Immunogenomics/immunopharmacogenomics: exploring our immune system

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Immunogenomics & Immunopharmacogenomics

Immunogenomics:

A field which uses genomics tools such as next generation sequencing to unravel the complexity of the human immune system including TCR, BCR and HLA

+

Pharmacogenomics:

A field which applies genetic/genomic information (germline variation, somatic mutation, gene expression etc.) for better understanding of drug response

↓

Immunopharmacogenomics

Immune system

First line of defense

Protect the body from harmful substances

Innate Immunity

- Epithelial Barrier
- Phagocytes
- Natural Killer cells

Adaptive Immunity

- T cells
- B cells

T cell receptor (TCR)

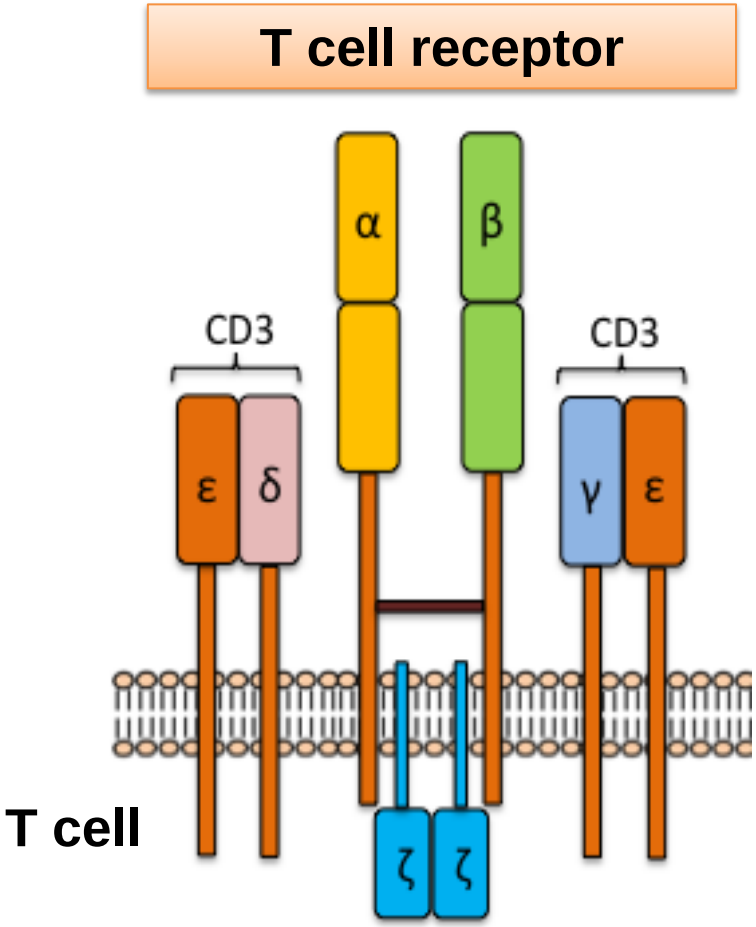
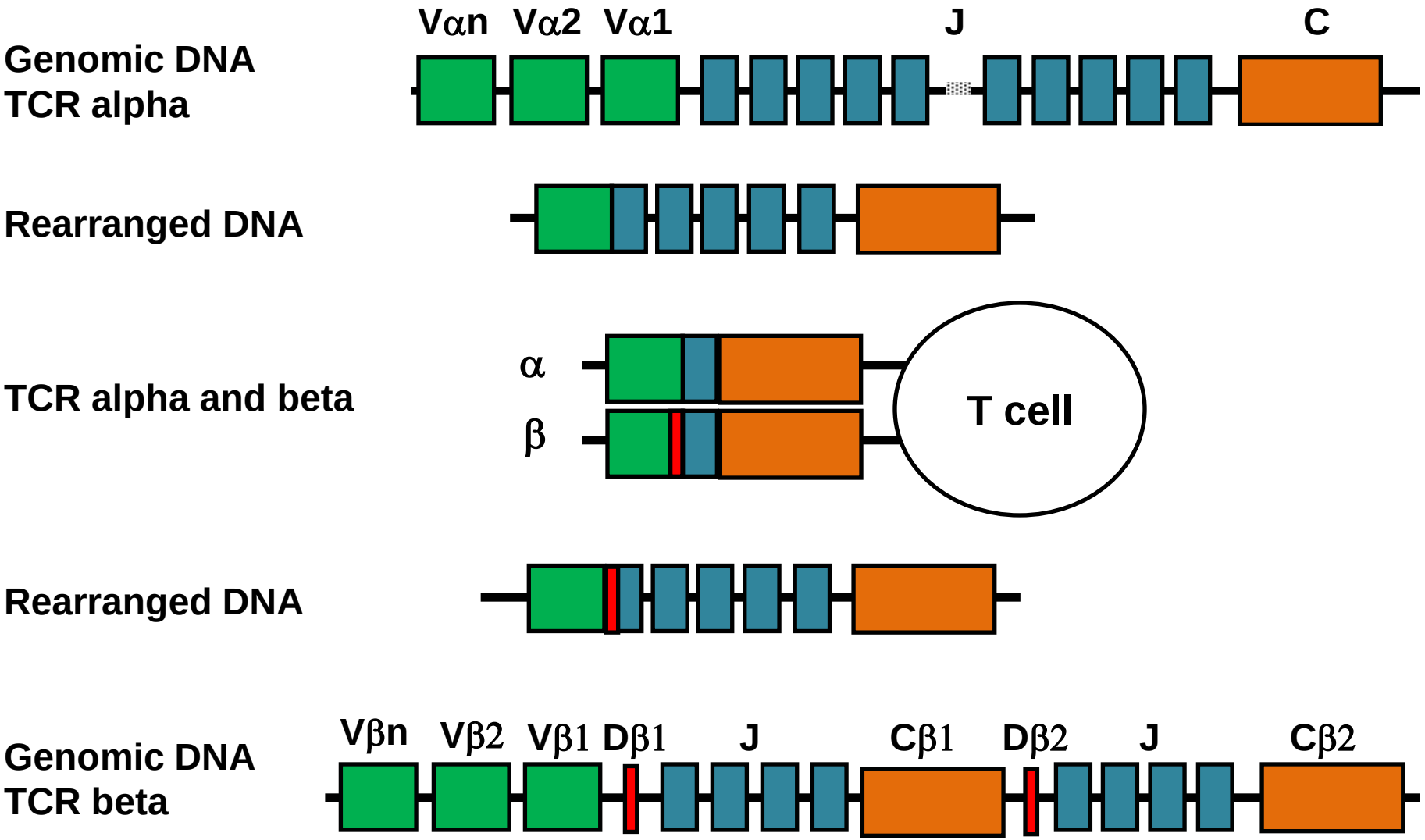


Figure from Immunopharmacogenomics, Springer

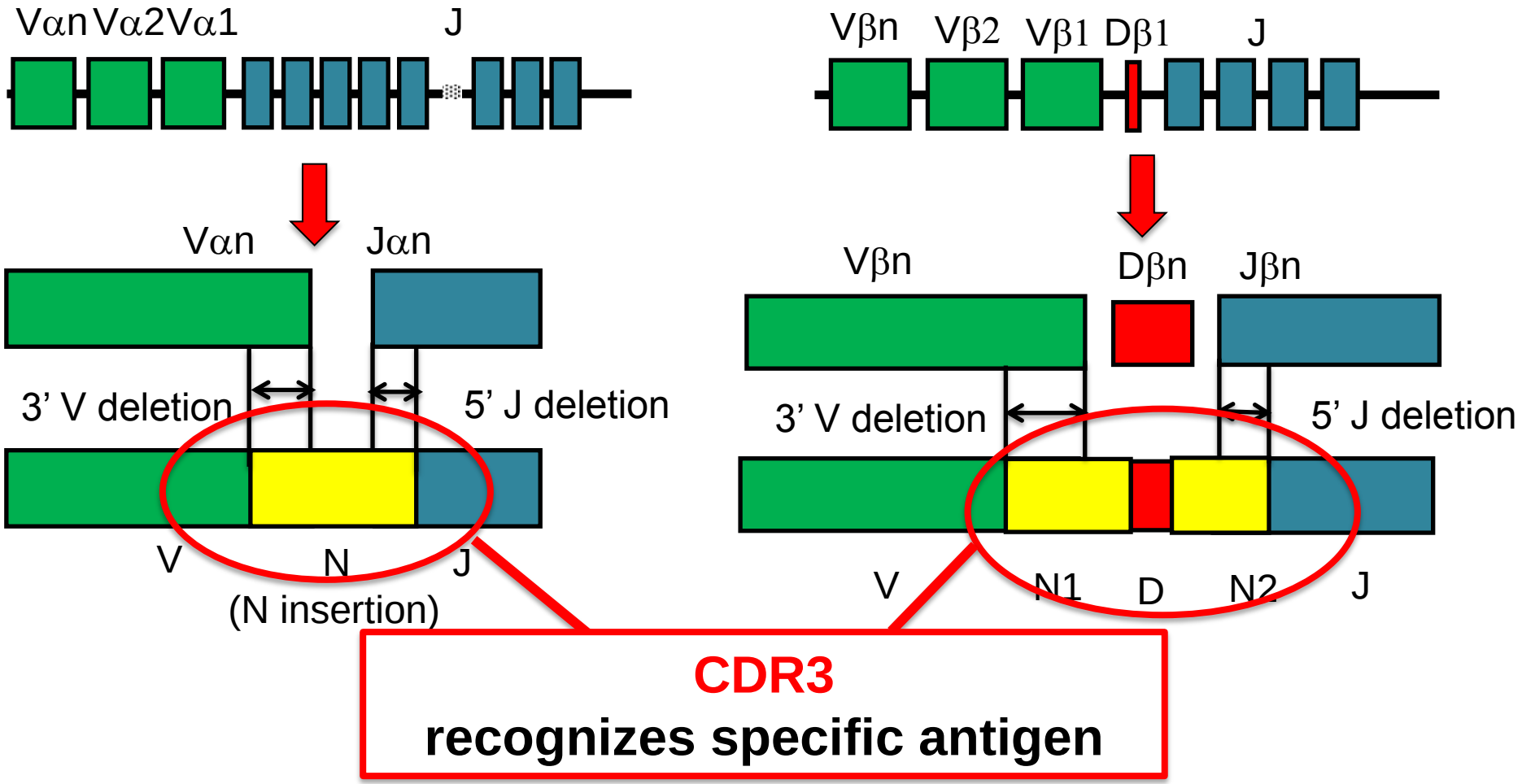
- **T cells**
  - involved in immune system
- **T cell receptor (TCR)**
  - Expressed on the surface of T cells
  - Recognition of antigen
  - Heterodimer (α+β or δ+γ linked together by a disulfide bridge)

Rearrangement of TCR

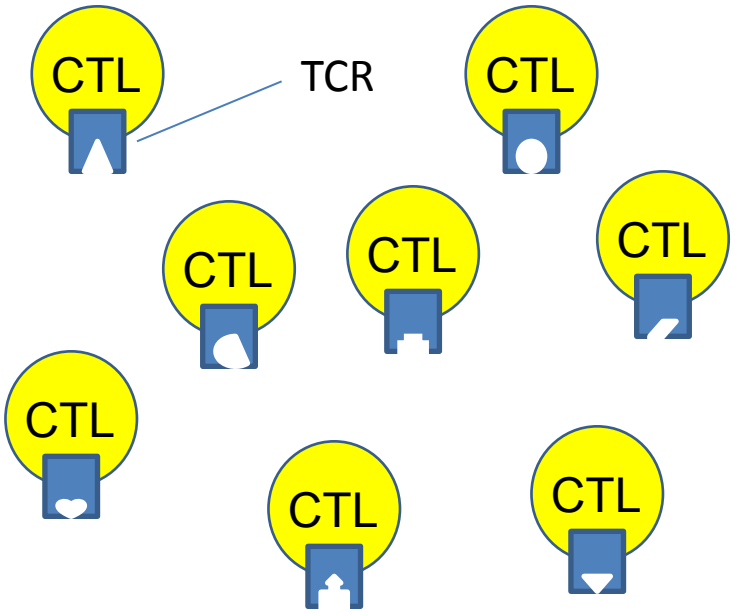


Rearrangement of TCR

- During rearrangement, nucleotides are deleted from V(D)J exons and/or inserted between VJ (alpha), or VD and DJ (beta) junctions.



Characterization of enormous individual differences in our immune responses



Each CTL has a unique TCR.

Millions of different T cells with unique TCRs

Number of unique T cells in our body = ???

The differences in T cell repertoire influence the response of various cancer treatments and are associated with various human autoimmune diseases.

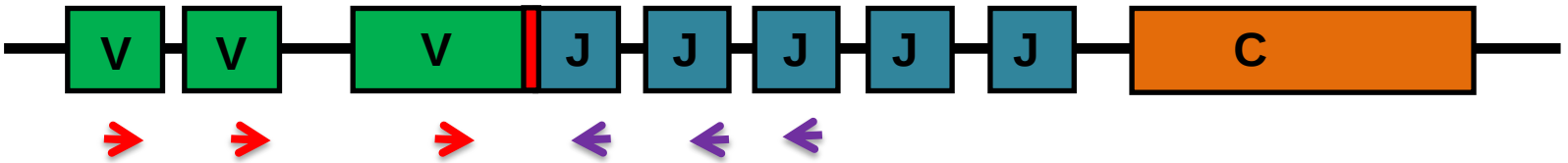
Next Generation Sequencing (NGS)

- **characterize millions of TCRs**

gDNA-based vs cDNA-based TCR sequencing

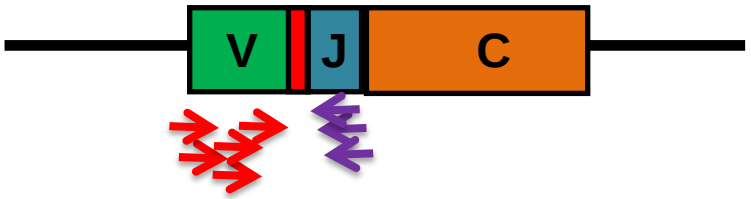
(a) Genomic DNA

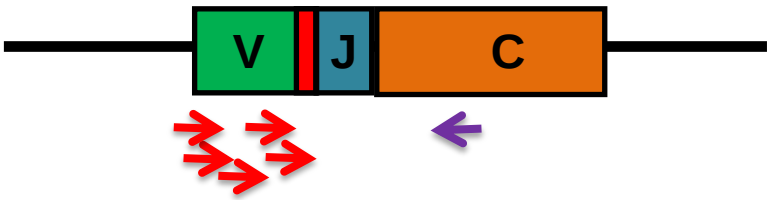
Multiplex PCR:
V and J specific primers



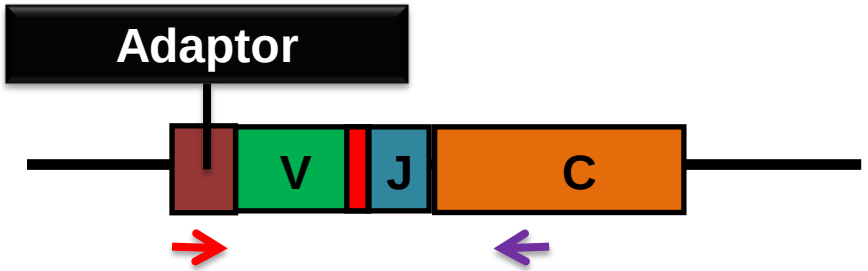
(b) mRNA / cDNA

Multiplex PCR:
V and J specific primers OR
V and C specific primers





5' RACE PCR:
Adaptor and C specific primer

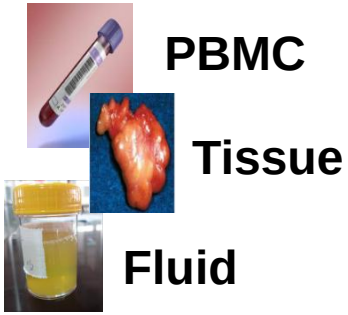


gDNA-based vs cDNA-based TCR sequencing

	Genomic DNA	mRNA・cDNA	
TCR-specific PCR Amplification	Multiplex PCR (V and J specific primers)	Multiplex PCR (V and J specific primers or V and C specific primers)	5’ RACE PCR (C and adaptor specific primers)
PCR bias	High	High	Low
Novel exons	Not detectable	Not detectable	Detectable
T cells in Tissue	High background	Low background	Low background
Functionality	No	Reflected	Reflected
Quantification of T cells	Yes	Low	Low
Analysis of paraffin-fixed tissue	Yes	Hard	Hard

