

MRD Analysis in Paediatric T-cell acute lymphoblastic leukaemias

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St. Jude Children's Research Hospital*

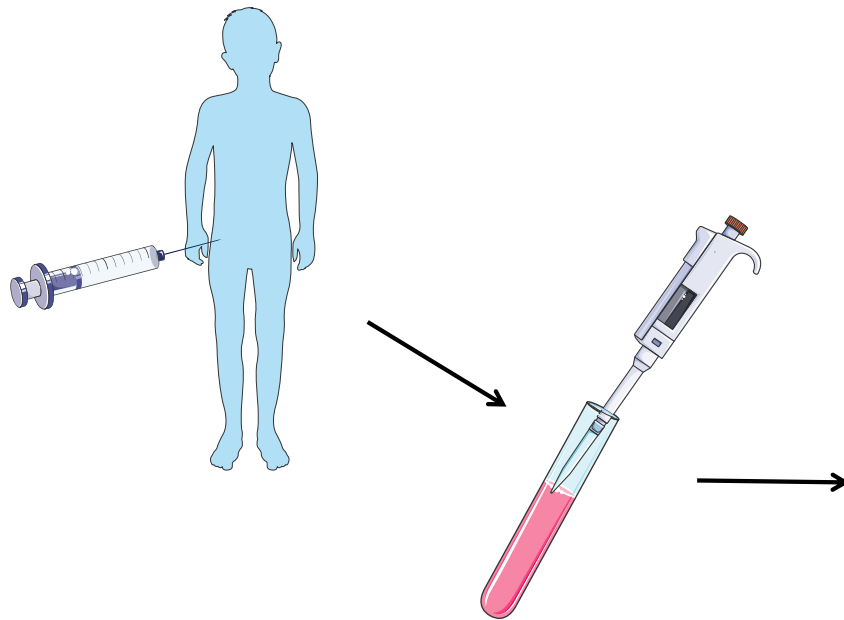


Non-profit ~100 bed hospital for catastrophic diseases
1st NIH-designated Comprehensive Cancer Center for kids
5,000+ employees, ½ clinical and ½ research
Families never get a bill from St Jude (medical, travel, housing ...)

Conflicts of Interest ...

Nothing to declare

St Jude New Leukemia Work Up



Core Lab

CBC, Chemistry, etc

Special Hematology

Morphology review, blast count

Cytochemistry (MPO, NSE)

Immunopathology Lab

Flow cytometry

Immunophenotyping & MRD baseline

Cytogenetics

Karyotype

Fluorescence in situ hybridization (FISH)

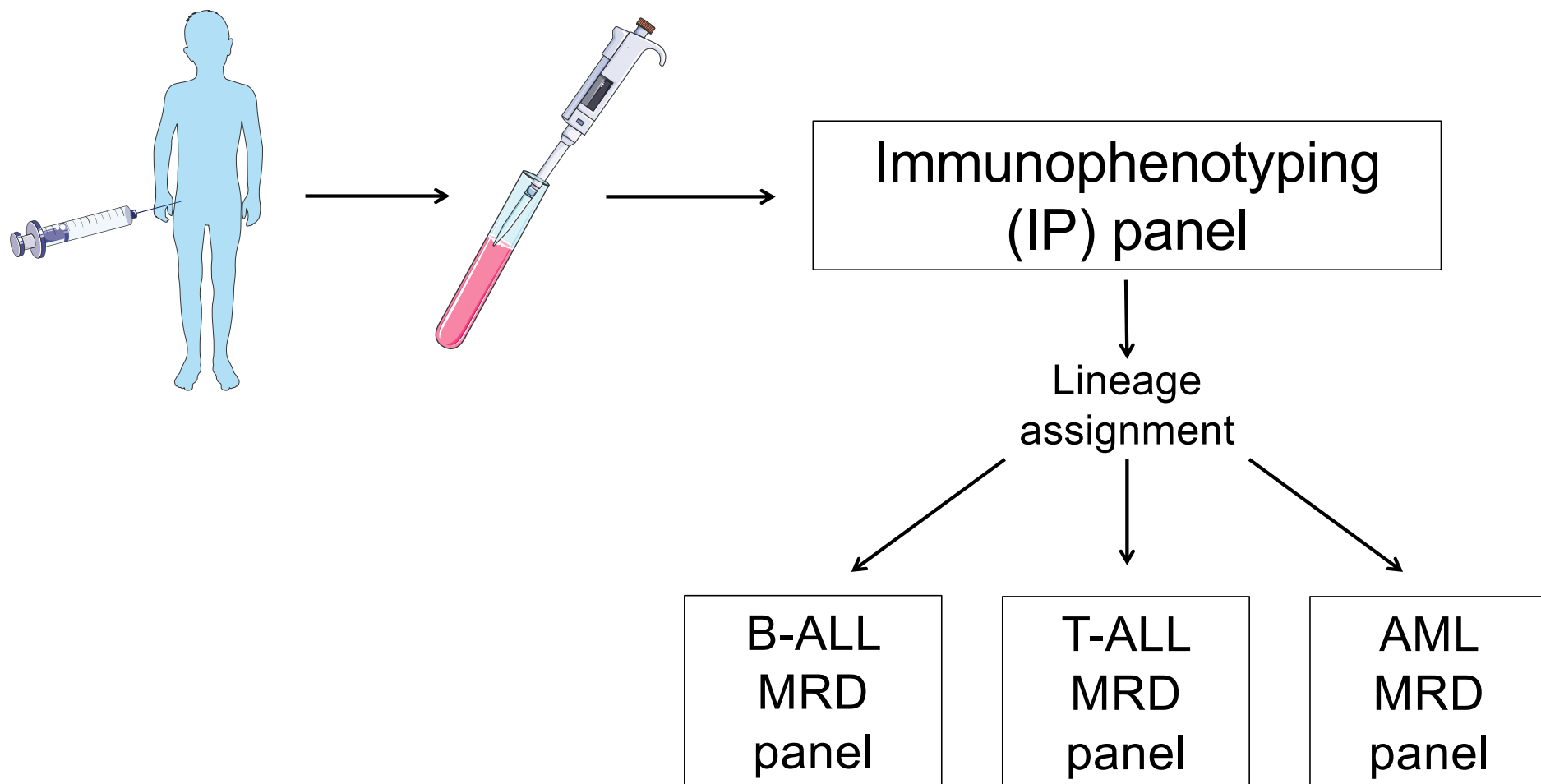
Molecular Lab

RT-PCR

ClinGen (WGS, WES, RNA-seq)

Capture-based NGS panel test

St Jude Immunopathology Lab Workflow

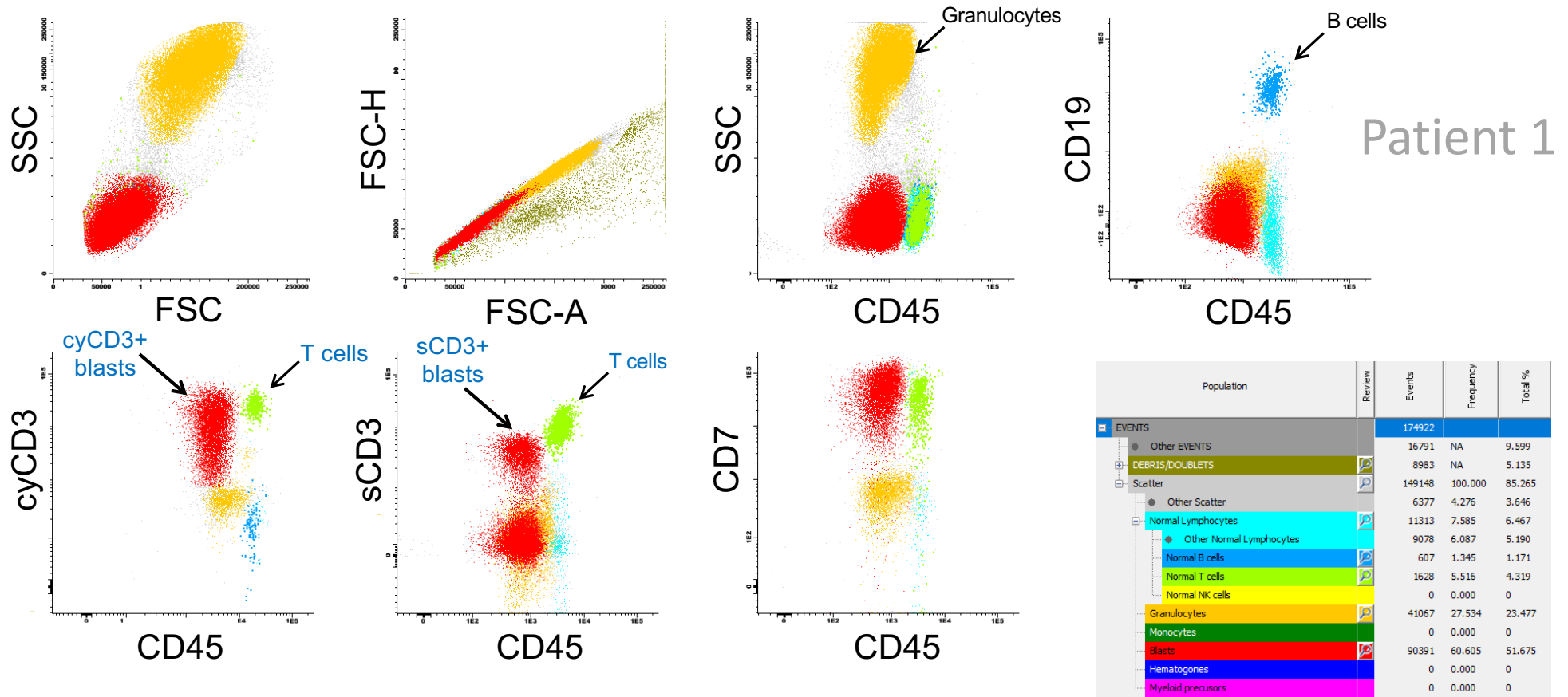


St Jude Immunophenotyping Panel

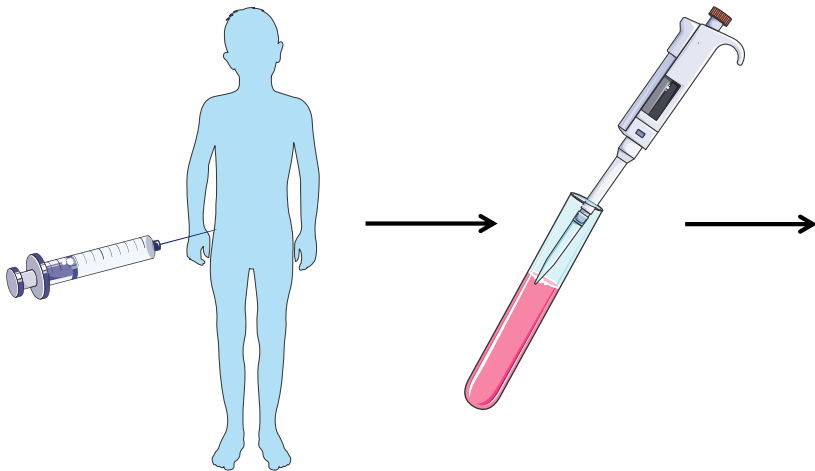
	Tube	FITC	PE	PerCP	PE-Cy7	APC	APC-H7	V450	V500
Surface	1	IgG1	IgG1	IgG1	IgG1	IgG1	45	IgG1	IgG1
	2	235a	TSLP-R	14	33	11c	45	61	64
	3	13	NG2	34	117	19	45	133	7
	4	7	1a	8	5		45	11b	DR
	5	$\alpha\beta$	$\gamma\delta$	3	5	7	45	2	56
	6	IgG2a	IgG2a	IgG2a	IgG2a	IgG2a	45	44 surface markers	
	7	λ	κ	3	19	22	45	8	4
	8	38	73	34	10	19	45	58	20
	9	66c	123	86	200	19	45	33	15
	10	41	42b		19	22	45	25	90
Cyto	11	cyIgG1	cyIgG1	45		19			
	12	MPO	cy3	45		19		4 intracellular markers	
	13	cyTdT	cy79a	45		19			

3-laser 8-colour BD Cantoll cytometer

St Jude Immunophenotyping Panel



St Jude Immunopathology Lab Workflow



Tube	FITC	PE	PerCP	PE-Cy7	APC	APC-H7	V450	V500
1	IgG1	IgG1	IgG1	IgG1	IgG1	45	IgG1	IgG1
2	235a	TSLP-R	14	33	11c	45	61	64
3	13	NG2	34	117	19	45	133	7
4	7	1a	8	5		45	11b	DR
5	$\alpha\beta$	$\gamma\delta$	3	5	7	45	2	56
6	IgG2a	IgG2a	IgG2a	IgG2a	IgG2a	45	IgG2a	IgG2a
7	λ	κ	3	19	22	45	8	4
8	38	73	34	10	19	45	58	20
9	66c	123	86	200	19	45	33	15
10	41	42b			22	45	25	90
11	cyIgG1	cyIgG1	45		19	IP panel		
12	MPO	cy3	45		19			
13	cyTdT	cy79a	45		19			

Lineage assignment

Tube	FITC	PE	V450	PerCP	PE-Cy7	APC	APC-H7	BV510
1	IgG1	IgG1	IgG1	34	10	22	45	19
2	38	13	15	34	10	22	45	19
3	66c	24	20	34	10	22	45	19
4	72	58	33	34	10	22	45	19
5		73	44	34	10	22	45	19
6		200	86	34	10	22	45	19
7		123		34	10	22	45	19
8		133		34	10	22	45	19
9		NG2		34	10	22	45	19

B panel

Tube	FITC	PE	V450	PerCP	PE-Cy7	APC	APC-H7	V500
1	nTdT	99	s3	cy3	5	19 + 33	DR	45
2	IgG1	99	s3	cy3	5	19 + 33	DR	45
3	7	IgG1	s3	cy3	5	19 + 33	DR	45
4	7	34	s3	cy3	5	19 + 33	DR	45
5	4	8	s3	cy3	5	19 + 33	DR	45
6	2	gd	s3	cy3	5	19 + 33	DR	45
7	7	1a	s3	cy3	5	19 + 33	DR	45
8	7	56	s3	cy3	5	19 + 33	DR	45

T panel

Tube	FITC	PE	PerCP	PE-Cy7	APC	APC-H7	BV421	BV510
1	IgG2a	IgG1	34	33	117	45	IgG3	IgG2a
2	7	13	34	33	117	45	11b	4
3	13	54	34	33	117	45	19	7
4	15	56	34	33	117	45	38	DR
5	235a	133	34	33	117	45	41b	56
6		NG2	34	33	117	45		
7			34	33	117	45		

AML panel

St Jude T-ALL MRD Diagnostic Panel

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
2	cyIgG1	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
3	CD7	IgG1	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

Variable markers

"Add On"

Surface marker cocktail

Cytoplasmic and Surface cocktail

St Jude T-ALL MRD Diagnostic Panel

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
2	cyIgG1	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
3	CD7	IgG1	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

Variable markers

"Add On"

Identify LAIP

Leukemia-Associated ImmunoPhenotype

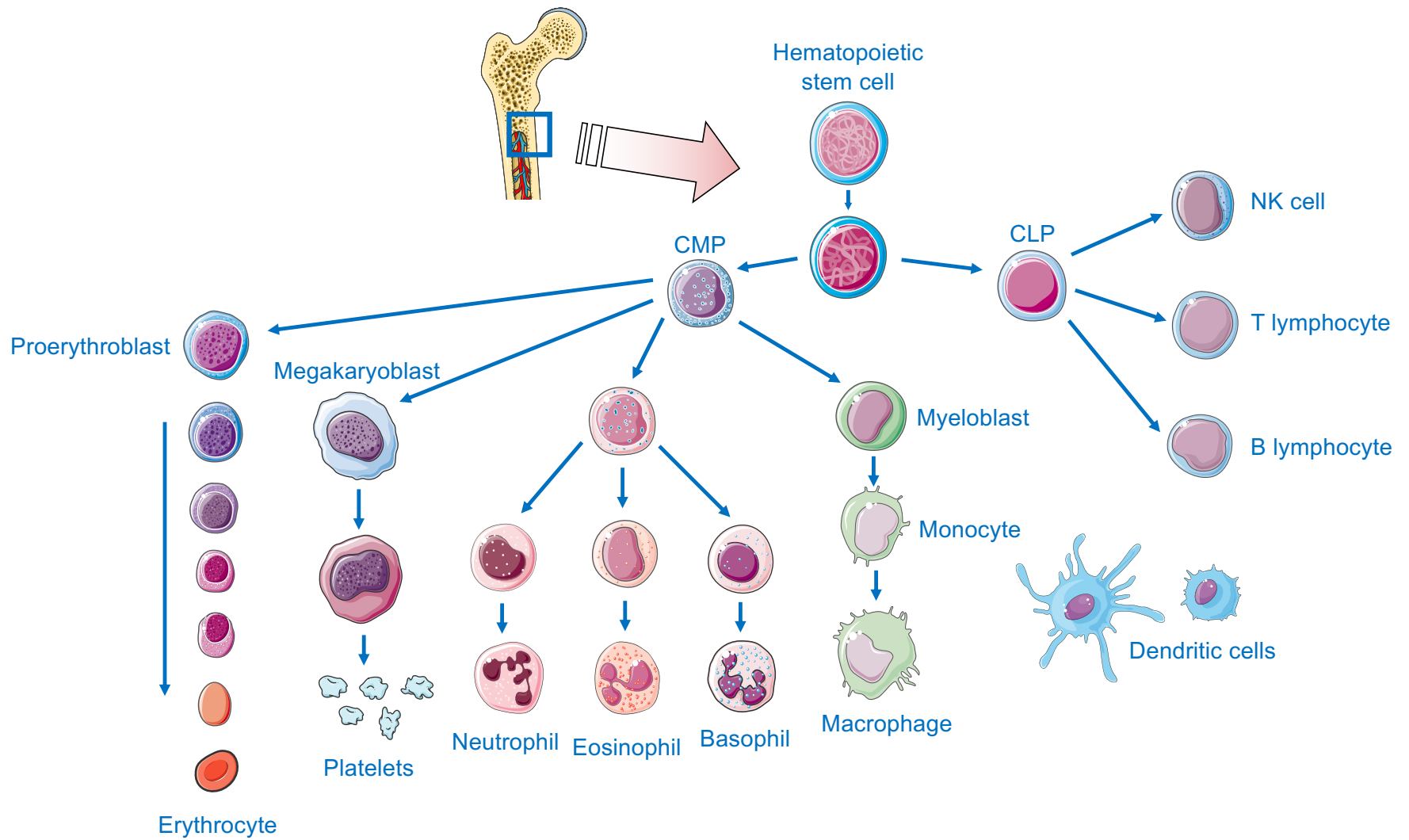
Surface marker cocktail

Cytoplasmic and Surface cocktail

Identify blasts

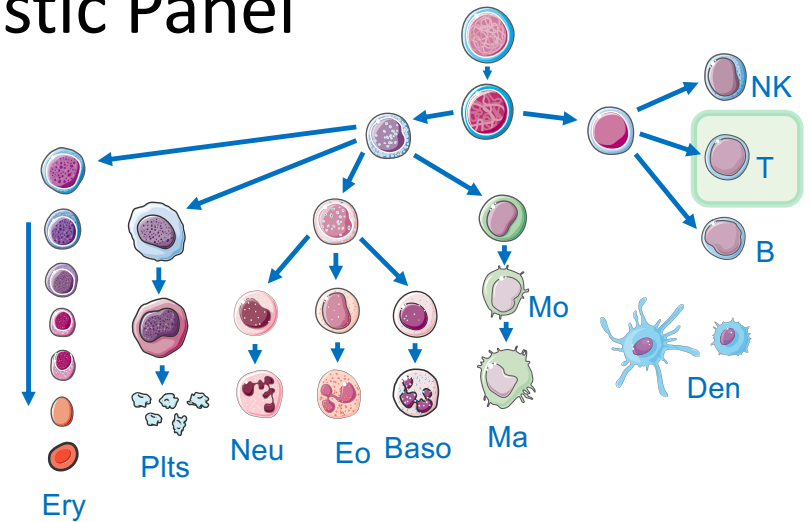
Understanding Normal is Key to Identifying Abnormal



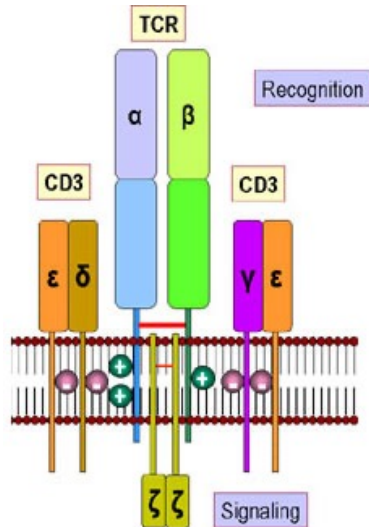


St Jude T-ALL Diagnostic Panel

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
2	cyIgG1	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
3	CD7	IgG1	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

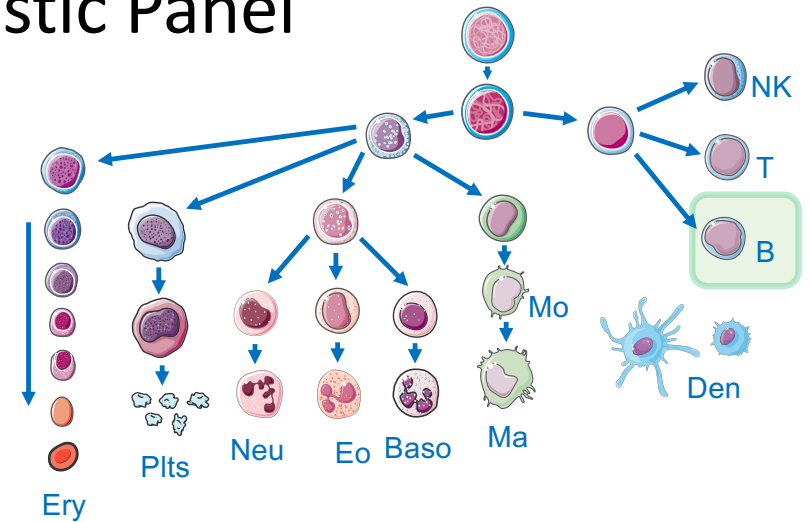


cyCD3: specific marker for T-cells



St Jude T-ALL Diagnostic Panel

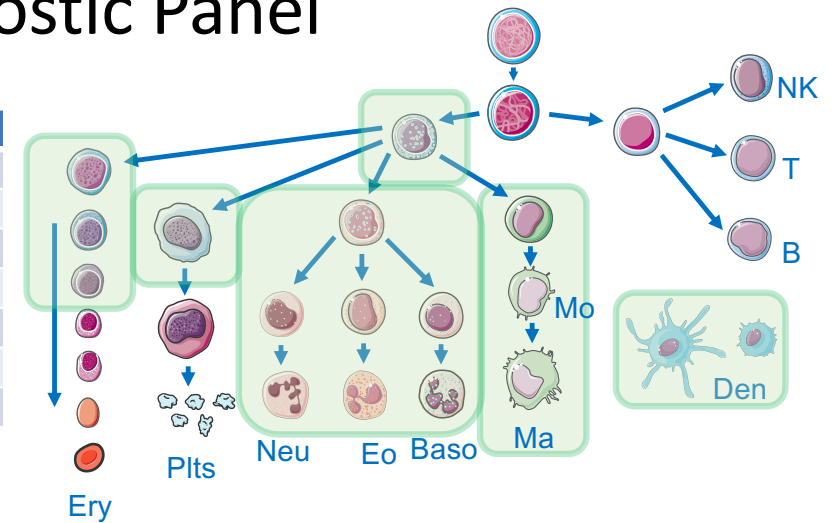
Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
2	cyIgG1	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
3	CD7	IgG1	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



CD19: B-cells

St Jude T-ALL Diagnostic Panel

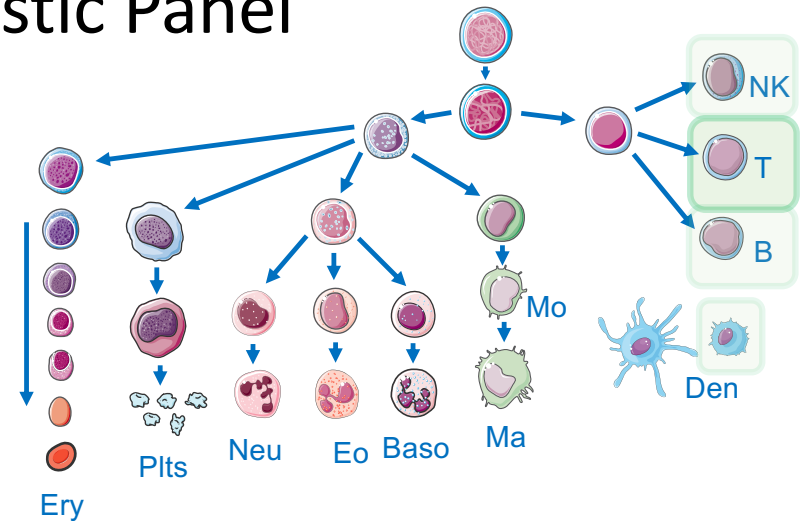
Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
2	cyIgG1	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
3	CD7	IgG1	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



CD33: expressed on myeloid cells
- sometimes on lymphoid cells

St Jude T-ALL Diagnostic Panel

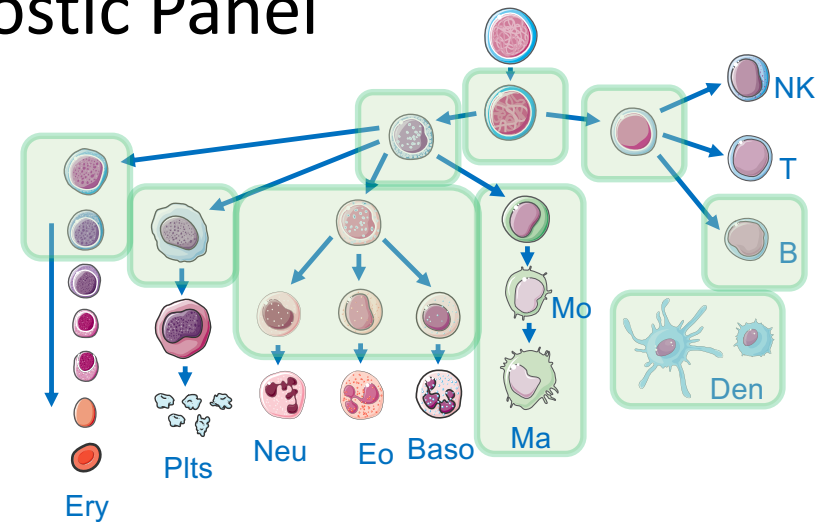
Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
2	cyIgG1	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
3	CD7	IgG1	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



CD5: T cells are predominantly CD5-positive
 CD8+ cells are often dim for CD5 compared to CD4+ cells
 CD5 also expressed on: subset of B cells
 late stage hematogones
 subsets of dendritic cells
 subsets of NK cells

St Jude T-ALL Diagnostic Panel

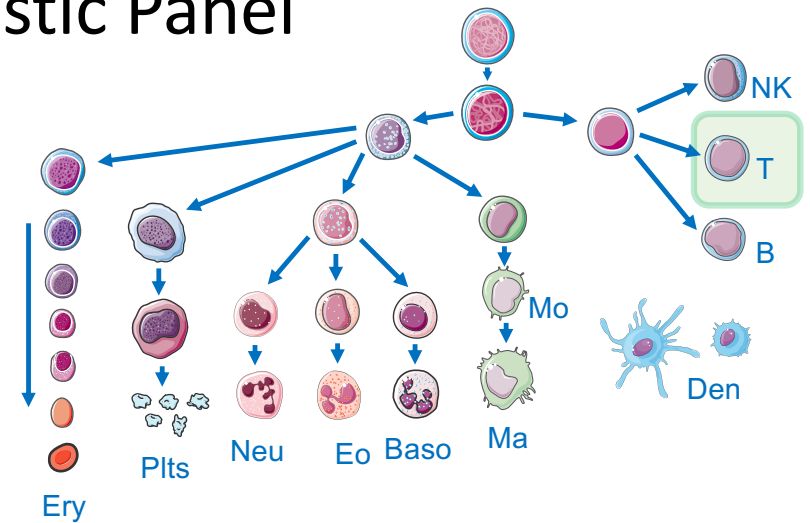
Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
2	cyIgG1	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
3	CD7	IgG1	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



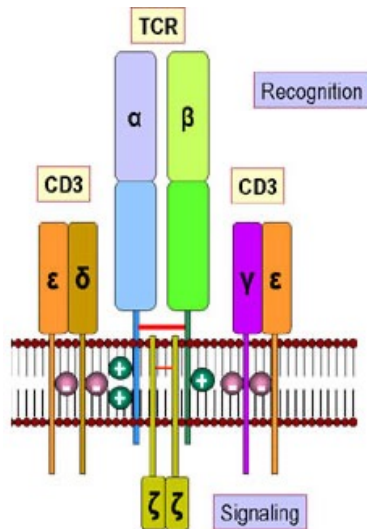
HLA-DR: most benign T cells and most T cell neoplasms are **negative** for HLA-DR; except activated benign T cells can be HLA-DR **positive**. Also expressed on B cells (except plasma cells), hematopoietic progenitors, myeloid, erythroid, and megakaryocytic precursors, monocytes/macrophages, dendritic cells, and immature T cells.

St Jude T-ALL Diagnostic Panel

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
2	cyIgG1	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
3	CD7	IgG1	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

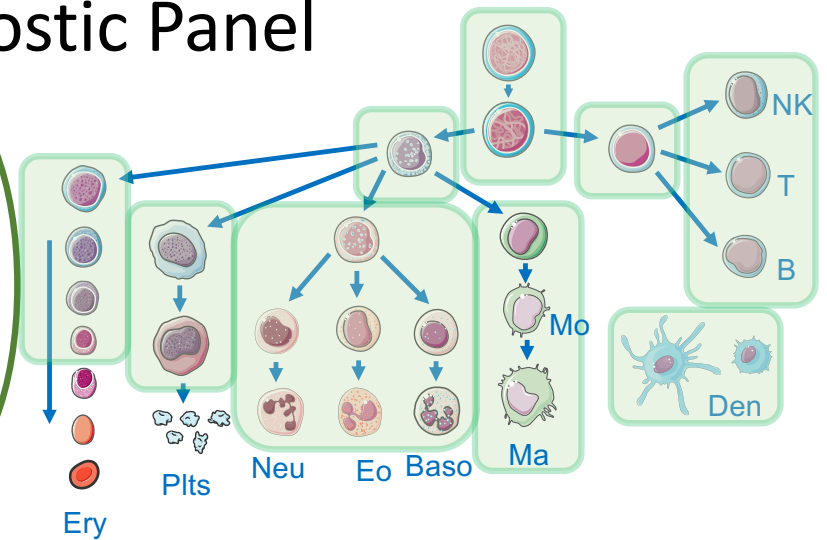


sCD3: specific marker for T-cells



St Jude T-ALL Diagnostic Panel

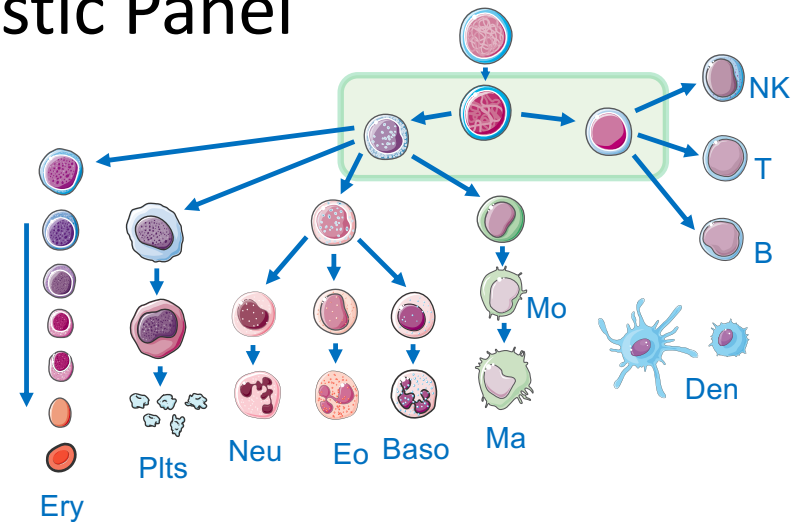
Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
2	cyIgG1	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
3	CD7	IgG1	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



CD45: present in all cells of the hematopoietic lineage except erythrocytes and plasma cells.

St Jude T-ALL Diagnostic Panel

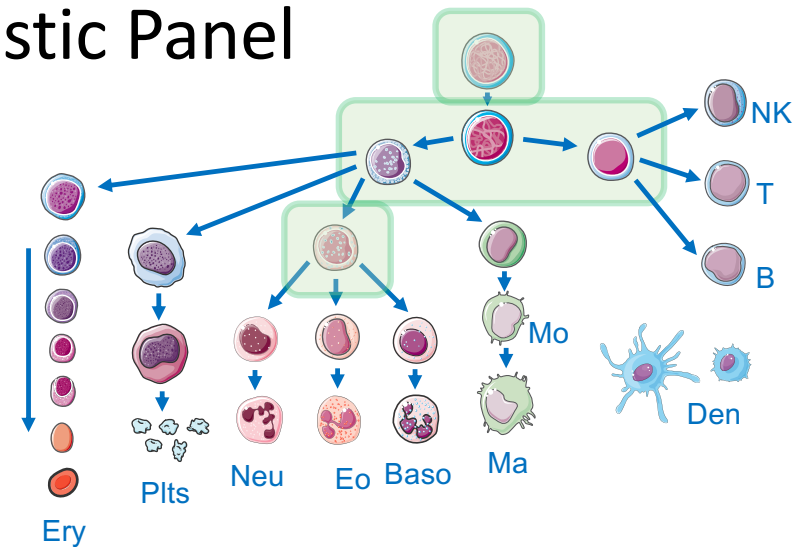
Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
2	cyIgG1	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
3	CD7	IgG1	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



cyTdT: lymphoid and some myeloid primitive precursors
ALL (>90%, stronger in B-ALL than T-ALL), some AMLs

St Jude T-ALL Diagnostic Panel

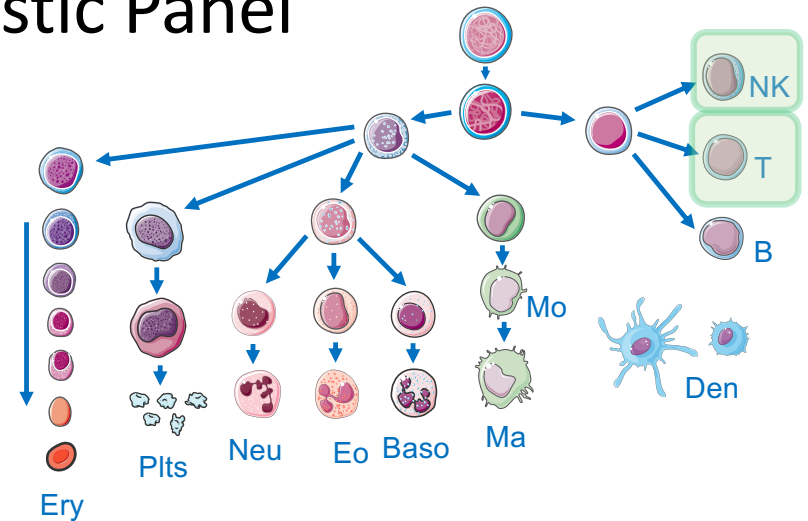
Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
2	cyIgG1	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
3	CD7	IgG1	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



CD99: ubiquitously expressed in almost all human cell types at low levels, strong expression in cortical thymocytes, bone marrow CD34+ cells, stromal lymphocytes and a broad range of hematopoietic cells, with the highest expression in the most immature lymphocytes and granulocytes, such as immature thymic T-lineage cells and tonsillar lymphoid progenitor cells.

