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

Biologic Insights | # 127

Exploring the role of Clonal T-LGL Proliferations in CTCL

Yumeng “Julia” Zhang MD

Assistant Member

Department of Malignant Hematology

H. Lee Moffitt Cancer Center and Research Institute

USA

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.

Cellular Immunity in CTCL

Early Stage

- Presence of reactive CD8+ cytotoxic T cells in tissue and blood
- CD4+ T cells with Th1 phenotype

Advanced Stage

- Reduction of reactive CD8+ cytotoxic T cells in tissue and blood and exhausted CD8+ T cells
- Malignant T cells with Th2 phenotype
- Reduction in DCs

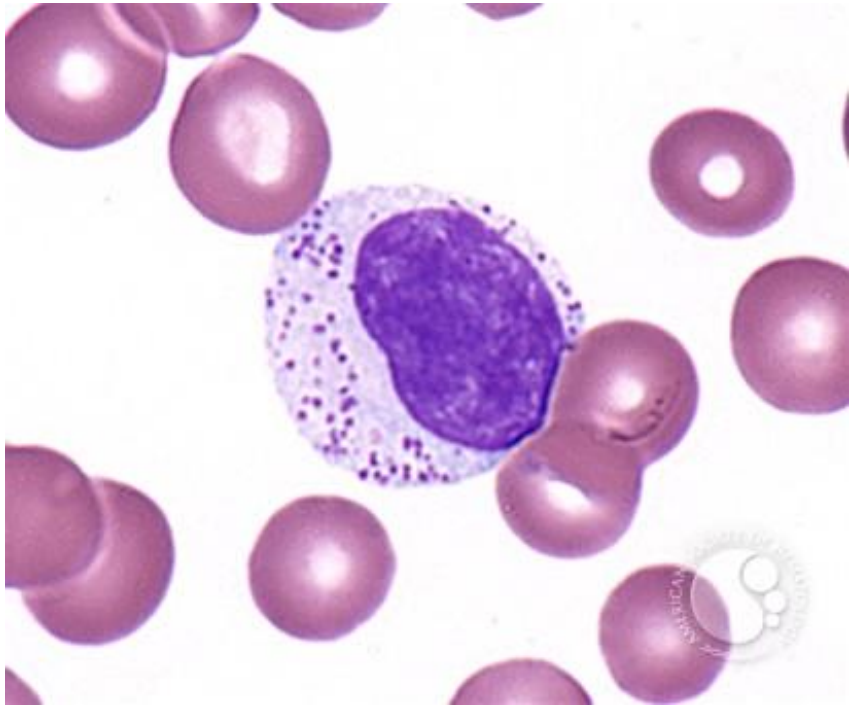
(Durgin et al., 2021)

Clonal Large Granular Lymphocyte Proliferations

- T-LGL cells are a subset of lymphocytes characterized by larger size, **azurophilic** cytoplasmic granules, and expression of surface makers such as CD3, CD8, and CD57.

- Clonal T-LGL proliferations in peripheral blood can be seen in T-LGL leukemia, reactive conditions with immune dysregulations, hematologic malignancies such as MDS, post-TKI therapy for CML, or post-allogeneic stem-cell transplant.

The goal of this study is to understand the role of clonal LGL proliferations in CTCL and explore their molecular underpinning.

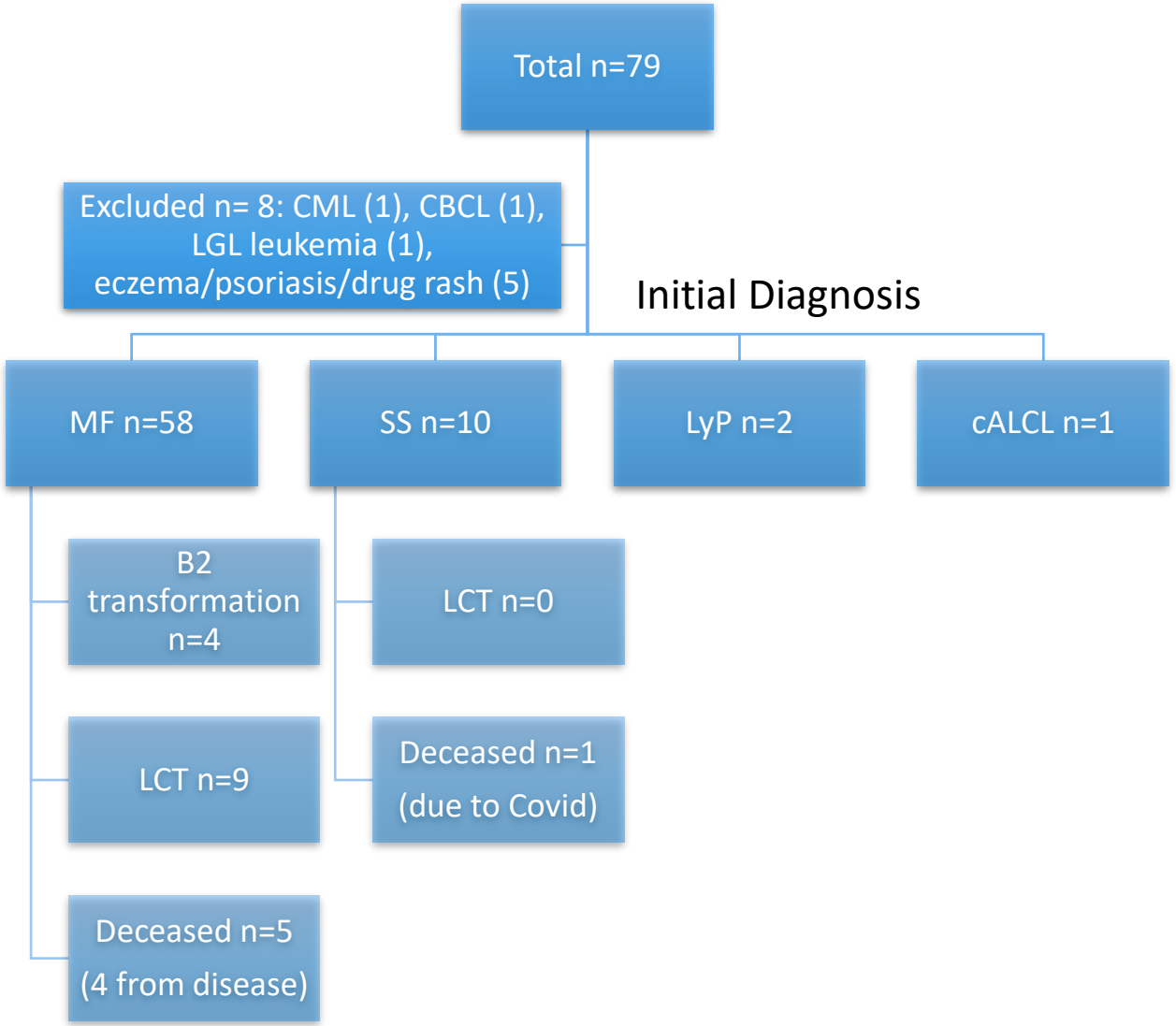


(ASH Image Bank)

Methods

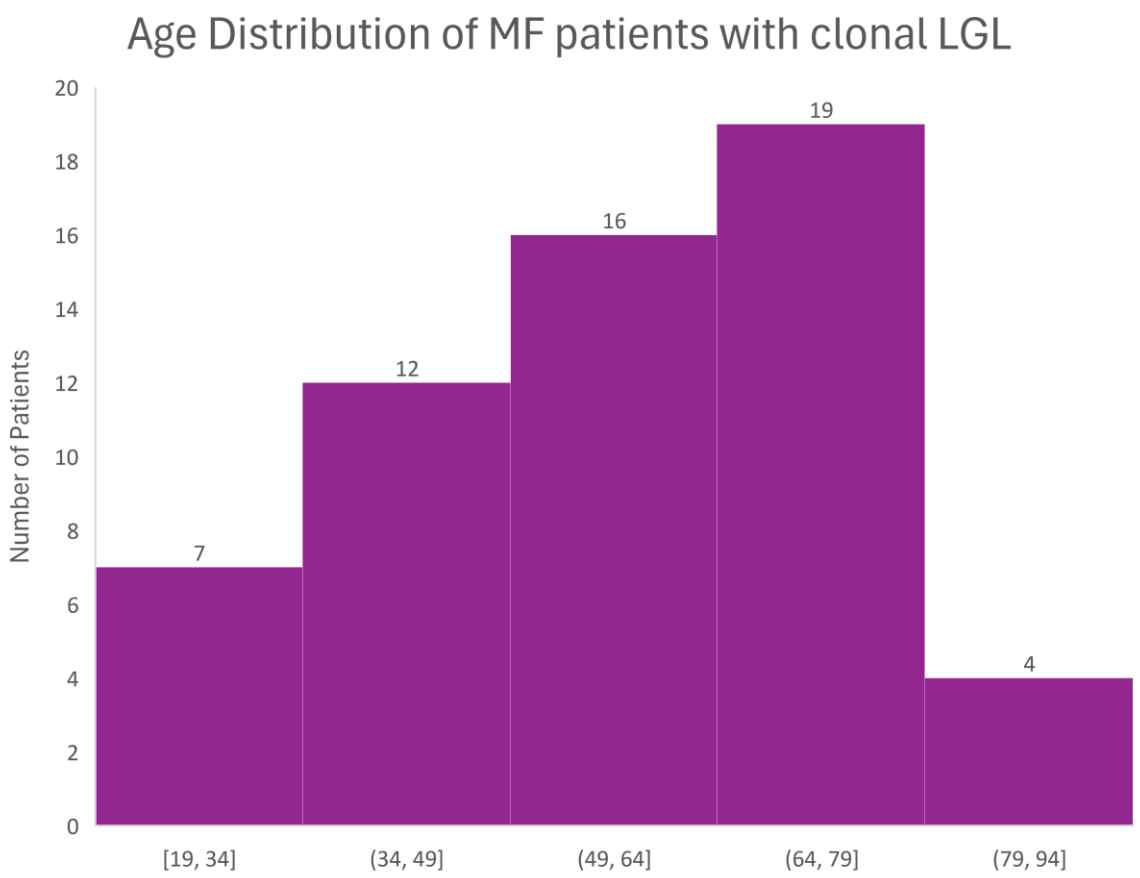
- We retrospectively and prospectively identified 79 patients seen at MCC multidisciplinary CTCL clinic with T-LGL proliferations in the peripheral blood based on flow cytometry and monoclonality based on TCR gene rearrangement.
- Custom multicolor flow cytometry included 25 T, B, and NK cell markers.
- The TCR gene rearrangement (TCRGR) test by PCR on DNA from paraffin-embedded skin tissue or cell suspensions uses multiplex PCR and capillary electrophoresis to detect clonal T-cell receptor gene rearrangements.