TCR sequencing - workflow

Samples collection

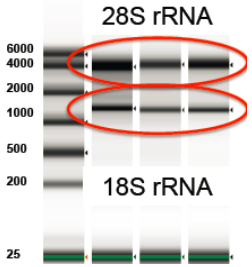


PBMC

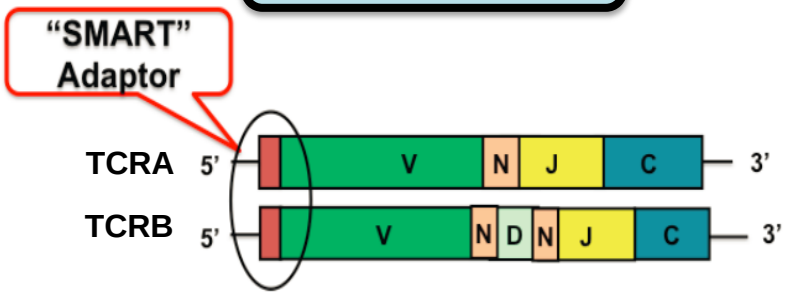
Tissue

Fluid

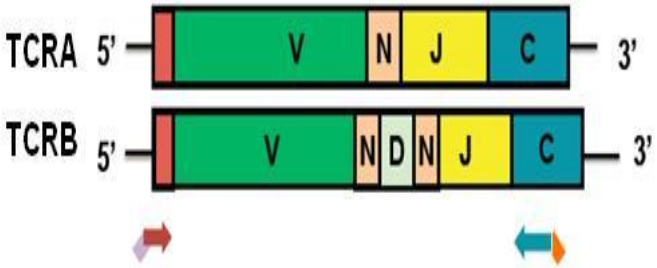
RNA extraction



cDNA synthesis



PCR



Sequencing

Machine: Illumina MiSeq
Read length: 2 X 300bp
Run time: ~ 56 hours

TCR analysis - CDR3 determination

Reads (fastq file)

V(D)J decomposition

- Reference = IMGT database
- Mapping to V, D, J, C exons
- Analysis of junction sequences between V, D and J

Mapped reads

CDR3 determination and clonotyping

TCRA analysis

VNJ decomposition of TCRA

TRAV	TRAJ	VdelNum	JdelNum	N
TRAV26-1	TRAJ17	1	3	-
TRAV10	TRAJ42	4	7	GG

CDR3 determination of TCRA

TRAV	TRAJ	CDR3 sequences	Count
TRAV26-1	TRAJ17	CIVRVKAAGNKLTF	12320
TRAV10	TRAJ42	CVVGGGSQGNLIF	2031

- **VdelNum = number of nucleotide deleted at 3' of V segment**
- **JdelNum = number of nucleotide deleted at 5' of J segment**
- **N = the nucleotides added during VJ rearrangement**
- **Count = The observed reads for a specific combination of V, J and CDR3 sequences**

TCRB analysis

VNJ decomposition of TCRB

TRBV	TRBD	TRBJ	Vdel Num	Jdel Num	N1	D	N2
TRBV6-1	TRBD2	TRBJ2-2	6	1	A	*GGACTAG*******	T
TRBV4-1	TRBD1	TRBJ2-7	4	1	TTCTCCGG	GGGACAGGG***	-

CDR3 determination of TCRB

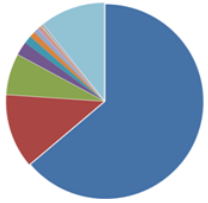
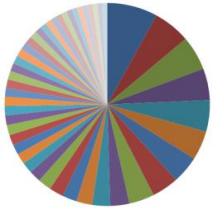
TRBV	TRBJ	CDR3 sequences	Count
TRBV6-1	TRBJ2-1	CASRGLVNTGELFF	10500
TRBV4-1	TRBJ2-7	CASSLLRGTGSYEQYF	2230

- **VdelNum = number of nucleotide deleted at 3' of V segment**
- **JdelNum = number of nucleotide deleted at 5' of J segment**
- **N1 = the nucleotides added during VD rearrangement.**
- *** in the D segment indicated the deleted nucleotides during rearrangement.**
- **N2 = the nucleotides added during DJ rearrangement.**
- **Count = The observed reads for a specific combination of V, J and CDR3 sequences**

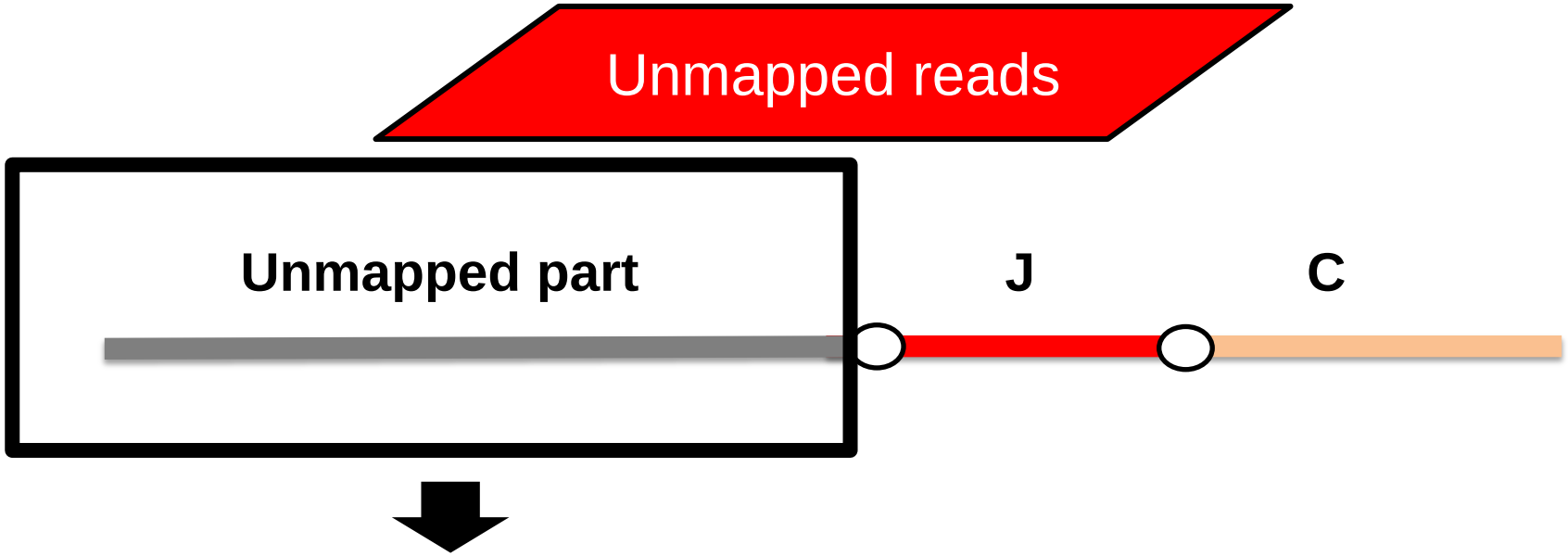
Evaluation of TCR diversity and clonality

- **Diversity Index:**
  - A quantitative measure that reflects how many different types (unique clones) there are in a dataset

$$D_S = \left[\frac{\sum_{i=1}^{K} n_i(n_i - 1)}{N(N-1)}\right]^{-1}$$

		
Clonal expansion	**High**	**Low**
Diversity (*1/D*$_S$)	**Low**	**High**

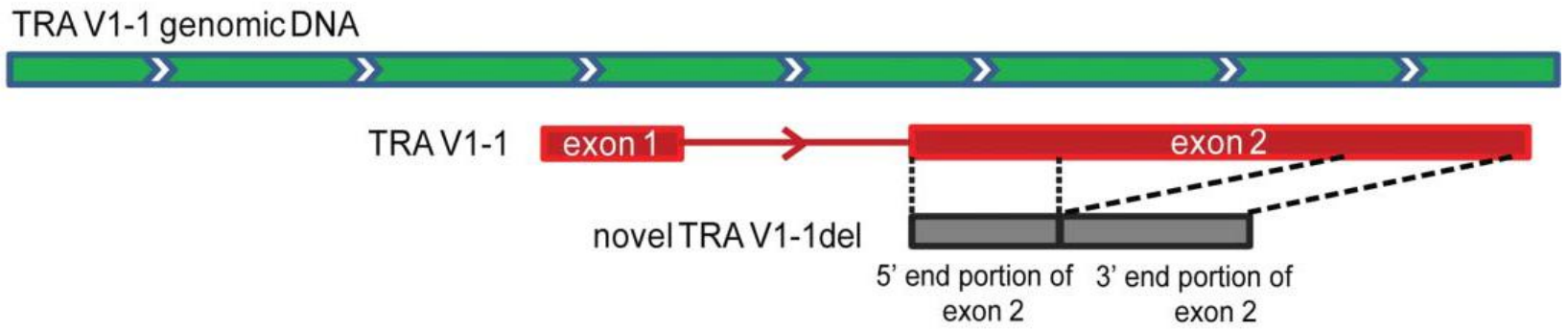
TCR analysis – Unmapped reads



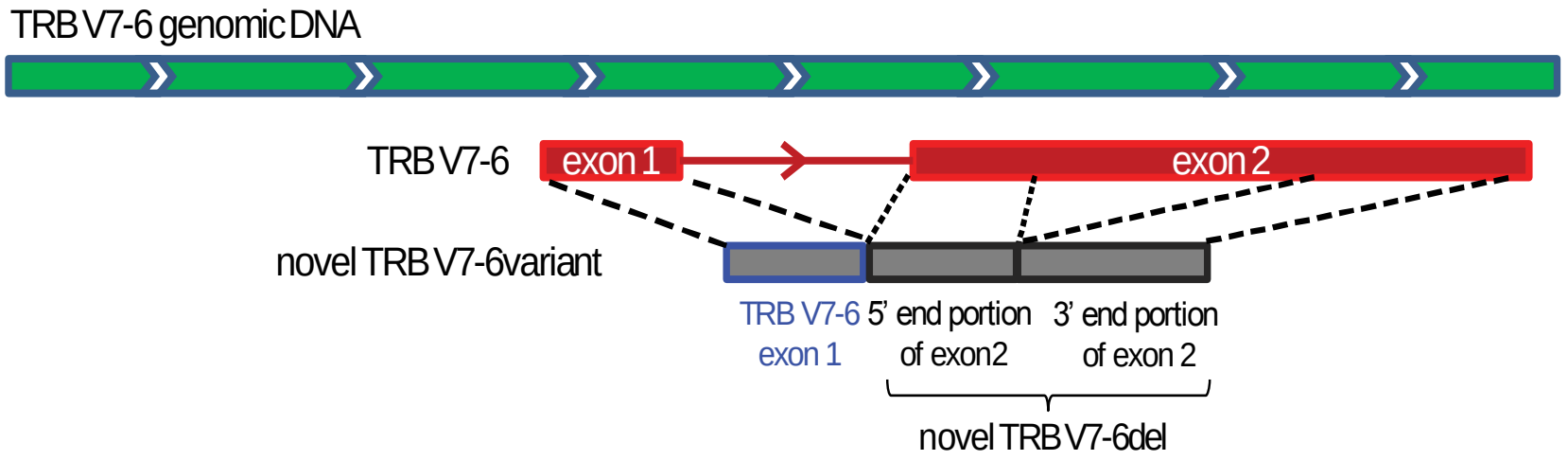
- Remapped to reference genome including intronic region
- May identify novel exon which are not deposited in the reference database
- May discover abnormalities in V(D)J recombination

Examples: Unmapped part analysis

a)



b)



Fang H., et al. Oncoimmunology, 2014

Publications from the University of Chicago

TCR analysis of cancer patients treated with cancer peptide vaccines

Fang H, Yamaguchi R, Liu X, et al. OncoImmunology, 2014
Quantitative T Cell Repertoire Analysis by Deep cDNA Sequencing of T Cell Receptor α and β Chains using Next-Generation Sequencing (NGS)

Tamura K, Hazama S, Yamaguchi R, et al. Oncology Letters, 2015
Characterization of T cell repertoire in tumor tissues and blood in advanced colorectal cancers through deep T cell receptor sequencing.

TCR analysis of cancer patients

Jang M, Yew PY, Hasegawa K, et al. OncoImmunology, 2015
Characterization of T cell repertoire of blood, tumor and ascites in ovarian cancer patients using next generation sequencing.

Liu X, Venkataraman G, Lin J, et al. OncoImmunology, 2015
Highly clonal T cell receptor repertoire among regulatory T cells in follicular lymphoma tissues – correlation with the CD8+ T cell receptor repertoire

Choudhury NJ, Kiyotani K, Yap KL et al. European Urology Focus, 2015
Low T-cell Receptor Diversity, High Somatic Mutation Burden, and High Neoantigen Load as Predictors of Clinical Outcome in Muscle-invasive Bladder Cancer

TCR analysis of hematopoietic stem cell transplant recipients

Yew PY, Alachkar H, Yamaguchi R, et al. Bone Marrow Transplantation, 2015
Quantitative characterization of T cell repertoire in allogeneic hematopoietic stem cell transplant recipients.

TCR analysis of autoimmune diseases

Chapman CG, Yamaguchi R, Tamura K, et al. Inflammatory Bowel Diseases, in press, 2016
Characterization of T-cell Receptor Repertoire in Inflamed Tissues of Patients with Crohn's Disease through Deep Sequencing

Review paper

Choudhury NJ and Nakamura Y. Cancer Science, in press, 2016
Importance of immunopharmacogenomics in cancer treatment: Patient selection and monitoring for immune checkpoint antibodies

TCR sequencing projects: Characterizing T cell repertoire in:

1. Allogeneic hematopoietic stem cell transplant (HSCT) recipients
2. Patients with Crohn's Disease
3. Patients with Follicular lymphoma
4. Patients with Muscle-invasive Bladder Cancer

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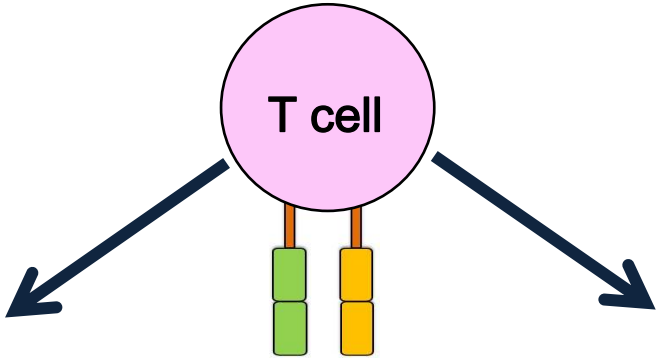
Quantitative characterization of T cell repertoire in allogeneic hematopoietic stem cell transplant (HSCT) recipients

Yew PY, Alachkar H, Yamaguchi R, Kiyotani K, Fang H, Yap KL, Liu HT, Wickrema A, Artz A, van Besien K, Imoto S, Miyano S, Bishop MR, Stock W, Nakamura Y.

