Erasmus MC
University Medical Center Rotterdam

laboratorium
medische
immunologie

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SKML IMCD nabespreking, online

TRBC2: ervaring in routinediagnostiek

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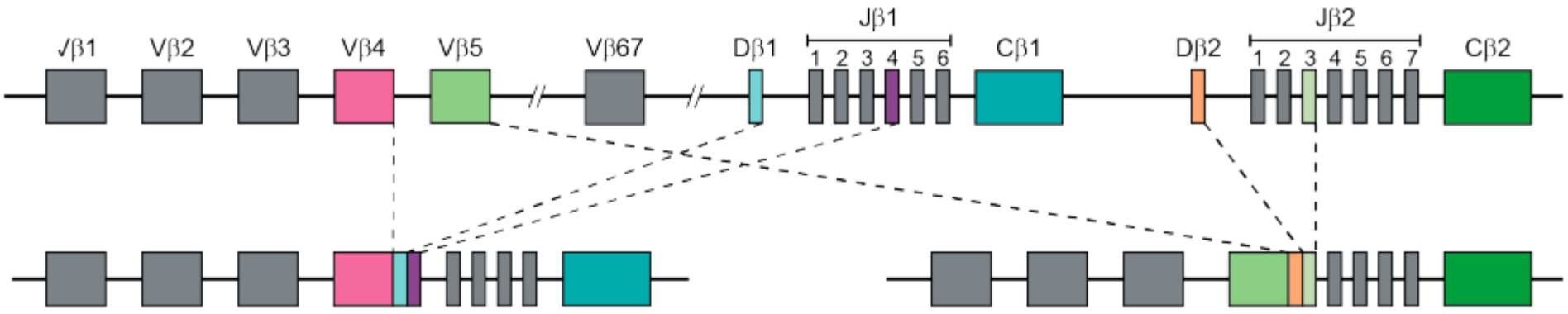
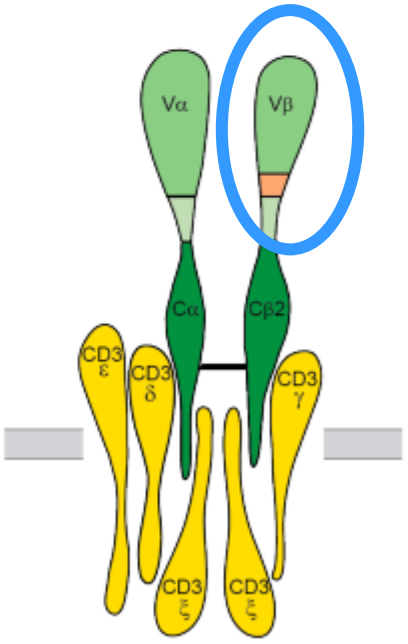
Analyse T-cel ‘klonaliteit’ : flow vs. moleculair