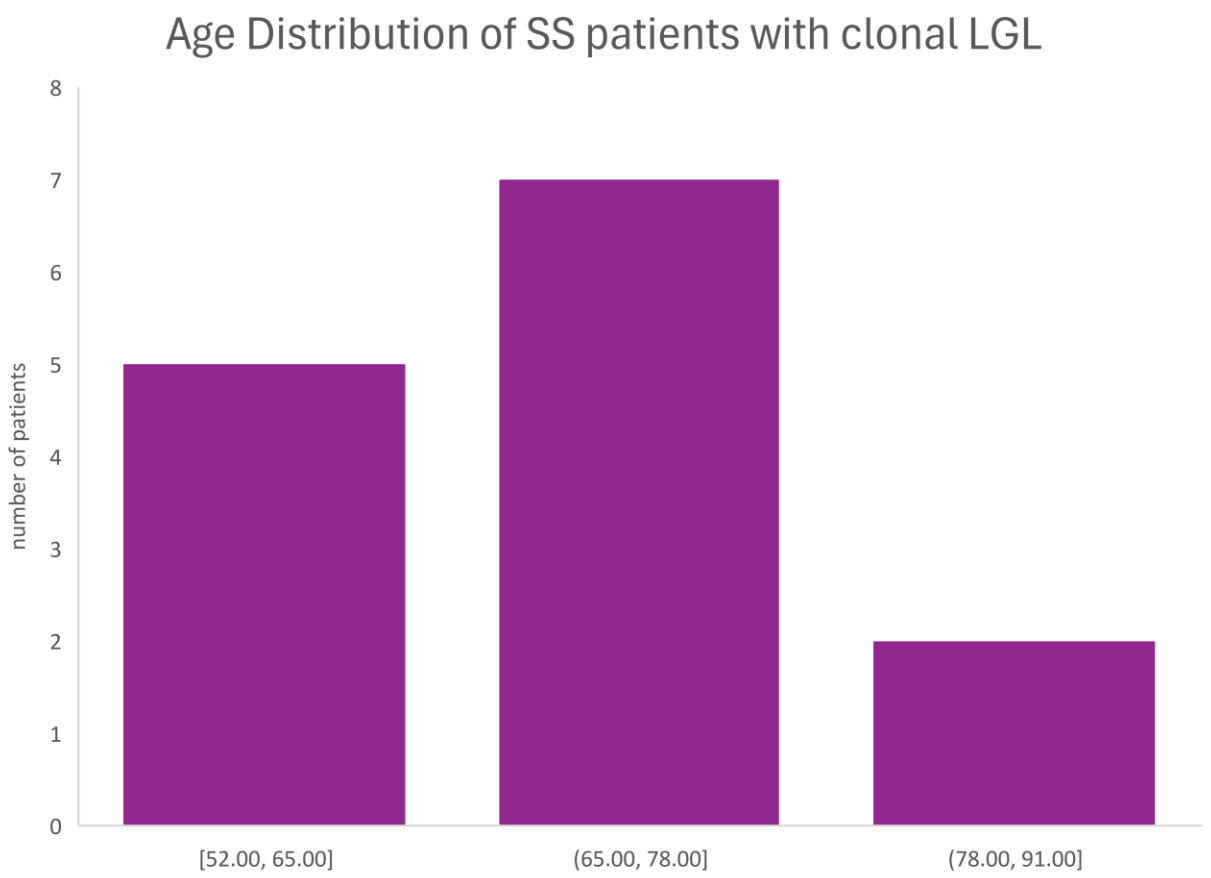
Full T-cell panel (peripheral blood)									
CD2	CD26	CD3	CD5	CD7	CD30	CD19	CD4	CD8	CD45
TCR γ/δ	TCR α/β	CD3	CD279	CD10	CD194	CD25	CD4	CD8	CD45
CD45RA	CD52	CD62L	CD56	CD7	CD16	CD4	CD3	CD57	CD45
TCR γ/δ	TCR α/β	CD8	CD5	CD7	CD16	CD56	HLA-DR	CD57	CD45
Kappa	Lambda	CD8	CD5	CD10	CD20	CD19	CD4	CD3	CD45



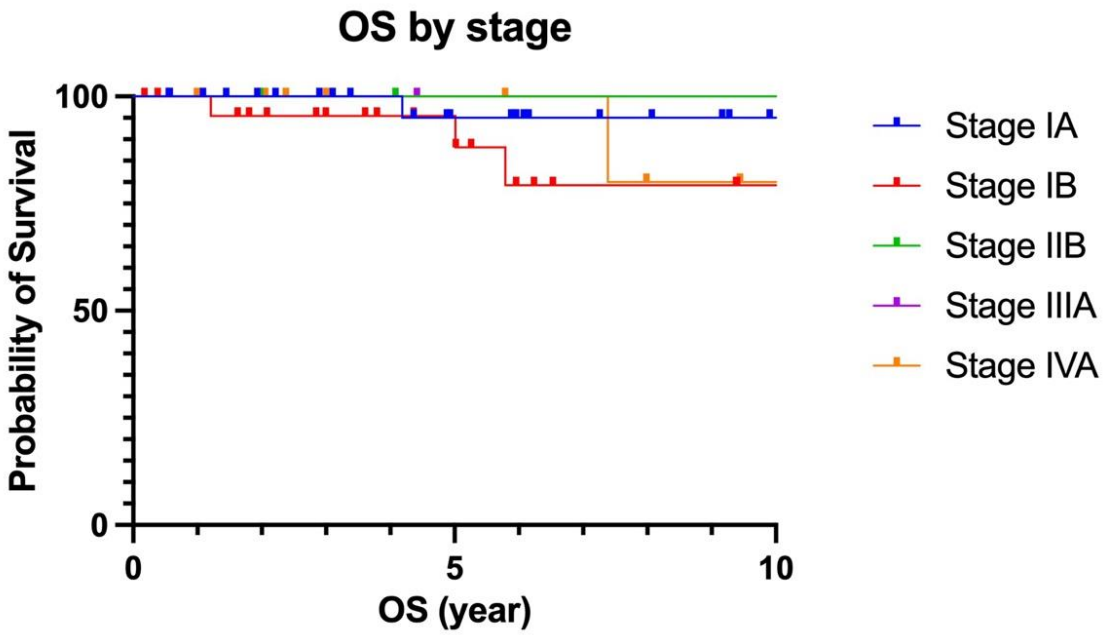
Initial Diagnosis		Mycosis Fungoides n=58	Sezary Syndrome n=14 (4 transformed)
Age, years, median (range)		61 (19-89)	67 (52-83)
Sex			
	Male	39	9
	Female	19	5
Race			
	White	42	11
	Black	9	2
	Hispanic	4	1
	Asian	3	0
IHC			
	CD4+	52	14
	CD8+	3	0
	CD4+CD8+	1	0
	CD4-CD8-	1	0
Initial Stage			
	IA	29	1
	IB	24	3
	IIB	3	0
	IIIA	2	0
	IVA1	0	10
B stage			
	B1 at diagnosis	14	
	B1 at anytime	20	
LCT			
	present	9	3 (only in secondary SS)