Bone Marrow Transplantation, 2015, 50(9):1227-1234

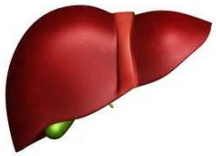
Hematopoietic Stem Cell Transplantation (HSCT)

- **HSCT = the most effective therapy for patients with AML**



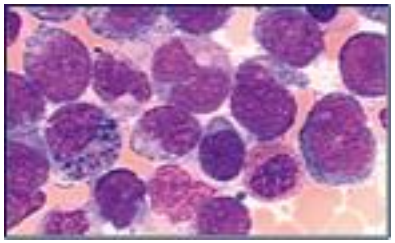
GVHD

Damage



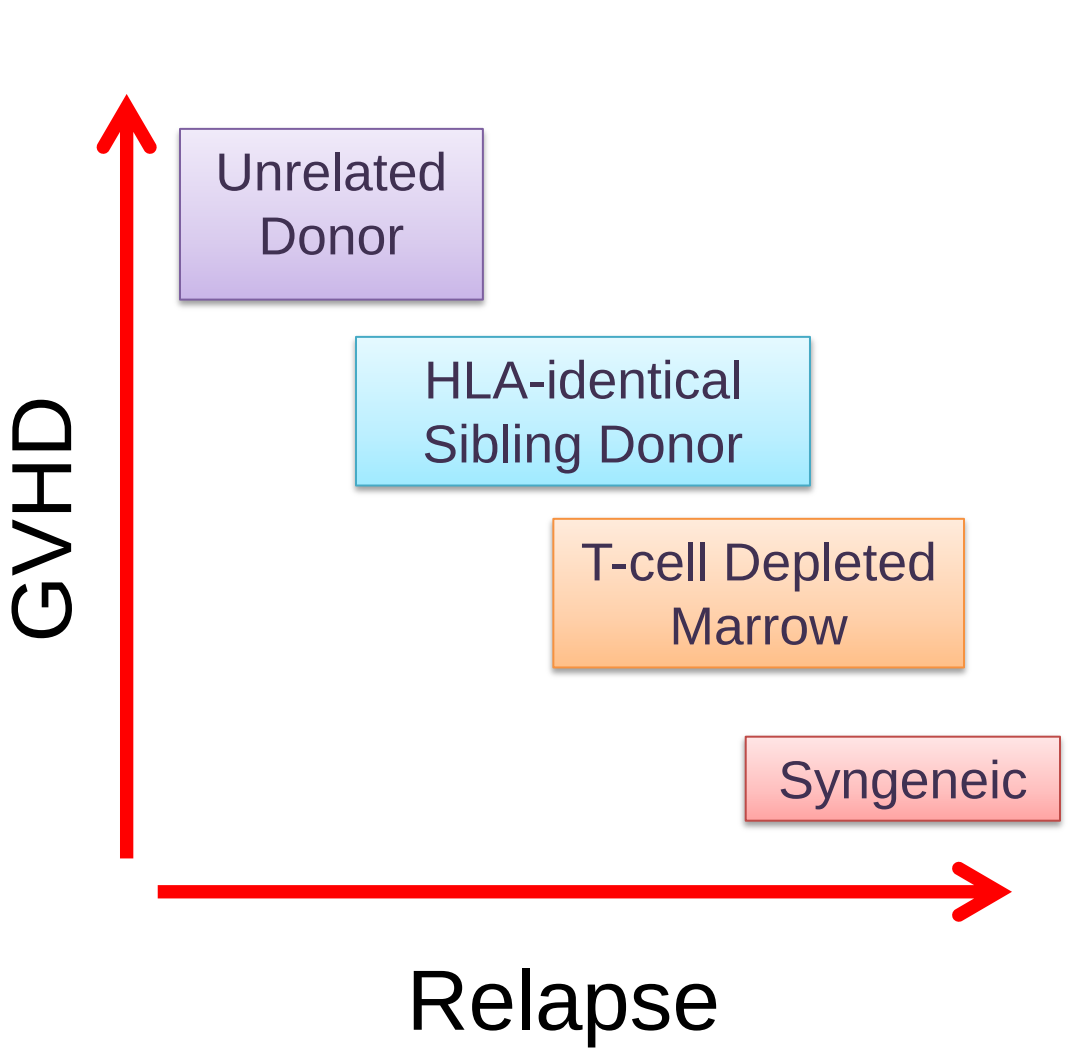
GVL

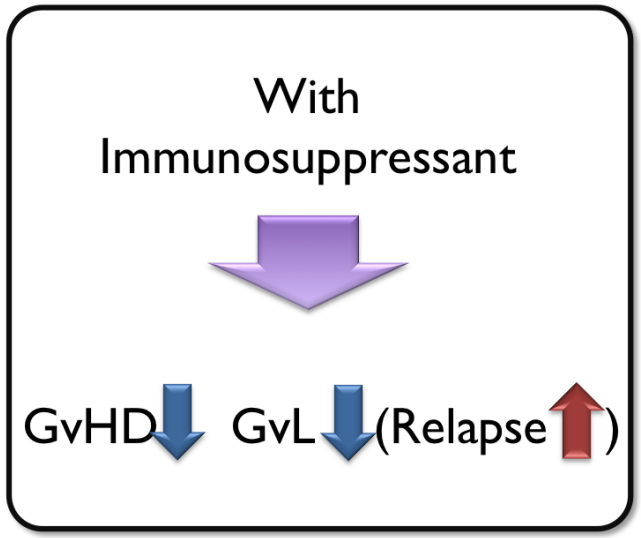
Attack



Leukemic cells

GVL vs GVHD vs Relapse





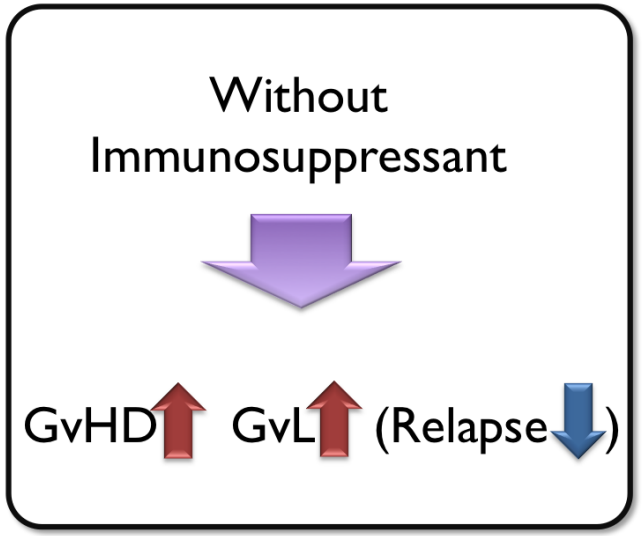


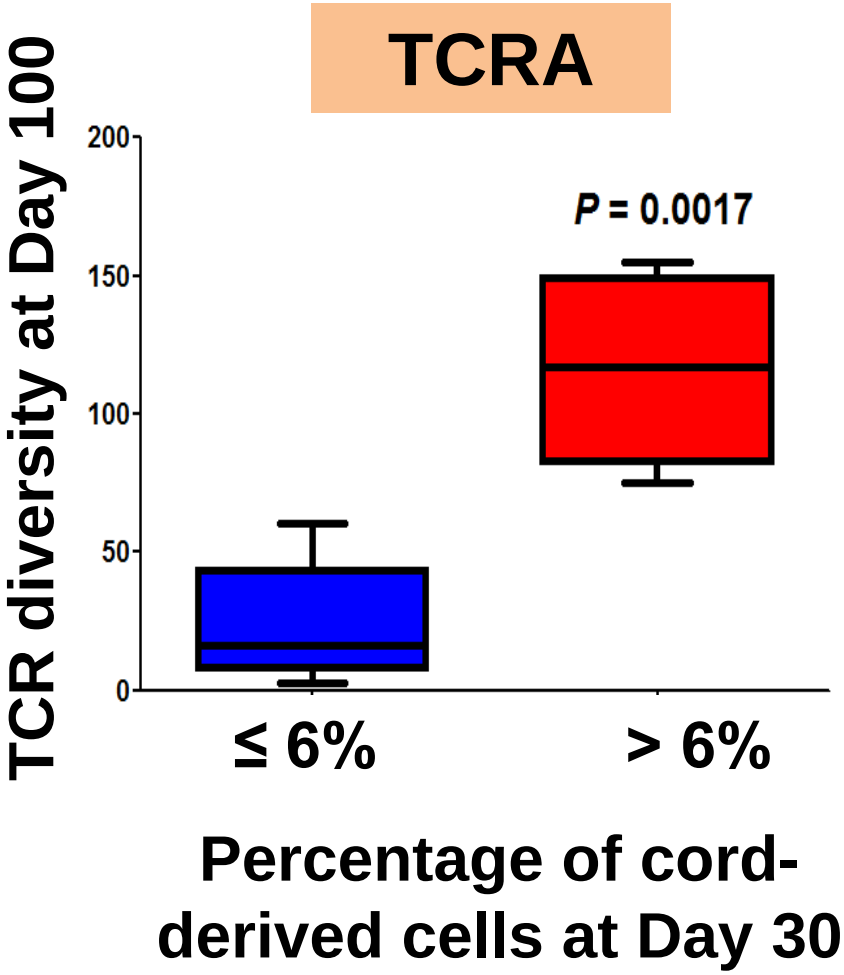
Figure from http://www.regimmune.com/product-pipeline/gvhd/

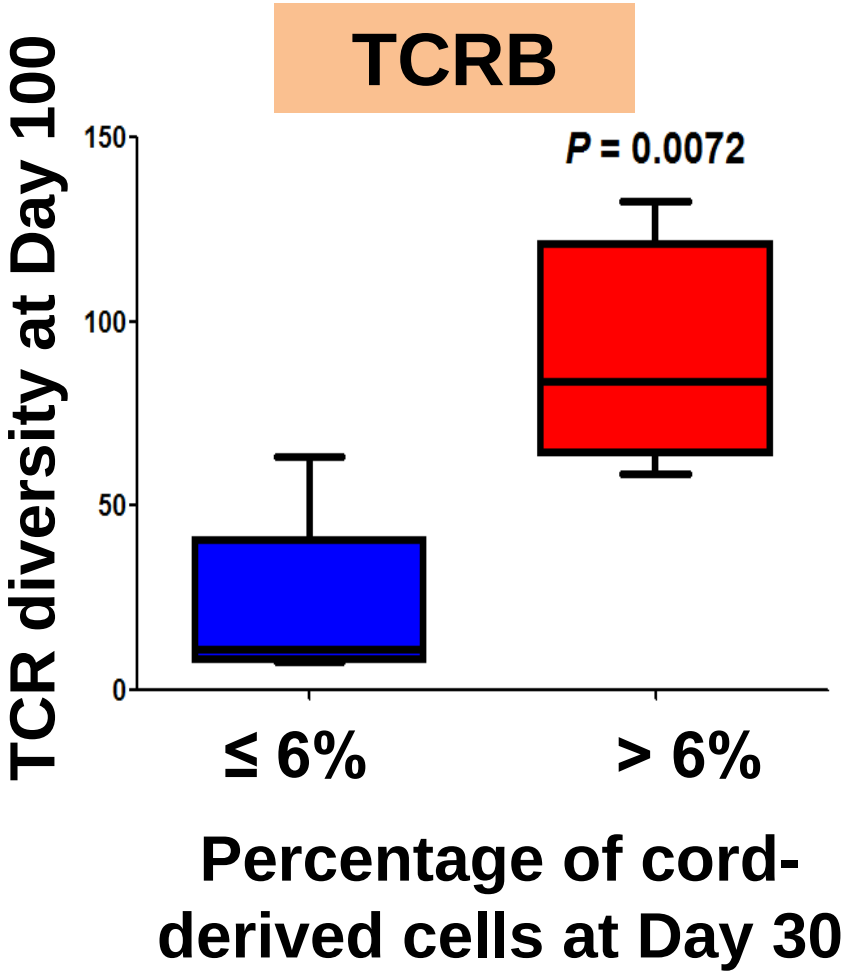
Patients Characteristic

	Matched Donor	Haplo-cord
Donor	12	9
HLA identical relative	5	-
HLA identical unrelated	7	-
Age	42-73	26-67
Conditioning regimen	Flu/Mel/Campath or Clo/Mel/Campath	Flu/Mel/anti-thymocyte globulin
Acute GVHD	9	2
Relapsed	5	3

GVHD, Graft-versus-host disease; Flu, fludarabine; Mel, melphalan

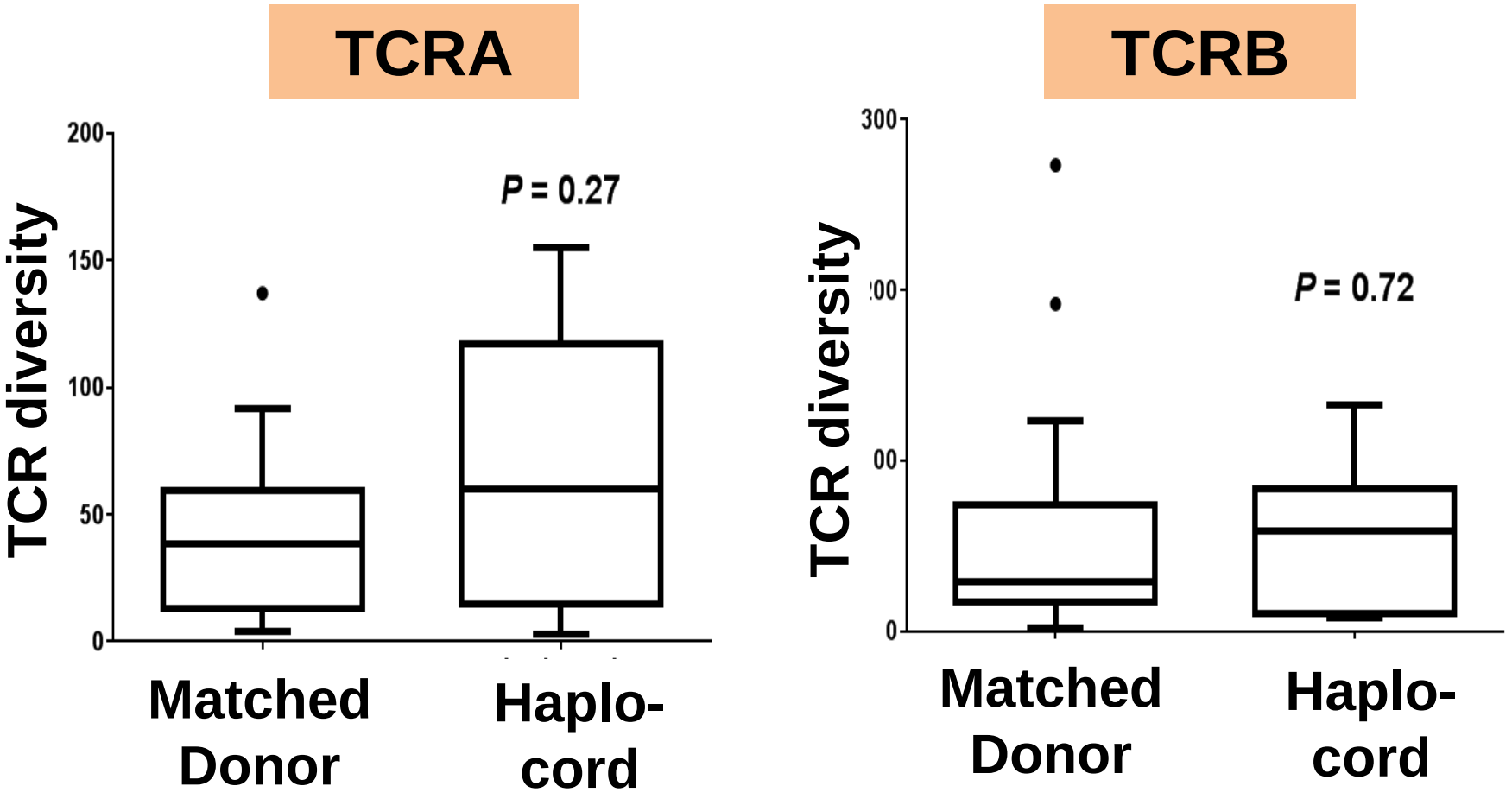
TCR diversity in haplo-cord transplanted patients