St Jude T-ALL Diagnostic Panel

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
2	cyIgG1	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
3	CD7	IgG1	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



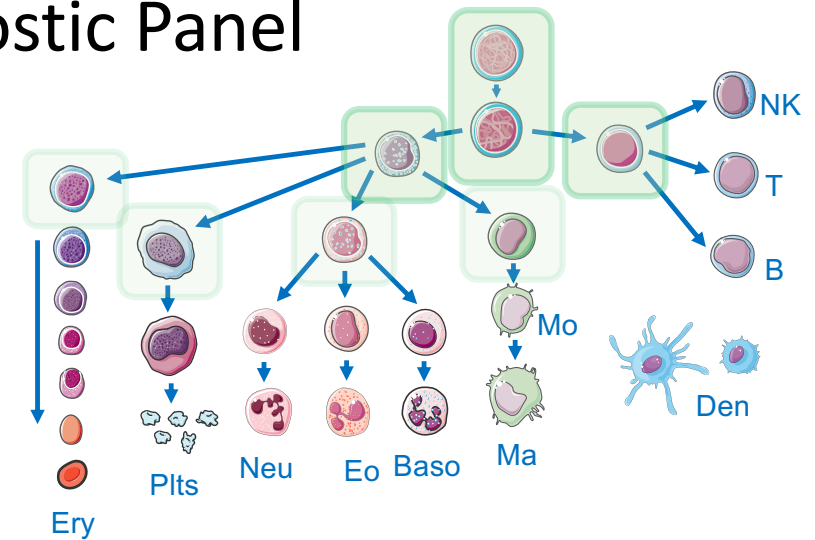
CD7: pan T-cell marker, not specific to T cells

T cell populations are predominantly CD7-positive, but there will typically be a proportion of CD7 dim to negative T cells

Also expressed in NK cells (CD3-negative, CD7-positive, CD56-positive)

St Jude T-ALL Diagnostic Panel

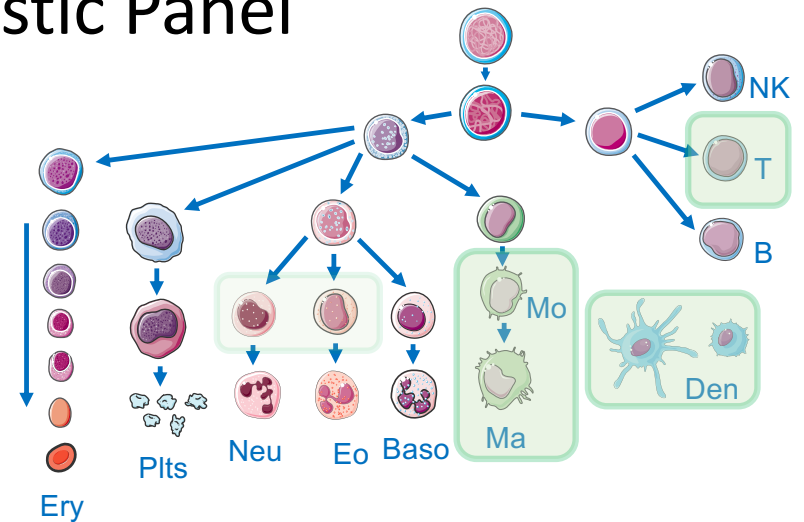
Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
2	cyIgG1	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
3	CD7	IgG1	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



CD34: lymphoid and some myeloid primitive precursors

St Jude T-ALL Diagnostic Panel

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
2	cyIgG1	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
3	CD7	IgG1	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

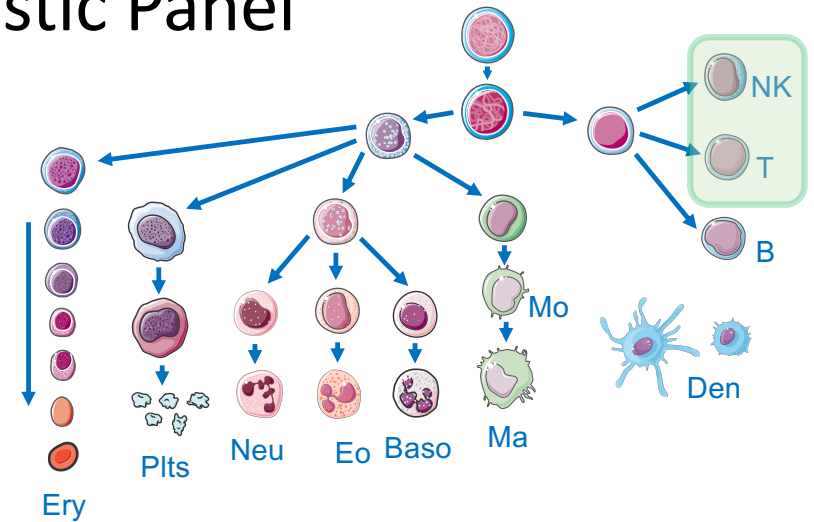


CD4: Helper T cells

Also expressed on monocytes, macrophages, and dendritic cells and a subset of granulocytes depending on activation state

St Jude T-ALL Diagnostic Panel

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
2	cyIgG1	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
3	CD7	IgG1	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

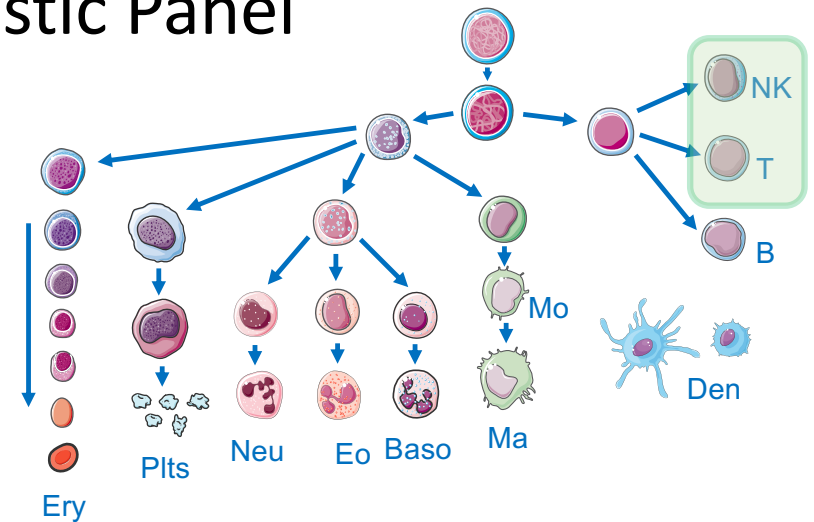


CD8: Cytotoxic T cells

Also expressed on subset of NK cells

St Jude T-ALL Diagnostic Panel

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
2	cyIgG1	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
3	CD7	IgG1	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

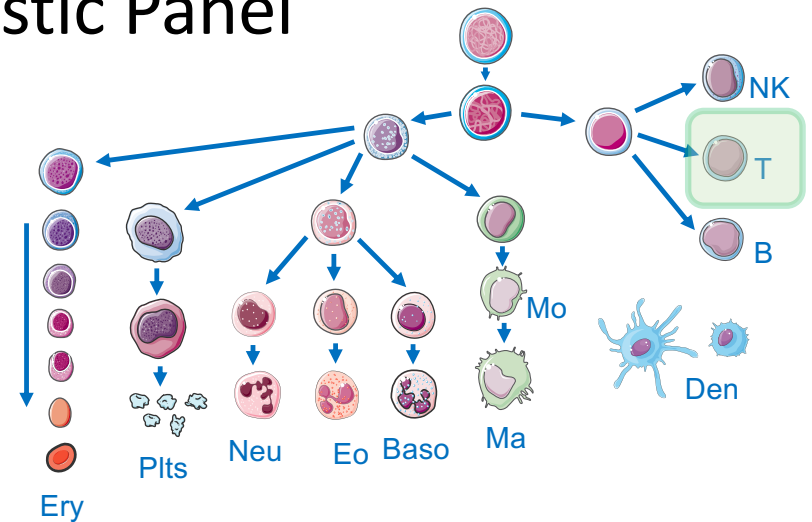


CD2: Pan T cell marker

Also expressed on subset of NK cells

St Jude T-ALL Diagnostic Panel

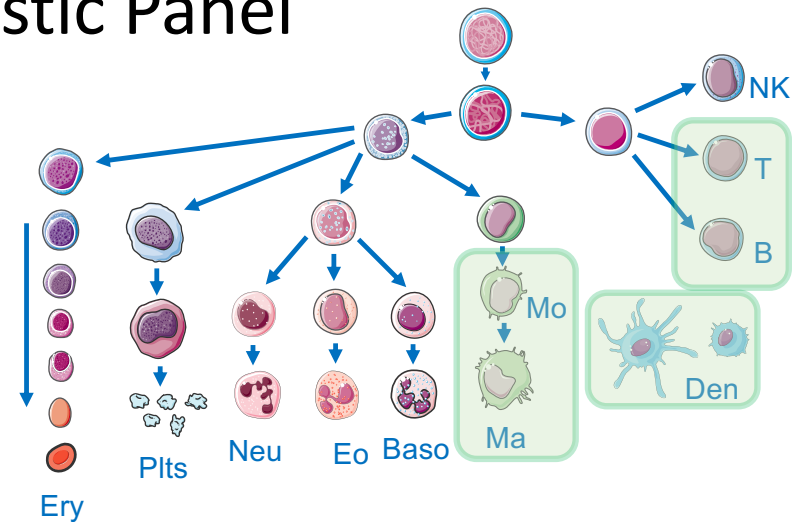
Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
2	cyIgG1	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
3	CD7	IgG1	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



γ/δ : a unique T-cell receptor (TCR) of low abundance T cells, found in the gut mucosa, skin, lungs and uterus.

St Jude T-ALL Diagnostic Panel

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
2	cyIgG1	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
3	CD7	IgG1	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



CD1a: found on professional antigen-presenting cells (APC) such as dendritic cells, on a subset of B cells, and strongly expressed by cortical thymocytes.

St Jude T-ALL MRD Diagnostic Panel

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
2	cyIgG1	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
3	CD7	IgG1	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

Variable markers

Surface marker cocktail

Identify
LAIP

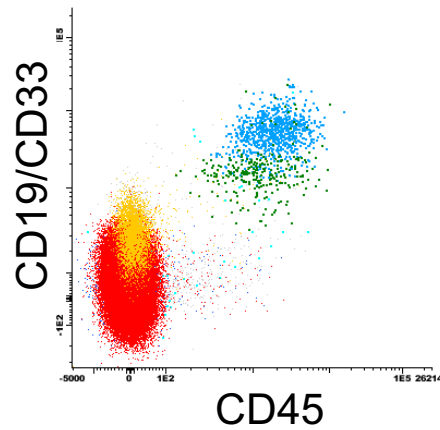
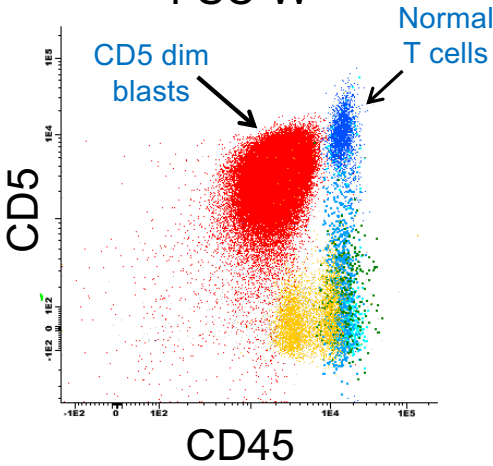
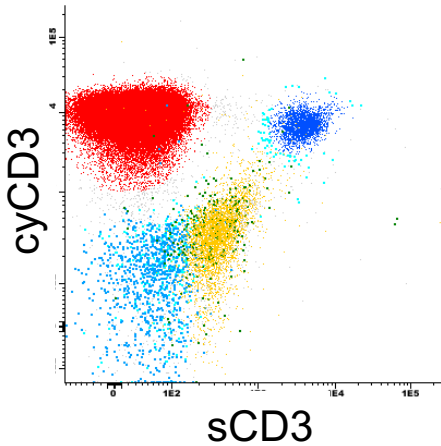
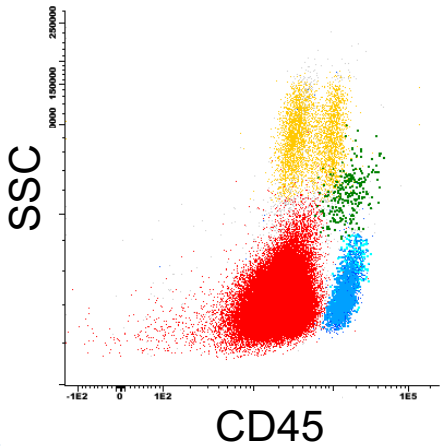
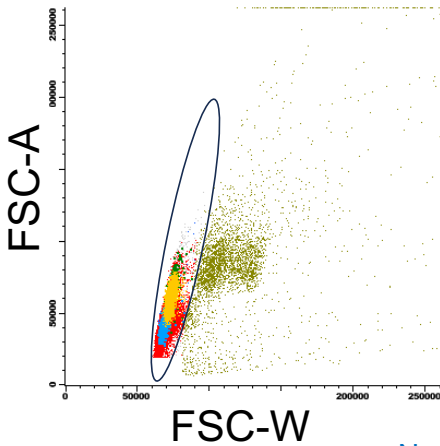
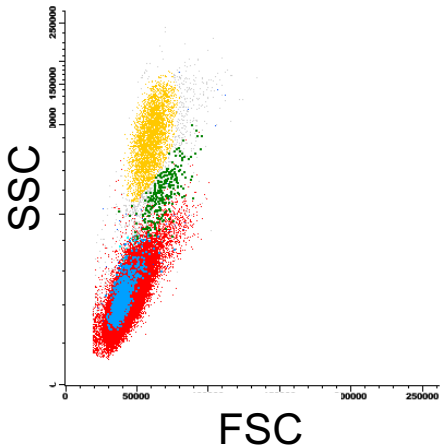
Cytoplasmic and Surface cocktail

Identify blasts

St Jude T-ALL Diagnostic Panel – Backbone Markers

Identify blasts

Patient 1



PerCP	APC	PE-Cy7	APC-H7	V450	V500
cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45
cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

Surface marker cocktail

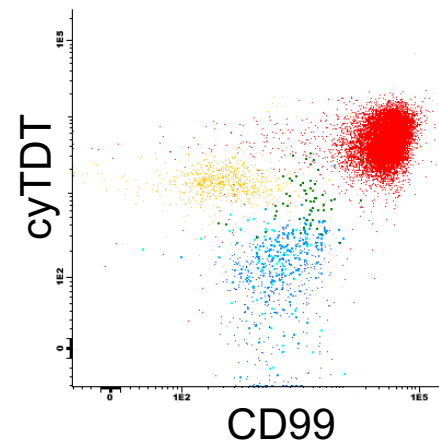
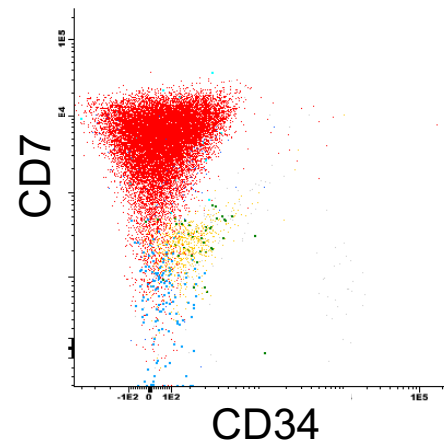
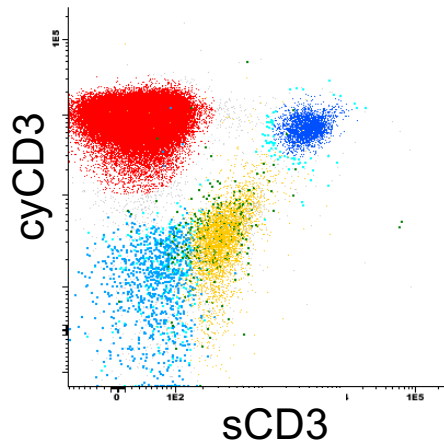
Cytoplasmic and Surface cocktail

Population	Review	Events	Frequency	Total %
EVENTS		120000		
Other EVENTS		1227	NA	1.022
DEBRIS/DOUBLETS		3770	NA	3.142
Scatter		115003	100.000	95.836
Other Scatter		1633	1.420	1.361
Granulocytes		3762	3.271	3.135
Cytoplasmic CD3 positive		108194	94.079	90.162
Other Cytoplasmic CD3 positive		0	0.000	0
Normal T cells		2482	2.158	2.068
Blasts		105712	91.921	88.093
CD5 dim/CD45dim blasts		0	0.000	0
Normal lymphocytes		1156	1.005	0.963
Other Normal lymphocytes		337	0.293	0.281
B cells		819	0.712	0.682
Monocytes		258	0.224	0.215

St Jude T-ALL Diagnostic Panel –Add On Markers

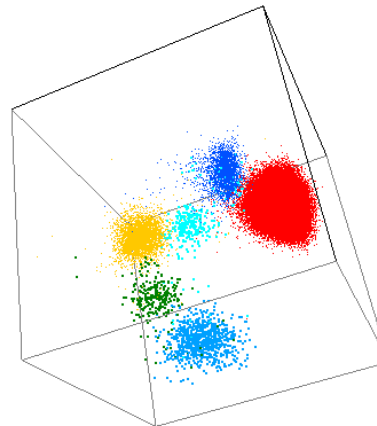
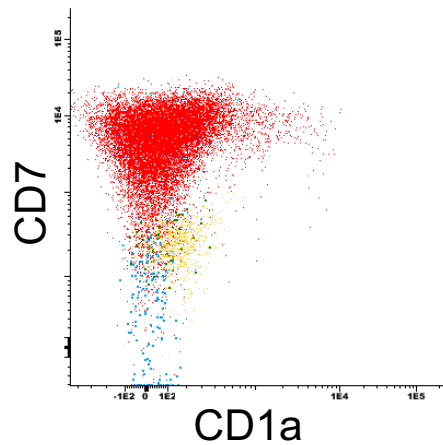
Identify Leukemia Associated Immunophenotype

Patient 1



Tube	FITC	PE
1	cyTdT	CD99
2	cyIgG1	CD99
3	CD7	IgG1
4	CD7	CD34
5	CD4	CD8
6	CD2	γ/δ
7	CD7	CD1a

Variable markers
"Add On"



Population	Events	Frequency	Total %
EVENTS	120000		
Other EVENTS	1227	NA	1.022
DEBRIS/DOUBLETS	3770	NA	3.142
Scatter	115003	100.000	95.836
Other Scatter	1633	1.420	1.361
Granulocytes	3762	3.271	3.135
Cytoplasmic CD3 positive	108194	94.079	90.162
Other Cytoplasmic CD3 positive	0	0.000	0
Normal T cells	2482	2.158	2.068
Blasts	105712	91.921	88.093
CD5 dim/CD45dim blasts	0	0.000	0
Normal lymphocytes	1156	1.005	0.963
Other Normal lymphocytes	337	0.293	0.281
B cells	819	0.712	0.682
Monocytes	258	0.224	0.215

Gating Strategies - Examples

Start with four simple cases that are easy to follow at later treatment stages

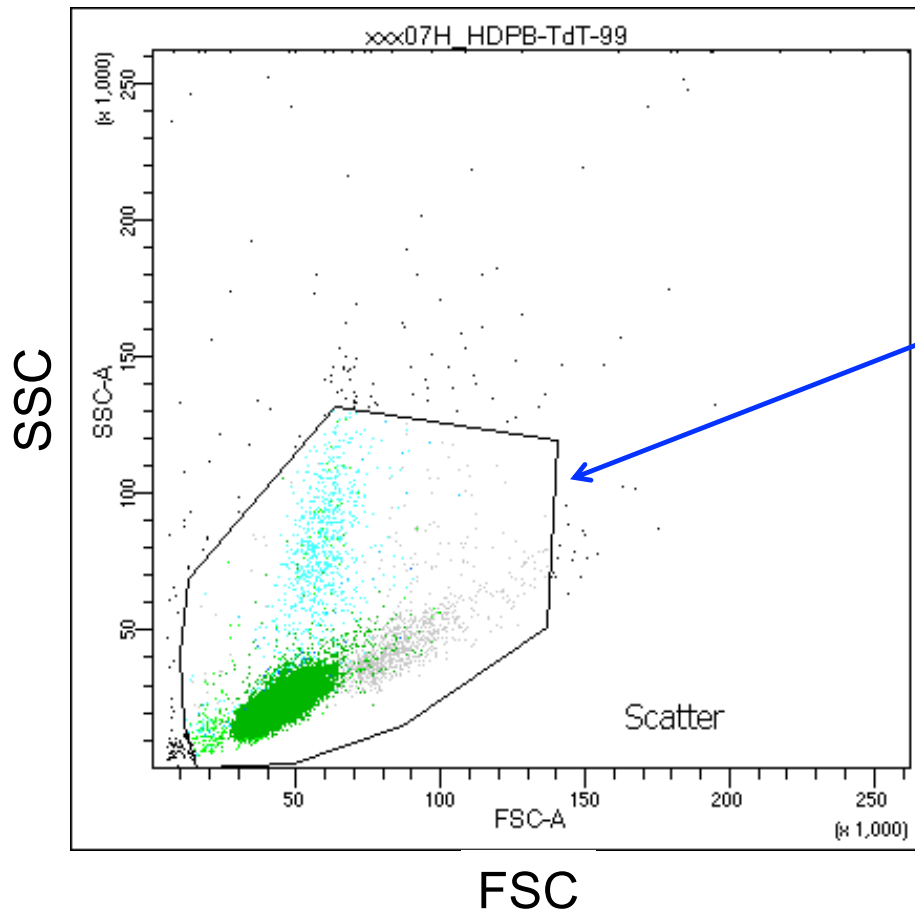
Move on to progressively more difficult cases

- loss of aberrant marker expression with treatment
- aberrantly low expression becomes more like normal with treatment

Patient 1 TdT+/CD99+

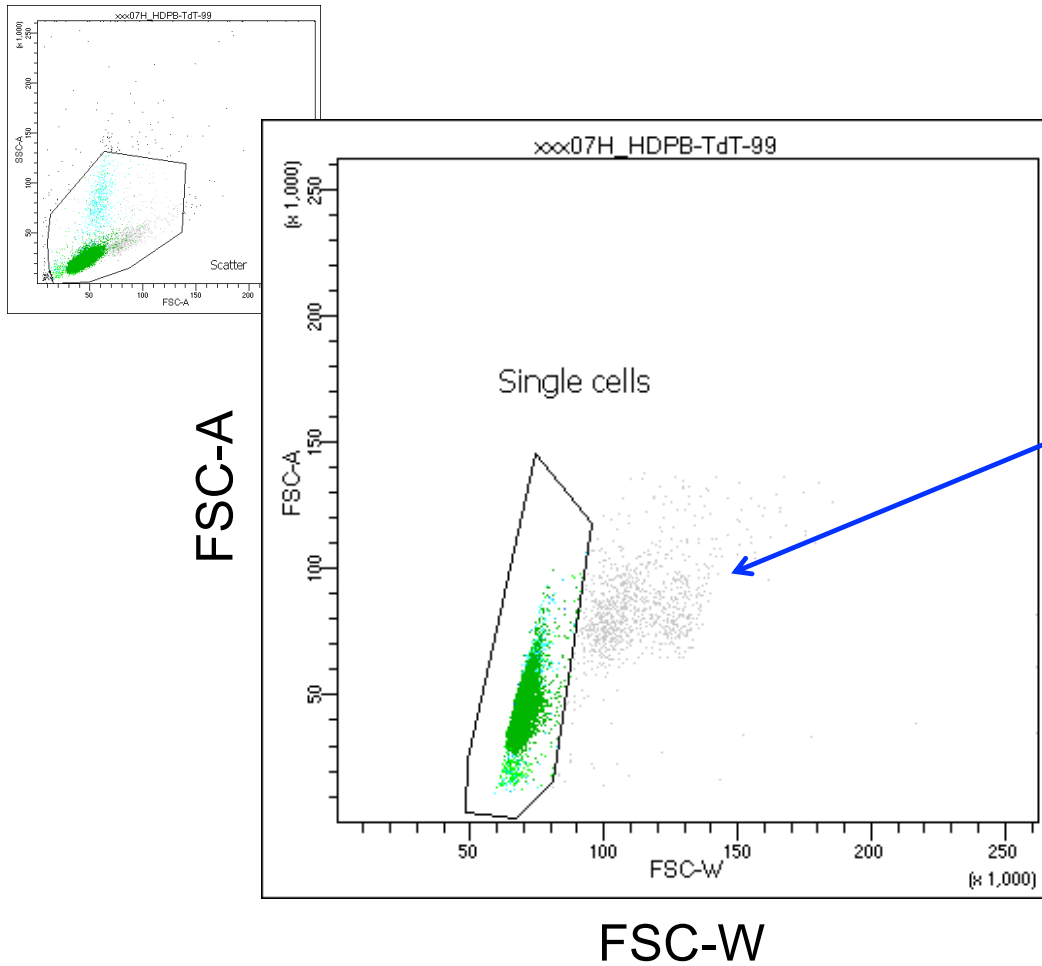
Gating Strategy (Diagnostic sample)

Patient 1



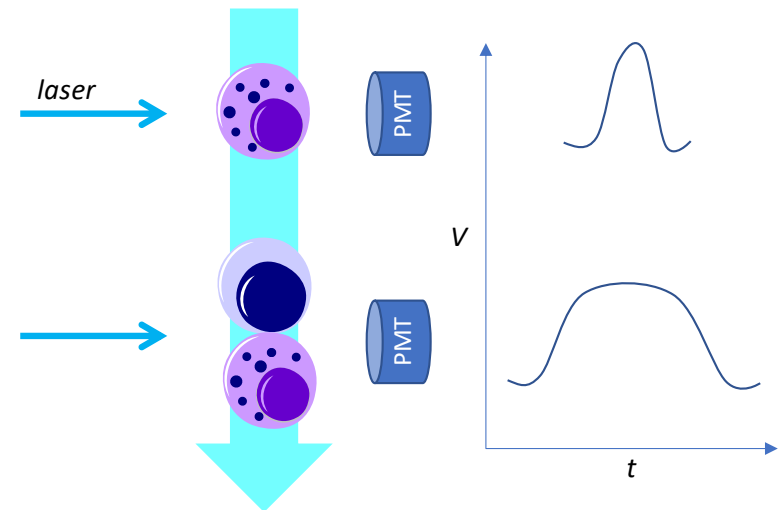
Start with a Scatter Gate SSC vs FSC

Eliminate debris from the gate, but use care not to exclude small blasts

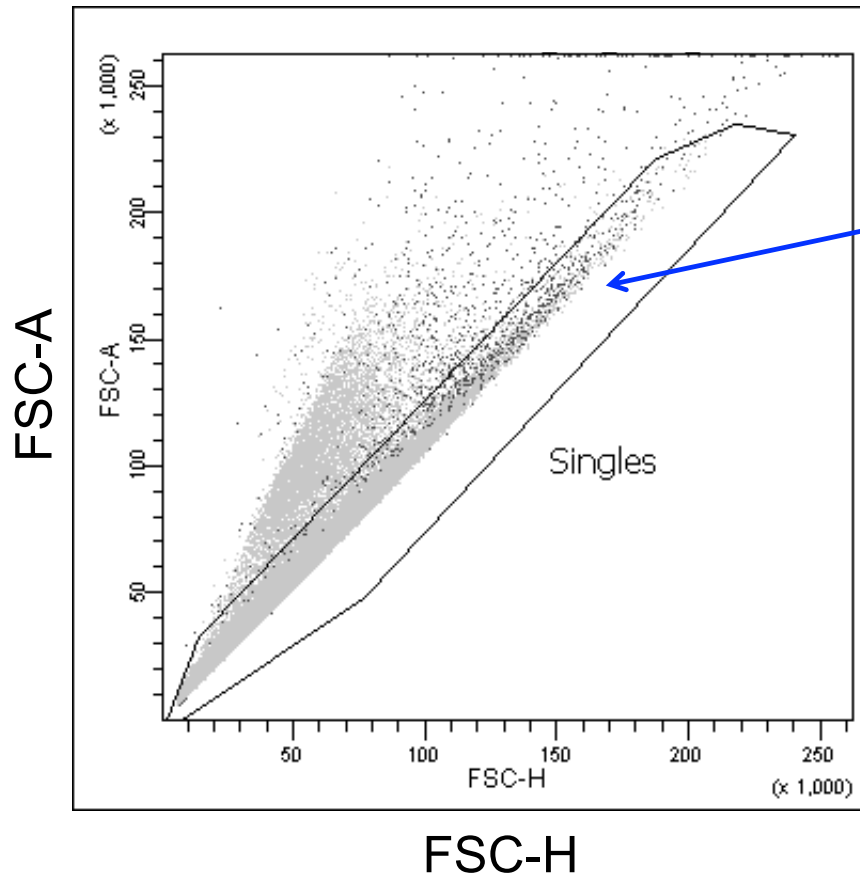


Diagnostic Sample (PB)

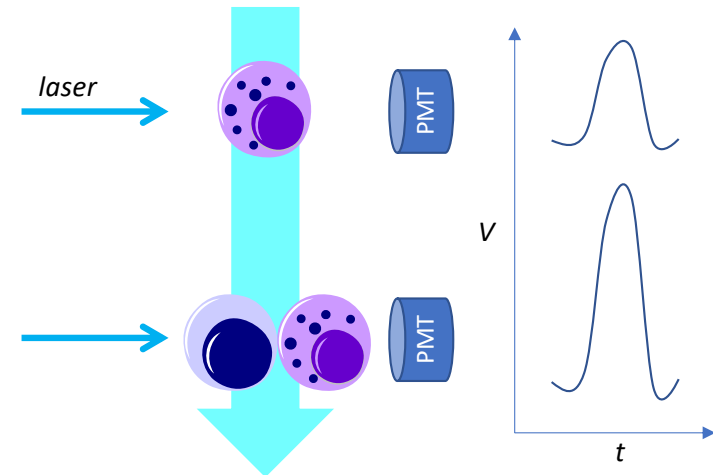
Exclude doublets using FSC-A vs FSC-W gate



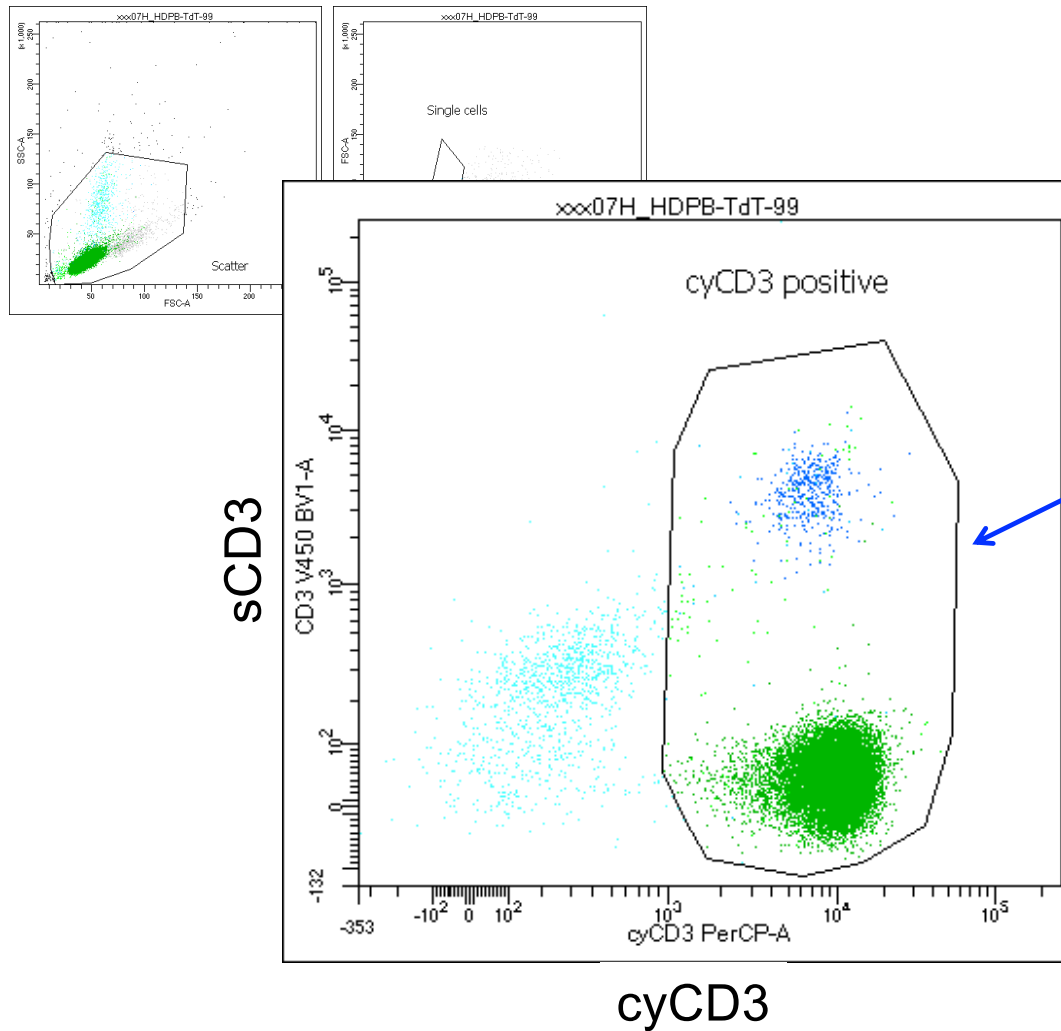
Not shown in this example
but recommend using both H and W doublet discrimination



Eliminate doublets using
FSC-A vs FSC-H gate

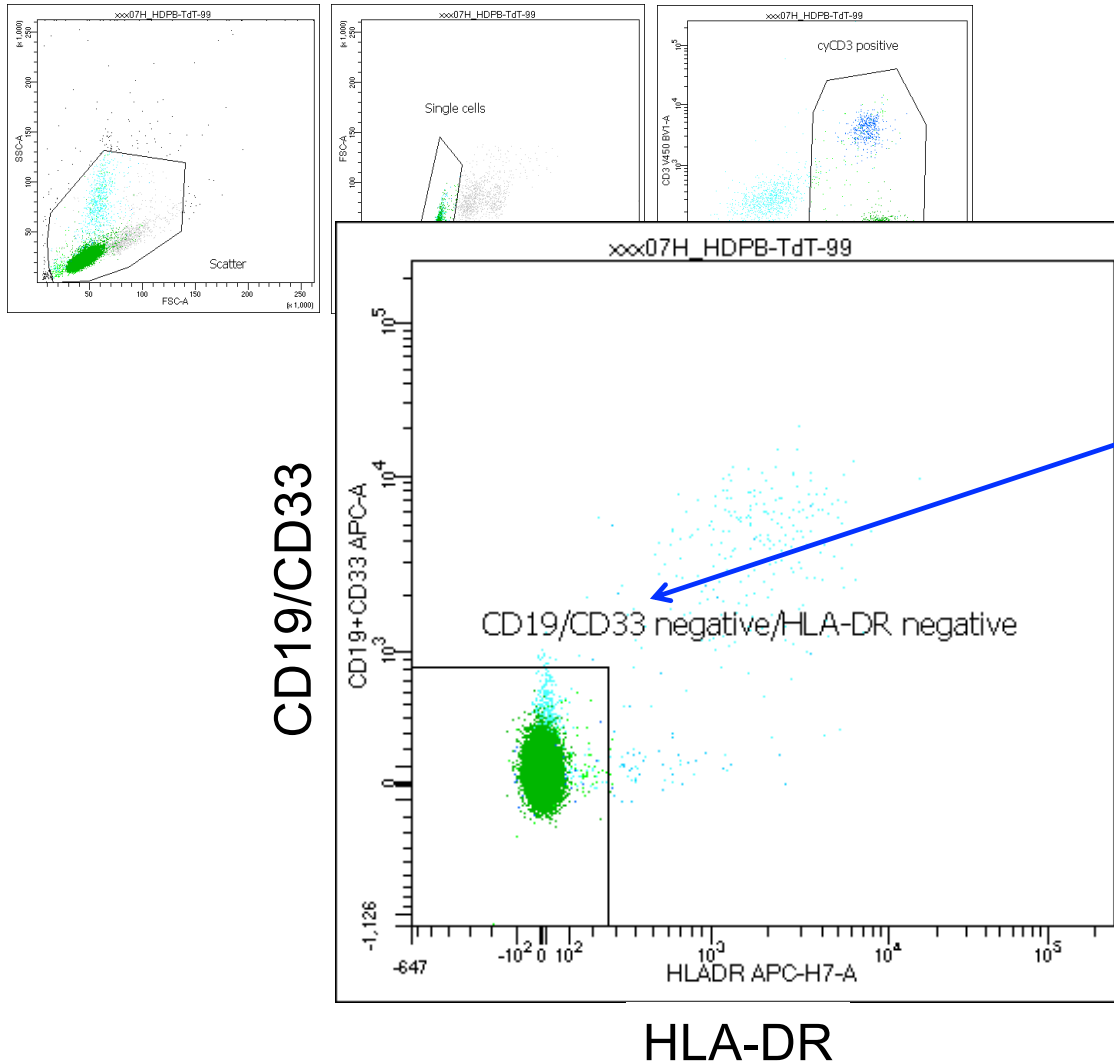


Diagnostic Sample (PB)



Gate on cyCD3 positive cells
to identify both normal and
blast cells

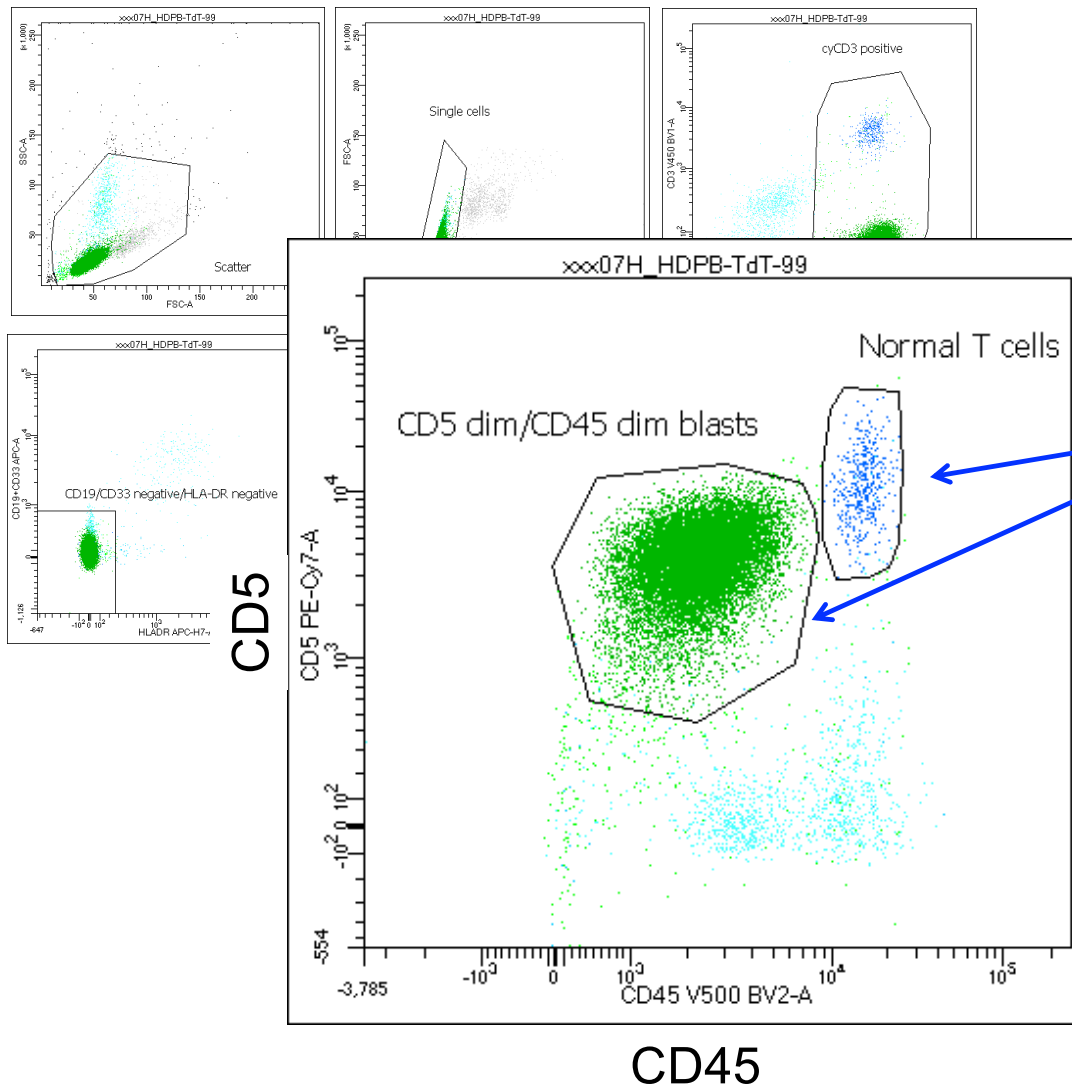
Diagnostic Sample (PB)



Use CD19/CD33 vs HLA-DR plot to gate on triple-negative population to exclude myeloid and B-cells

T cells are typically negative for HLA-DR, but check back gates to ensure that you are not excluding blasts

Diagnostic Sample (PB)

