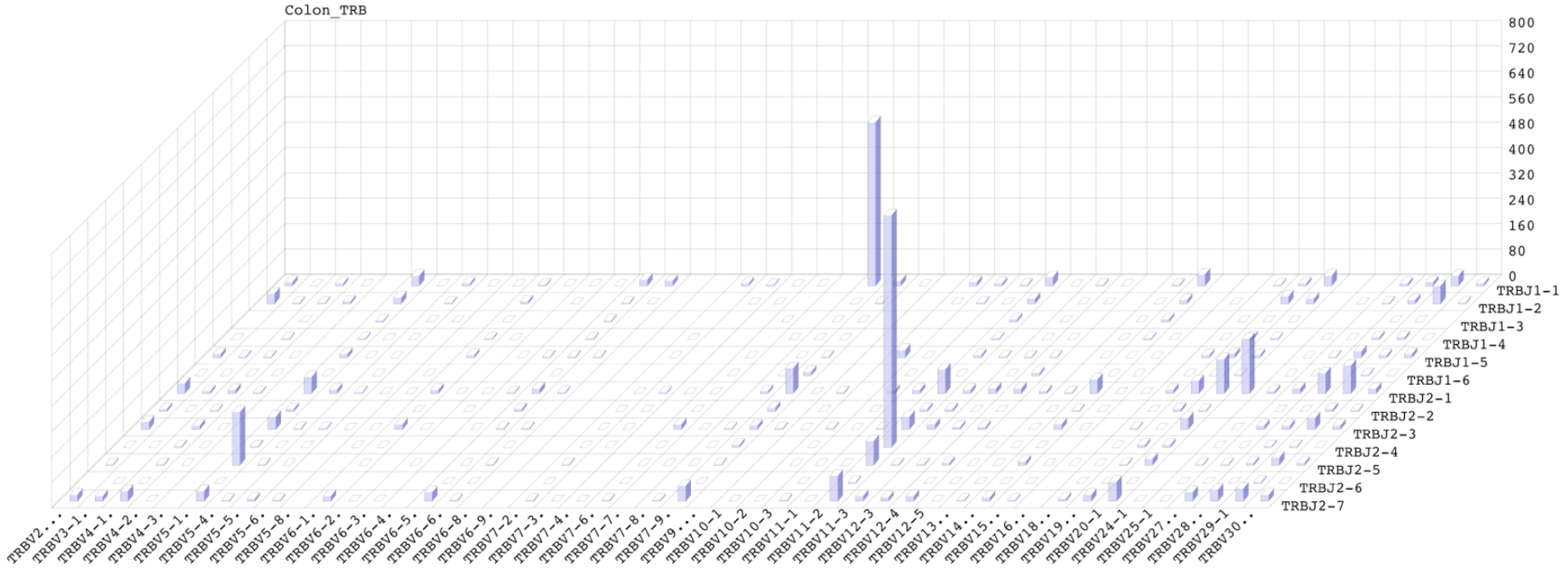
Neutrophil



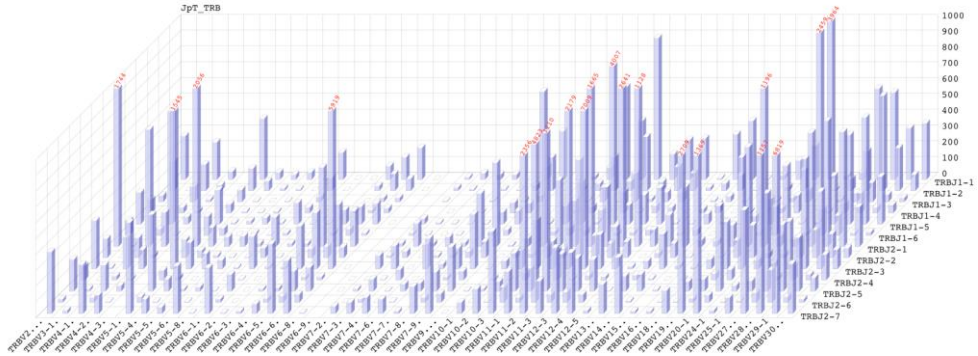
Normal Male Pan T cell TCRB



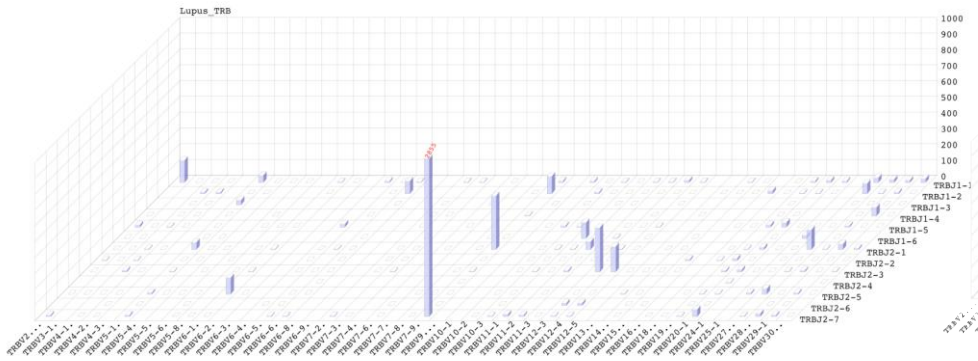
Lupus patient Pan T cell TCRB



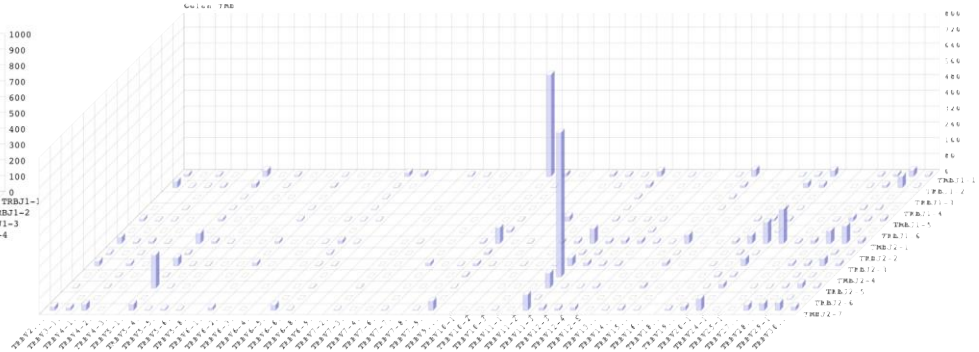
Colon cancer patient Pan T cell TCRB



Normal



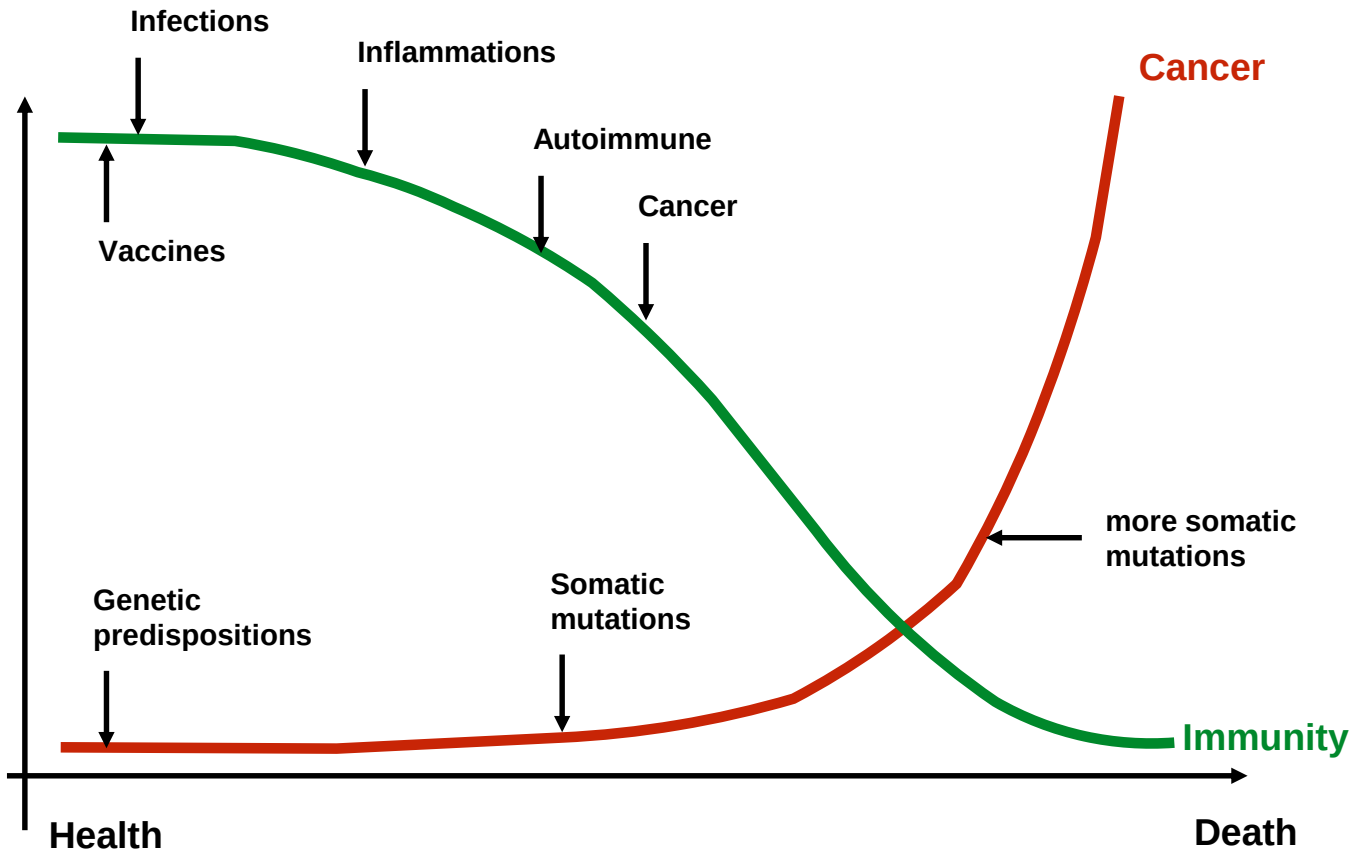
Lupus



Colon Cancer

TCRB

Re-thinking cancer



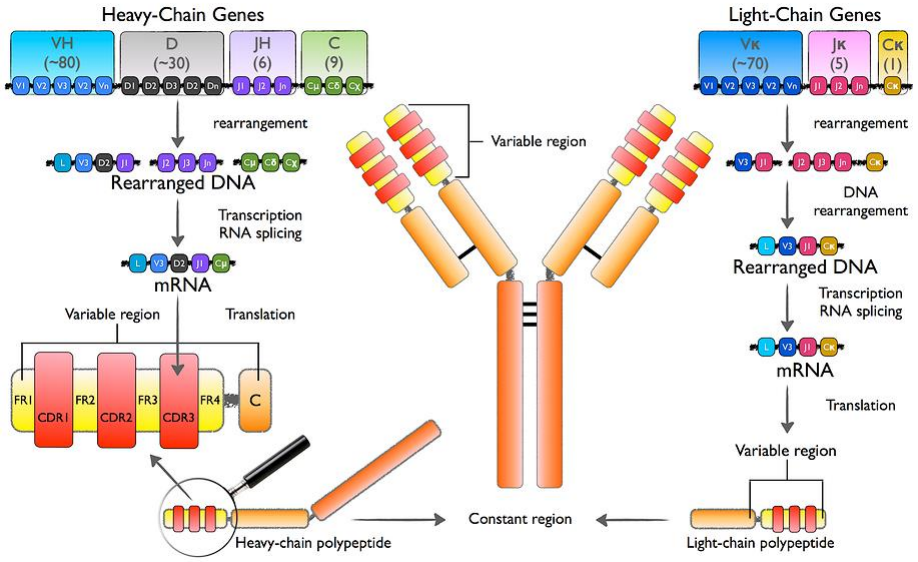
Re-thinking cancer

- Are “driver mutations” really driving cancer development? or are they “passengers” of a runaway car with a broken immune system?

New needs

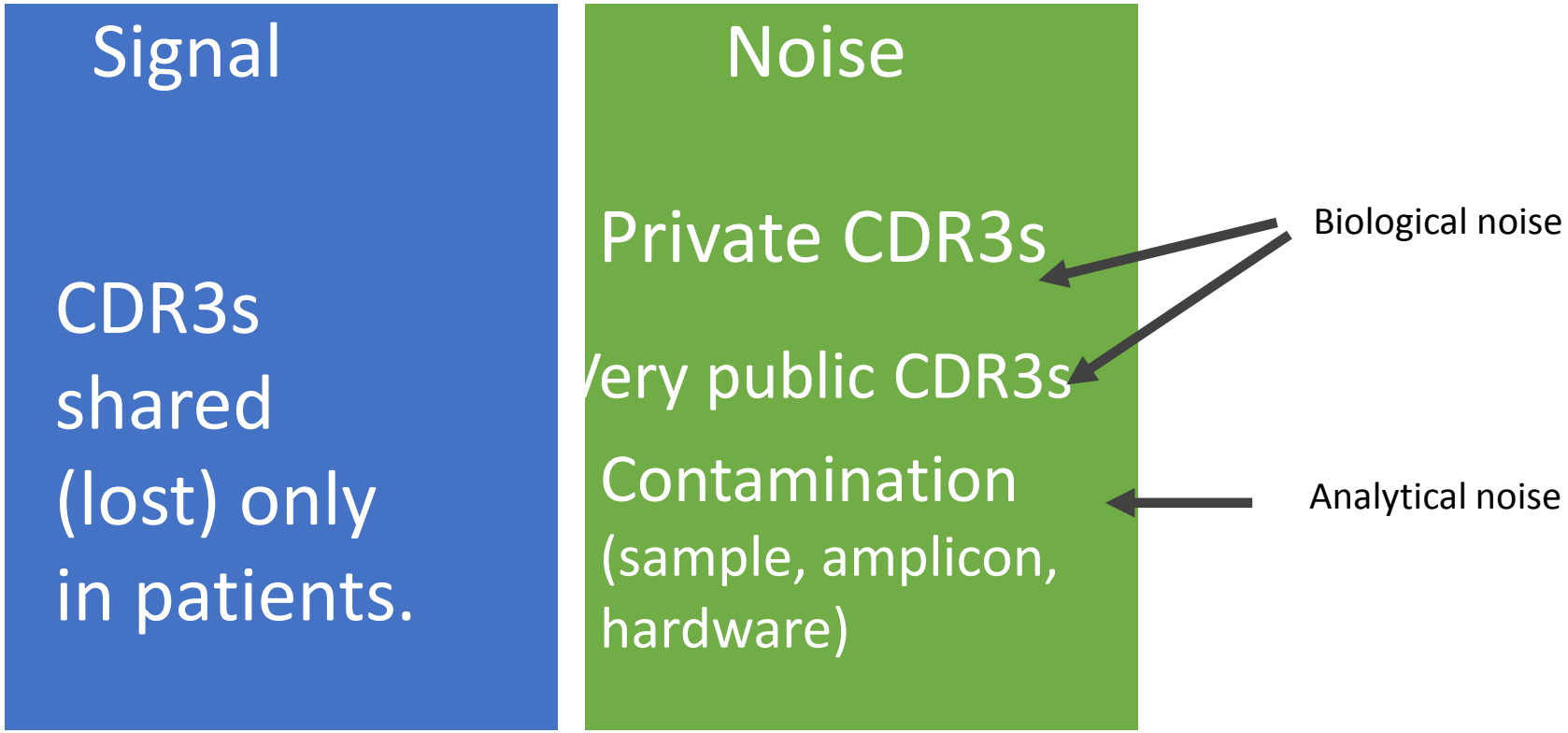
- More accurate and comprehensive evaluation of immune status: overall force (number) and capabilities (diversity), and deployment (tissue distribution).