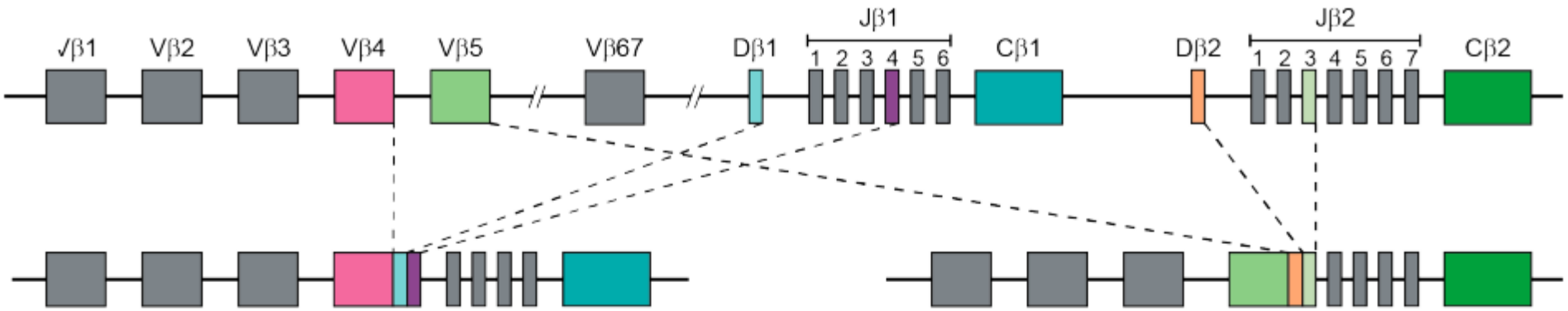


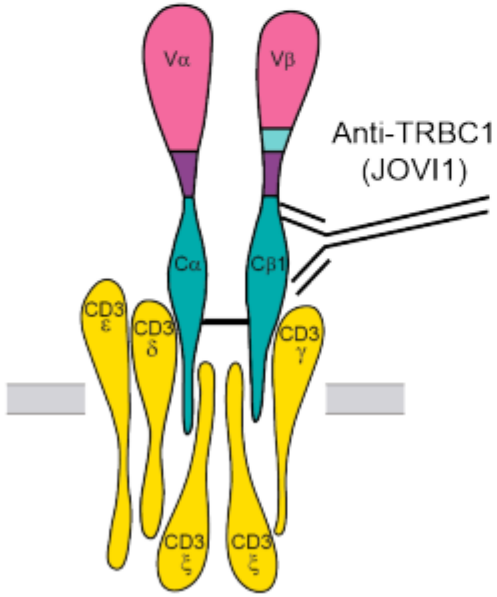


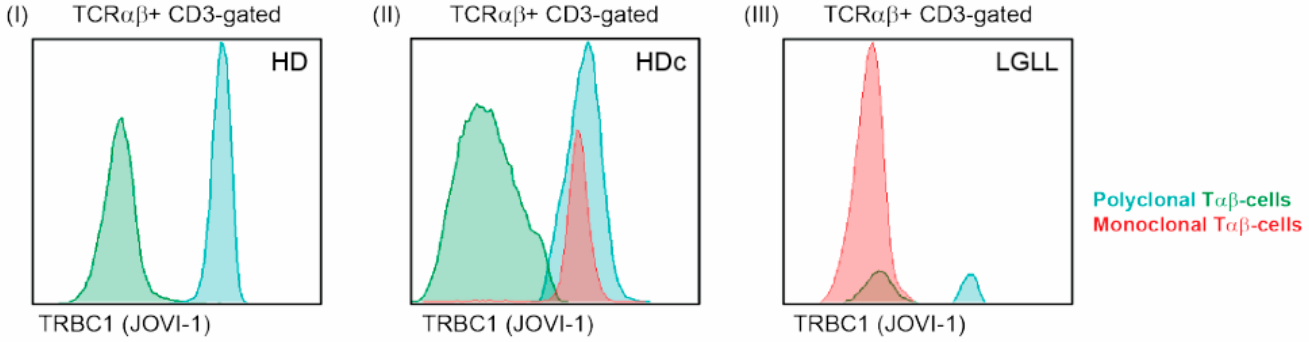
- Vb antistoffen
- TCR PCR

Munoz-Garcia et al, Cancers 2021;13:4379

Analyse T-cel ‘klonaliteit’ : flow vs. moleculair

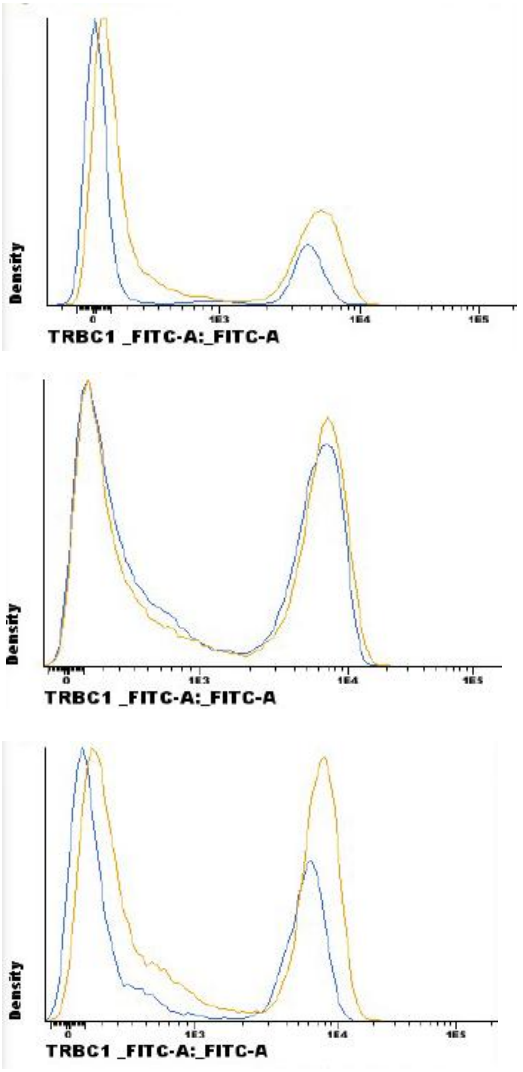


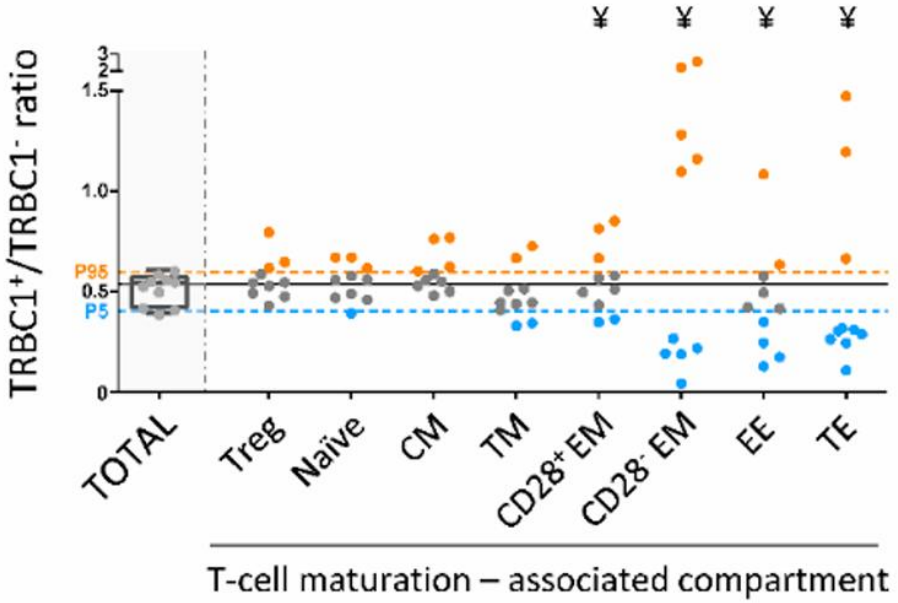




Munoz-Garcia et al, Cancers 2021;13:4379

Biologische variatie referentiewaarde TRBC1+ / TRBC1- ratio





Tαβ-Cell Subset	% TRBC1+ Cells *		TRBC1+/TRBC1− Ratio	
	Mean ± 1 SD	Range (Mean ± 3 SD)	Mean ± 1 SD	Range (Mean ± 3 SD)
Tαβ cells	40 ± 6.7	20–60	0.66 ± 0.071	0.25–1.4
Tαβ CD4+	43 ± 6.3	24–62	0.75 ± 0.067	0.31–1.6
Tαβ CD8+	35 ± 8.8	8.3–61	0.53 ± 0.096	0.091–1.6
Tαβ DP	36 ± 12	1.6–71	0.57 ± 0.13	0.016–2.5
Tαβ DN	29 ± 10	0-61	0.41 ± 0.12	0–1.5

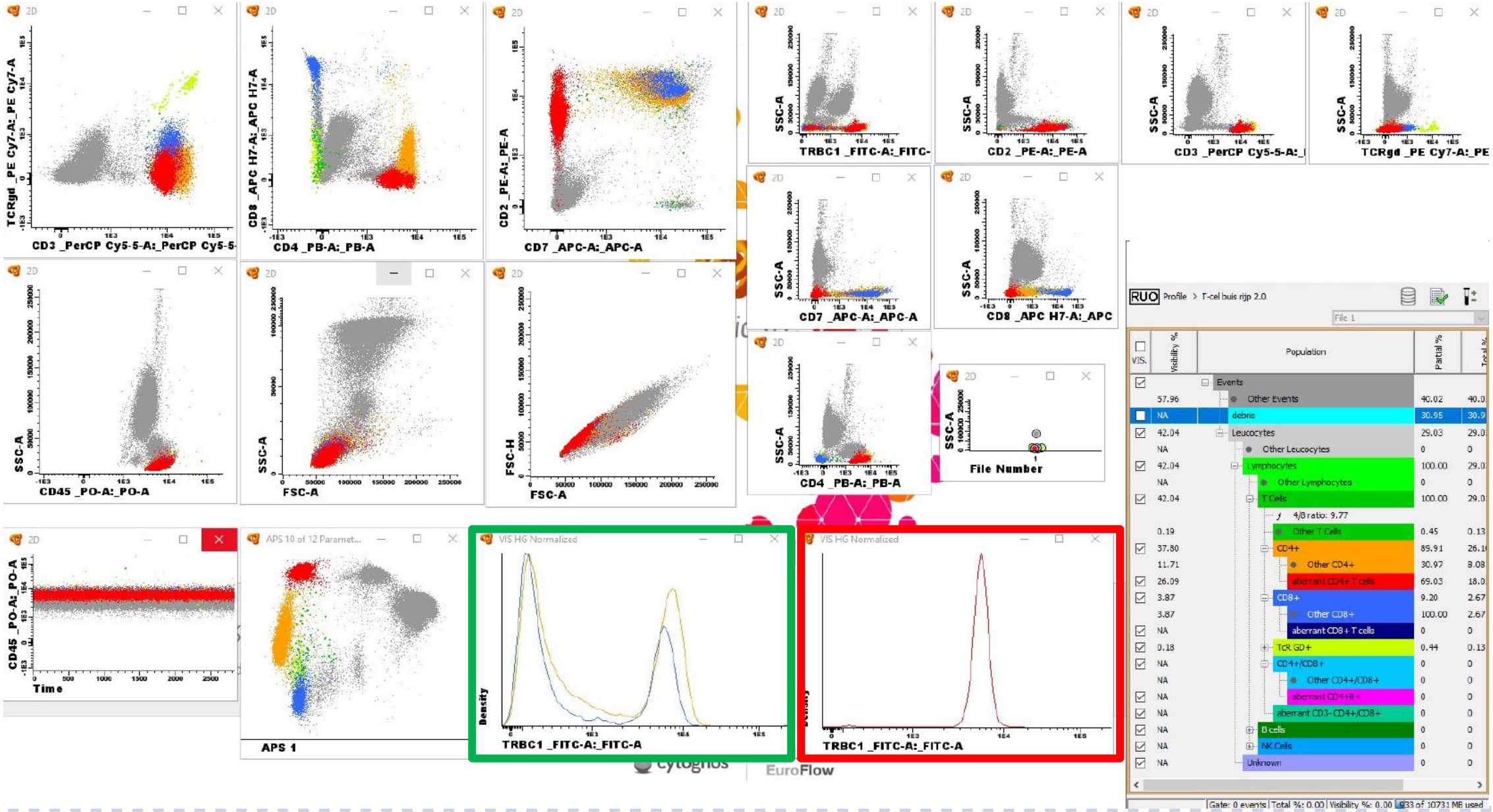
Munoz-Garcia et al, Cancers 2021;13:4379

