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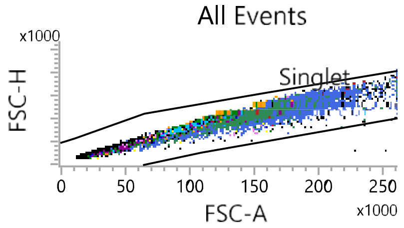
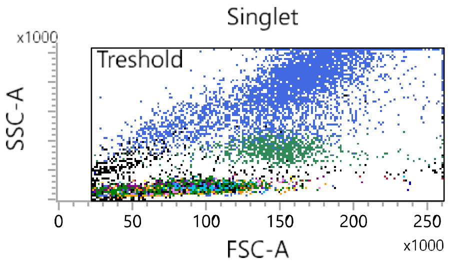
T-CUS
Small Populations
Growing Insights!

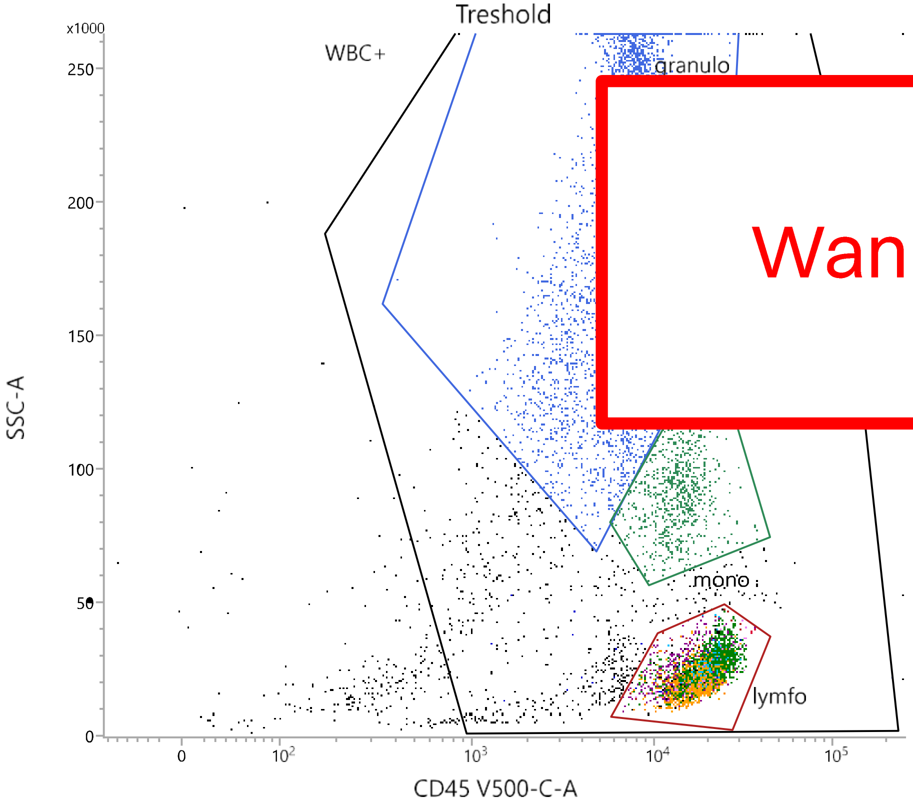
Nathan Debunne

Introduction

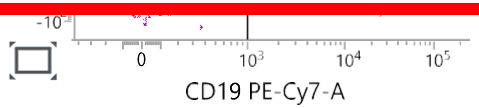
T-lymphoma

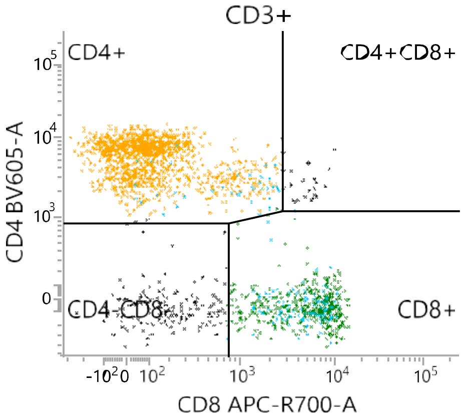


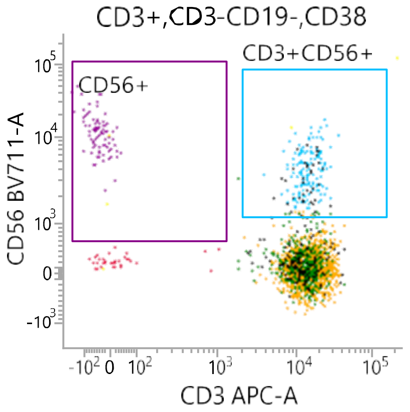




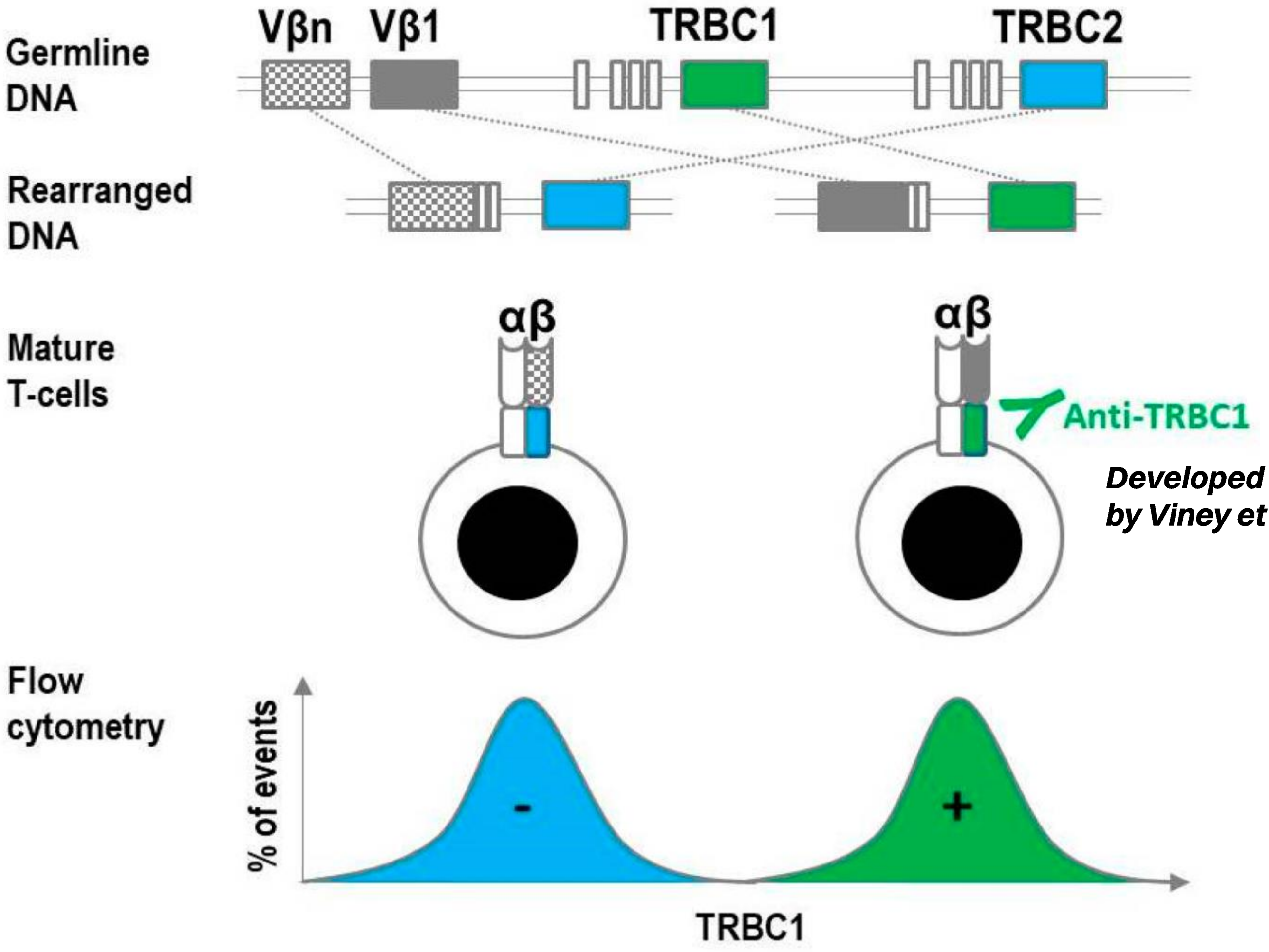
Wanneer klonaal?







TRBC1



Developed in 1992 by Viney et al.

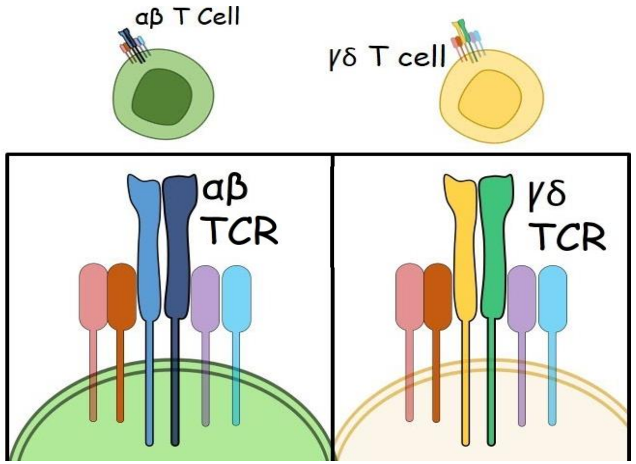
Horna et al. 2021

TRBC1: Limitations

1) No clonality interpretation of γδ T-cells
   → TCR rearrangements

2) No clonality interpretation of sCD3- T-cells
   → cyTRBC1

3) No aberrant phenotype
   → extra markers or TCR rearrangements



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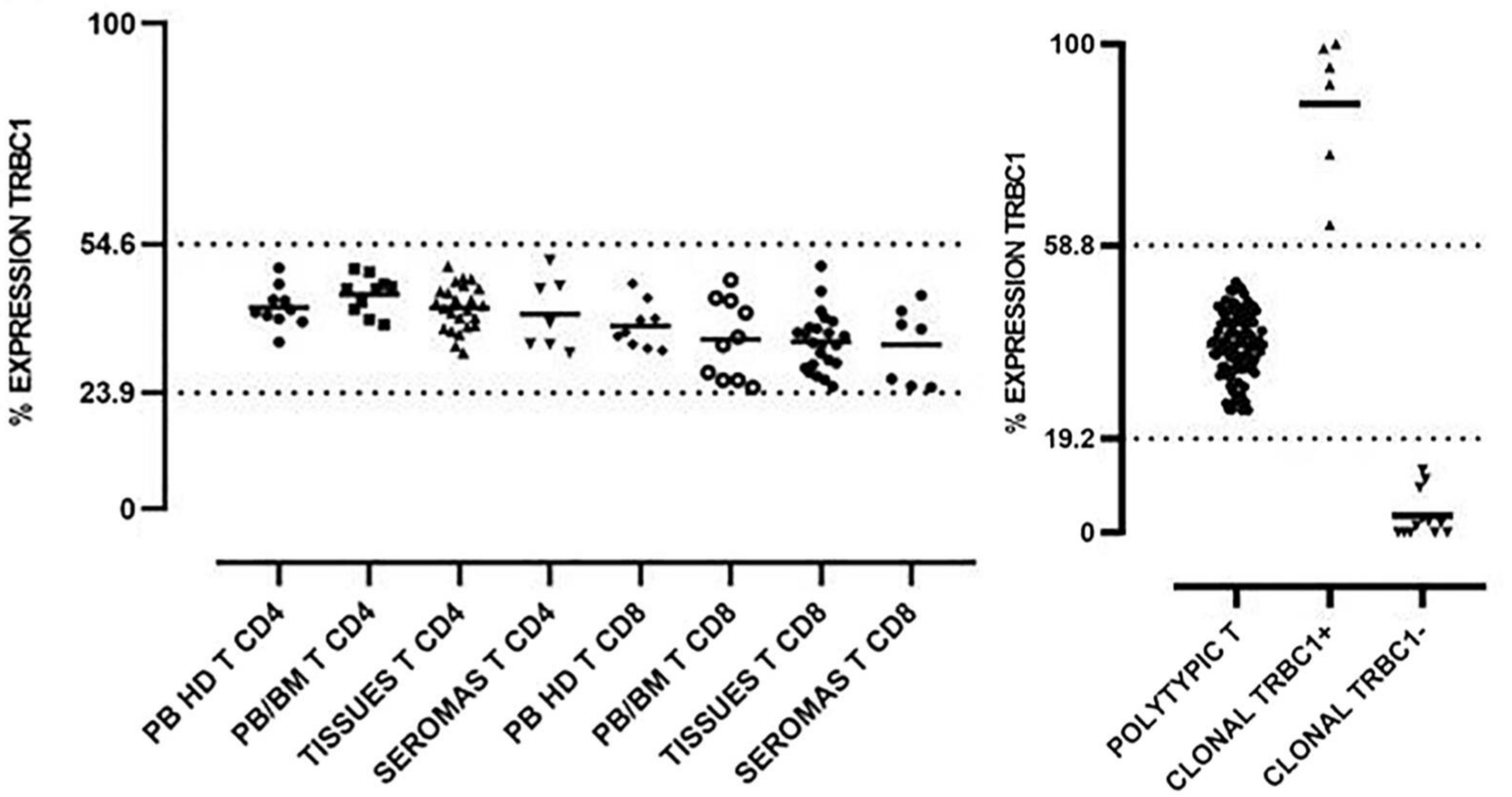
TRBC1: Clonality?



