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International Society for Cutaneous Lymphomas ISCL

5TH World Congress of Cutaneous Lymphomas

Enhancing the Ability to Diagnose, Interpret and Apply Best Treatment Options for Cutaneous Lymphomas

Pathogenesis and Biomarkers | # 89

High-throughput Sequencing of the T-cell Receptor β and γ Genes Correlates with Aggressive Subtypes of Mycosis Fungoides and Sezary Syndrome and Immune Checkpoint Expression

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Disclosures

- I do not have any relevant financial relationships.

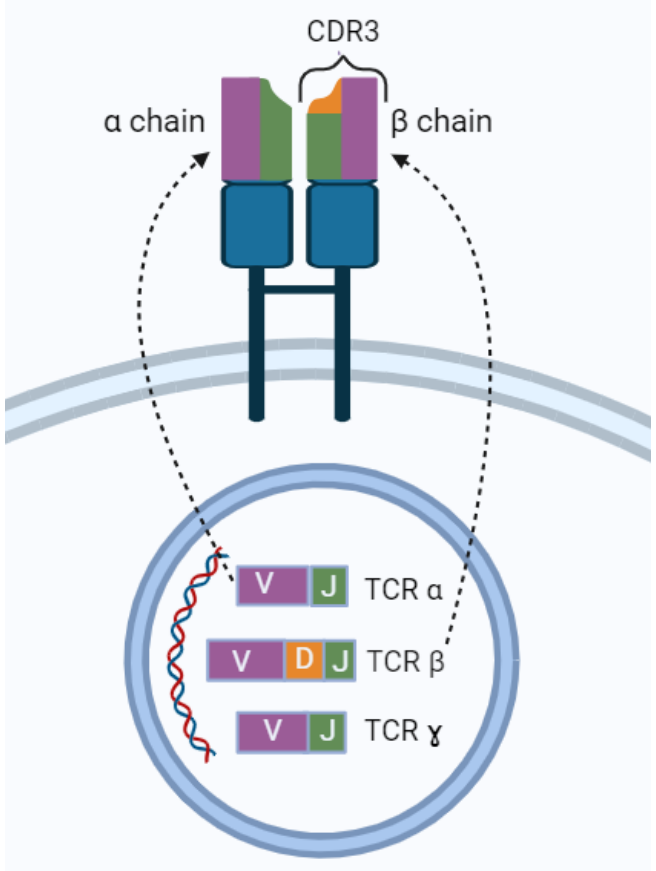
This presentation and/or comments will provide a balanced, non-promotional, and evidence-based approach to all diagnostic, therapeutic and/or research related content.

Background

- Mycosis fungoides (MF) and Sezary syndrome (SS) are clonal T cell malignancies
- Folliculotropism (FT) and large cell transformation (LCT) are histological subtypes with poor outcome [1,2]
- Detection of clonal T cell receptor β and γ (TCRB and TCRG) gene rearrangements facilitates MF/SS diagnosis
- Next-generation sequencing (NGS) provides detailed evaluation of TCR rearrangements and the possibility to quantify individual clone frequency compared to multiplex PCR technique
- It was shown that TCRVb20 is the most frequent β clone, and a total clone frequency for TCRVB >25% is associated with worst prognosis[3]
- Correlation between distinct clones and histological subtypes in MF and SS has not been investigated.

[1]Gerami et al., Arch Derm 2008; [2]Benner et al., Blood 2012;
[3] De Masson et al., Science Translational Med, 2018

Background (cont'd)



V=variable, D=diversity, J=joining

- TCR in MF/SS is typically composed of αβ chains
- CDR3 is the antigen-binding site encoded by rearranged V-(D)-J segments
- Rearranged TCRG segments are usually maintained in αβ T cells

Goals

- To investigate the TCR repertoire in MF and SS
- To identify association of TCR signature with stage, histological subtypes, checkpoints proteins, and prognosis in patients diagnosed with MF and SS

Methods

- **Data collection**:
  - Identified patients with MF and SS registered at City of Hope
  - Retrospectively reviewed patients' charts
- **TCR sequencing**: NGS on skin biopsies at initial diagnosis or after a treatment failure
- **Study duration**: August 2020 to December 2023
- **Clonality definition**: Detection of 1 or 2 segments ≥ to 2.5% of total reads (PTR) at least 5-fold-increased over polygonal background
- **PTR**=total clone frequency= # reads for a specific sequence/# reads for all rearranged sequences X 100
- **Statistical analysis methods**: Fisher's exact test, Kaplan-Meier estimator, and Cox's proportional hazards model
- **Analysis:** by stage , FT and LCT

Patient Characteristics

Characteristics	MF & SS, n=108* (%)
Gender	
Male	65 (60%)
Female	43 (40%)
Ethnicity	
White	67 (62%)
Hispanic/Latino	24 (23%)
African-American	10 (9%)
Asian	7 (6%)
Lesion Type, Biopsy	
Patch	16 (15%)
Plaque	53 (49%)
Tumor	20 (19%)
Erythrodermic	19 (17%)
Histology	
Classic	55 (51%)
Folliculotropism without LCT	30 (28%)
LCT without folliculotropisn	8 (7%)
Folliculotropism & LCT	15 (14%)

- **Number of patients**: 108 out of 400 patients with MF and SS had TCR analysis on skin biopsies by NGS