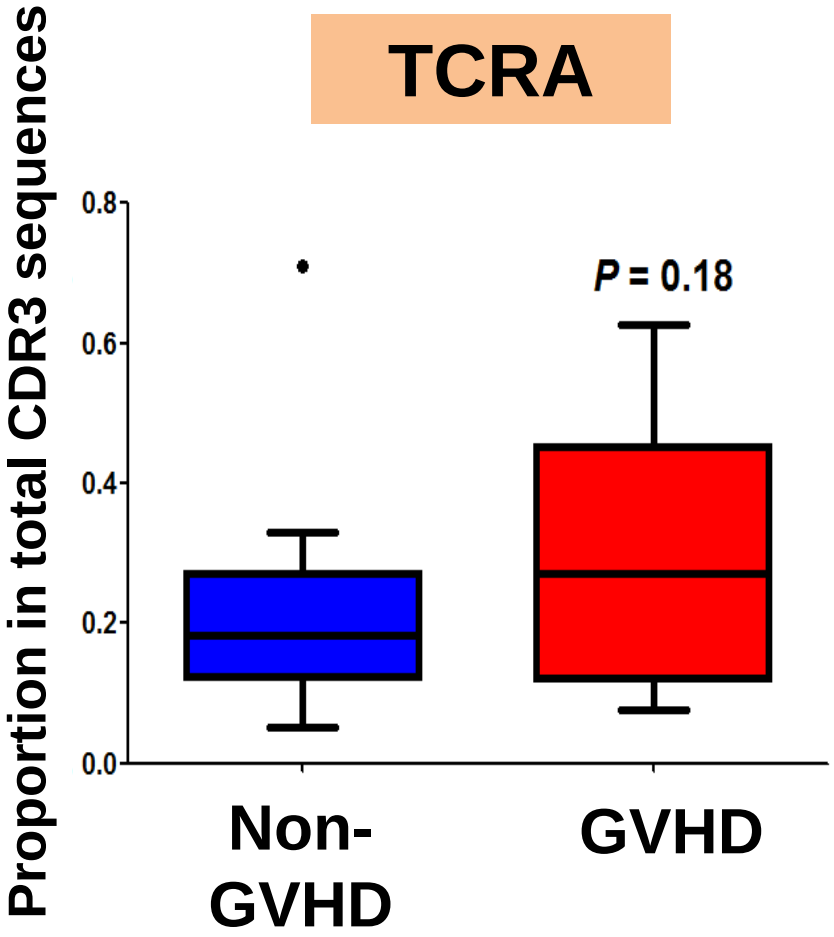
Patients with higher % of cord-derived cells at Day 30 had significantly higher TCR diversity at Day 100

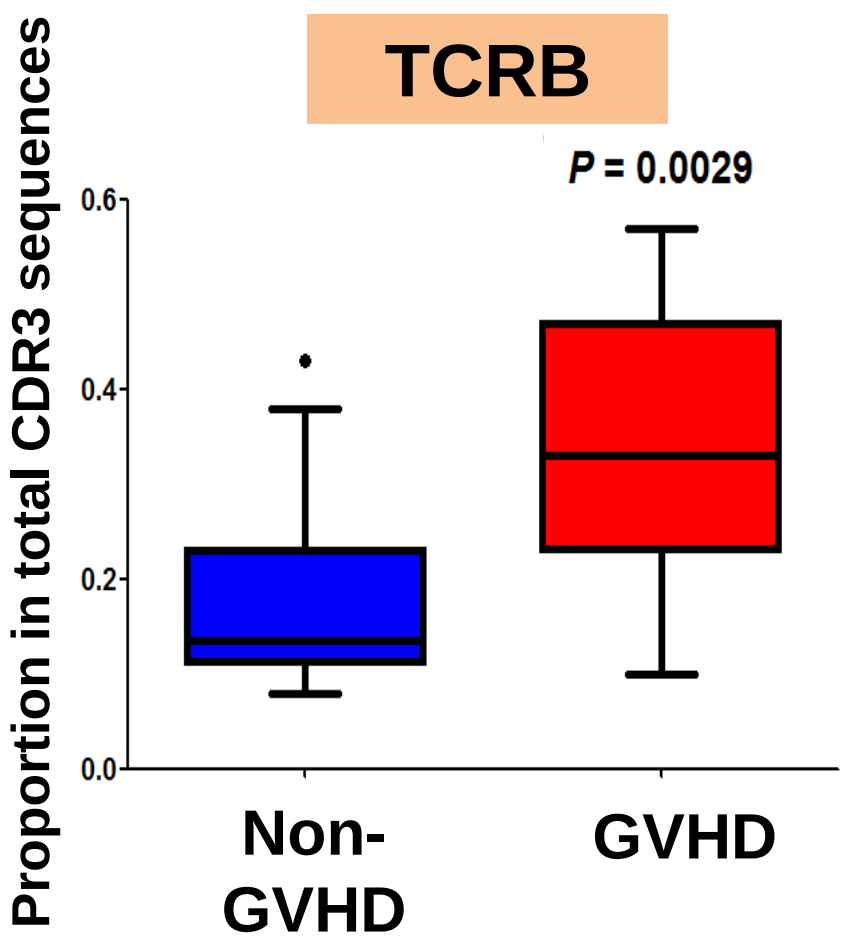
TCR repertoire diversity and source of donor stem cells



No significant difference between patients underwent MD and haplo-cord transplant

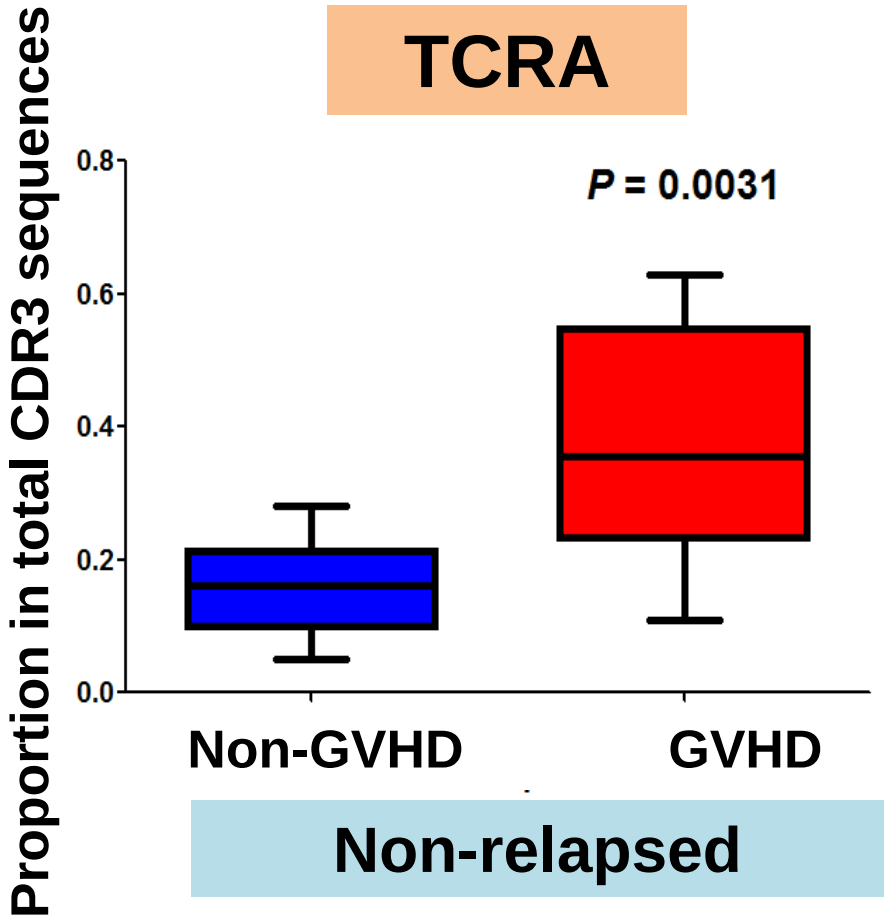
Proportions of 10 most abundant CDR3: GVHD vs non-GVHD

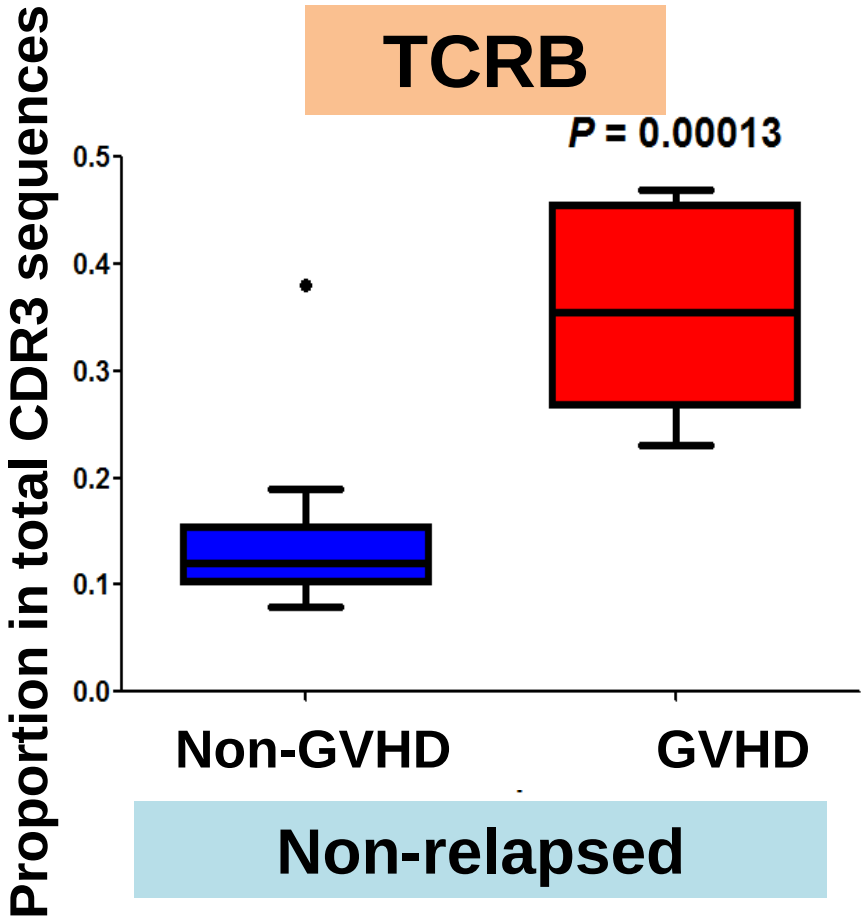




Significant Expansion of TCRB Clones in GVHD Patients

Proportions of 10 most abundant CDR3: Non-relapsed - GVHD vs non-GVHD



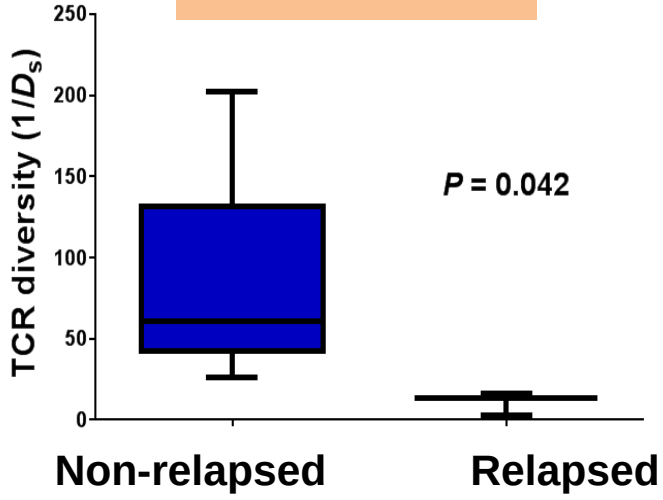


GVHD significantly correlates with expansion of TCRA and TCRB clones

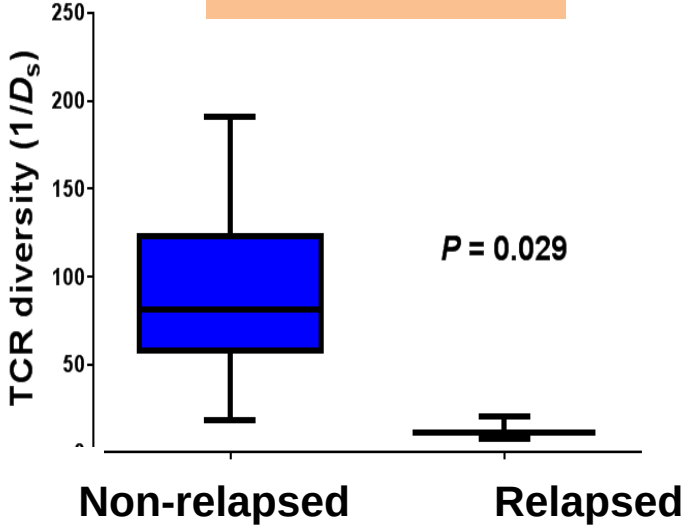
TCR repertoire and correlation with relapse

Non-GVHD

TCRA

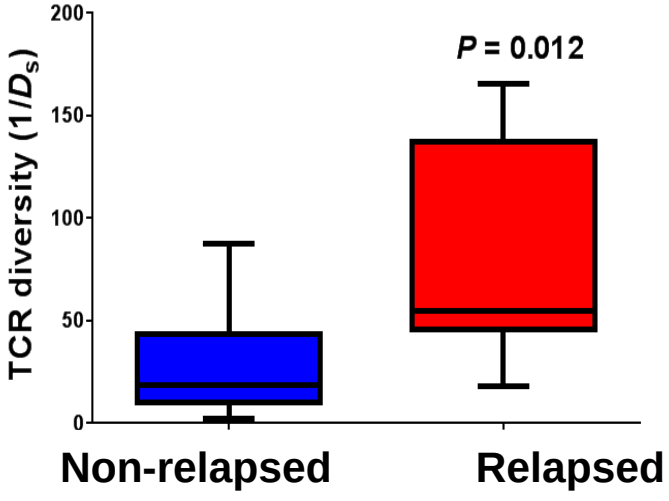


TCRB

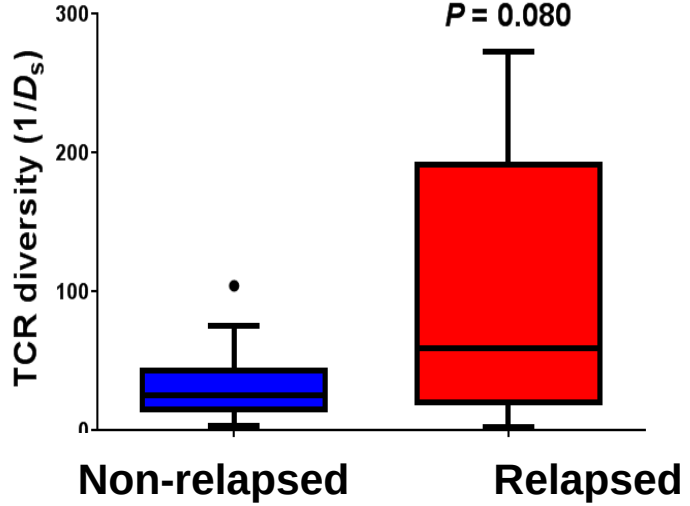


GVHD

TCRA



TCRB



Summary

- TCR repertoire of patients with higher % cord cells on day 30 after haplo-cord transplant were significantly more diverse on day 100 compared to TCRs in patients with lower % of cord cells

- GVHD patients:
  - Lower TCR diversity, expansion of certain clones

- Non-GVHD and non-relapsed patients
  - Higher TCR diversity

- TCR analysis of hematopoietic stem cell transplant recipients:
  - Understanding of the immunological response of patients after transplantation
  - Understanding the immune reconstitution after transplantation

TCR sequencing projects: Characterizing T cell repertoire in:

1. Allogeneic hematopoietic stem cell transplant (HSCT) recipients
2. Patients with Crohn's Disease
3. Patients with Follicular lymphoma
4. Patients with Muscle-invasive Bladder Cancer