Castillo et al. (2023)

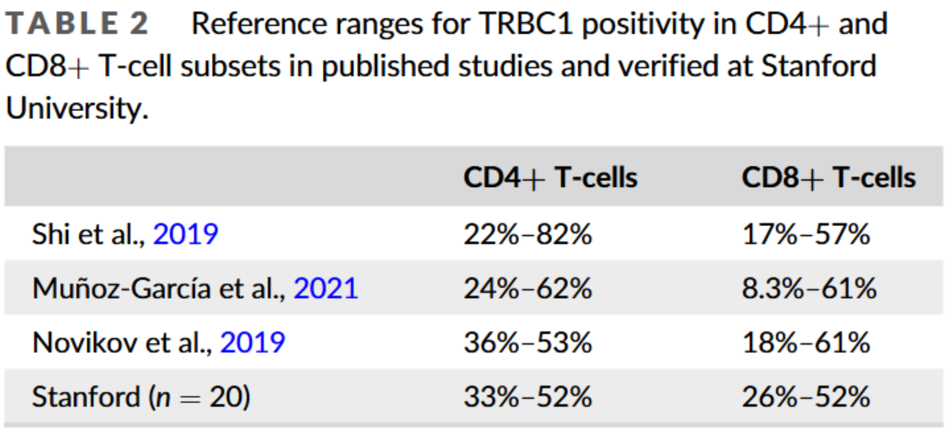


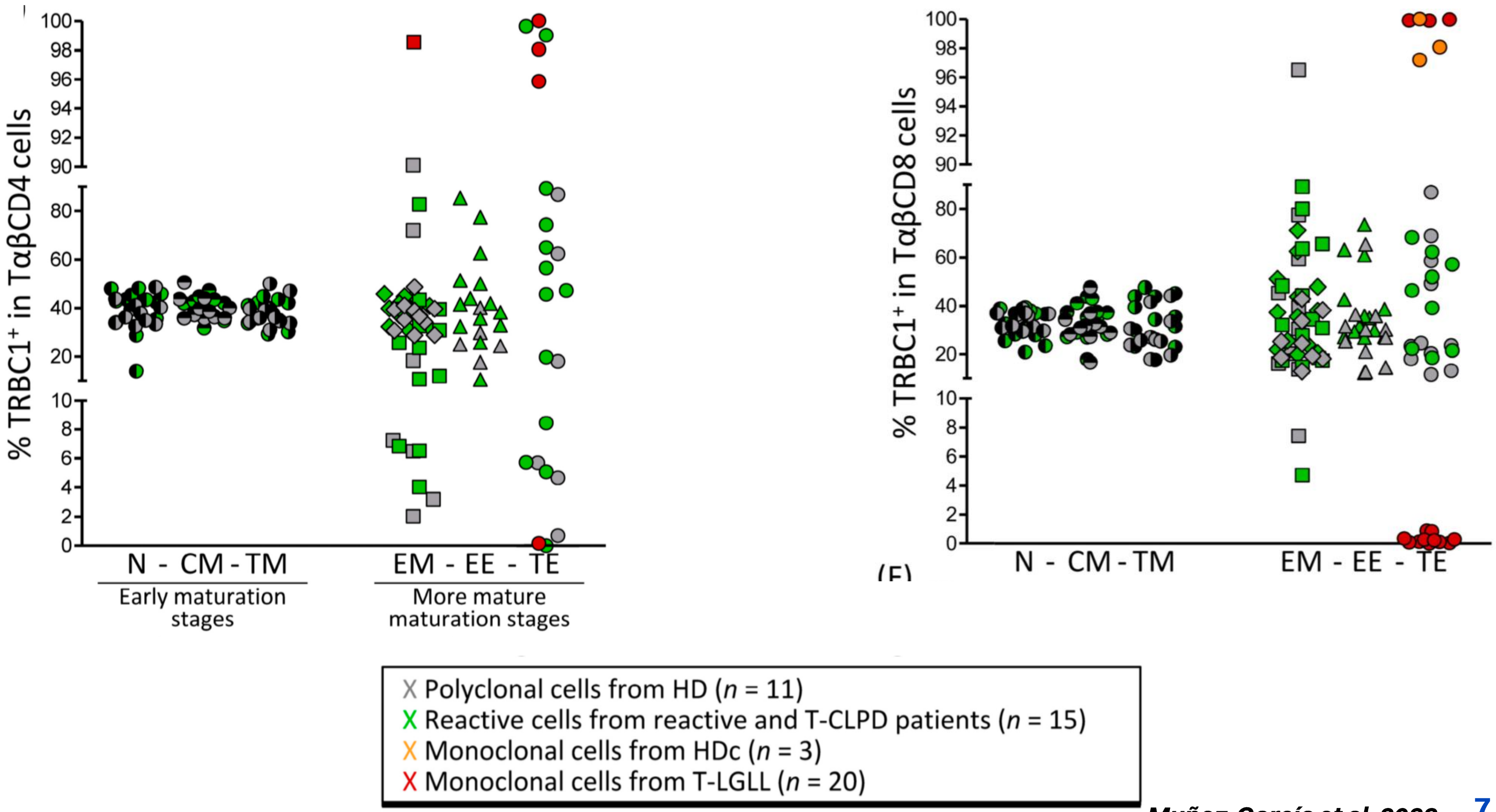
TABLE 2 Reference ranges for TRBC1 positivity in CD4+ and CD8+ T-cell subsets in published studies and verified at Stanford University.

	CD4+ T-cells	CD8+ T-cells
Shi et al., 2019	22%–82%	17%–57%
Muñoz-García et al., 2021	24%–62%	8.3%–61%
Novikov et al., 2019	36%–53%	18%–61%
Stanford (*n* = 20)	33%–52%	26%–52%

Devitt et al. (2024)

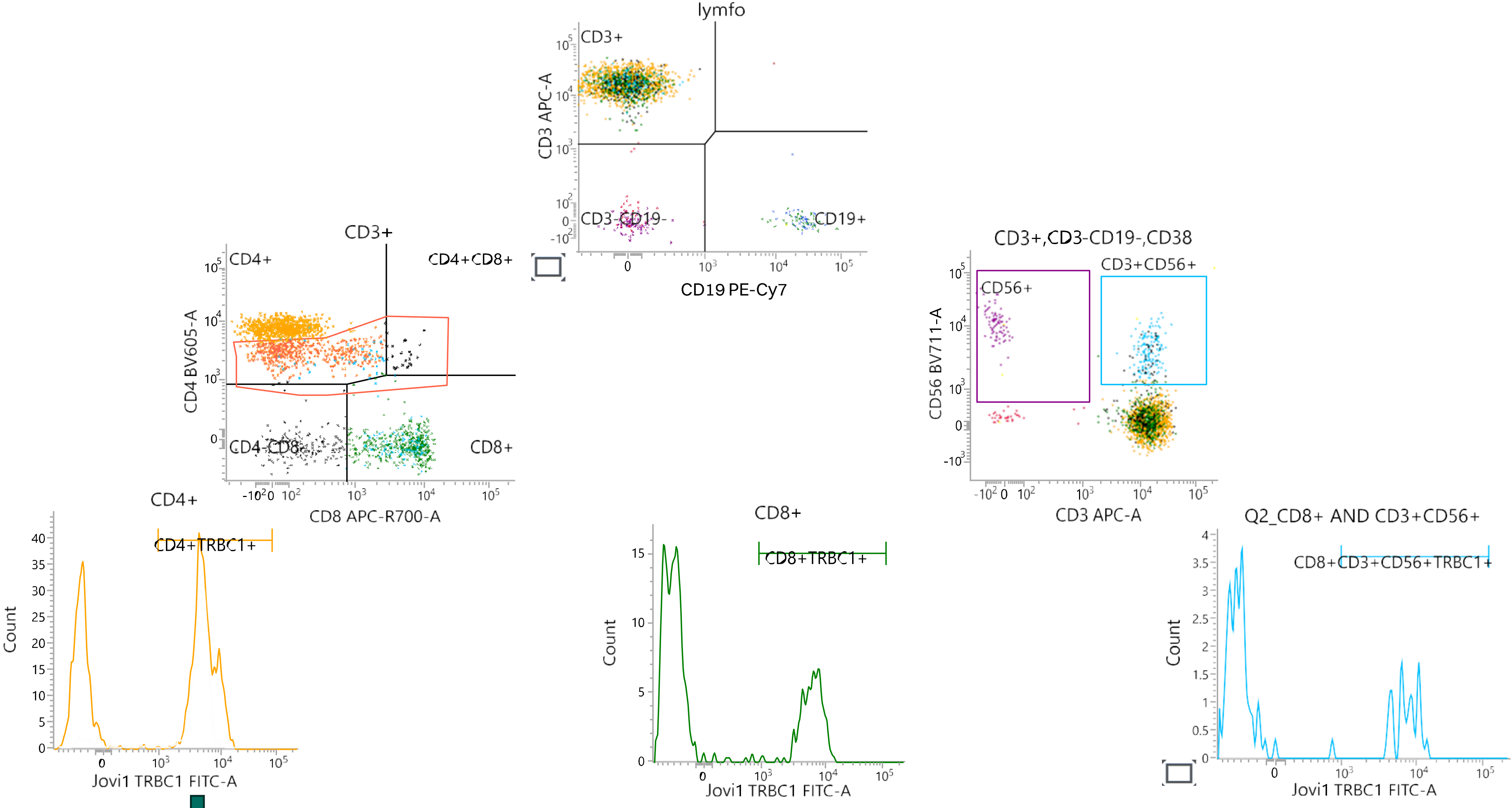
<15% of >85%

TRBC1: Clonality?



Muñoz-García et al. 2023

TRBC1: Clonality?



T-cell clones of uncertain significance

T-CUS

Shi et al. 2019

Prevalence

Prevalence

Patient population
8-37%

Prevalence	Reference
P: 16% (n=44)	Shi et al. 2019
P: 26% (n=159) / H: 13% (3/24)	Shi et al. 2020
P: 8% (n=26)	Capone et al 2022
P: 37% (n=148)	Buček et al. 2024
P: 13% (n=166)	Yu et al. 2025

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Prevalence

Reporting limit

Monotypic?