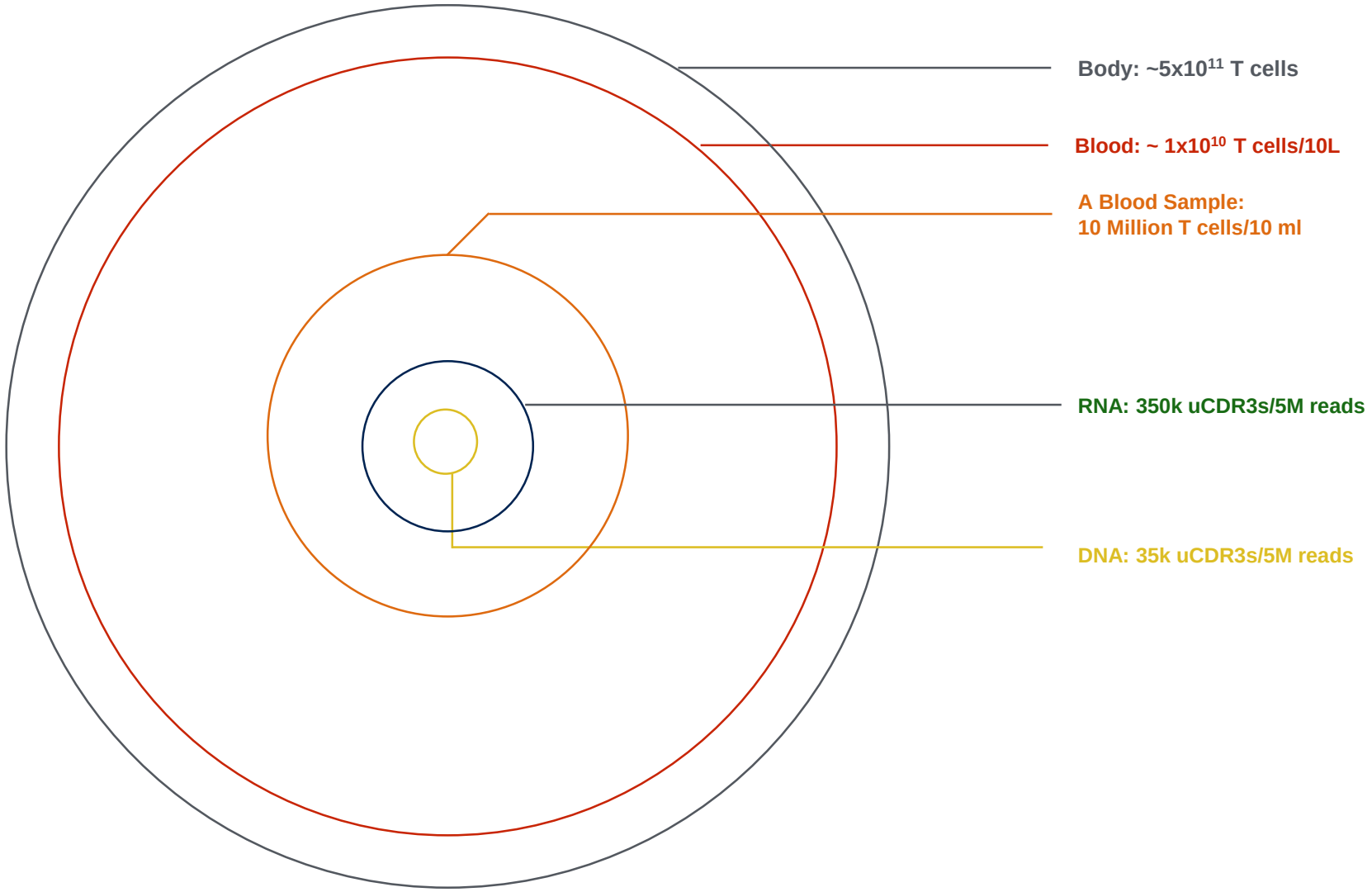
Different from sequencing genome



- De novo sequencing, no “standard repertoire”
- Private and dynamic repertoire.
- Don’t know the size of repertoire universe
- Noise everywhere.

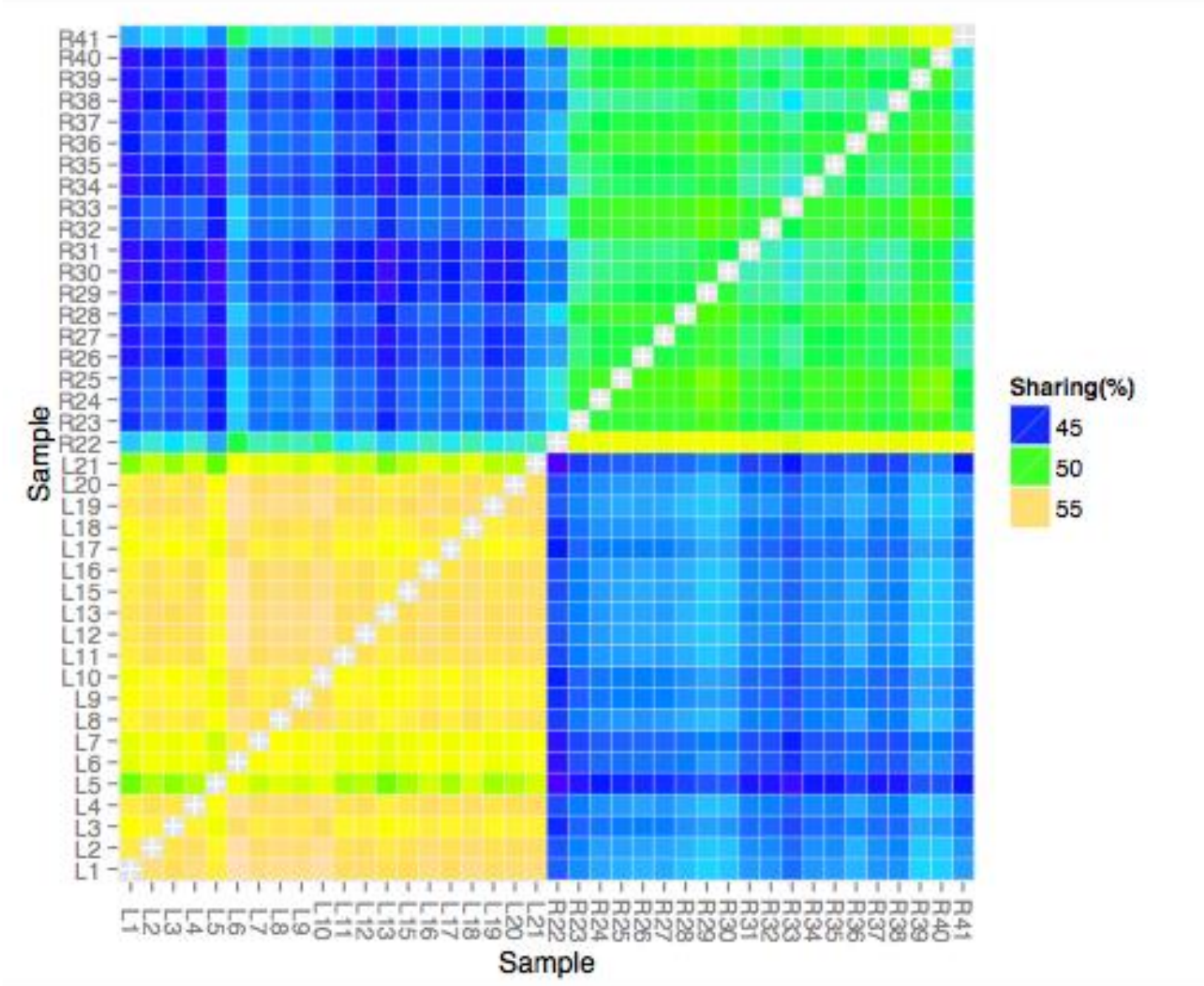
Signals Vs. Noise



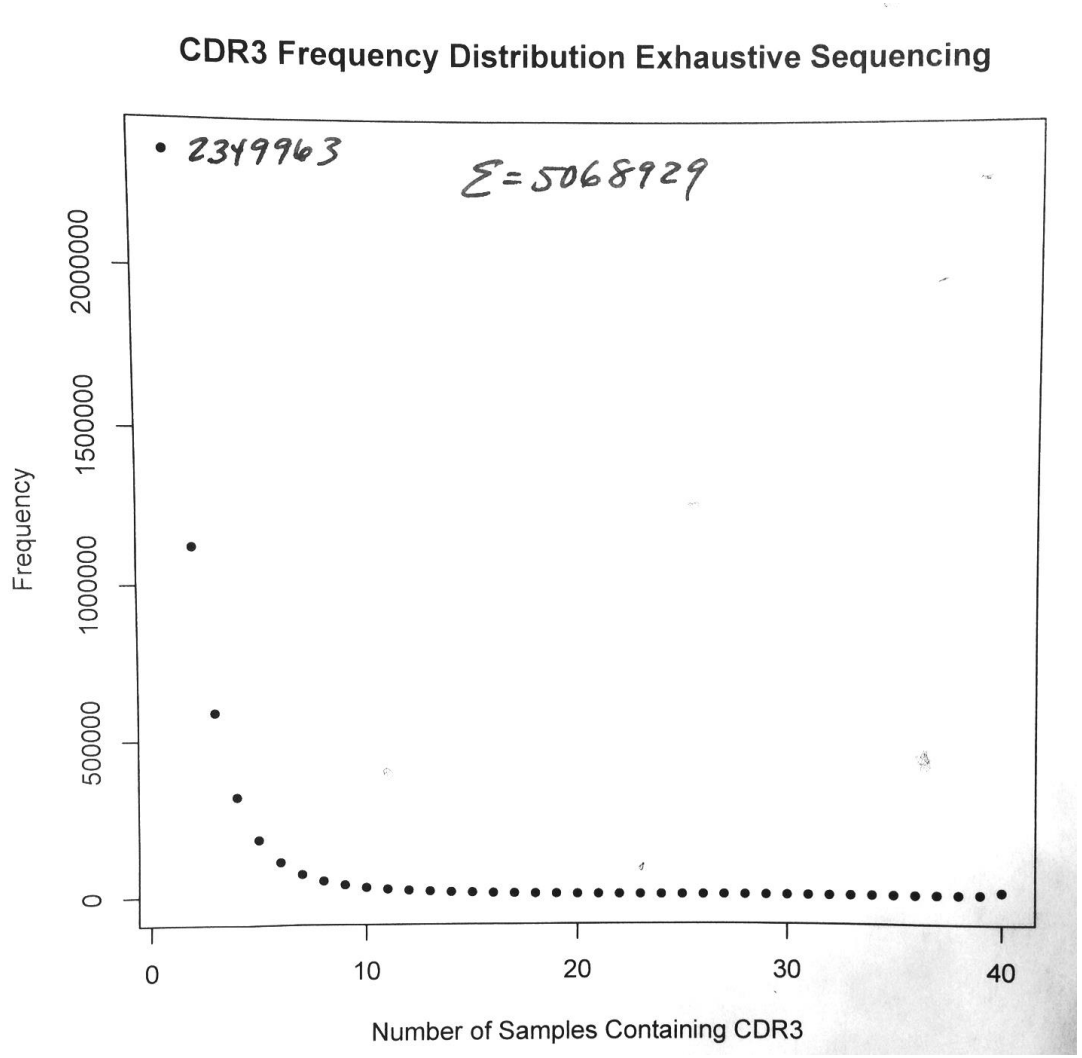


Is your (sample) data representative enough and include disease signal?

Exhaustive sequencing of one individual

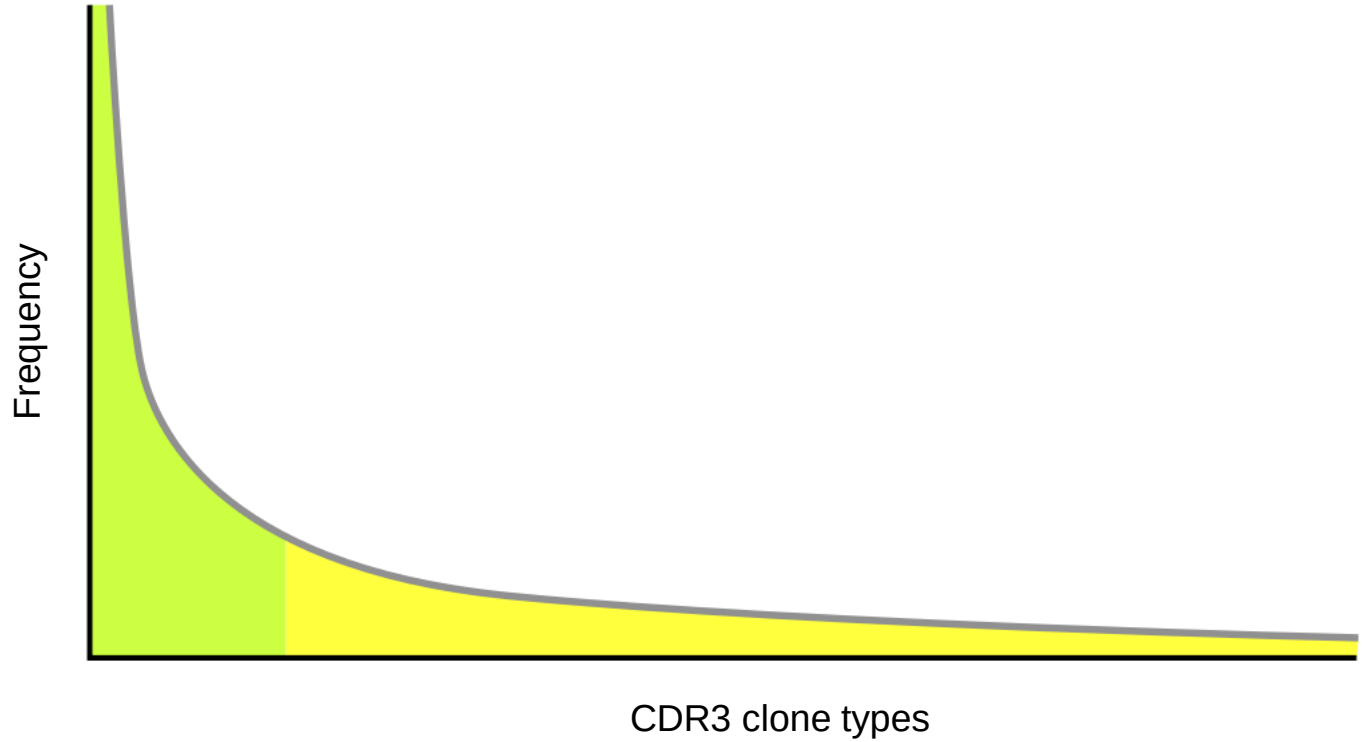


The distribution of CDR3s in one individual



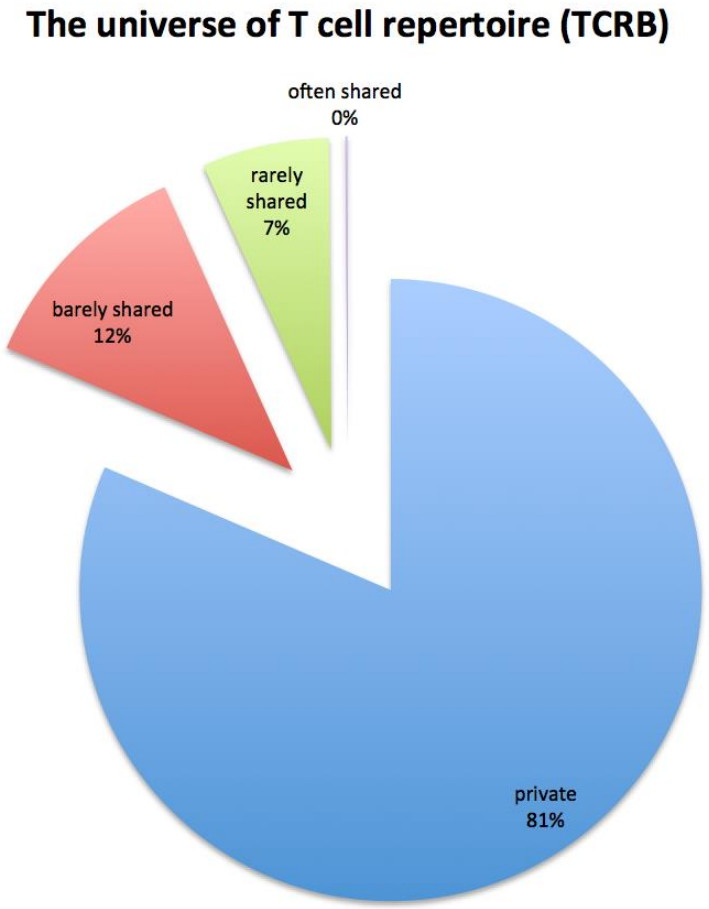
Of the ~300k CDR3s found in one sample, only the top ~10K is constantly found in all 40 samples from the same individual.

In a person, repertoire (CDR3) distribution is long tailed.



Repertoire distribution in a **population**.