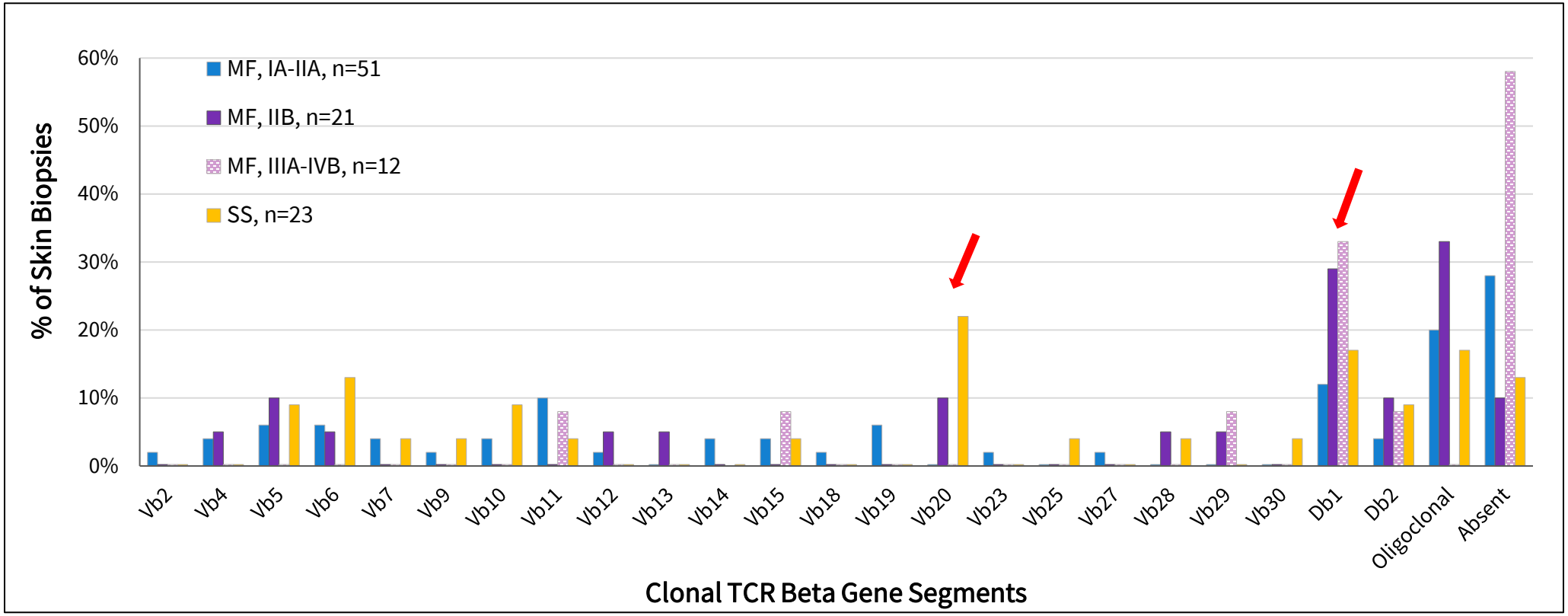
*1 case missing value for TCRB

Results

Overall:

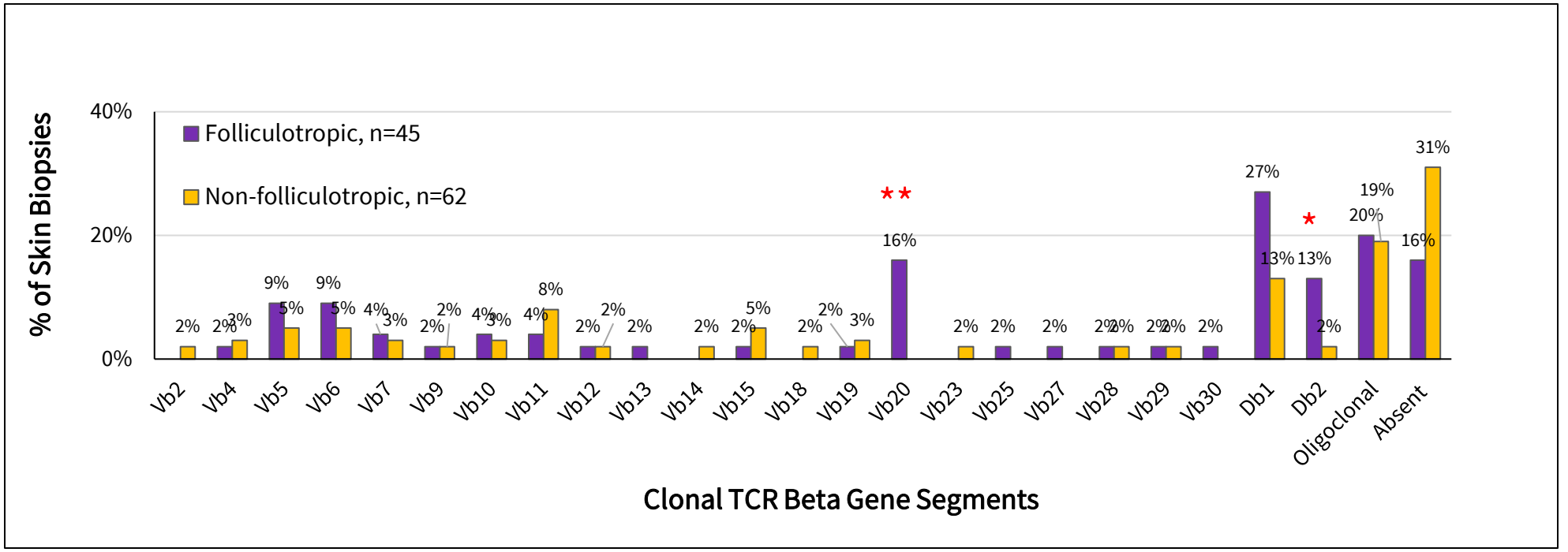
- Clonal TCRB: 56% patients
- Clonal TCRG: 71% patients
- Clonal any TCRB and/or TCRG: 76% patients