CD Markers

sTRBC1 vs cyTRBC1

Patient group

Gating strategy

Dim TRBC1

Reporting threshold	Prevalence	Reference
0.05%	16%	Shi et al. 2019
0.01%	26%	Shi et al. 2020
0.05%	8%	Capone et al 2022
0.01%	37%	Buček et al. 2024
0.025%	13%	Yu et al. 2025

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Prevalence

Reporting limit

Monotypic?

CD Markers

sTRBC1 vs cyTRBC1

Patient group

Gating strategy

Dim TRBC1

Clonality threshold	Prevalence	Reference
<15% or >85%	16%	Shi et al. 2019
<15% or >85%	26%	Shi et al. 2020
3SD of normal	8%	Capone et al 2022
<15% or >85%	37%	Buček et al. 2024
<10% or >90%	13%	Yu et al. 2025

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Prevalence

- Reporting limit
- Monotypic?
- **CD Markers**
- sTRBC1 vs cyTRBC1
- Patient group
- Gating strategy
- Dim TRBC1

CD markers	Prevalence	Reference
CD2/CD3/CD4/ CD5/CD7/CD8/	16%	Shi et al. 2019
	26%	Shi et al. 2020
	8%	Capone et al 2022
	37%	Buček et al. 2024
CD2/CD3/CD4/ CD5/CD7/CD8/CD56	13%	Yu et al. 2025

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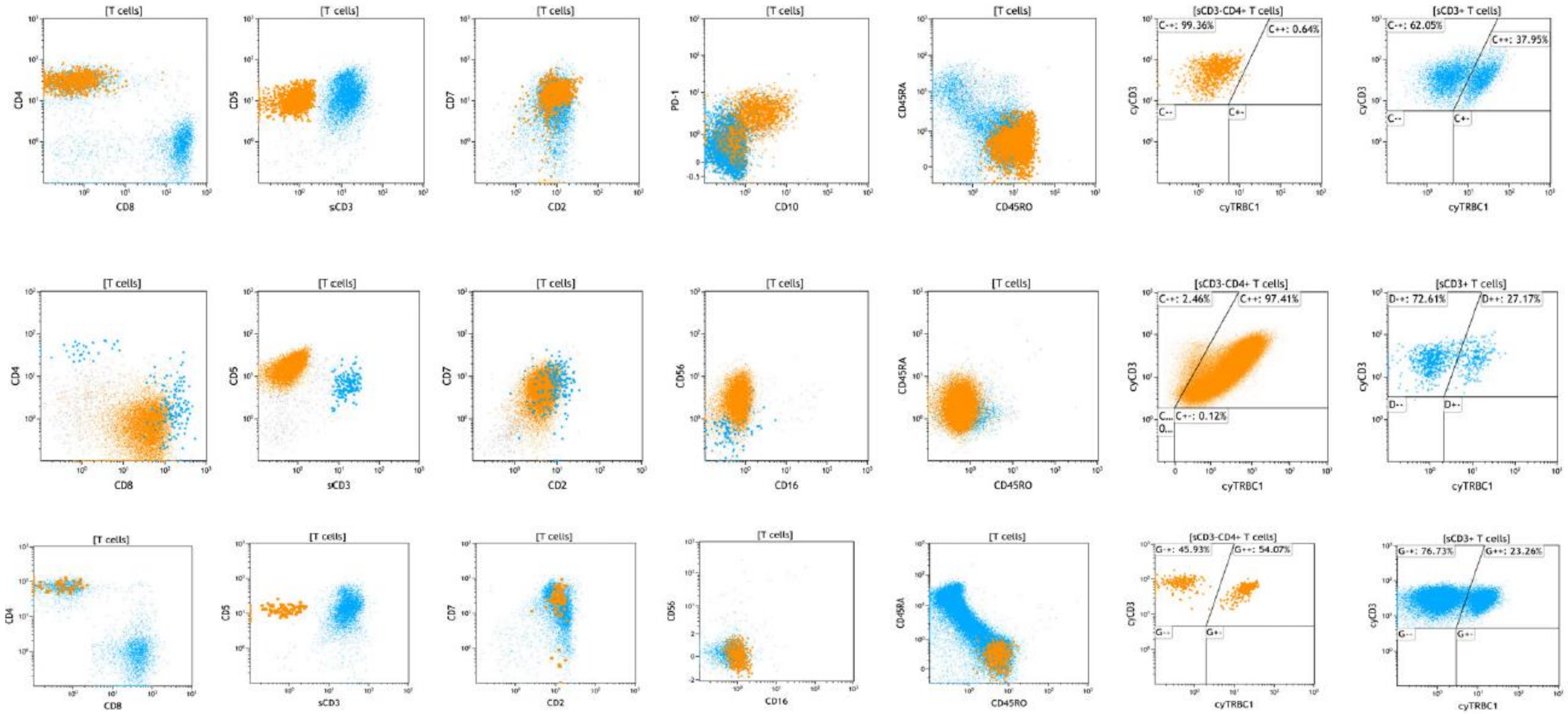
Prevalence

- Reporting limit
- Monotypic?
- CD Markers
- **sTRBC1 vs cyTRBC1**
- Patient group
- Gating strategy
- Dim TRBC1



+1% T-CUS
sCD3(-)

Lu et al. 2024

Prevalence

- Reporting limit
- Monotypic?
- CD Markers
- sTRBC1 vs cyTRBC1
- **Patient group**
- Gating strategy
- Dim TRBC1

Comorbidities

Age

Gender

“T-CUS was not associated with age, gender, comorbidities, or peripheral blood counts”
Shi et al. 2020

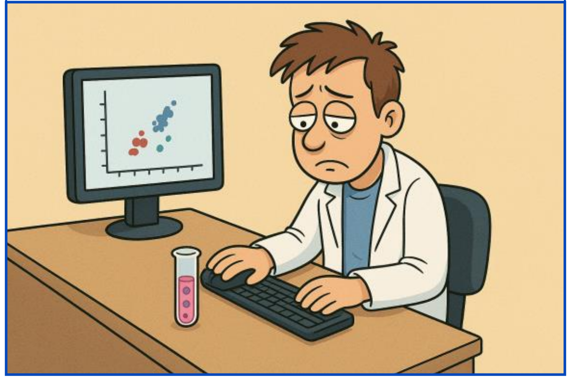
Disease	Age (mean)	Gender (F/M)	Prevalence	Reference
“Patients”	68 y (20-90)	1.2:1	16%	Shi et al. 2019
“Patients”	46 y (24-76)	1:1	26%	Shi et al. 2020
“Patients”	49 y (SD±18)	1.6:1	8%	Capone et al 2022
B-lymphoma’s	*N.A.*	*N.A.*	37%	Buček et al. 2024
“Patients”	*N.A.*	*N.A.*	13%	Yu et al. 2025

a) B-lymfoom: 37%
b) T-Lymfoom: 24%
c) Reactive Lymphocytic Proliferation: 16%

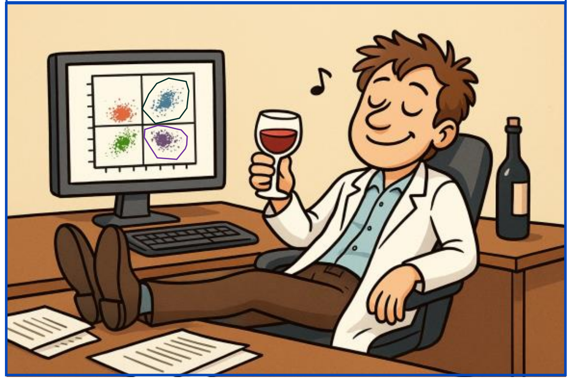
Prevalence

- Reporting limit
- Monotypic?
- CD Markers
- sTRBC1 vs cyTRBC1
- Patient group
- **Gating strategy**
- Dim TRBC1

Manual gating



Algorithm gating



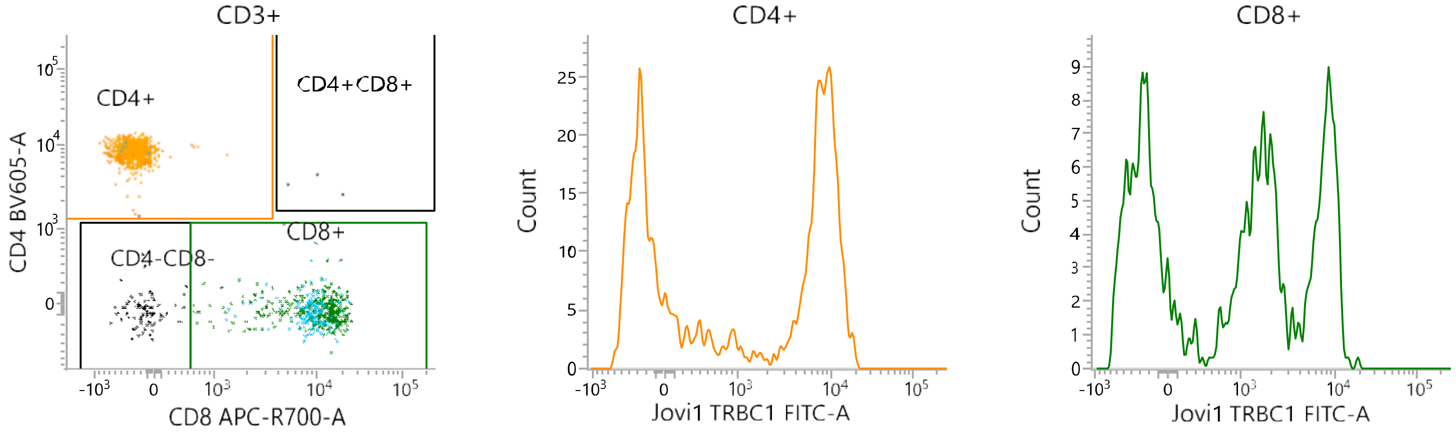
+7% T-CUS

Average size of overlooked monotypic populations
0.25% of CD45-positive cells

Buček et al. 2024

Prevalence

- Reporting limit
- Monotypic?
- CD Markers
- sTRBC1 vs cyTRBC1
- Patient group
- Gating strategy
- **Dim TRBC1**



"The use of JOVI,1 antibody recognizing TRBC1 has been shown to be useful in assessing T-cell clonality by flow cytometry with proposed cutoffs of <15%, >85%, or homogenous dim TRBC1 expression as evidence of clonality"

ICC 2025

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Prevalence

Reporting limit

Monotypic?