The distribution of CDR3s in 1000 individuals



1000 samples analyzed

3,428,836,771 reads obtained (pair-end, 150bp)

75 million unique CDR3s were found

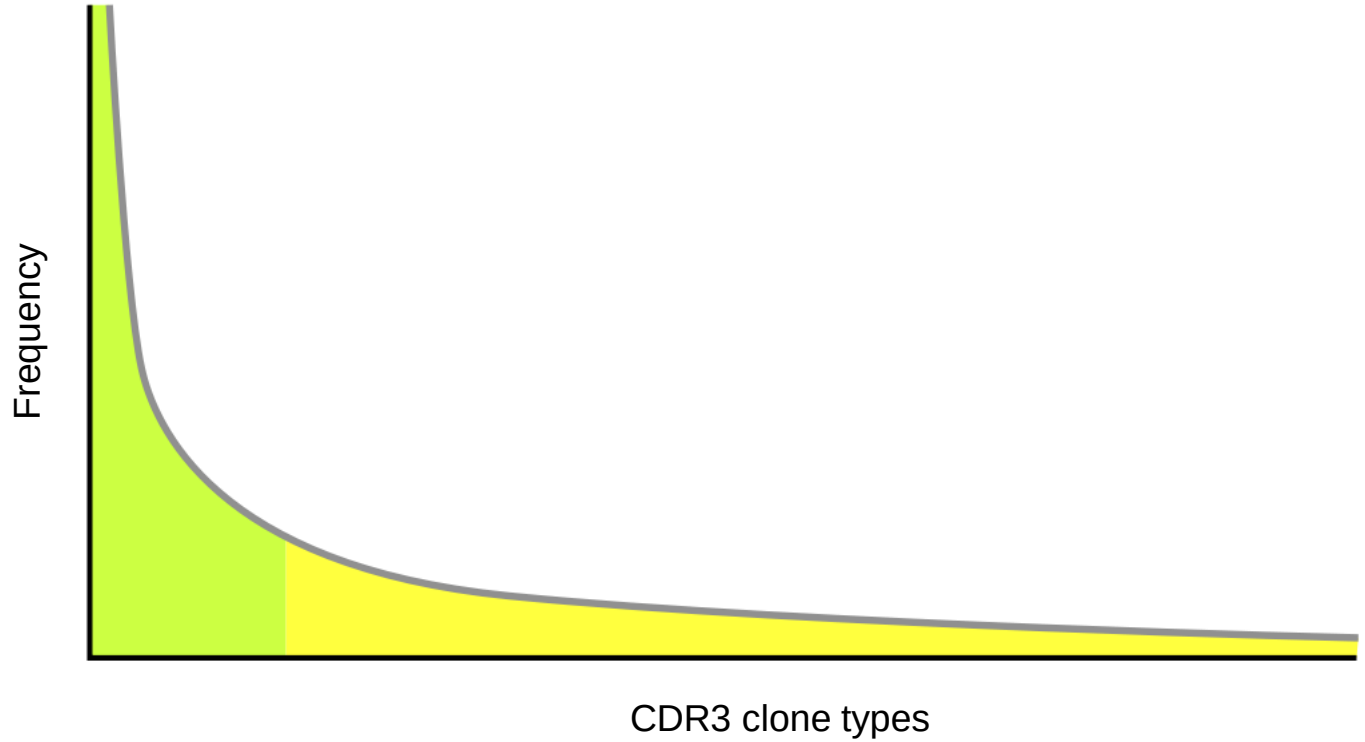
81% not shared

12% shared by 2-3 people

7% shared by 4-100 people

0.01% shared by >50% of the people!

In a population, repertoire distribution is also long tailed.

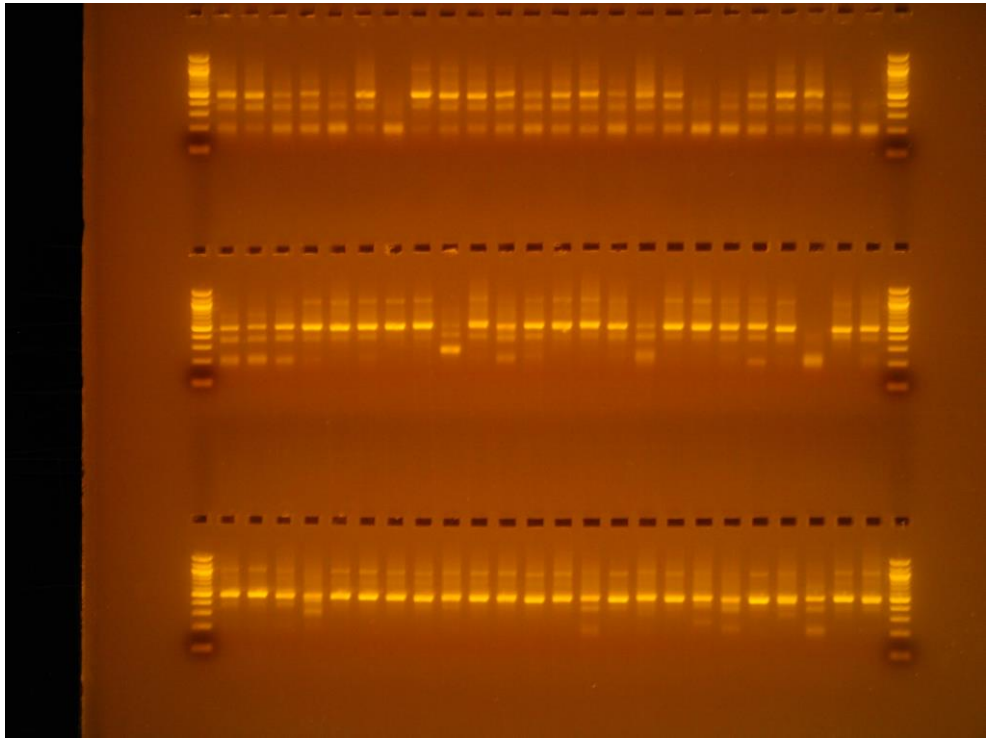


The long tail is largely noise.

Quantitative analysis is the key.

Single cell isolation methods

Sony sorter + dam-PCR+NGS



7-Chain damPCR Method

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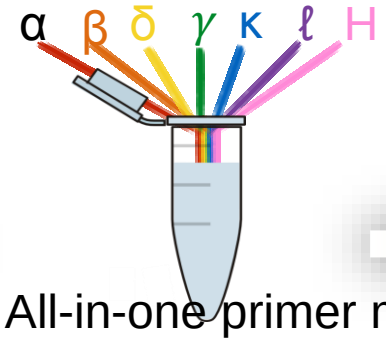
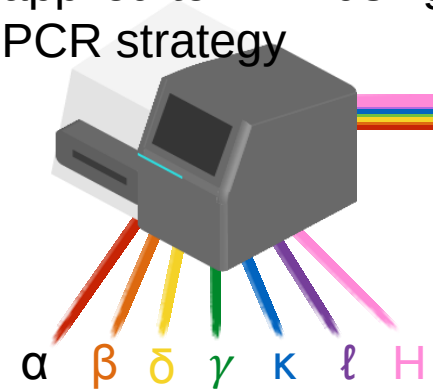


2. All-in-one primer mix covering TCR alpha, beta, delta gamma and BCR IgH applied to RNA using dam-PCR strategy

3. Library clean-up

4. Next Generation Sequencing



5-Chain damPCR Method

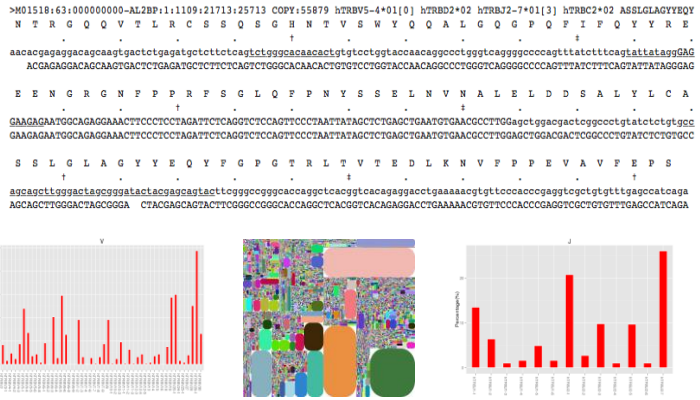
5. iRMap Data Analysis Pipeline

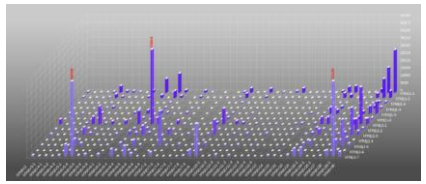
Top 10 most frequent alignments listed !

CDR3 list

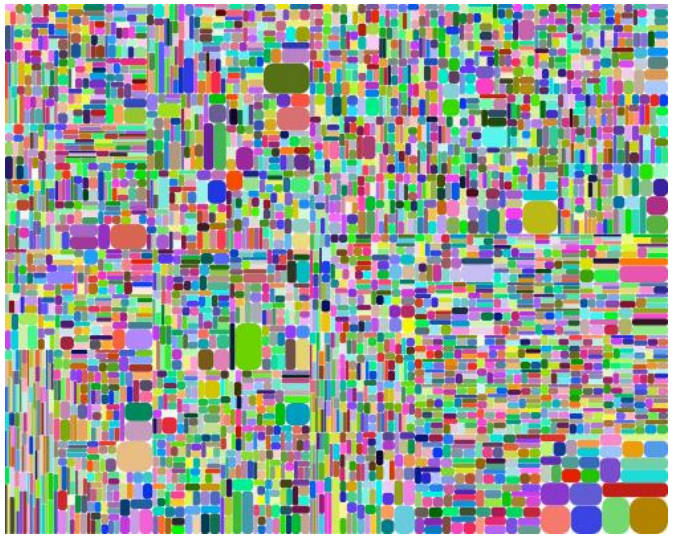
Show 100 entries Search:

Frequency	Peptide	V	J
56217	ASSLGLAGYYEQY	hTRBV5-4	hTRBJ2-7
48511	ASTKWAGGRNEQF	hTRBV6-5	hTRBJ2-1
45383	SAAAVASYEQY	hTRBV29-1	hTRBJ2-7
19766	ASSLDPSGSETQY	hTRBV7-2	hTRBJ2-5
15661	AWSVLTTEAF	hTRBV30	hTRBJ1-1
11373	ASSGTSGGAAYEQY	hTRBV9	hTRBJ2-7
9476	ASSPQVSGEQF	hTRBV5-4	hTRBJ2-1
7198	ASSWYMNTEAF	hTRBV6-5	hTRBJ1-1
6260	AWRADRAPMFAEAF	hTRBV30	hTRBJ1-1
5514	ASGPKPGAF	hTRBV6-3	hTRBJ1-1
3189	AWGRQDSNQPQH	hTRBV30	hTRBJ1-5
3107	ASSGHVAGGYTQY	hTRBV9	hTRBJ2-3

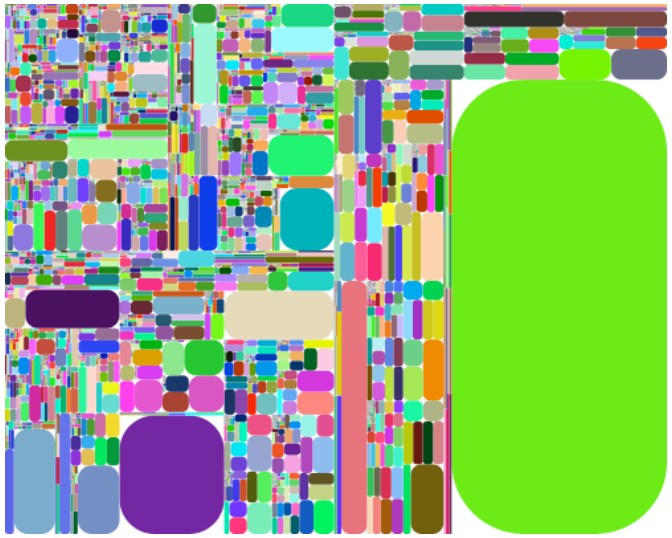




Diversity Index

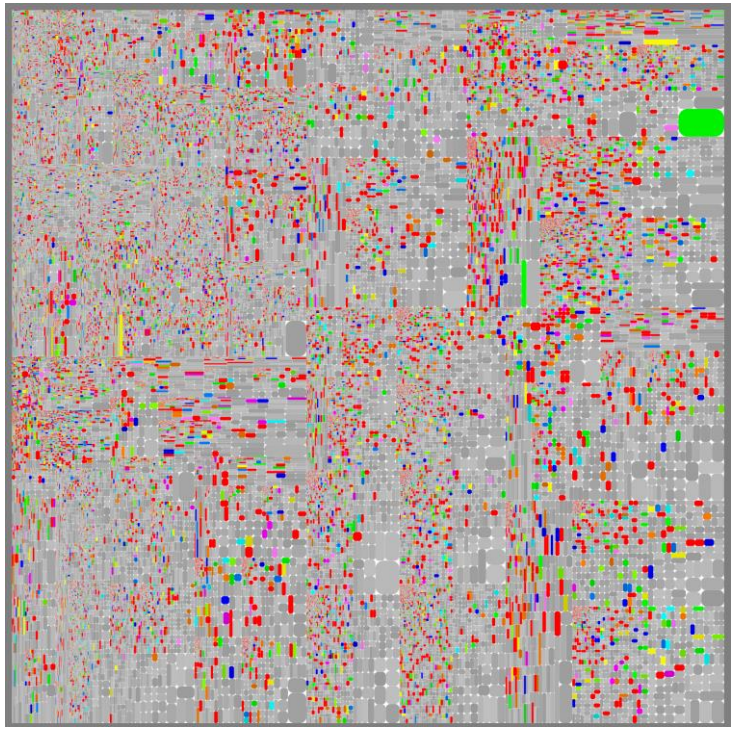


D50: 28

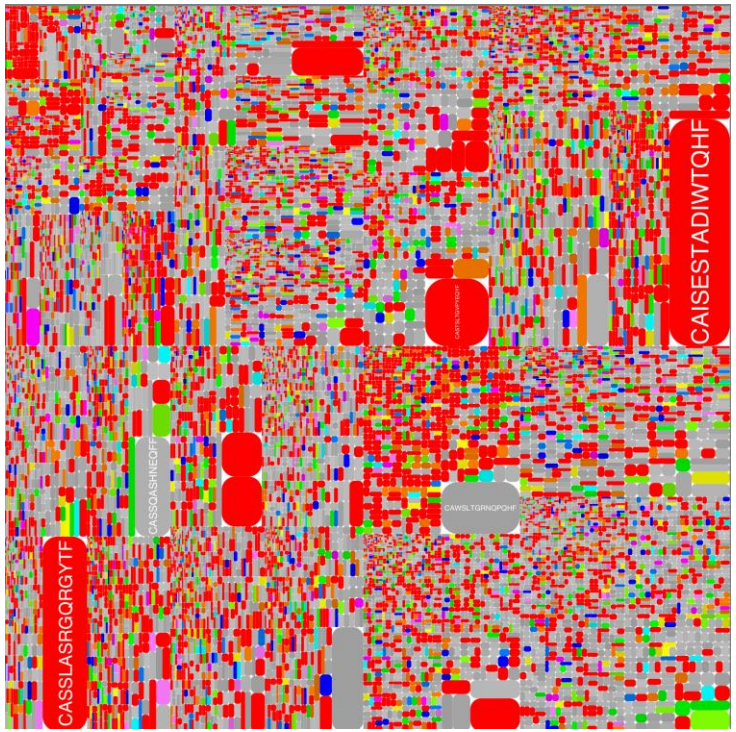


D50: 0.2

Publicity Index

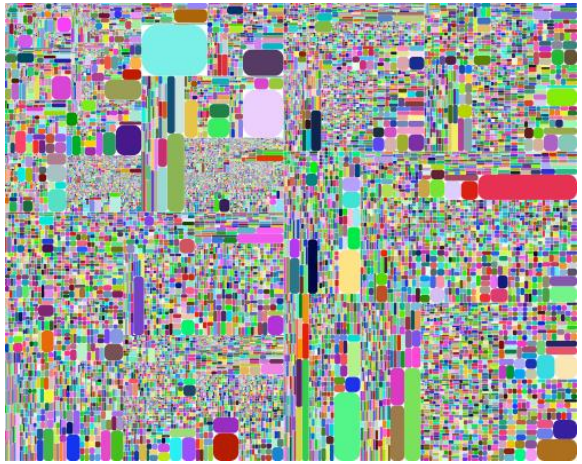


Cord blood

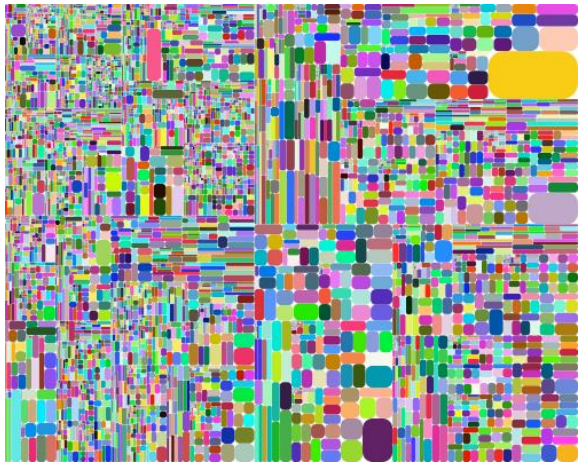


Healthy adult blood

Wellness Index: Number of top 1000 publicCDR3s in 100,000 reads. Average 0.35.