Skin TCR β repertoire in 107 patients with MF and SS by stage shows distinct clones for advanced MF & SS



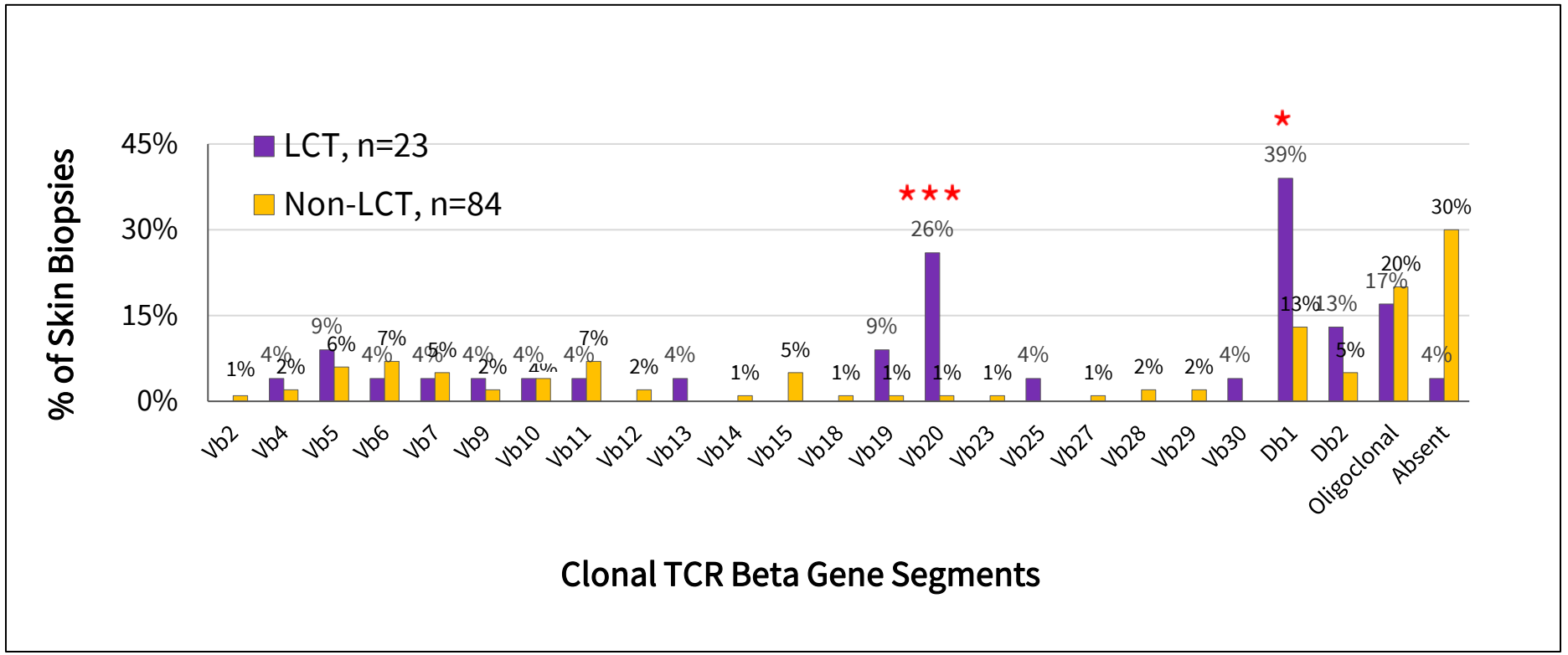
- Vb20 was detected only in patients with MF stage IIB (2/21) and SS (5/23, all with tumors)
- Db1 was detected more frequently in advance-stage MF vs. early-stage MF

TCR beta repertoire in 107 patients with MF and SS shows association of Vb20 and Db2 with folliculotropism