Age Distribution

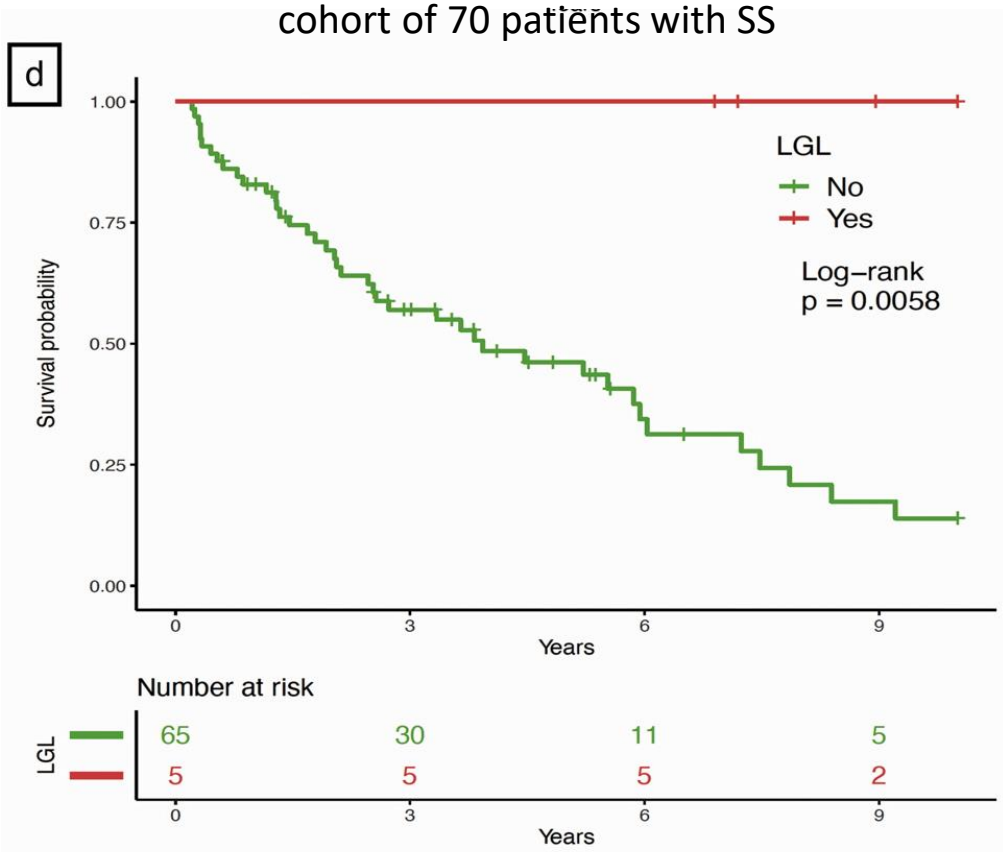




Survival



Overall survival based on the presence of clonal T-LGL cells in Moffitt institutional cohort of 70 patients with SS



(Zhang et al., 2022)