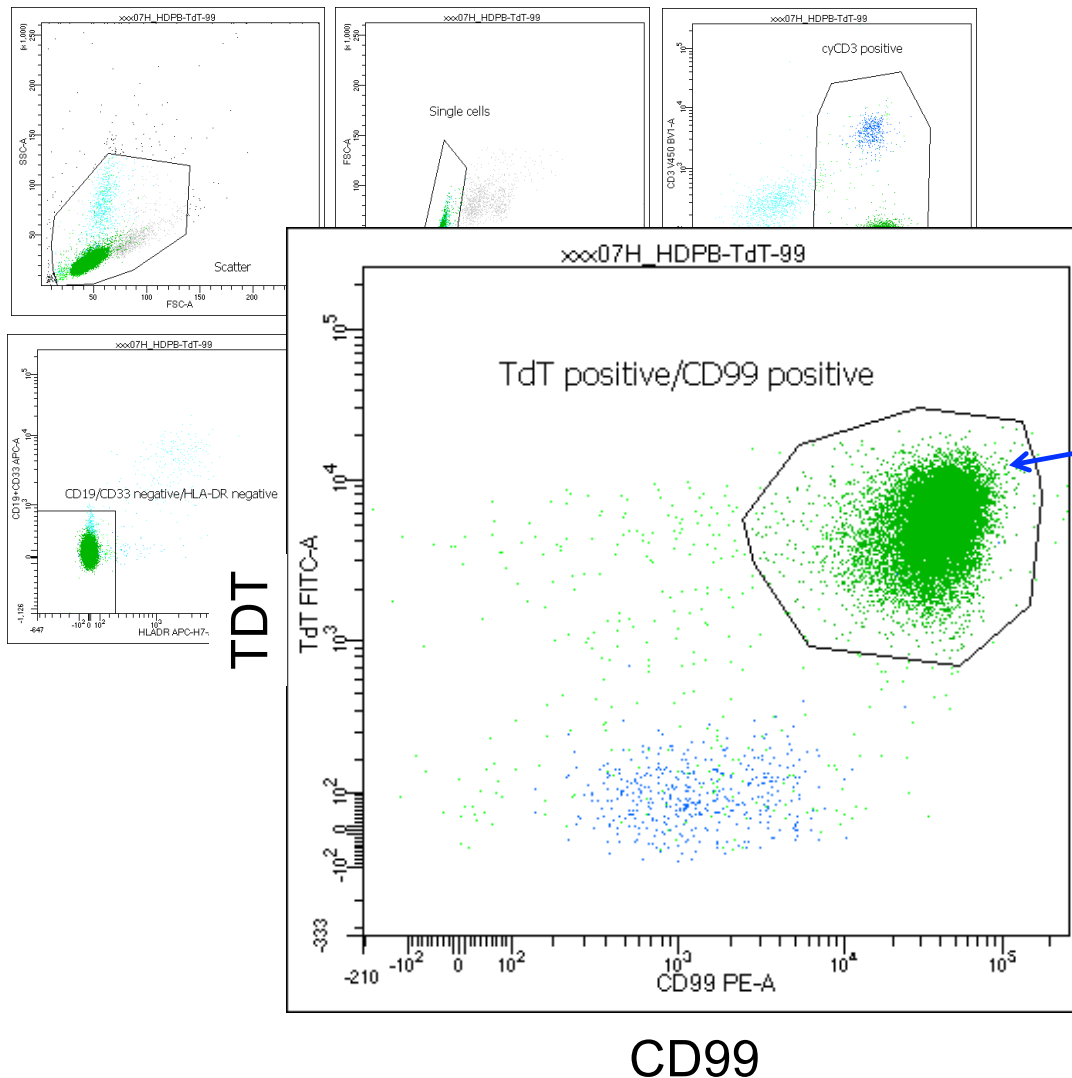


Can use CD5 vs CD45 gate to identify blasts and normal T cells

Used a PB sample here to show presence of normal T cells

Blasts are frequently dim for both CD45 and CD5 compared to normal T cells

Diagnostic Sample (PB)

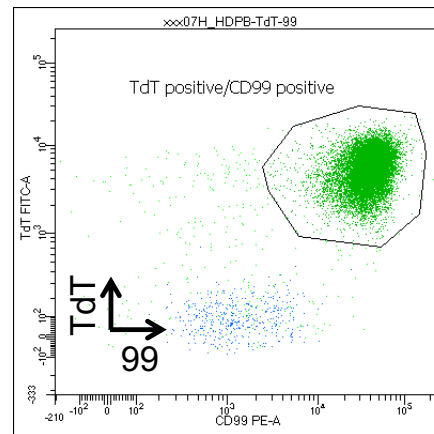
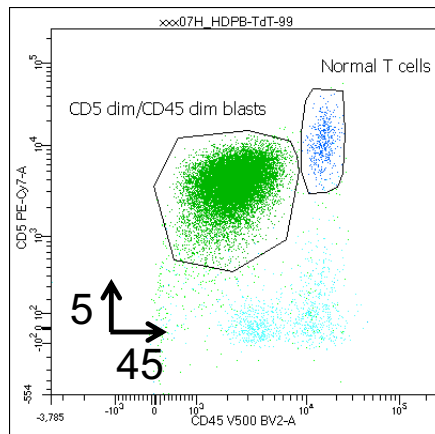
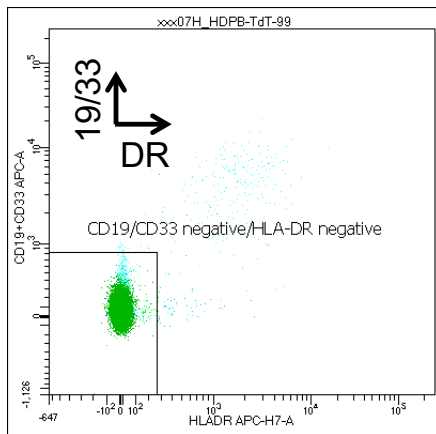
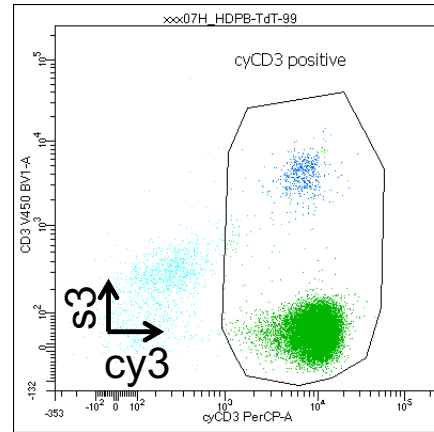
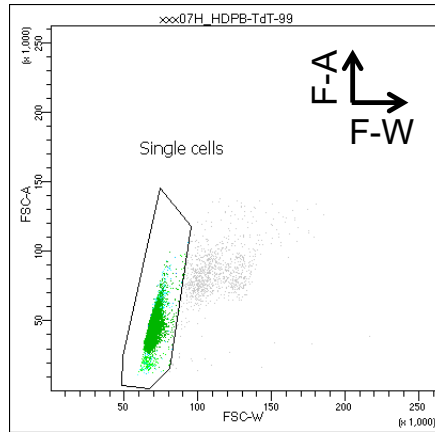
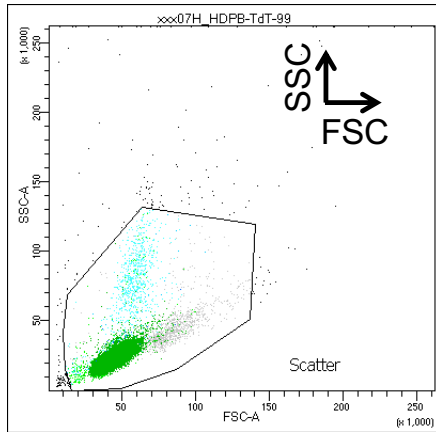


Use 'best' combination of markers to identify blasts

In this case, blasts are positive for both TdT and CD99

This leukemia should be simple to follow

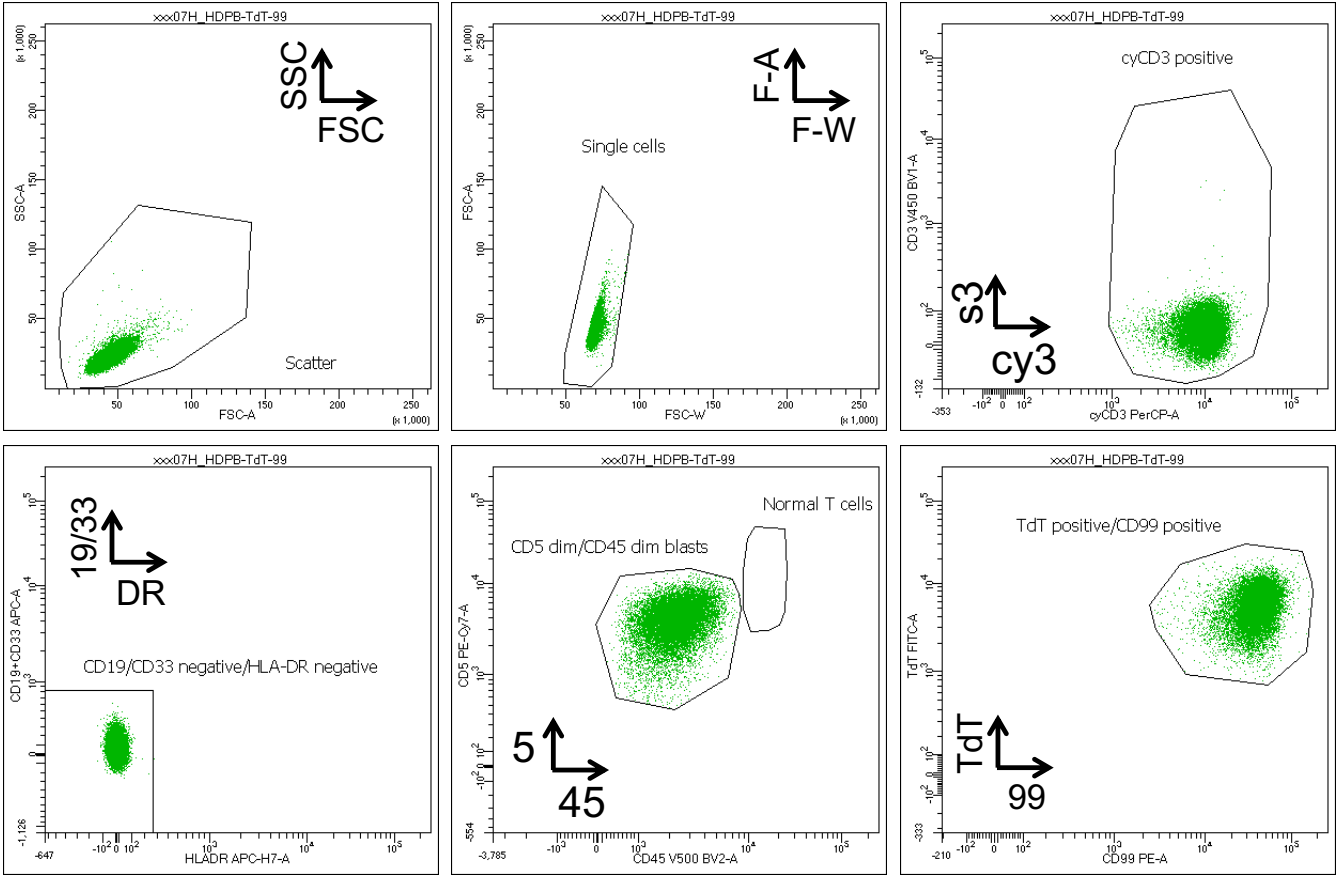
Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



Diagnostic Sample (PB)

Tube: TdT-99			
Population	#Events	%Parent	%Total
All Events	20,000	###	100.0
Scatter	19,779	98.9	98.9
Single cells	18,795	95.0	94.0
cyCD3 positive	17,702	94.2	88.5
CD19/CD33 negative/HLA-DR negative	17,660	99.8	88.3
Normal T cells	420	2.4	2.1
CD5 dim/CD45 dim blasts	16,951	96.0	84.8
TdT positive/CD99 positive	16,810	99.2	84.0

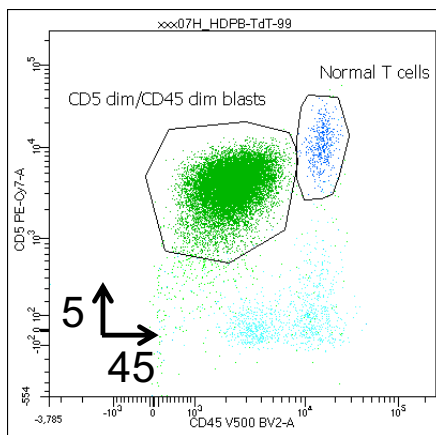
Back gate to ensure blast population is not cut off



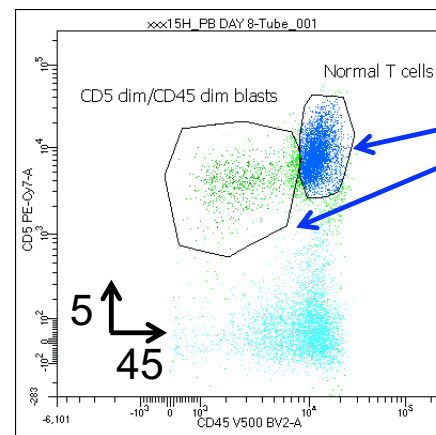
Diagnostic Sample (PB)

Tube: TdT-99			
Population	#Events	%Parent	%Total
■ All Events	20,000	###	100.0
■ Scatter	19,779	98.9	98.9
■ Single cells	18,795	95.0	94.0
■ cyCD3 positive	17,702	94.2	88.5
■ CD19/CD33 negative/HLA-DR negative	17,660	99.8	88.3
■ Normal T cells	420	2.4	2.1
■ CD5 dim/CD45 dim blasts	16,951	96.0	84.8
■ TdT positive/CD99 positive	16,810	99.2	84.0

Follow up samples



Diagnostic PB



Day 8 PB

Gates will need adjusting

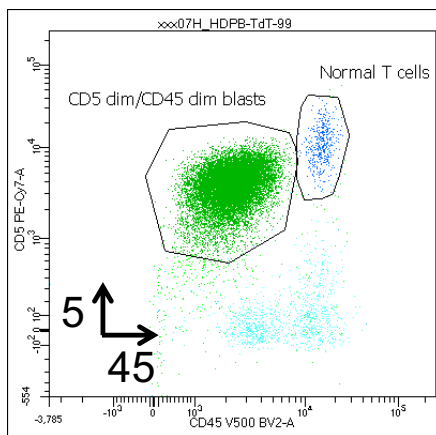
Probably more so than you are used to with B-ALL MRD analysis

Due to the variability in the intracellular staining protocol

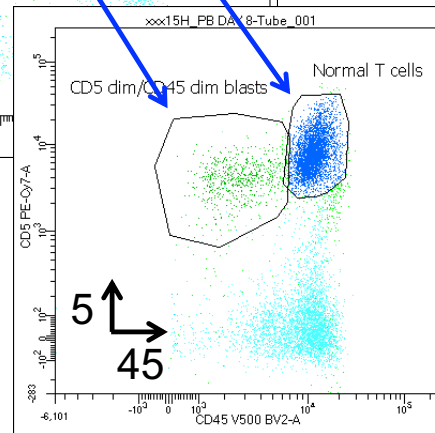
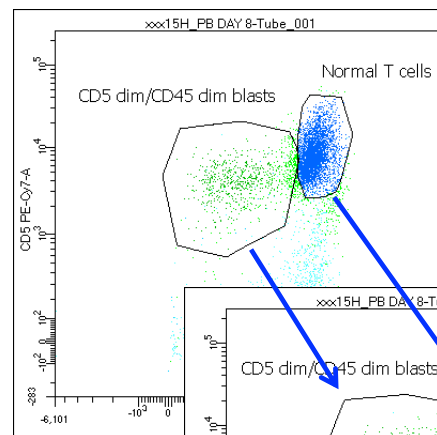
We can not draw 'normal gates' to help identify difference from normal

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

Follow up samples



Diagnostic PB



Day 8 PB

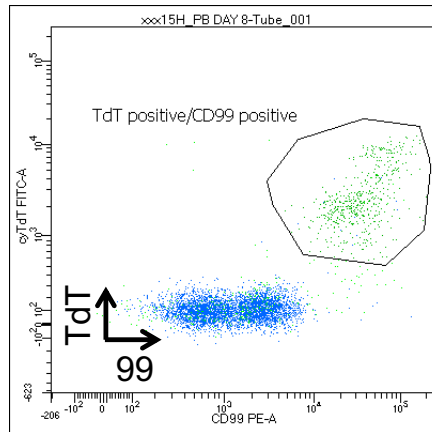
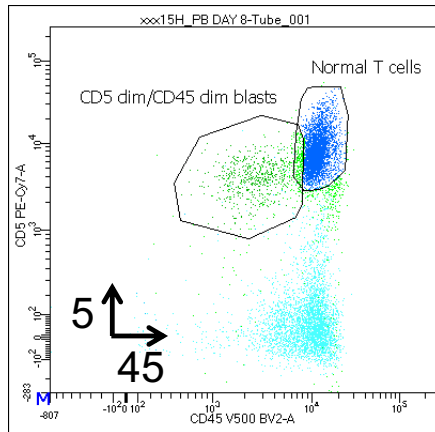
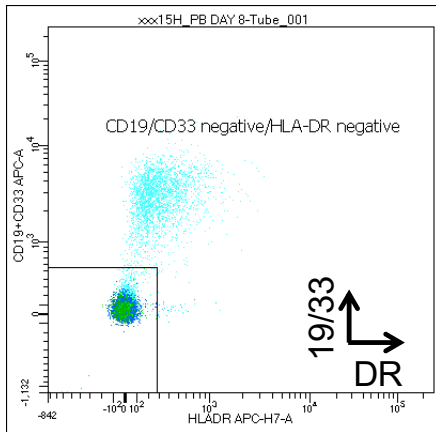
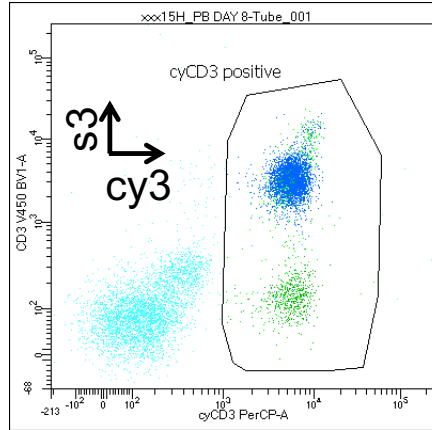
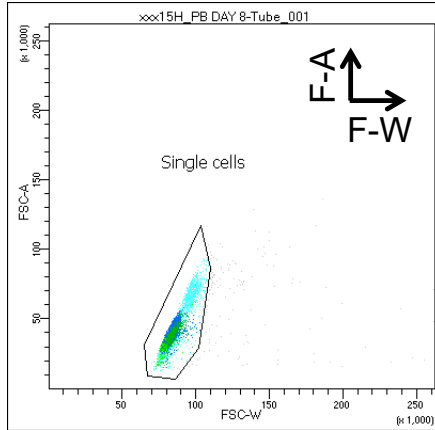
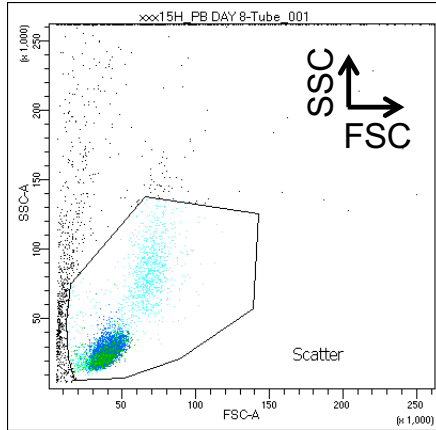
Gates will need adjusting

Probably more so that you are used to with B-ALL MRD analysis

Due to the variability in the intracellular staining protocol

We can not draw 'normal gates' to help identify difference from normal

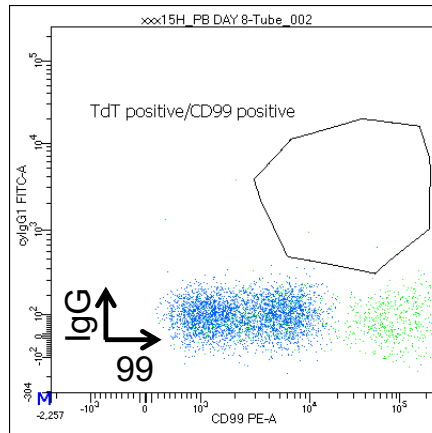
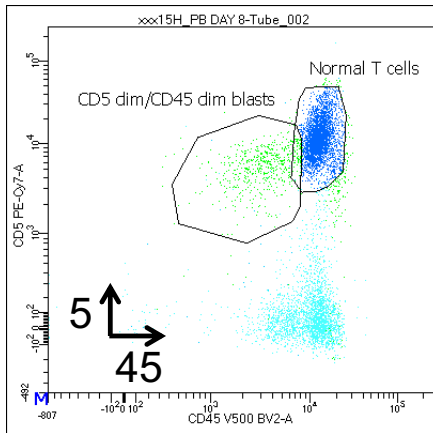
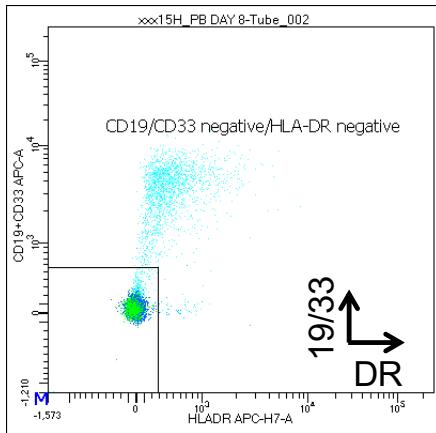
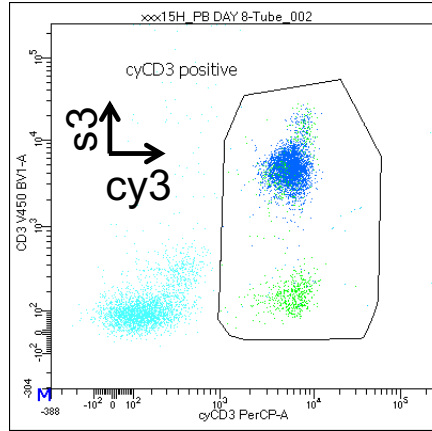
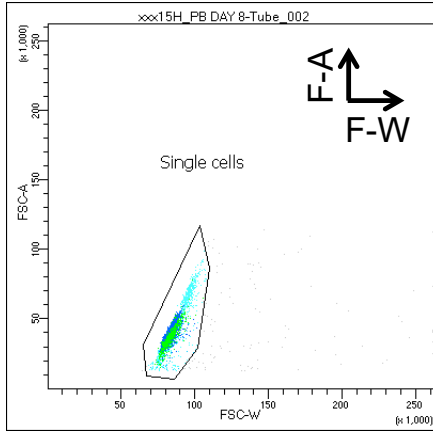
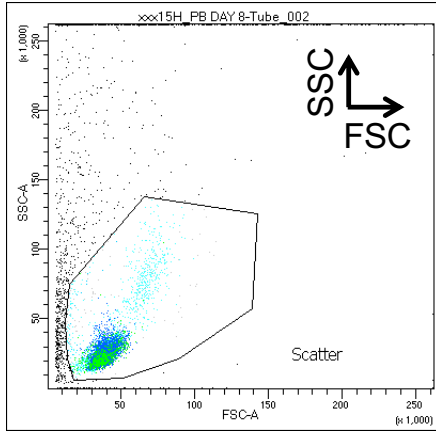
Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



Day 8 (PB)

Tube: Tube_001			
Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
□ Scatter	8,803	88.0	88.0
■ Single cells	8,671	86.7	86.7
■ cyCD3 positive	4,804	55.4	48.0
■ CD19/CD33 negative/HLA-DR negative	4,785	99.6	47.8
■ Normal T cells	3,903	81.6	39.0
■ CD5 dim/CD45 dim blasts	658	13.8	6.6
■ TdT positive/CD99 positive	513	78.0	5.1

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
2	cyIgG1	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



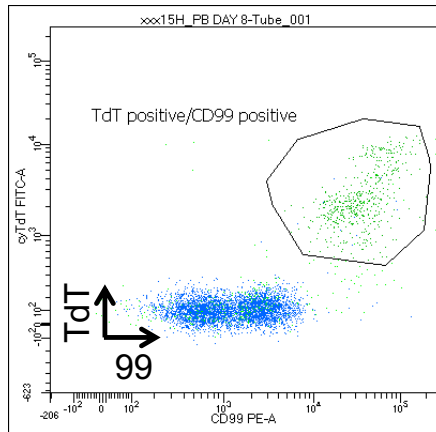
Day 8 (PB)

IgG1 Control Tube

Tube: Tube_002			
Population	#Events	%Parent	%Total
■ All Events	8,606	###	100.0
■ Scatter	7,060	82.0	82.0
■ Single cells	6,957	98.5	80.8
■ cyCD3 positive	4,195	60.3	48.7
■ CD19/CD33 negative/HLA-DR negative	4,154	99.0	48.3
■ Normal T cells	3,475	83.7	40.4
■ CD5 dim/CD45 dim blasts	574	13.8	6.7
■ TdT positive/CD99 positive	0	0.0	0.0

MRD calculation

Day 8 (PB)



Tube: Tube_001			
Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ Scatter	8,803	88.0	88.0
■ Single cells	8,671	98.5	86.7
■ cyCD3 positive	4,804	55.4	48.0
■ CD19/CD33 negative/HLA-DR negative	4,785	99.6	47.8
■ Normal T cells	3,903	81.6	39.0
■ CD5 dim/CD45 dim blasts	658	13.8	6.6
■ TdT positive/CD99 positive	513	78.0	5.1

$$\text{MRD} = \frac{\text{TdT+CD99+ blast count}}{\text{Total events recorded}} \times \frac{100}{1}$$

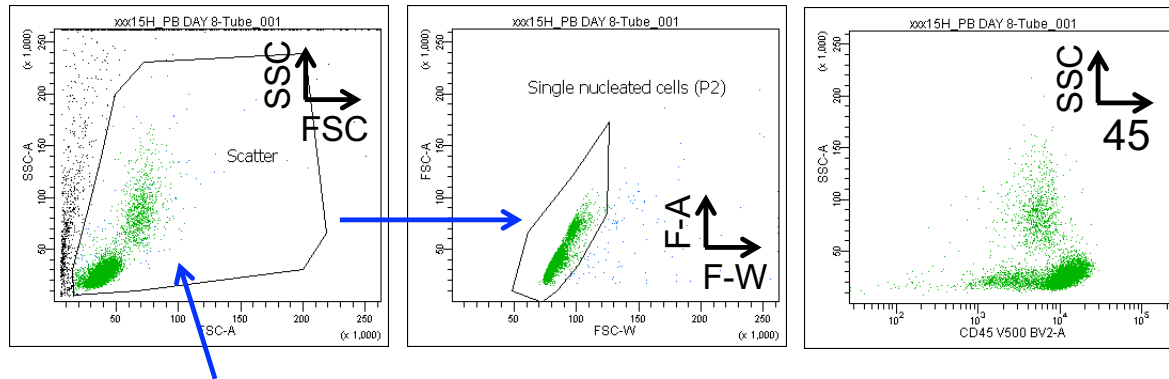
$$= \frac{513}{10,000} \times \frac{100}{1}$$

$$= 0.0513 \times 100$$

$$= 5.13 \% \text{ of ALL events}$$

The denominator is the **Total (ALL) Events** acquired by the cytometer, and includes debris, RBCs, dead cells, and doublets etc. We need the MRD to represent the fraction of leukemic blasts per nucleated cells, and thus we need to apply a correction factor to determine the nucleated cell count from the total event count for each sample.

Correction Factor calculation



Day 8 (PB)
Run 10,000 ungated events

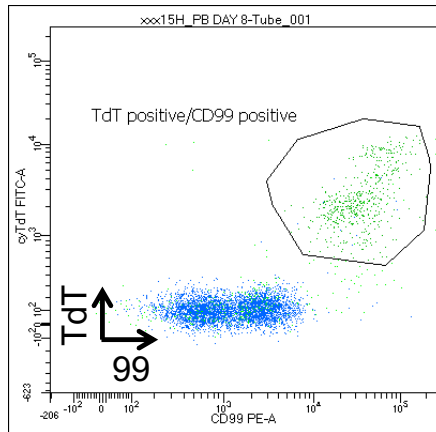
Tube: Tube_001			
Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ Scatter	8,843	88.4	88.4
■ Single nucleated cells (P2)	8,737	98.8	87.4

Draw scatter gate to eliminate dead cells and debris.
Use FSC-A vs FSC-W plot to eliminate doublets = approximates single nucleated cells.

$$\begin{aligned}
 \text{Correction Factor (CF)} &= 10,000 / \text{Single nucleated cells (P2)} \\
 &= 10,000 / 8,737 \\
 &= 1.145
 \end{aligned}$$

MRD calculation

Day 8 (PB)



Tube: Tube_001

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ Scatter	8,803	88.0	88.0
■ Single cells	8,671	98.5	86.7
■ cyCD3 positive	4,804	55.4	48.0
■ CD19/CD33 negative/HLA-DR negative	4,785	99.6	47.8
■ Normal T cells	3,903	81.6	39.0
■ CD5 dim/CD45 dim blasts	658	13.8	6.6
■ TdT positive/CD99 positive	513	78.0	5.1

$$\text{MRD} = \frac{\text{TdT+CD99+ blast count}}{\text{Total events recorded}} \times \frac{100}{1}$$

$$= \frac{513}{10,000} \times \frac{100}{1}$$

$$= 0.0513 \times 100$$

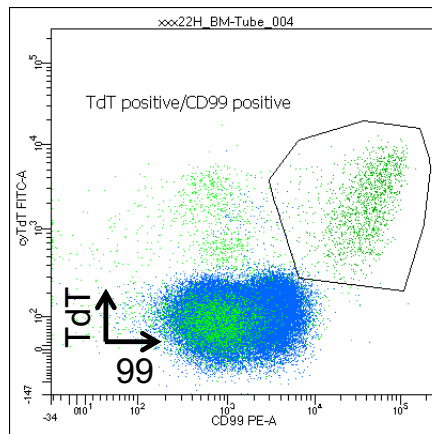
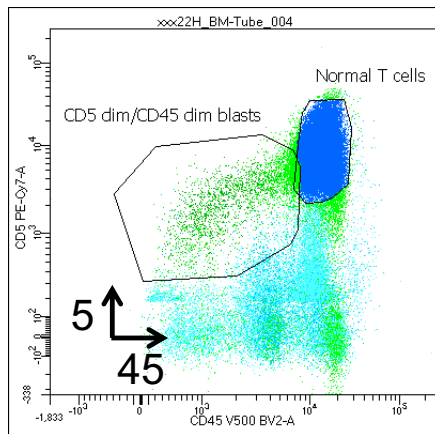
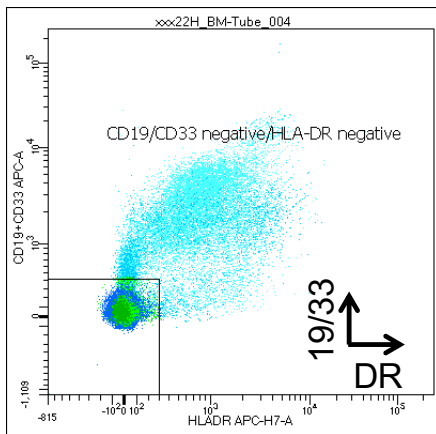
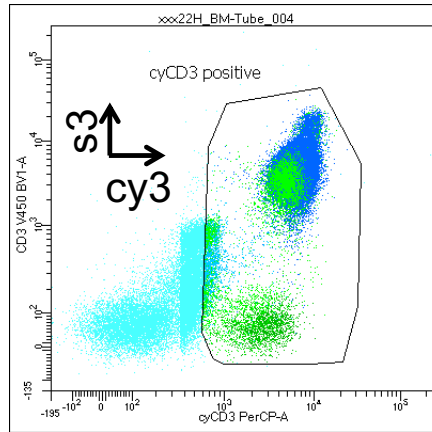
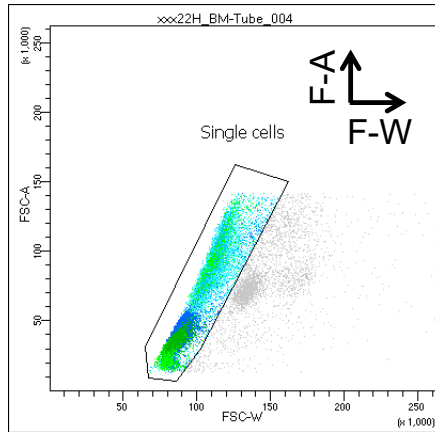
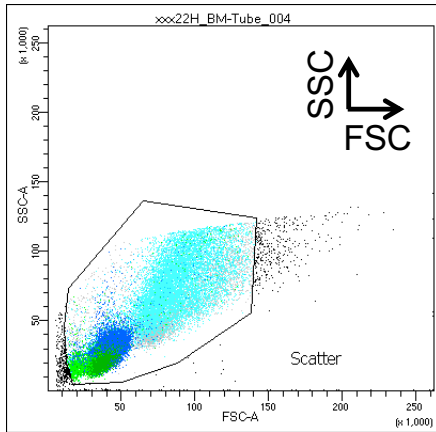
$$= 5.13 \% \text{ of ALL events} \times \text{Correction Factor}$$

$$= 5.13 \times 1.145$$

$$= \mathbf{5.87\% \text{ MRD Positive}}$$

For T-ALL MRD, the threshold for positivity is 0.01%

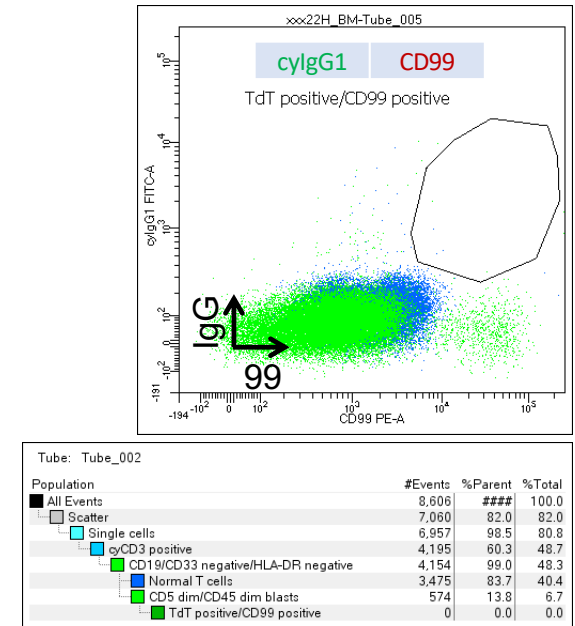
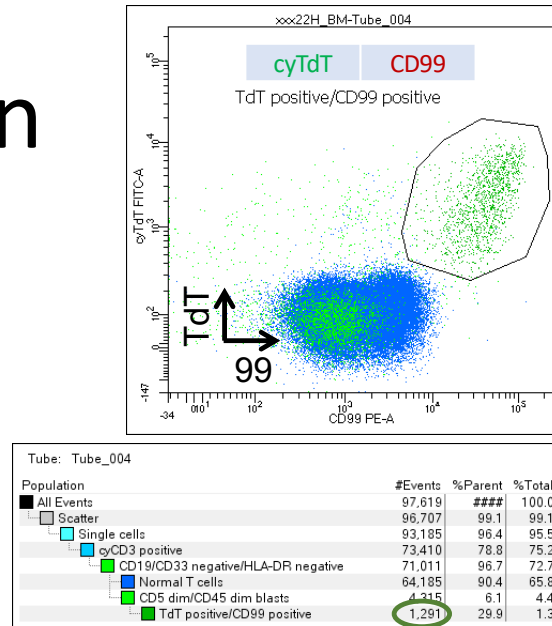
Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



Day 15 (BM)

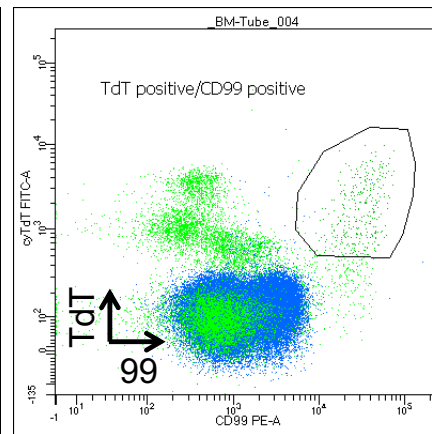
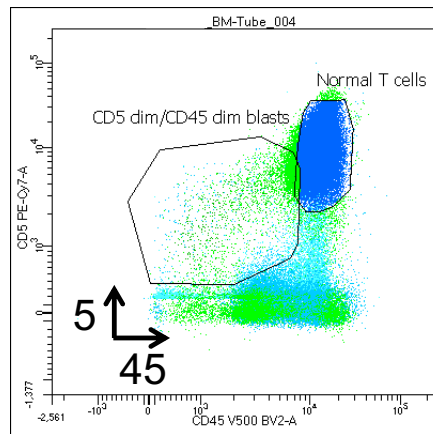
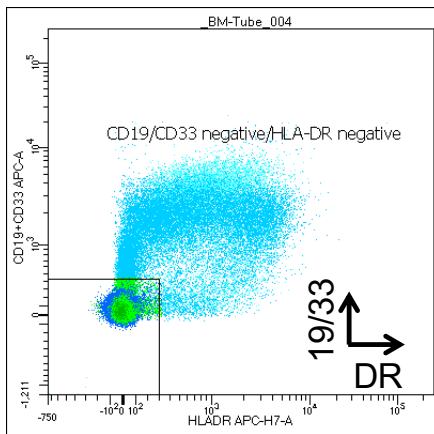
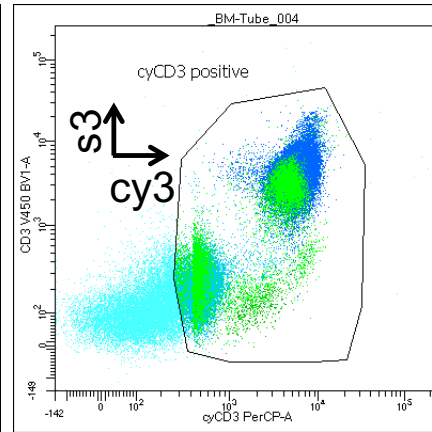
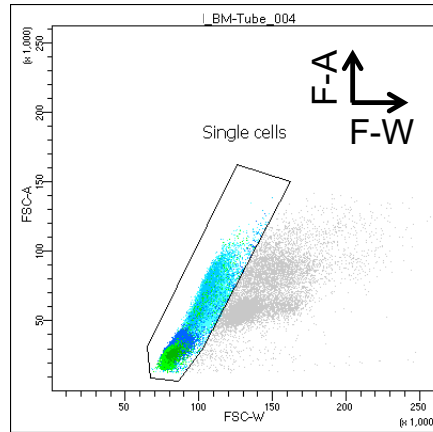
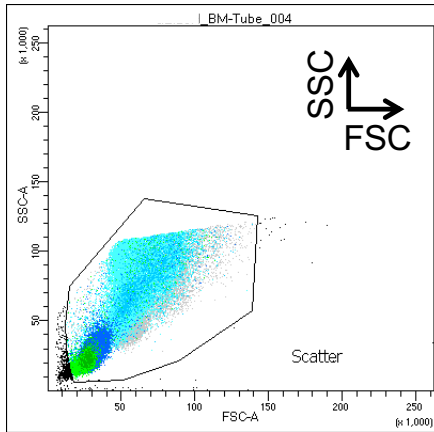
Tube: Tube_004			
Population	#Events	%Parent	%Total
All Events	97,619	####	100.0
Scatter	96,707	99.1	99.1
Single cells	93,185	96.4	95.5
cyCD3 positive	73,410	78.8	75.2
CD19/CD33 negative/HLA-DR negative	71,011	96.7	72.7
Normal T cells	64,185	90.4	65.8
CD5 dim/CD45 dim blasts	4,315	6.1	4.4
TdT positive/CD99 positive	1,291	29.9	1.3