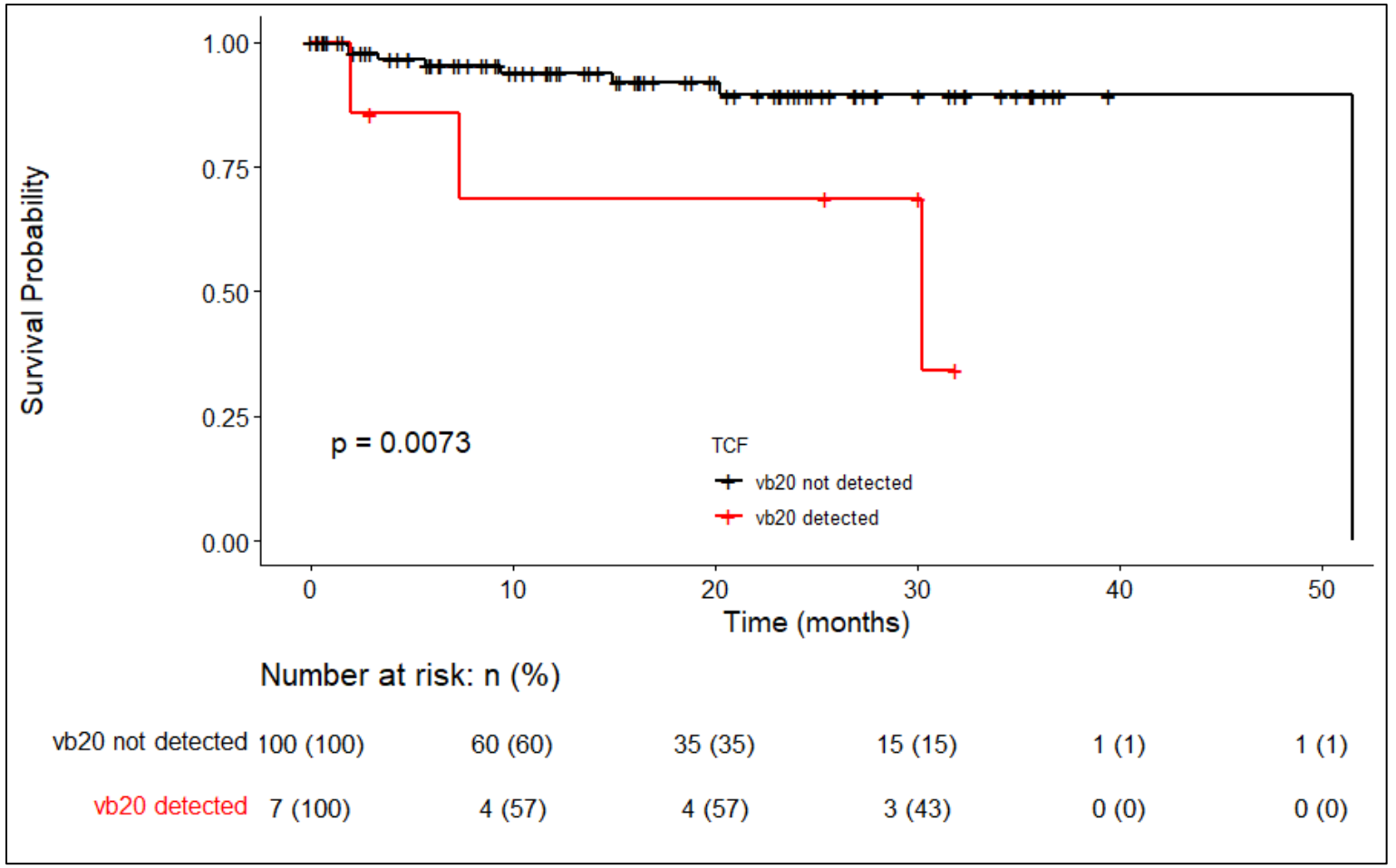
- Vb20 was detected only in FT (7/45) but not in non-FT (0/62) MF and SS (p=0.0017)
- Db2 was more frequently detected in those with vs. without folliculotropism (6/45 vs. 1/61, p=0.03)

TCR beta repertoire in 107 patients with MF and SS shows association of Vb20 and Db1 with large cell transformation