T cel screening tube 2.0

Tube	PB	PO	FITC	PE	PerCP Cy5.5	PC7	APC	APC-H7
T 2.0	CD4	CD45	TRBC1	CD2	CD3	TCRγδ	CD7	CD8

Rationale voor markerkeuze: CD5, CD56 evt. uit LST te halen

TRBC1 – afwijkend patroon



Pilot TRBC1 analyse vs. moleculaire TCR analyse

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- Materiaal (PB, BM, BAL, CSF, biopt); inclusie n=20
- LST (vanwege verdenking afwijkende T-celpopulatie)
- Bij afwijkende CD4/CD8 ratio of CD marker

  1) T cel screening tube; 2) TCR genherschikking
- Resultaat

TCR genherschikking	**TRBC1 patroon**	
	polytypisch (n=8)	**monotypisch** (n=12)
polyklonaal (n=5)	5/5 (100%)	0/5 (0%)
monoklonaal (n=15)	3/15 (20%) *	12/15 (80%)

** 3x lage CD4/CD8 ratio in LST, geen duidelijk afwijkende marker*

- Sensitiviteit 80%; specificiteit 100%
- PPV 100%; NPV 62,5%

TRBC1 analyse vs. moleculaire klonaliteitsanalyse

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Table 4. Comparison between TRBC1 assay by FCM and the reference molecular and FCM techniques used to assess Tαβ-cell clonality (n = 117).

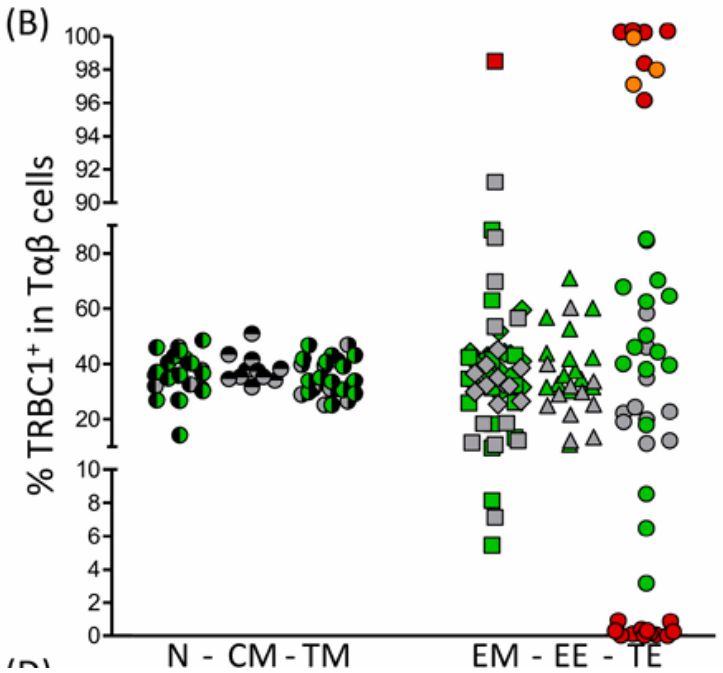
Clonality Status by Other Techniques *	TRBC1 Expression Pattern by FCM		p-Value
	Polytypic (n = 23)	Monotypic (n = 94)	
Poly/oligoclonal (n = 24)	**21/24 (87%)**	3/24 (13%)	<0.0001
Monoclonal (n = 93)	2/93 (2%)	**91/93 (98%)**	

Concordant cases are highlighted in bold. * Clonality assessed by PCR, TCRVβ family expression by FCM and/or gene mutation assays. Abbreviation: FCM, flow cytometry; PCR, polymerase chain region; TR, T-cell receptor.

Munoz-Garcia et al, Cancers 2021;13:4379

Beperkingen 8-kleuren TRBC1 analyse

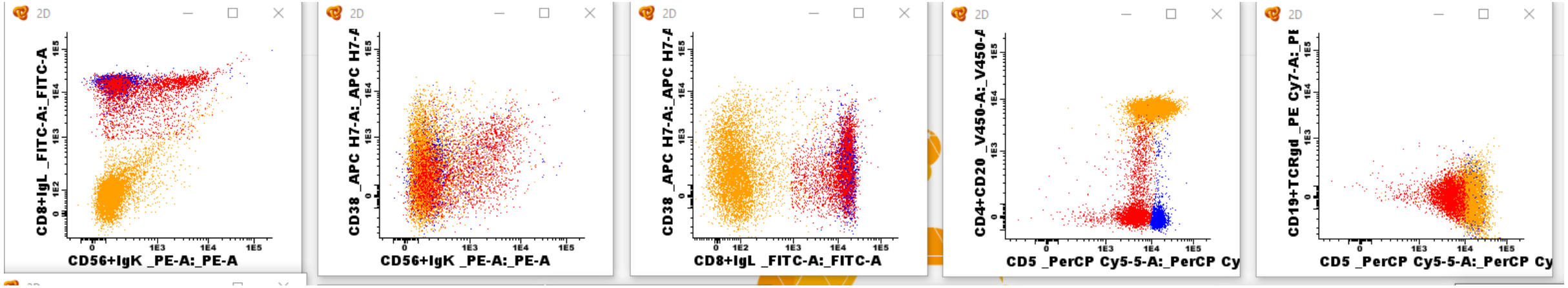
- aanname dat TRBC1- cellen TRBC2+ cellen zijn
- ratio TRBC1+/TRBC1- sterk afhankelijk van context
  - T celmaturatie
  - CD4 vs CD8
  - leeftijd individu
- interpretatie TRBC1 test: skewing van TCRab T cellen



Munoz-Garcia et al, Cancers 2022

Beperkingen 8-kleuren TRBC1 analyse (cont.)