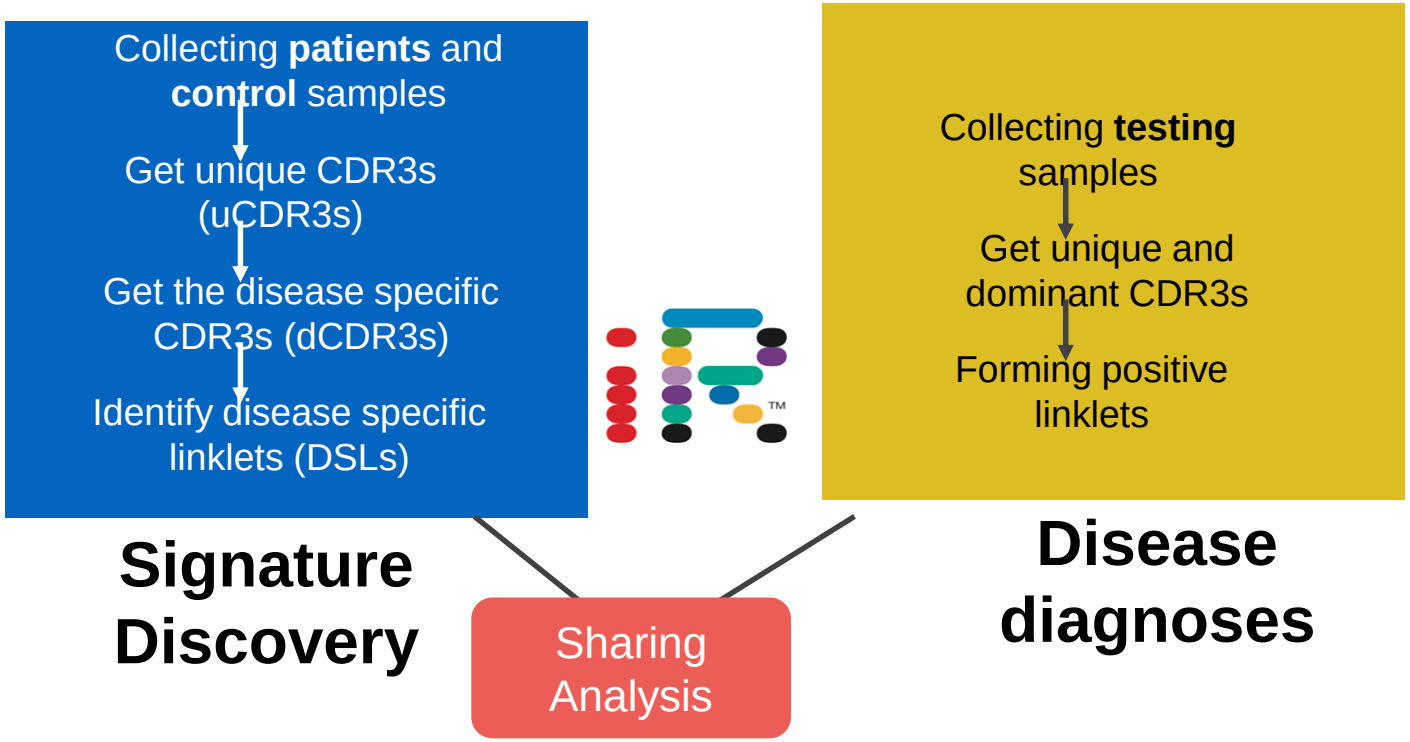


24 yr. 214k uCDR3. Health Index 0.618



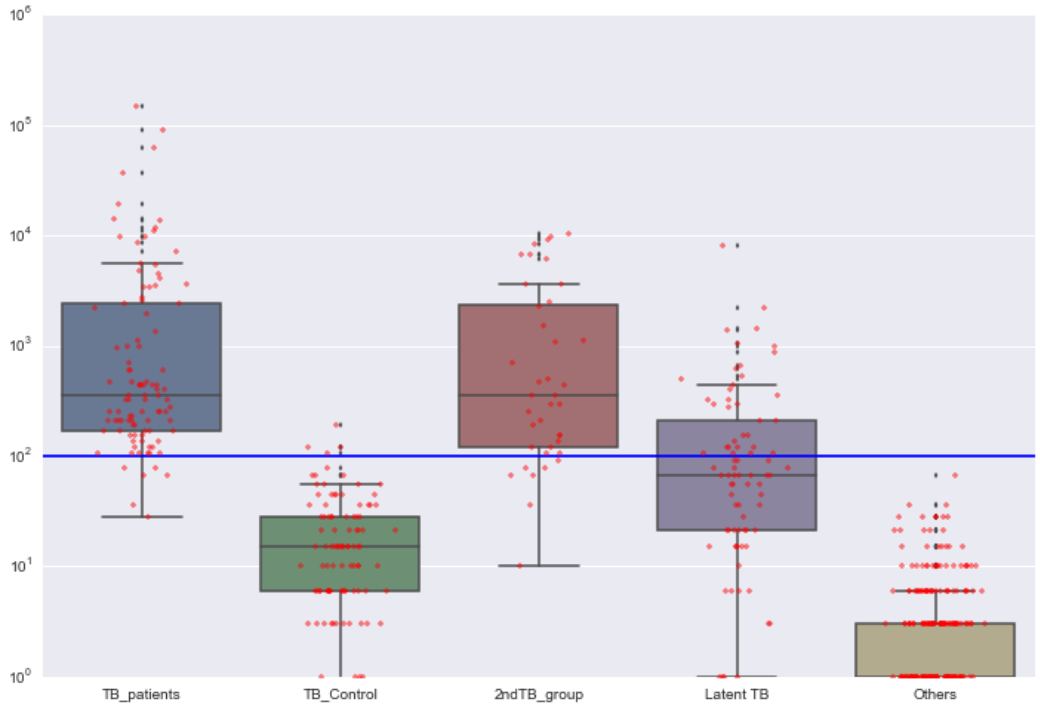
64 yr. 14k uCDR3. Health Index 0.062

Sharing Index
Identify disease specific linklet analysis (DSLA)



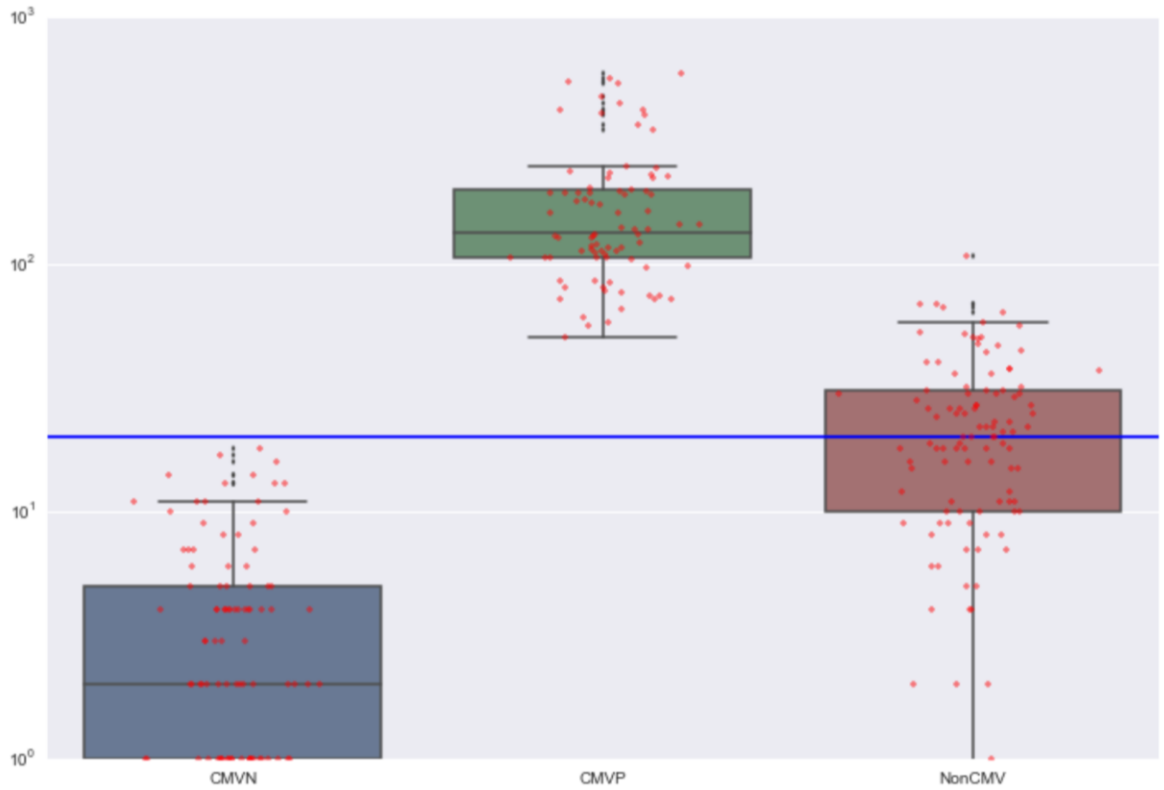
“ How many DSLs can be found in a test sample’s top ranked CDR3s? ”