MRD calculation



Minimal Residual Disease = Corrected number of Blasts/Total number of Events
 = (1,291/180,492) x CF X 100/1
 = 0.007153 x 1.073 x 100
 = 0.767% **MRD positive**

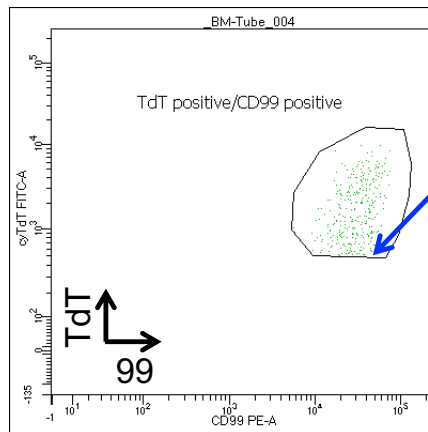
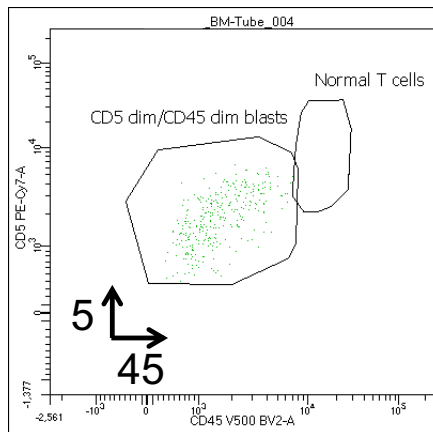
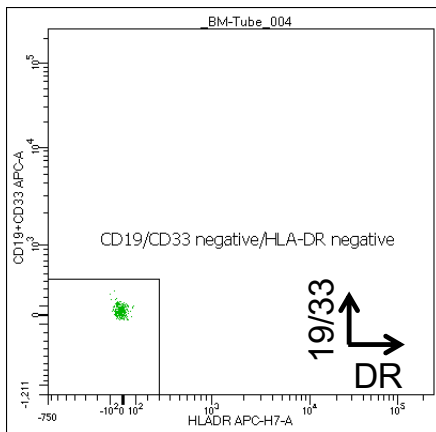
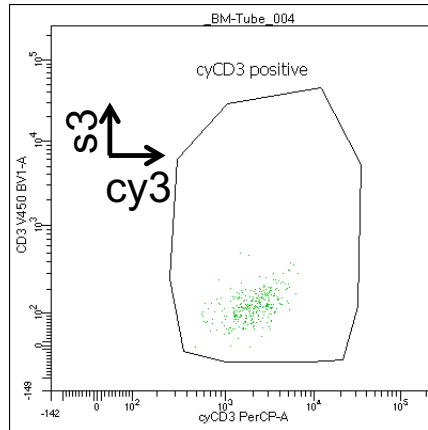
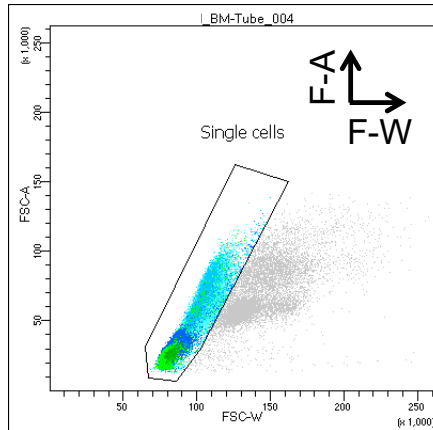
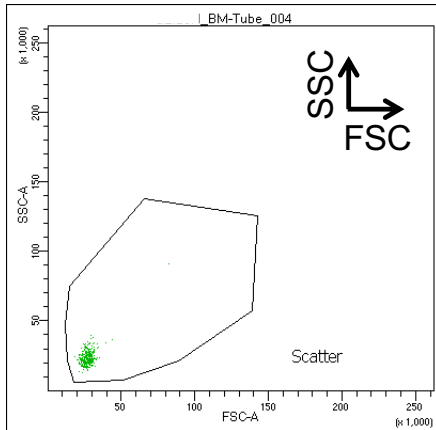
Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



Day 22 (BM)

Tube: Tube_004			
Population	#Events	%Parent	%Total
All Events	123,684	###	100.0
Scatter	122,918	99.4	99.4
Single cells	112,675	91.7	91.1
cyCD3 positive	97,959	86.9	79.2
CD19/CD33 negative/HLA-DR negative	78,772	80.4	63.7
Normal T cells	68,567	87.0	55.4
CD5 dim/CD45 dim blasts	5,297	6.7	4.3
TdT positive/CD99 positive	317	6.0	0.3

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



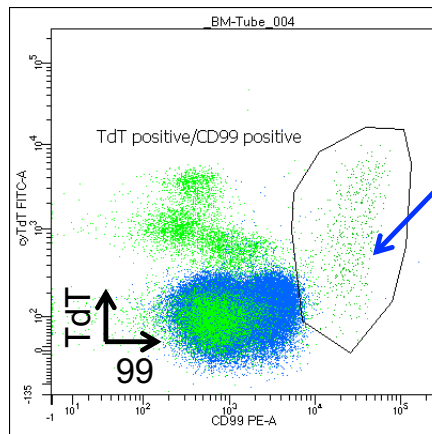
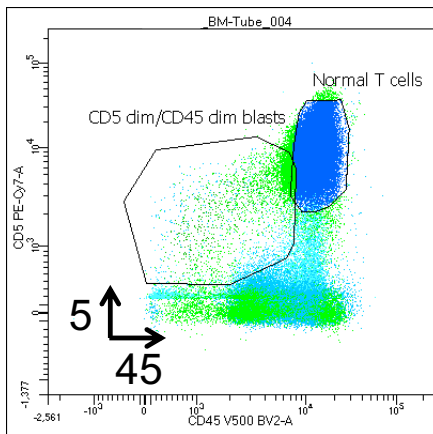
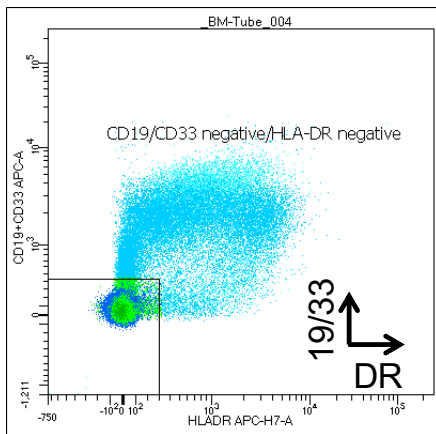
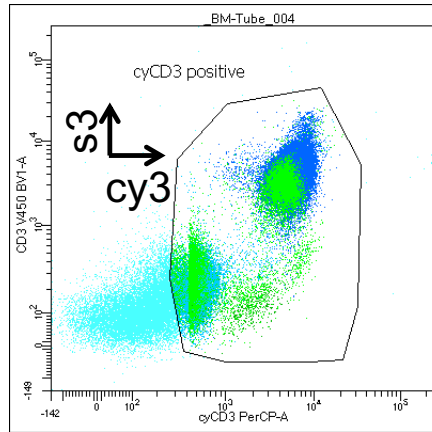
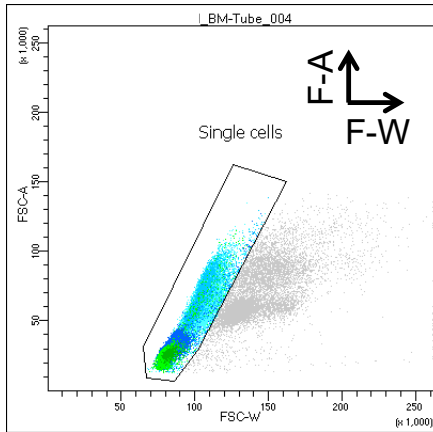
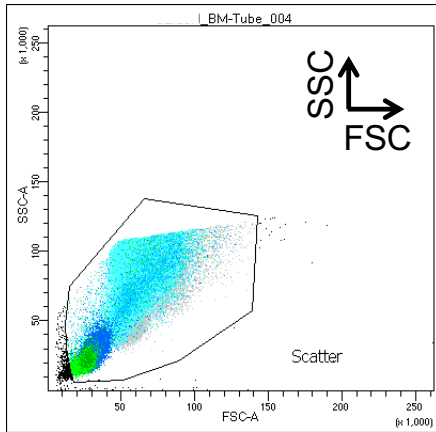
Day 22 (BM) Back gating

Tube: Tube_004			
Population	#Events	%Parent	%Total
■ All Events	123,684	###	100.0
■ Scatter	122,918	99.4	99.4
■ Single cells	112,675	91.7	91.1
■ cyCD3 positive	97,959	86.9	79.2
■ CD19/CD33 negative/HLA-DR negative	78,772	80.4	63.7
■ Normal T cells	68,567	87.0	55.4
■ CD5 dim/CD45 dim blasts	5,297	6.7	4.3
■ TdT positive/CD99 positive	317	6.0	0.3

Bottom part of population is missing

In this patient, cyTdT staining has decreased progressively since diagnosis. CD99 still high.

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

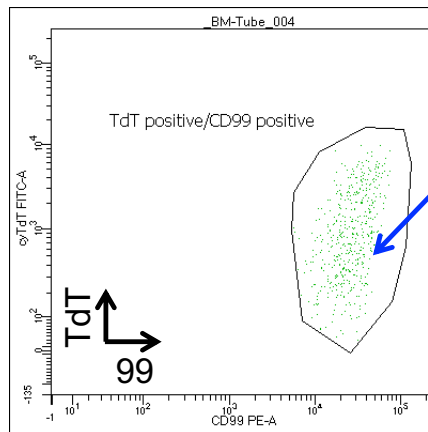
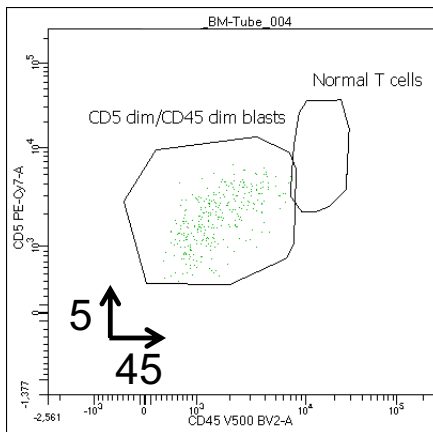
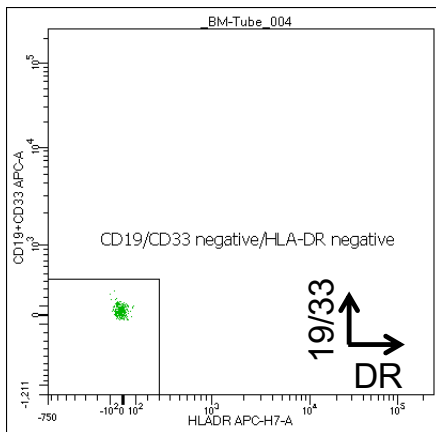
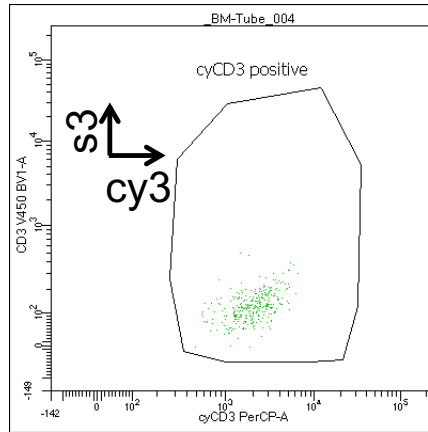
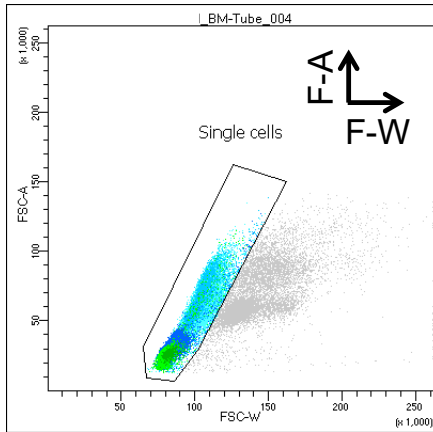
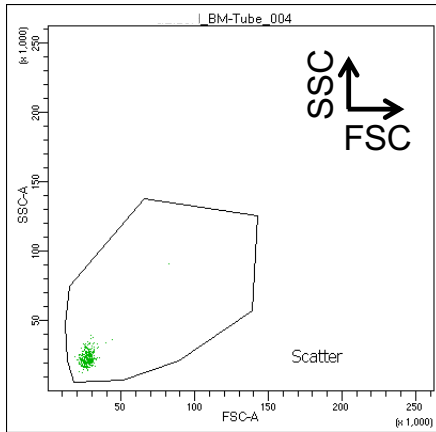


Day 22 (BM)

Tube: Tube_004			
Population	#Events	%Parent	%Total
■ All Events	123,684	###	100.0
■ Scatter	122,918	99.4	99.4
■ Single cells	112,675	91.7	91.1
■ cyCD3 positive	97,959	86.9	79.2
■ CD19/CD33 negative/HLA-DR negative	78,772	80.4	63.7
■ Normal T cells	68,567	87.0	55.4
■ CD5 dim/CD45 dim blasts	5,297	6.7	4.3
■ TdT positive/CD99 positive	483	9.1	0.4

Extend gate to include cyTdT negative blasts

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



Day 22 (BM)

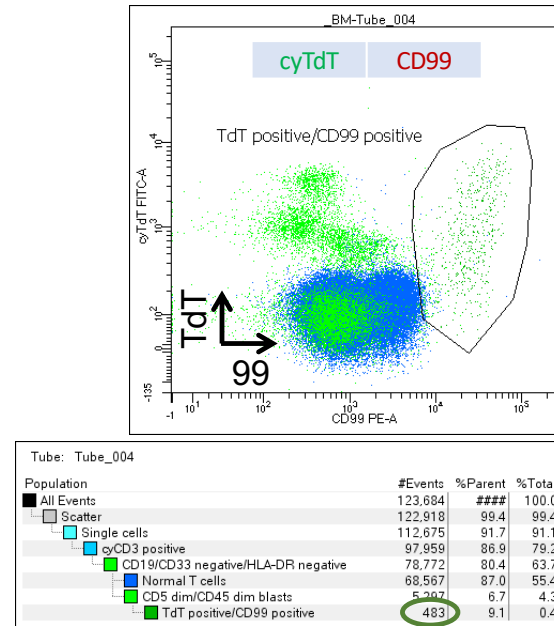
Back gating

Tube: Tube_004			
Population	#Events	%Parent	%Total
■ All Events	123,684	###	100.0
■ Scatter	122,918	99.4	99.4
■ Single cells	112,675	91.7	91.1
■ cyCD3 positive	97,959	86.9	79.2
■ CD19/CD33 negative/HLA-DR negative	78,772	80.4	63.7
■ Normal T cells	68,567	87.0	55.4
■ CD5 dim/CD45 dim blasts	5,297	6.7	4.3
■ TdT positive/CD99 positive	483	9.1	0.4

Extend gate to include
cyTdT negative blasts

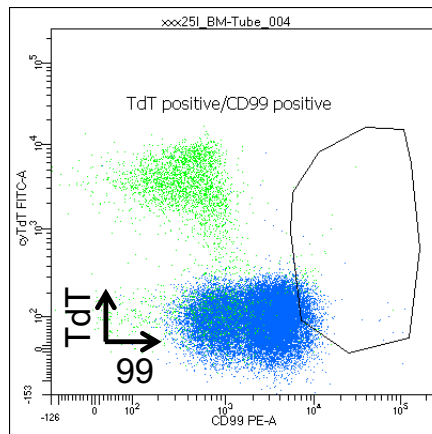
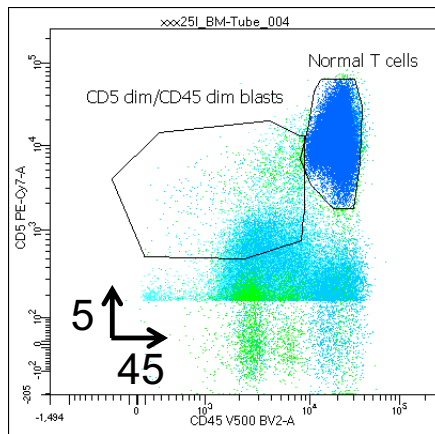
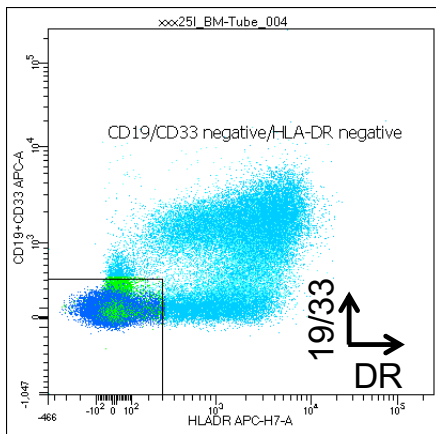
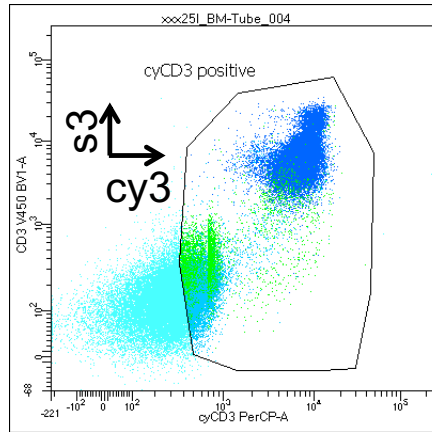
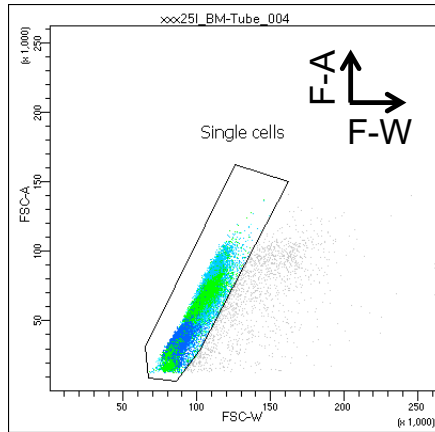
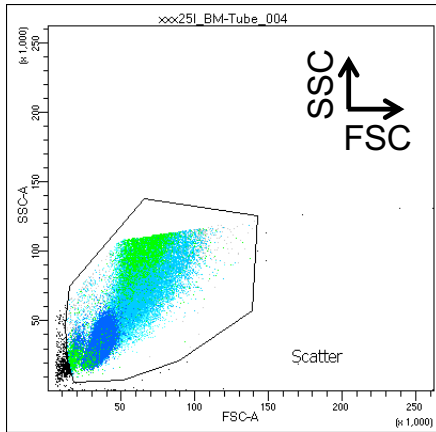
Now have all the blasts

MRD calculation



Minimal Residual Disease = Corrected number of Blasts/Total number of Events
 = (483/368,472) x CF X 100/1
 = 0.001311 x 1.080 x 100
 = 0.142% MRD positive

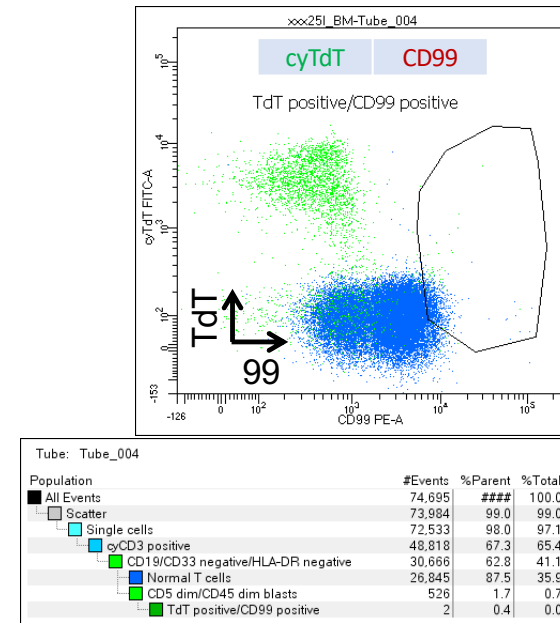
Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



Day 42 (BM)

Tube: Tube_004			
Population	#Events	%Parent	%Total
All Events	74,695	###	100.0
Scatter	73,984	99.0	99.0
Single cells	72,533	98.0	97.1
cyCD3 positive	48,818	67.3	65.4
CD19/CD33 negative/HLA-DR negative	30,666	62.8	41.1
Normal T cells	26,845	87.5	35.9
CD5 dim/CD45 dim blasts	526	1.7	0.7
TdT positive/CD99 positive	2	0.4	0.0

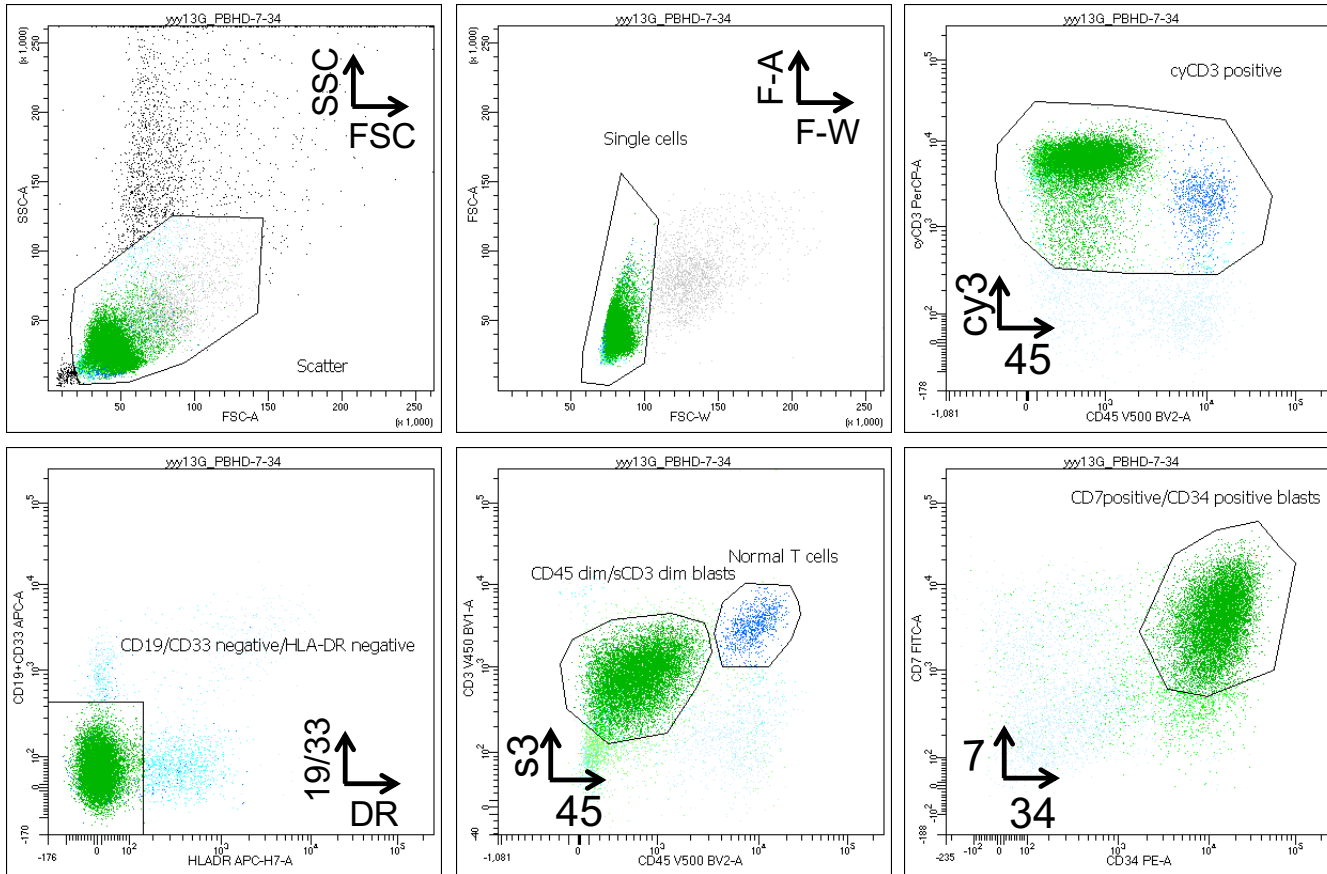
MRD calculation



Minimal Residual Disease = Corrected number of Blasts/Total number of Events
 = $(2/449,982) \times CF \times 100/1$
 = $0.0000044 \times 1.047 \times 100$
 = <0.01% MRD negative

Patient 2 CD7+/CD34+

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

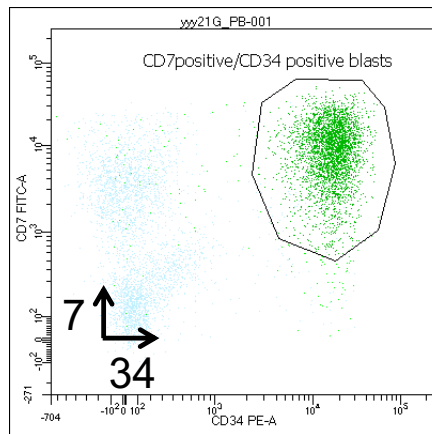
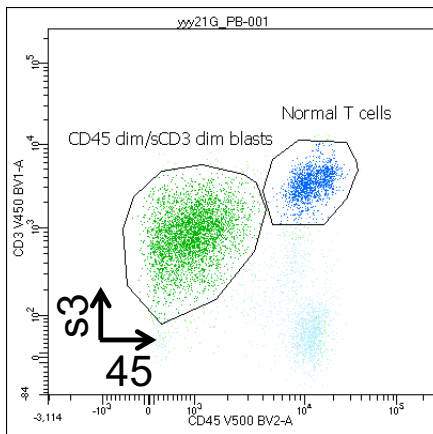
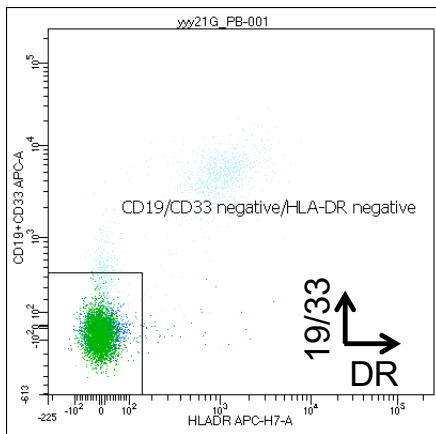
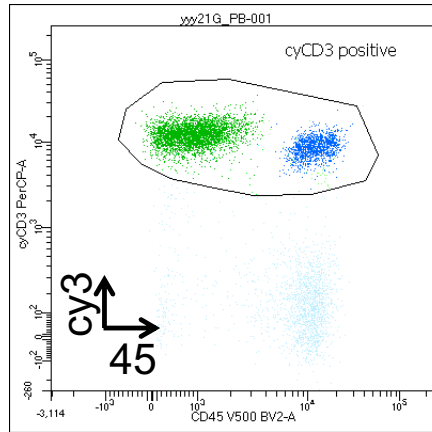
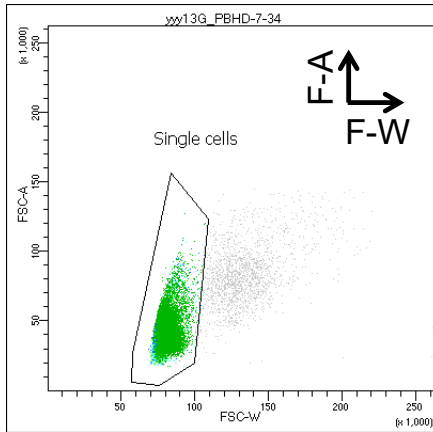
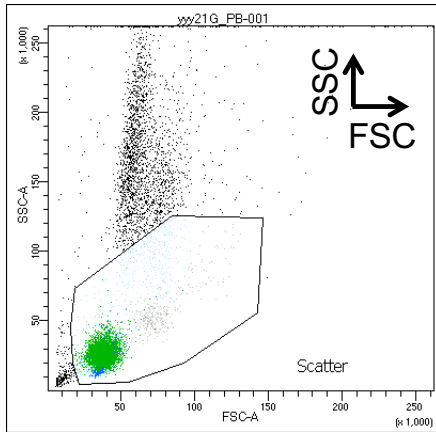


Patient 2 Diagnostic (PB)

Tube: 7-34			
Population	#Events	%Parent	%Total
■ All Events	20,000	###	100.0
■ Scatter	18,157	90.8	90.8
■ Single cells	16,417	90.4	82.1
■ cyCD3 positive	15,061	91.7	75.3
■ CD19/CD33 negative/HLA-DR negative	13,899	92.3	69.5
■ CD45 dim/sCD3 dim blasts	12,137	87.3	60.7
■ CD7positive/CD34 positive blasts	11,128	91.7	55.6
■ Normal T cells	845	5.6	4.2

Blasts are cyCD3+, sCD3dim to negative, CD45dim, CD7 positive, CD34 positive, CD5 positive, CD19/CD33 neg, and HLA-DR negative

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



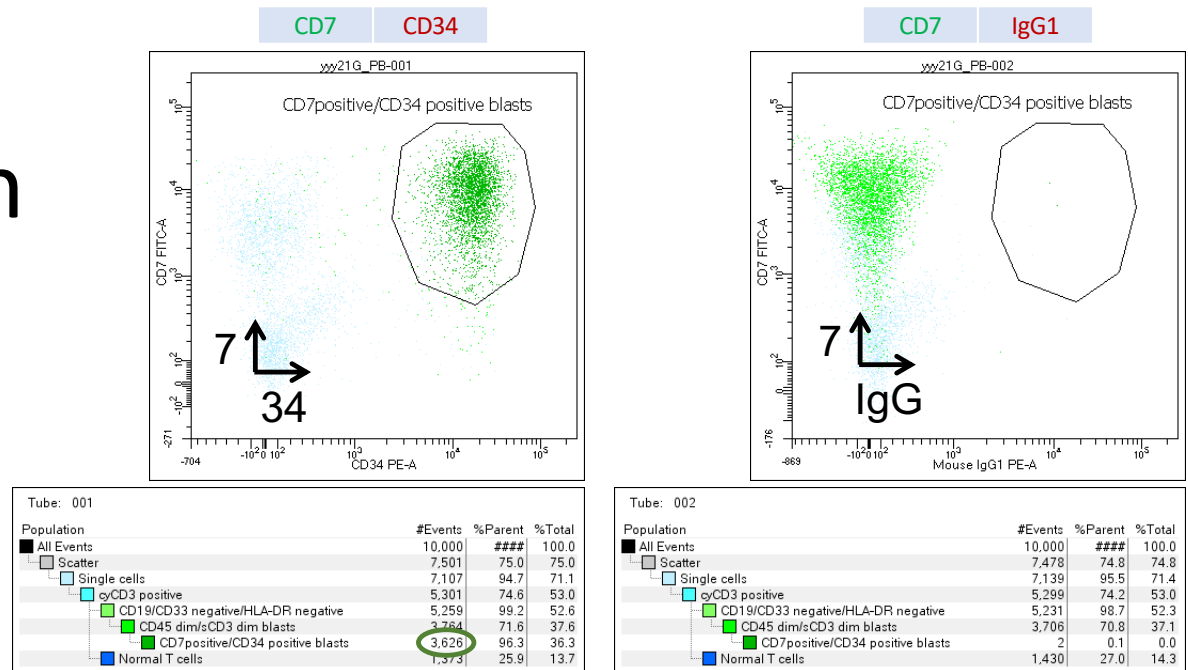
Patient 2 Day 8 (PB)

Tube: 001			
Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ Scatter	7,501	75.0	75.0
■ Single cells	7,107	94.7	71.1
■ cyCD3 positive	5,301	74.6	53.0
■ CD19/CD33 negative/HLA-DR negative	5,259	99.2	52.6
■ CD45 dim/sCD3 dim blasts	3,764	71.6	37.6
■ CD7positive/CD34 positive blasts	3,626	96.3	36.3
■ Normal T cells	1,373	25.9	13.7

Blasts are cyCD3+, sCD3dim to negative, CD45dim, CD7 positive, CD34 positive, CD5 positive, CD19/CD33 neg, and HLA-DR negative

MRD calculation

Day 8 (PB)



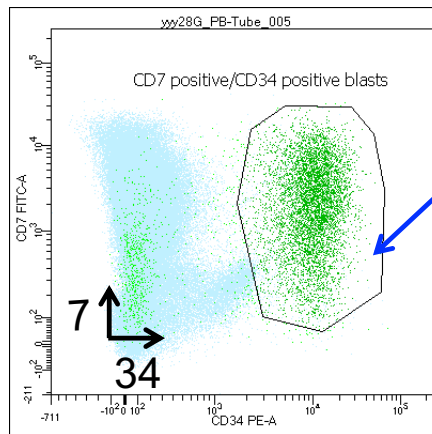
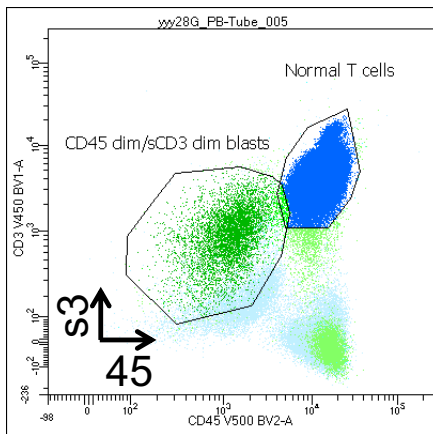
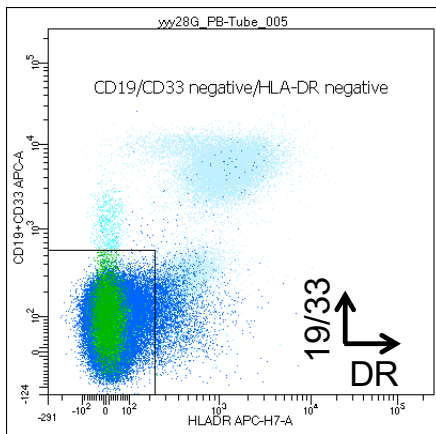
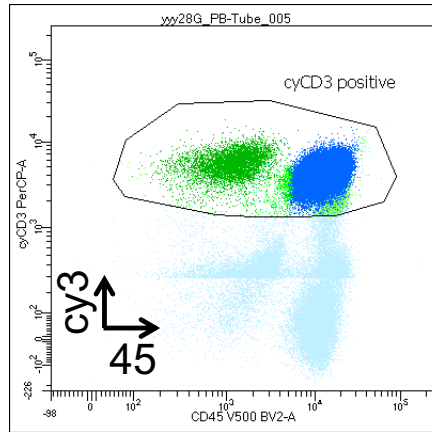
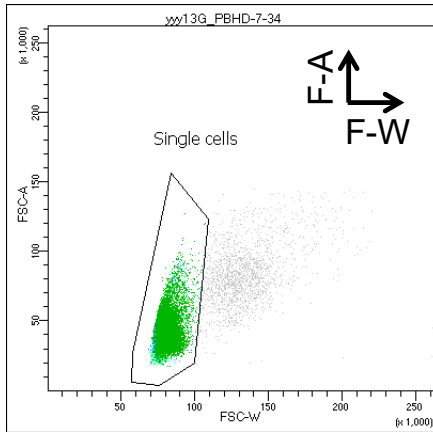
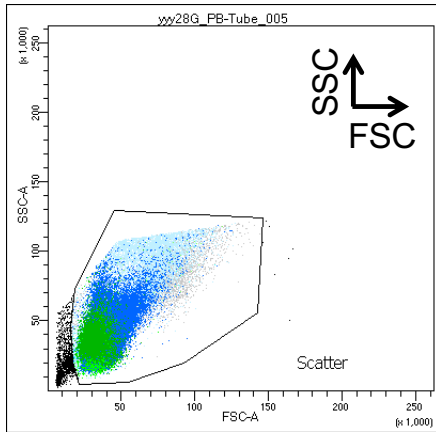
Minimal Residual Disease = Corrected number of Blasts/Total number of Events

$$= ((3,626 - 2) / 10,000) \times CF \times 100 / 1$$

$$= 0.3634 \times 1.099 \times 100$$

$$= 39.83\% \text{ MRD positive}$$

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



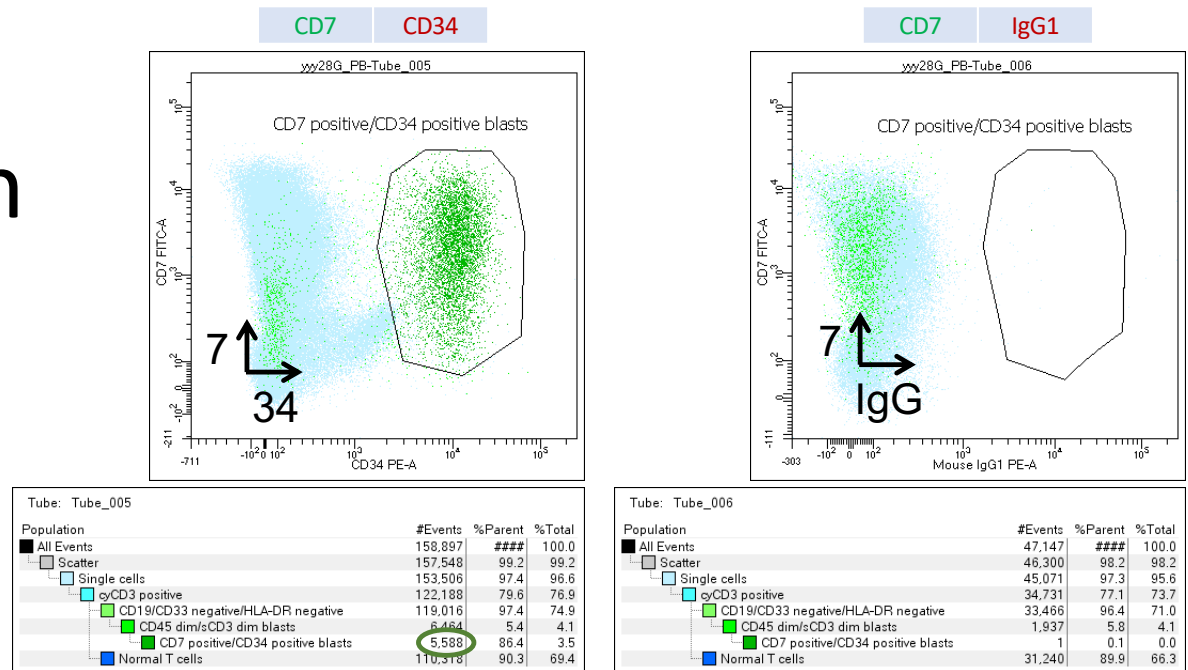
Patient 2 Day 15 (PB)

Tube: Tube_005			
Population	#Events	%Parent	%Total
All Events	158,897	###	100.0
Scatter	157,548	99.2	99.2
Single cells	153,506	97.4	96.6
cyCD3 positive	122,188	79.6	76.9
CD19/CD33 negative/HLA-DR negative	119,016	97.4	74.9
CD45 dim/sCD3 dim blasts	6,464	5.4	4.1
CD7 positive/CD34 positive blasts	5,588	86.4	3.5
Normal T cells	110,318	90.3	69.4

CD7 expression on blasts is decreasing with treatment, CD34 remains high.