- niet alle / te weinig aberrante markers in zelfde labeling om goed in te zoomen op afwijkende cellen

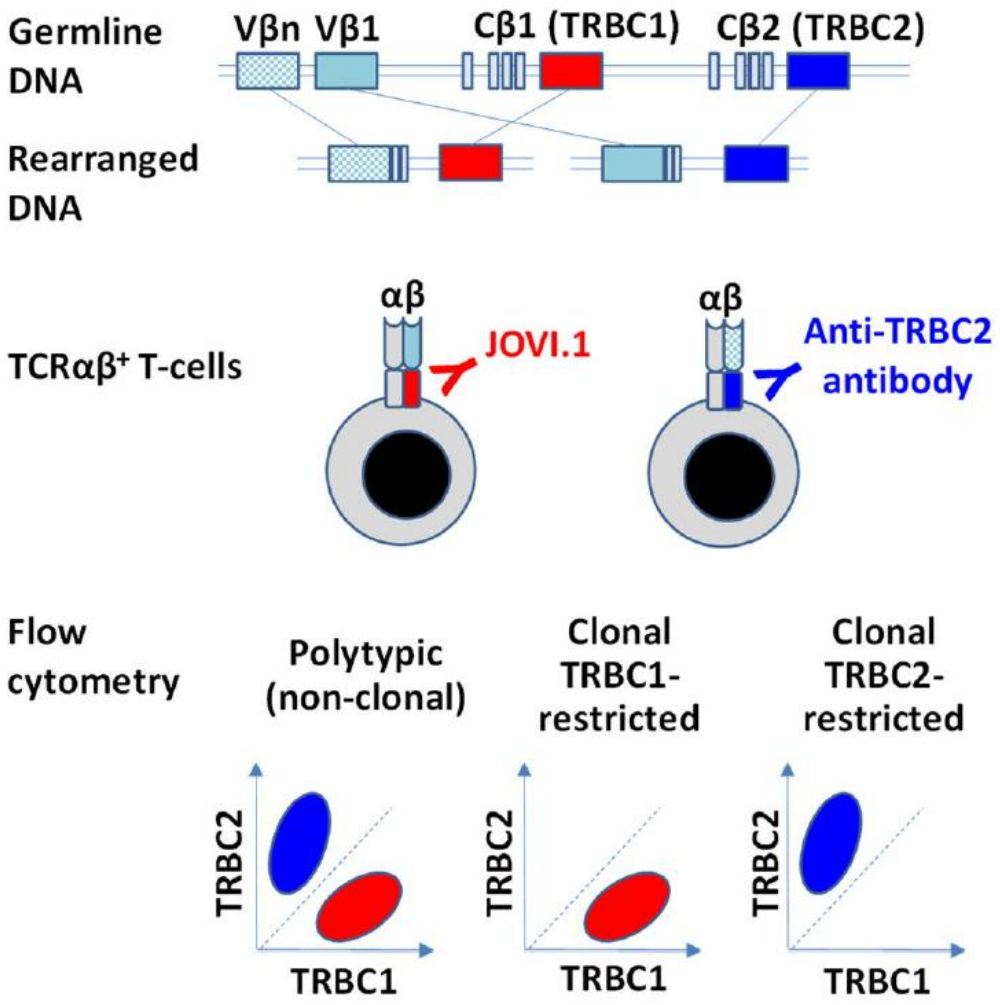


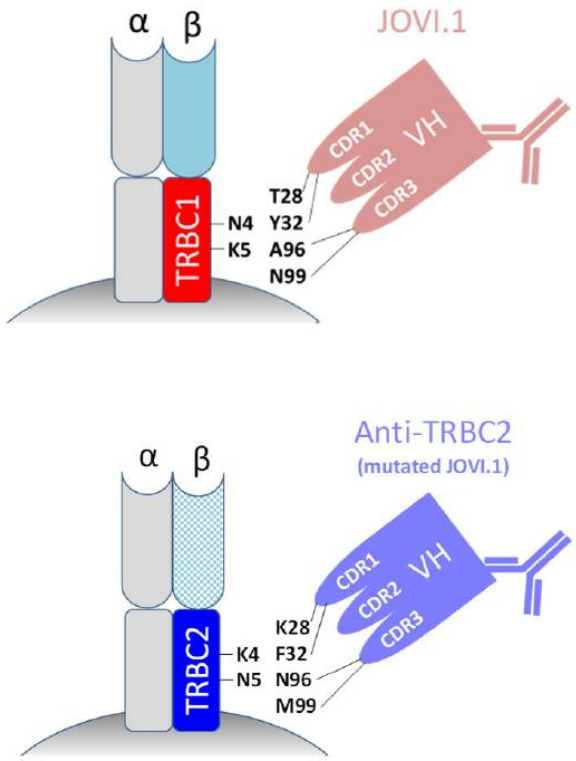
Populatie CD3+/CD8+/CD5 dim/deels CD56+

→ kans op zowel fout-positieve als fout-negatieve interpretaties

→ ruimte voor verbetering

Gecombineerde TRBC1-TRBC2 flowcytometrische test





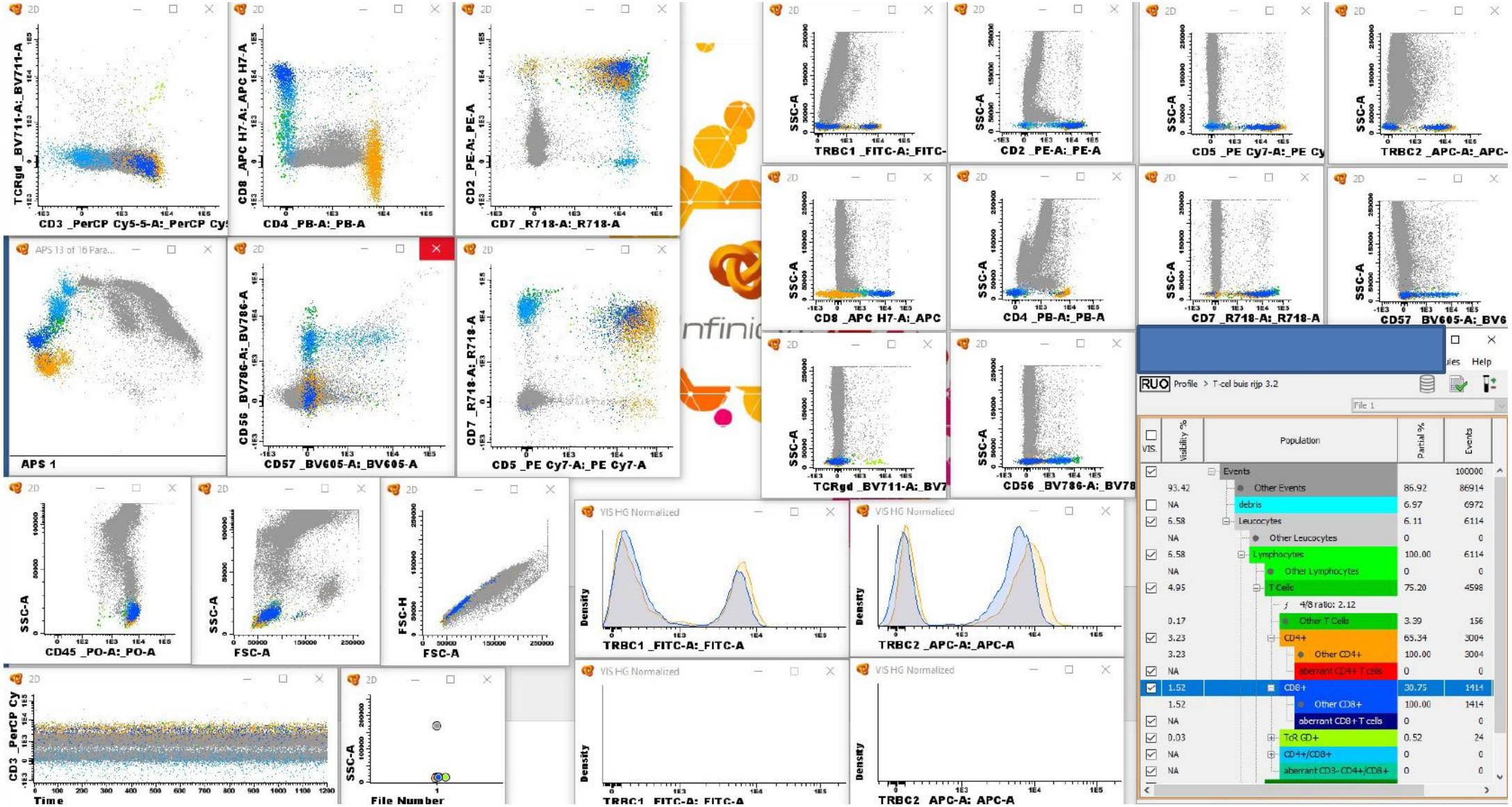
Horna et al, Blood Cancer J 2024;14