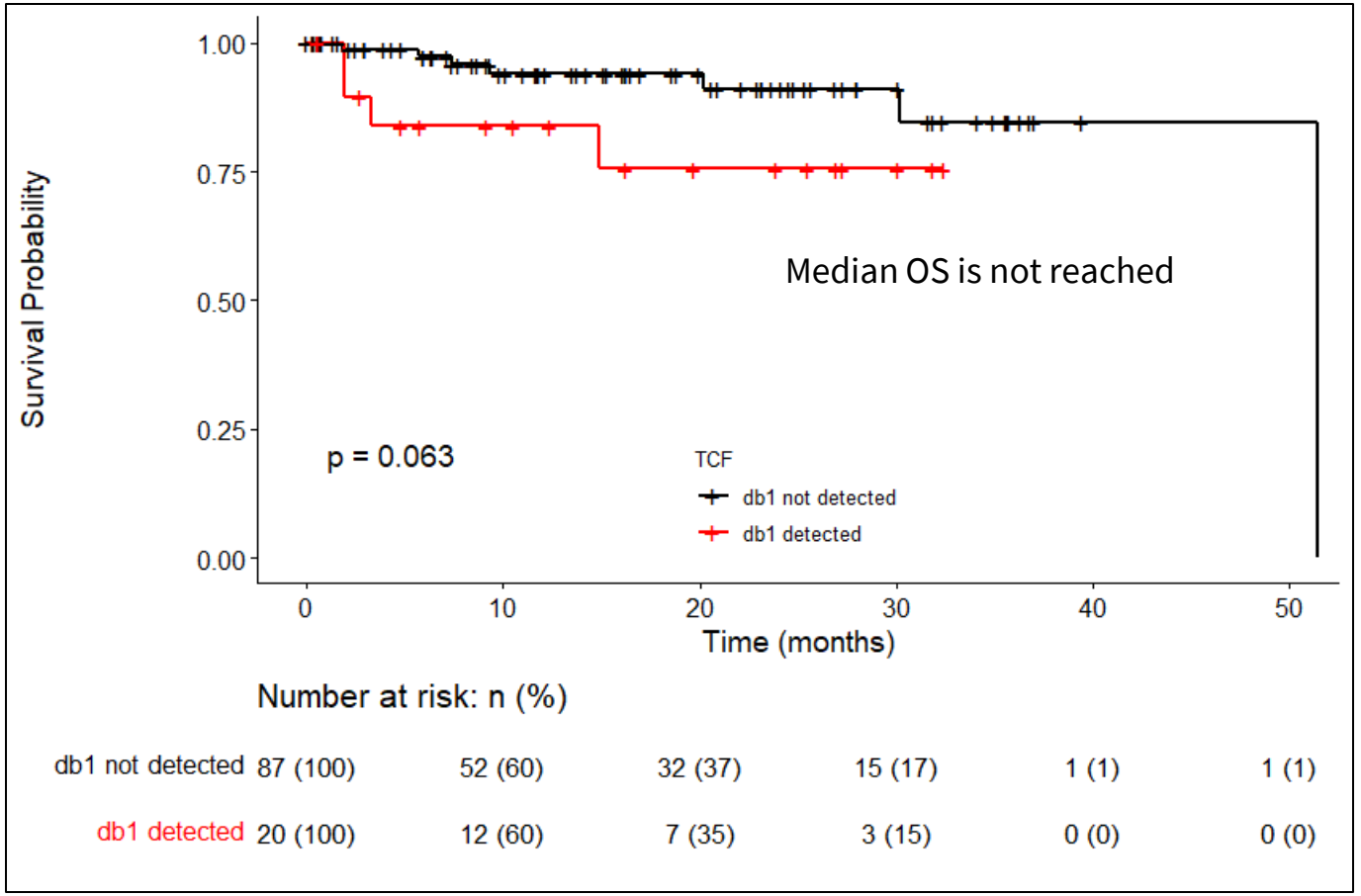


- Vb20 was detected in 6/23 patients with LCT and in 1/84 patients without LCT (p=0.0003)
- Db1 was detected more frequently in LCT vs. non-LCT (9/23 vs. 11/84, p=0.01)

Survival probability by Kaplan-Meier estimate - TCRVb20 is significantly associated with worse overall survival

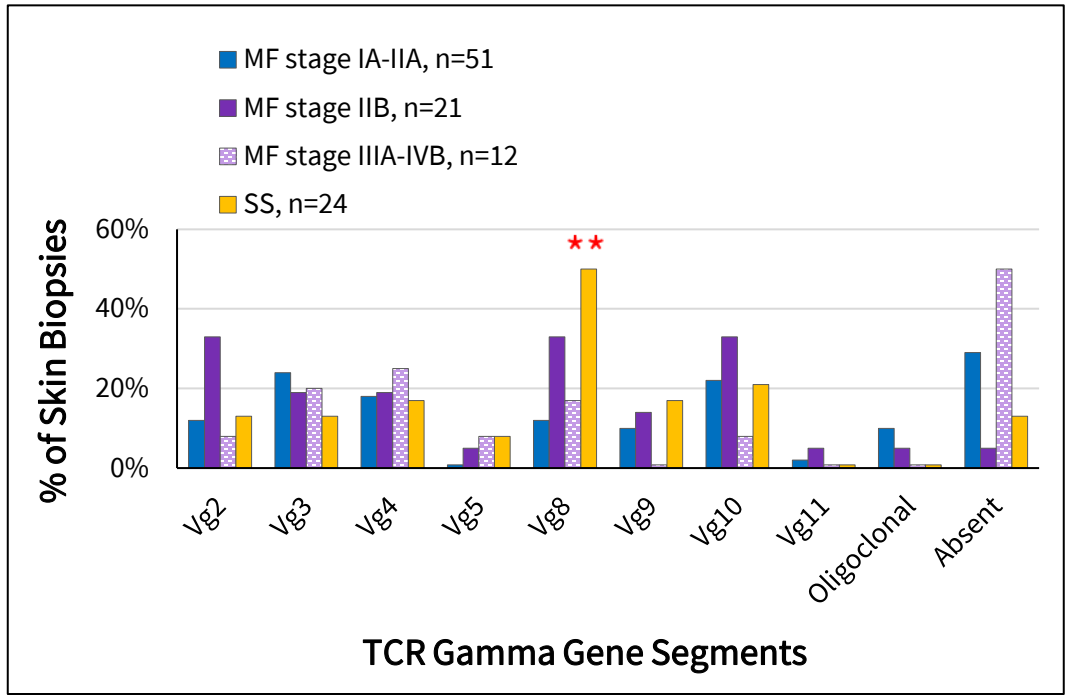


Survival probability by Kaplan-Meier estimate shows lower overall survival associated with TCRDb1