DSLA for TB



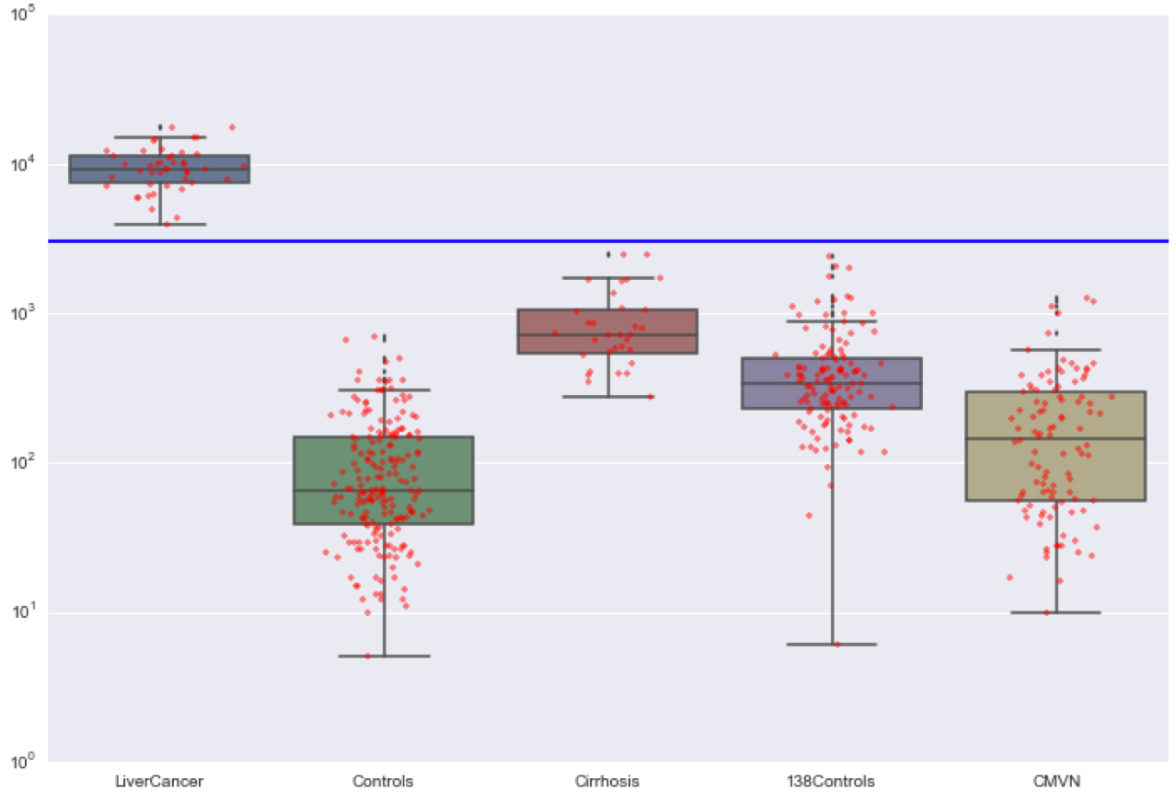
R10K collaborator: Dr. XinChun Chen

DSLA of CMV Negative and Positives



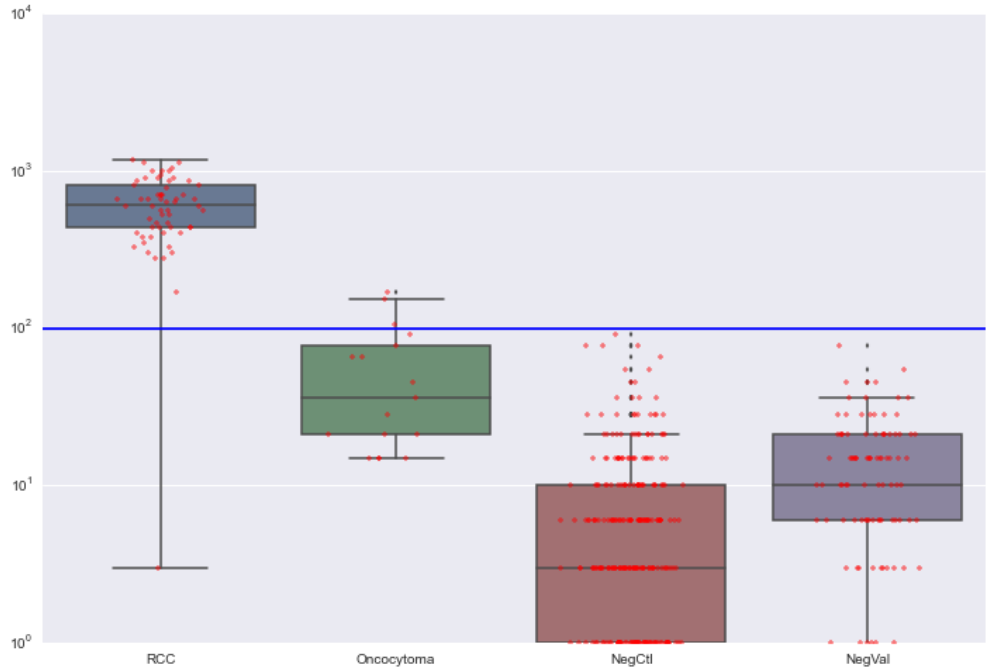
R10K collaborator: Dr. Antoine Blancher

DSLA for Liver Cancer

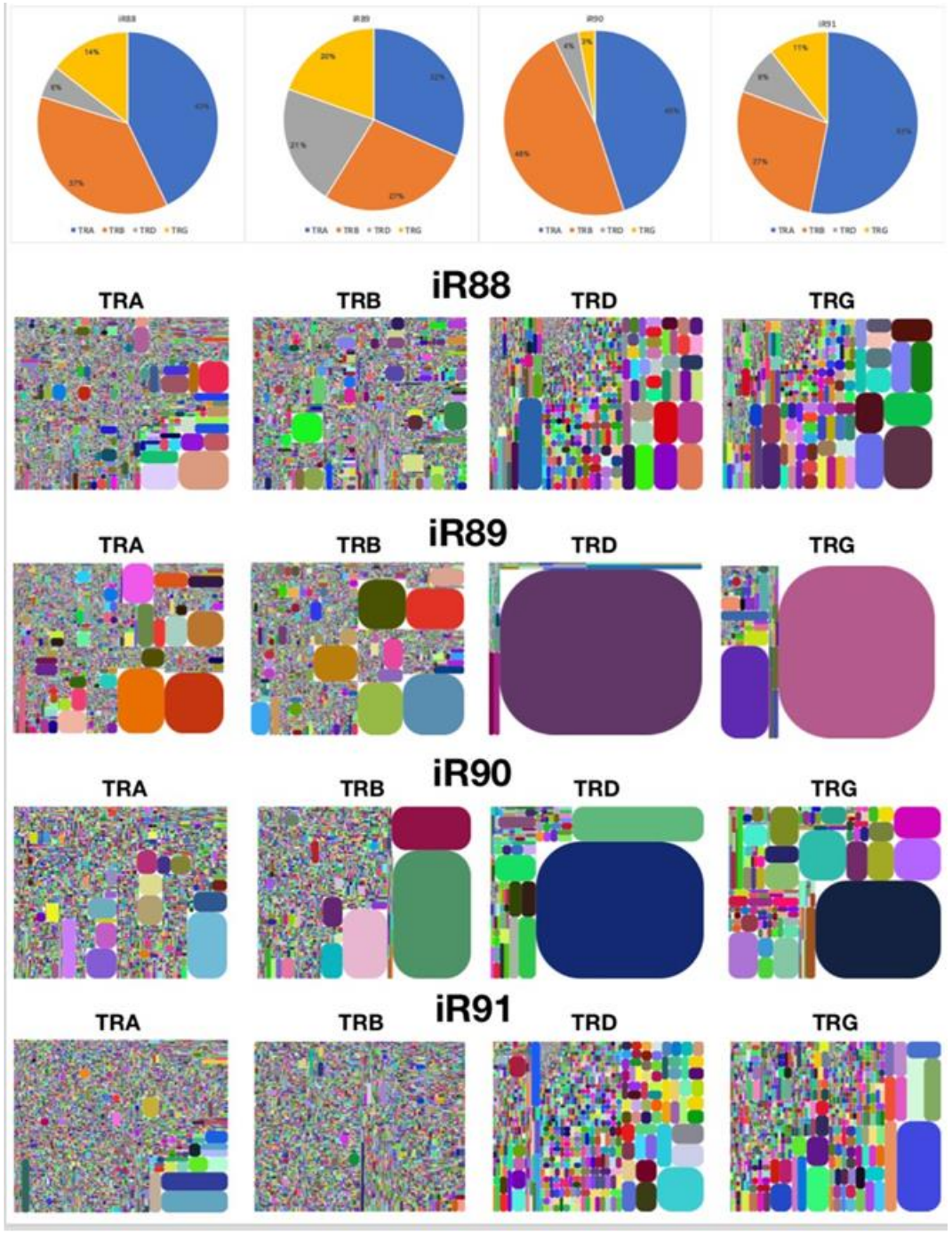


R10K collaborator: Dr. James Yang

DSLA for Renal Cancer

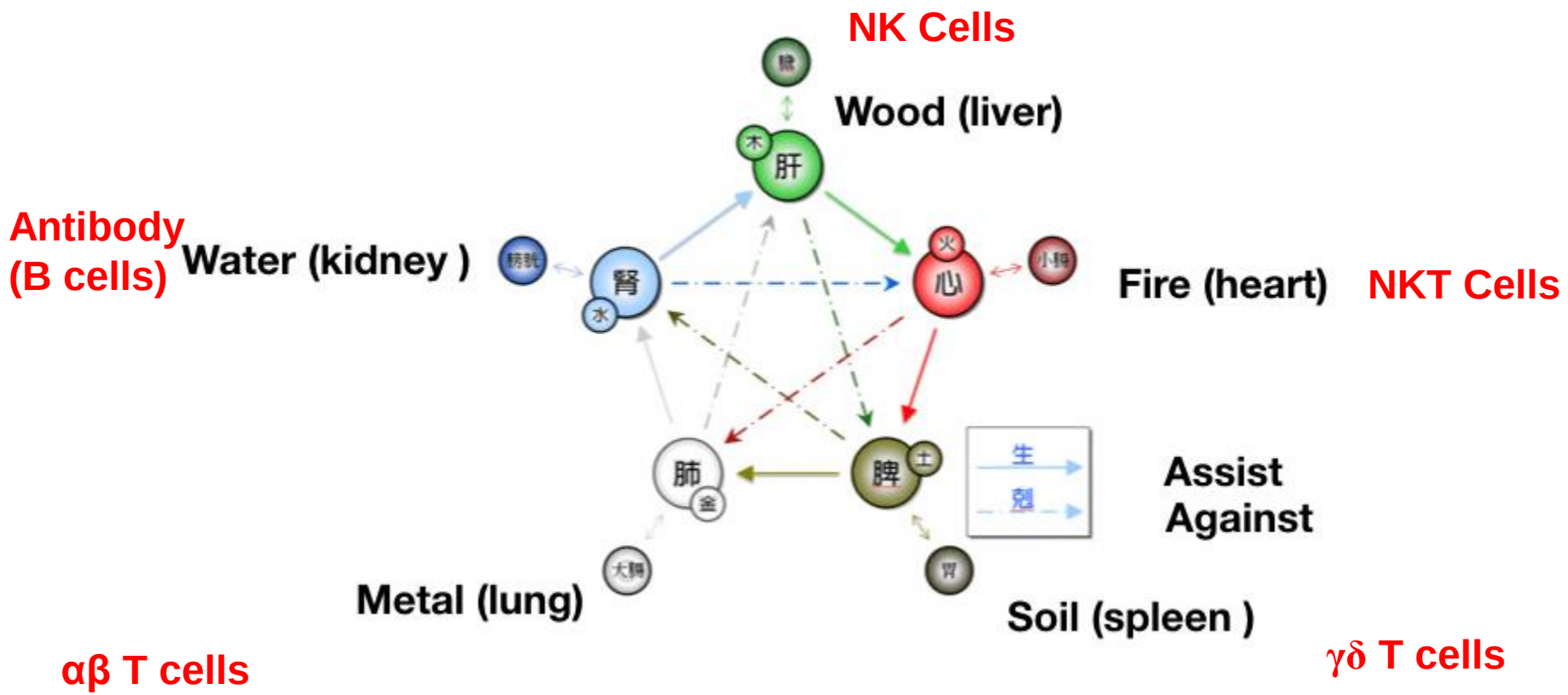


R10K collaborator: Dr. Michael Gorin



Four Normal Individuals Have Grossly Different Distribution Of TCRs

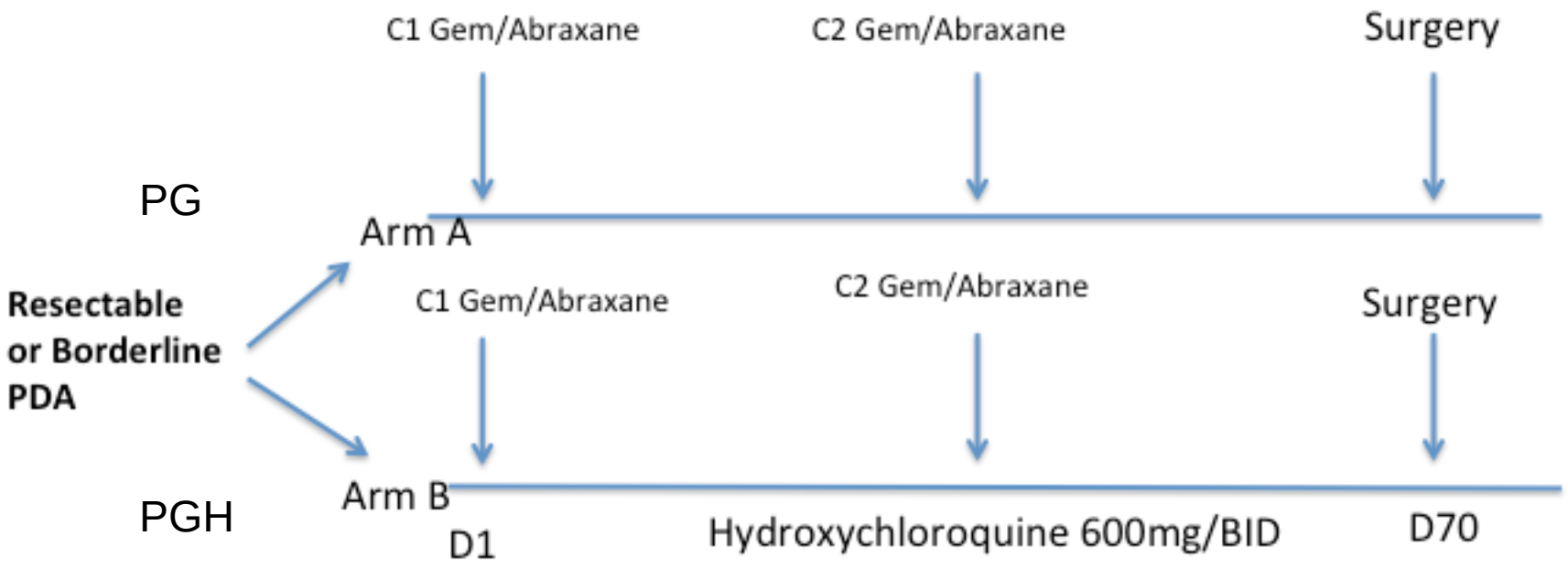
500 Million Years of Adaptive Immunity-Wu Xing
The Immunologic Big Bang



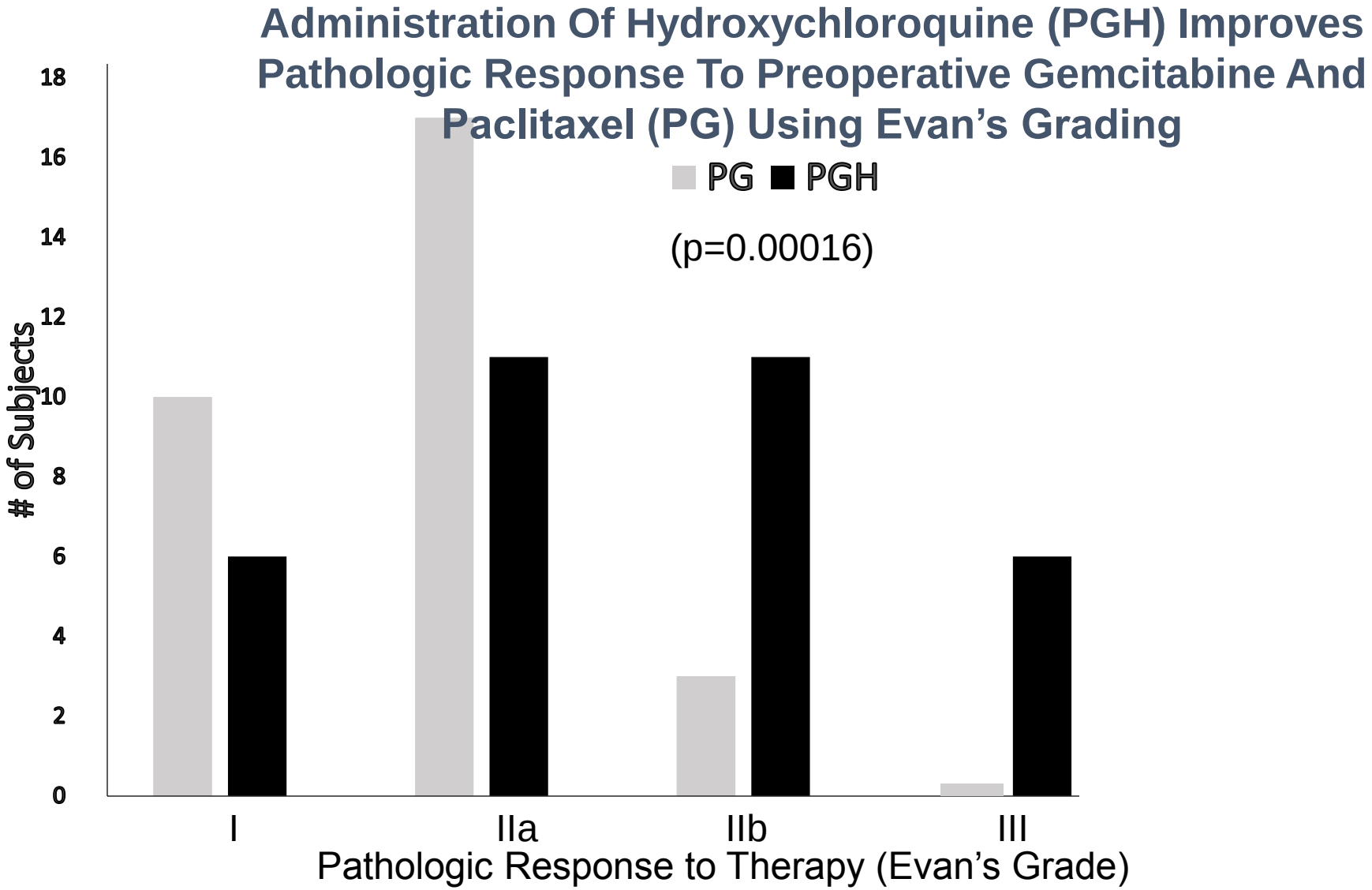
"Wǔ zhǒng liúxíng zhī qì" (五種流行之氣) or "the five types of chi dominating at different times".

Randomized Phase II Trial of Pre-Operative nab-Paclitaxel and Gemcitabine+/- Autophagy Inhibition with Hydroxychloroquine In Pancreatic Adenocarcinoma=PGH

(R01 CA160417)