T cel screening tube 3.2

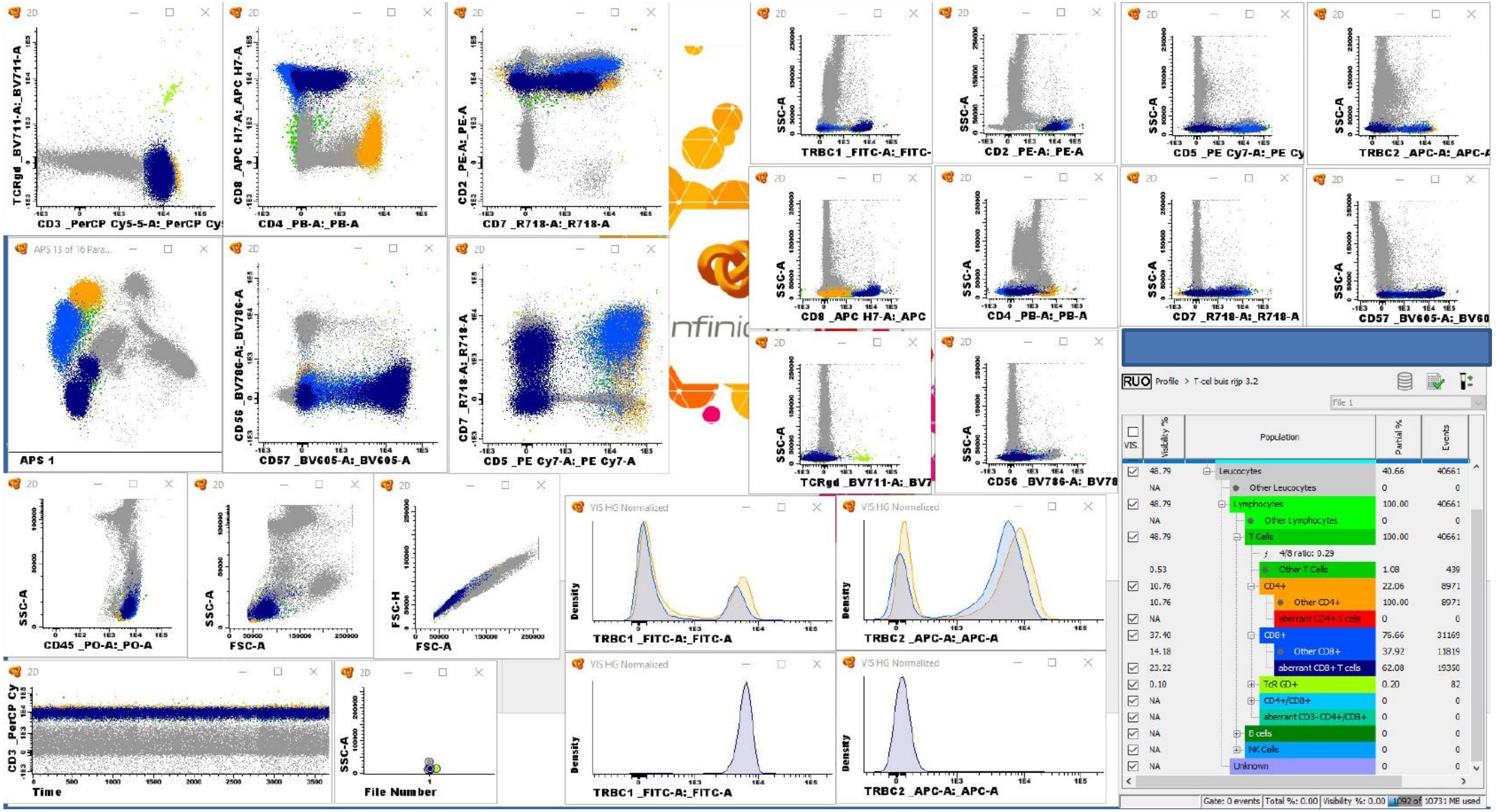
Tube	BV605	BV711	BV786	R718	PB	PO	FITC	PE	PerCP Cy5.5	PC7	APC	APC-H7
T 3.2	CD57	TCRγδ	CD56	CD7	CD4	CD45	TRBC1	CD2	CD3	CD5	TRBC2	CD8

- volgorde toevoegen antistoffen (1e mix: BV Abs + stain buffer, dan 2e mix met overige Abs; geldt voor combinatie van TRBC1 FITC en TRBC2 APC conjugaten)
- validatie vs. T 2.0 labeling (n=10) → zie dia 15
- validatie vs. TCR klonaliteitsanalyse (n=28; lopend) → zie dia 15