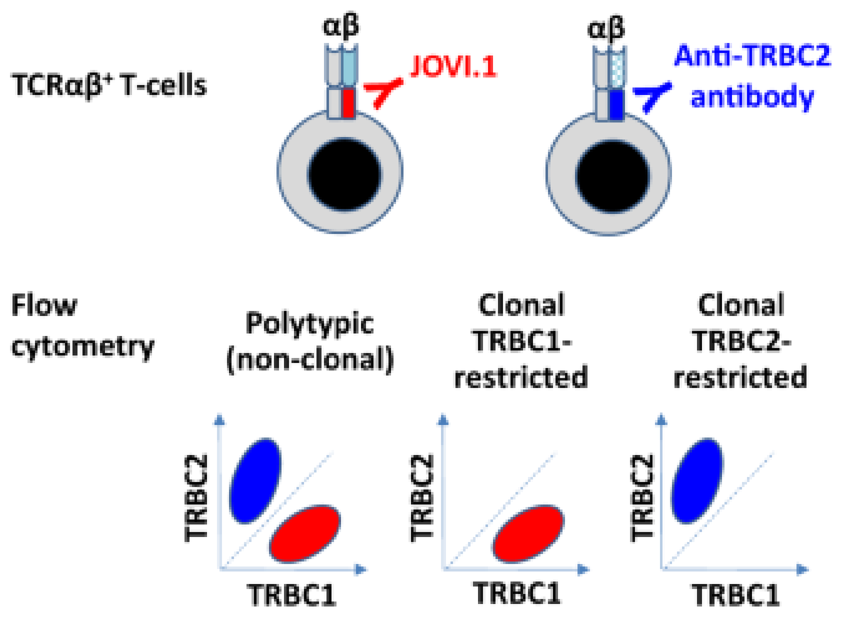
CD Markers

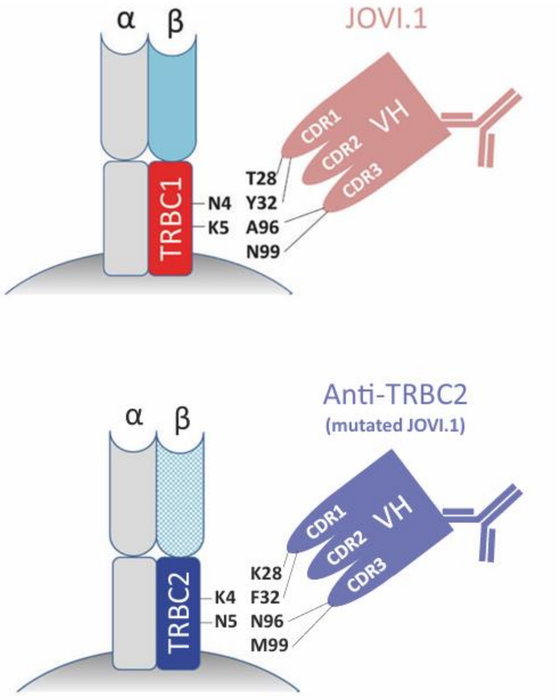
sTRBC1 vs cyTRBC1

Patient group

Gating strategy

Dim TRBC1

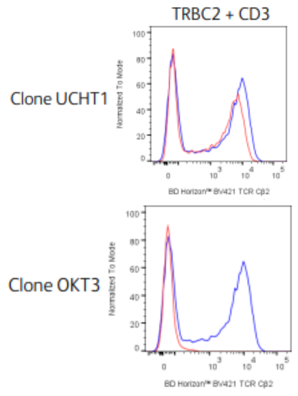




Horna et al. 2024

Caution:
Steric hindrance

Specificity	Clone	TRBC2 + TCR	TRBC2 10min then TCR	TCR 10min then TRBC2
CD3	UCHT1	✓	✓	✓
CD3	OKT3	X	X	X
CD3	SP34-2	✓	✓	✓
CD3	HIT3a	X	X	X
CD3	SK7	X	✓	X
TCRαβ	IP26	X	X	X
TCRαβ	T10B9	X	X	X
TCRαβ	WT31	X	X	X
TCRγδ	11F2	✓	✓	Not tested



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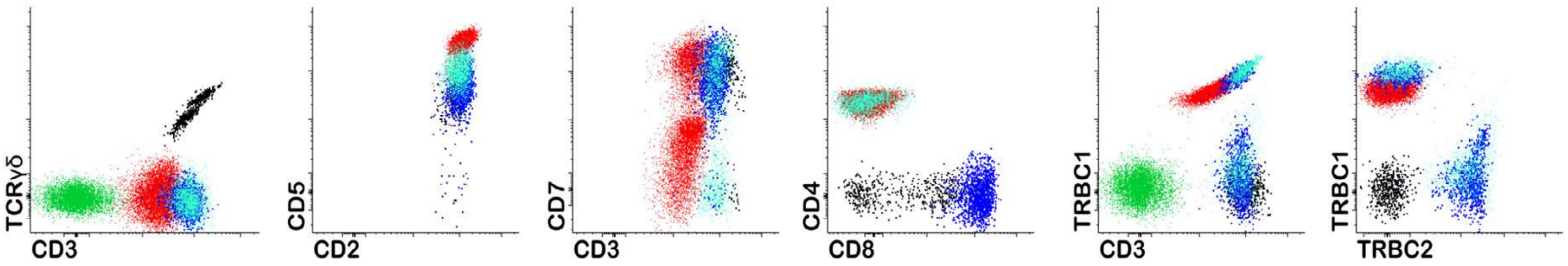
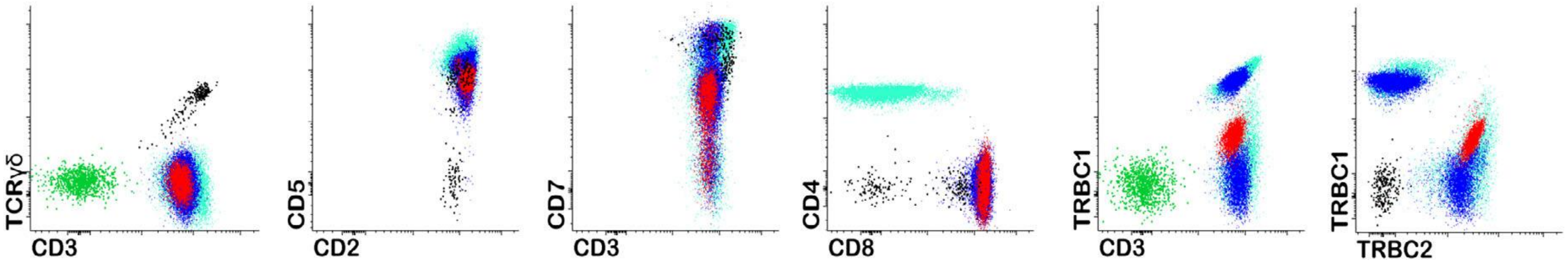
Prevalence

- Reporting limit
- Monotypic?
- CD Markers
- sTRBC1 vs cyTRBC1
- Patient group
- Gating strategy
- **Dim TRBC1**

Clonal TRBC1 dim with CD3 dim → ***TRBC1***



Clonal TRBC1 dim with CD3+ → ***TRBC2***

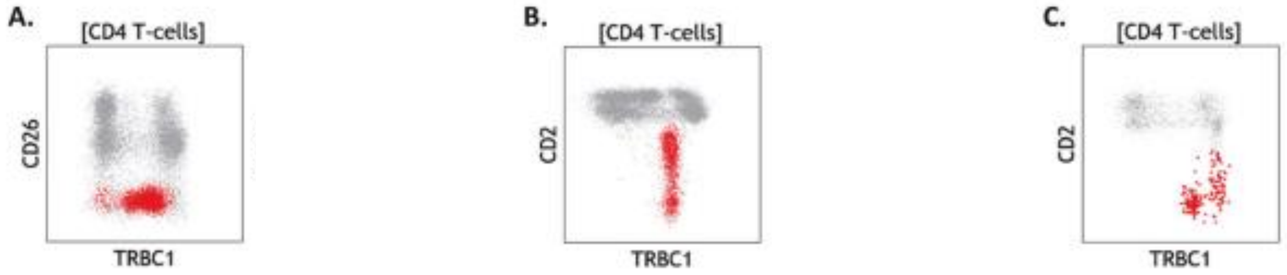


Shi et al. 2024

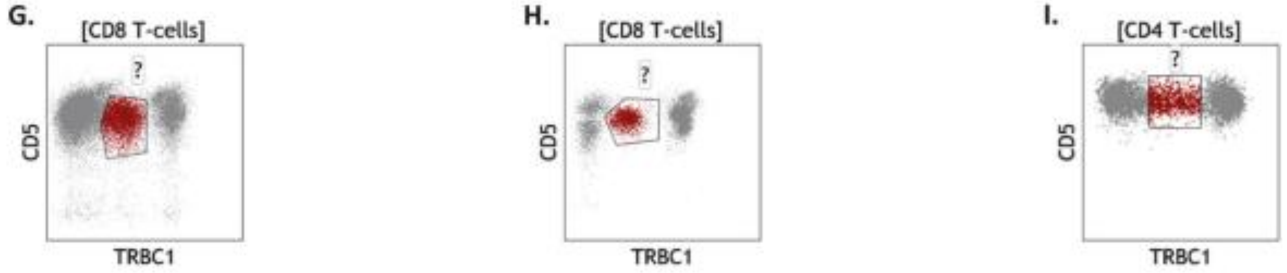
Prevalence

- Reporting limit
- Monotypic?
- CD Markers
- sTRBC1 vs cyTRBC1
- Patient group
- Gating strategy
- **Dim TRBC1**

MONOTYPIC



POLYTYPIC?



No abnormal fenotype

Horna et al. 2024

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Correlation TCR rearrangements

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Correlation TCR rearrangements

Kleine T-LGL populaties 2-5%

		TRBC1 expression		Total
		Polytypic	Monotypic	
TCR rearrangment	Poly/oligoclonal	21	3	24
	Monoclonal	2	91	93
Total		23	94	117
Kappa agreement		0.87 [95%CI:0.75-98]		
Sensitivity		97% [95%CI:92-99.9]		
Specificity		91% [95%CI:72-99]		

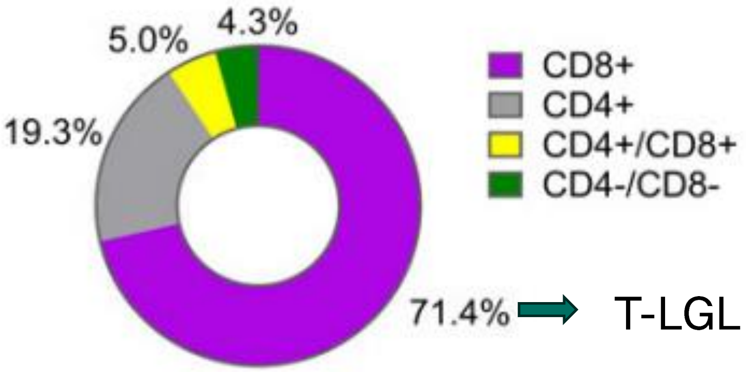
Monoclonal	PCR (whole PB)	Polytypic (0.45)	CD2^{+} CD3^{+} CD4^{-} CD5^{+} CD7^{het} CD8^{+} CD28^{het} CD38^{++} CD45RA^{-} CD45RO^{+} CD56^{-} CD57^{-}	34%	Acute EBV infection	Reactive T-cell lymphocytosis
Monoclonal / Polyclonal	PCR (whole PB) / TCRVβ by FCM	Polytypic (0.37)	CD2^{lo} CD3^{+} CD4^{-} CD5$^{-/lo}$ CD7$^{-/lo}$ CD8^{lo} CD11c^{+} CD28^{-} CD45RA^{+} CD45RO^{-} CD56^{-} CD57^{-}	62%	Neutropenia + lymphocytosis	Unclassifiable