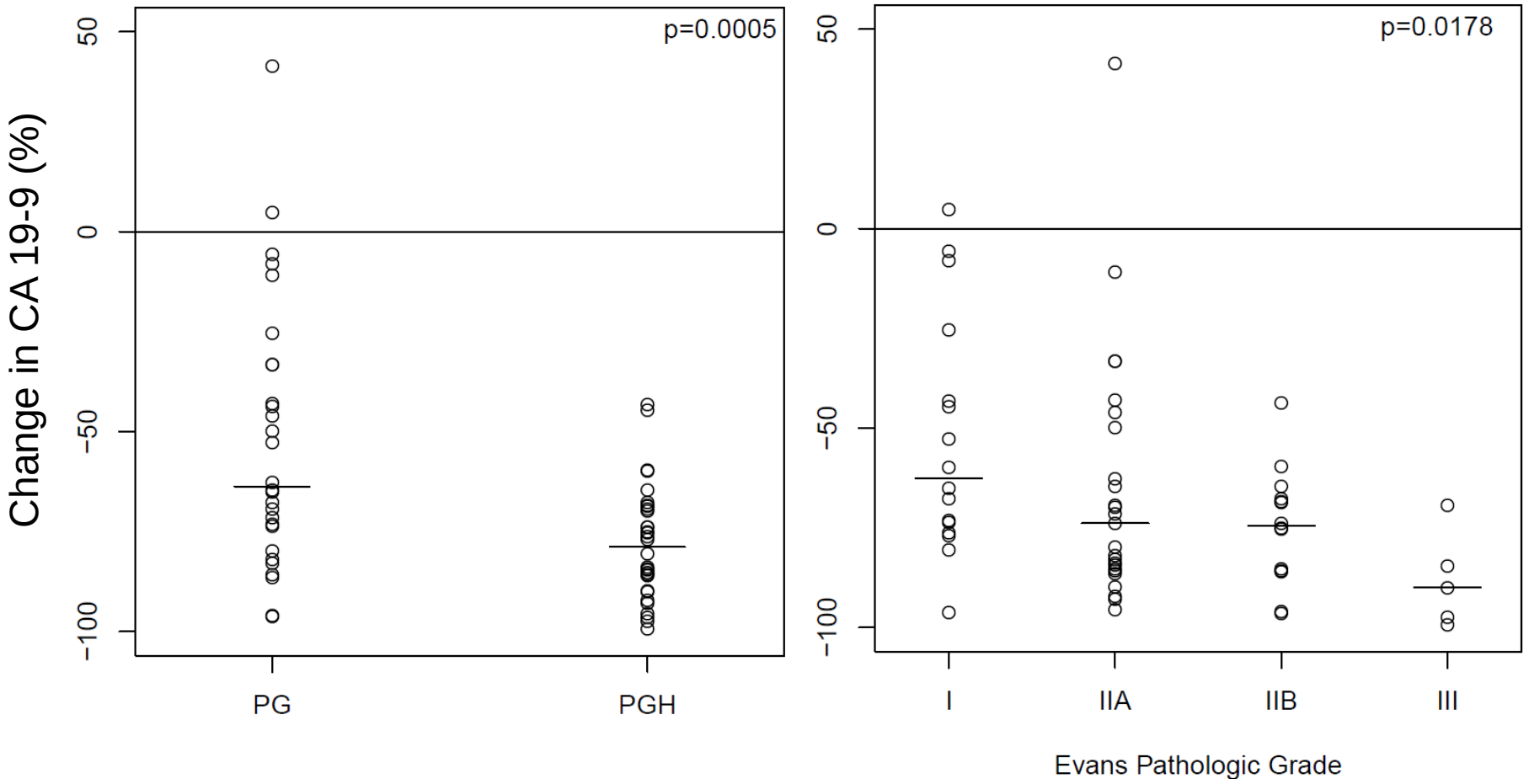


120 Patients Consented, 64 completed

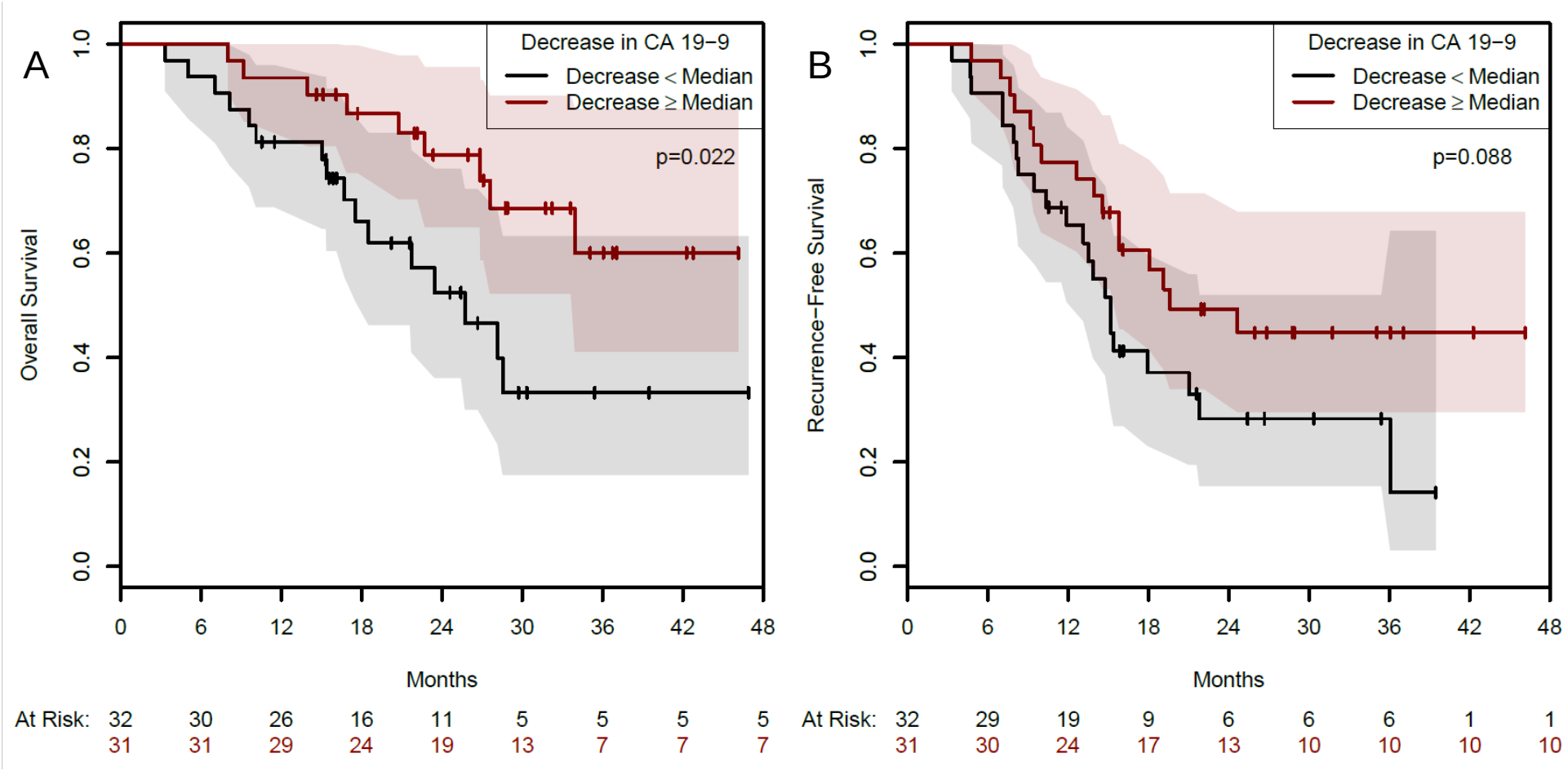


Evan's grade defines the pathologic response to chemotherapy

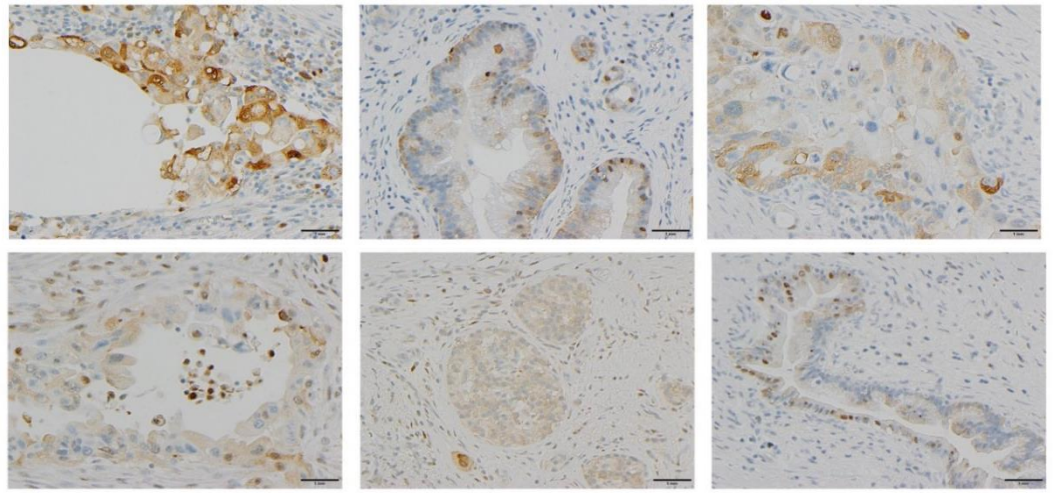
Administration Of Hydroxychloroquine (PGH) Improves CA 19-9 Response To Preoperative Gemcitabine And Nab-paclitaxel (PG) Treatment



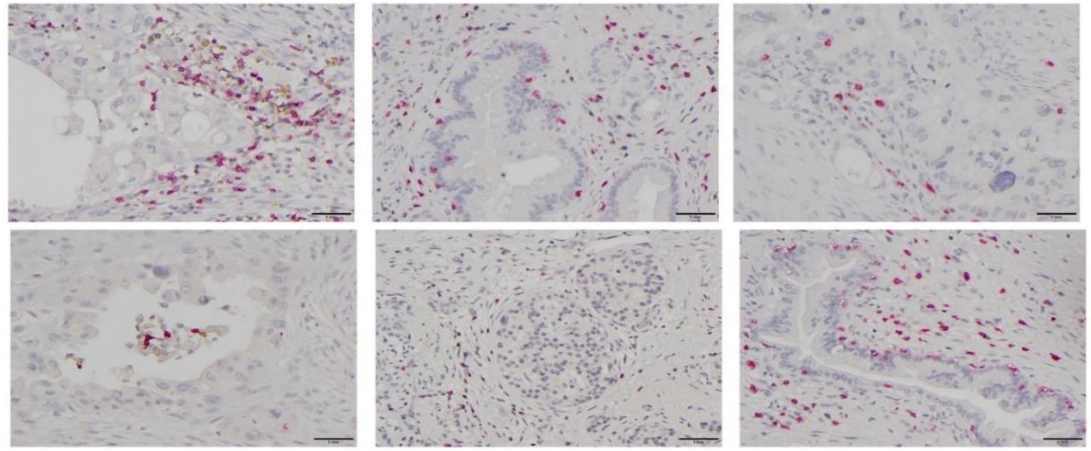
Decrease in CA 19-9 Correlates with Overall Survival



A. SQSTM1/p62



B. CD4/CD8

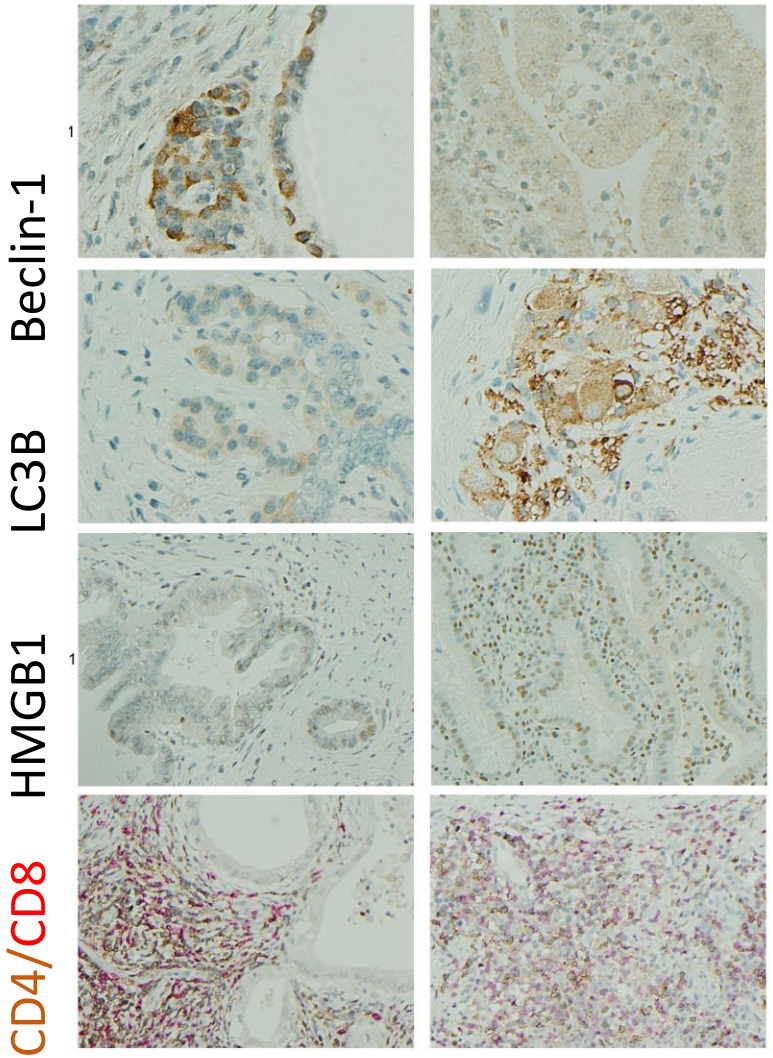


Resected Patients with increased SQSTM1/p62 expression ($p<0.001$) and tumor infiltration with lymphoid cells ($p<0.001$)

Autophagy Inhibited Based on Cytosolic SQSTM1/p62 Increase

Mean Value Reported	PG n=30	PGH n=32	p
% Cytoplasm SQSTM1	27.5	43.6	**0.028**
% Nuclear SQSTM1	28.6	36.9	0.367
% PCNA +	41.3	43.8	0.735
% CC3 +	0.84	0.83	0.960
% HMGB1 +	42.3	42.7	0.989
% Beclin 1 +	52.5	50.9	0.860
% Atg7 +	50.9	48.6	0.746
% LC3B +	31.2	28.8	0.506

A.



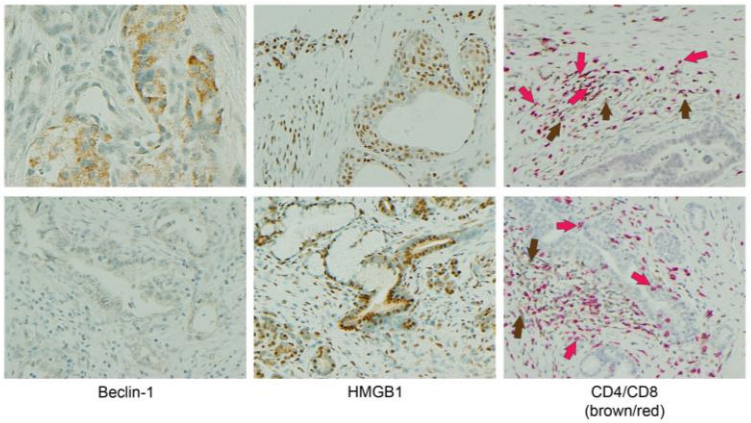
PG
Evans I
68% ↓ CA 19-9

PGH
Evans IIb
86% decrease CA 19-9

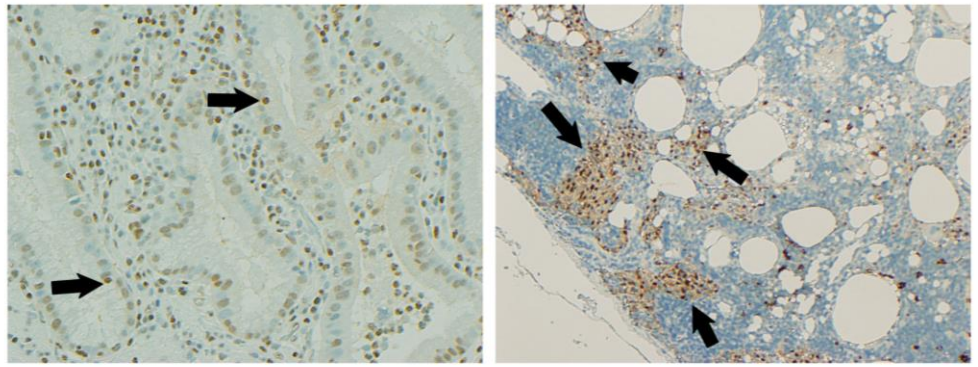
B.

PG
Evans I
8% ↓ Ca 19-9

PG
Evans IIb
96% ↓ Ca 19-9



C.



PGH
Evans IIb
86% ↓ CA 19-9
HGMB1
20x magnification

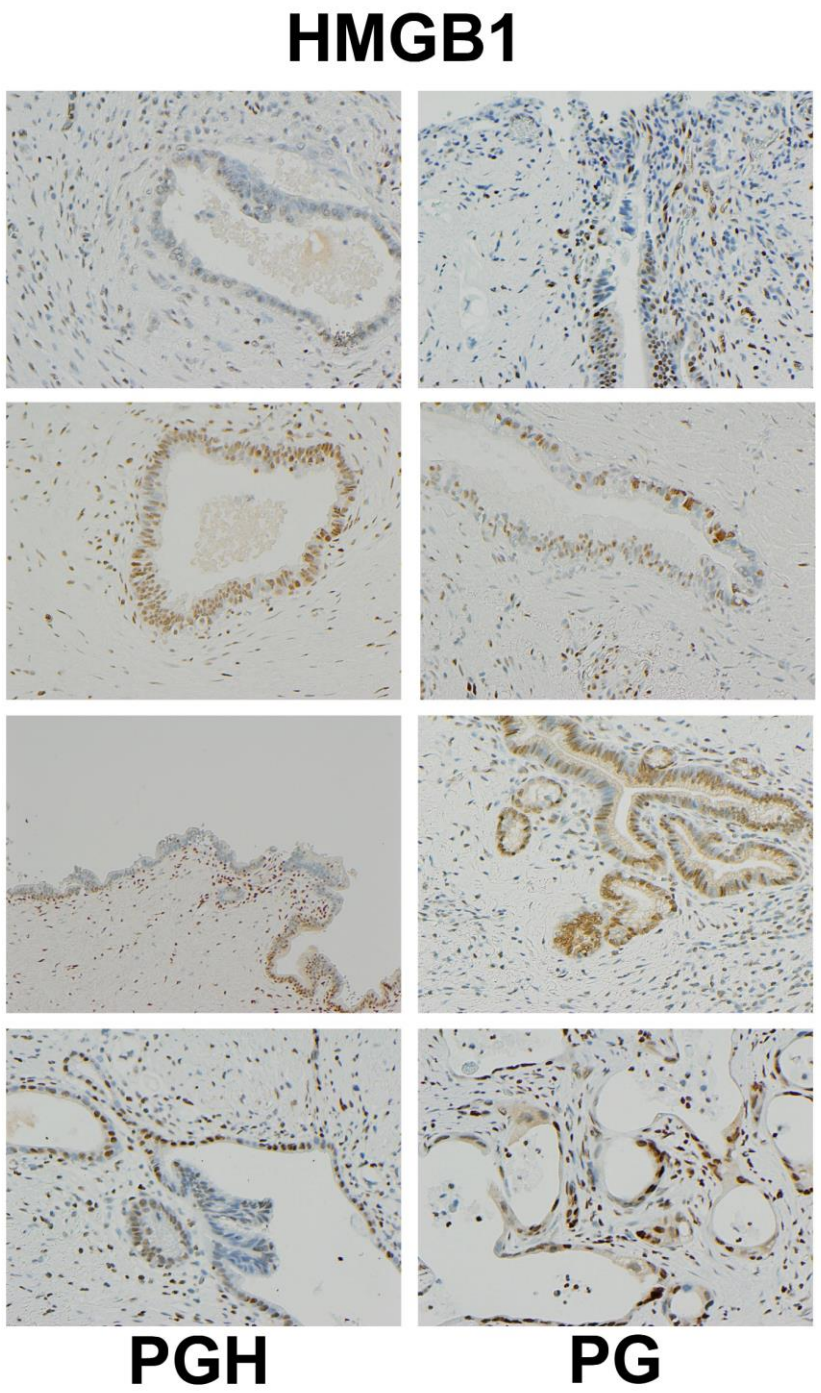
PGH
Evans III
CA 19-9 not a marker
SQSTM1/p62
10x magnification

Retained nuclear HMGB1, increased SQSTM1, tumor CD4, and tumor immune infiltration

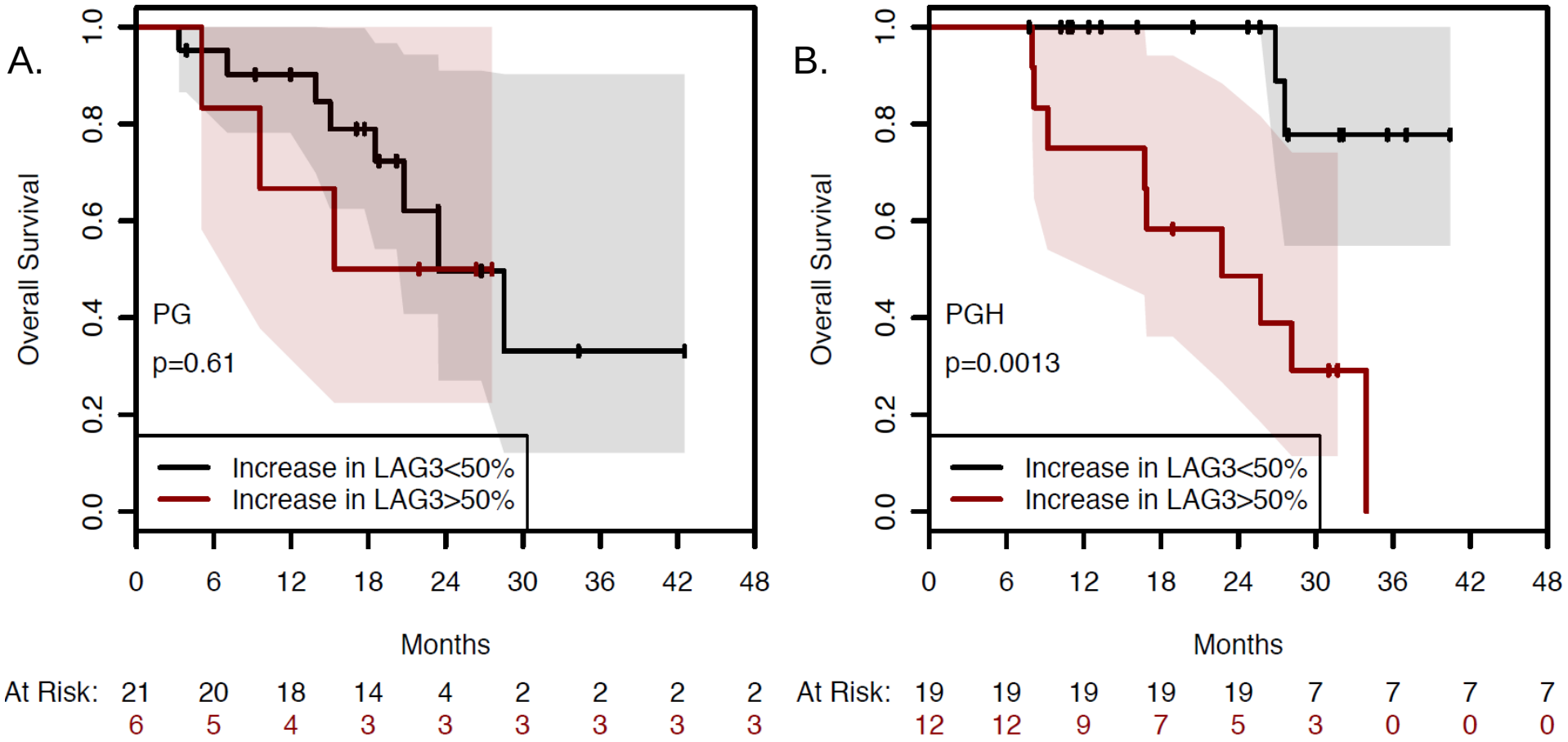
Retained Nuclear HMGB1 is Prognostic for Outcome Independent of Treatment Arm

HMGB1 has distinct compartmental roles with a nuclear role to promote DNA damage repair and sequestered with apoptotic death; cytosolic and extracellular HMGB1 is associated with autophagy and necrosis.