LGL information

Total n=7

No longitudinal follow up

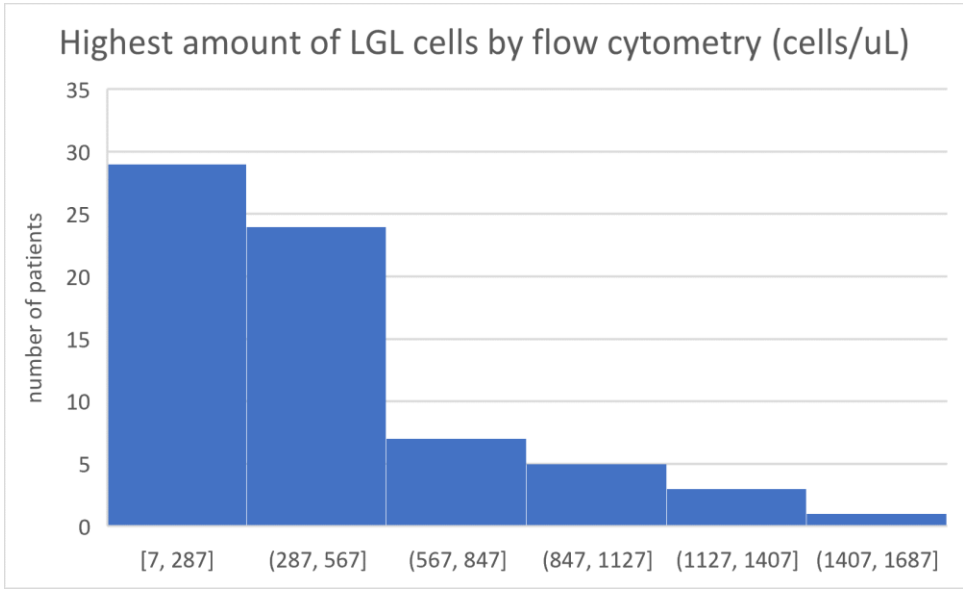
n=17

T-LGL proliferation persistent

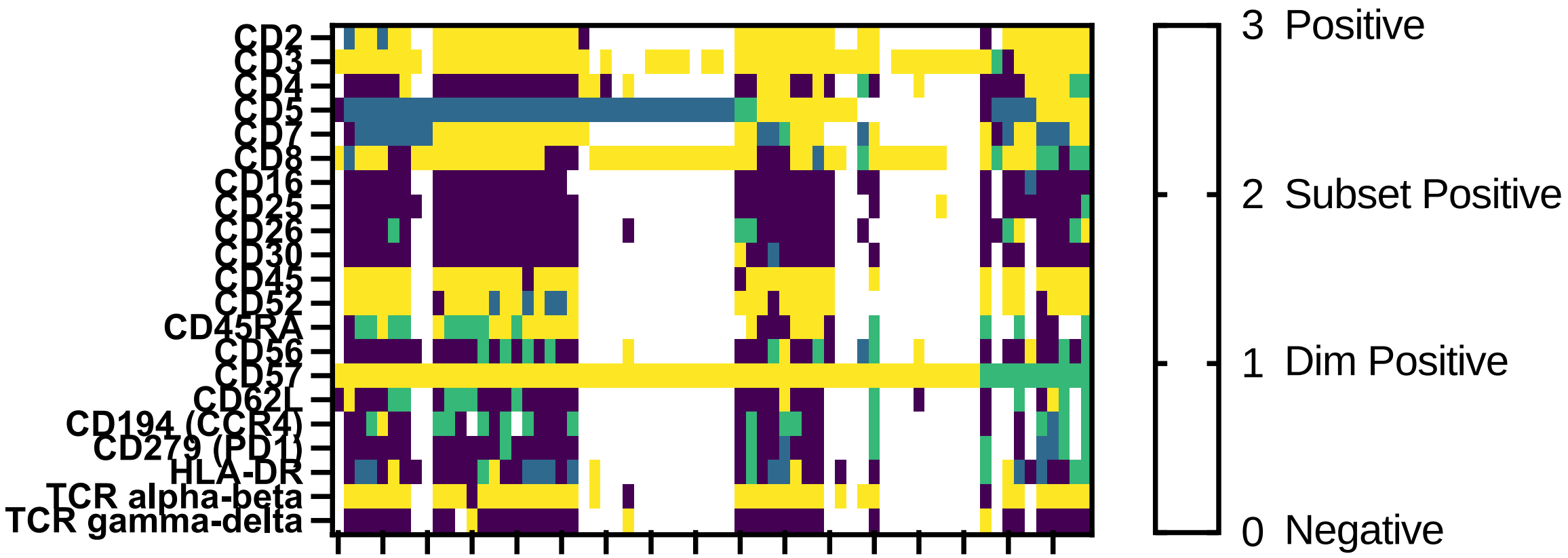
n=5

The median duration: 30 months

The longest duration: 140 months



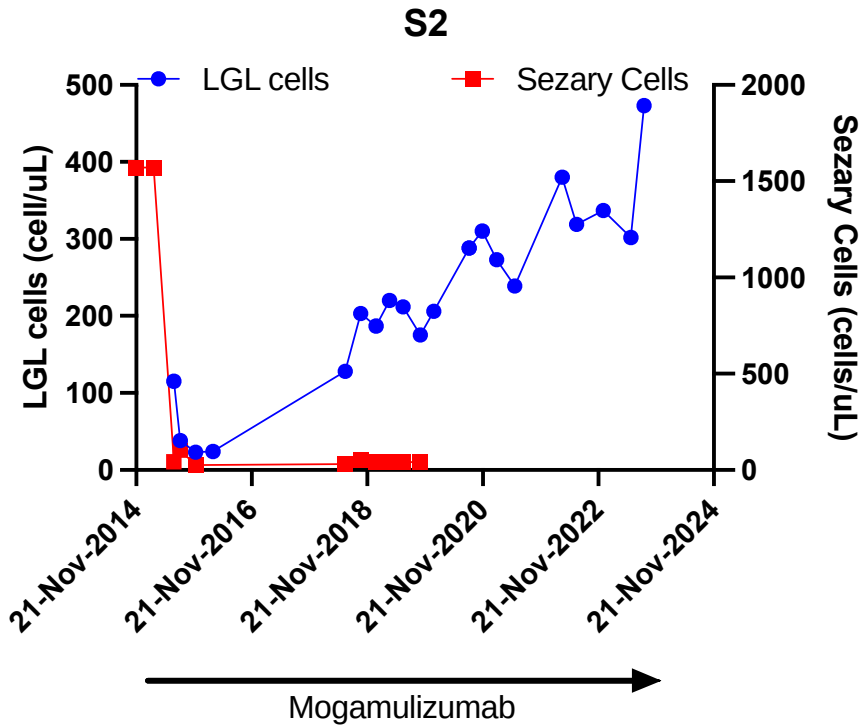
T-LGL Immunophenotype by Flow Cytometry

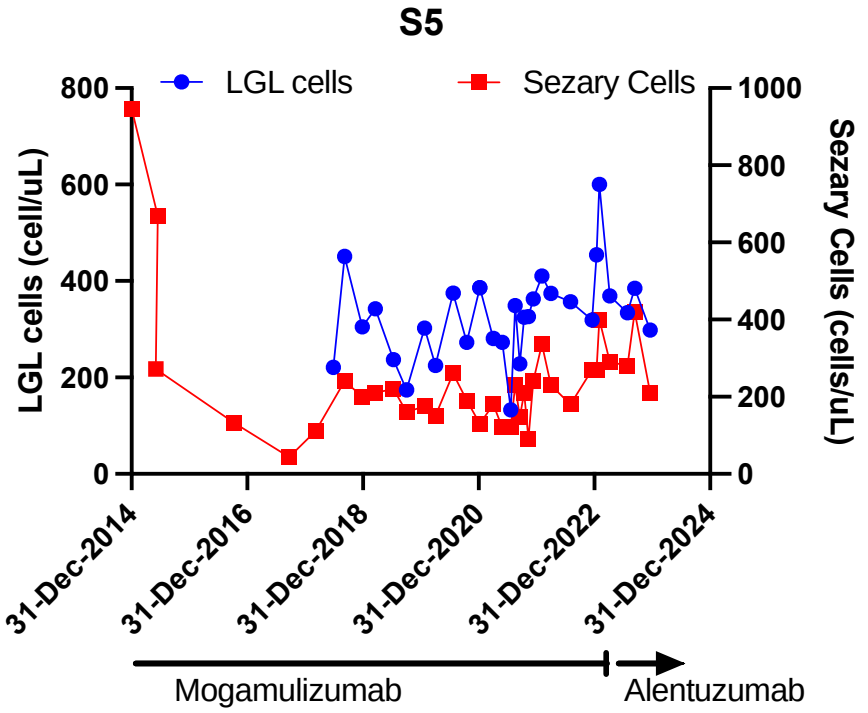


Outcome of LGLs

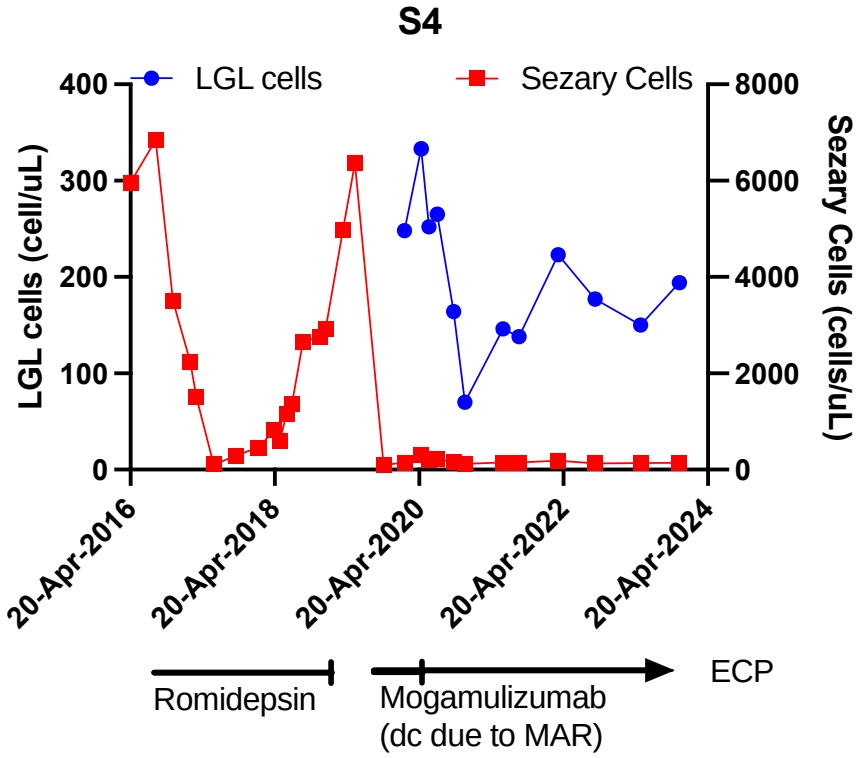
- All T-LGL cells showed distinct TCR clonotypes from those observed in Sezary cells (n=11, research effort ongoing)
- Among 12 patients who had custom myeloid NGS done, none showed STAT3/STAT5B mutations.
- None of the patients developed neutropenia or splenomegaly related to LGL clones.
- Four patients had autoimmune conditions: 2 with rheumatoid arthritis, 1 with multiple sclerosis, and 1 with x chronic inflammatory demyelinating polyradiculoneuropathy.
- Two patients were clinically defined as possibly having LGL leukemia: one based on the PB LGL count persistently above 2000 cells/uL and one based on the presence of RA, which improved with MTX treatment, but the LGL count did not decrease with treatment.