T-CUS phenotype

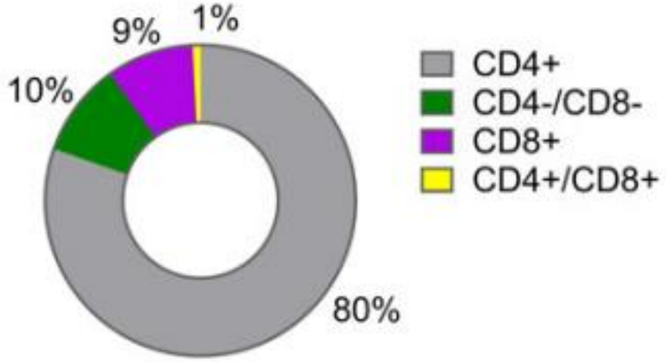
T-CUS phenotype

sCD3+ T-CUS

sCD3- T-CUS

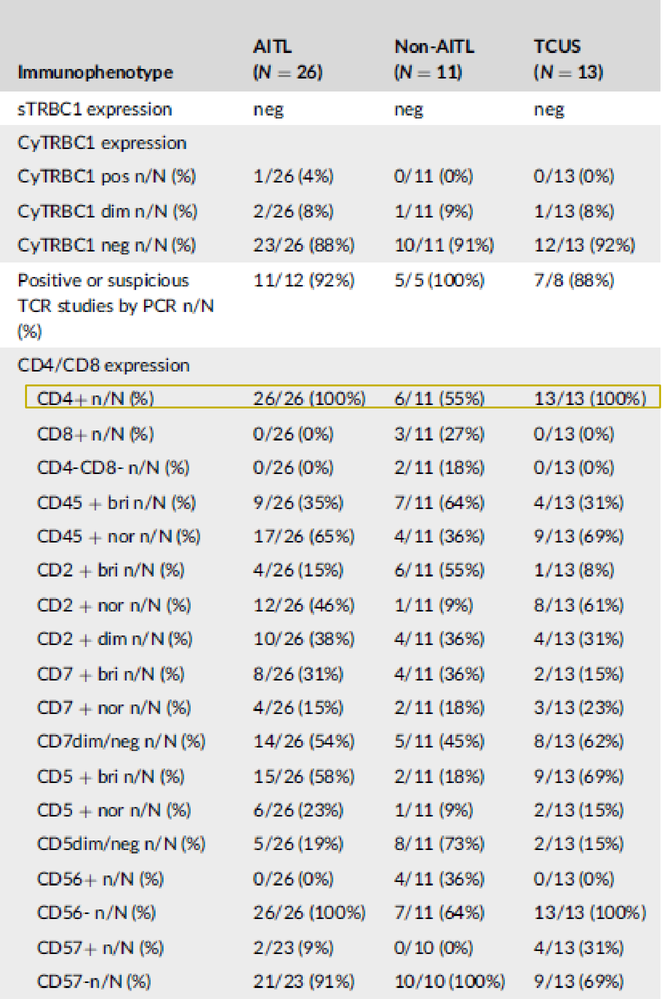


N = 140 T-CUS populations



N=122 cases of T-cell lymphoma

Buček et al. 2024

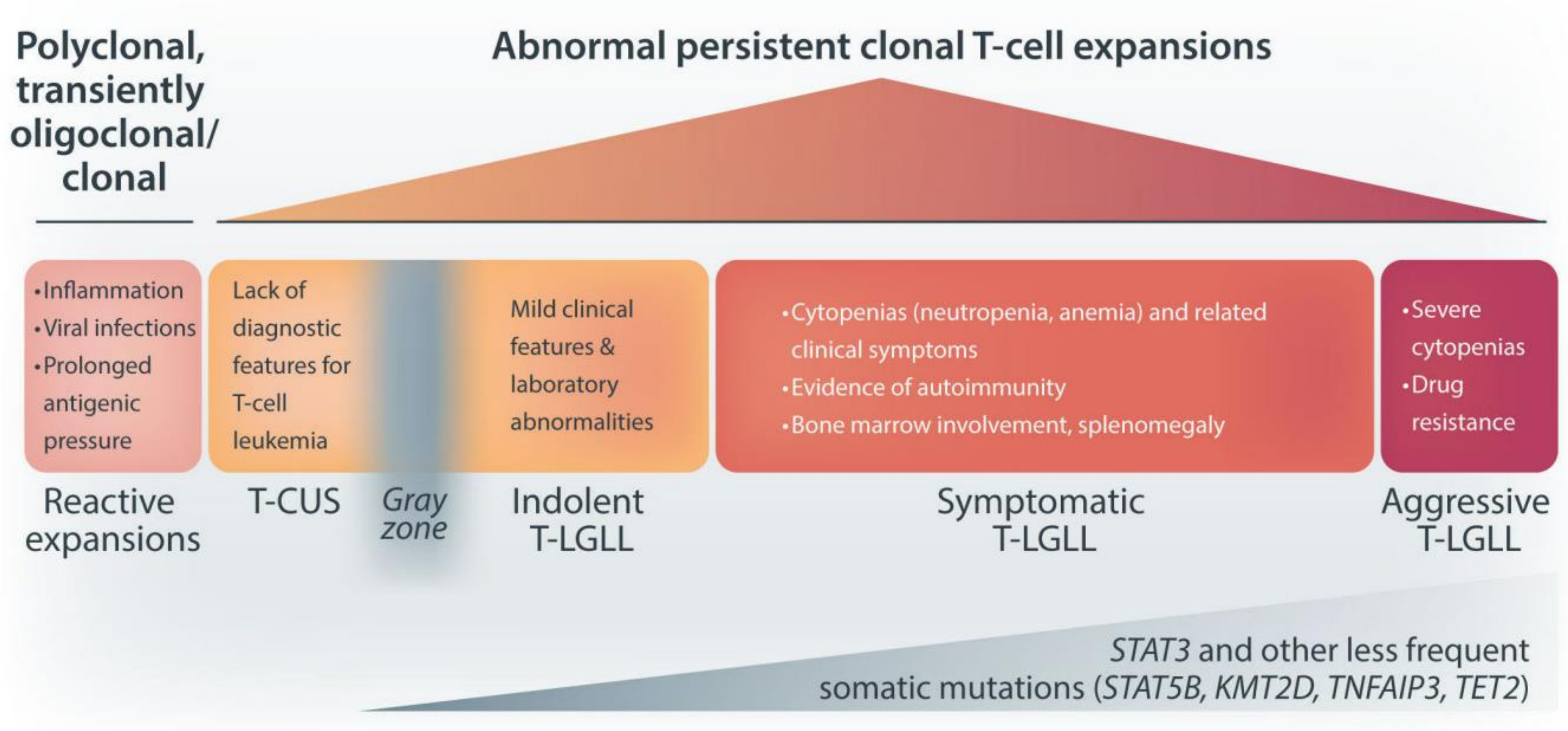


Immunophenotype	AITL ($N = 26$)	Non-AITL ($N = 11$)	TCUS ($N = 13$)
sTRBC1 expression	neg	neg	neg
CyTRBC1 expression			
CyTRBC1 pos n/N (%)	1/26 (4%)	0/11 (0%)	0/13 (0%)
CyTRBC1 dim n/N (%)	2/26 (8%)	1/11 (9%)	1/13 (8%)
CyTRBC1 neg n/N (%)	23/26 (88%)	10/11 (91%)	12/13 (92%)
Positive or suspicious TCR studies by PCR n/N (%)	11/12 (92%)	5/5 (100%)	7/8 (88%)
CD4/CD8 expression			
CD4+ n/N (%)	26/26 (100%)	6/11 (55%)	13/13 (100%)
CD8+ n/N (%)	0/26 (0%)	3/11 (27%)	0/13 (0%)
CD4-CD8- n/N (%)	0/26 (0%)	2/11 (18%)	0/13 (0%)
CD45 + bri n/N (%)	9/26 (35%)	7/11 (64%)	4/13 (31%)
CD45 + nor n/N (%)	17/26 (65%)	4/11 (36%)	9/13 (69%)
CD2 + bri n/N (%)	4/26 (15%)	6/11 (55%)	1/13 (8%)
CD2 + nor n/N (%)	12/26 (46%)	1/11 (9%)	8/13 (61%)
CD2 + dim n/N (%)	10/26 (38%)	4/11 (36%)	4/13 (31%)
CD7 + bri n/N (%)	8/26 (31%)	4/11 (36%)	2/13 (15%)
CD7 + nor n/N (%)	4/26 (15%)	2/11 (18%)	3/13 (23%)
CD7dim/neg n/N (%)	14/26 (54%)	5/11 (45%)	8/13 (62%)
CD5 + bri n/N (%)	15/26 (58%)	2/11 (18%)	9/13 (69%)
CD5 + nor n/N (%)	6/26 (23%)	1/11 (9%)	2/13 (15%)
CD5dim/neg n/N (%)	5/26 (19%)	8/11 (73%)	2/13 (15%)
CD56+ n/N (%)	0/26 (0%)	4/11 (36%)	0/13 (0%)
CD56- n/N (%)	26/26 (100%)	7/11 (64%)	13/13 (100%)
CD57+ n/N (%)	2/23 (9%)	0/10 (0%)	4/13 (31%)
CD57-n/N (%)	21/23 (91%)	10/10 (100%)	9/13 (69%)

Lu et al. 2024

T-CUS vs T-LGL

T-CUS vs T-LGL



Semenzato et al. 2025

T-CUS vs T-LGL

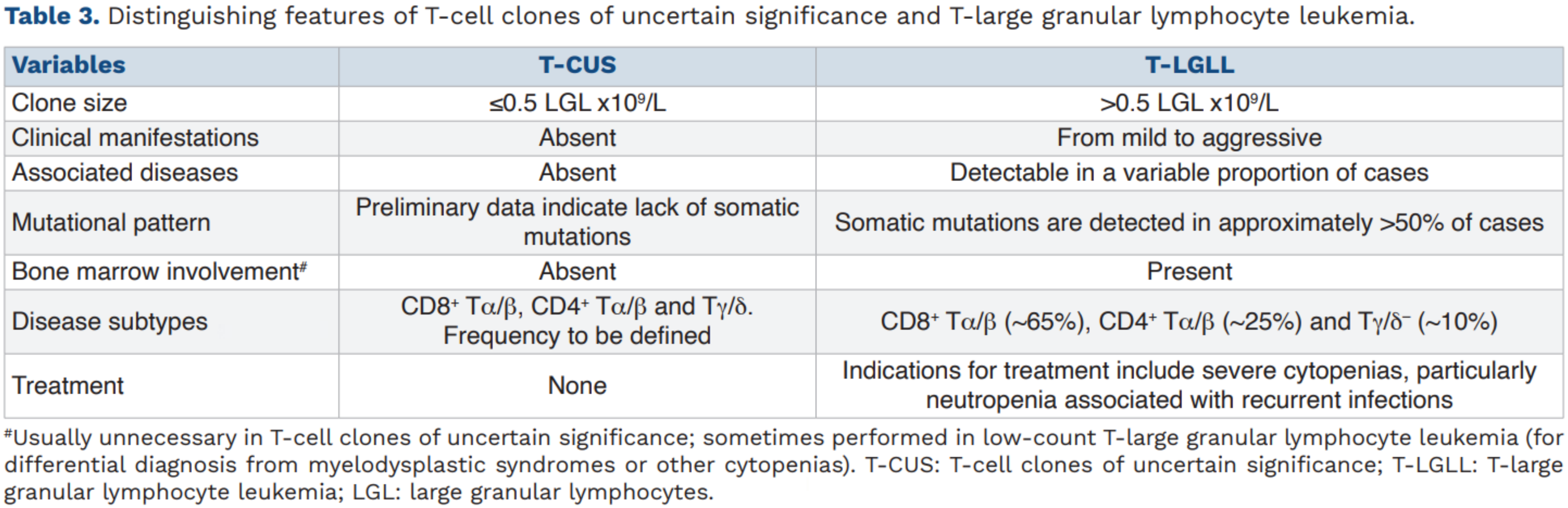


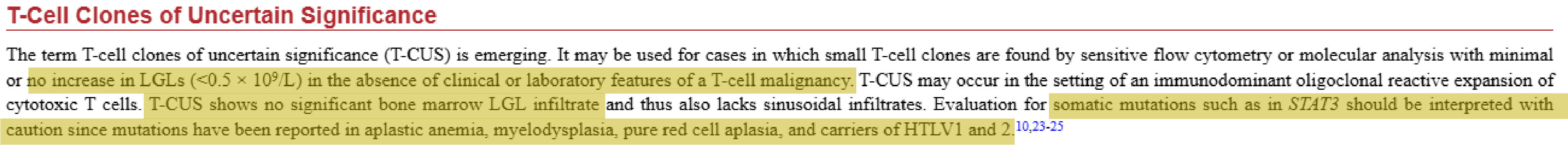
Table 3. Distinguishing features of T-cell clones of uncertain significance and T-large granular lymphocyte leukemia.