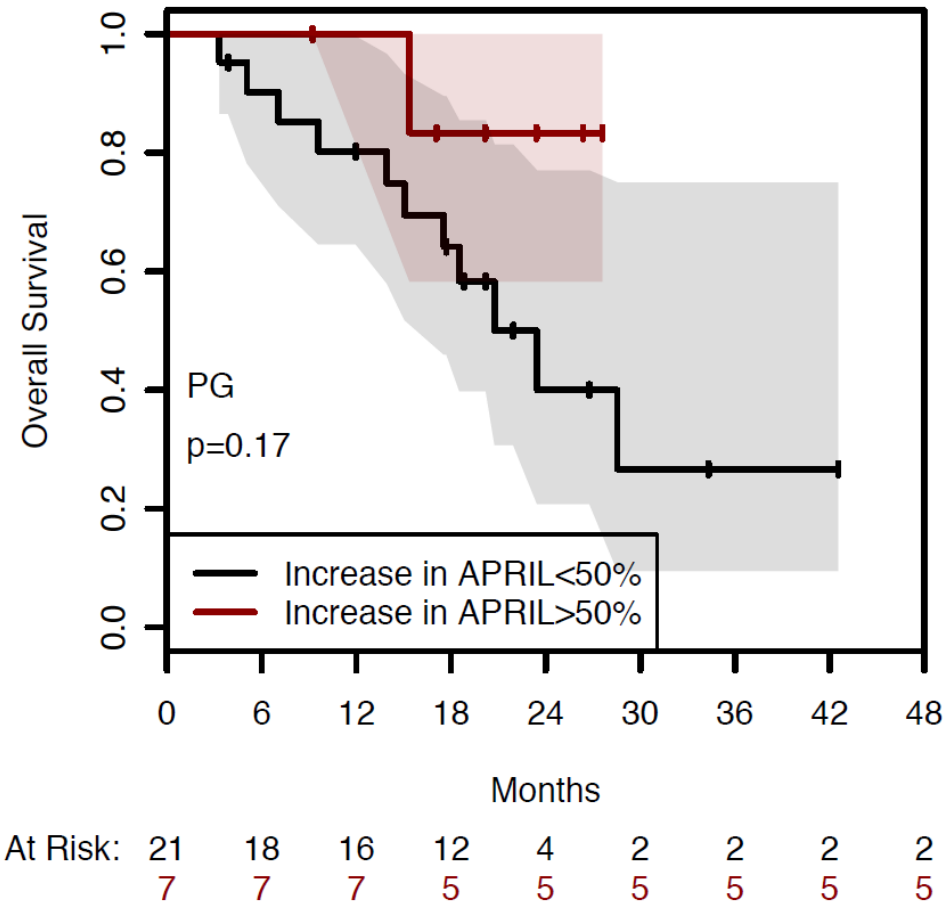
Marker	Hazard	95% CI	p-value
% CC3 +	0.36	(0.18, 0.73)	0.001
% HMGB1 +	**0.95**	**(0.93, 0.98)**	**2.08×10^{-5}**
% Atg7 +	1.02	(1.00, 1.04)	0.081
% Cyto p62 +	1.03	(1.01,1.05)	0.001
Tumor CD4	0.99	(0.97,1.00)	0.002
Tumor CD8	1.01	(1.00,1.02)	0.123
Tumor Infiltrate	0.10	(0.02,0.47)	0.001
Stroma Infiltrate	4.33	(1.82, 10.32)	0.0002
% PDL-1 +	0.77	(0.53, 1.10)	0.124

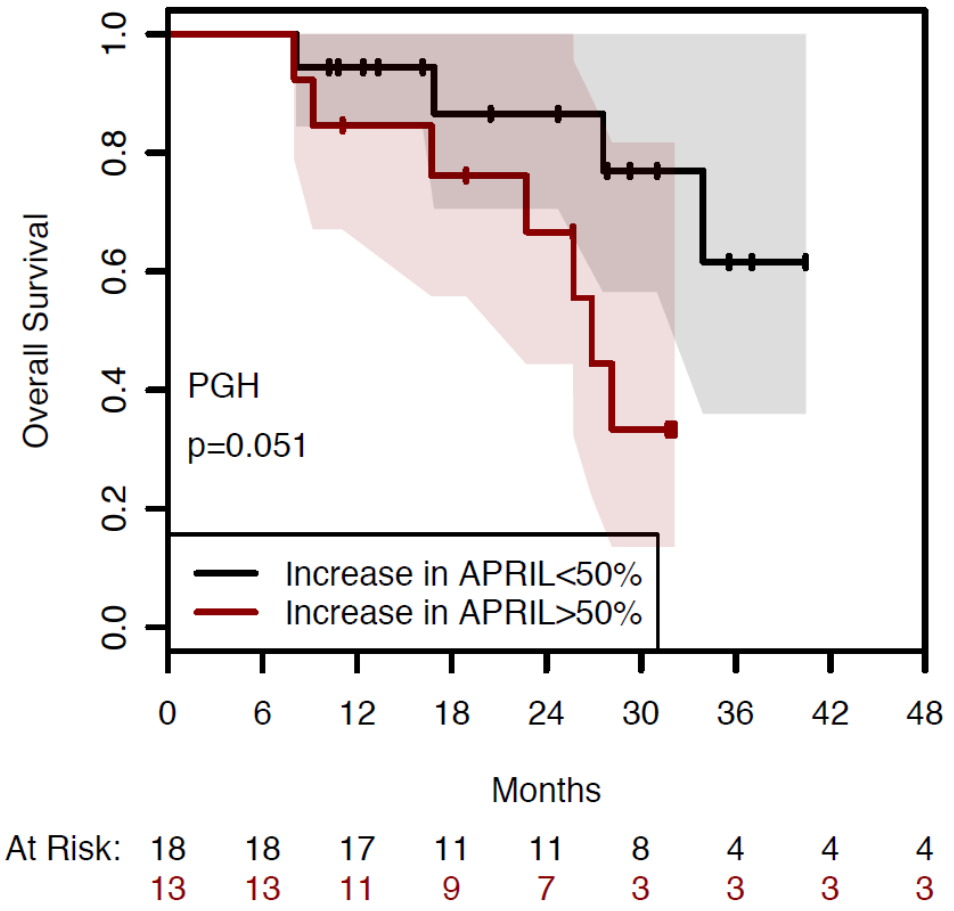


Serum soluble LAG3 Increases in PG Arm Associates with Diminished Overall Survival

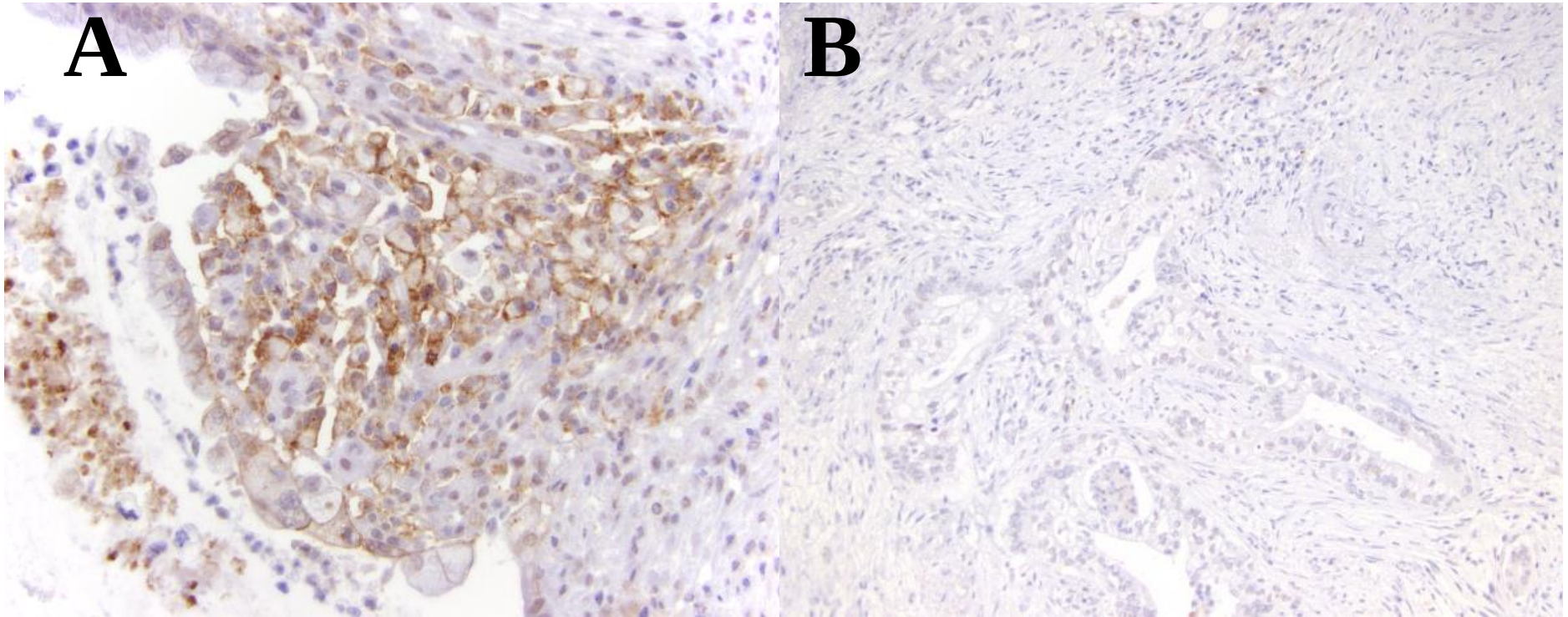


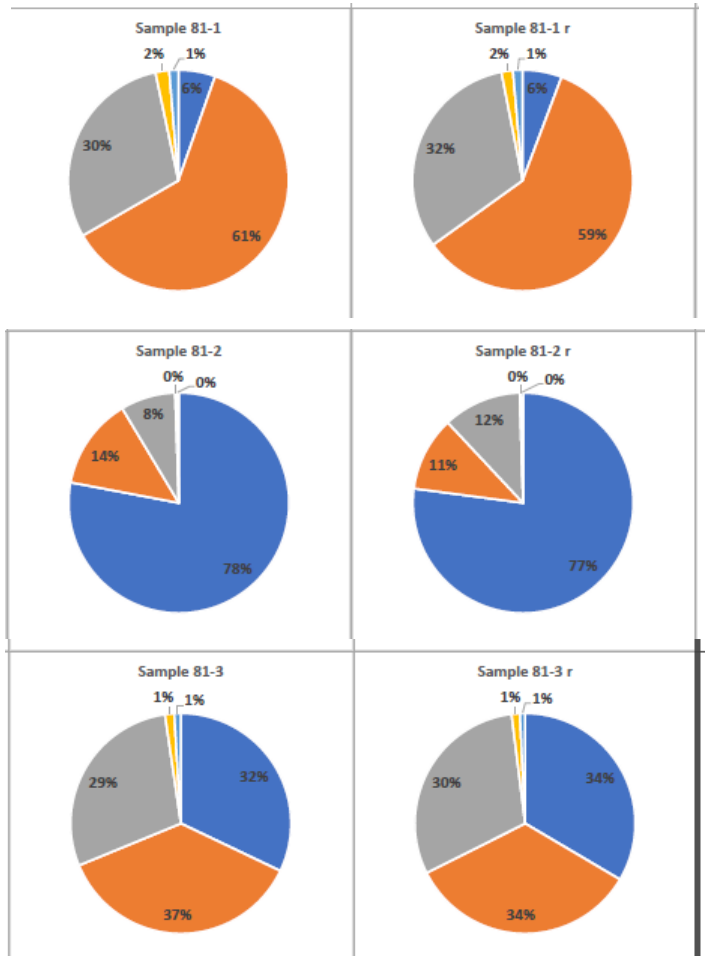
APRIL/BAFF Increases in the Serum associate with Less Survival only in PG Arm





Resected human tumor treated with chemotherapy + CQ (A) show increased PD-L1 expressing immune infiltration as compared to chemotherapy alone (B)





PreChemo

IgH mRNA
TRα
TRβ
TRγ
TRδ

PostChemo

PostOp

Immunologic Budget 13-074

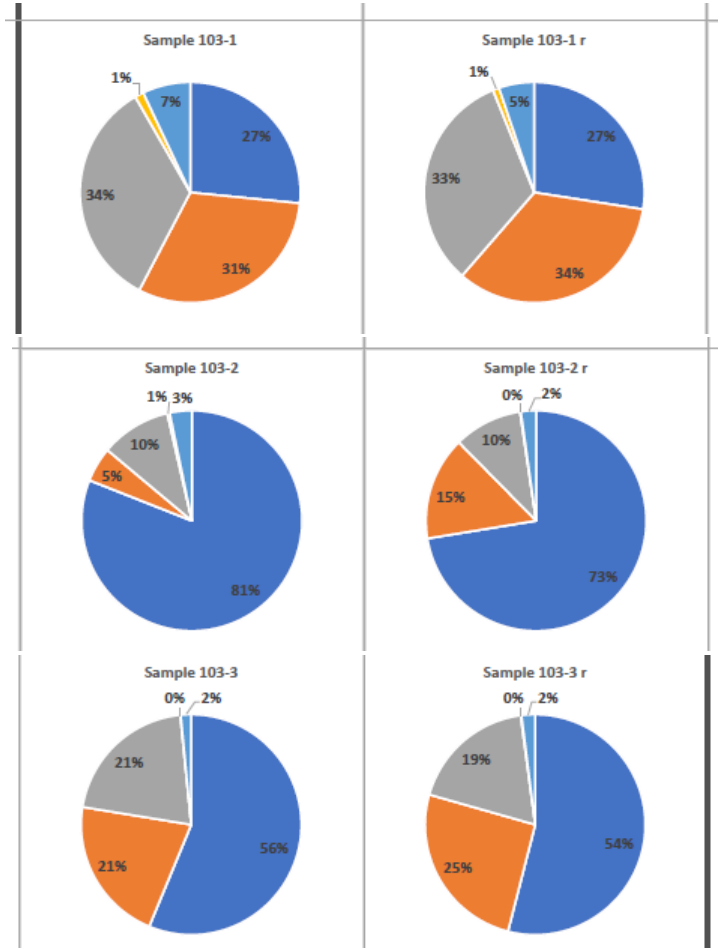
	Supplied Patient Information			
	Evans Grade Path response	Recurrence?	Death?	Resection Margin
Patient 81	No HCQ IIA	1	1	R1

Patient 81 Progressed Quickly and Died at 5 ½ Months

Patient 81 - unique CDR3 for TCR beta, with respect to time point 1

CDR3	81-1 Rank	81-1 Frequency	81-2 Rank	81-2 Frequency	81-3 Rank	81-3 Frequency
ASSSQERPNTEAF	1	2085421	1	1601175	2	1289557
ASSQEEGRVNGYT	2	1847743	2	1205289	3	648784
ASSMQGYTEAF	3	1301953	3	854743	1	1621080
SAIQGKGNEQF	4	399956	5	285054	4	230255
ASSPAGTGSNQPQH	5	320523	21	125104	5	200131
AISLGGYYEQY	6	179189	40	87741	6	174494
ASSQERGAYNEQF	7	144902	37	90680	14	39898
ASSPLKGFNEQY	8	118218	30	103274	9	102228
ASSENRFTDTQY	9	105651	7	225860	10	99024
ASSEALDEQY	10	103169	41	87321	8	118732