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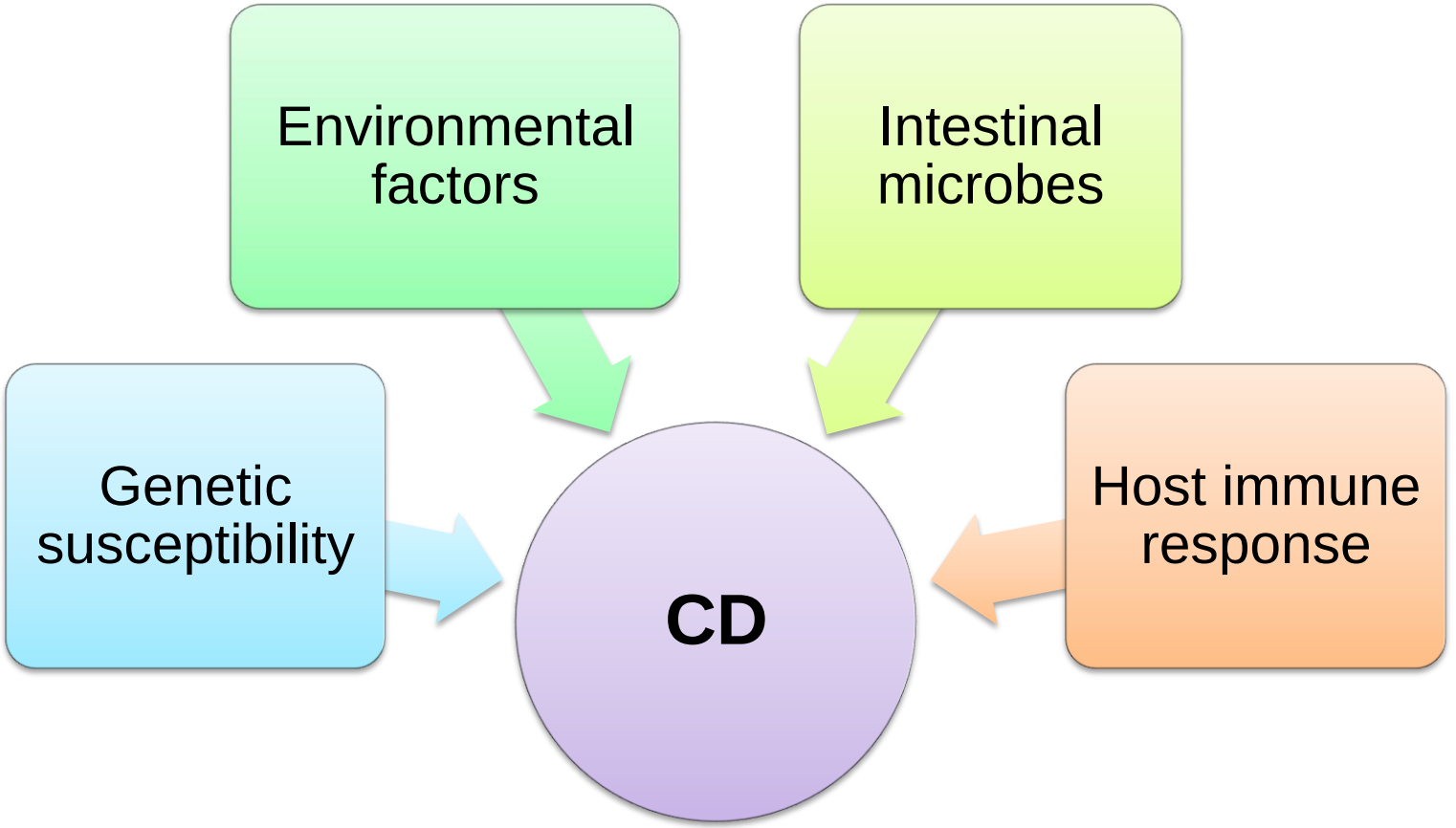
Characterization of T-cell Receptor Repertoire in Inflamed Tissues of Patients with Crohn's Disease through Deep Sequencing

Chapman CG, Yamaguchi R, Tamura K, Weidner J, Imoto S, Kwon J, Fang H, Yew PY, Marino SR, Miyano S, Nakamura Y, Kiyotani K

Inflammatory Bowel Diseases, 2016, in press

Crohn's disease (CD)

- A chronic, relapsing inflammatory bowel disease (IBD), characterized by an abnormal inflammatory response to intestinal microbes in a genetically susceptible patient



Patients characteristic

17 CD patients

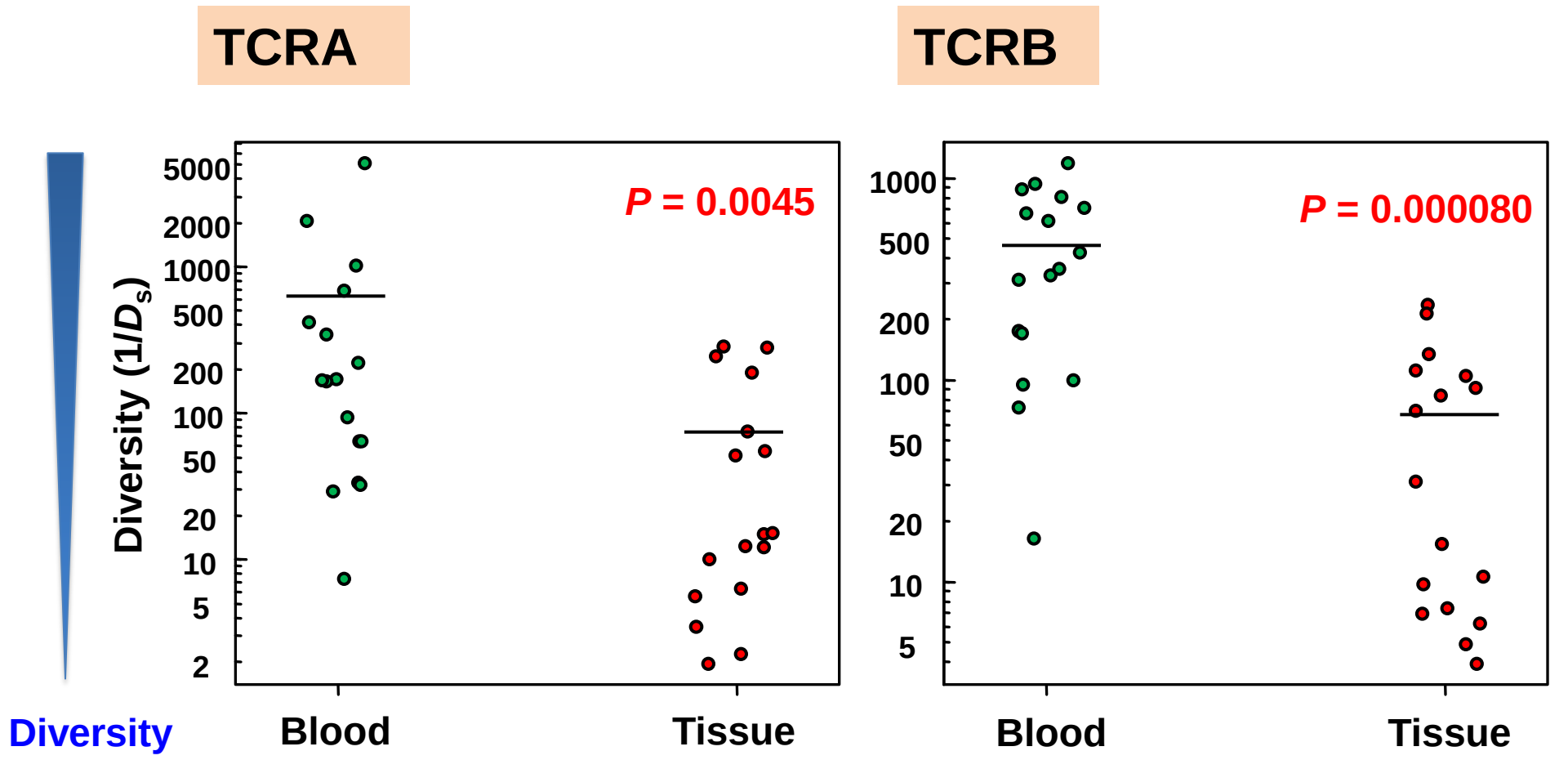
Tissue

- **12 biopsy from the neo-terminal ileum of post-operative recurrent CD patients**
- **5 surgical resections of terminal ileum of CD patients**

Blood

- **17 PBMC samples**

Comparison of TCR Diversity between Tissue and Blood in CD



The Neo-Terminal Ileum: Rutgeert's score: Colonoscopy 6 months after surgery to re-stratify

Rutgeerts 0

Normal ileal mucosa

Rutgeerts 1

<5 aphthous ulcers

Rutgeerts 2

>5 aphthous ulcers, normal intervening mucosa

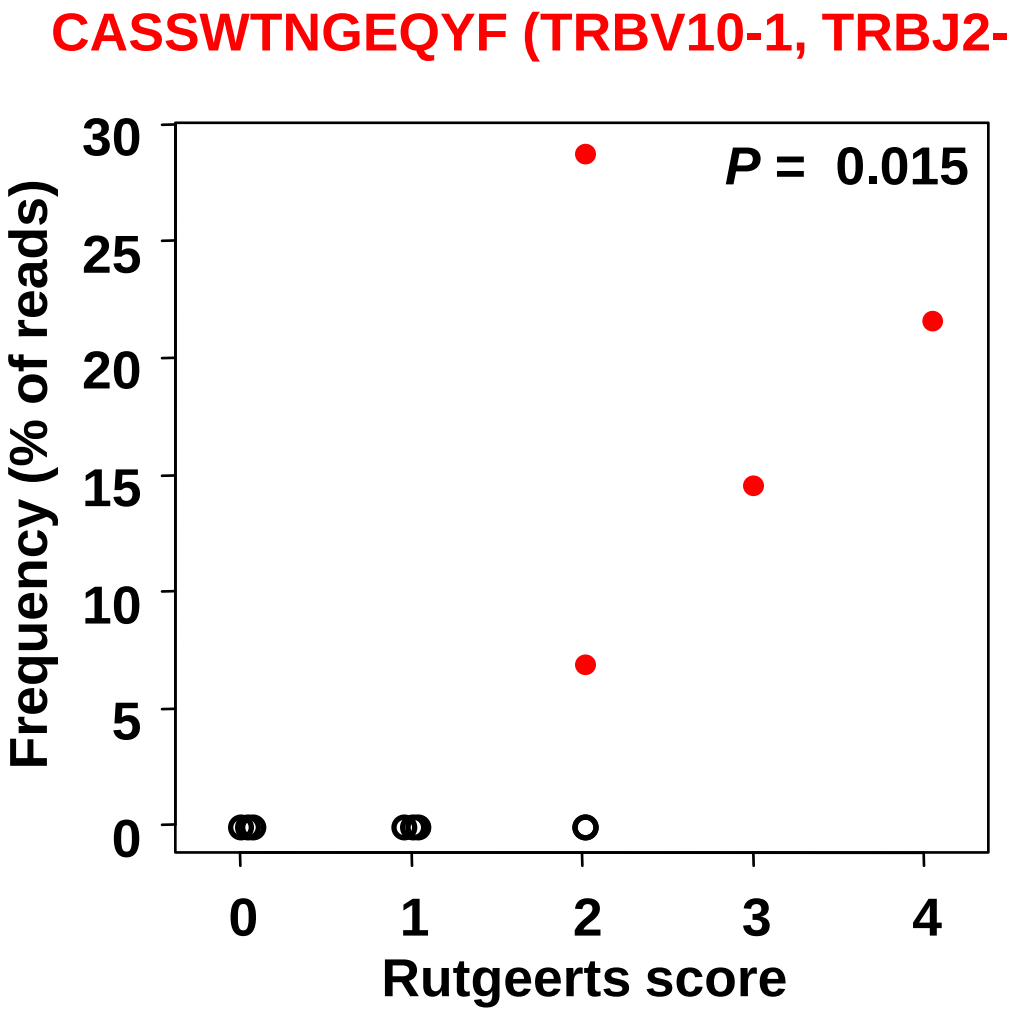
Rutgeerts 3

Ulceration without normal intervening mucosa

Rutgeerts 4

Severe ulceration with nodules, cobblestoning, or stricture

Correlation of one clonotype with Disease Severity



Summary

- **TCR diversity in mucosal tissue was significantly lower compared the matched PBMCs.**
  - **Expansion of certain T cell clones in the inflamed intestinal tissue.**

- **The abundance of one clonotype is correlated with severity of disease recurrence, based on Rutgeerts score.**

- **TCR analysis of Crohn's disease patients:**
  - **Understanding about the immunological reaction in CD**

TCR sequencing projects: Characterizing T cell repertoire in:

1. Allogeneic hematopoietic stem cell transplant (HSCT) recipients
2. Patients with Crohn's Disease
3. Patients with Follicular lymphoma
4. Patients with Muscle-invasive Bladder Cancer

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Highly clonal regulatory T-cell population in follicular lymphoma - inverse correlation with the diversity of CD8+ T cells.

Liu X, Venkataraman G, Lin J, Kiyotani K, Smith S, Montoya M, Nakamura Y, Kline J

OncoImmunology, 2015, 4(5):e1002728.

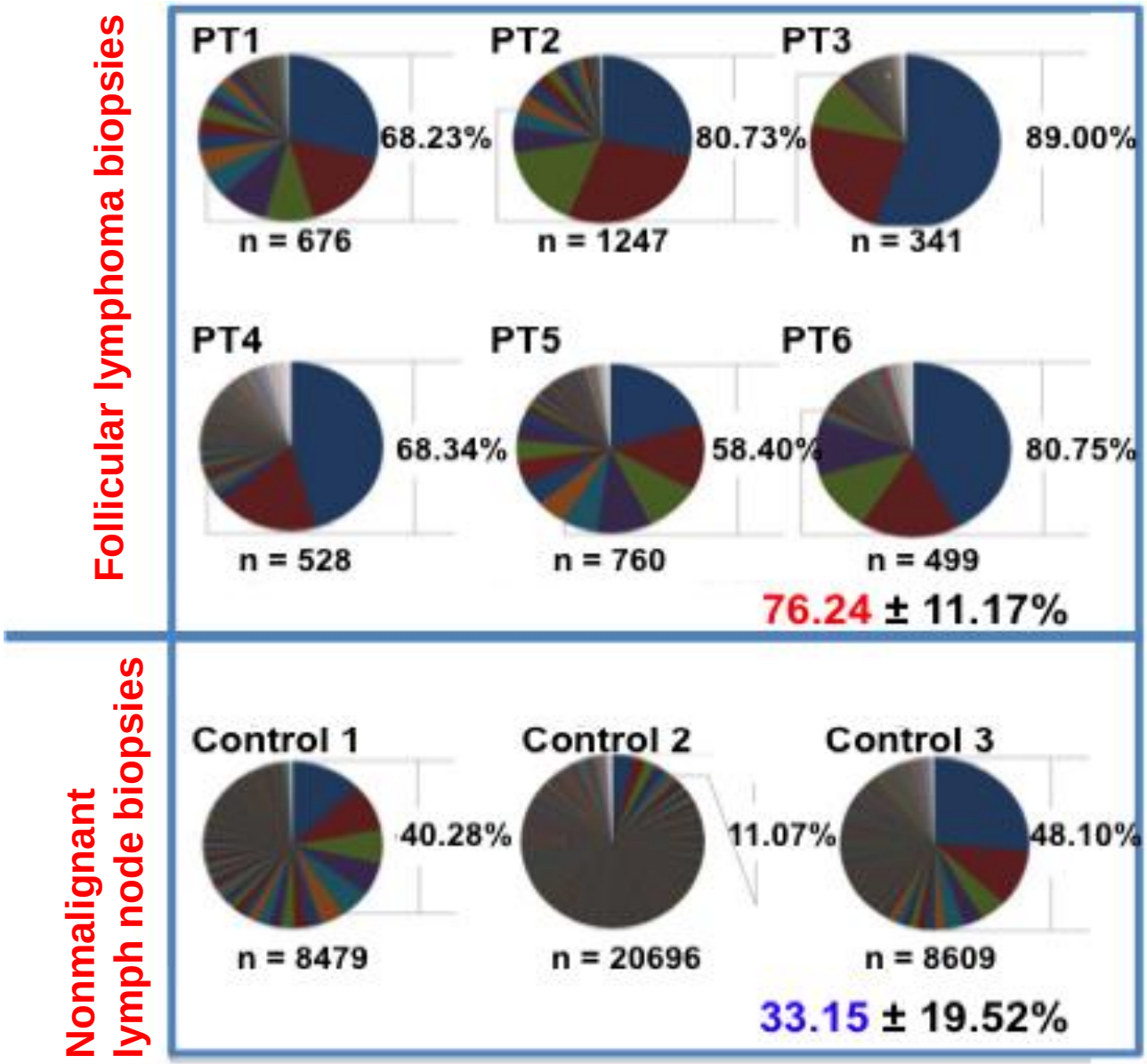
Patients characteristic

ID	Age	Age at Diagnosis	Grade	Stage at Diagnosis
PT 1	84	81	II-IIIA	III
PT 2	84	83	IIIA	IV
PT 3	76	70	II	I
PT 4	72	61	I-II	II
PT 5	77	77	I-II (80%), IIIA (20%)	IV
PT 6	71	55	I-II	I