Variables	**T-CUS**	**T-LGLL**
Clone size	$\leq$0.5 LGL x10^9/L	>0.5 LGL x10^9/L
Clinical manifestations	Absent	From mild to aggressive
Associated diseases	Absent	Detectable in a variable proportion of cases
Mutational pattern	Preliminary data indicate lack of somatic mutations	Somatic mutations are detected in approximately >50% of cases
Bone marrow involvement[#]	Absent	Present
Disease subtypes	CD8$^+$ Tα/β, CD4$^+$ Tα/β and Tγ/δ. Frequency to be defined	CD8$^+$ Tα/β (~65%), CD4$^+$ Tα/β (~25%) and Tγ/δ^- (~10%)
Treatment	None	Indications for treatment include severe cytopenias, particularly neutropenia associated with recurrent infections

[#] Usually unnecessary in T-cell clones of uncertain significance; sometimes performed in low-count T-large granular lymphocyte leukemia (for differential diagnosis from myelodysplastic syndromes or other cytopenias). T-CUS: T-cell clones of uncertain significance; T-LGLL: T-large granular lymphocyte leukemia; LGL: large granular lymphocytes.

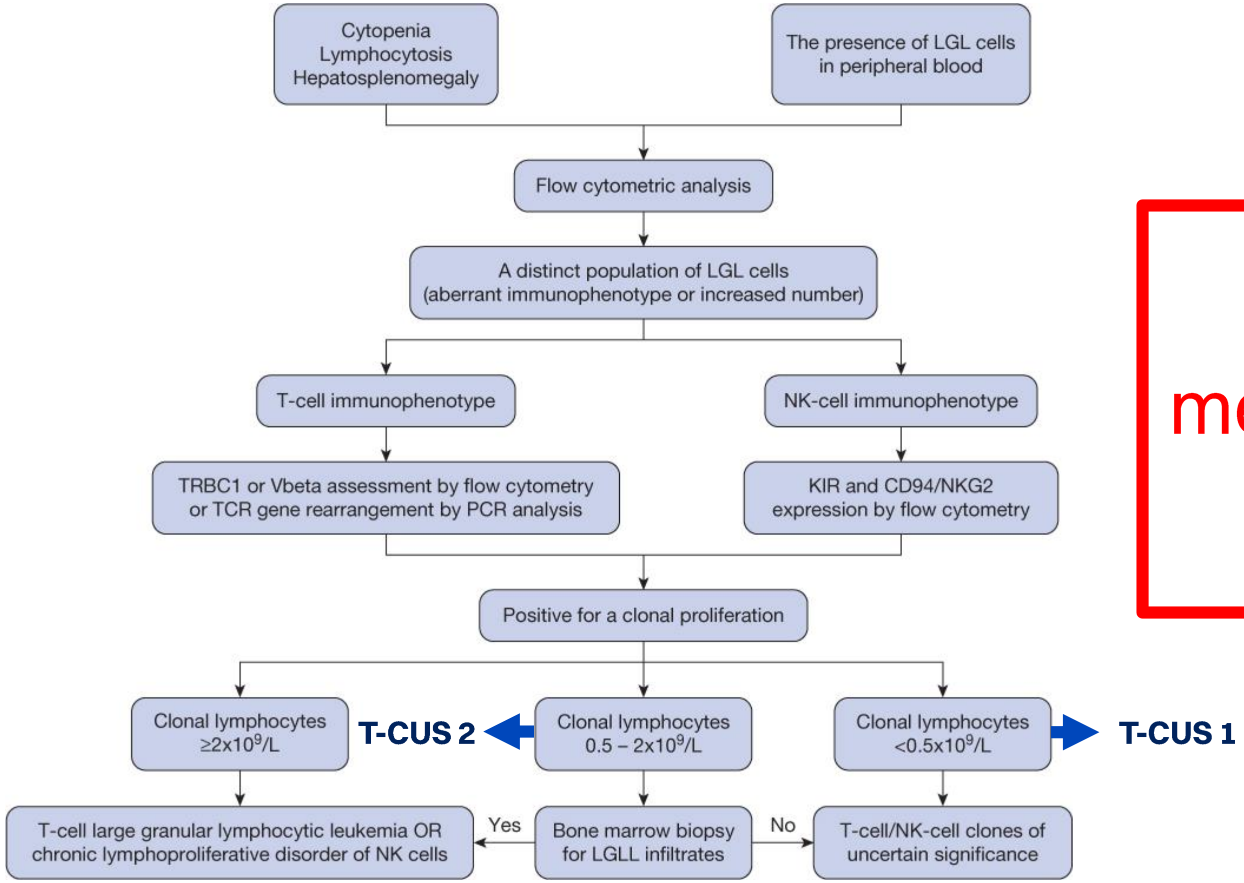
Semenzato et al. 2025

T-CUS vs T-LGL



T-Cell Clones of Uncertain Significance

The term T-cell clones of uncertain significance (T-CUS) is emerging. It may be used for cases in which small T-cell clones are found by sensitive flow cytometry or molecular analysis with minimal or no increase in LGLs (<0.5 × 10^9/L) in the absence of clinical or laboratory features of a T-cell malignancy. T-CUS may occur in the setting of an immunodominant oligoclonal reactive expansion of cytotoxic T cells. T-CUS shows no significant bone marrow LGL infiltrate and thus also lacks sinusoidal infiltrates. Evaluation for somatic mutations such as in *STAT3* should be interpreted with caution since mutations have been reported in aplastic anemia, myelodysplasia, pure red cell aplasia, and carriers of HTLV1 and 2.[10,23-25]



Cytopenia
Lymphocytosis
Hepatosplenomegaly

The presence of LGL cells in peripheral blood

Flow cytometric analysis

A distinct population of LGL cells (aberrant immunophenotype or increased number)

T-cell immunophenotype

NK-cell immunophenotype

TRBC1 or Vbeta assessment by flow cytometry or TCR gene rearrangement by PCR analysis

KIR and CD94/NKG2 expression by flow cytometry

Positive for a clonal proliferation

Clonal lymphocytes ≥$2x10^9$/L

T-CUS 2

Clonal lymphocytes 0.5 – $2x10^9$/L

Clonal lymphocytes <$0.5x10^9$/L

T-CUS 1

T-cell large granular lymphocytic leukemia OR chronic lymphoproliferative disorder of NK cells

Yes

Bone marrow biopsy for LGLL infiltrates

No

T-cell/NK-cell clones of uncertain significance

Not mentioned in WHO

T-CUS vs T-LGL

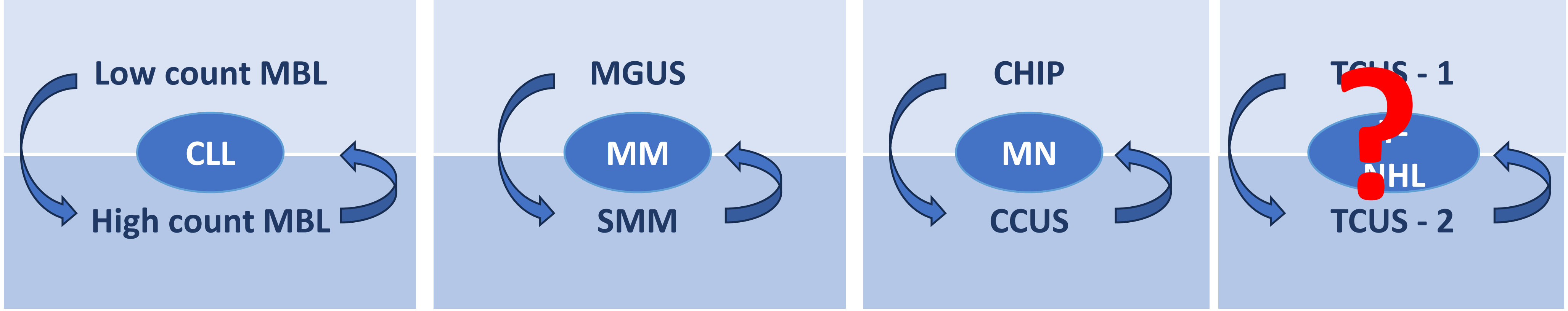
Non-IgG/M-prot >1.5g/abn FLC ratio

No increased risk
of progression

3RF: 58% in 20 years
2RF : 37% in 20 years
1RF : 21% in 20 years
0RF : 5% in 20 years

Clonal hematopoiesis risk score

Low risk: no progression
High risk: 1% per year



1% per year

3-4RF: 63% in 2 years
2RF : 45% in 2 years
1RF : 23% in 2 years
0RF : 6% in 2 years

100% progresses

BM>20%/M-prot >2g/FLC ratio>20/MOL

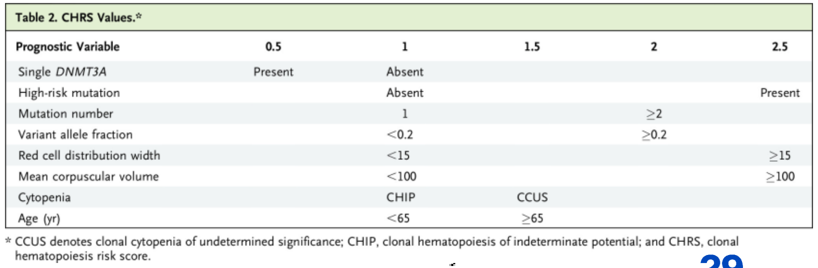
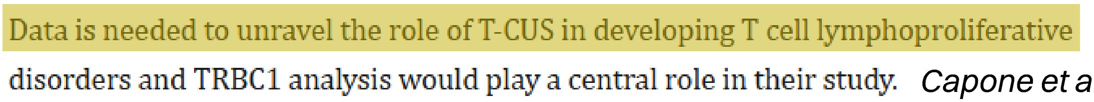


Table 2. CHRS Values.*

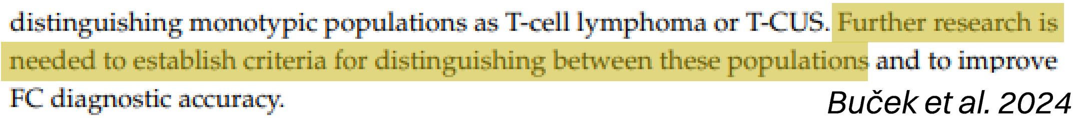
Prognostic Variable	0.5	1	1.5	2	2.5
Single *DNMT3A*	Present	Absent			
High-risk mutation		Absent			Present
Mutation number		1		≥2	
Variant allele fraction		<0.2		≥0.2	
Red cell distribution width		<15			≥15
Mean corpuscular volume		<100			≥100
Cytopenia		CHIP	CCUS		
Age (yr)		<65	≥65		

* CCUS denotes clonal cytopenia of undetermined significance; CHIP, clonal hematopoiesis of indeterminate potential; and CHRS, clonal hematopoiesis risk score.

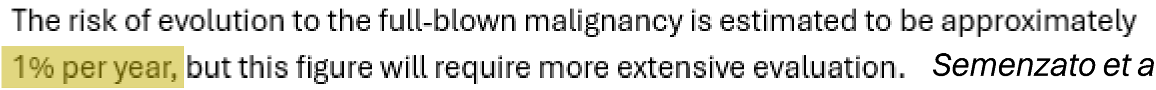
T-CUS vs T-LGL


Data is needed to unravel the role of T-CUS in developing T cell lymphoproliferative disorders and TRBC1 analysis would play a central role in their study.

Capone et al 2022


distinguishing monotypic populations as T-cell lymphoma or T-CUS. Further research is needed to establish criteria for distinguishing between these populations and to improve FC diagnostic accuracy.

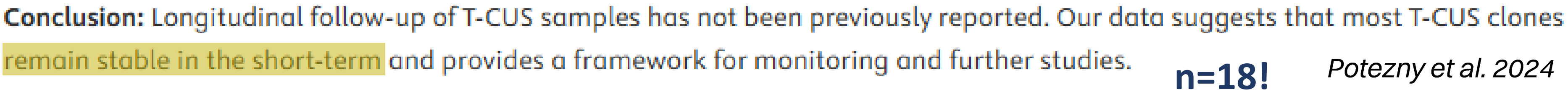
Buček et al. 2024


The risk of evolution to the full-blown malignancy is estimated to be approximately 1% per year, but this figure will require more extensive evaluation.