MRD calculation

Day 15 (PB)



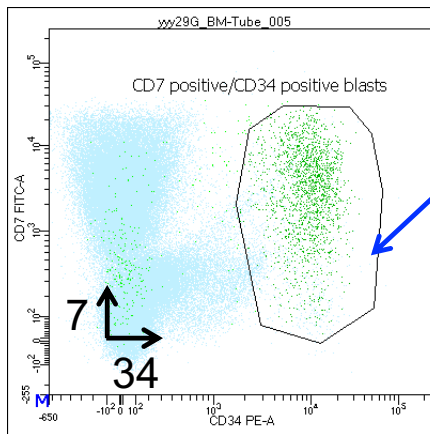
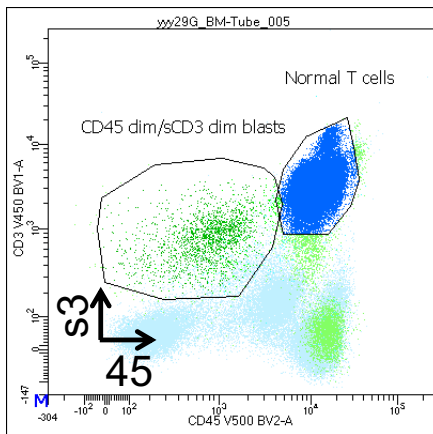
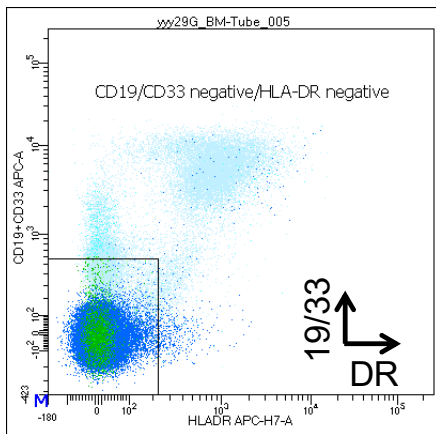
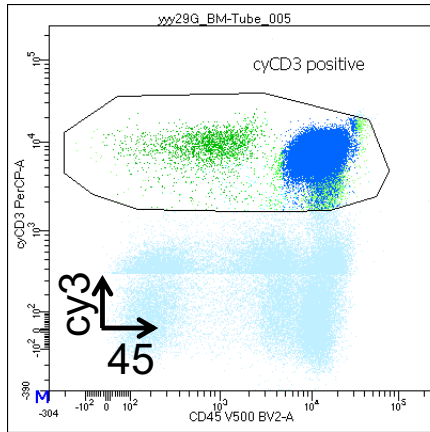
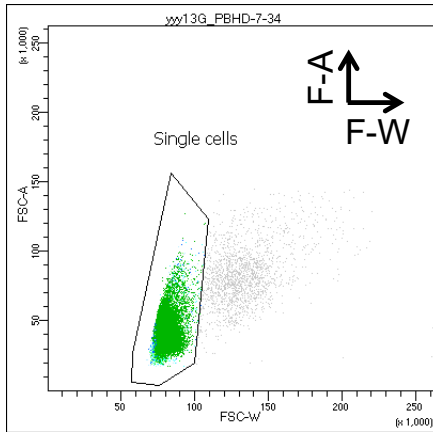
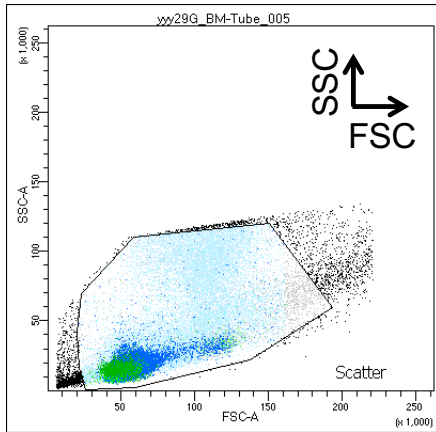
Minimal Residual Disease = Corrected number of Blasts/Total number of Events

$$= ((5,588-1)/748,774) \times CF \times 100/1$$

$$= 0.007461 \times 1.373 \times 100$$

$$= 1.02\% \text{ MRD positive}$$

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



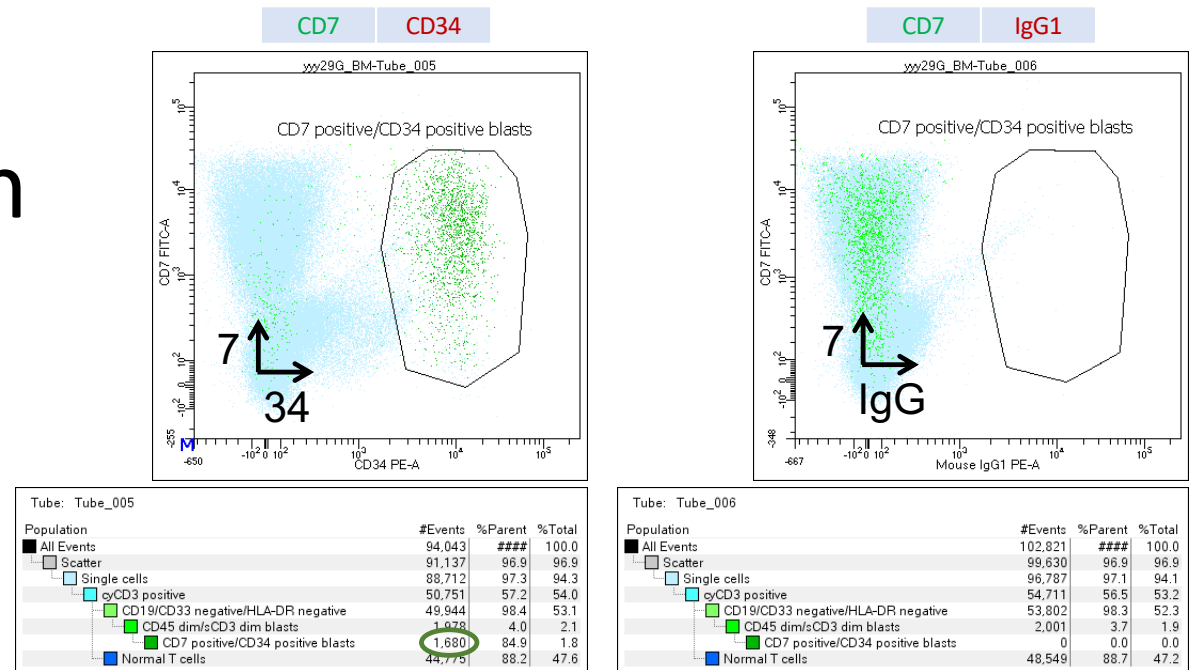
Tube: Tube_005			
Population	#Events	%Parent	%Total
All Events	94,043	###	100.0
Scatter	91,137	96.9	96.9
Single cells	88,712	97.3	94.3
cyCD3 positive	50,751	57.2	54.0
CD19/CD33 negative/HLA-DR negative	49,944	98.4	53.1
CD45 dim/sCD3 dim blasts	1,978	4.0	2.1
CD7 positive/CD34 positive blasts	1,680	84.9	1.8
Normal T cells	44,775	88.2	47.6

Patient 2 Day 15 (BM)

CD7 expression on blasts is decreasing with treatment, CD34 remains high.

MRD calculation

Day 15 (BM)



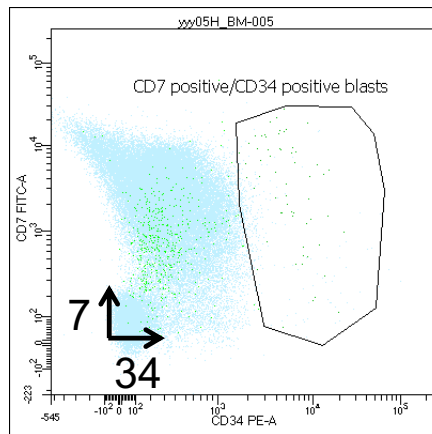
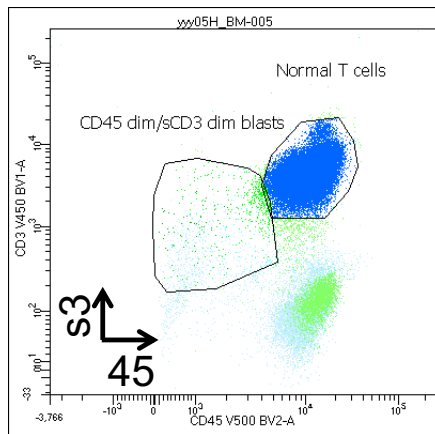
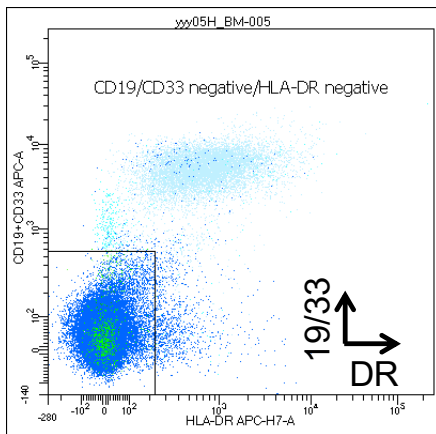
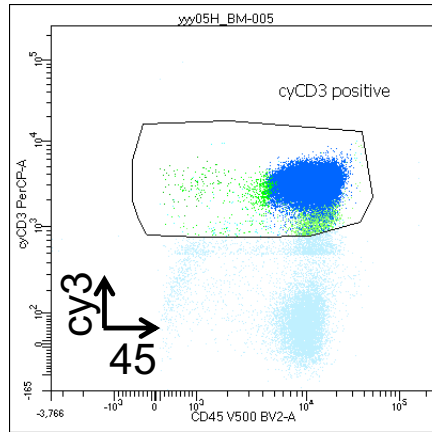
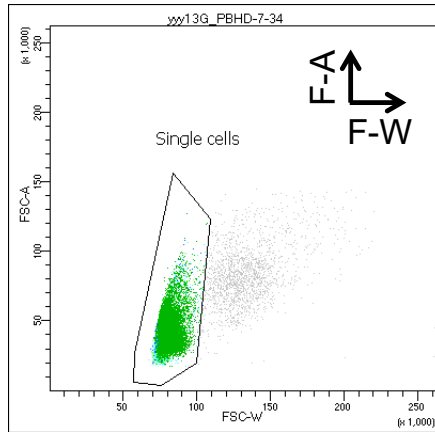
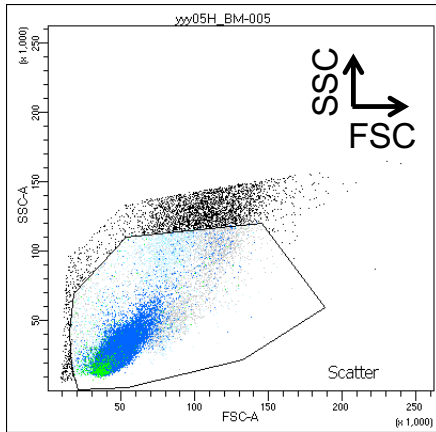
Minimal Residual Disease = Corrected number of Blasts/Total number of Events

$$= ((1,680)/282,750) \times CF \times 100/1$$

$$= 0.005942 \times 1.357 \times 100$$

$$= 0.81\% \text{ MRD positive}$$

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

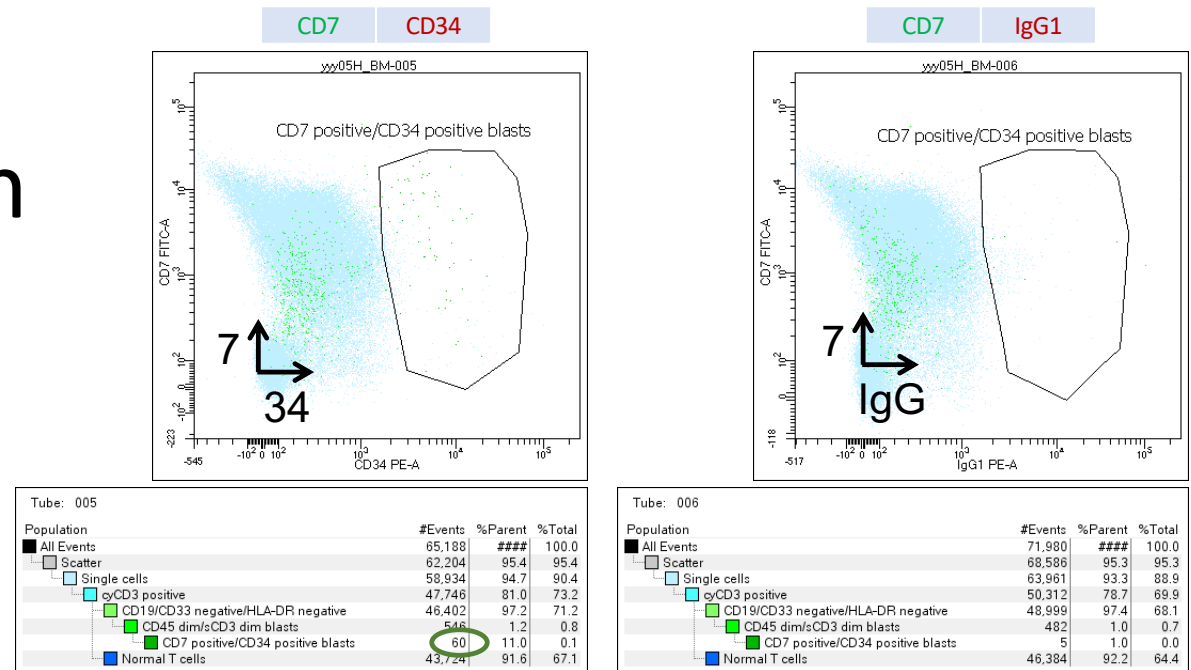


Patient 2 Day 22 (BM)

Tube: 005			
Population	#Events	%Parent	%Total
■ All Events	65,188	###	100.0
■ Scatter	62,204	95.4	95.4
■ Single cells	58,934	94.7	90.4
■ cyCD3 positive	47,746	81.0	73.2
■ CD19/CD33 negative/HLA-DR negative	46,402	97.2	71.2
■ CD45 dim/sCD3 dim blasts	546	1.2	0.8
■ CD7 positive/CD34 positive blasts	60	11.0	0.1
■ Normal T cells	43,724	91.6	67.1

MRD calculation

Day 22 (BM)



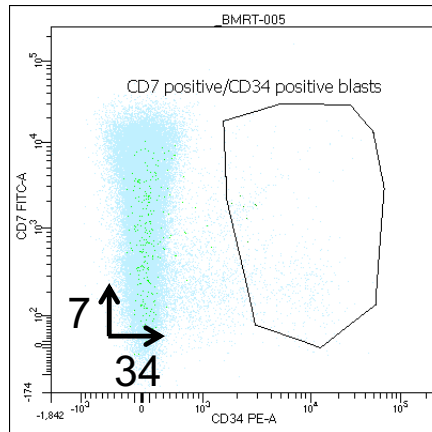
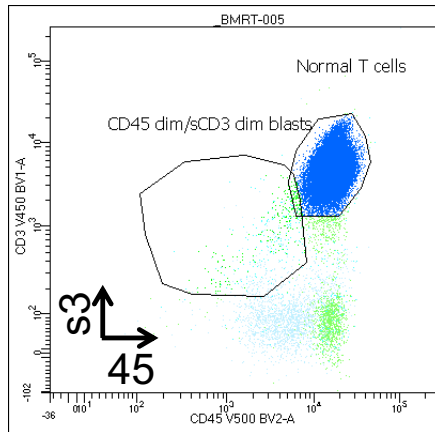
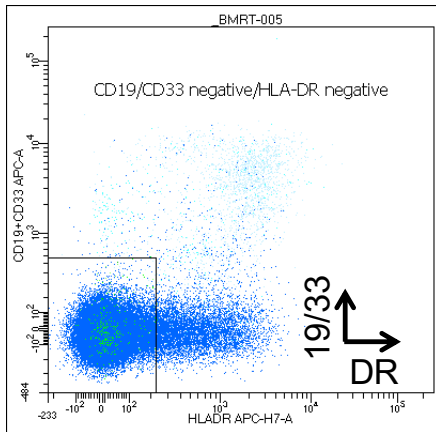
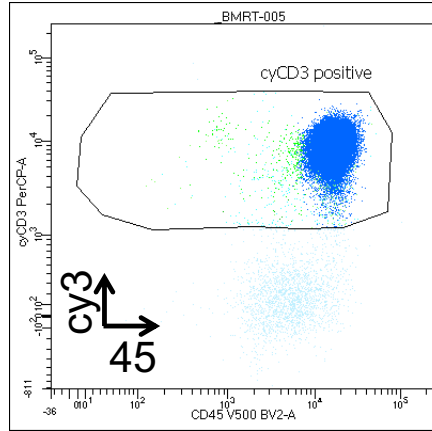
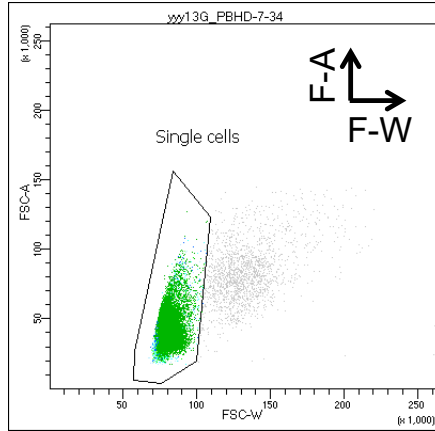
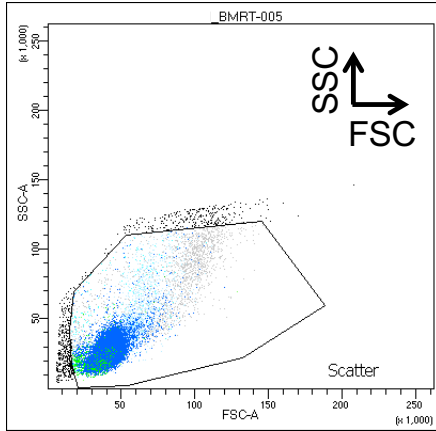
Minimal Residual Disease = Corrected number of Blasts/Total number of Events

$$= ((60-5)/693,966) \times CF \times 100/1$$

$$= 0.00007925 \times 1.541 \times 100$$

$$= 0.012\% \text{ MRD positive}$$

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

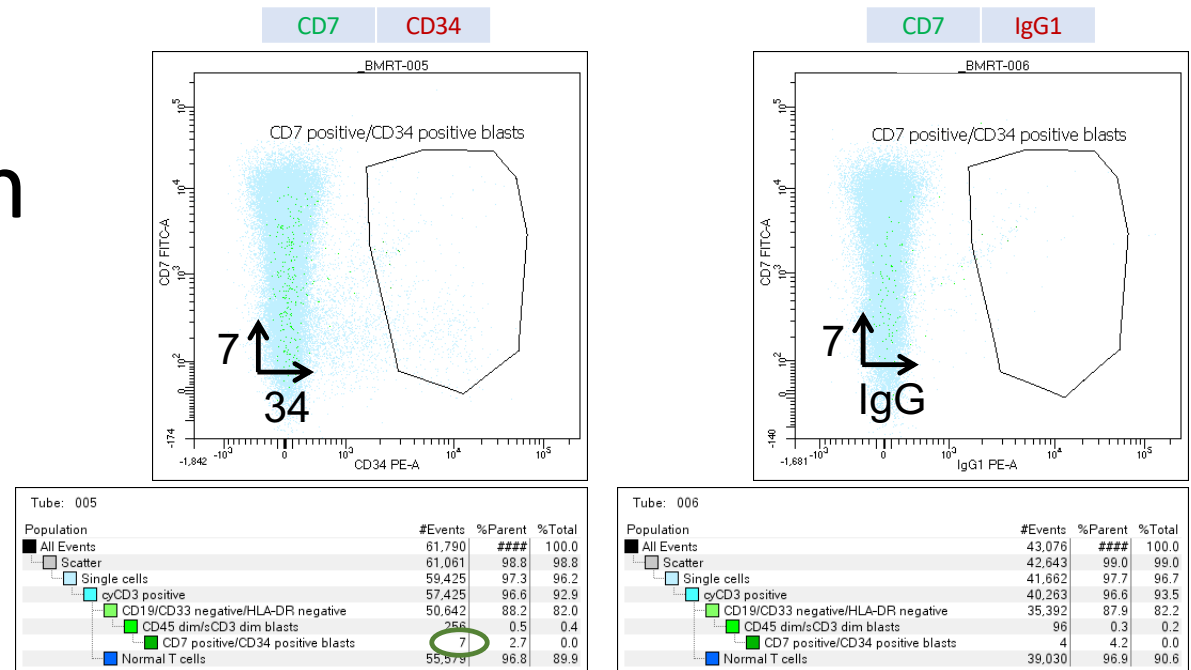


Patient 2 Day 51 (BM)

Tube: 005			
Population	#Events	%Parent	%Total
■ All Events	61,790	###	100.0
■ Scatter	61,061	98.8	98.8
■ Single cells	59,425	97.3	96.2
■ cyCD3 positive	57,425	96.6	92.9
■ CD19/CD33 negative/HLA-DR negative	50,642	88.2	82.0
■ CD45 dim/sCD3 dim blasts	256	0.5	0.4
■ CD7 positive/CD34 positive blasts	7	2.7	0.0
■ Normal T cells	55,579	96.8	89.9

MRD calculation

Day 51 (BM)



Minimal Residual Disease = Corrected number of Blasts/Total number of Events

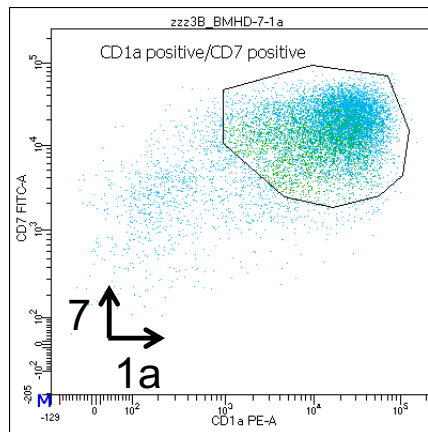
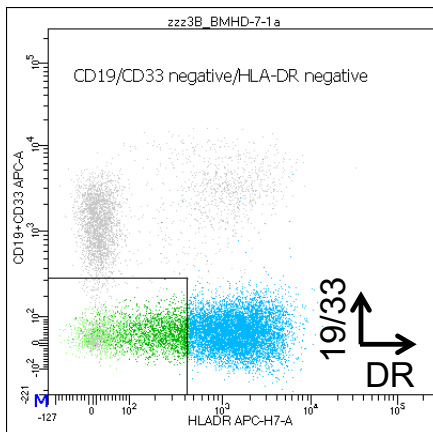
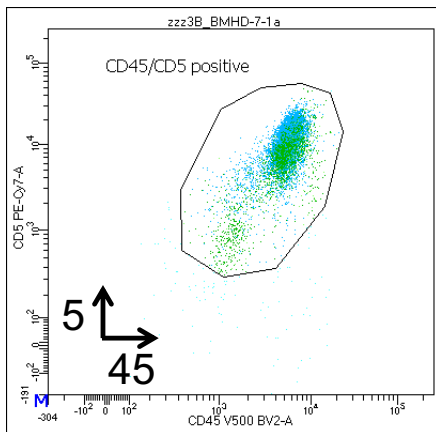
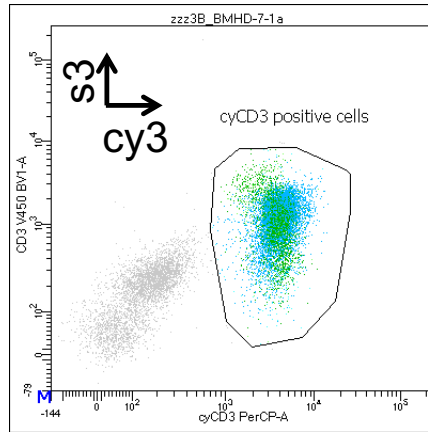
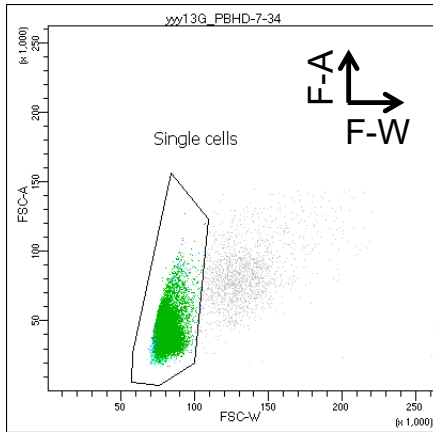
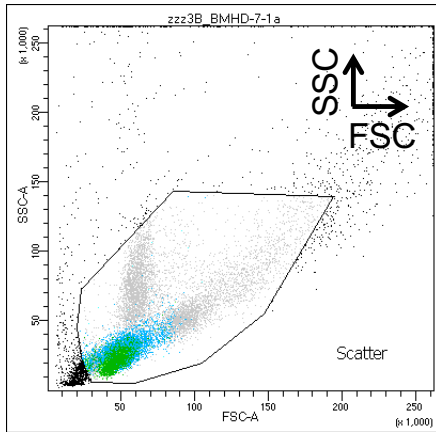
$$= ((7-4)/376,428) \times CF \times 100/1$$

$$= 0.000008 \times 1.256 \times 100$$

$$= <0.01\% \text{ MRD negative}$$

Patient 3 HLA-DR+

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

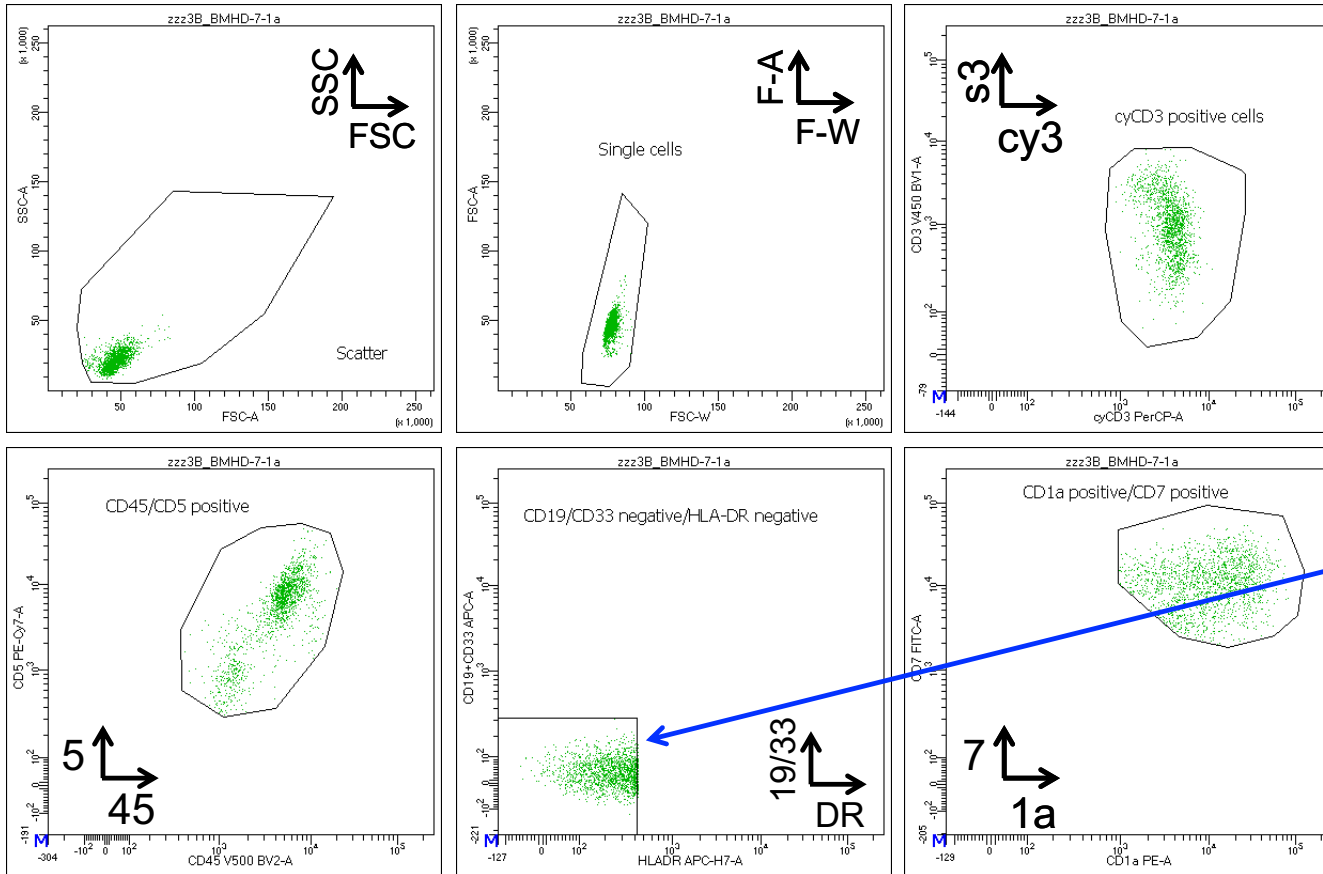


Patient 3 Diagnostic (PB)

Tube: 7-1a			
Population	#Events	%Parent	%Total
■ All Events	20,000	###	100.0
■ Scatter	17,792	89.0	89.0
■ Single cells	14,870	83.6	74.4
■ cyCD3 positive cells	10,909	73.4	54.5
■ CD45/CD5 positive	10,798	99.0	54.0
■ CD19/CD33 negative/HLA-DR negative	1,784	16.5	8.9
■ CD1a positive/CD7 positive	1,645	92.2	8.2

Blasts are cyCD3+, sCD3var, CD45positive, CD7 positive, CD1a positive, CD5 positive, CD19/CD33 negative, and HLA-DR looks strange

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

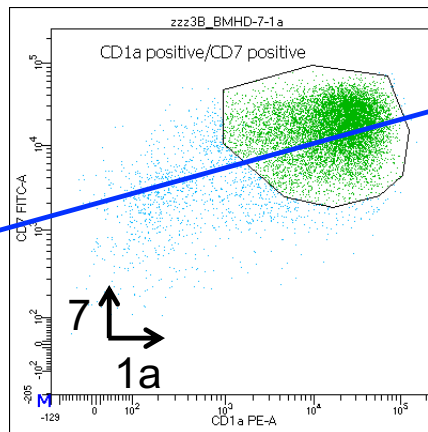
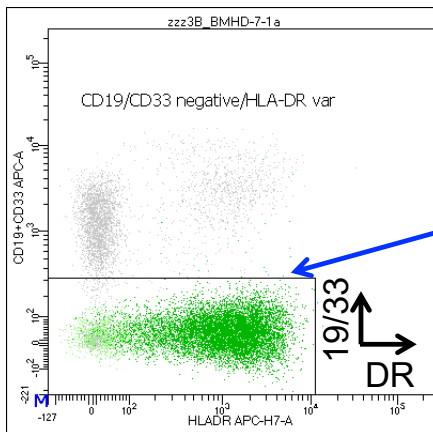
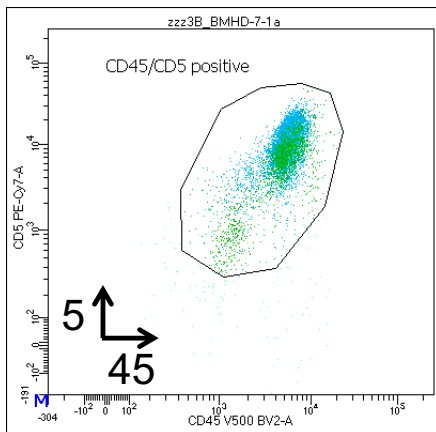
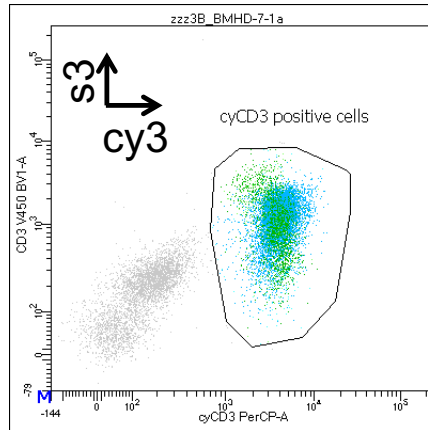
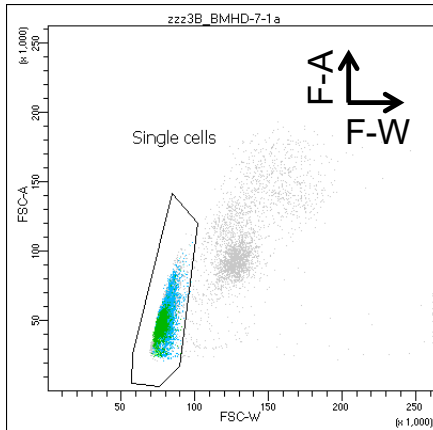
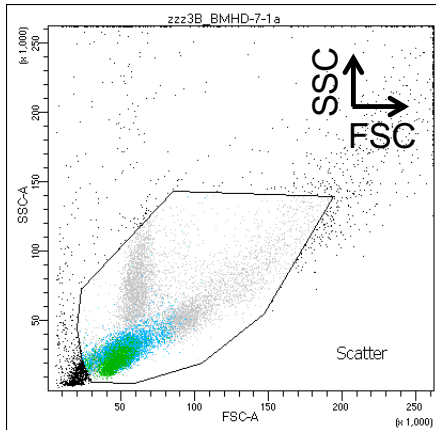


Patient 3 Diagnostic (PB) Back gating

Tube: 7-1a			
Population	#Events	%Parent	%Total
■ All Events	20,000	###	100.0
■ Scatter	17,792	89.0	89.0
■ Single cells	14,870	83.6	74.4
■ cyCD3 positive cells	10,909	73.4	54.5
■ CD45/CD5 positive	10,798	99.0	54.0
■ CD19/CD33 negative/HLA-DR negative	1,784	16.5	8.9
■ CD1a positive/CD7 positive	1,645	92.2	8.2

Something wrong with gating here, HLA-DR expression is cut off
→ re-gate

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



Patient 3 Diagnostic (PB) Re-gated

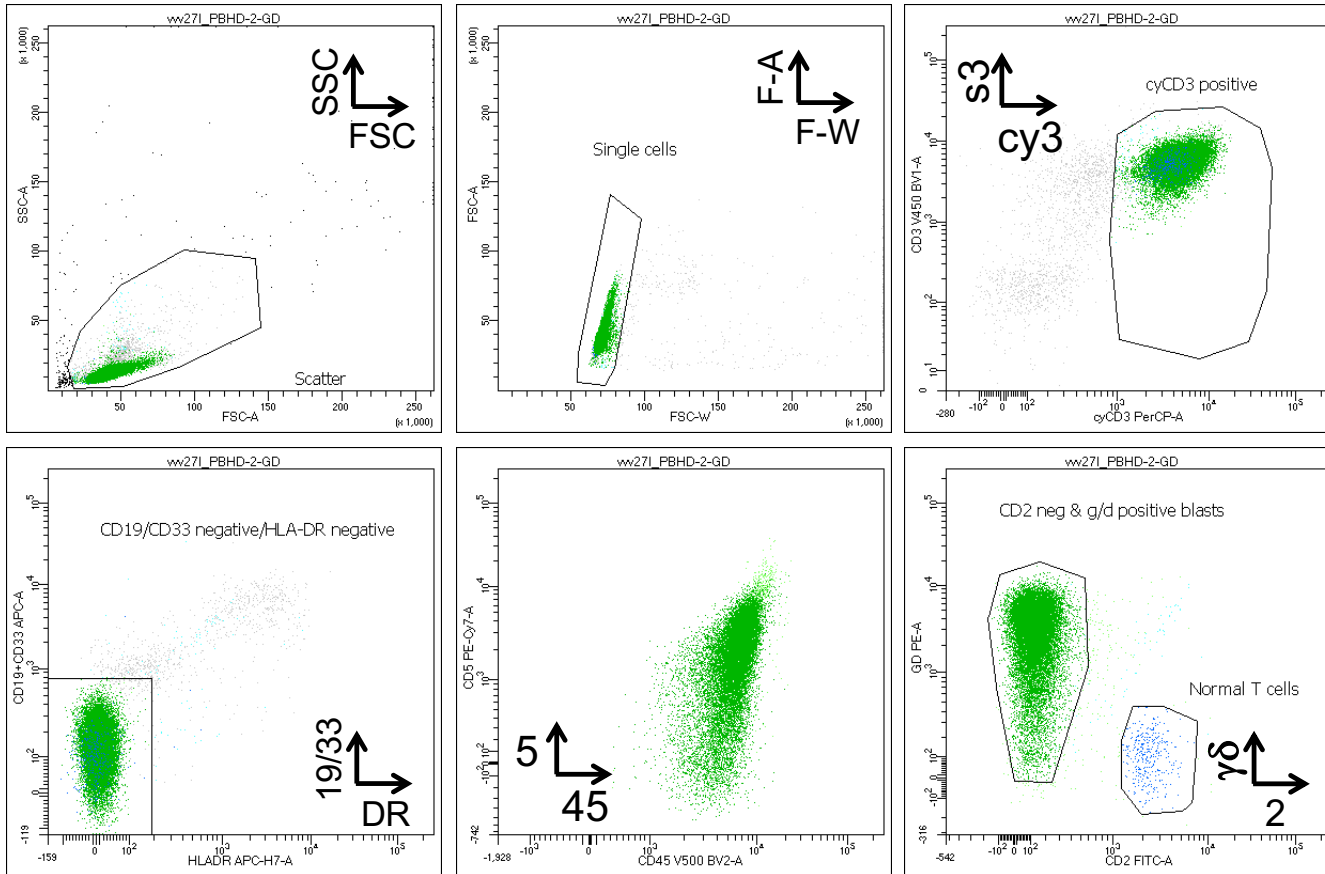
Tube: 7-1a			
Population	#Events	%Parent	%Total
■ All Events	20,000	###	100.0
■ Scatter	17,792	89.0	89.0
■ Single cells	14,870	83.6	74.4
■ cyCD3 positive cells	10,909	73.4	54.5
■ CD45/CD5 positive	10,798	99.0	54.0
■ CD19/CD33 negative/HLA-DR negative	1,784	16.5	8.9
■ CD1a positive/CD7 positive	1,645	92.2	8.2