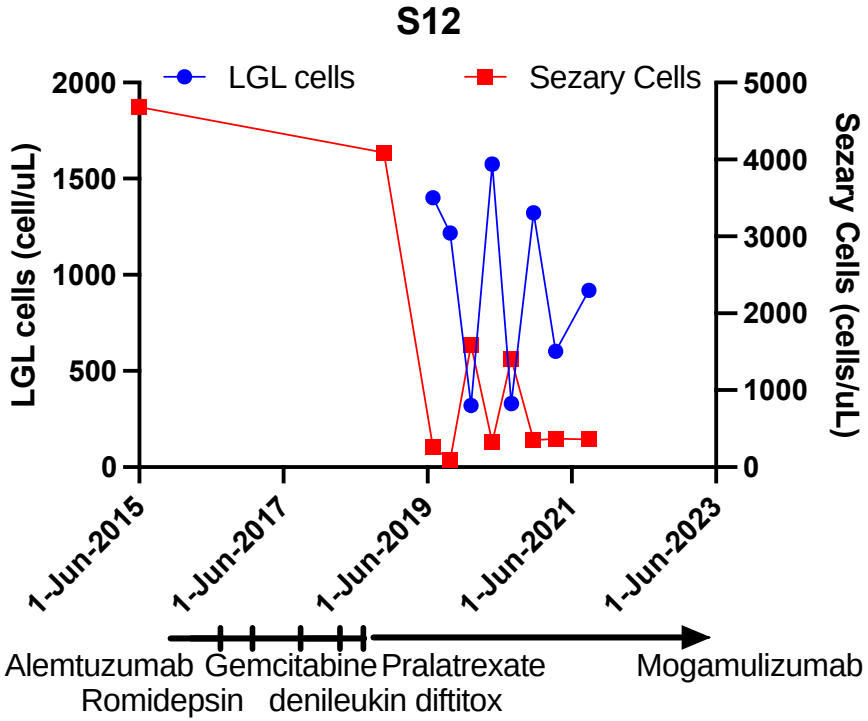
Dynamic Trend of T-LGL and SC





Dynamic Trend of T-LGL and SC





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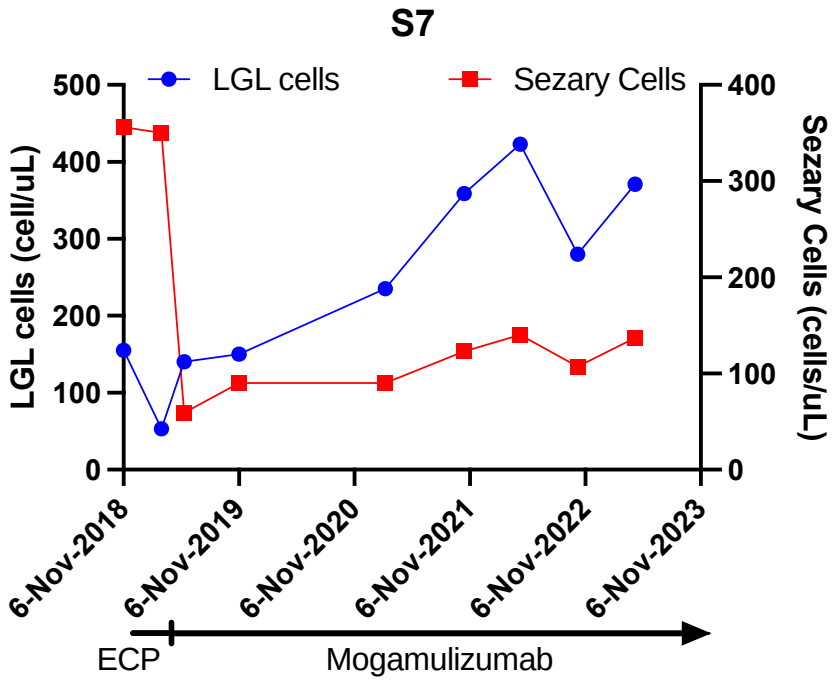
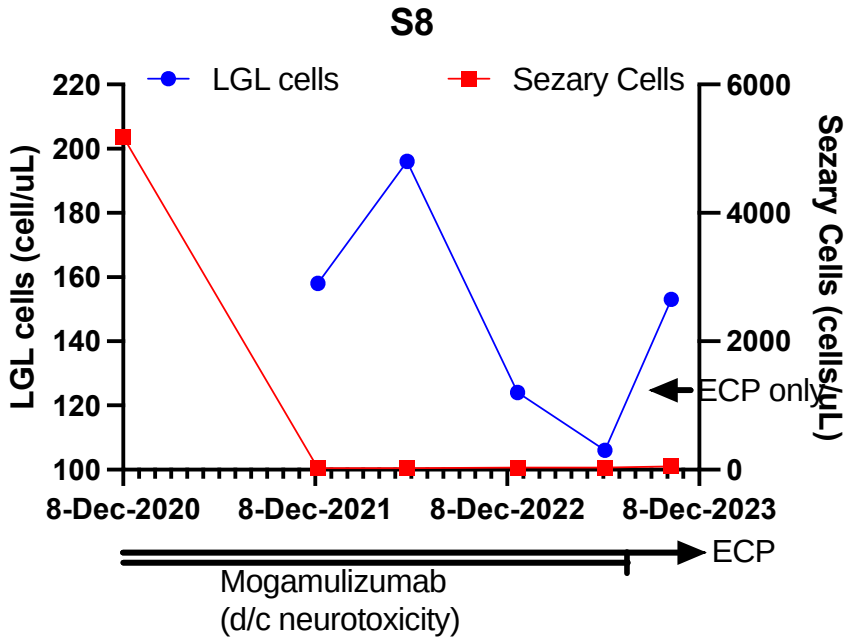
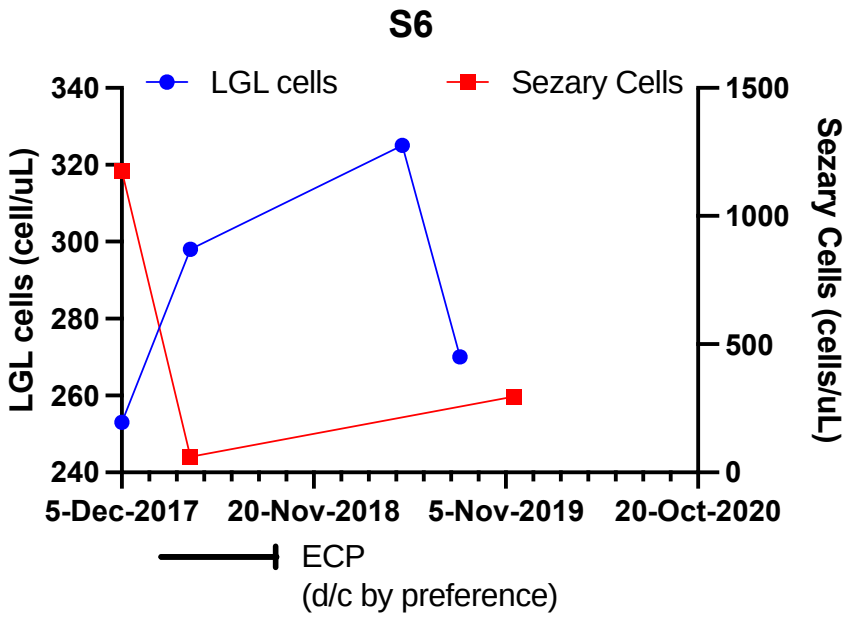
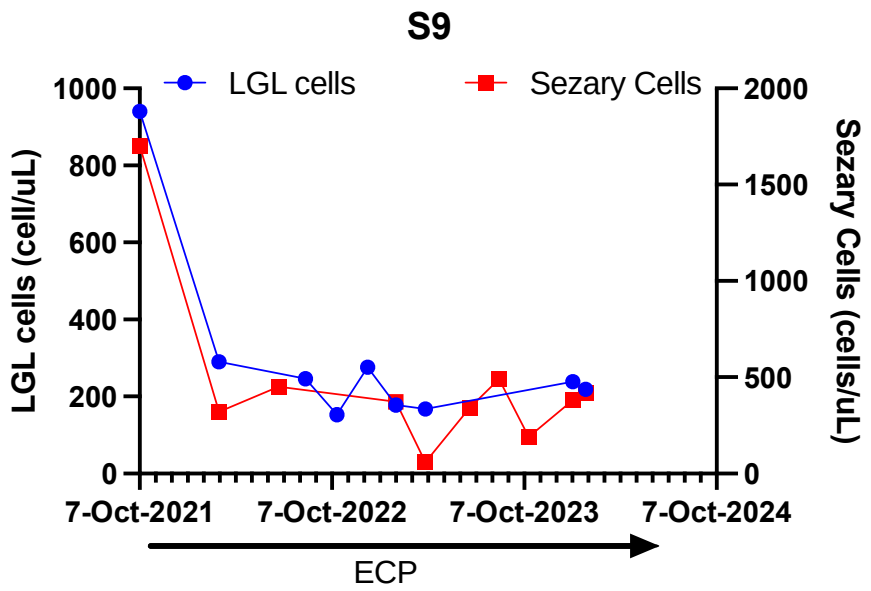