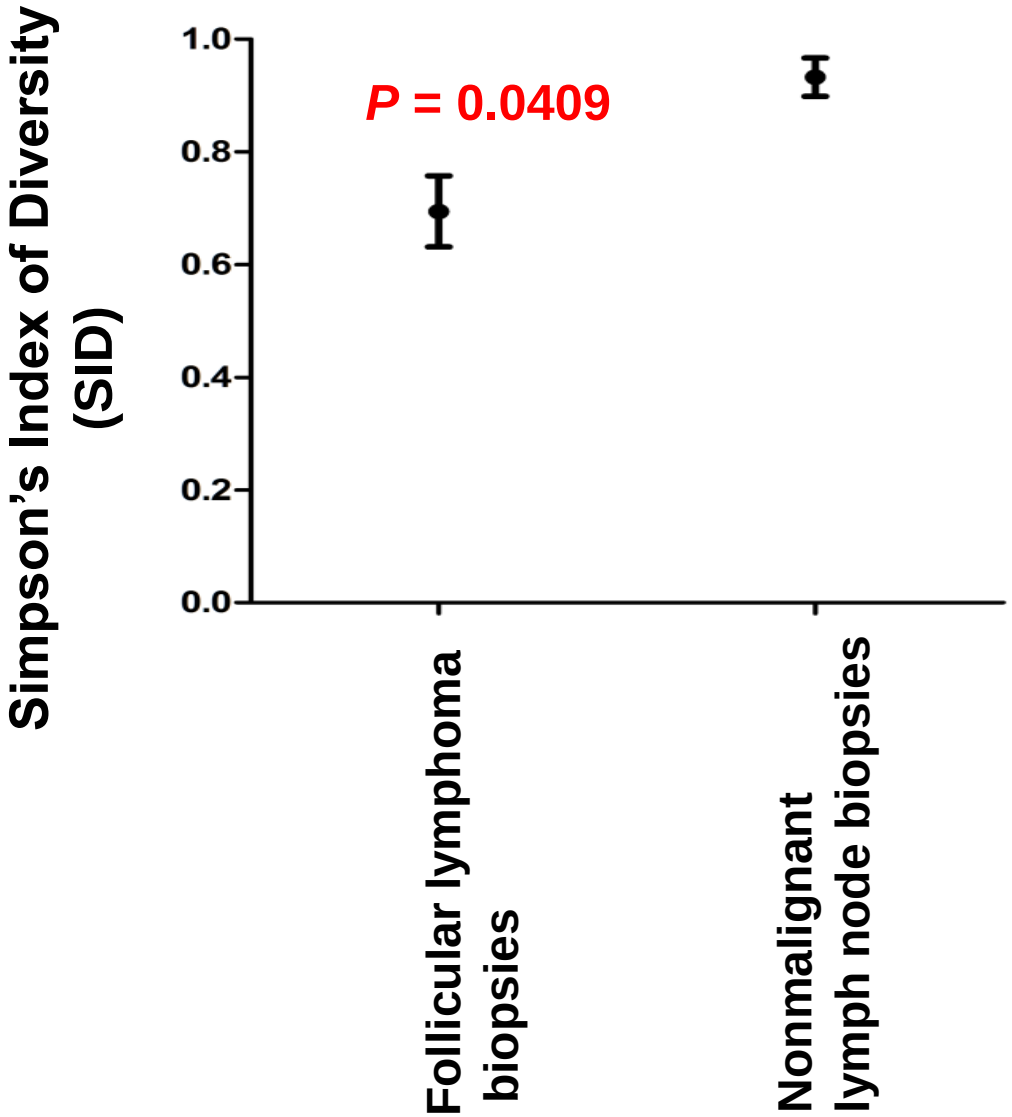
- The patient samples were single-cell suspensions derived from diagnostic FL biopsy specimens (pre-treatment lymph node).
- $CD8^+$, $CD4^+CD25^-$ and $CD4^+CD25^+$ were isolated.
- The Treg control samples were single-cell suspensions of nonmalignant lymph node biopsies from three nonfollicular lymphoma patients.

Oligoclonal enrichment of Treg TCRs in FL tumors

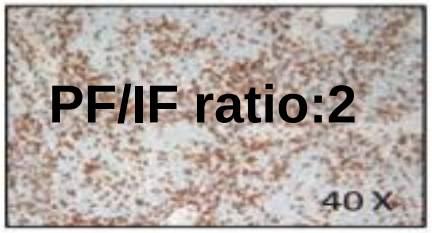
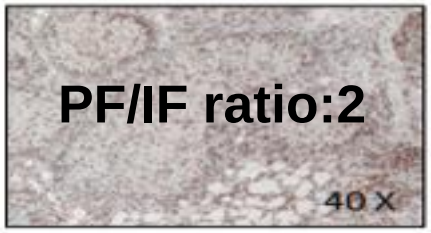
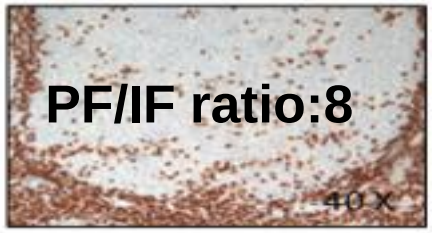
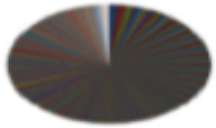
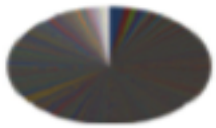
The % = The total percentage of 5 most abundant TCRB sequences

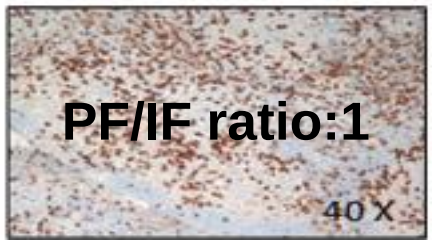
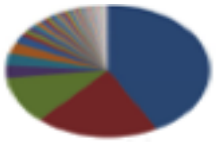


Diversity of Treg TCRs is lower in FL tumors



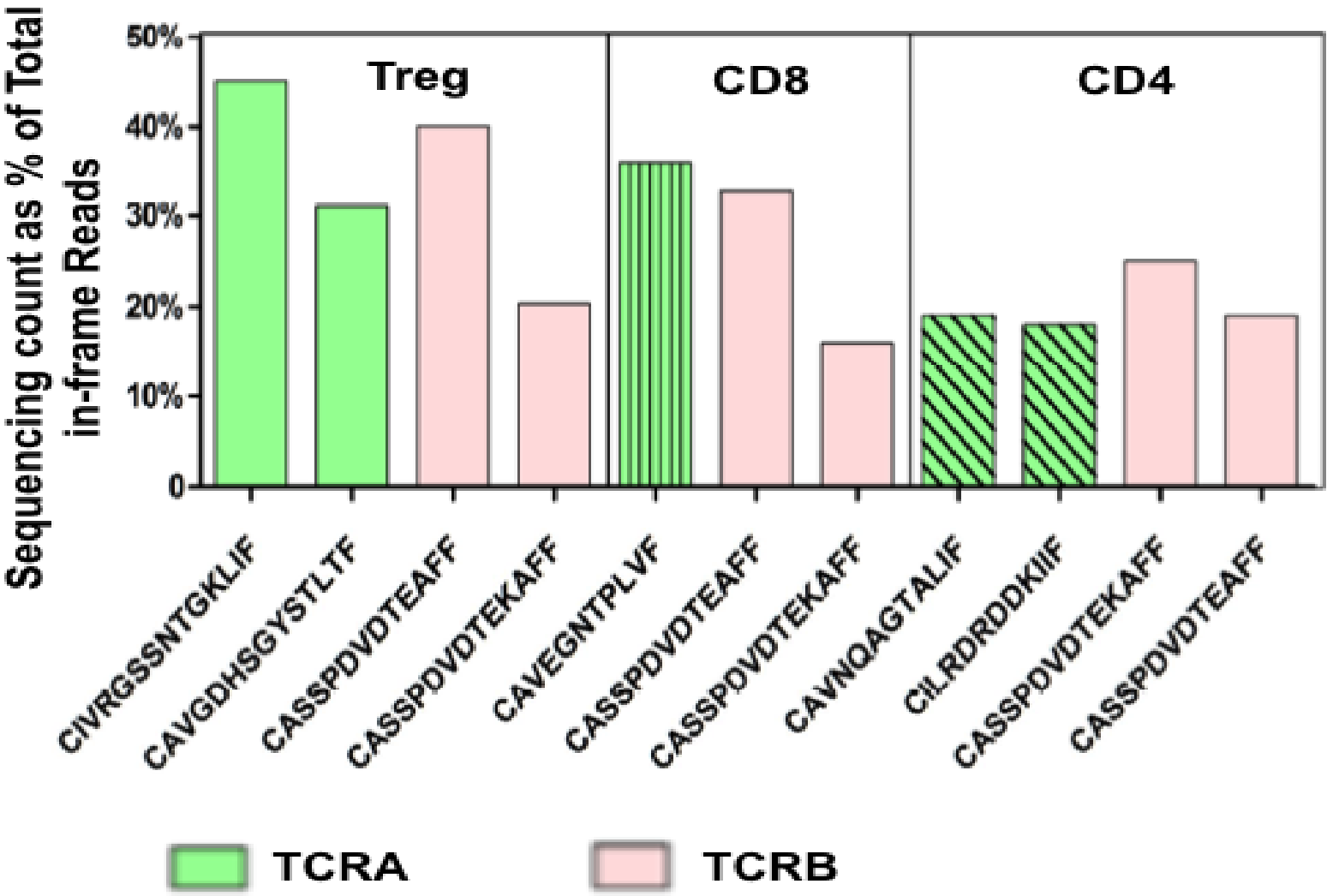
CD8+ T cell repertoire & infiltration pattern in FL tissue

	Pt 1 – CD8	Pt 2 – CD8	Pt 3 – CD8
CD8 staining			
CD8 T cell repertoire	 n = 12347	 n = 20907	n = 33

	Pt 4 – CD8	Pt 5 – CD8	Pt 6 – CD8
CD8 staining			
CD8 T cell repertoire	 n = 44	n = 82	n = 30

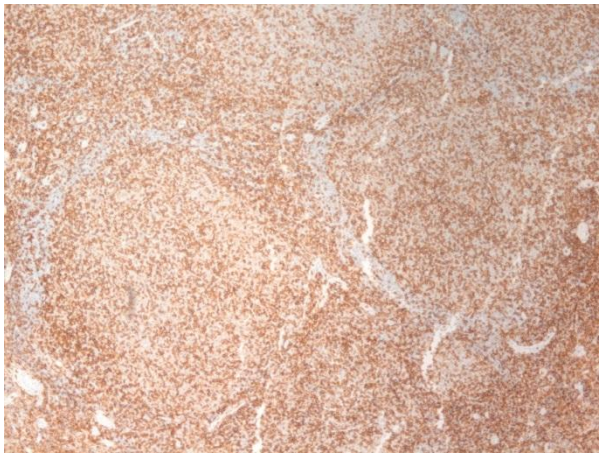
PF = perifollucular, IF = intrafollicular

A Special Case Study: Patient 4

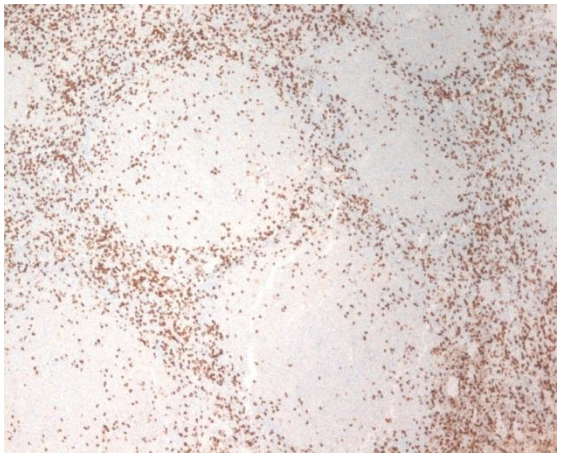


A Special Case Study: Patient 4

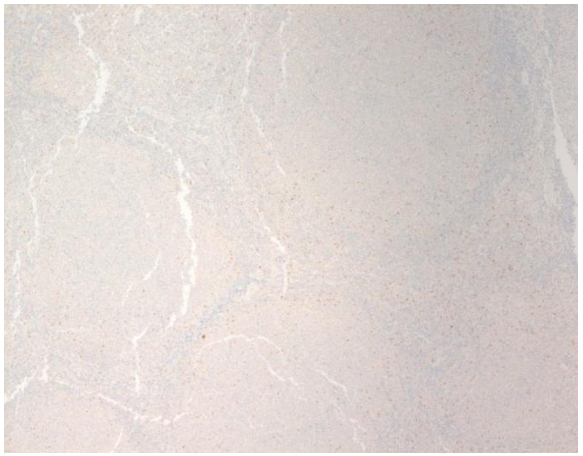
ID	Sex	Age	Age at Disease	Grade	Stage at Disease	Treatment	Response to treatment	Alive
PT 4	Male	72	61	I-II	II	No	NA	yes



CD4

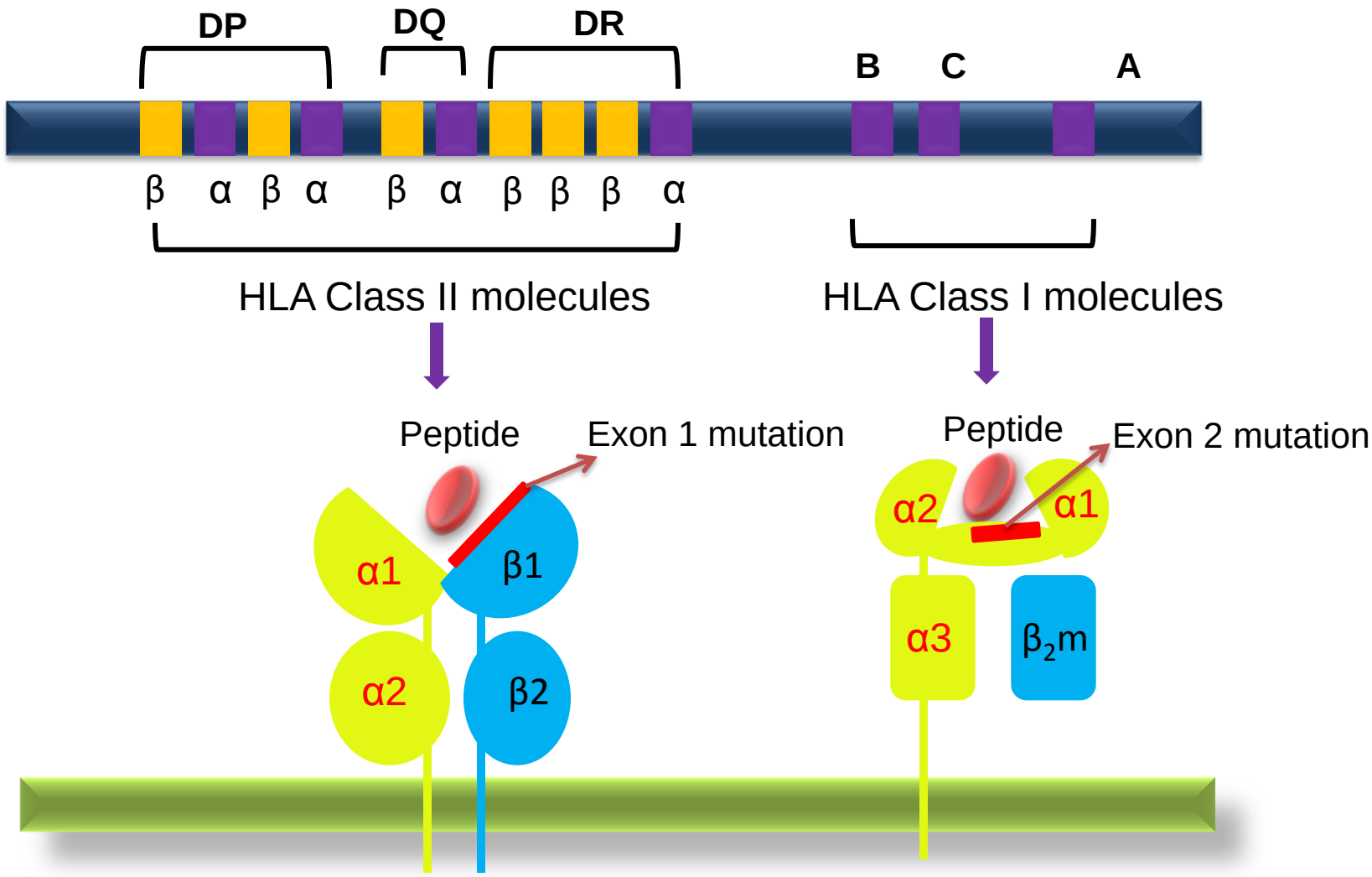


CD8



Treg (FOXP3)

Nonsynonmous SNV mutations were found in both HLA class II and class I molecules of patient 4



Summary

- **Strong enrichment of regulatory T cells was observed commonly in FL specimens**
- **Tumors with perifollicular $CD8^+$ T cell distribution tend to have stronger enrichment of $CD8^+$ T cell**
- **One interesting case (Patient 4):**
  - **Missense mutations at the peptide binding domains in both HLA class I and II molecules**
  - **May alter the peptide antigens displayed**
- **TCR sequencing combined with exome sequencing of FL patients**
  - **Understanding the immune microenvironment of FL patients**
  - **Identify the targetable antigens for T cell based therapeutic strategies**

TCR sequencing projects: Characterizing T cell repertoire in:

1. Allogeneic hematopoietic stem cell transplant (HSCT) recipients
2. Patients with Crohn's Disease
3. Patients with Follicular lymphoma
4. Patients with Muscle-invasive Bladder Cancer

THE UNIVERSITY OF CHICAGO

Low T-cell Receptor Diversity, High Somatic Mutation Burden, and High Neoantigen Load as Predictors of Clinical Outcome in Muscle-invasive Bladder Cancer

Choudhury NJ, Kiyotani K, Yap KL, Campanile A, Antic T, Yew PY, Steinberg G, Park JH, Nakamura Y, O'Donnell PH.