Blasts have variable
expression of HLA-DR
positive to negative

Not common, but back
gating helps catch these
unusual ones

Patient 4 TCR γ / δ +

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

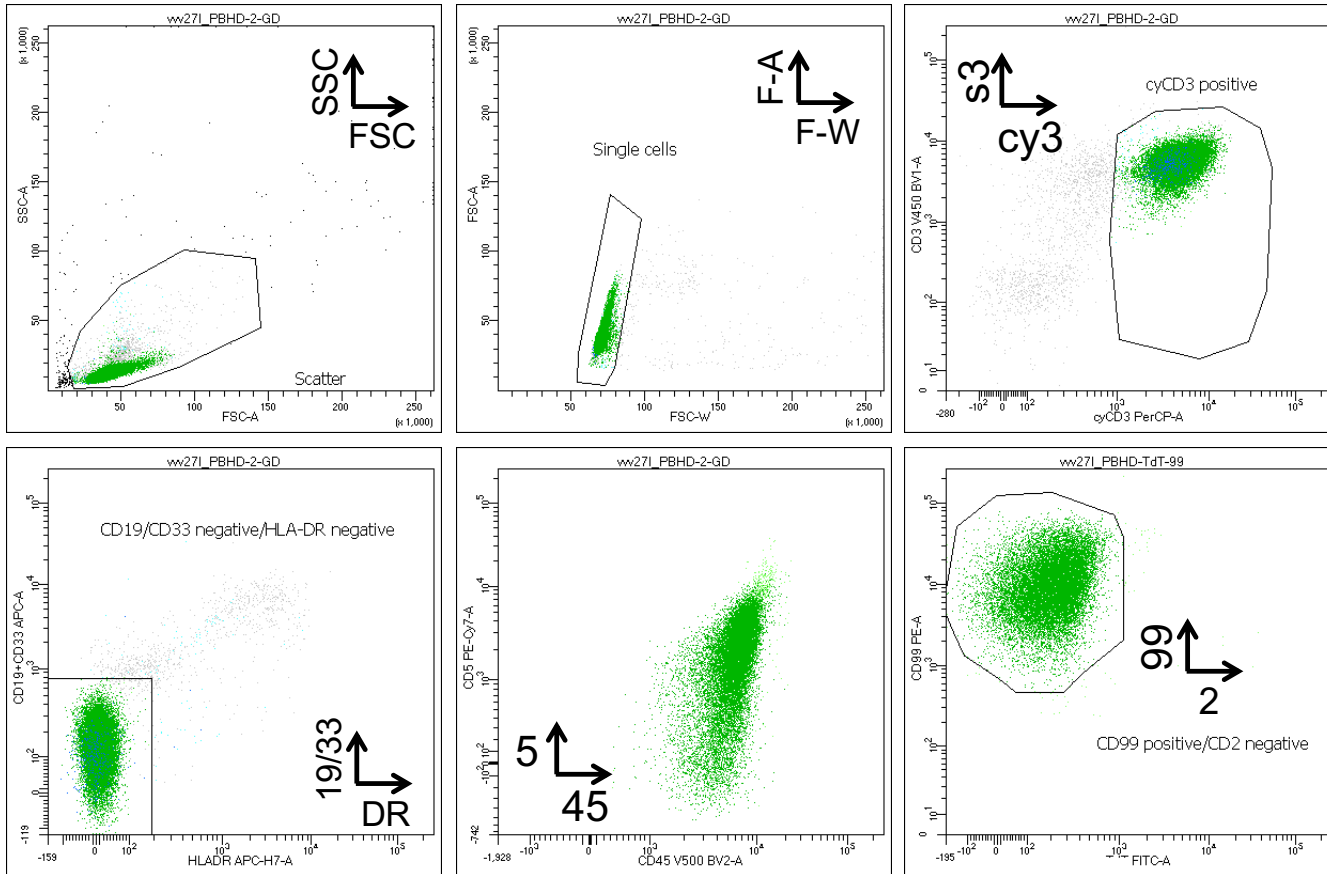


Patient 4 Diagnosis (PB)

Tube: 2-GD			
Population	#Events	%Parent	%Total
All Events	20,000	###	100.0
Scatter	19,695	98.5	98.5
Single cells	19,352	96.8	96.8
cyCD3 positive	18,053	93.3	90.3
CD19/CD33 negative/HLA-DR negative	17,967	99.5	89.8
CD2 neg & g/d positive blasts	17,025	94.8	85.1
Normal T cells	344	1.9	1.7

Blasts are cyCD3+, CD5var, CD45 positive, TCR γ/δ positive and CD2 negative

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
8	CD2	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



Patient 4 Diagnosis (PB)

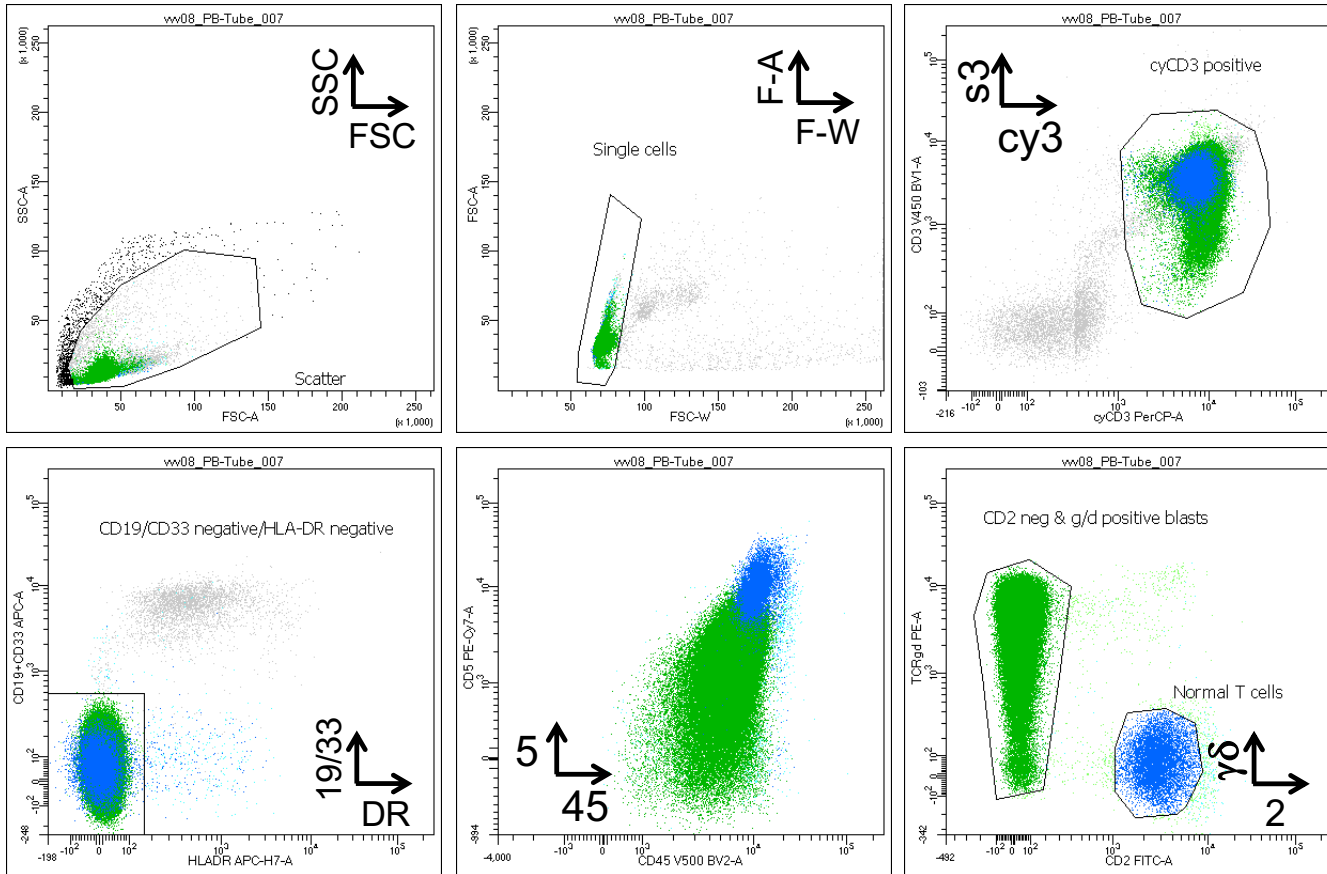
Tube: TdT-99			
Population	#Events	%Parent	%Total
■ All Events	20,000	###	100.0
□ Scatter	19,627	98.1	98.1
□ Single cells	19,283	96.2	96.4
■ cyCD3 positive	18,078	93.8	90.4
■ CD19/CD33 negative/HLA-DR negative	18,013	99.6	90.1
■ CD99 positive/CD2 negative	17,938	99.6	89.7
■ Normal T cells	16	0.1	0.1

Blasts are cyCD3+, CD5var,
CD45 positive, TCR γ/δ +,
CD2 neg, CD99 positive

Follow with two tubes:

CD2	γ/δ
CD2	CD99

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



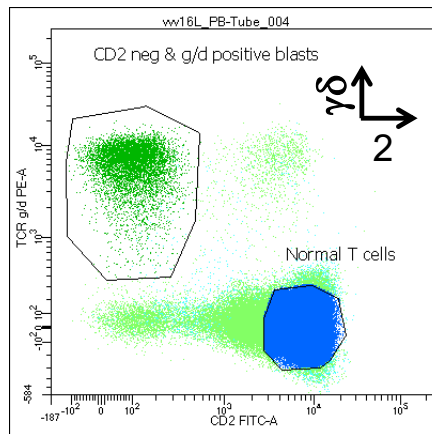
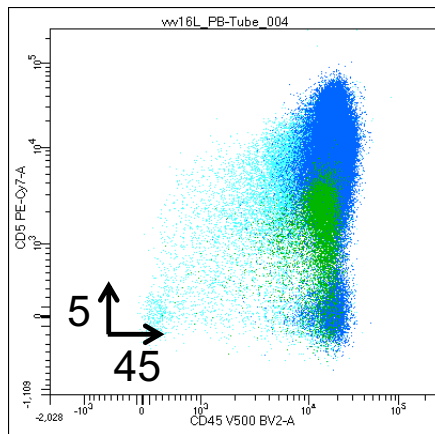
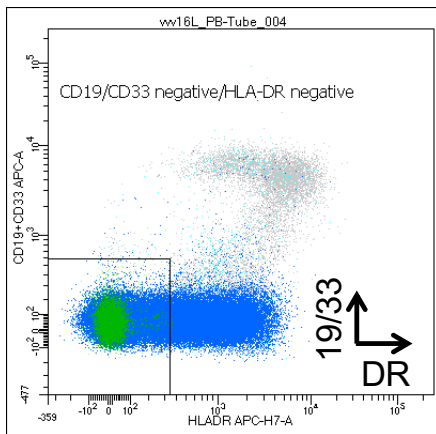
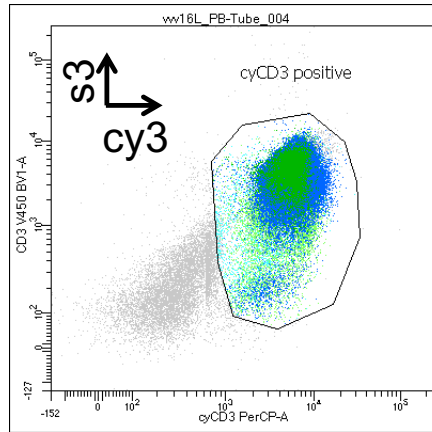
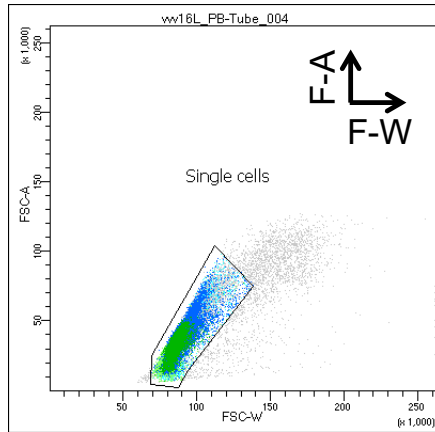
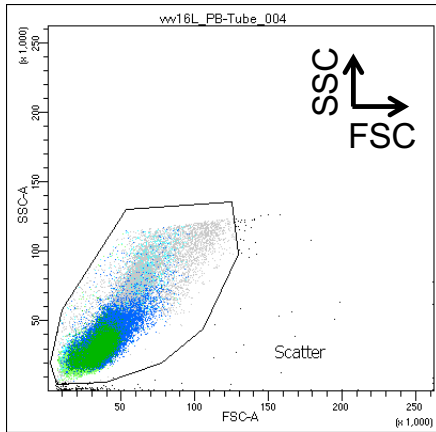
Patient 4 Day 8 (PB)

Tube: Tube_007			
Population	#Events	%Parent	%Total
■ All Events	141,023	###	100.0
□ Scatter	139,546	99.0	99.0
□ Single cells	137,526	98.6	97.5
■ cyCD3 positive	133,343	97.0	94.6
■ CD19/CD33 negative/HLA-DR negative	132,866	99.6	94.2
■ CD2 neg & g/d positive blasts	123,722	93.1	87.7
■ Normal T cells	8,373	6.3	5.9

Blasts are cyCD3+, CD5var,
CD45 positive, TCR γ/δ +,
CD2 neg, CD99 positive

MRD positive (85.53%)

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



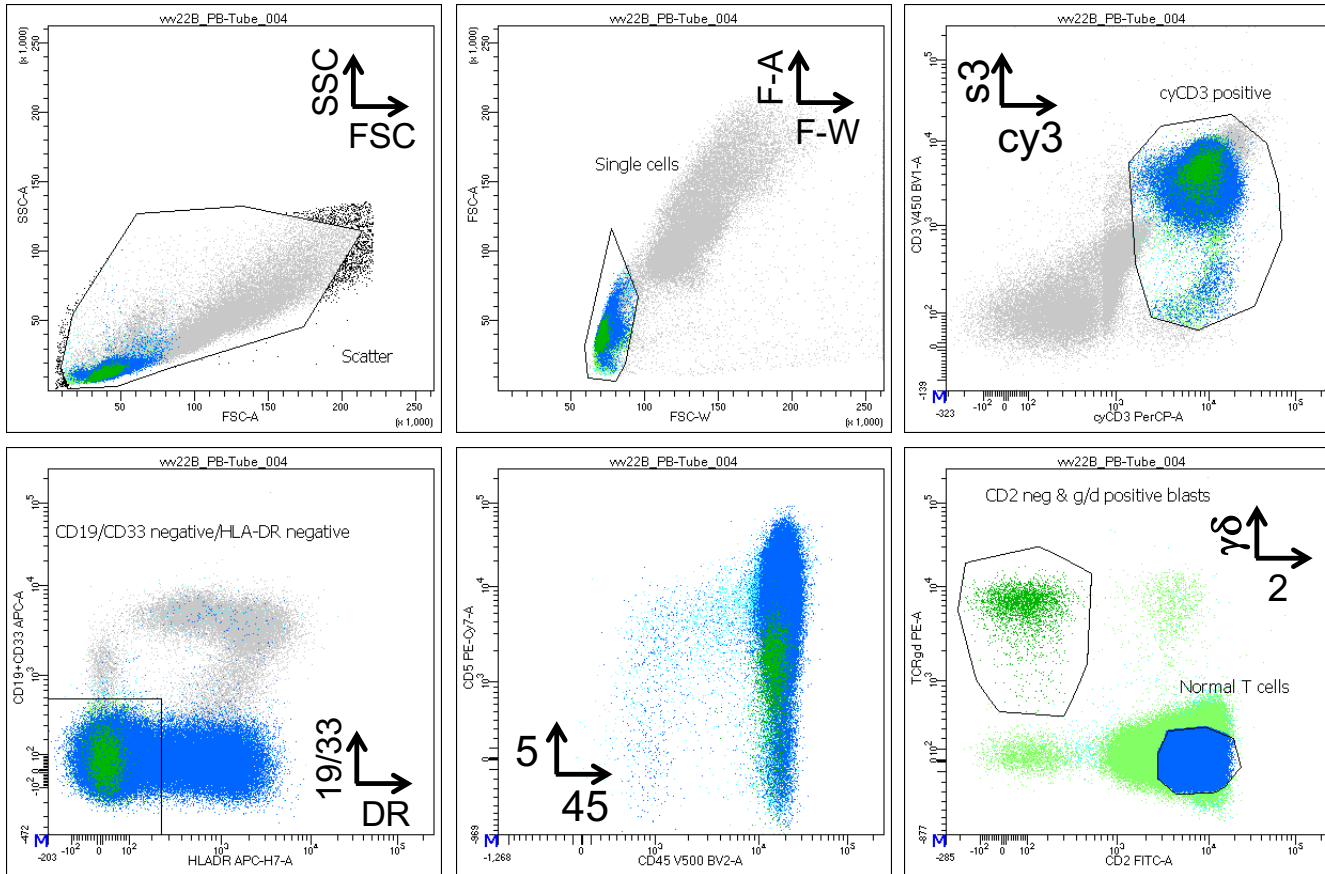
Tube: Tube_007			
Population	#Events	%Parent	%Total
■ All Events	141,023	###	100.0
■ Scatter	139,546	99.0	99.0
■ Single cells	137,526	98.6	97.5
■ cyCD3 positive	133,343	97.0	94.6
■ CD19/CD33 negative/HLA-DR negative	132,866	99.6	94.2
■ CD2 neg & g/d positive blasts	123,722	93.1	87.7
■ Normal T cells	8,373	6.3	5.9

Patient 4 Day 42 (PB)

Blasts are cyCD3+, CD5var,
CD45 positive, TCR γ/δ +,
CD2 neg, CD99 positive

MRD positive (13.01%)

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
6	CD2	γ/δ	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



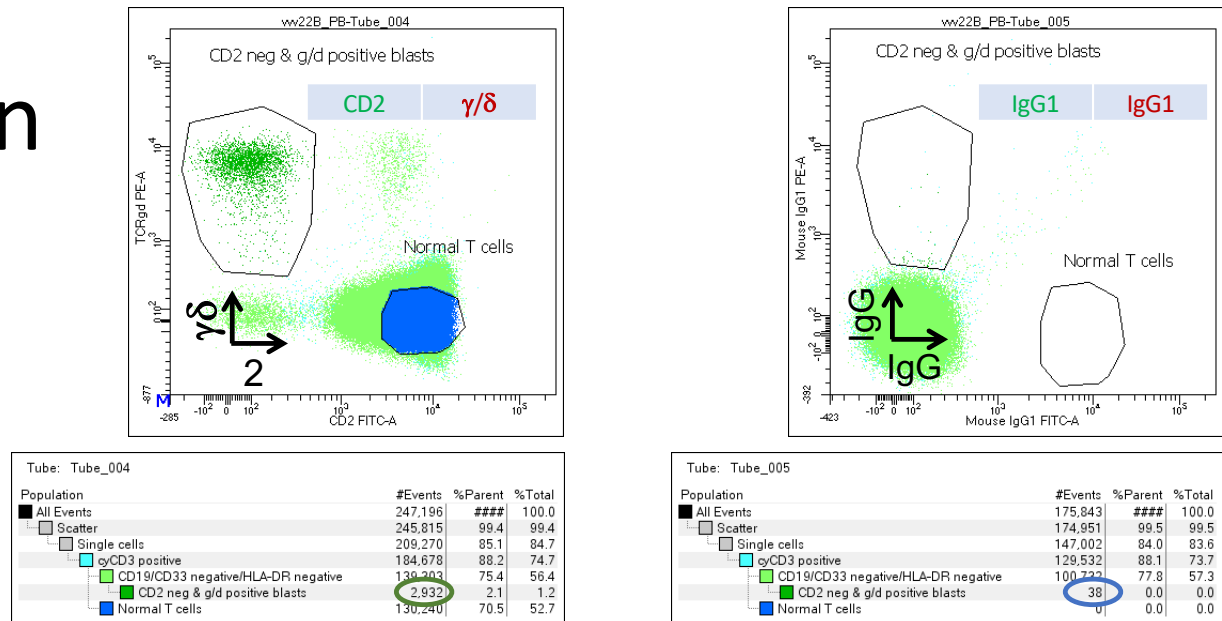
Patient 4 Day 148 (PB)

Tube: Tube_004			
Population	#Events	%Parent	%Total
■ All Events	247,196	###	100.0
■ Scatter	245,815	99.4	99.4
■ Single cells	209,270	85.1	84.7
■ cyCD3 positive	184,678	88.2	74.7
■ CD19/CD33 negative/HLA-DR negative	139,303	75.4	56.4
■ CD2 neg & g/d positive blasts	2,932	2.1	1.2
■ Normal T cells	130,240	70.5	52.7

Blasts are cyCD3+, CD5var,
CD45 positive, TCR γ/δ +,
CD2 neg, CD99 positive

MRD positive (0.79%)

MRD calculation



Minimal Residual Disease = Corrected number of Blasts/Total number of Events

$$= ((2,932/426,686) - (38/314,002)) \times CF \times 100/1$$

$$= 0.00675 \times 1.176 \times 100$$

$$= 0.794\% \text{ MRD positive}$$

Gating Strategies - Examples

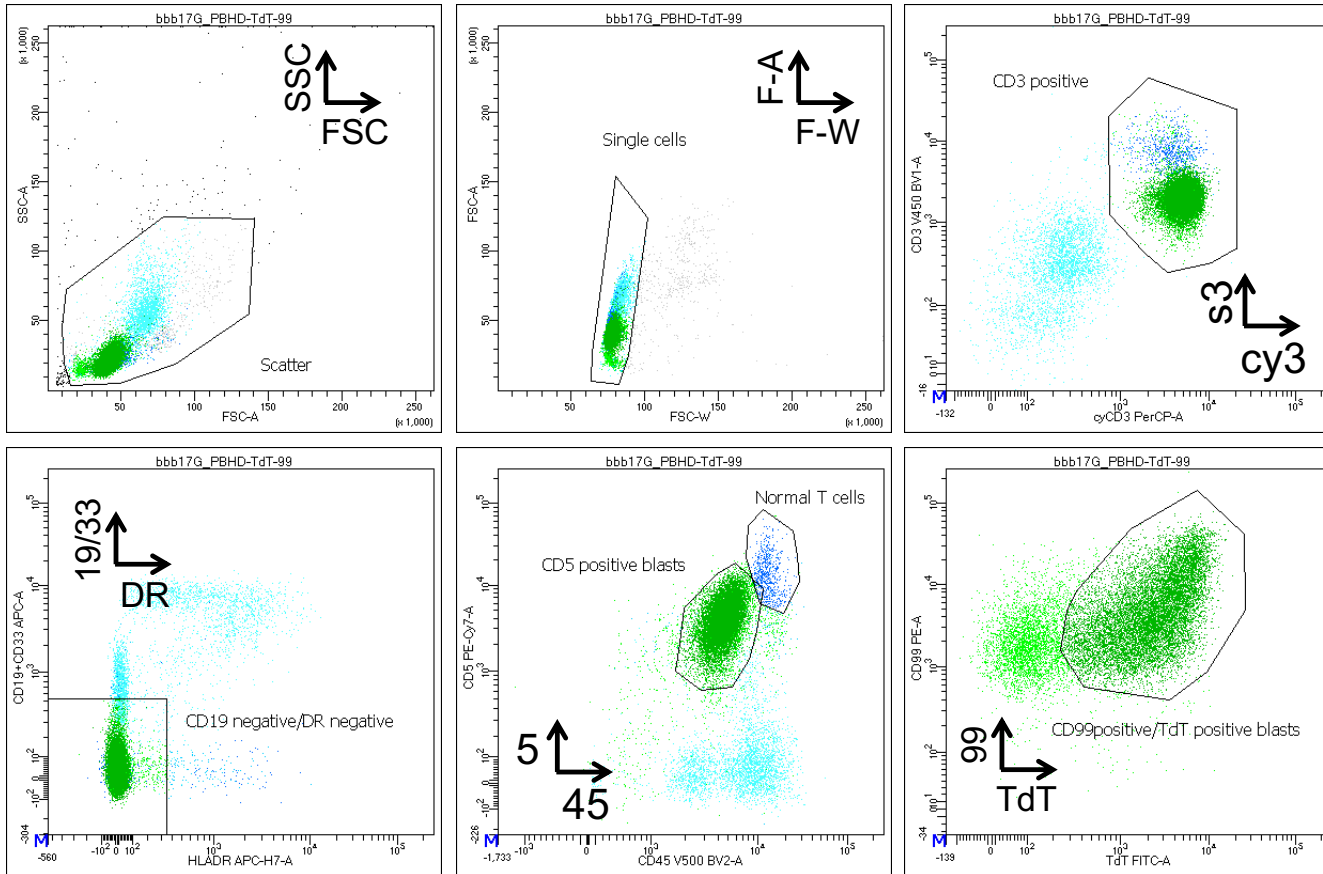
Start with four simple cases that are easy to follow at later treatment stages

Move on to progressively more difficult cases

- loss of aberrant marker expression with treatment
- aberrantly low expression on blast population becomes more like normal with treatment

Patient 5 Rapid Immunophenotypic Shift

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



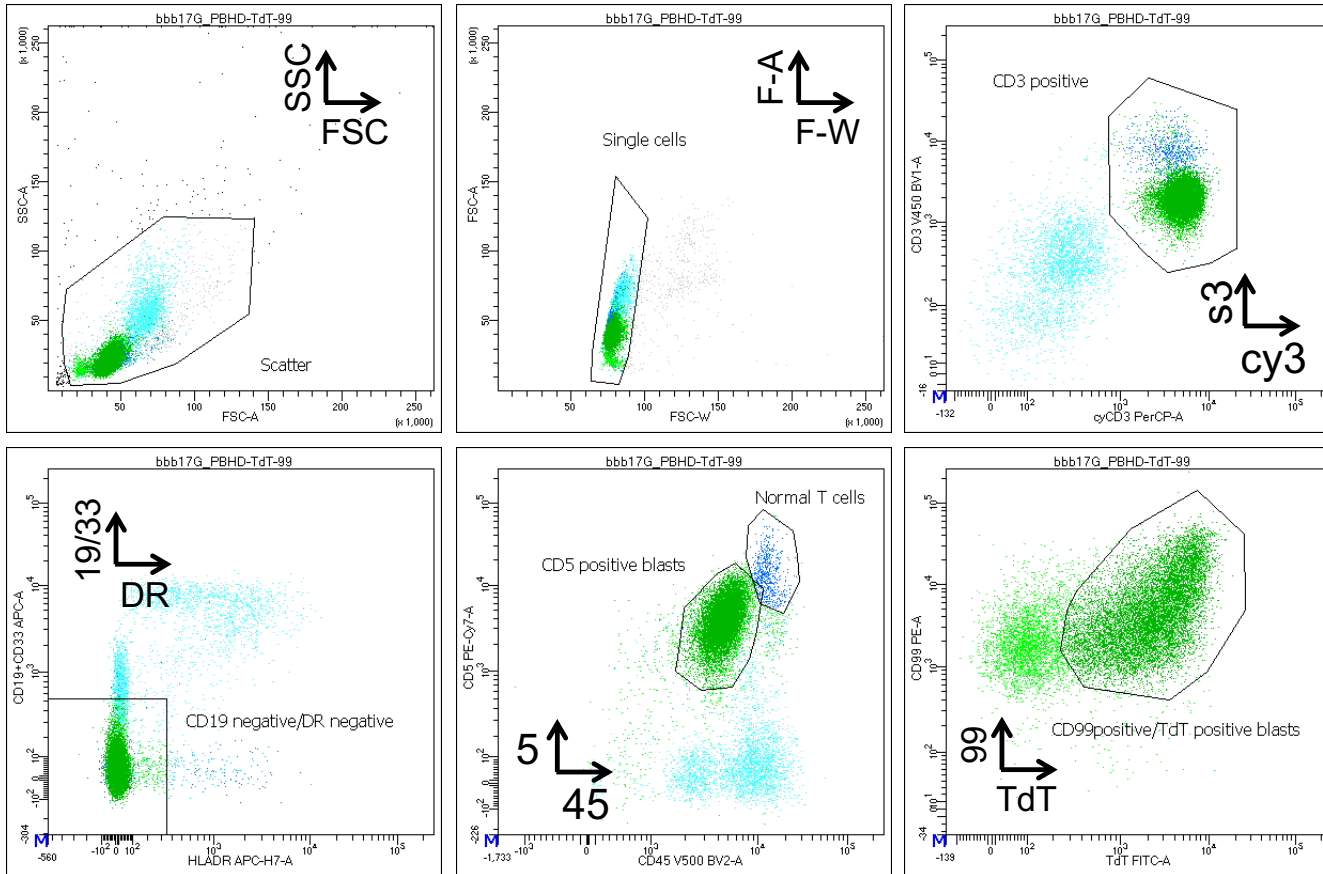
Patient 5 Diagnostic (PB)

Tube: TdT-99			
Population	#Events	%Parent	%Total
All Events	20,000	###	100.0
Scatter	19,775	98.9	98.9
Single cells	19,310	97.6	96.6
CD3 positive	16,152	83.6	80.8
CD19 negative/DR negative	15,914	98.5	79.6
CD5 positive blasts	14,802	93.0	74.0
CD99positive/TdT positive blasts	11,938	80.7	59.7
Normal T cells	657	4.1	3.3

Blasts are cyCD3+, sCD3dim, CD45dim, CD5dim, CD99+, TdT variable, CD19/CD33-, and HLA-DR negative

Should be easy to follow with this panel.

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

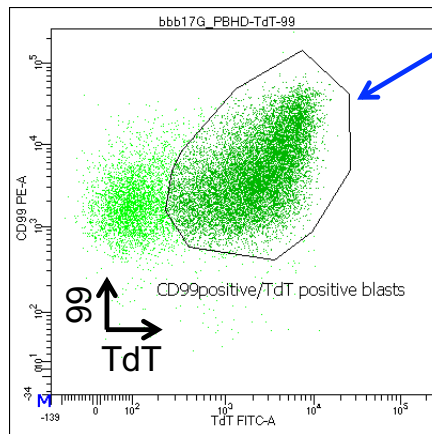
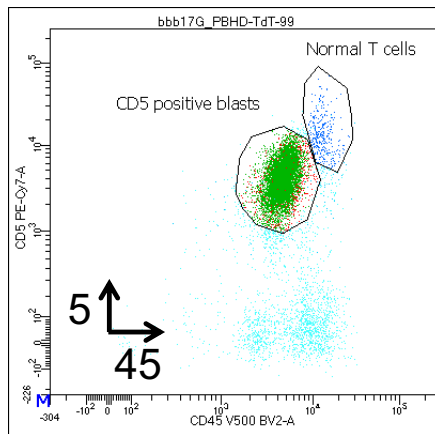
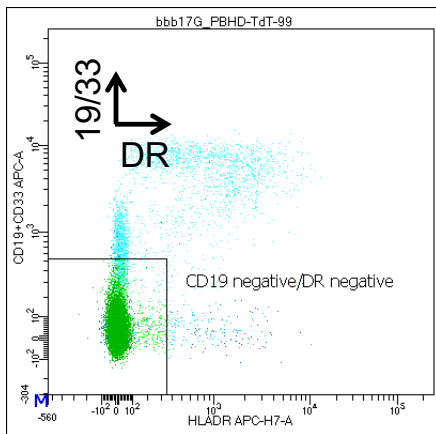
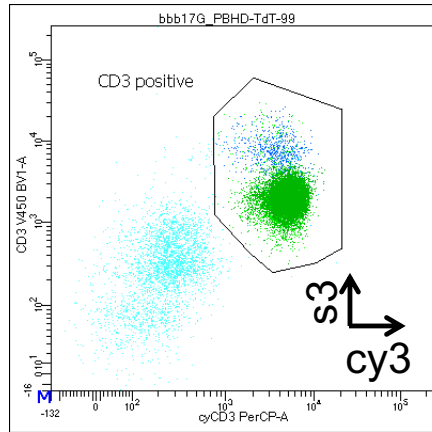
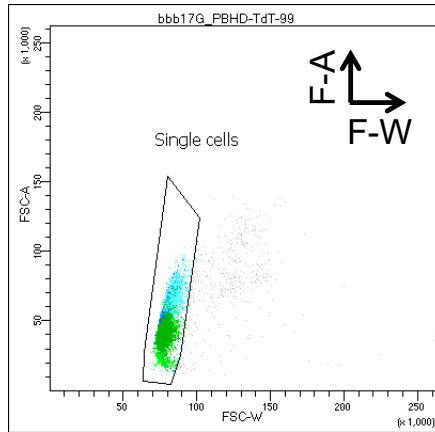
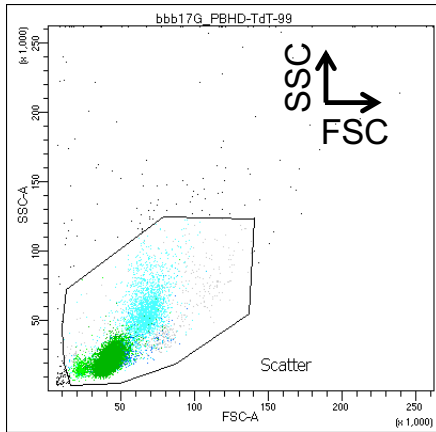


Patient 5 Diagnostic (PB)

Tube: TdT-99			
Population	#Events	%Parent	%Total
All Events	20,000	###	100.0
Scatter	19,775	98.9	98.9
Single cells	19,310	97.6	96.6
CD3 positive	16,152	83.6	80.8
CD19 negative/DR negative	15,914	98.5	79.6
CD5 positive blasts	14,802	93.0	74.0
CD99 positive/TdT positive blasts	11,938	80.7	59.7
Normal T cells	657	4.1	3.3

Not catching all the blasts
with this gate

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



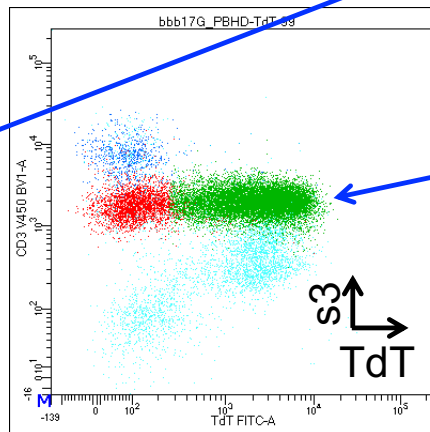
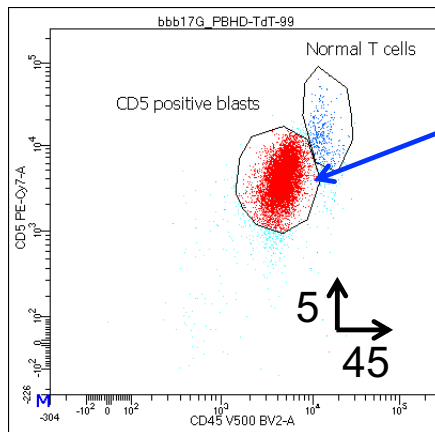
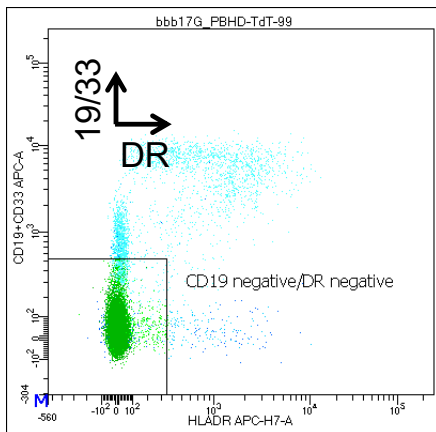
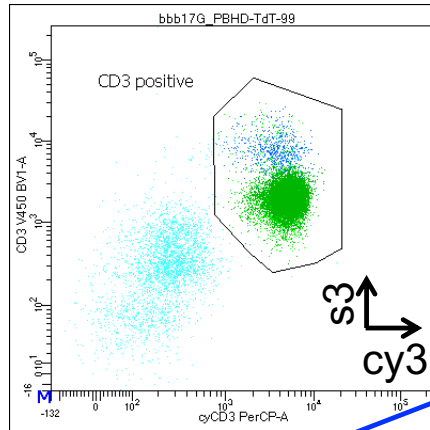
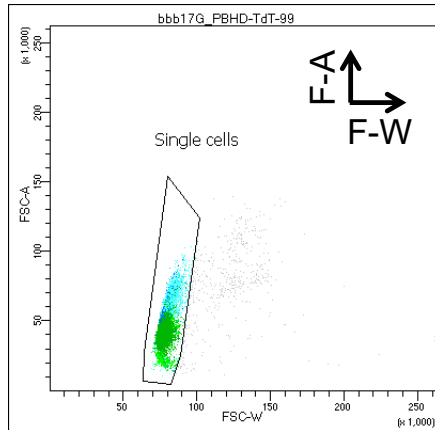
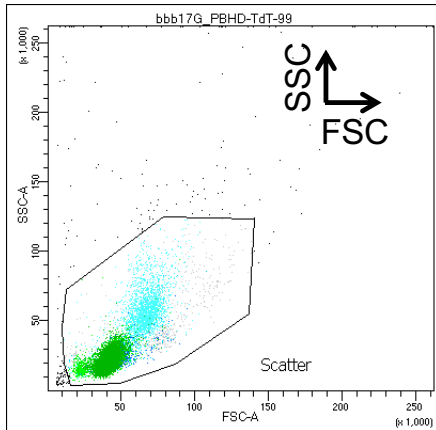
Patient 5 Diagnostic (PB)

Tube: TdT-99			
Population	#Events	%Parent	%Total
■ All Events	20,000	###	100.0
■ Scatter	19,775	98.9	98.9
■ Single cells	19,310	97.6	96.6
■ CD3 positive	16,152	83.6	80.8
■ CD19 negative/DR negative	15,914	98.5	79.6
■ CD5 positive blasts	14,802	93.0	74.0
■ CD99positive/TdT positive blasts	11,938	80.7	59.7
■ Normal T cells	657	4.1	3.3

Not catching all the blasts
with this gate

Subset are TdT negative

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



Patient 5 Diagnostic (PB)