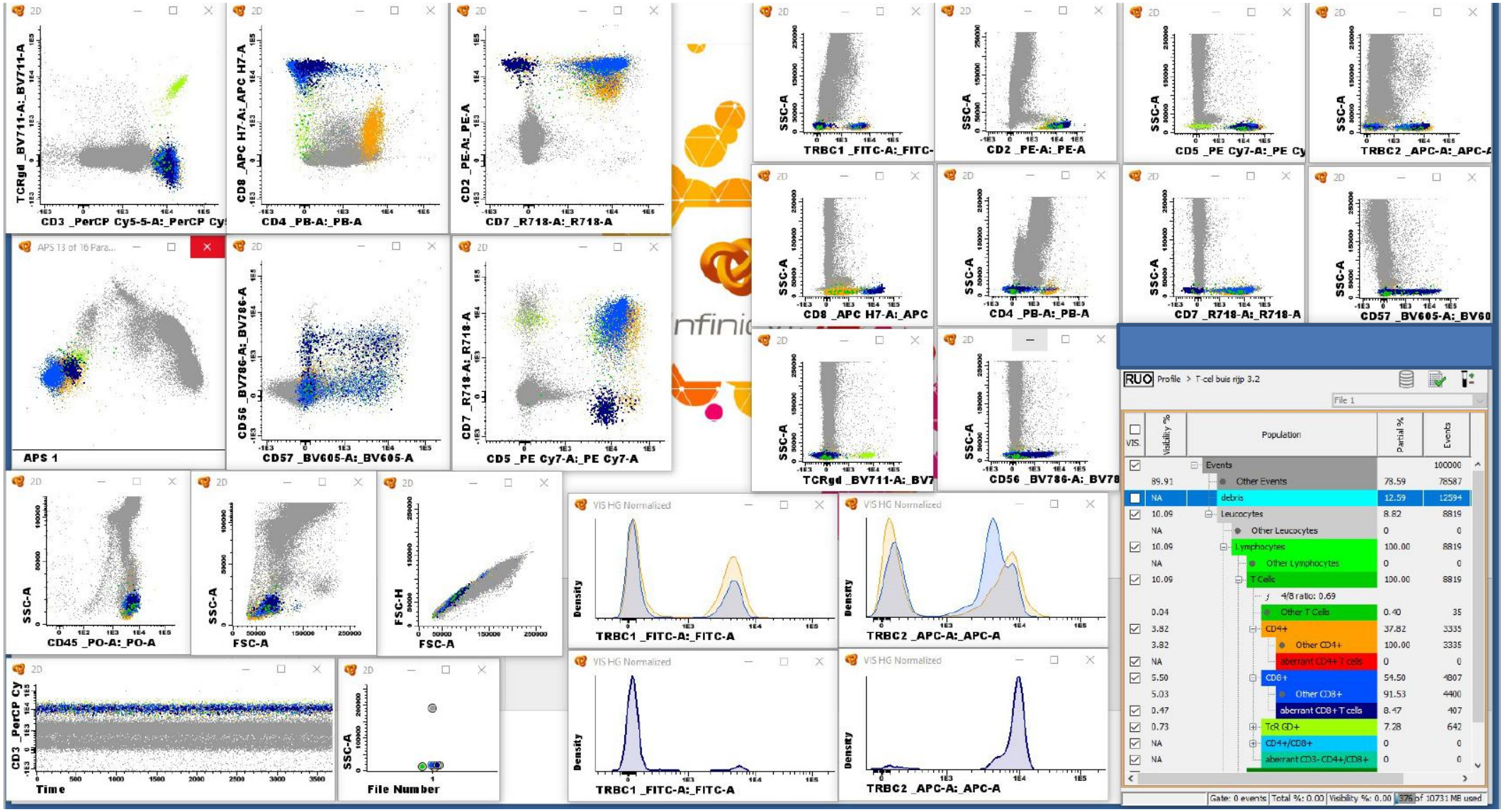
T cel screening tube 3.2 – normaal PB



T cel screening tube 3.2 – afwijkend PB (KL2025-356)



T cel screening tube 3.2 – afwijkend PB (KL2025-445)



Validatie T cel screening tube 3.2

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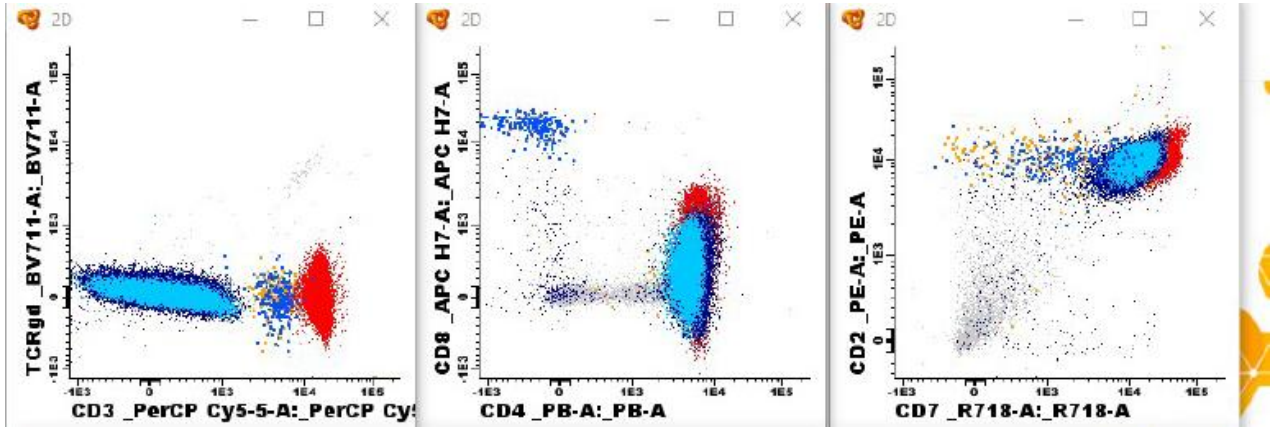
- **T 2.0 vs. T3.2 labeling (n=10)**

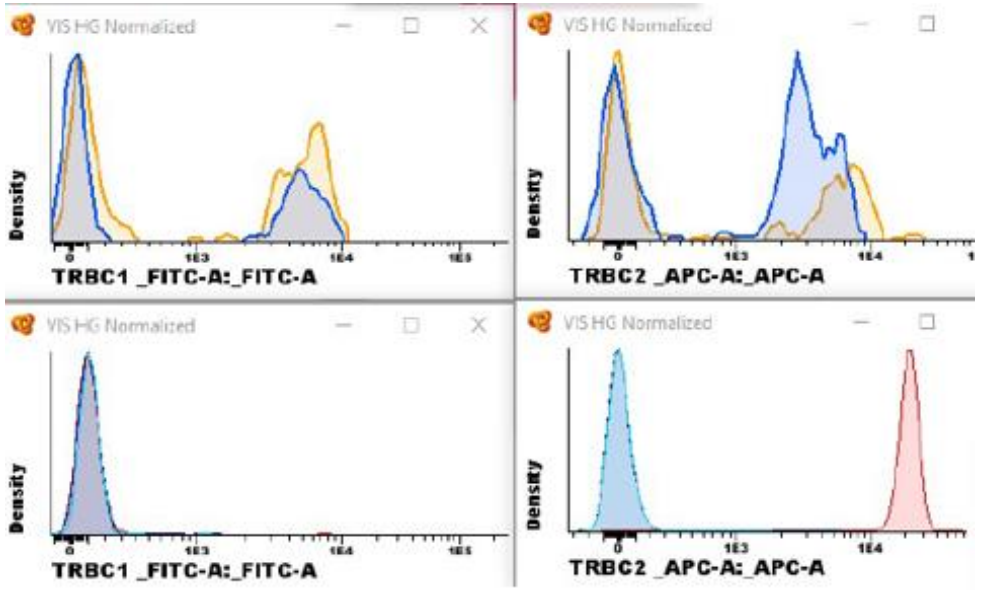
- n=8 concordant; n=2 met afwijkende T-celpopulatie in T 3.2 (niet in T 2.0; wel TCR klonaal)
- %CV totaal T / CD4 / CD8 / CD4-CD8 ratio vrijwel altijd <30%
  → toegevoegde waarde van nieuwe test