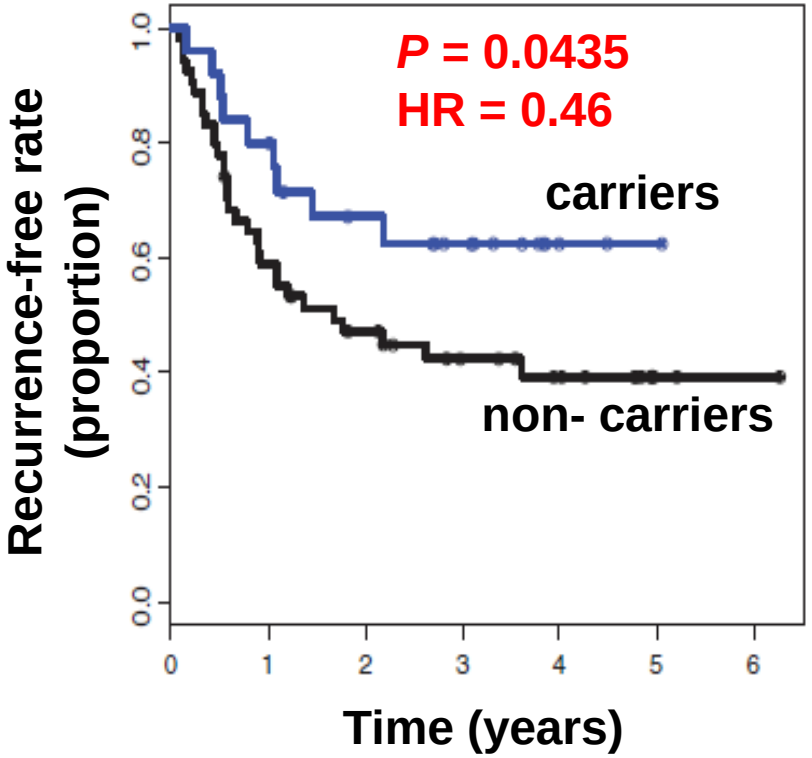
European Urology Focus, 2015, http://dx.doi.org/10.1016/j.euf.2015.09.007

Previous study: Exome sequencing of MIBC

Whole exome sequencing
(43 samples)
&
Targeted gene sequencing
(38 samples)

Somatic mutations in any of six DNA repair genes (*ATM, ERCC2, FANCD2, PALB2, BRCA1, and BRCA2*)

- **Carriers:**
  - **Higher overall somatic mutation burden (307.4 mutations/case)**
- **Non-carriers:**
  - **155.4 mutations/case**

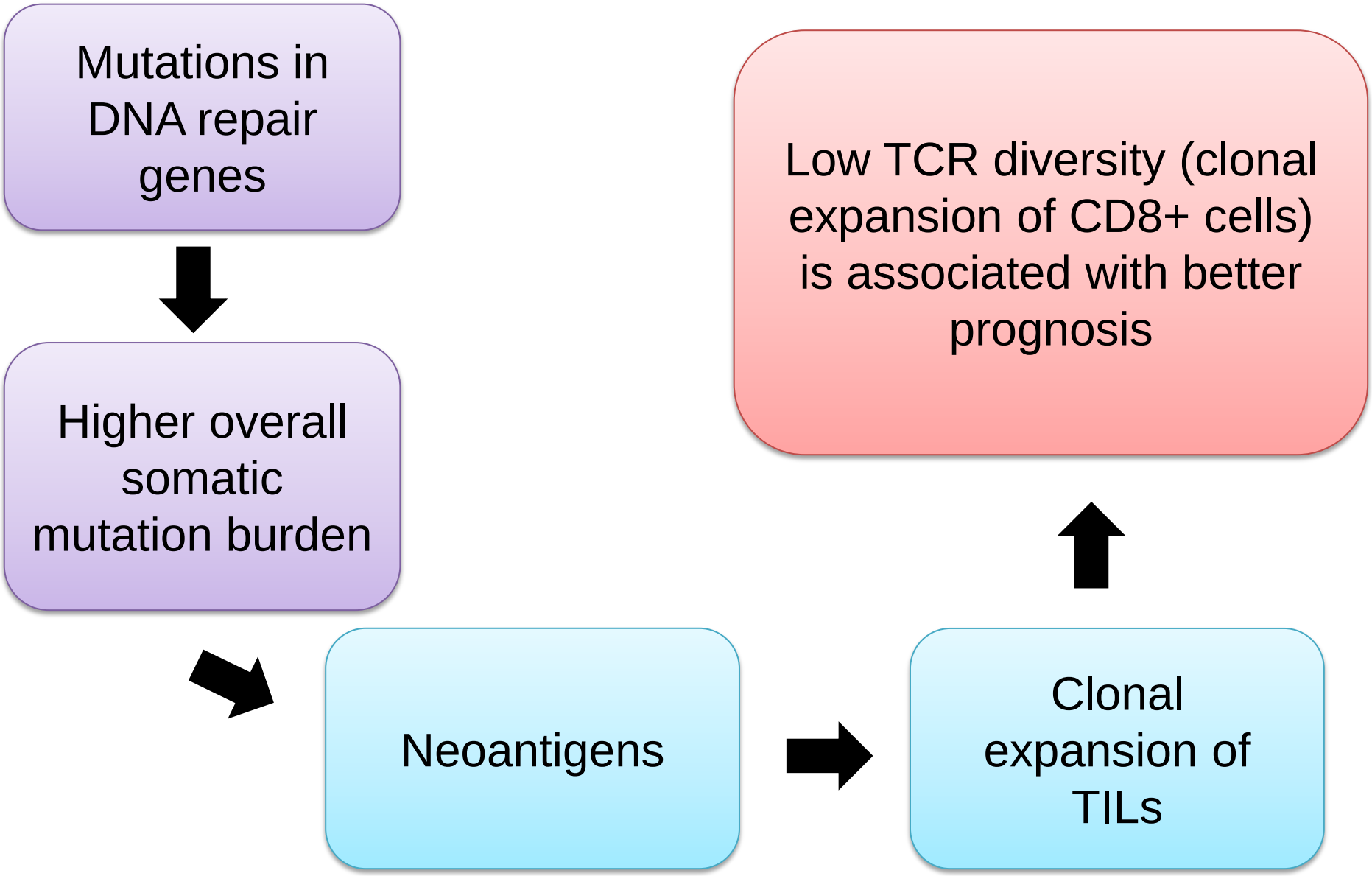


Carriers (n= 25)
Median RFS = 32.4 months

Non-carriers (n=54)
Median RFS = 14.8 months

K. Yap, et al. Clin Cancer Research 2014

Hypothesis


Mutations in DNA repair genes
Higher overall somatic mutation burden
Neoantigens
Clonal expansion of TILs
Low TCR diversity (clonal expansion of CD8+ cells) is associated with better prognosis

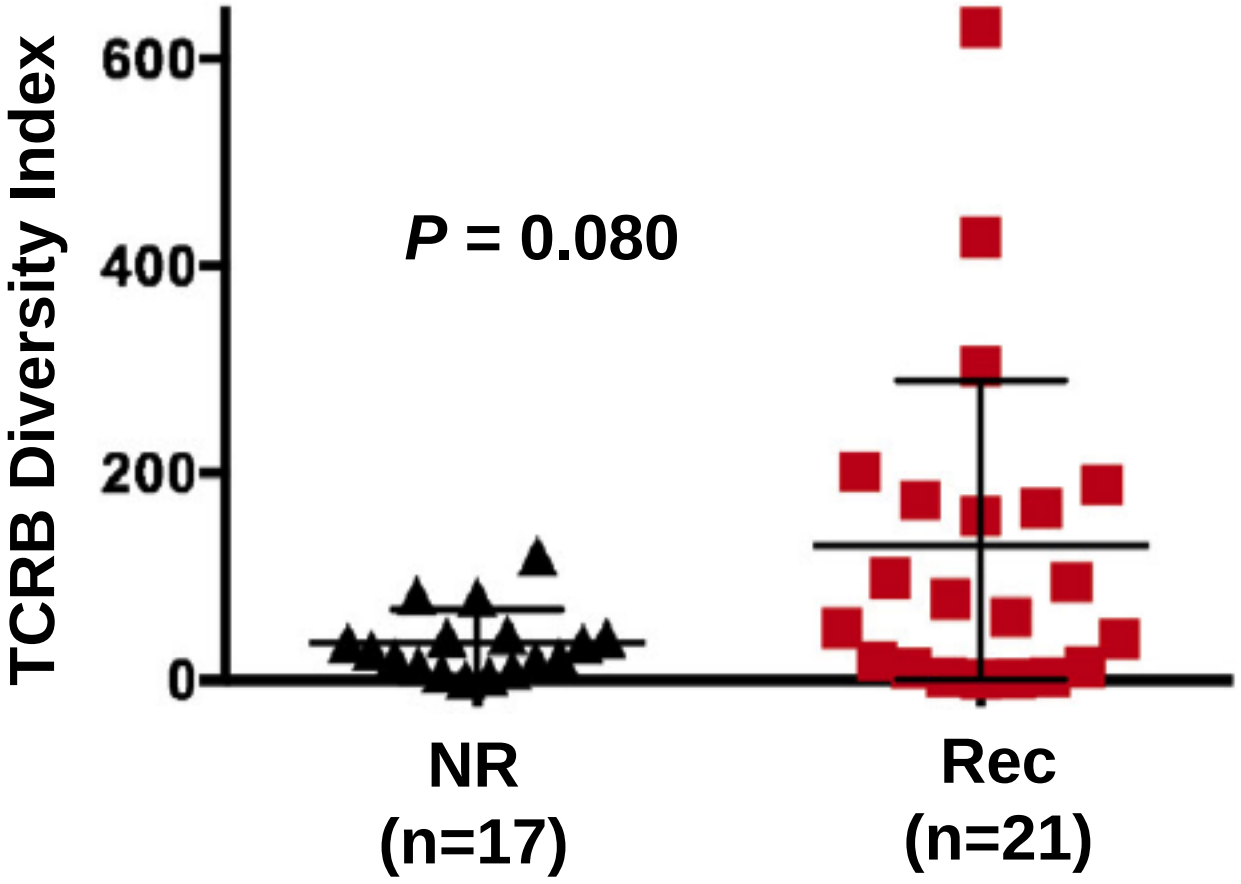
Workflow

TCR sequencing of 38 samples (Recurrent vs Non-recurrent)