Semenzato et al. 2025


Moreover, the ability to rapidly and routinely identify T-CUS using dual TRBC1 and TRBC2 stains should facilitate future studies to understand its biology and clinical significance

Horna et al. 2024


Conclusion: Longitudinal follow-up of T-CUS samples has not been previously reported. Our data suggests that most T-CUS clones remain stable in the short-term and provides a framework for monitoring and further studies.

n=18!

Potezny et al. 2024

T-CUS progression

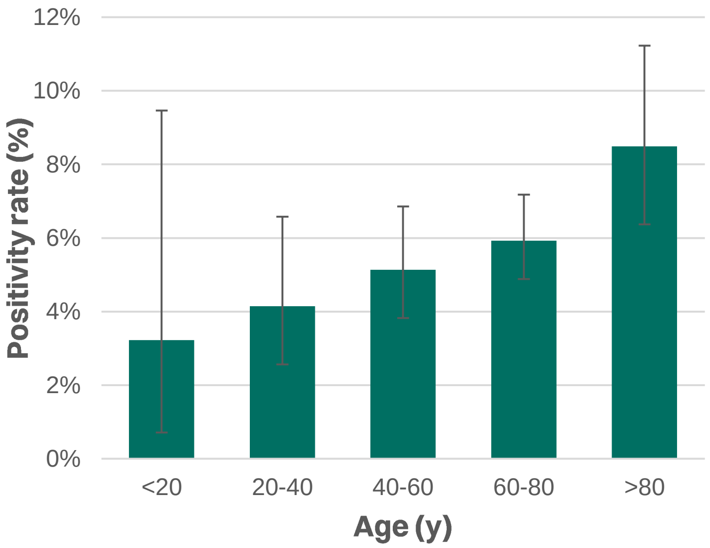
AZ
Sint-Jan
Brugge

T-CUS progression

Reporting limit	Niet geïncludeerd <50/µL T-CUS 1 = 50-500/µL T-CUS 2 = 500-2000/µL T-LGLL = >2000/µL
Monotypic?	<15% or >85%
CD Markers	CD3/CD4/CD5/CD8/CD56
sTRBC1 vs cyTRBC1	sTRBC1
Patient group	Patients of AZ Sint-Jan Brugge
Gating strategy	Manual gating
Dim TRBC1	Monotypic

T-CUS progression

2022-2024



Patiënts tested for TRBC1
(N=3495)

Polytypic
(N=3291)
94%
[93-95%]

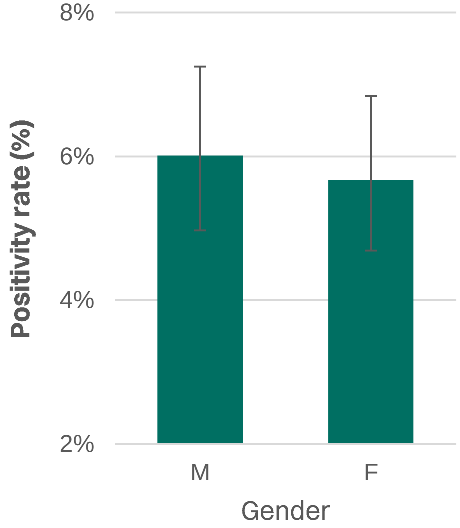
Monotypic
(N=204)
5.8%
[5.0-6.7%]

Non-LGL
(N=17/204)
8.3%
[4.9-13%]

Potential
LGL-phenotype
(N=187/204)
92%
[87-95%]

T-LGLL
(>2000/ µL)
(N=24/204)
12%
[7.7-17%]

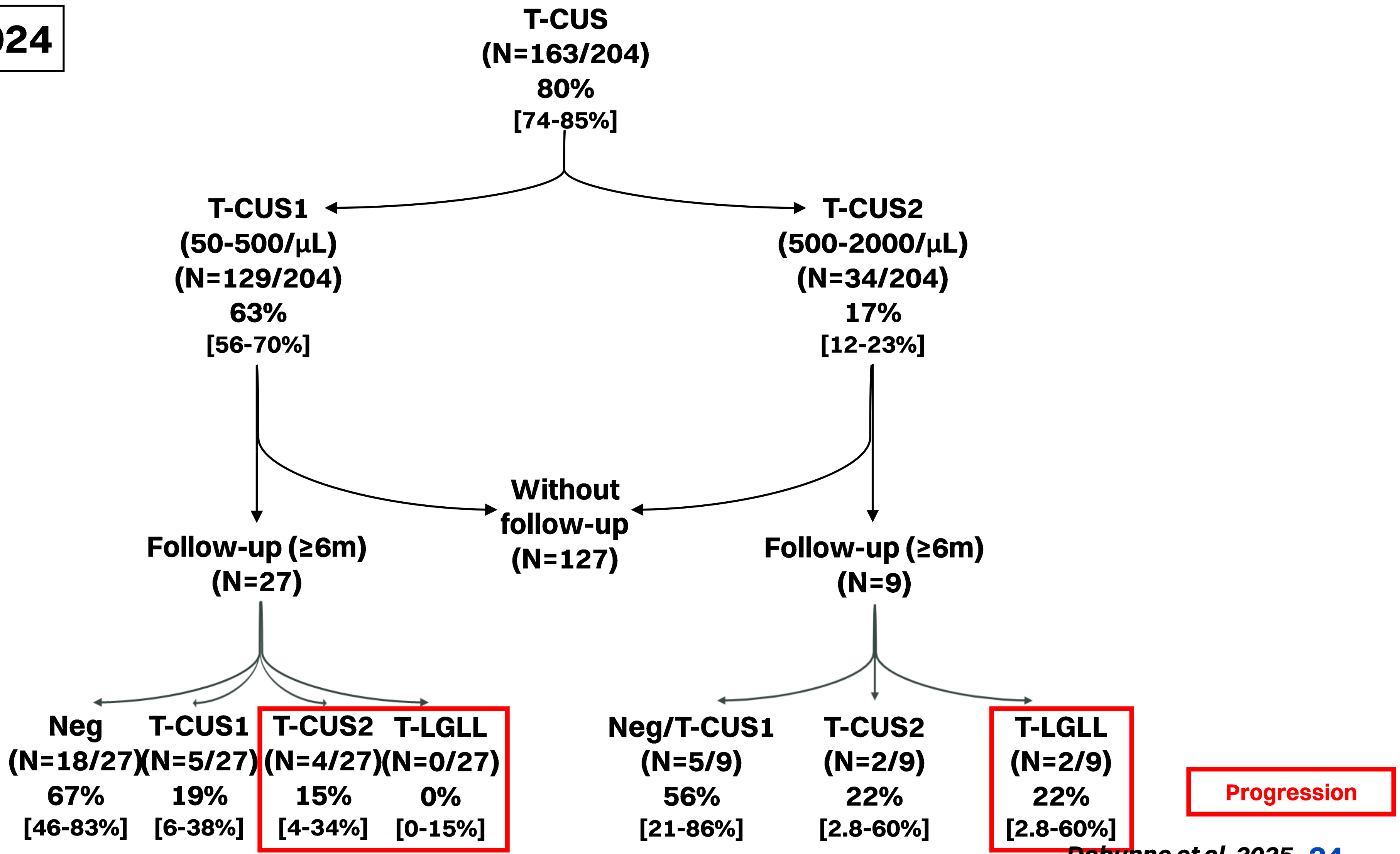
T-CUS
(N=163/204)
80%
[74-85%]



Debunne et al. 2025

T-CUS progression

2022-2024



T-CUS
(N=163/204)
80%
[74-85%]

T-CUS1
(50-500/μL)
(N=129/204)
63%
[56-70%]

T-CUS2
(500-2000/μL)
(N=34/204)
17%
[12-23%]

Follow-up (≥6m)
(N=27)

Without follow-up
(N=127)

Follow-up (≥6m)
(N=9)

Neg
(N=18/27)
67%
[46-83%]

T-CUS1
(N=5/27)
19%
[6-38%]

T-CUS2
(N=4/27)
15%
[4-34%]

T-LGLL
(N=0/27)
0%
[0-15%]

Neg/T-CUS1
(N=5/9)
56%
[21-86%]

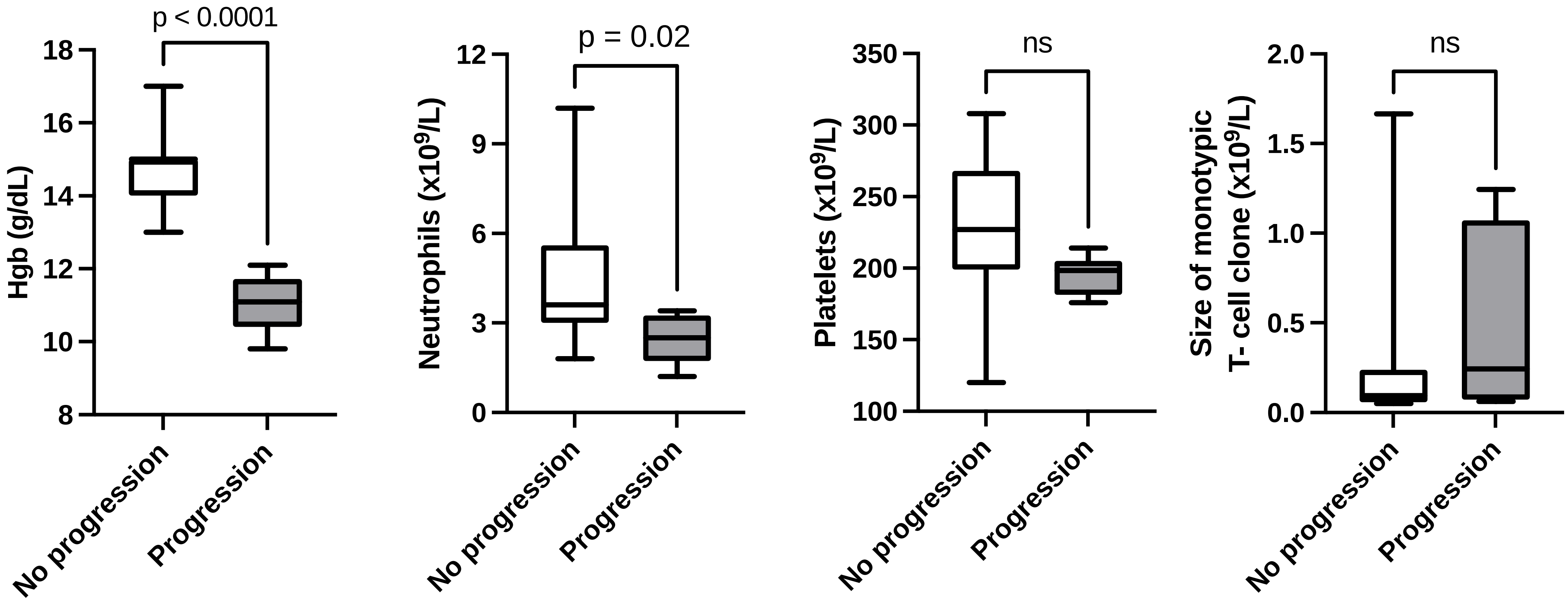
T-CUS2
(N=2/9)
22%
[2.8-60%]

T-LGLL
(N=2/9)
22%
[2.8-60%]