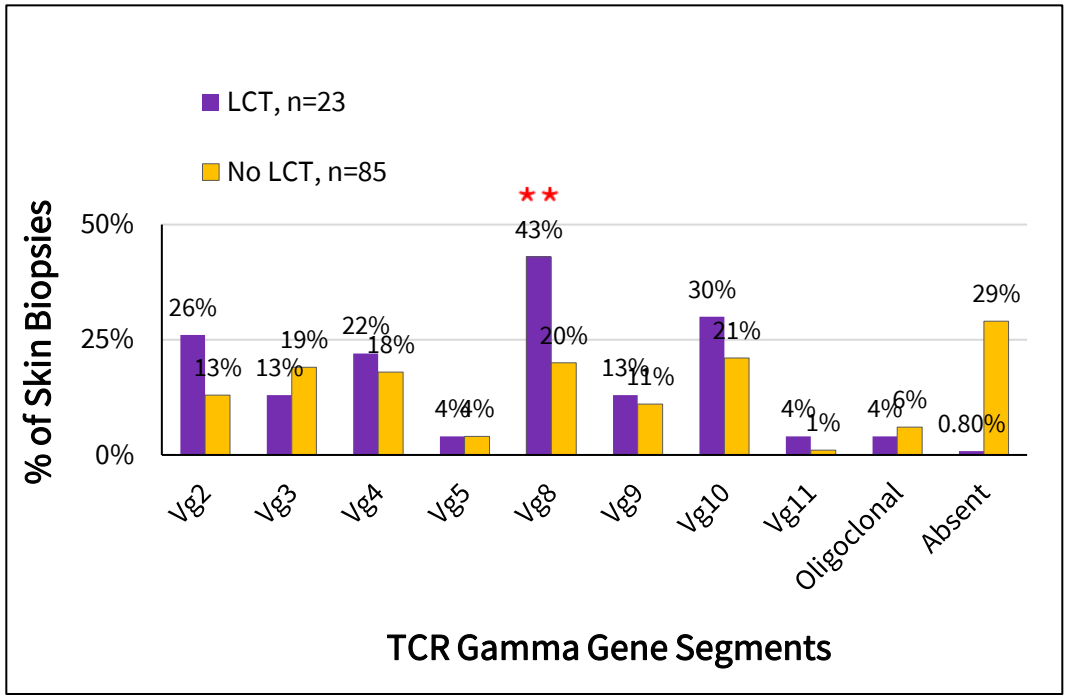
TCR gamma repertoire in 108 patient with MF/SS reveals association of Vg8 with SS (A) and large cell transformation (B)

A. TCR Gamma Repertoire by Stage



- Vg8 was detected more frequently in SS than MF (12/24 vs. 15/84, p=0.0027)

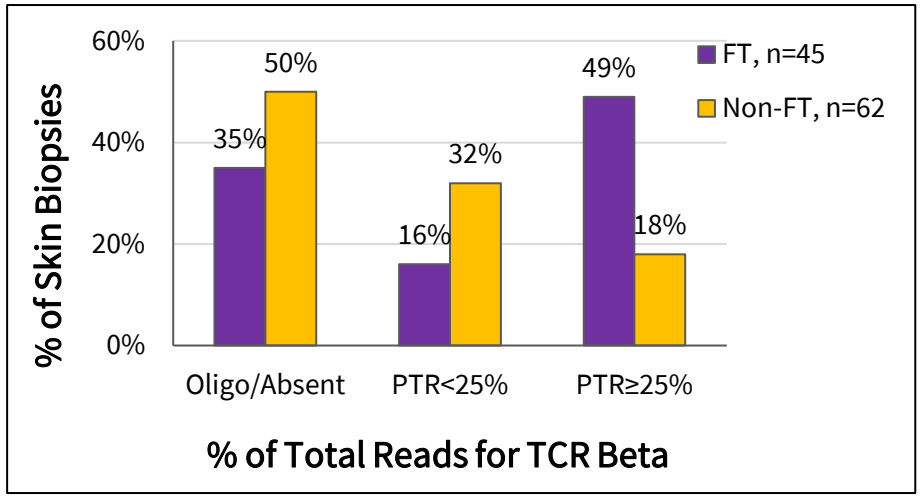
B. TCR Gamma Repertoire by LCT



- Vg8 was detected more frequently in LCT vs. non-LCT (10/23 vs. 17/85, p=0.029)

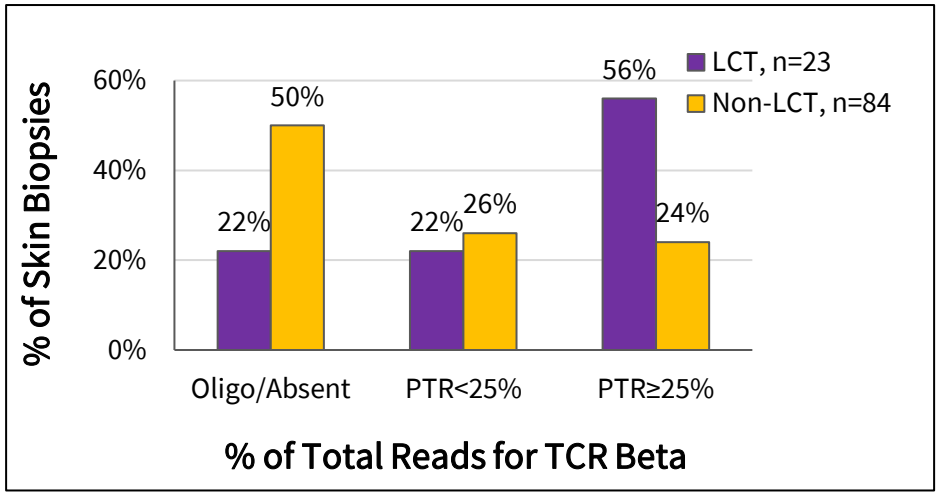
Folliculotropic and transformed MF/SS are associated with higher percentage of total reads (PTR) for TCR beta