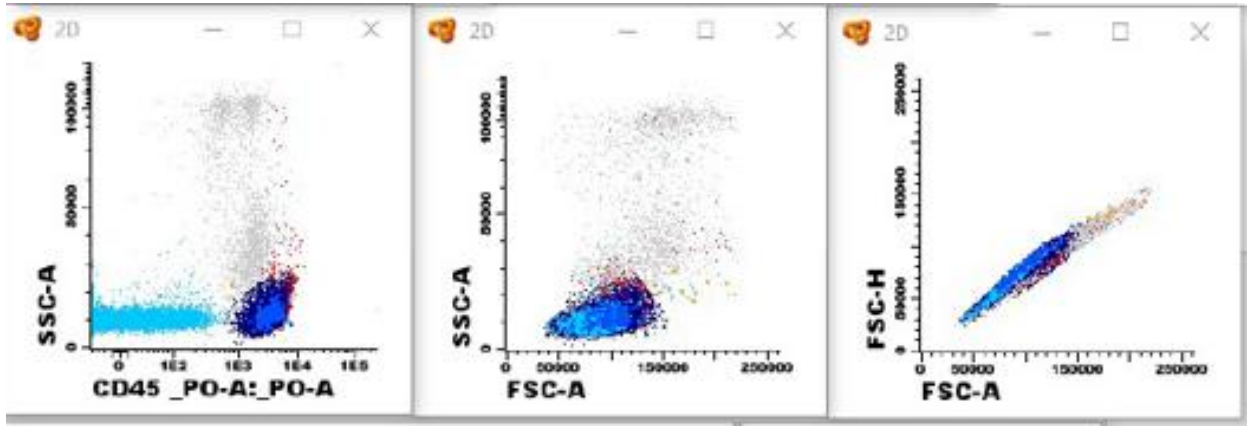
- **T 3.2 labeling vs. TCR klonaliteitsanalyse (momenteel n=28)**

- validatie loopt nog
- preliminaire data: goede specificiteit en hoge PPV, maar sensitiviteit kan nog beter

Casus 2025-058 : T cel screening tube 3.2



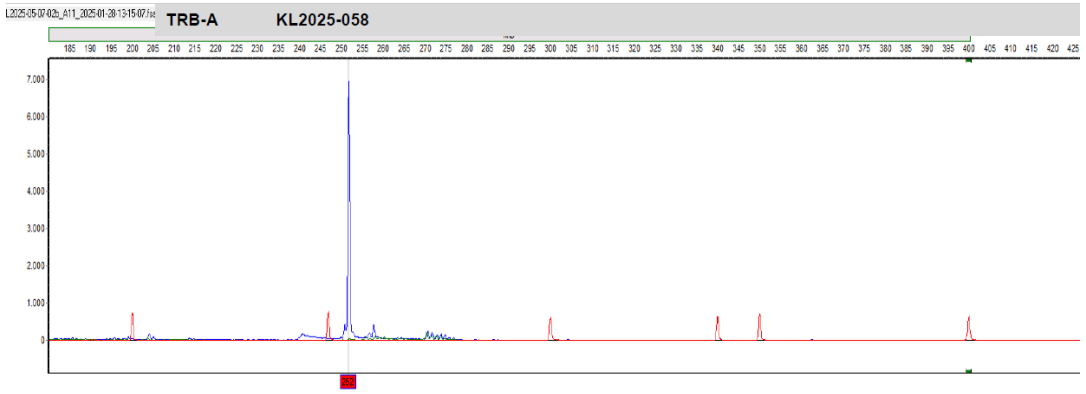


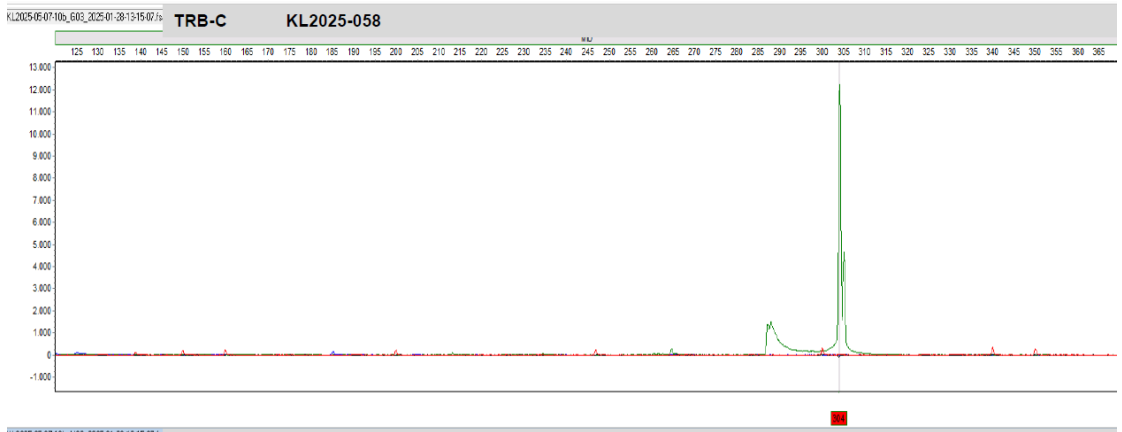


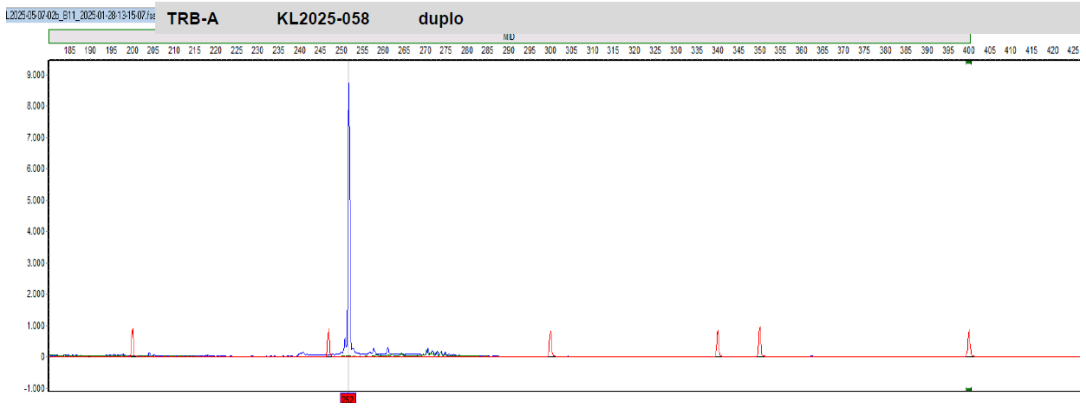
Pop 1 (32%): CD45+/3+s/4+/8-/2+/7+/5+/TRBC2
Pop 2 (52%): CD45+/3-/4+/8-/2+/7+/5+/TRBC-
Pop 3 (13%): CD45-/3-/4+/8-/2+/7+/5+/TRBC-