Pre-Chemo **Post-Chemo** **Post-op**



PreChemo

IgH mRNA
TRα
TRβ
TRγ
TRδ

PostChemo

PostOp

Immunologic Budget 13-074

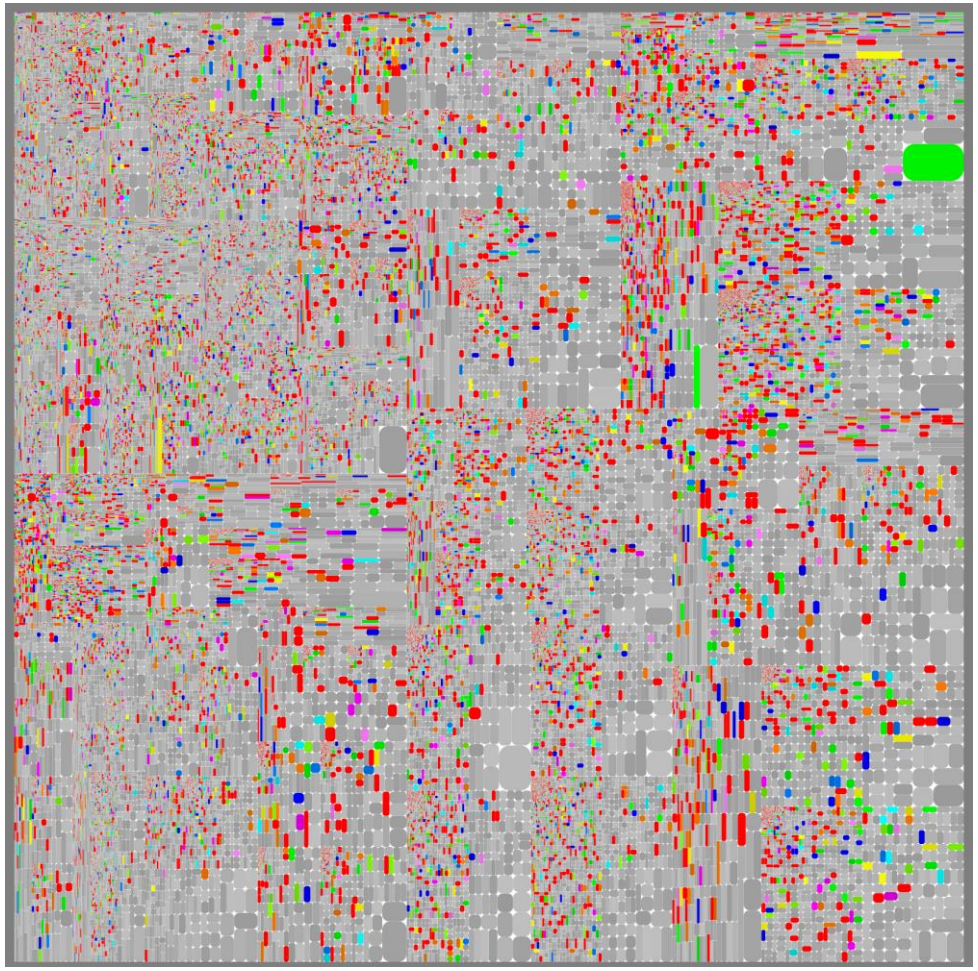
	Supplied Patient Information			
	Evans Grade Path response	Recurrence?	Death?	Resection Margin
Patient 103	+HCQ IIB	1	0	R1

Patient 103 Had an R0 Resection and Has Not Recurred

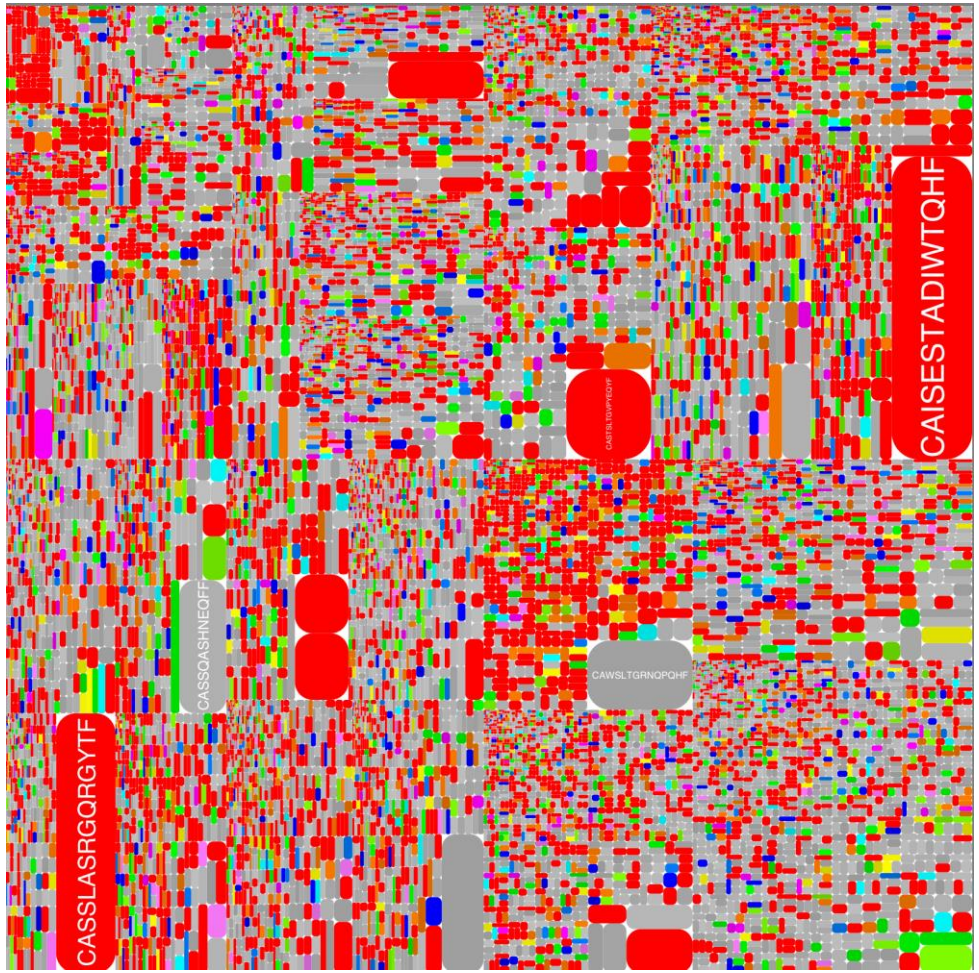
CDR3	Rank 103-1	103-1	Rank 103-2	103-2	Rank 103-3	103-3
SAGPGLEEYNEQF	1	1629720	1	3808674	104	395
ASSKQGSTEAF	2	505738	2	1059239		
SARDPSGPGYT	3	118236				
ASSDSLEKDNEQF	4	92643	7	629043		
ATSTQGPSSYNEQF	5	43722				
ASSITSGDYNEQF	6	43588				
SAGSTSGGTSSYNEQF	7	43055				
SVEGGTGITDTQY	8	37590				
SASRPGDQPQH	9	35590				
ASSLRENQPQH	10	33191				
AIIRDRGRNEKLF			3	731971		
ASSFLSAPLH			4	682897		
ASSEVSTNEQF			5	665052		
SVLRTFGQAF	40	21461	6	653898		
SARGSVANSNYGYT			8	506676		
SVRLQGVGNQPQH			9	412670		
ASSQDADGTGCGTYQRAPRTDTQY			10	346706		
ASSQAVNSNQPQH					1	374369
ASSLGYRPYSNQPQH					2	192623
AISEFQGFNEKLF					3	191436
AISYRVNYGYT					4	182932
ASSPGQGEGYEQY					5	128349
ASSLAGNNQPQH					6	97300
ASSVELAGSTDTQY					7	93938
AISVRQGRGYT					8	79106
ASSTGTGIYEQY					9	63087
ASSQEVRYEQY					10	56363

Pre-Chemo **Post-Chemo** **Post-op**

Publicity Index

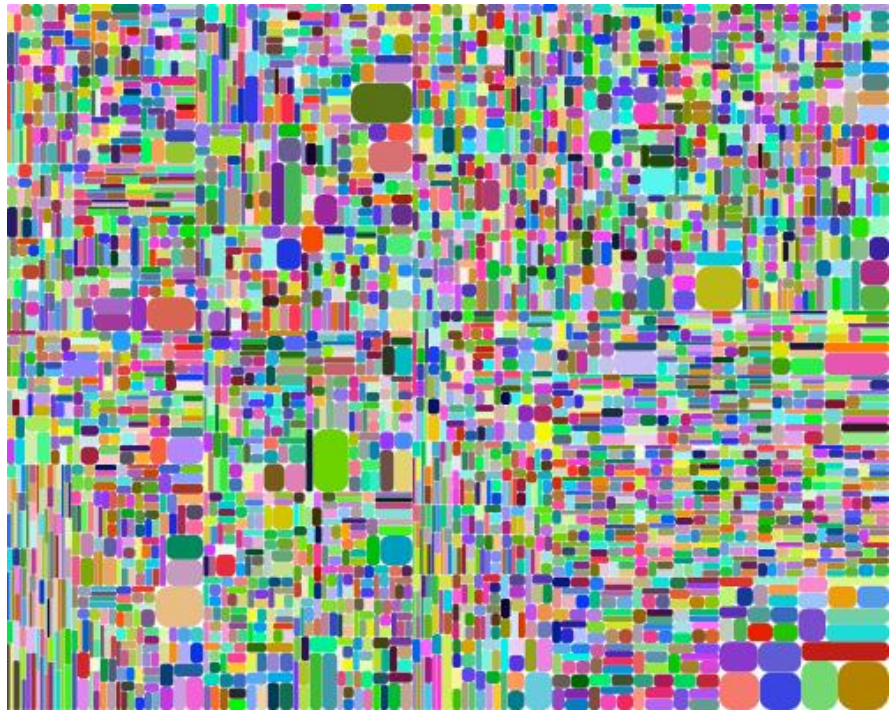


Cord blood

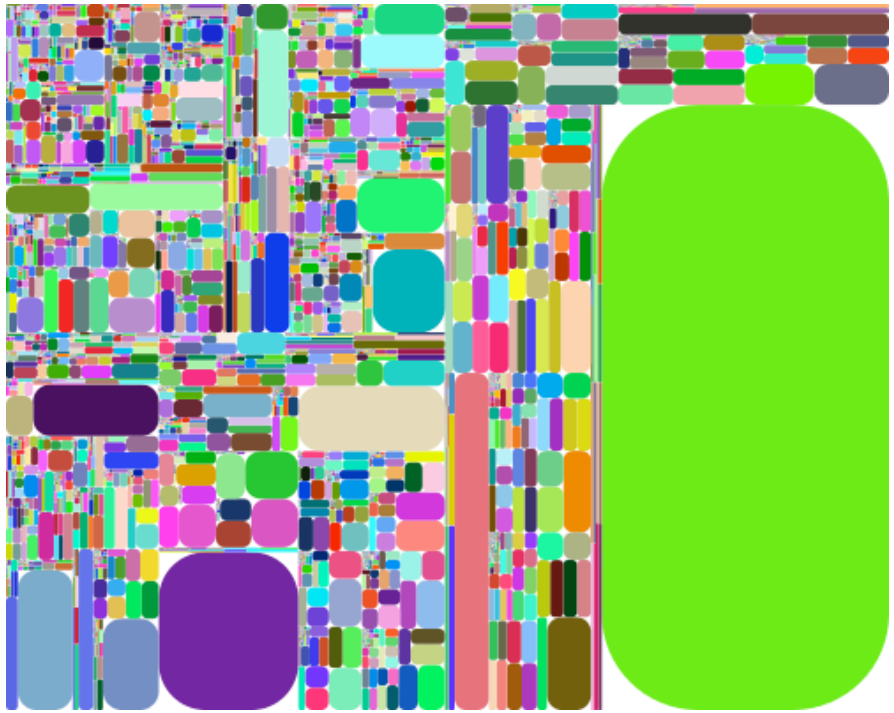


Healthy adult blood.

Diversity Index

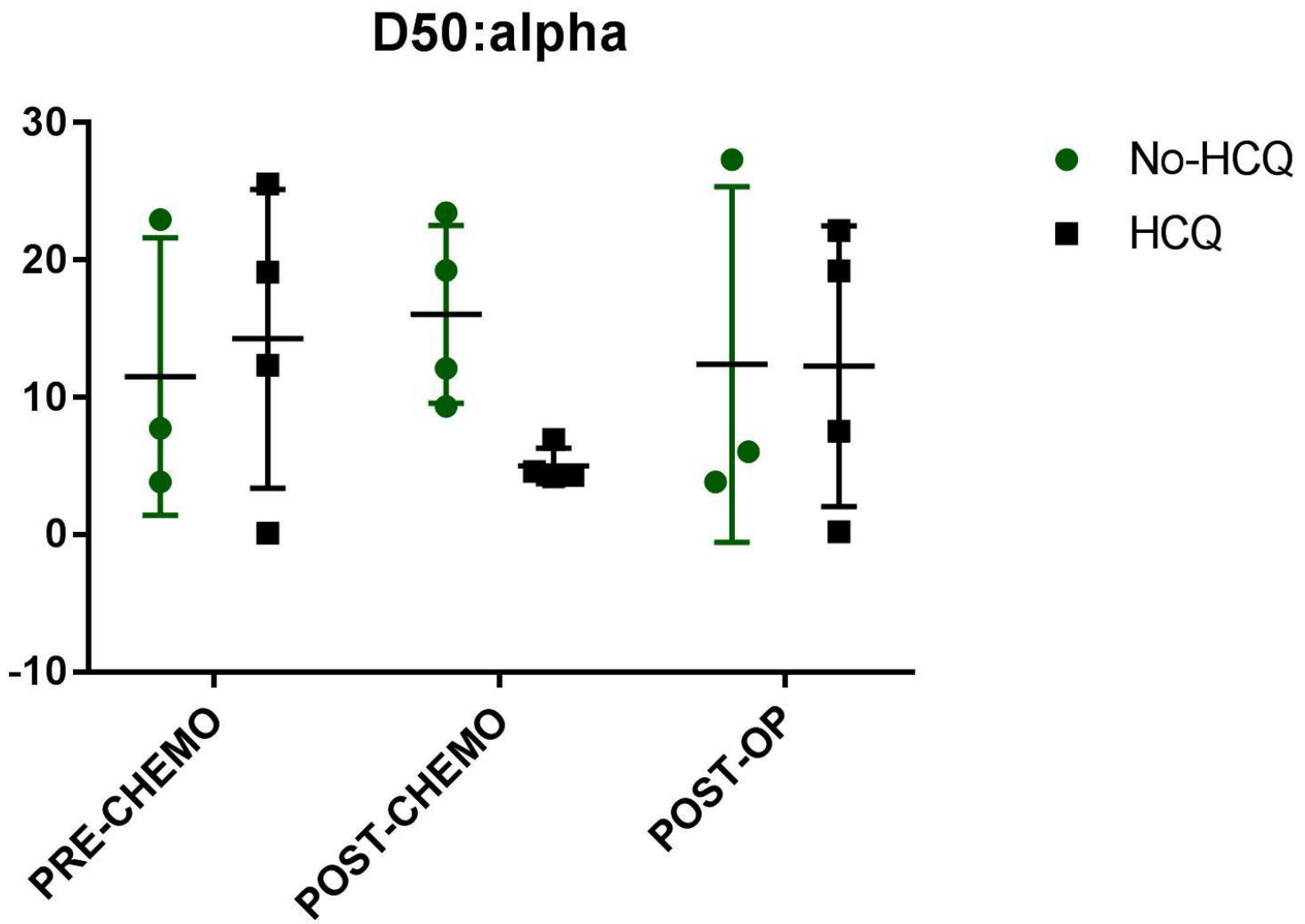


D50: 28



D50: 0.2

D50 Alpha Chain Falls During Chemotherapy in Those on HCQ



Summary

- Better mPCR, better NGS library, better results.
- Detecting signals require inclusive, quantitative, and complete repertoire analysis.
- Identify noise, avoid noise, remove noise.
- Macro- and Micro- indexes for clinical applications.
- “知己知彼，百戰不殆“—Victory is secured only when you know your own strength as well as your enemy’s weakness.

Acknowledgement

Dr. Chunlin Wang
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Dr. Peter Mannon (IBD)
Dr. Antoine Blancher (CMV)
Dr. JinXiong She (T1D)
Dr. XinChun Chen (TB)
Dr. James Yang (Liver Cancer)
Dr. Alex Vasquez (Heart Disease)