A. PTR for TCRB by folliculotropism



- 49% skin biopsies with FT vs. 18% without FT had PTR for TCRB ≥25%

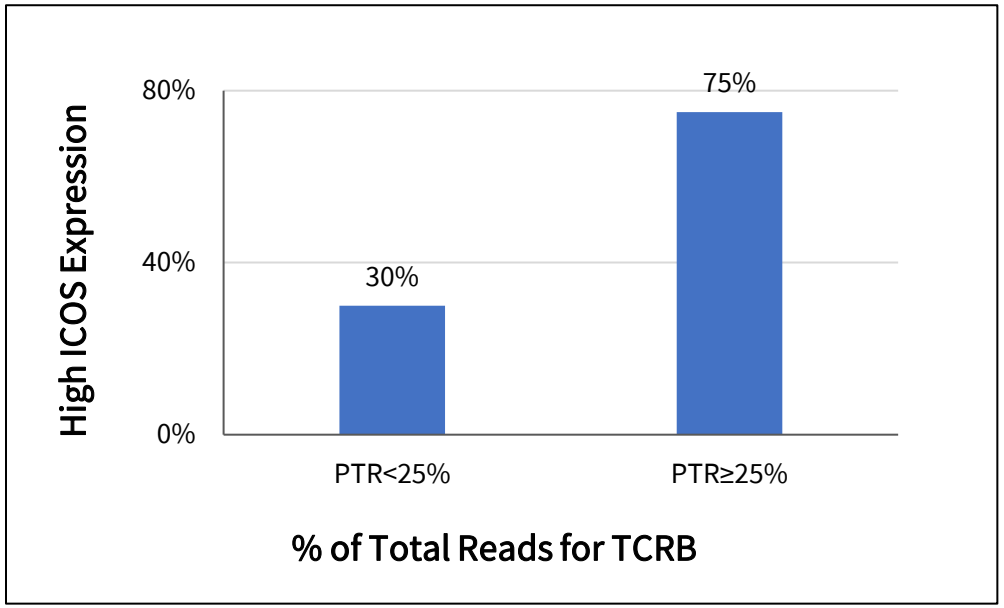
B. PTR for TCRB by LCT



- 56% skin biopsies with LCT vs. 24% with non-LCT had PTR for TCRB ≥25%

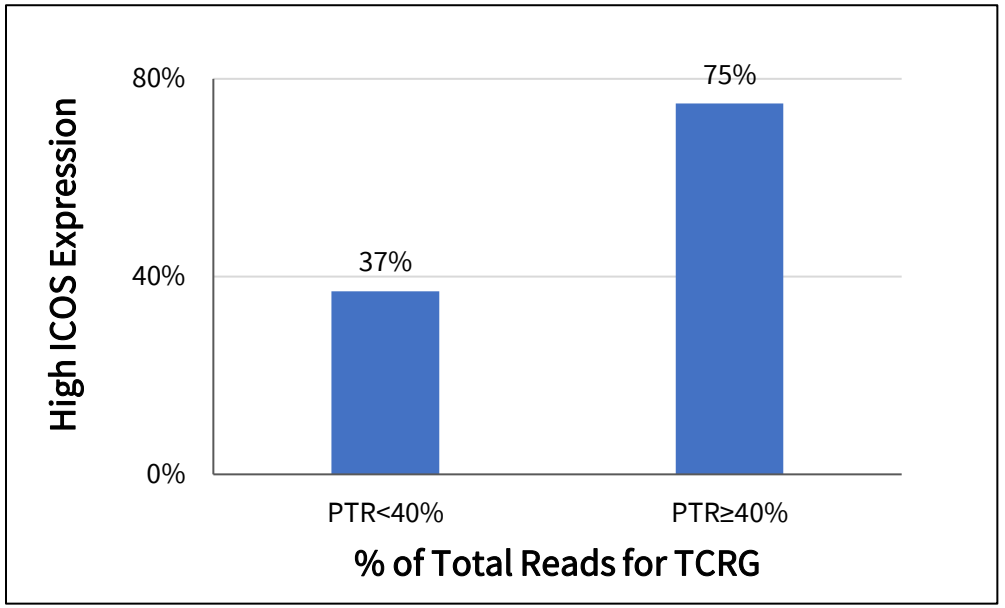
Percentage of total reads for TCRB and TCRG correlate with high ICOS expression