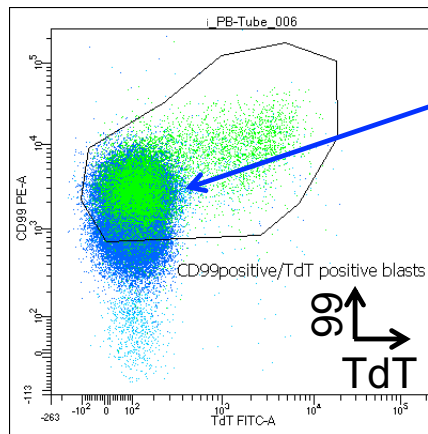
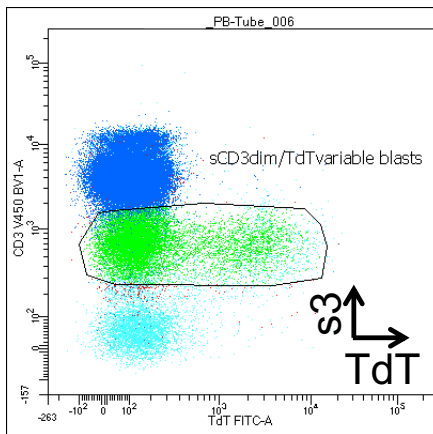
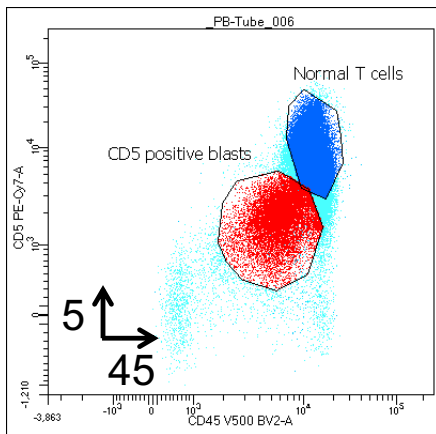
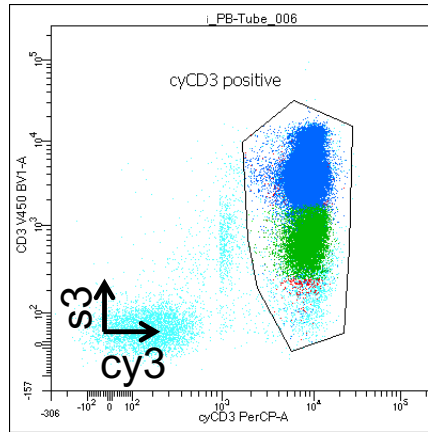
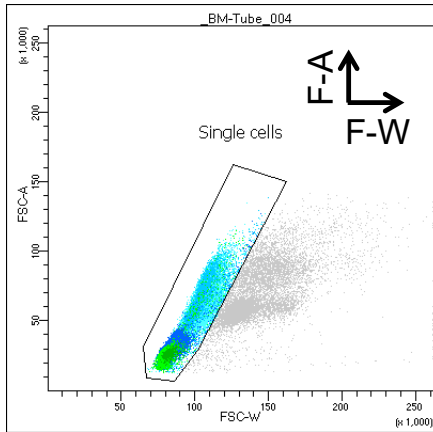
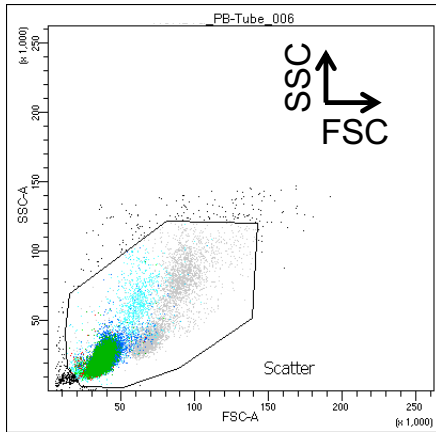
Tube: TdT-99			
Population	#Events	%Parent	%Total
■ All Events	20,000	###	100.0
■ Scatter	19,751	98.8	98.8
■ Single cells	19,325	97.8	96.6
■ CD3 positive	16,152	83.6	80.8
■ CD19 negative/DR negative	15,946	98.7	79.7
■ CD5 positive blasts	14,561	91.3	72.8
■ CD99positive/TdT positive blasts	11,702	80.4	58.5
■ Normal T cells	638	3.9	3.2

Look at the CD5dim/
CD45dim population

Subset are TdT **negative**

Surface CD3 dim/TdT var

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



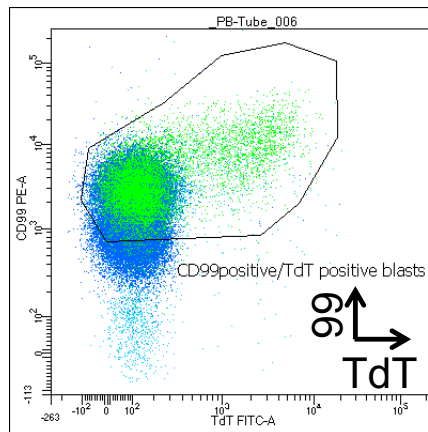
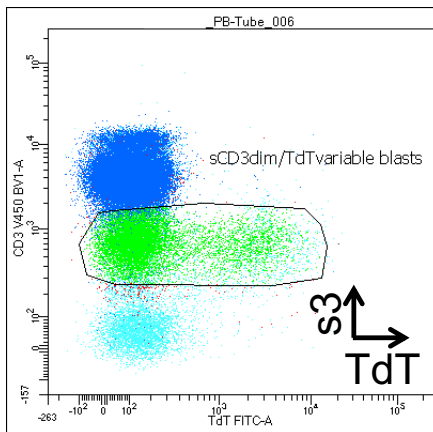
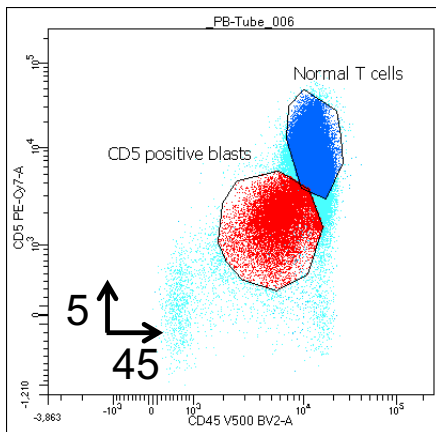
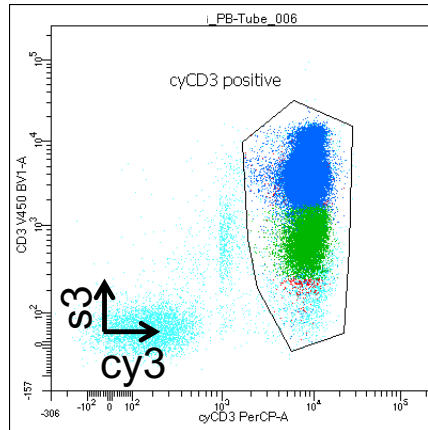
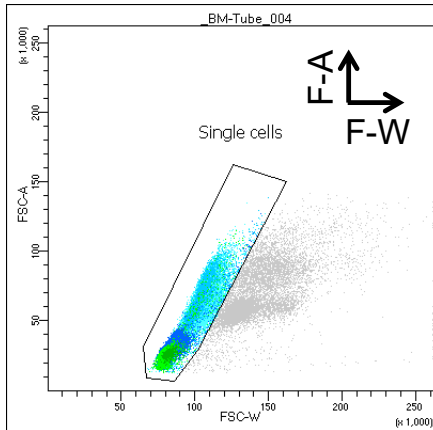
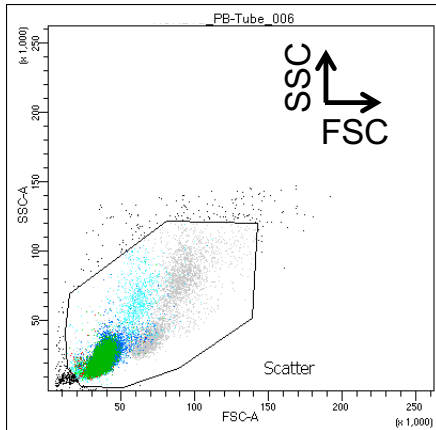
Patient 5 Day 8 (PB)

Tube: Tube_006			
Population	#Events	%Parent	%Total
■ All Events	273,682	###	100.0
■ Scatter	271,834	99.3	99.3
■ Single cells	256,105	94.2	93.6
■ cyCD3 positive	237,386	92.7	86.7
■ CD19 negative/DR negative	235,017	99.0	85.9
■ CD5 positive blasts	46,209	19.7	16.9
■ sCD3dim/TdT variable blasts	37,543	81.2	13.7
■ CD99positive/TdT positive blasts	36,688	97.7	13.4
■ Normal T cells	165,269	69.6	60.4

At day 8, most of blasts are now TdT negative (~85%), and substantial overlap with normal T cells

MRD positive (9.8%)

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	cyTdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



Patient 5 Day 8 (PB)

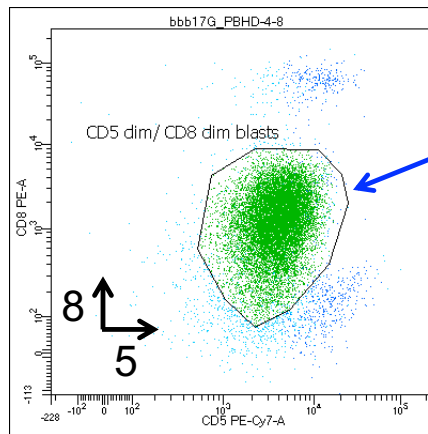
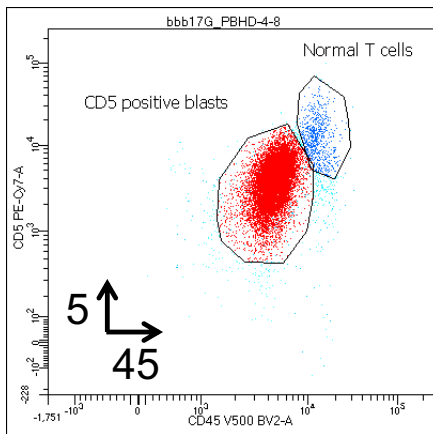
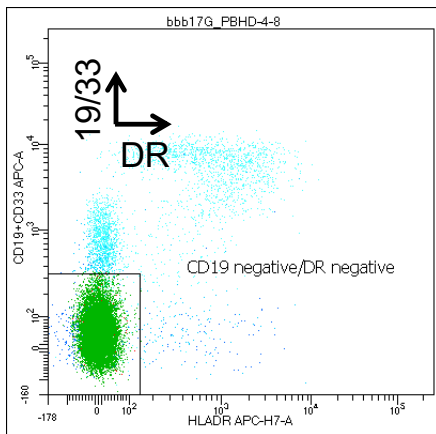
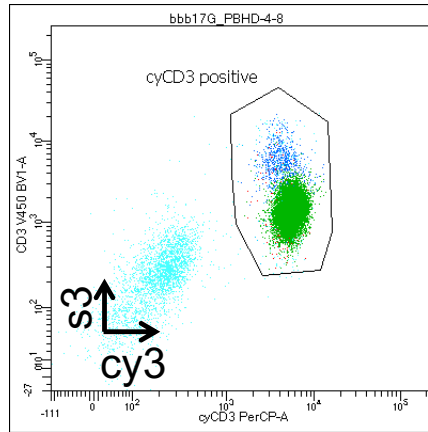
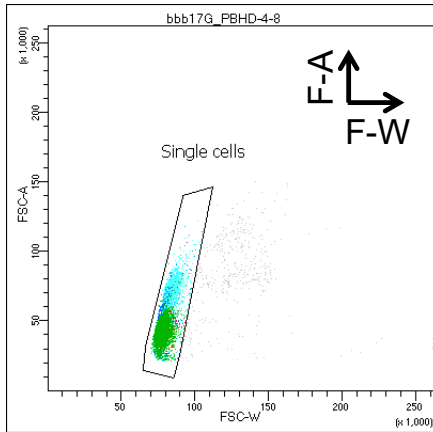
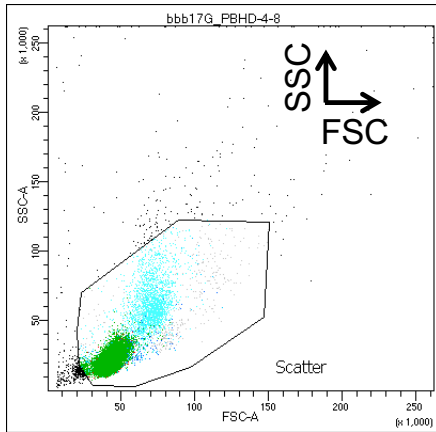
Tube: Tube_006			
Population	#Events	%Parent	%Total
■ All Events	273,682	###	100.0
■ Scatter	271,834	99.3	99.3
■ Single cells	256,105	94.2	93.6
■ cyCD3 positive	237,386	92.7	86.7
■ CD19 negative/DR negative	235,017	99.0	85.9
■ CD5 positive blasts	46,209	19.7	16.9
■ sCD3dim/TdT variable blasts	37,543	81.2	13.7
■ CD99positive/TdT positive blasts	36,688	97.7	13.4
■ Normal T cells	165,269	69.6	60.4

Switch to another tube as
the primary tube

CD4/CD8

This turned out to be a
good idea, as by day 15,
the blasts also lost the
bright CD99 subset

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

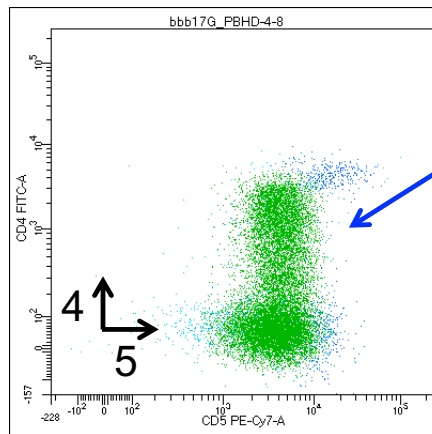
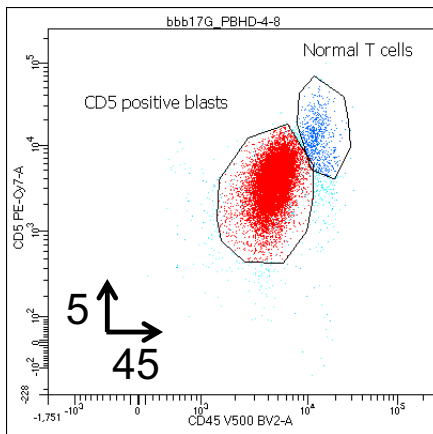
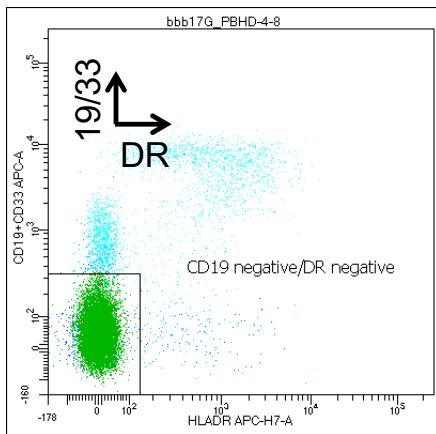
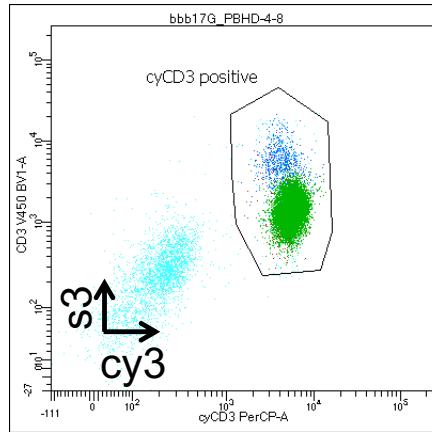
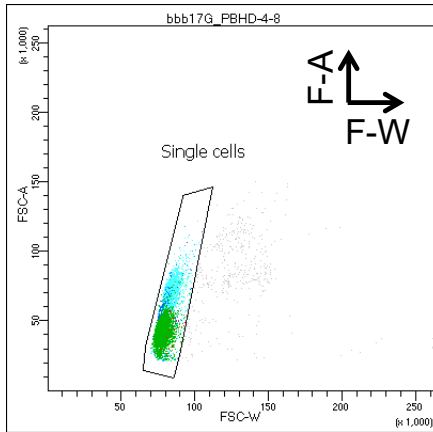
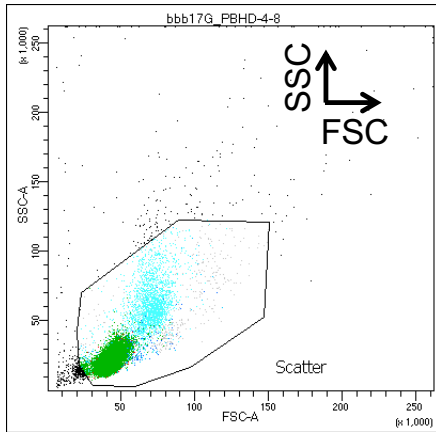


Patient 5 Diagnostic (PB)

Tube: 4-8			
Population	#Events	%Parent	%Total
■ All Events	20,000	###	100.0
■ Scatter	19,372	96.9	96.9
■ Single cells	18,978	98.0	94.9
■ cyCD3 positive	16,164	85.2	80.8
■ CD19 negative/DR negative	15,802	97.8	79.0
■ CD5 positive blasts	14,847	94.0	74.2
■ CD5 dim/ CD8 dim blasts	14,359	96.7	71.8
■ Normal T cells	702	4.3	3.5

Blasts are cyCD3+, sCD3dim, CD45dim, CD5dim, and CD8 dim

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

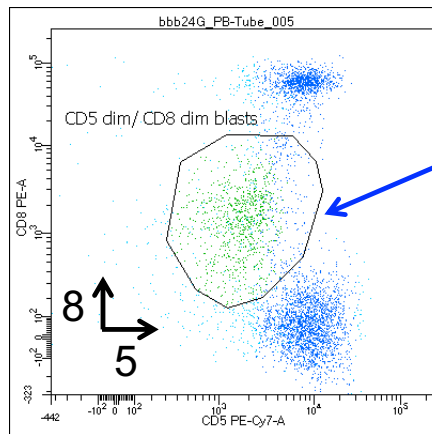
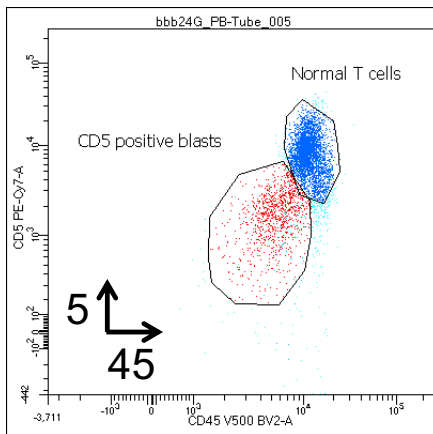
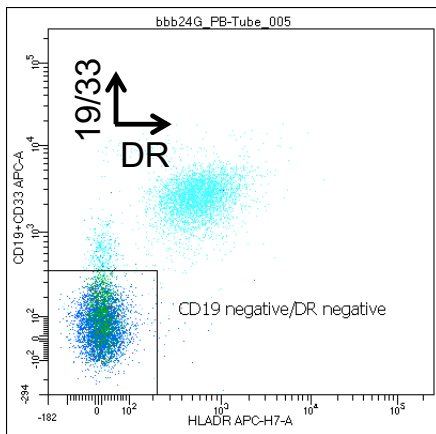
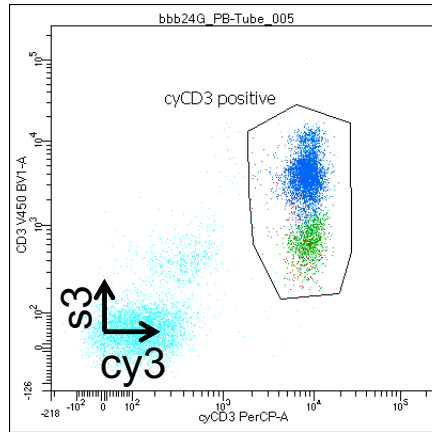
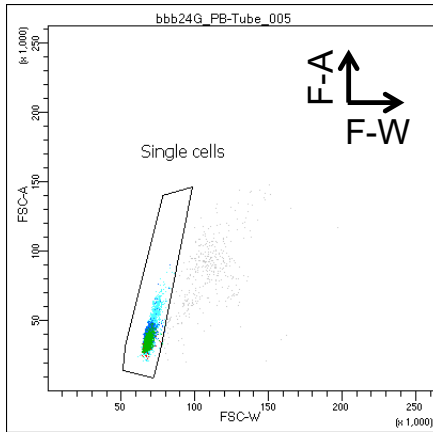
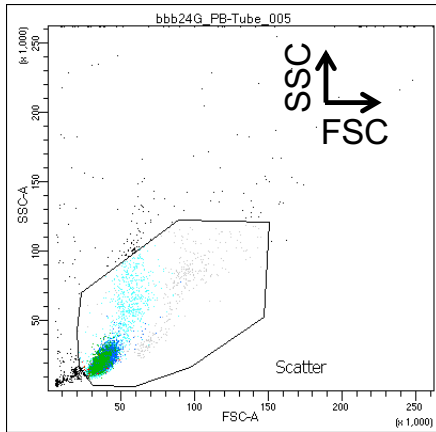


Patient 5 Diagnostic (PB)

Tube: 4-8			
Population	#Events	%Parent	%Total
■ All Events	20,000	###	100.0
□ Scatter	19,372	96.9	96.9
■ Single cells	18,978	98.0	94.9
■ cyCD3 positive	16,164	85.2	80.8
■ CD19 negative/DR negative	15,802	97.8	79.0
■ CD5 positive blasts	14,847	94.0	74.2
■ CD5 dim/ CD8 dim blasts	14,359	96.7	71.8
■ Normal T cells	702	4.3	3.5

Blasts are cyCD3+, sCD3dim, CD45dim, CD5dim, CD8 dim, CD4 negative to positive

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

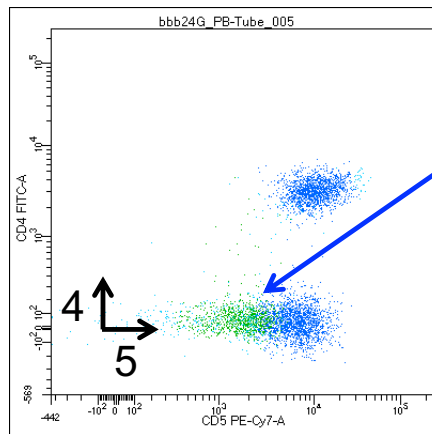
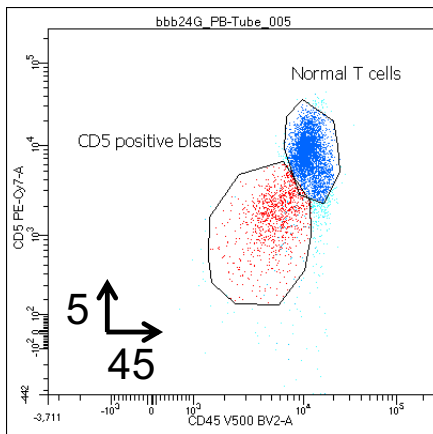
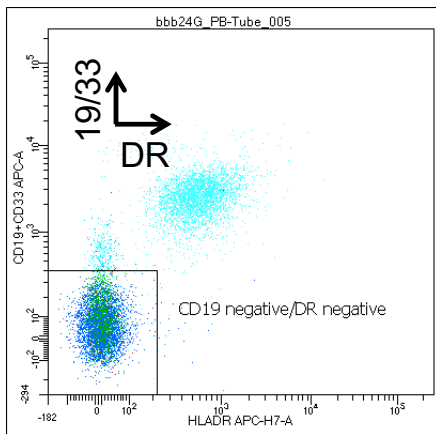
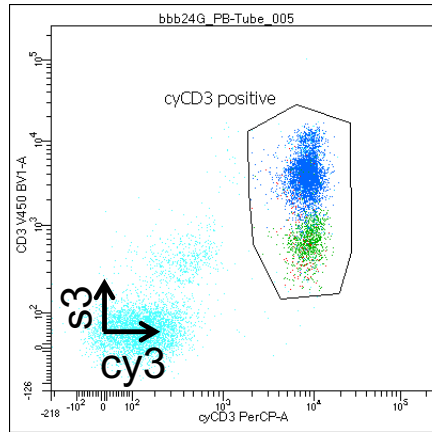
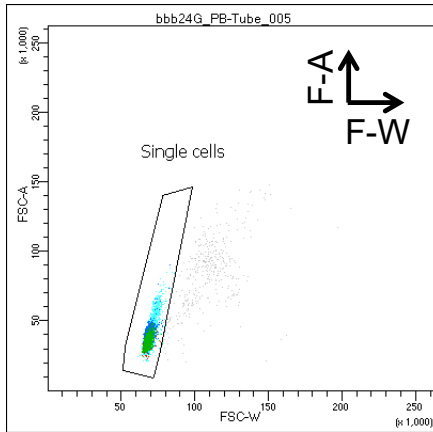
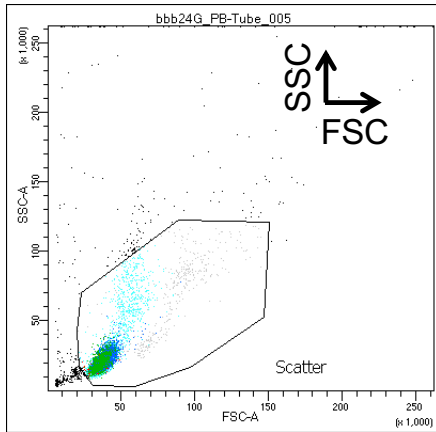


Patient 5 Day 8 (PB)

Tube: Tube_001			
Population	#Events	%Parent	%Total
All Events	10,000	###	100.0
Scatter	8,836	88.4	88.4
Single cells	8,211	92.9	82.1
cyCD3 positive	2,534	30.9	25.3
CD19 negative/DR negative	2,357	93.0	23.6
CD5 positive blasts	1,021	43.3	10.2
CD5 dim/ CD8 dim blasts	318	31.1	3.2
Normal T cells	914	36.1	9.1

Blasts are cyCD3+, sCD3dim, CD45dim, CD5dim, and remain CD8 dim

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

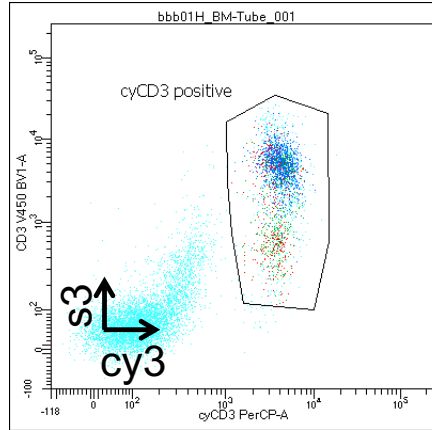
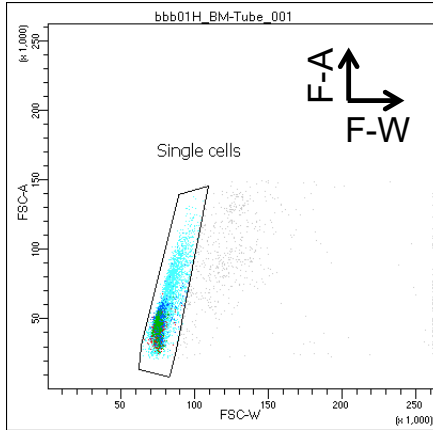
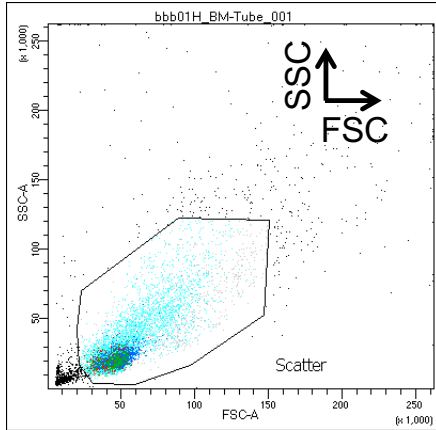


Patient 5 Day 8 (PB)

Tube: Tube_001			
Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ Scatter	8,836	88.4	88.4
■ Single cells	8,211	92.9	82.1
■ cyCD3 positive	2,534	30.9	25.3
■ CD19 negative/DR negative	2,357	93.0	23.6
■ CD5 positive blasts	1,021	43.3	10.2
■ CD5 dim/CD8 dim blasts	318	31.1	3.2
■ Normal T cells	914	36.1	9.1

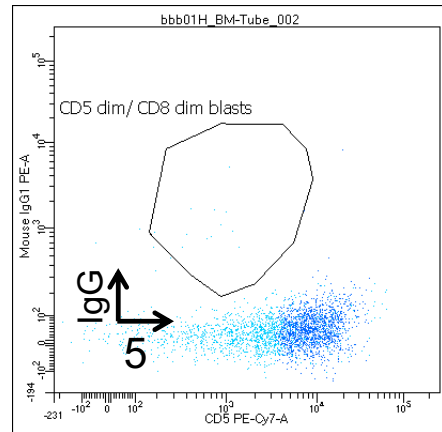
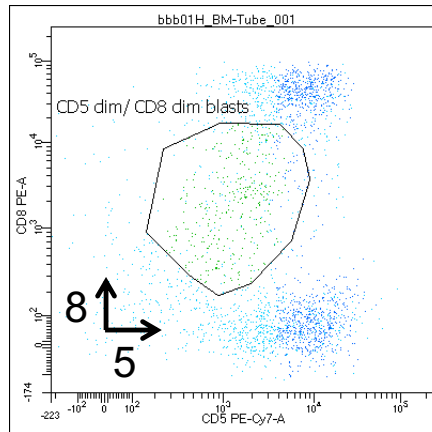
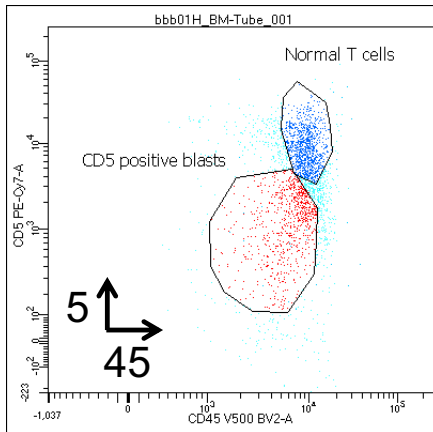
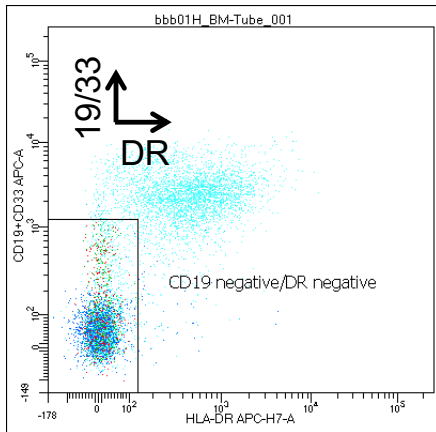
Blasts are cyCD3+, sCD3dim, CD45dim, CD5dim, CD8 dim, but have now lost CD4 expression

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



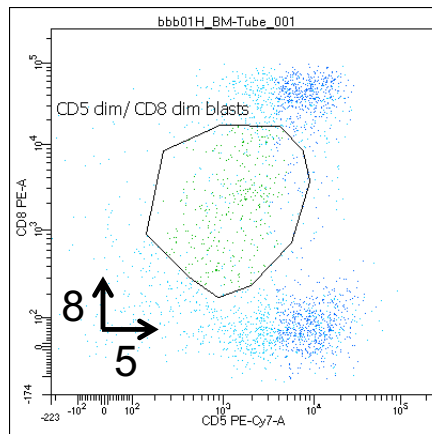
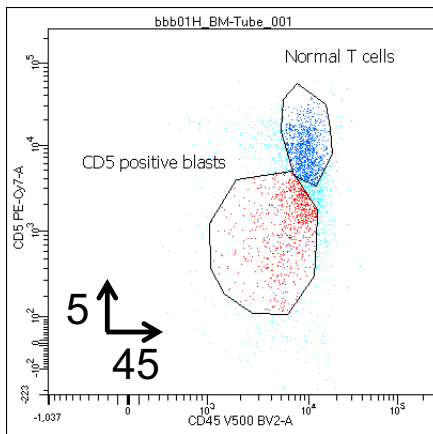
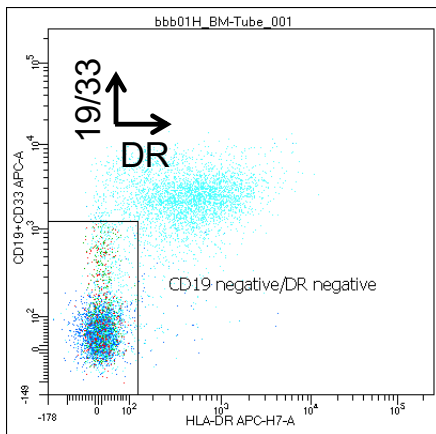
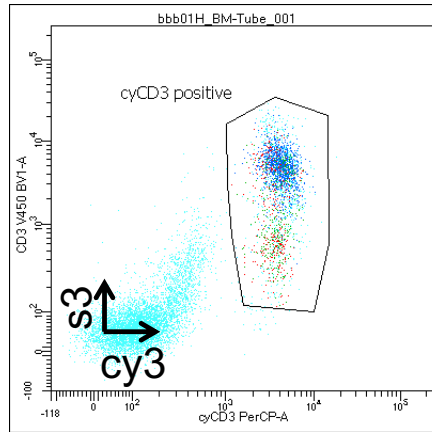
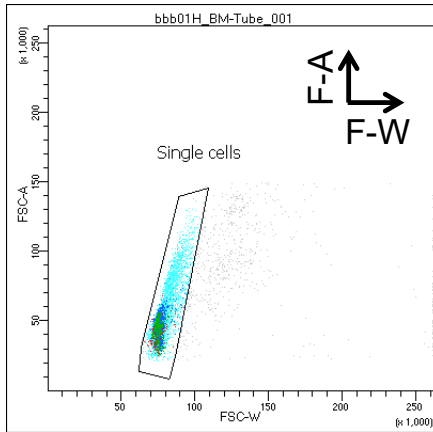
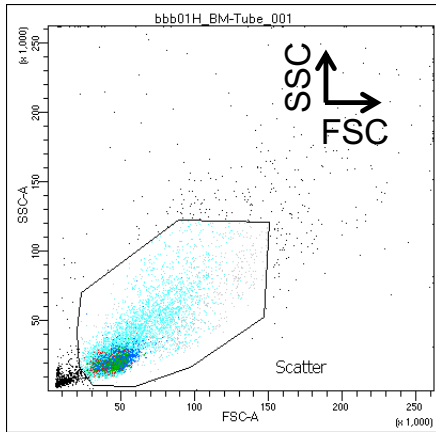
Tube: Tube_001

Population	#Events	%Parent	%Total
All Events	10,000	###	100.0
Scatter	8,836	88.4	88.4
Single cells	8,211	92.9	82.1
cyCD3 positive	2,534	30.9	25.3
CD19 negative/DR negative	2,467	97.4	24.7
CD5 positive blasts	768	31.1	7.7
CD5 dim/ CD8 dim blasts	313	40.8	3.1
Normal T cells	977	38.6	9.8



Patient 5
Day 15 (BM)

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



Patient 5 Day 15 (BM)

Tube: Tube_001			
Population	#Events	%Parent	%Total
All Events	10,000	###	100.0
Scatter	8,836	88.4	88.4
Single cells	8,211	92.9	82.1
cyCD3 positive	2,534	30.9	25.3
CD19 negative/DR negative	2,467	97.4	24.7
CD5 positive blasts	768	31.1	7.7
CD5 dim/CD8 dim blasts	313	40.8	3.1
Normal T cells	977	38.6	9.8

Blasts are cyCD3+, sCD3dim, CD45dim, CD5dim, CD8 dim

MRD positive (3.6%)

Gating Strategies - Examples

Start with four simple cases that are easy to follow at later treatment stages

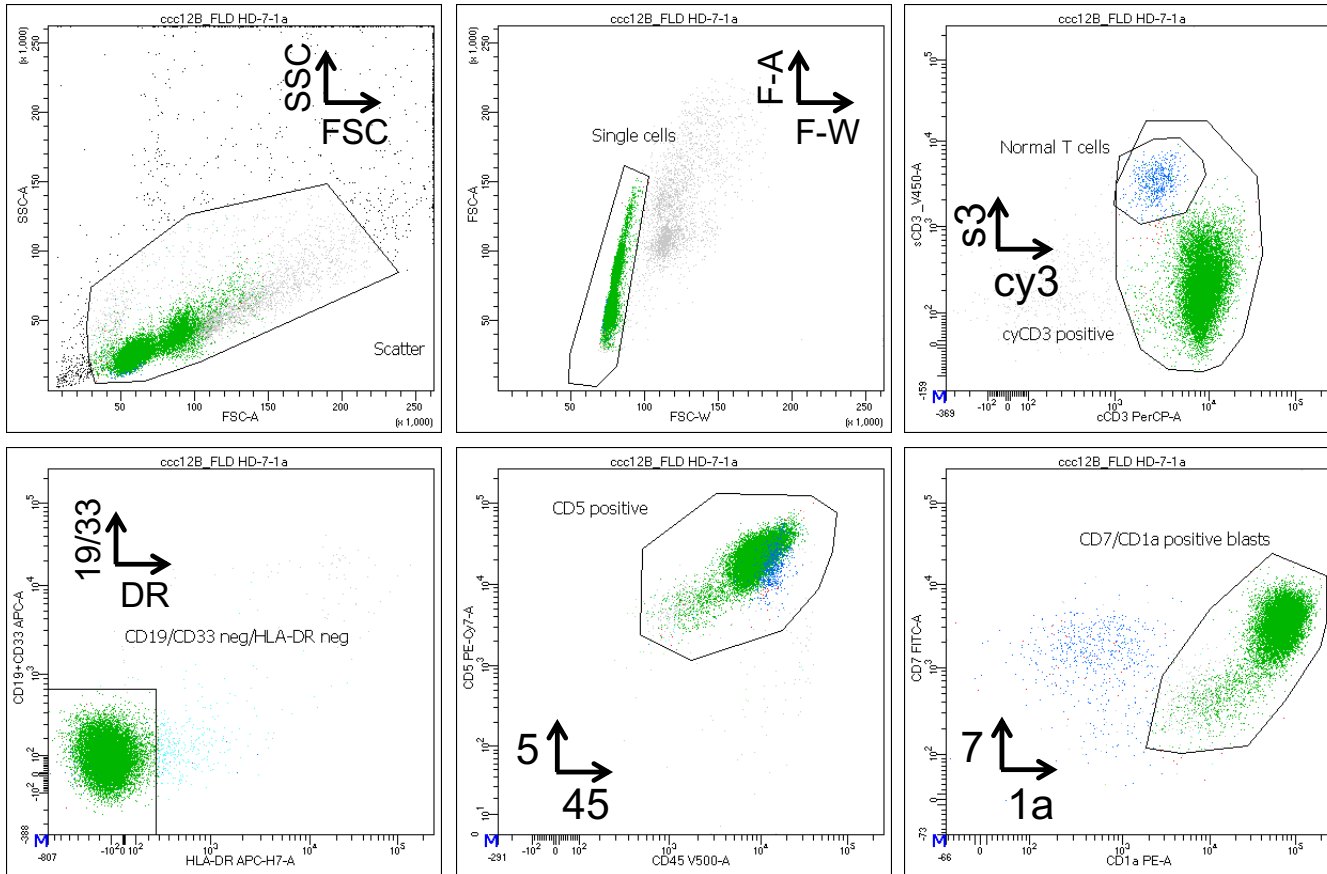
Move on to progressively more difficult cases