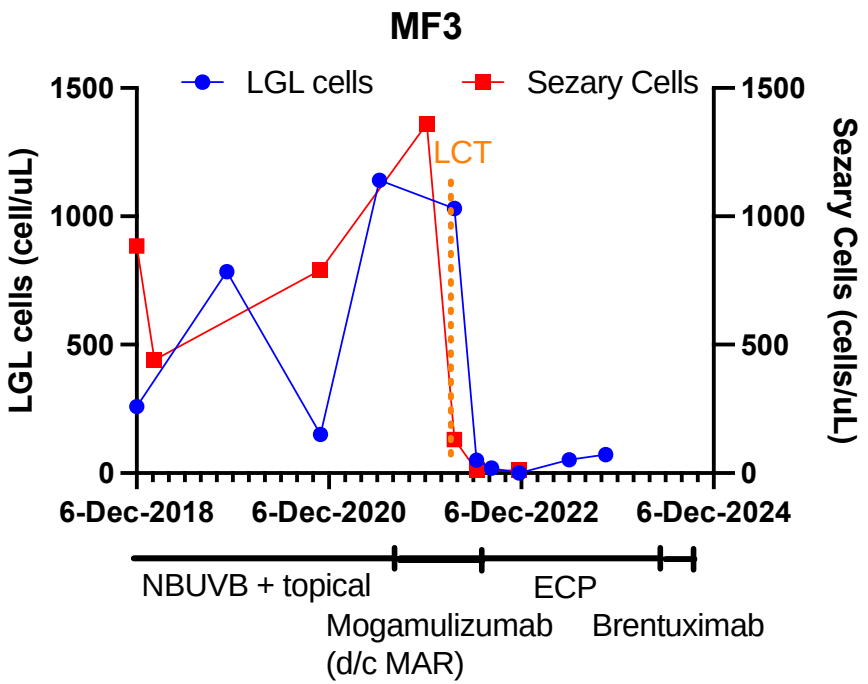


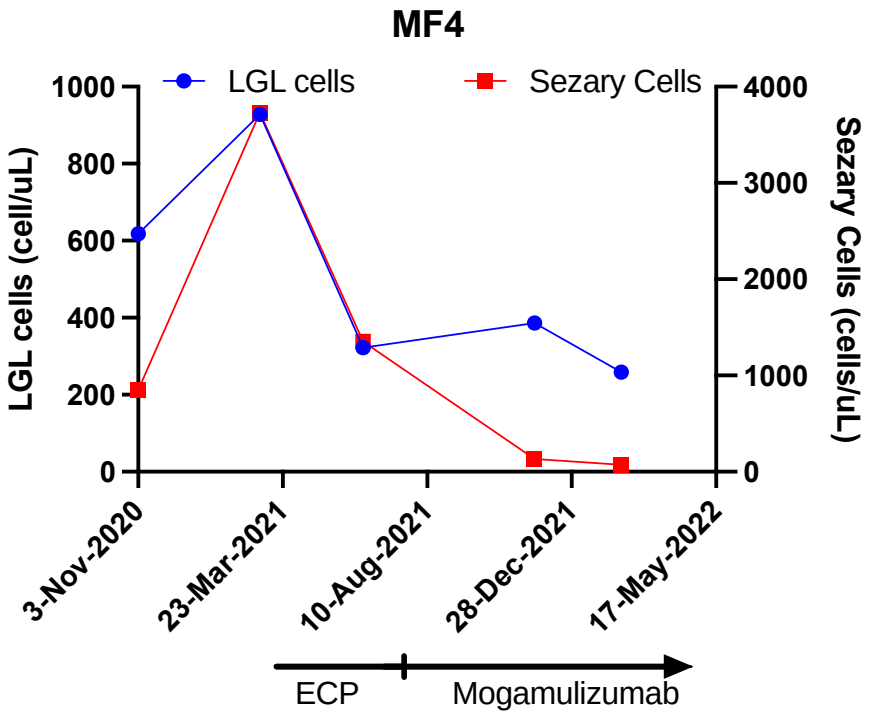
Dynamic Trend of T-LGL and SC



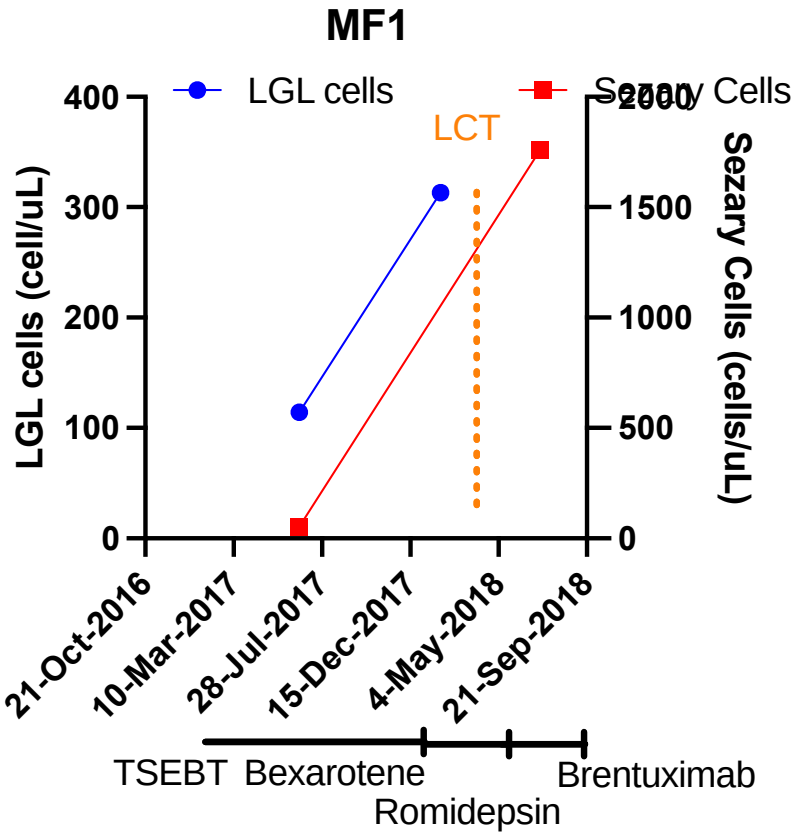


Dynamic Trend of T-LGL and SC



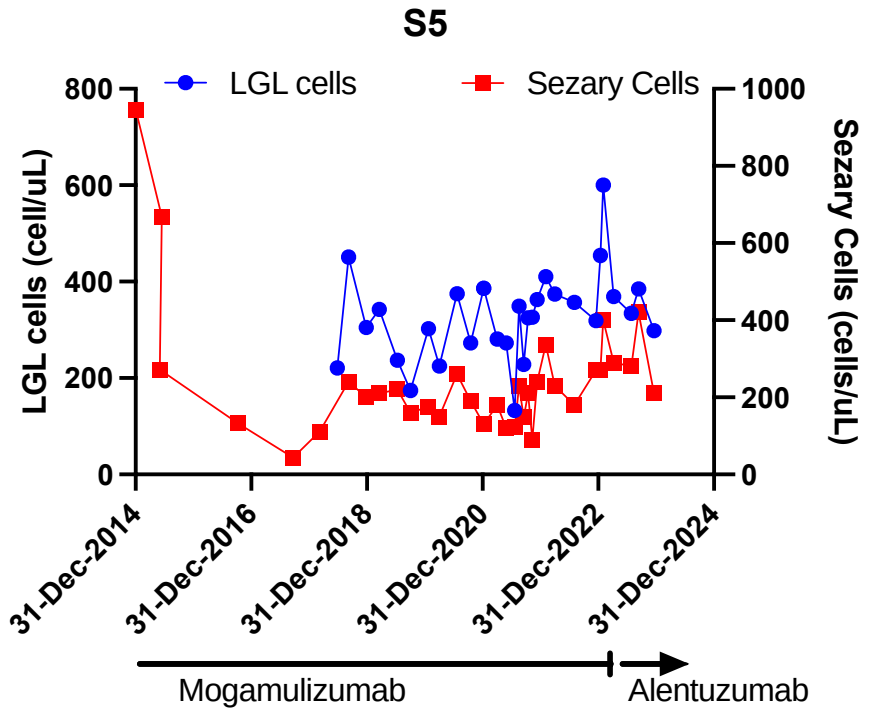


Dynamic Trend of T-LGL and SC

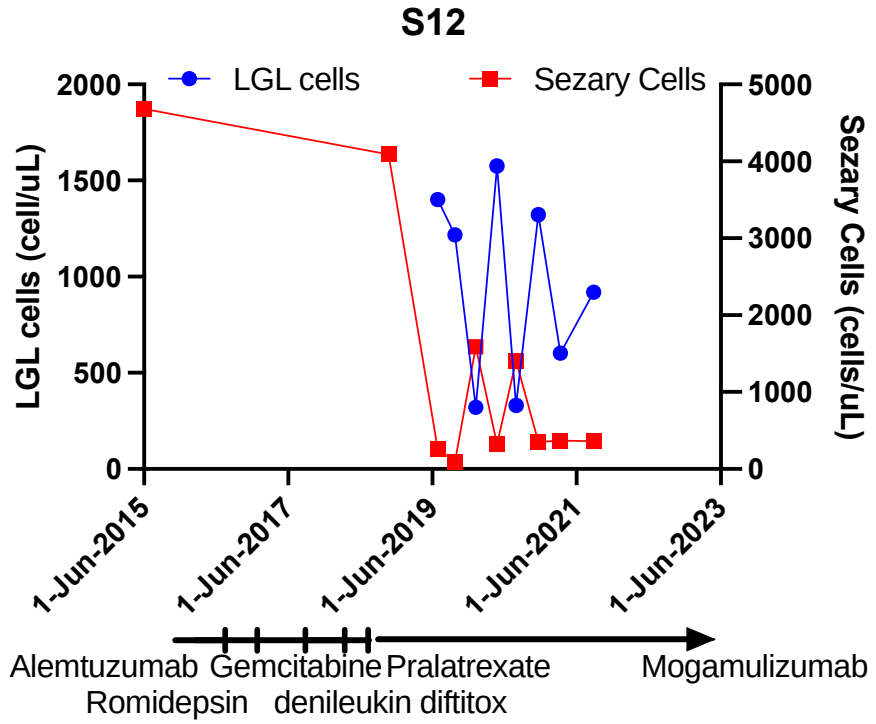


Paired single-cell RNA and TCR sequencing

S5 is a 62-year-old white man who was diagnosed with SS in 2014. Blood samples were collected on 2/20/2020.



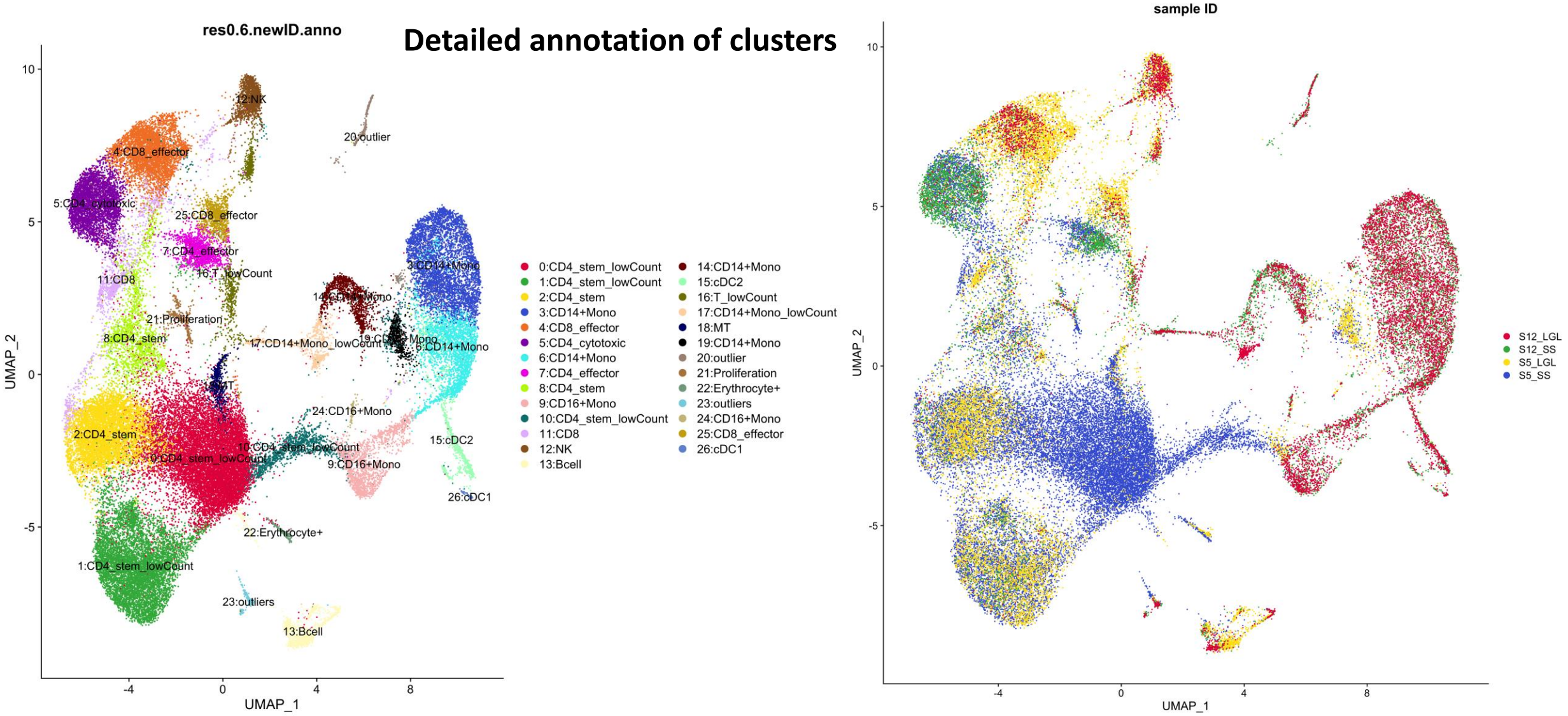
S12 is a 73-year-old white man who was diagnosed with SS in 2015. Blood samples were collected on 8/18/2020.