Whole exome sequencing

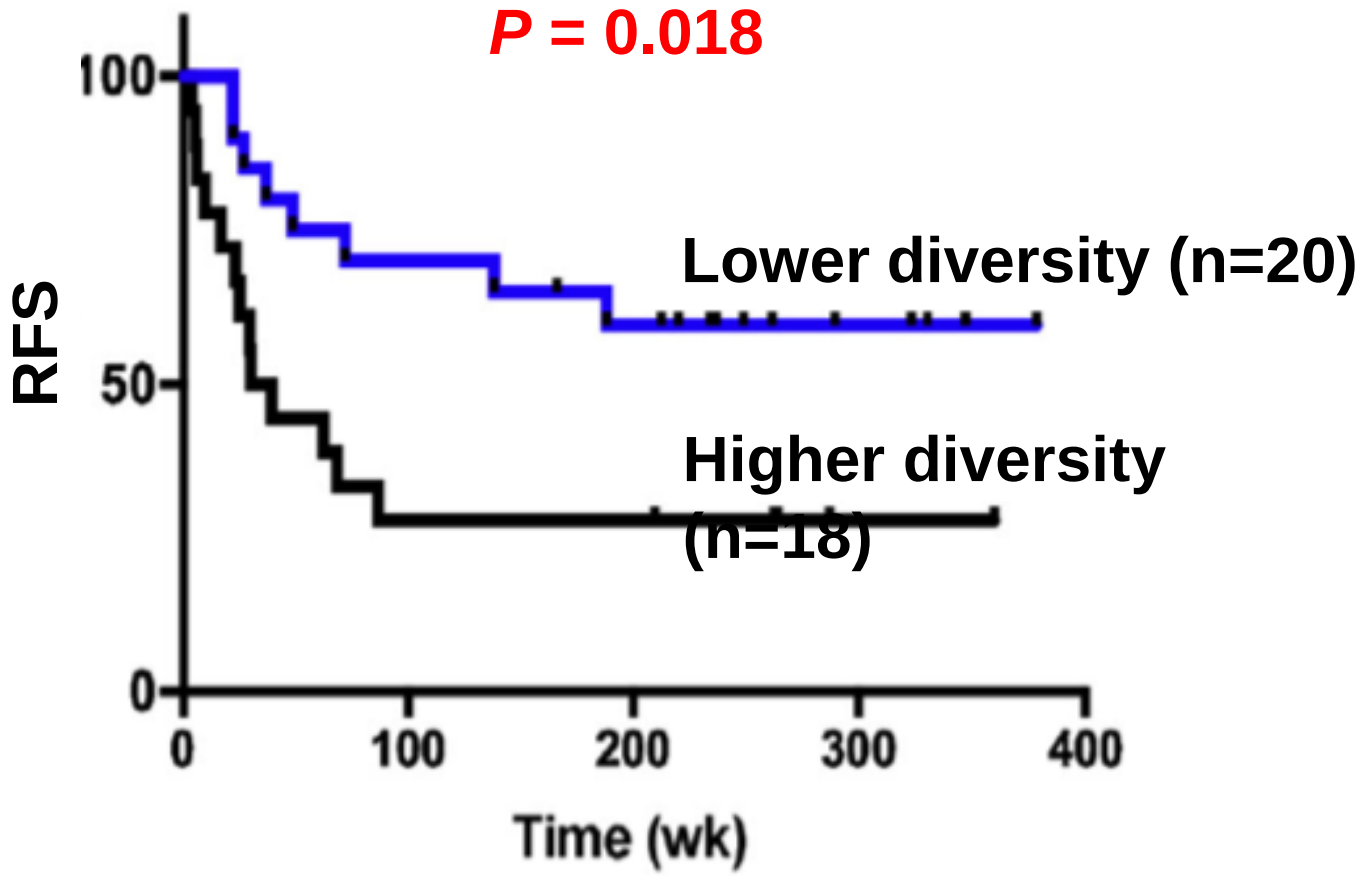
Neoantigens prediction

TCR diversity and recurrence risk



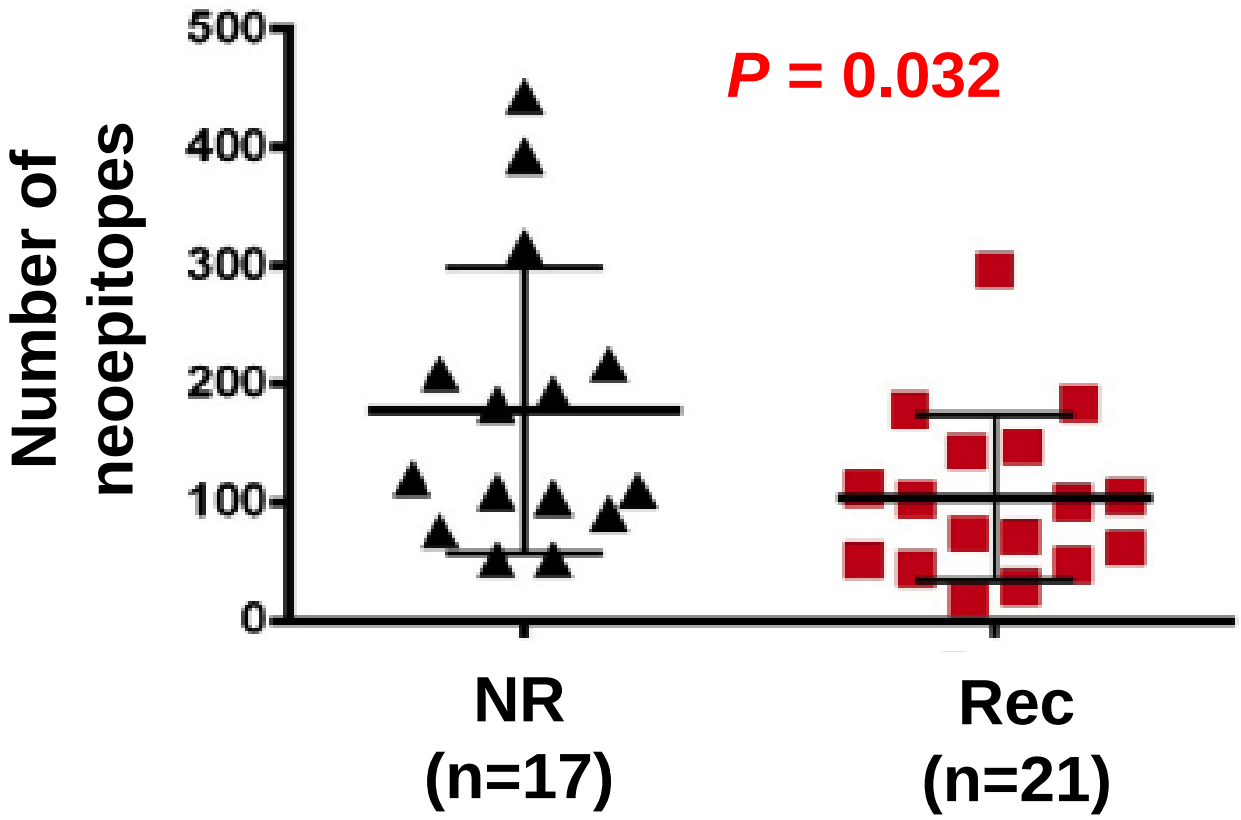
Non-recurrent patients had lower TCRB diversity index

TCR diversity and RFS



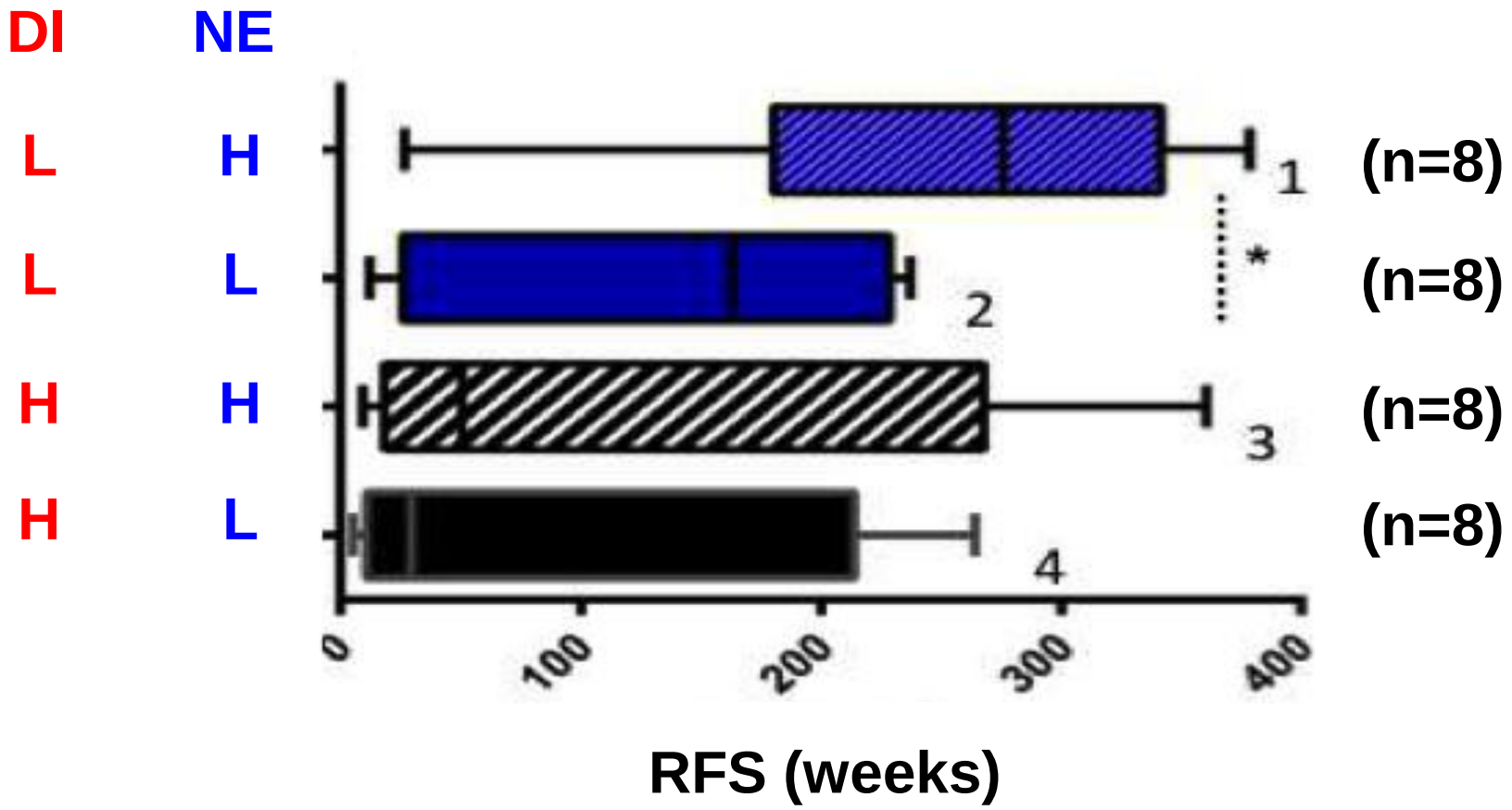
Patients with lower TCRB diversity index had significantly longer RFS

Recurrence and number of predicted neoantigens



Non-recurrent Patients had higher average number of predicted neoantigens

RFS, neoantigen load and TCRB diversity index



Patients with high antigen load and low TCRB diversity index had longer RFS

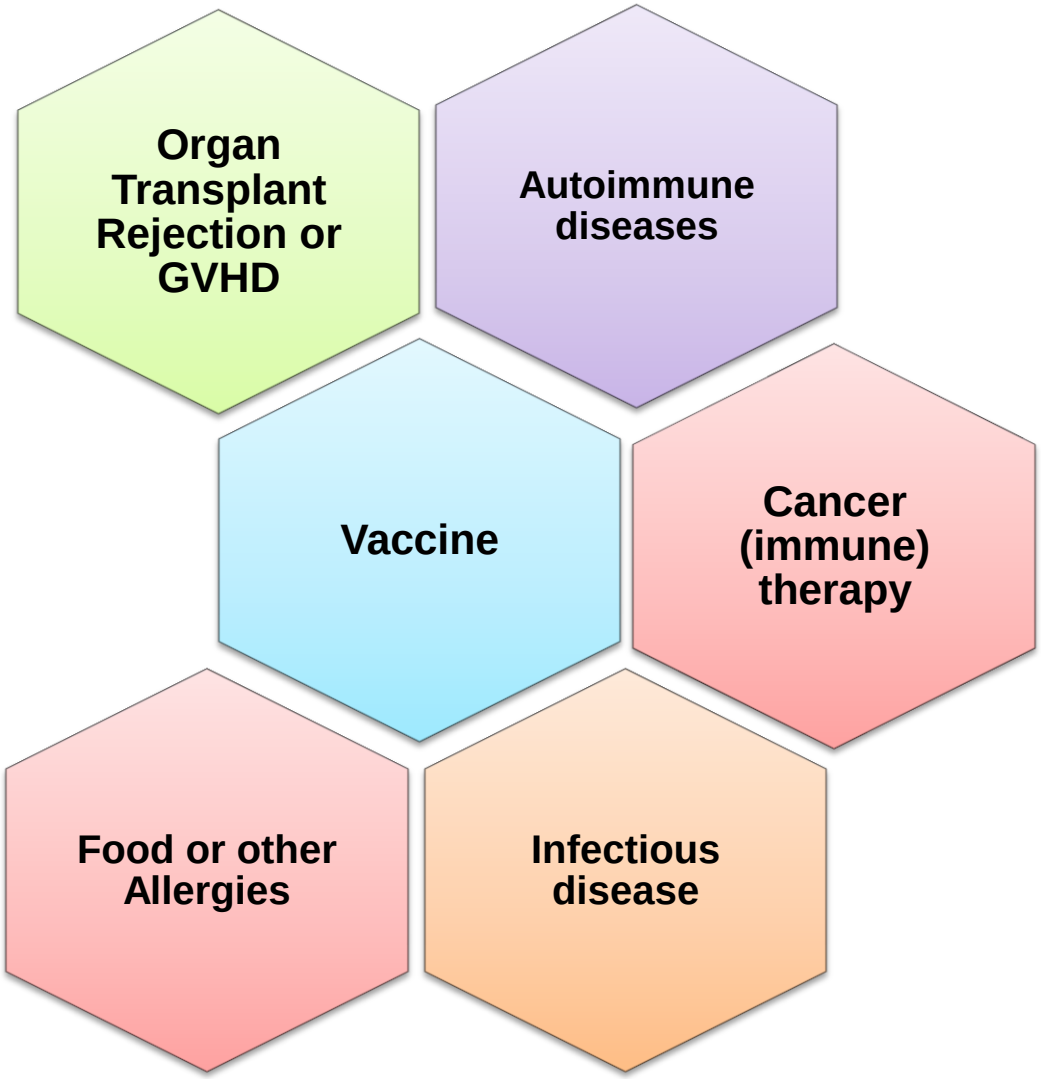
Summary

- **Low TCRB diversity index correlate with oligoclonal TIL expansion and longer RFS**

- **Patients with a high number of neoantigens and low TCRB diversity had longer RFS**

- **TCR analysis and exome sequencing of MIBC patients**
  - **Understanding the molecular patterns of antitumor immune response in MIBC**
  - **Provide us the valuable prognostic information on the clinical course of MIBC**

TCR analysis will contribute to:

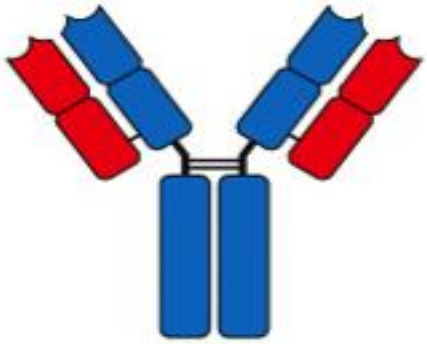


- understanding of complex interaction between **cancer and the immune system**,
- understanding of **cancer therapy mechanism**, either in the setting of human studies or mouse models,
- **patient selection** - characterizing the best responders for cancer therapy,
- **monitoring and assessment** of ongoing cancer therapy.

Future directions

BCR sequencing

• To obtain a better understanding in the fundamental of immunology and the pathophysiology of various disease such as autoimmune diseases, food allergy etc.

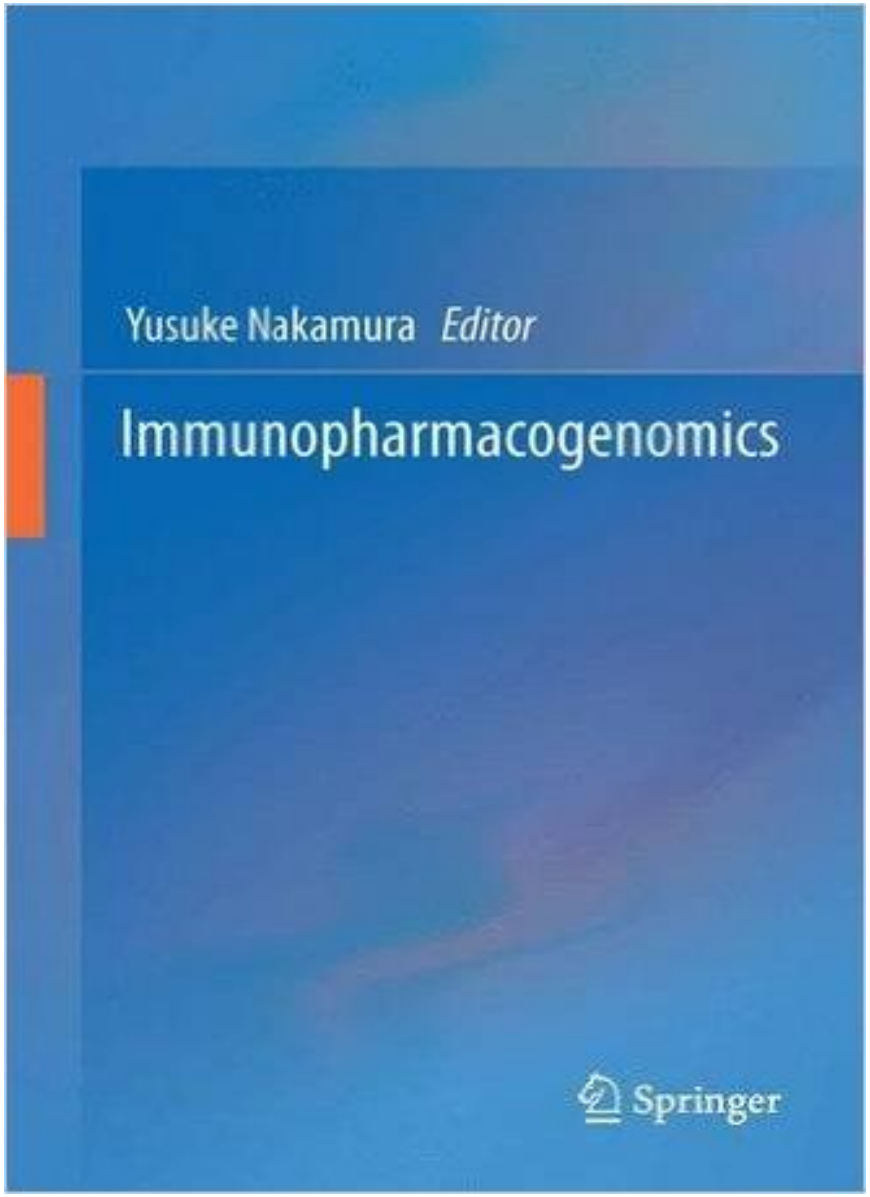


Single cell analysis

• Identify the pair in BCR or TCR
  • important for subsequent functional analysis

• Investigate the heterogeneity in gene expression among T cells.

Reference



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Acknowledgements

THE UNIVERSITY OF CHICAGO

- **Prof. Yusuke Nakamura**
- Members from Nakamura lab, the University of Chicago

東京大学
THE UNIVERSITY OF TOKYO

- Prof. Satoru Miyano
- Prof. Seiya Imoto
- Dr. Rui Yamaguchi

Thank you very much for your attention!!

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