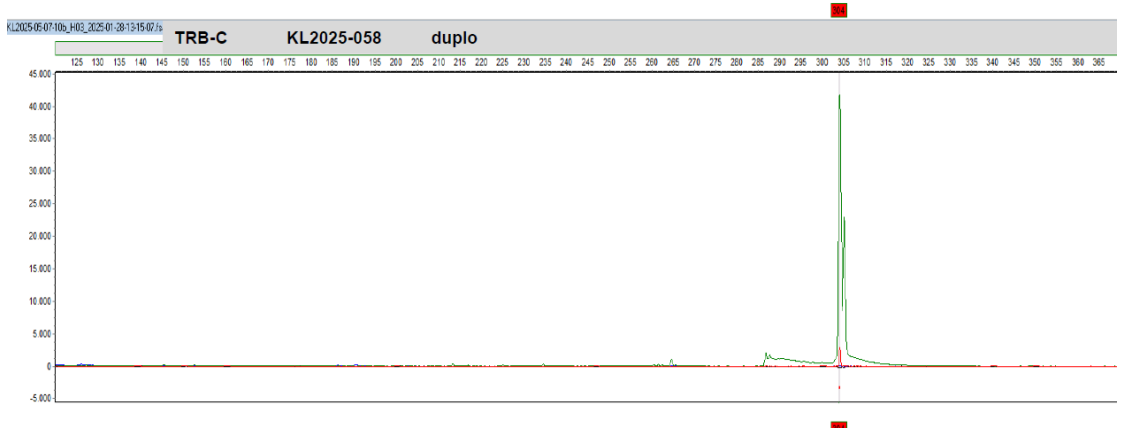
Meerdere klonen?

Casus 2025-058 : TCRB analyse



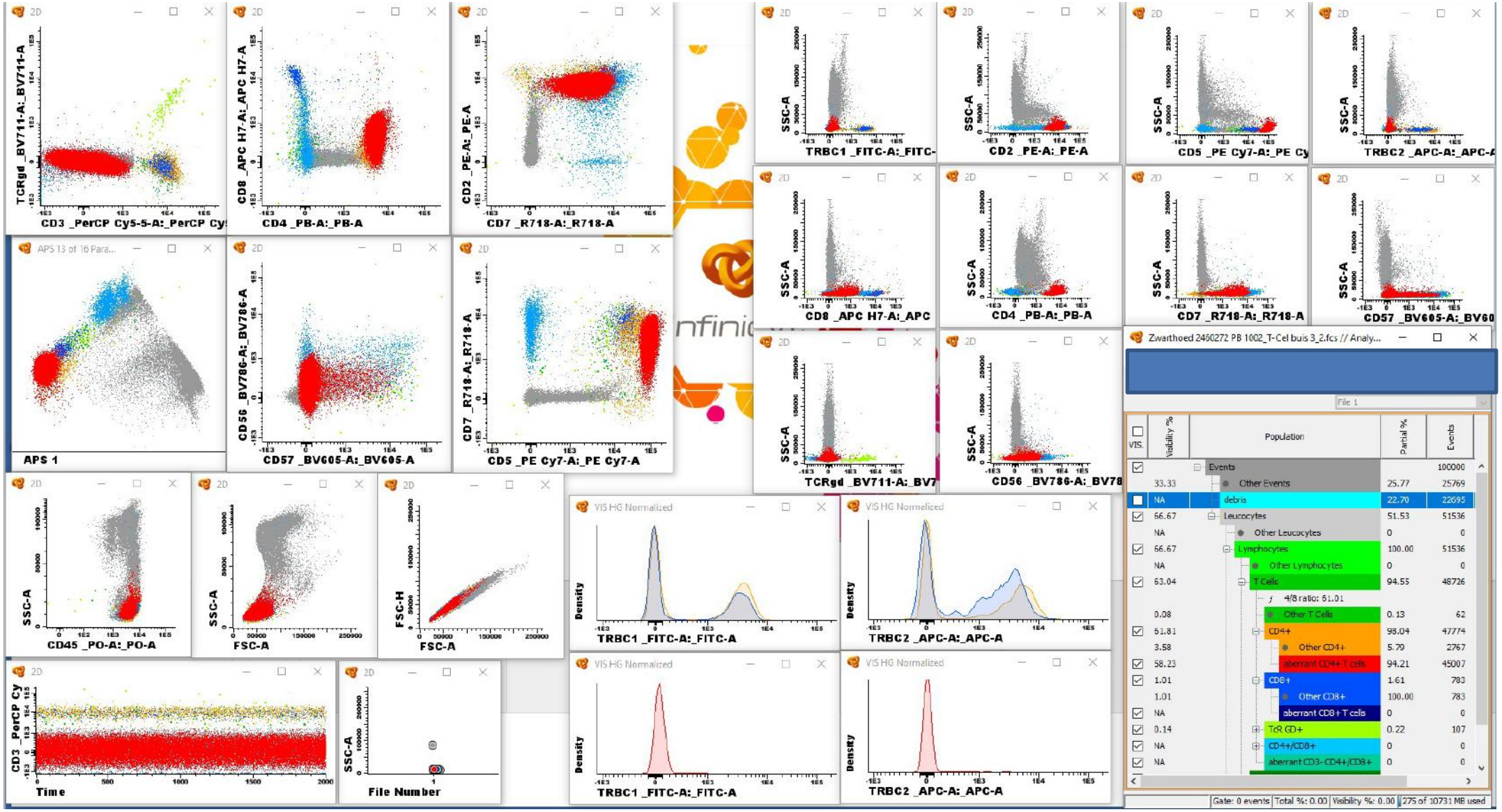






Conclusie: 1 monoklonale celpopulatie
NB bij membraan CD3 verlies, ook geen TCR complex op membraan, dus TRBC1- en TCRB2-

T cel screening tube 3.2 – afwijkend PB (KL2025-125)



Take home lessons

- TRBC1 + TRBC2 screening geeft completer inzicht in skewing T cel (sub)populatie(s)

- Toegevoegde waarde van TRBC1 vs. TRBC2 analyse ligt bij toepassing binnen <u>aberrante</u> celpopulatie
  - skewing + aberrantie = proxy voor klonaliteit
  - NB vermijd analyse van TRBC1 / TRBC2 ratio (zonder aberrantie)

- Valkuilen
  - TRBC1 en TRBC2 negativiteit bij SmCD3- T-celpopulatie of TCRgd T-celpopulatie
  - fout-negativiteit TRBC1 + TRBC2 test (op basis van preliminaire data)
  - biologische / klinische betekenis van zeer kleine T-celklonen (zowel flow als mol.)

laboratorium medische immunologie

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