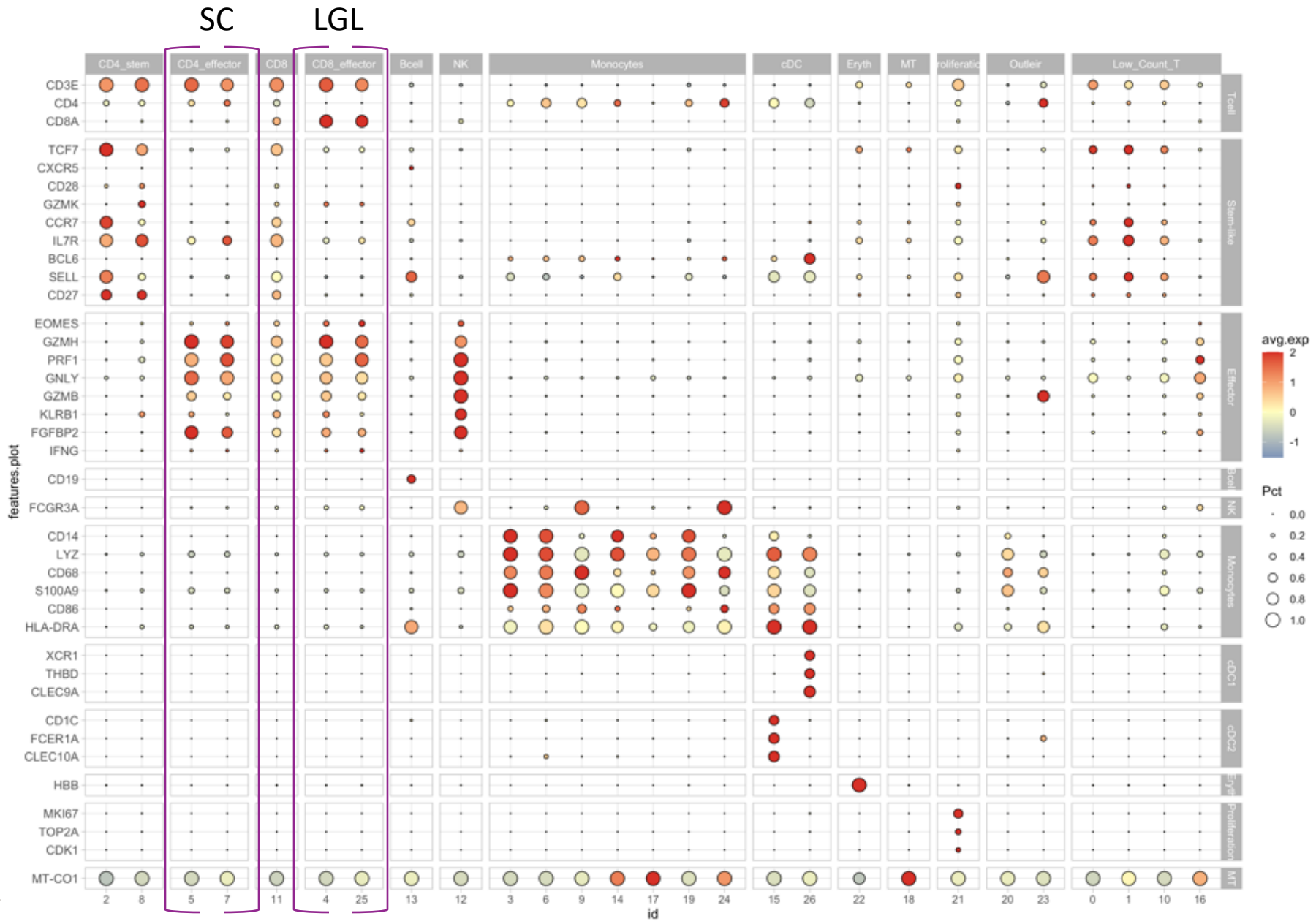
LGL and Sezary Cell Selection and Preparation for Single-Cell RNA Sequencing

- Thawed cryopreserved cells, washed and resuspended in PBS.
- Performed CD3+ and CD4+ dual selection using an EasySep kit and RapidSpheres
- Executed CD57 positive and CD26 negative selections to sort out both subpopulations
- Utilized a magnet to facilitate the separation process, collecting CD4-CD57+ (LGL) and CD4+CD26- (SC) cell fractions
- Counted cells using AOPI staining and prepared LGL and SC cell fractions for single-cell RNA sequencing.

Figure 1: UMAP plots illustrate the heterogeneity within the SS and LGL compartments with clear distinction between the clonal T-LGL populations and the Sezary cells.



	scRNA cluster			4 CD8 Effec	5 CD4 effec	6 Monocyt	7 CD4 effec	11 - CD8	25 - CD8 Ef	aa
	LGL	clonotype71	14%	**75%**	0%	0%	1%	**4%**	**17%**	CAVWMDSSYKLIF;CAYEGTDKLIF_CASSLGTLSILGYTF
S5	SS	clonotype1	5%	0%	**71%**	0%	**17%**	4%	0%	CAGGLLGSVGNEKLTF_CASSLLGSGNTIYF
		clonotype2	2%	0%	**62%**	0%	**19%**	6%	0%	CAASETSGSRLTF_CASSVRGVLSPLHF
		clonotype3	2%	0%	**38%**	0%	**20%**	1%	0%	_CASSLERGLKQTQYF
		clonotype4	2%							_CASSLERGLKQTQYF
		clonotype5	2%							_CASSLERGLKQTQYF
		clonotype6	2%							_CASSLERGLKQTQYF
		clonotype7	1%	0%	**60%**	0%	**24%**	3%	0%	CAVEDAPTYKYIF_CSATGTGAHVGEQYF
		clonotype8	1%	1%	**45%**	0%	**30%**	2%	0%	CAGGLLGSVGNEKLTF_
		clonotype9	1%							CAGGLLGSVGNEKLTF_
		clonotype10	1%							CAGGLLGSVGNEKLTF_
		clonotype11	1%							CAGGLLGSVGNEKLTF_
		clonotype12	1%							CAGGLLGSVGNEKLTF_
		clonotype13	1%							CAGGLLGSVGNEKLTF_
		clonotype14	1%	0%	**61%**	0%	**23%**	**5%**	0%	CAASRRSNDYKLSF_CSVEVTQGGNEQFF
S12	LGL	clonotype11	35%	**73%**	0%	**5%**	1%	1%	**13%**	CGSAPAAGNKLTF_CASSIFGEQFF
	SS	clonotype11	35%	**3%**	**68%**	**5%**	**17%**	1%	1%	CAFSPPPLRNTGKLIF;CAVQVNGNKLVF_CASSLVGSTEAFF
		clonotype11	12%	**2%**	**66%**	**4%**	**19%**	0%	0%	CAVRDRTAGNKLTF;CVVNDPAGGFKTIF_CASSHTGSLYNEQFF


SC
LGL
CD4_stem
CD4_effector
CD8
CD8_effector
Bcell
NK
Monocytes
cDC
Eryth
MT
Outleir
Low_Count_T
CD3E
CD4
CD8A
TCF7
CXCR5
CD28
GZMK
CCR7
IL7R
BCL6
SELL
CD27
EOMES
GZMH
PRF1
GNLY
GZMB
KLRB1
FGFBP2
IFNG
CD19
FCGR3A
CD14
LYZ
CD68
S100A9
CD86
HLA-DRA
XCR1
THBD
CLEC9A
CD1C
FCER1A
CLEC10A
HBB
MKI67
TOP2A
CDK1
MT-CO1
Tcell
Stem-like
Effector
Bcell
NK
Monocytes
cDC1
cDC2
Eryth
Proliferation
MT
features.plot
2 8 5 7 11 4 25 13 12 3 6 9 14 17 19 24 15 26 22 18 21 20 23 0 1 10 16
id
avg.exp
2
1
0
-1
Pct
0.0
0.2
0.4
0.6
0.8
1.0