Progression

Debunne et al. 2025

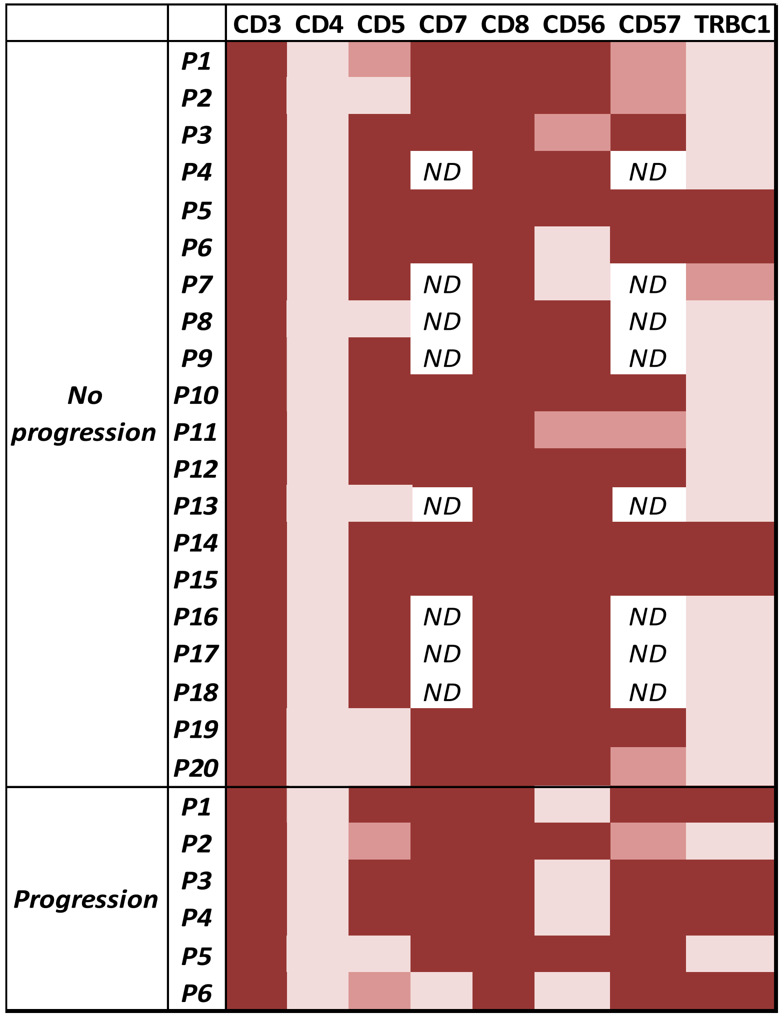
T-CUS progression



AZ Sint-Jan Brugge

Debunne et al. 2025

T-CUS progression



		CD3	CD4	CD5	CD7	CD8	CD56	CD57	TRBC1
No progression	*P1*	■	□	▨	■	■	■	▨	□
	P2	■	□	□	■	■	■	▨	□
	P3	■	□	■	■	■	▨	■	□
	P4	■	□	■	*ND*	■	■	*ND*	□
	P5	■	□	■	■	■	■	■	■
	P6	■	□	■	■	■	□	■	■
	P7	■	□	■	*ND*	■	□	*ND*	▨
	P8	■	□	□	*ND*	■	■	*ND*	□
	P9	■	□	■	*ND*	■	■	*ND*	□
	P10	■	□	■	■	■	■	■	□
	P11	■	□	■	■	■	▨	▨	□
	P12	■	□	■	■	■	■	■	□
	P13	■	□	□	*ND*	■	■	*ND*	□
	P14	■	□	■	■	■	■	■	■
	P15	■	□	■	■	■	■	■	■
	P16	■	□	■	*ND*	■	■	*ND*	□
	P17	■	□	■	*ND*	■	■	*ND*	□
	P18	■	□	■	*ND*	■	■	*ND*	□
	P19	■	□	□	■	■	■	■	□
	P20	■	□	□	■	■	■	▨	□
Progression	*P1*	■	□	■	■	■	□	■	■
	P2	■	□	▨	■	■	■	▨	□
	P3	■	□	■	■	■	□	■	■
	P4	■	□	■	■	■	□	■	■
	P5	■	□	□	■	■	■	■	□
	P6	■	□	▨	□	■	□	■	■

ND Not determined
□ Negative
▨ Weakly expressed
■ Positive

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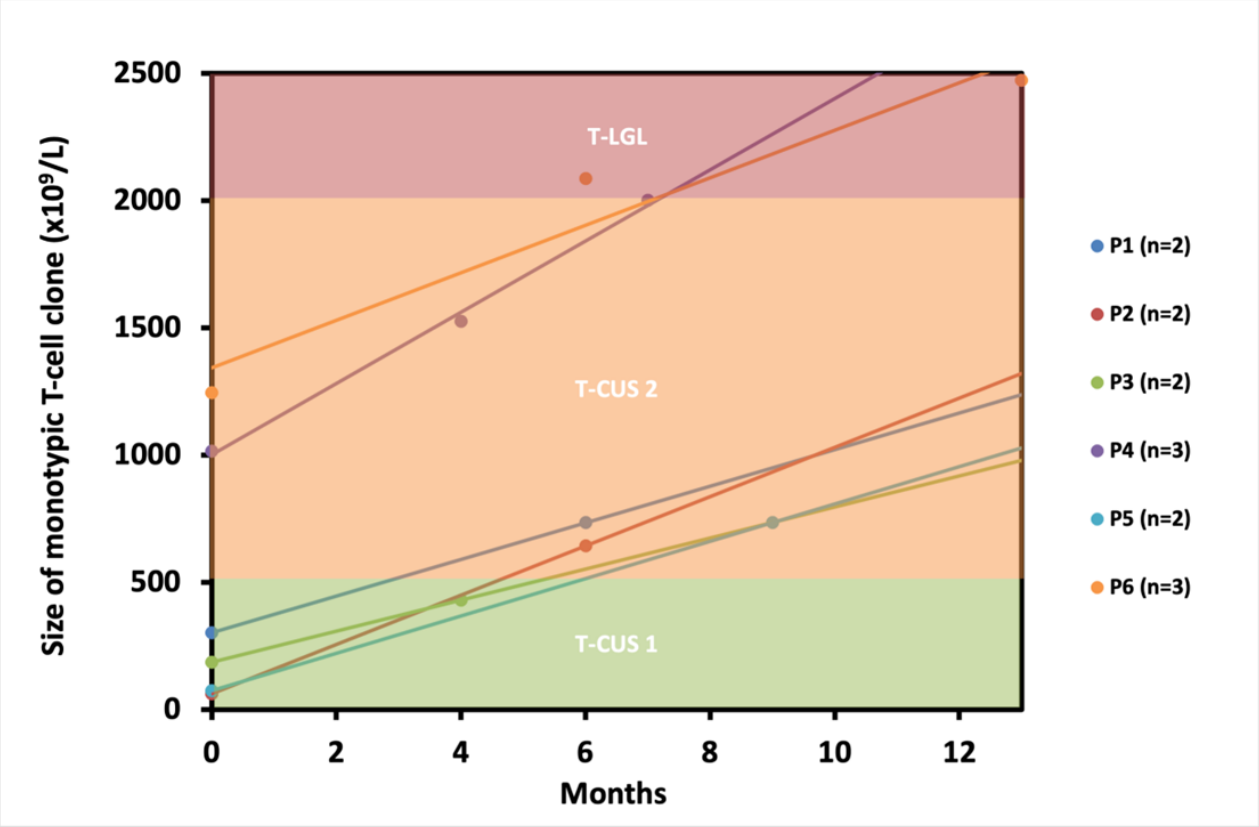
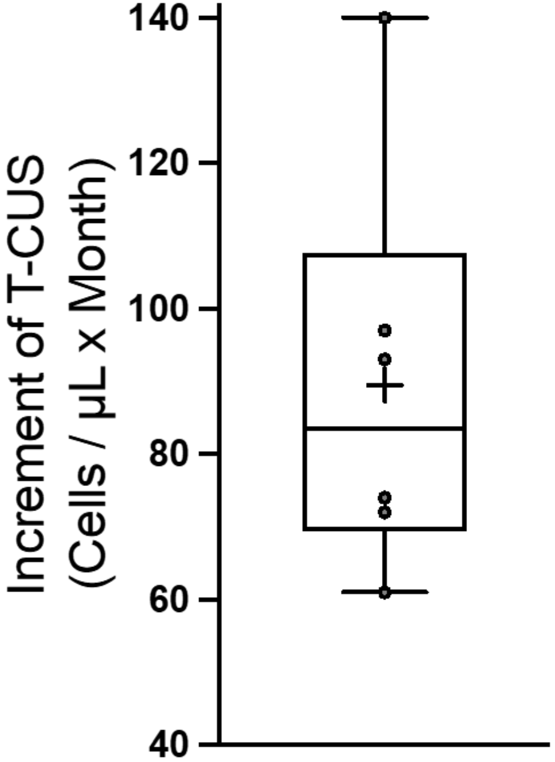
T-CUS progression





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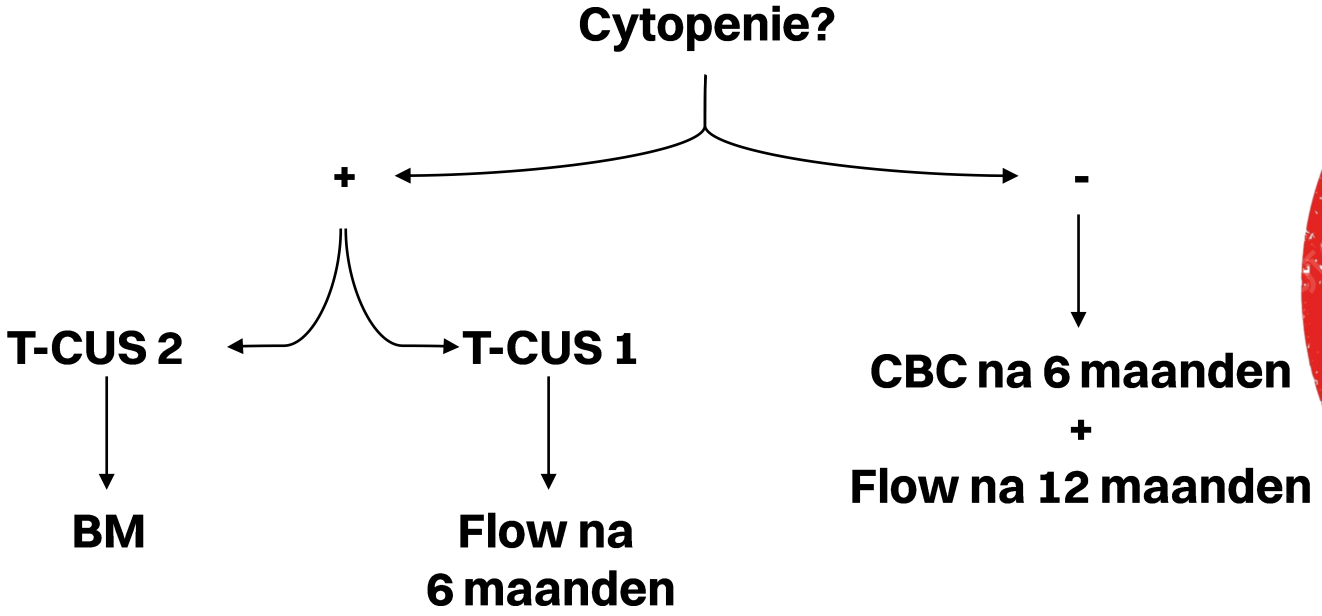
T-CUS progression

Limitations

1) Lack of uniform molecular analysis (beware of detection limit)
2) Low number of follow-up samples
3) Relatively short follow-up period (6 to 30 months)
4) CD57 expression not determined in all samples

T-CUS: Follow-up algoritme



Cytopenie?

+

T-CUS 2

BM

T-CUS 1

Flow na
6 maanden

-

CBC na 6 maanden
+
Flow na 12 maanden

HAS NOT BEEN VALIDATED

Conclusion

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Sint-Jan
Brugge

Conclusion

1) Majority (2/3) of T-CUS disappear

2) No T-CUS1 evolved into a T-LGL (6–30 months)

3) Anemia is correlated with increased risk of progression

4) Average increase of monotypic cells: 90 cells/µL per month

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Brugge

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Bedankt!

"Nothing in life is to be feared,
it is only to be understood."
- Marie Curie -