A. High ICOS Expression by PTR for TCRB



- Percentage of total reads for TCRB correlates positively with ICOS. *P*=0.04

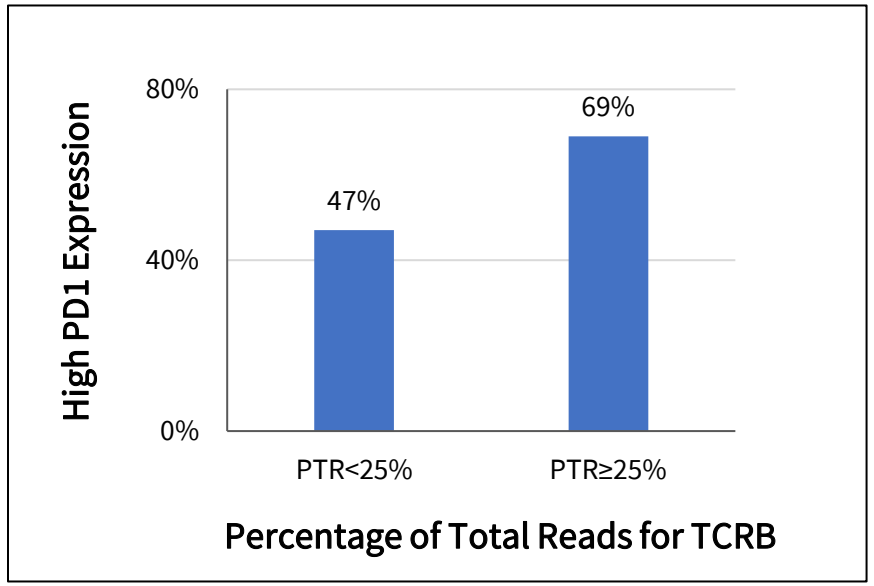
B. High ICOS Expression by PTR for TCRG



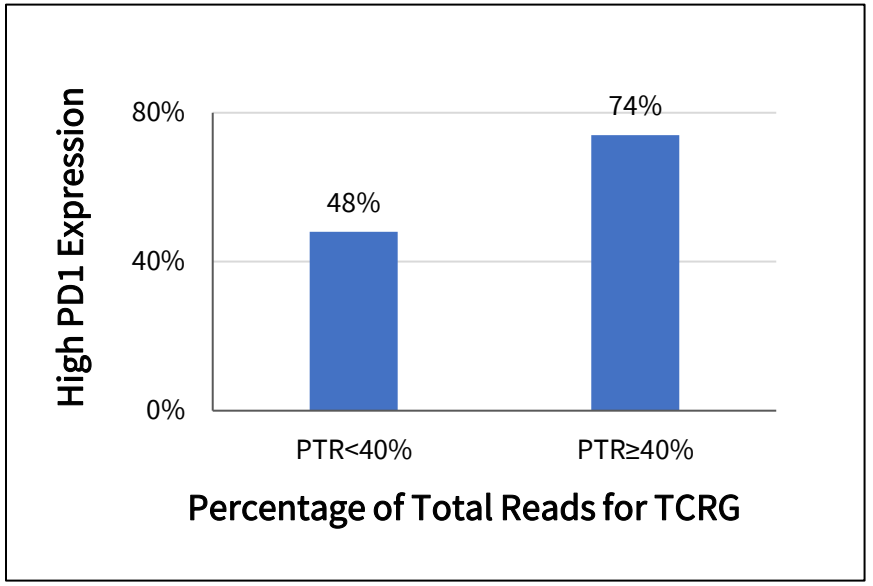
- Percentage of total reads for TCRG correlates positively with ICOS. *P*=0.012

Percentage of total reads for TCRB and TCRG correlation with high PD1 expression in skin biopsies

A. High PD1 Expression by PTR for TCRB



B. High PD1 Expression by PTR for TCRG



- There is a trend for positive association of high PD1 expression with percentage of total reads for TCRB (A) and TCRG (B), but the results are not statistically significant

Uni- and multivariate analysis on overall survival in MF and SS patients

Variables	Univariate			Multivariate		
	Hazard Ratio	95% CI	*P value*	Hazard Ratio	95% CI	*P value*
PTR for TCRB ≥25%	10.23	2.16-48.45	**<0.01**	6.21	1.14-33.79	**0.03**
PTR for TCRG ≥50%	12.76	3.19-51.05	**<0.01**	7.98	1.75-36.40	**<0.01**
FT vs. non-FT	5.71	1.21-26.91	**0.03**	2.31	0.41-13.15	0.34
LCT vs. non-LCT	4.08	1.18-14.11	**0.03**	1.77	0.45-7.00	0.41
Clonal Vb20	5.31	1.36-20.74	**0.02**			
Clonal Db1	3.12	0.88-11.11	0.08			
Male vs Female	0.78	0.22-2.69	0.69			
Age≥60	2.15	0.55-8.37	0.27			
Non-white vs white	1.65	0.48-5.7	0.43			

Subgroup analysis on folliculotropism

Gene	PTR	HR	95% CI	*P value*
TCRB	≥25%	7.98	0.96-66.16	**0.05**
	≥40%	8.98	1.08-74.75	**0.04**
	≥50%	3.69	0.81-16.6	0.09
TCRG	≥25%	4.21	0.51-34.93	0.18
	≥40%	8.84	1.07-72.97	**0.04**
	≥50%	16.31	1.96-135.9	**<0.01**

- No sufficiently large number of events for calculating the stable estimates of Hazard Ratio for LCT

Conclusions

- TCRB and TCRG repertoires reveal distinct clones for aggressive subtypes, and reduced overall survival in MF and SS.
- The percentage of total reads for TCRB>25% and TCRG>40% correlates with immune checkpoint profile.

Acknowledgements

Co-authors:

- Jeffrey Li, PhD
- Michelle Afkhami, MD
- Raju Pillai, MD
- Joycelynne Palmer, PhD
- Niloufer Khan, MD
- Jasmine Zain, MD
- Steven Rosen, MD
- **Christiane Querfeld, MD, PhD (Mentor)**

Thank you!

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