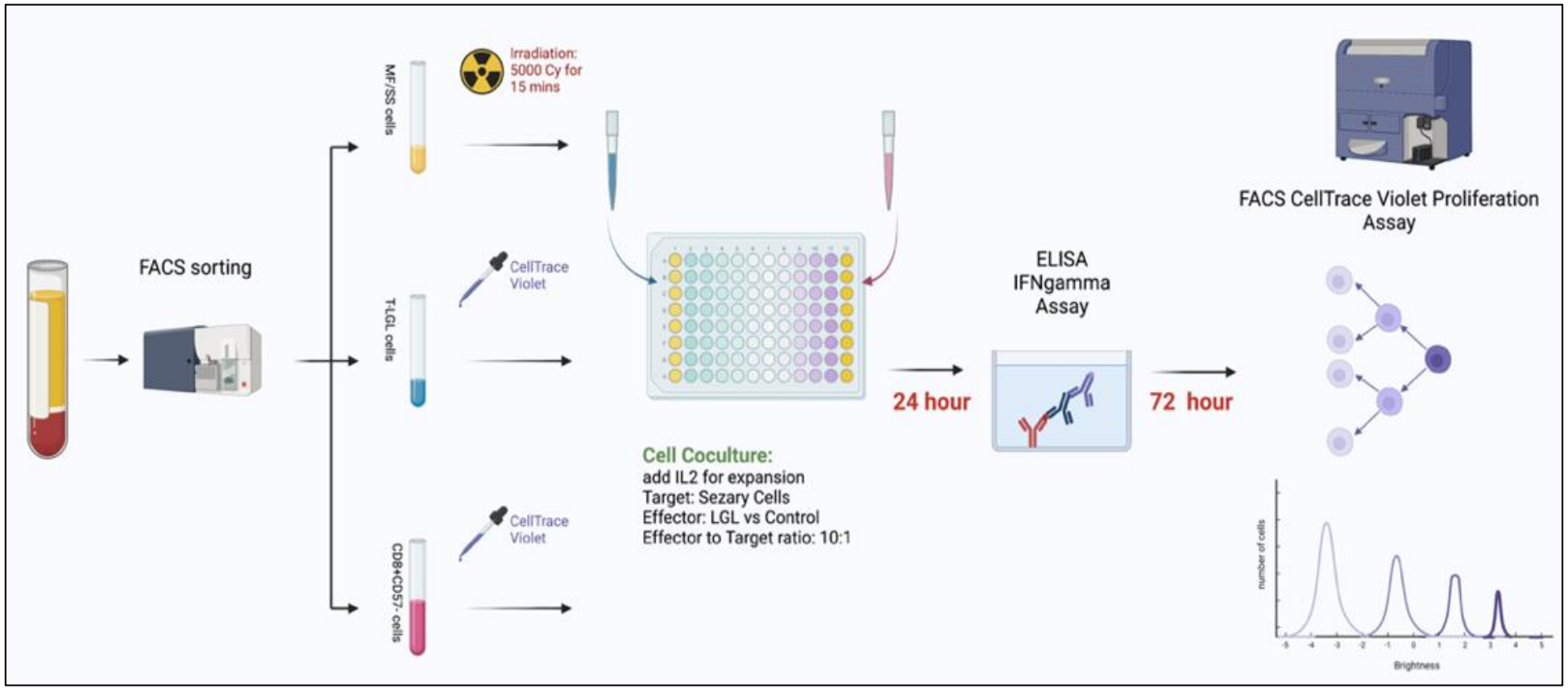
T-LGL proliferation is an oligoclonal process with a clear dominant clonotype (TRAV12-2, TDV8, TRAJ12 / TRBV9, TRBJ1-2; and TRAV12-2, TRAJ12 / TRBV9, TRBJ1-2).

T-LGL exhibits the phenotype of CD8+ effector T- cells with high expression of cytotoxic markers such as granzyme H and perforin-1.

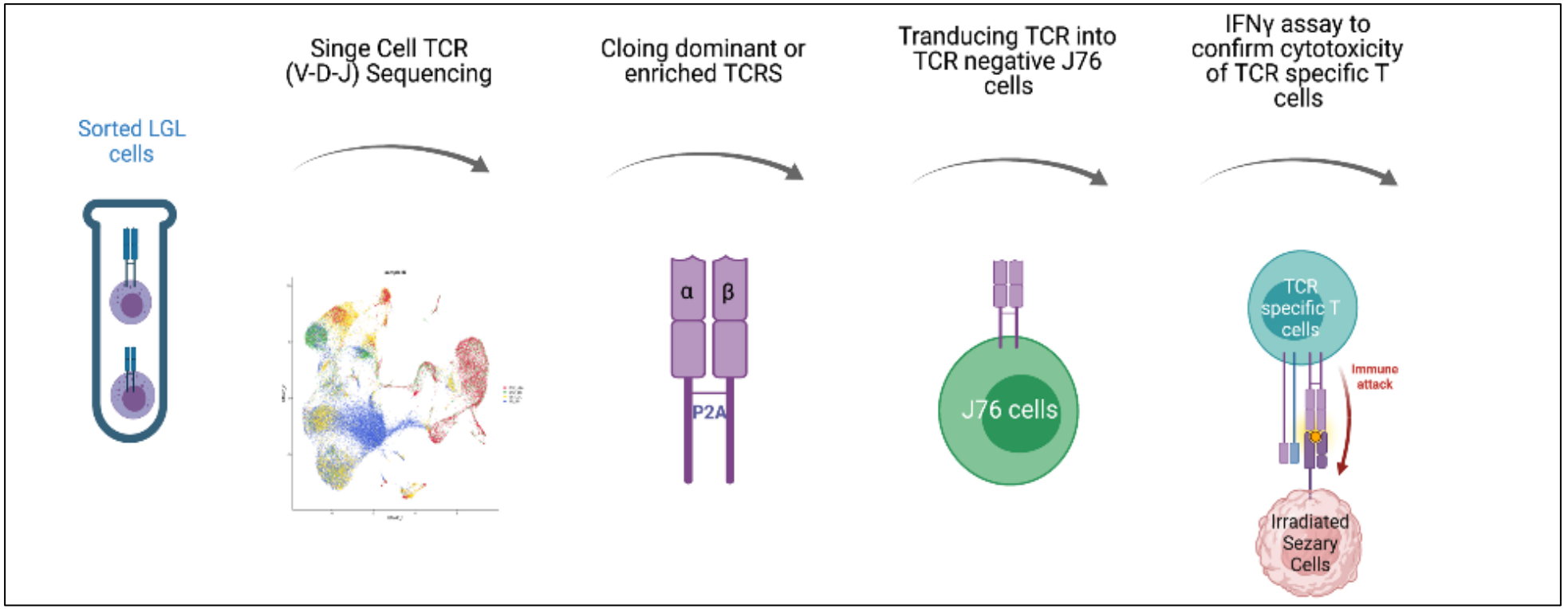
Maybe…

Clonal LGLs are activated by neoantigens expressed by SS cells and have cytotoxic effect on SS cells

Aim 1: To determine if autologous T-LGL cells exhibit cytotoxicity against MF/SS cells.



Aim 2: To identify neoantigen-specific TCR sequences recognized by clonal T-LGLs using high-throughput single-cell TCR V(D)J sequencing and validate the functionality of those TCRs against tumor samples.



Other hypotheses

- LGL and SS may originate from a common mutated hematopoietic stem cell/progenitor, and clonal proliferations represent a skewed hematopoiesis

- Specific autocrine cytokines drive the clonal expansion of SS and LGL

- Mogamulizumab bound to SS cells can be recognized via the CD16 receptor expressed on the surface of LGLs and enhance their expansion and/or cytotoxic activities

Lubomir Sokol
MD PhD
Malignant Hematology

Jose Conejo-Garcia
MD PhD
Immunology (Duke University)

Javier Pinilla
MD PhD
Malignant Hematology
Immunology

Clinical team
Frank Glass MD (Derm)
Ling Zhang MD (Heme path)
Mohammad Hussaini MD (Heme Path)
Sara Niyazi MD (Heme Path)
Pei-Ling Chen MD PhD (Derm Path)
Dahui Qin MD (Molecular Path)

Conejo Lab
Carly Harro PhD
John Powers PhD
Kimberly Sprenger PhD

Bioinformatics
Xiaoqing Yu PhD

Research Project Manager
Kimberly Ward MS
Angela Reagan

Pinilla Lab
Eva Sahakian PhD
Melanie Mediavilla-Varela PhD

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Tissue Core

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Profound thanks to our Moffitt patients, whose participation and courage inspire our ongoing research and dedication to excellence.


"We know too much and believe
too little."