- loss of aberrant marker expression with treatment
- aberrantly low expression on blast population becomes more like normal with treatment

Note 1: Expression of candidate markers to follow the leukemia can change quickly with treatment = when cells are available, run multiple tubes, particularly in the early weeks of treatment

Patient 6 Relapse T-ALL

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



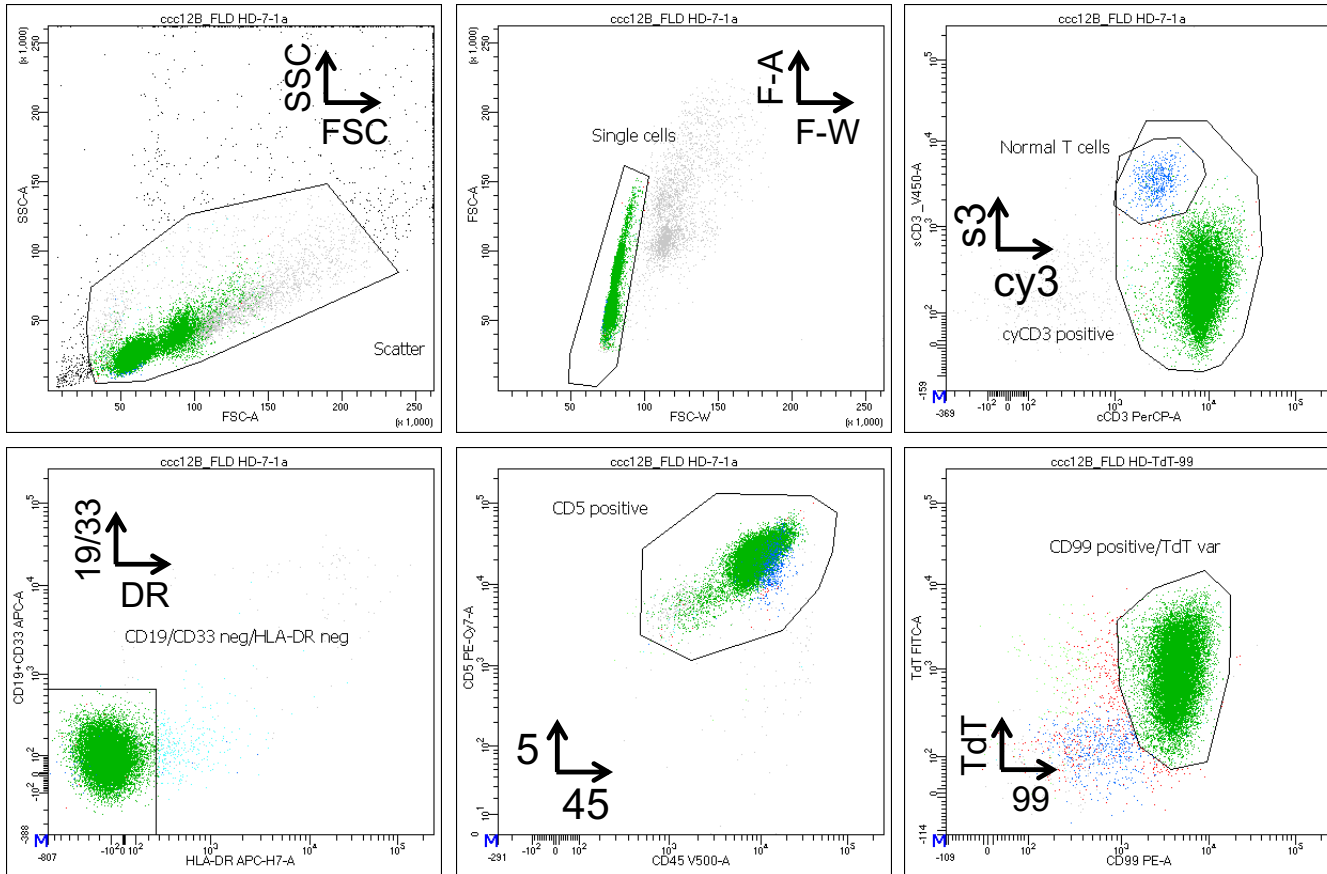
Patient 6 Dx (pleural fluid)

Tube: 7-1a			
Population	#Events	%Parent	%Total
■ All Events	20,000	###	100.0
■ Scatter	18,972	94.9	94.9
■ Single cells	16,353	86.2	81.8
■ cyCD3 positive	15,966	97.6	79.8
■ CD19/CD33 neg/HLA-DR neg	15,678	98.2	78.4
■ CD5 positive	15,667	99.9	78.3
■ CD7/CD1a positive blasts	15,100	96.4	75.5
■ Normal T cells	567	3.6	2.8

Blasts are cyCD3+, sCD3dim, CD45+, CD5+, CD19/CD33-, and HLA-DR negative, CD1a bright and CD7+

Should be simple to follow with this panel

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
1	TdT	CD99	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

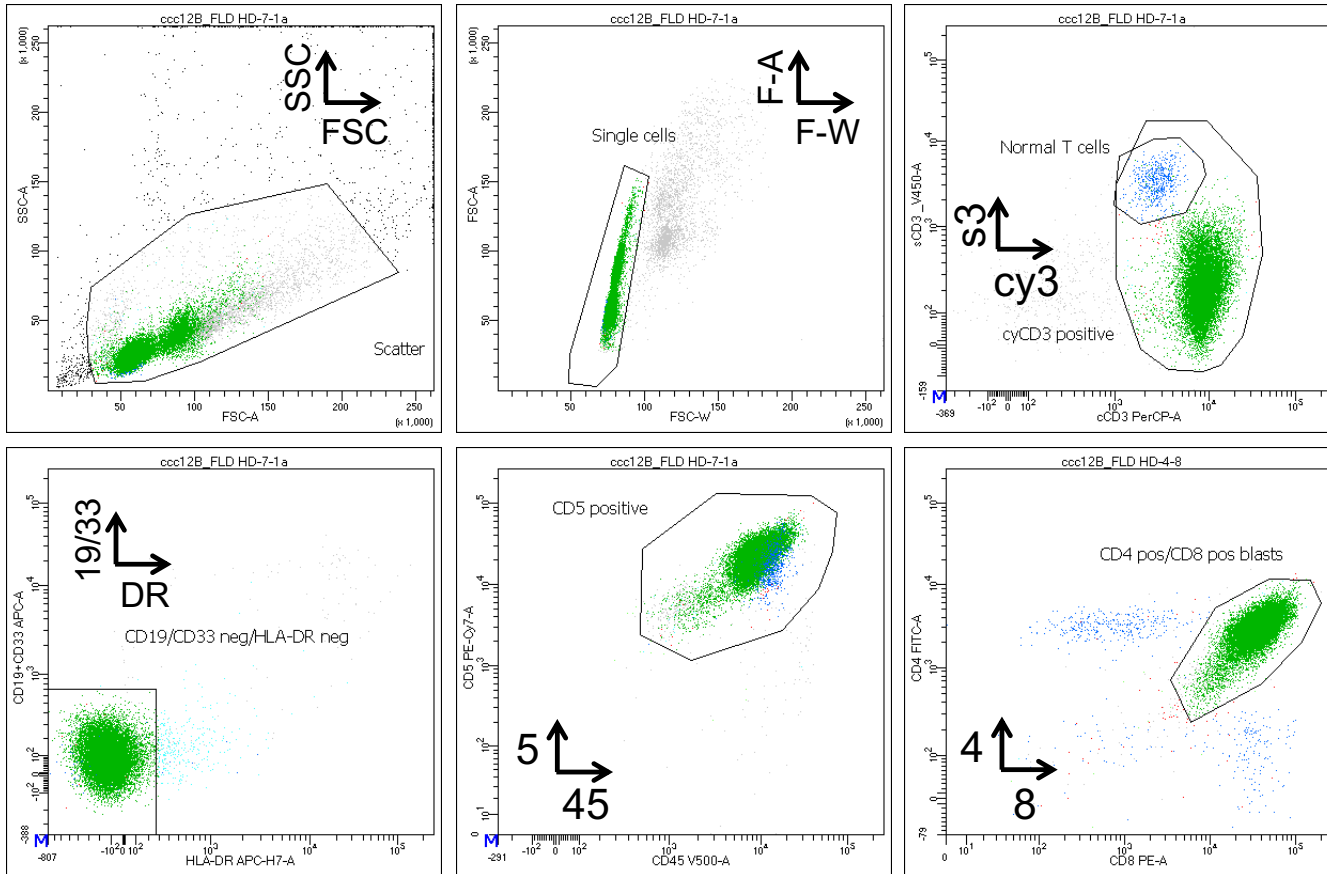


Patient 6 Dx (pleural fluid)

Tube: 7-1a			
Population	#Events	%Parent	%Total
■ All Events	20,000	###	100.0
■ Scatter	18,972	94.9	94.9
■ Single cells	16,353	86.2	81.8
■ cyCD3 positive	15,966	97.6	79.8
■ CD19/CD33 neg/HLA-DR neg	15,678	98.2	78.4
■ CD5 positive	15,667	99.9	78.3
■ CD7/CD1a positive blasts	15,100	96.4	75.5
■ Normal T cells	567	3.6	2.8

Blasts are cyCD3+, sCD3dim, CD45+, CD5+, CD19/CD33-, and HLA-DR negative, CD1a bright and CD7+, also CD99 positive and TdT variable

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



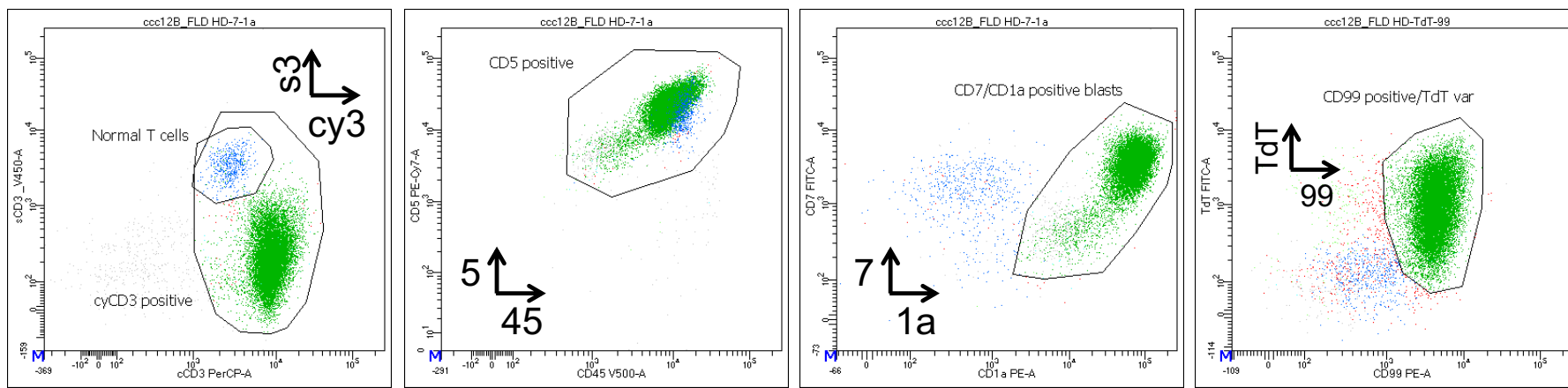
Patient 6 Dx (pleural fluid)

Tube: 4-8			
Population	#Events	%Parent	%Total
■ All Events	20,000	###	100.0
□ Scatter	19,236	96.2	96.2
□ Single cells	18,476	96.0	92.4
□ cyCD3 positive	18,192	98.5	91.0
□ CD19/CD33 neg/HLA-DR neg	17,989	98.9	89.9
□ CD5 positive	17,949	99.8	89.7
□ CD4 pos/CD8 pos blasts	17,373	96.8	86.9
□ Normal T cells	538	3.0	2.7

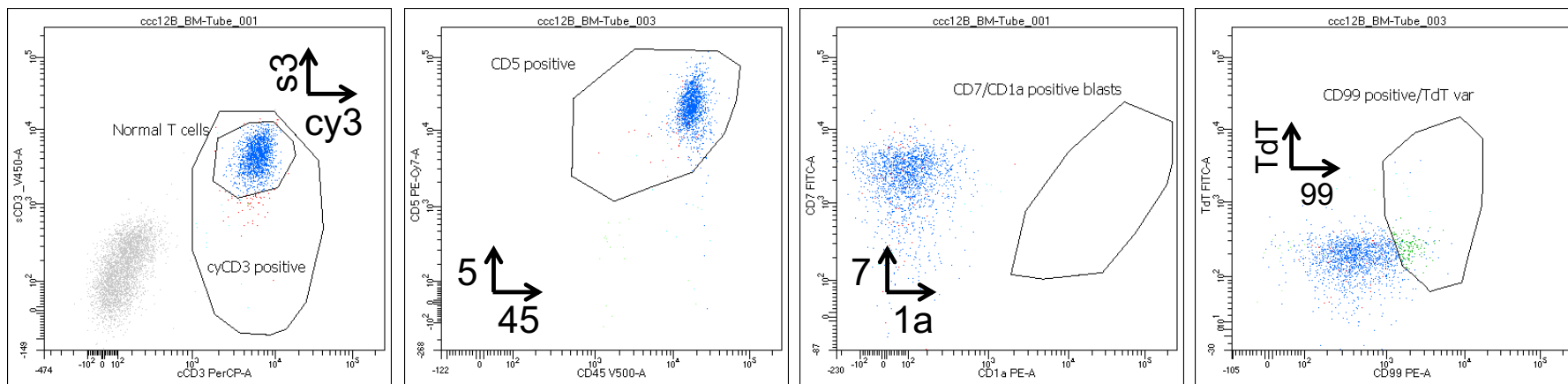
Blasts are cyCD3+, sCD3dim, CD45+, CD5+, CD19/CD33-, and HLA-DR negative, CD1a bright and CD7+, CD99 positive, TdT variable, CD4 positive & CD8 positive

Patient 6

Dx (pleural fluid)

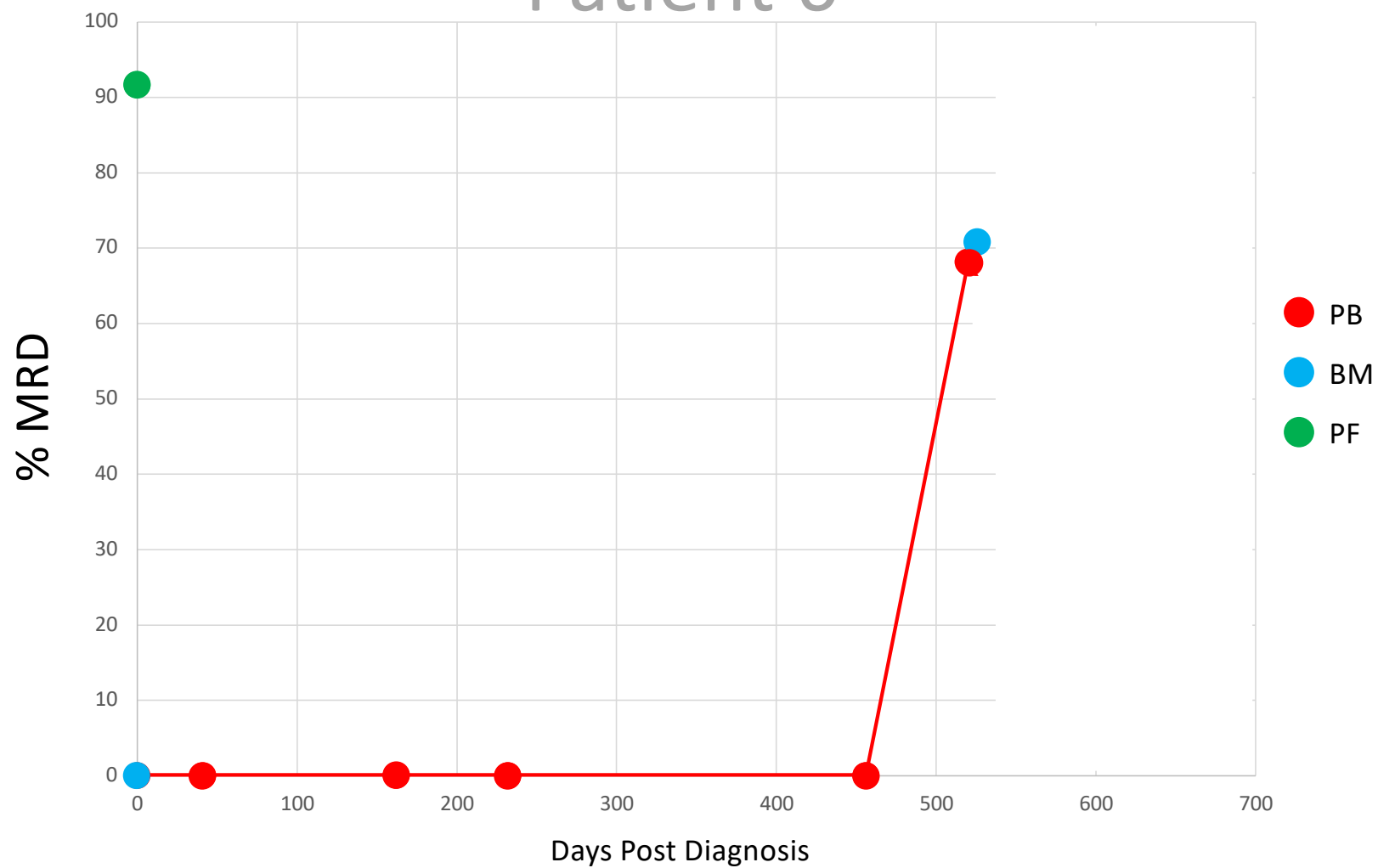


Dx (BM)



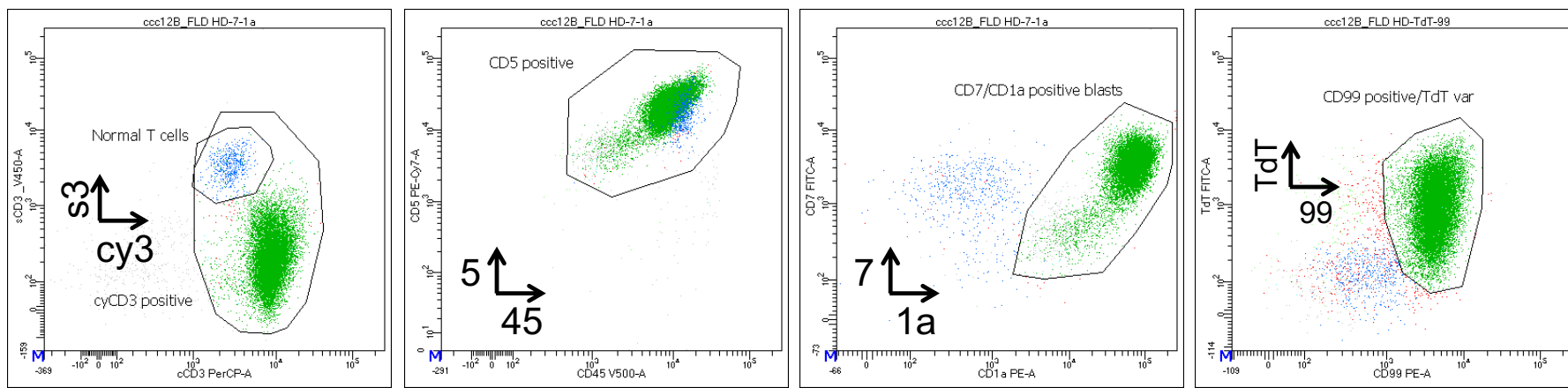
No evidence of disease in bone marrow at diagnosis (Day 0)

Patient 6

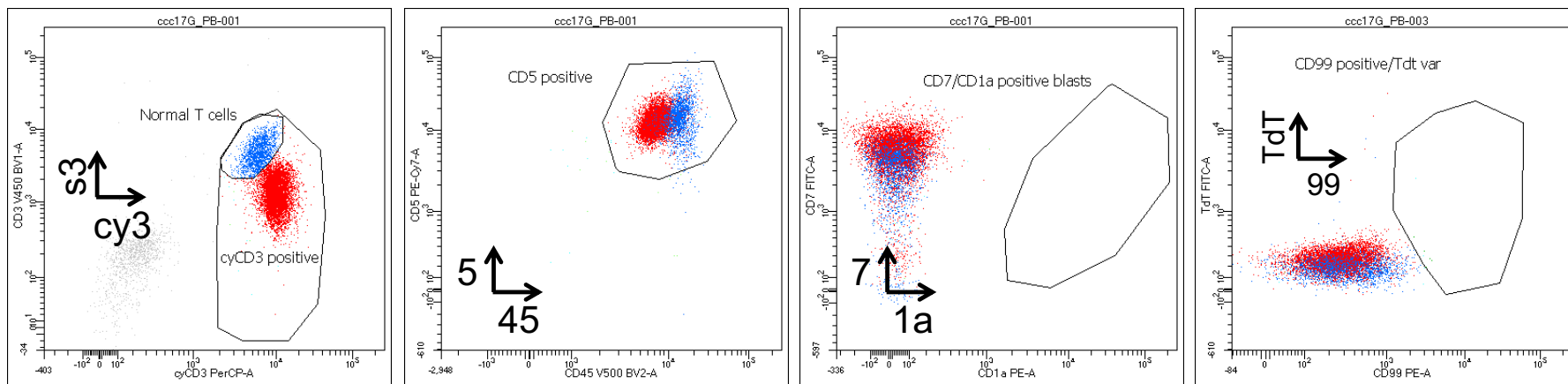


Patient 6

Dx (pleural fluid)



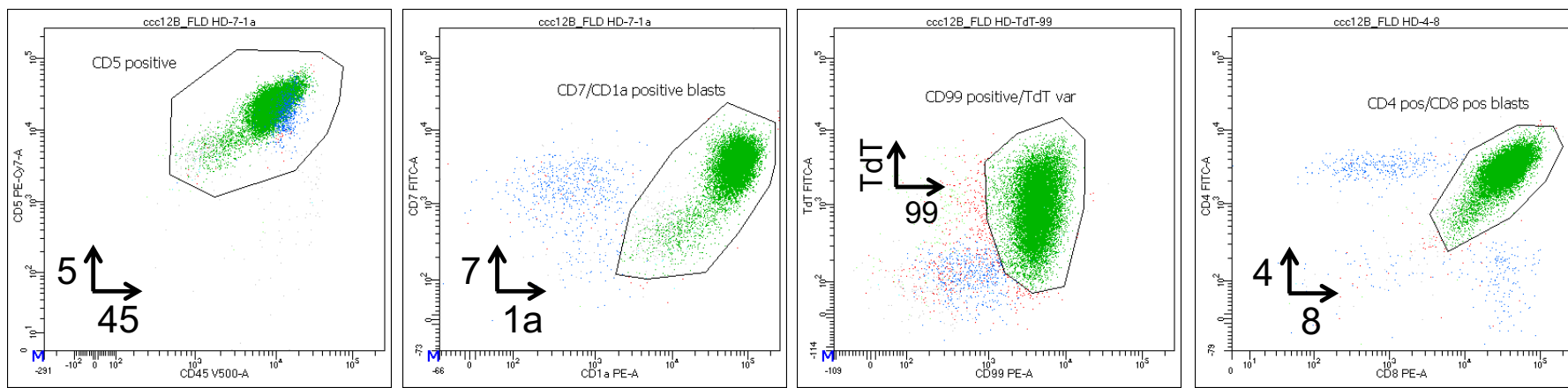
Relapse (PB)



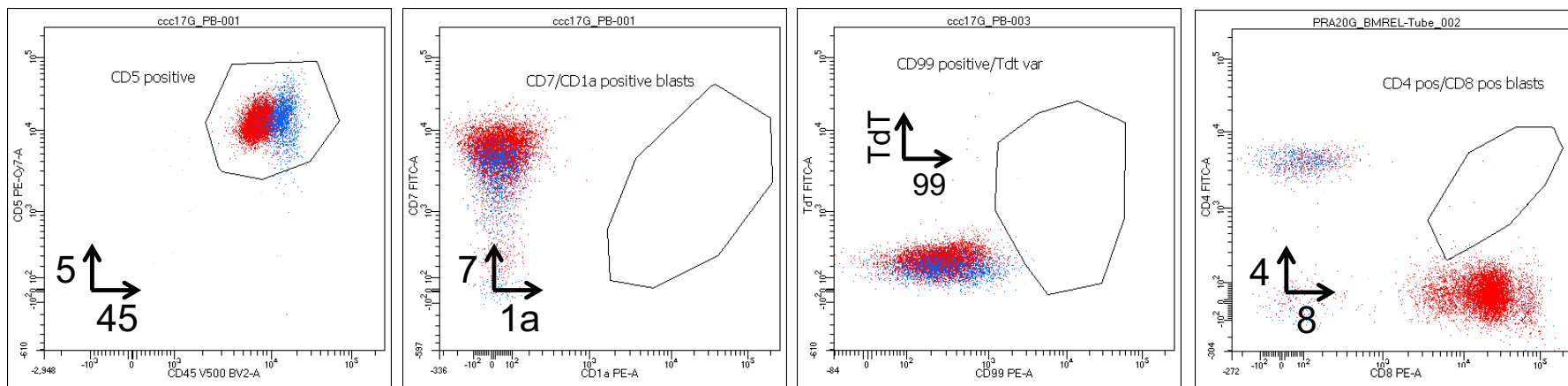
Phenotypic shift at relapse (Day 520)

Patient 6

Dx (pleural fluid)

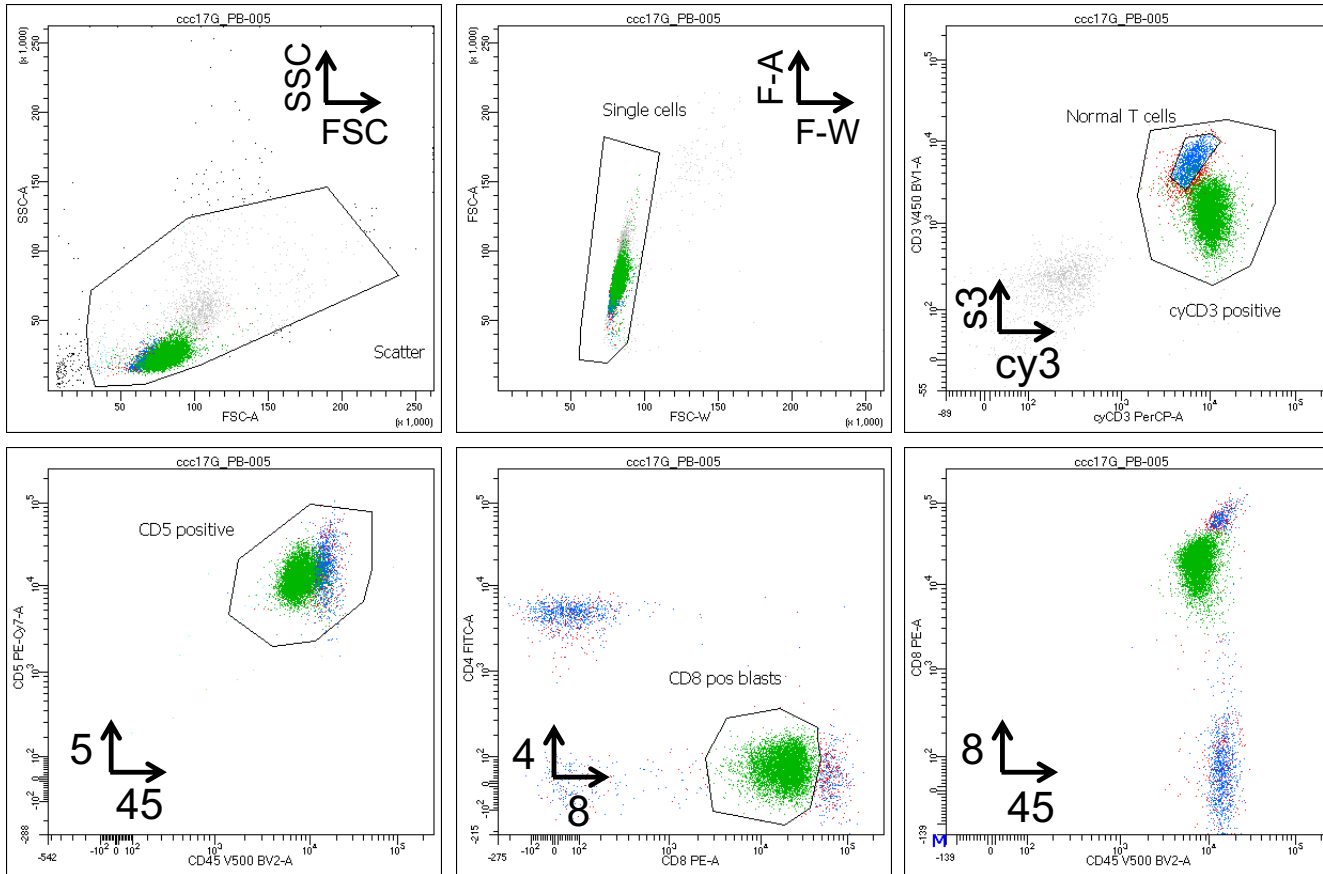


Relapse (PB)



Phenotypic shift at relapse (Day 520)

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
5	CD4	CD8	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



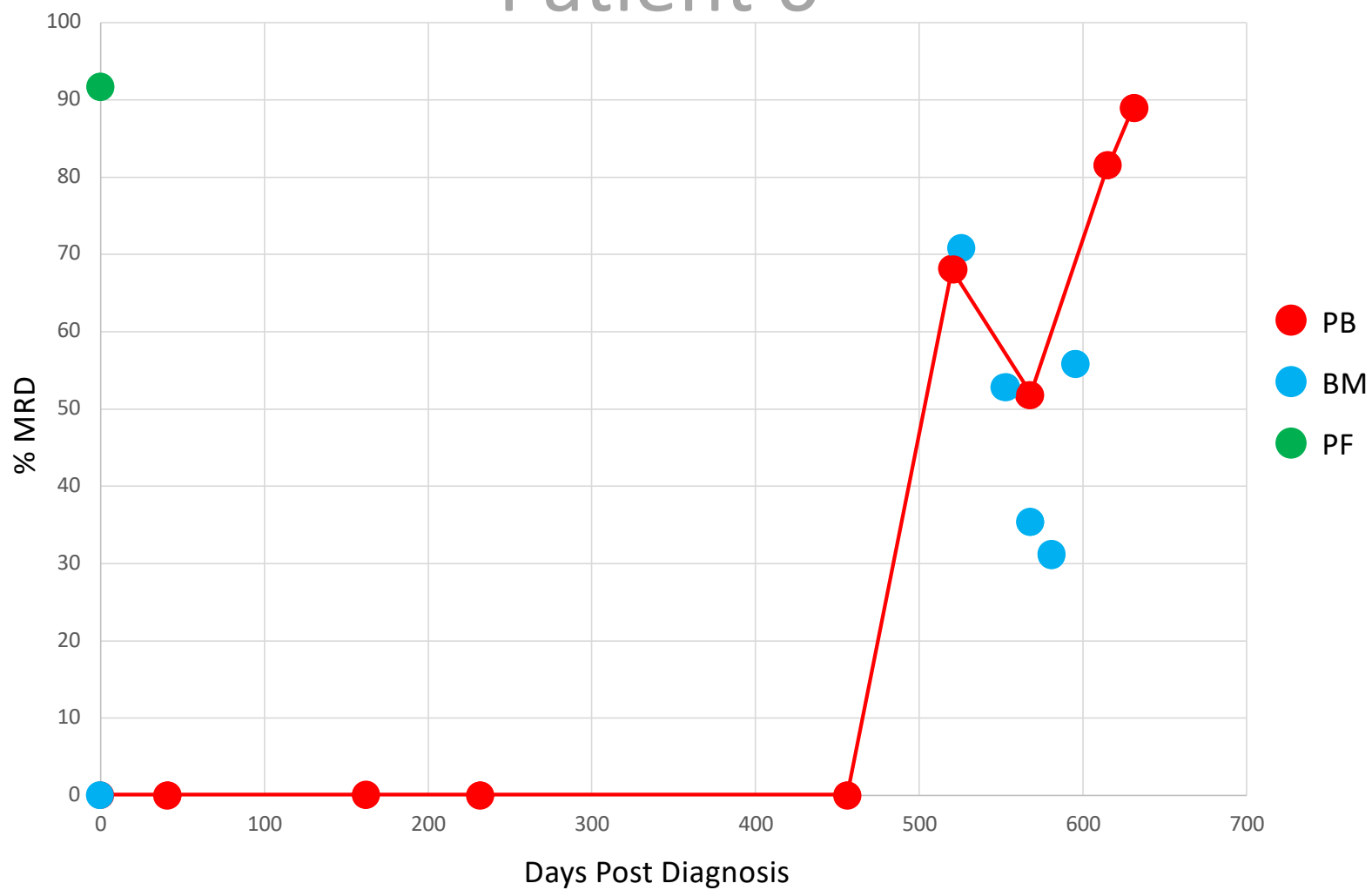
Patient 6 Relapse (PB)

Tube: 005			
Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
□ Scatter	9,760	97.6	97.6
□ Single cells	9,614	98.5	96.1
□ cyCD3 positive	8,498	88.4	85.0
□ CD19/CD33 neg/HLA-DR neg	8,448	99.4	84.5
□ CD5 positive	8,424	99.7	84.2
□ CD8 pos blasts	6,755	80.2	67.6
□ Normal T cells	1,251	14.7	12.5

At relapse, blasts are now cyCD3+, sCD3dim, CD45+, CD5+, CD4 negative, CD8+

Using a CD8 vs CD45 plot can help, but still only useful when blast percentage is high

Patient 6



Gating Strategies - Examples

Start with four simple cases that are easy to follow at later treatment stages

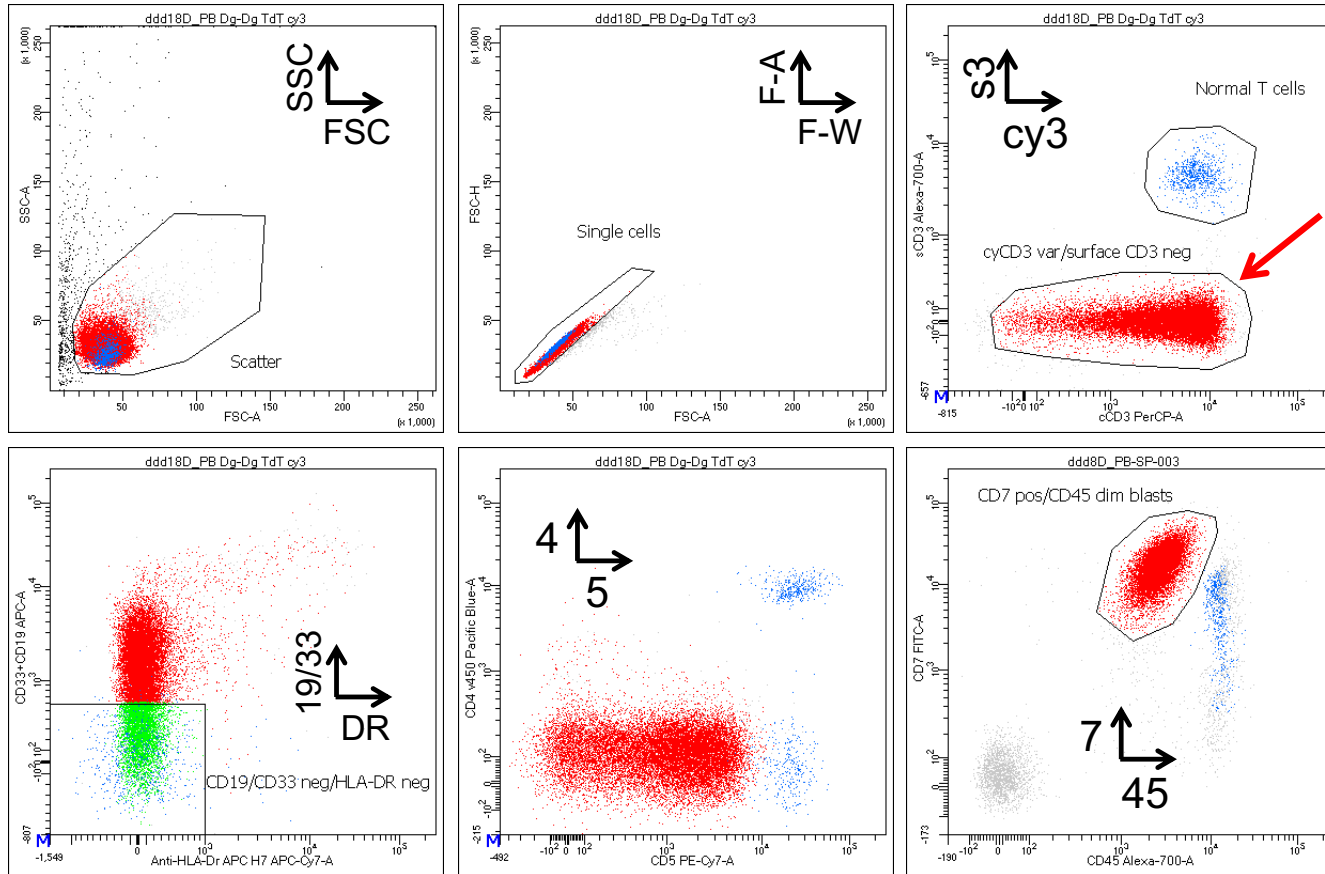
Move on to progressively more difficult cases

- loss of aberrant marker expression with treatment
- aberrantly low expression on blast population becomes more like normal with treatment

Note 2: Upon relapse in T-ALL, anything can happen! Much more so than is usually observed in B-ALLs or AMLs

Patient 7 CD7+/CD34+

An interesting case



Patient 7 Diagnostic (PB)

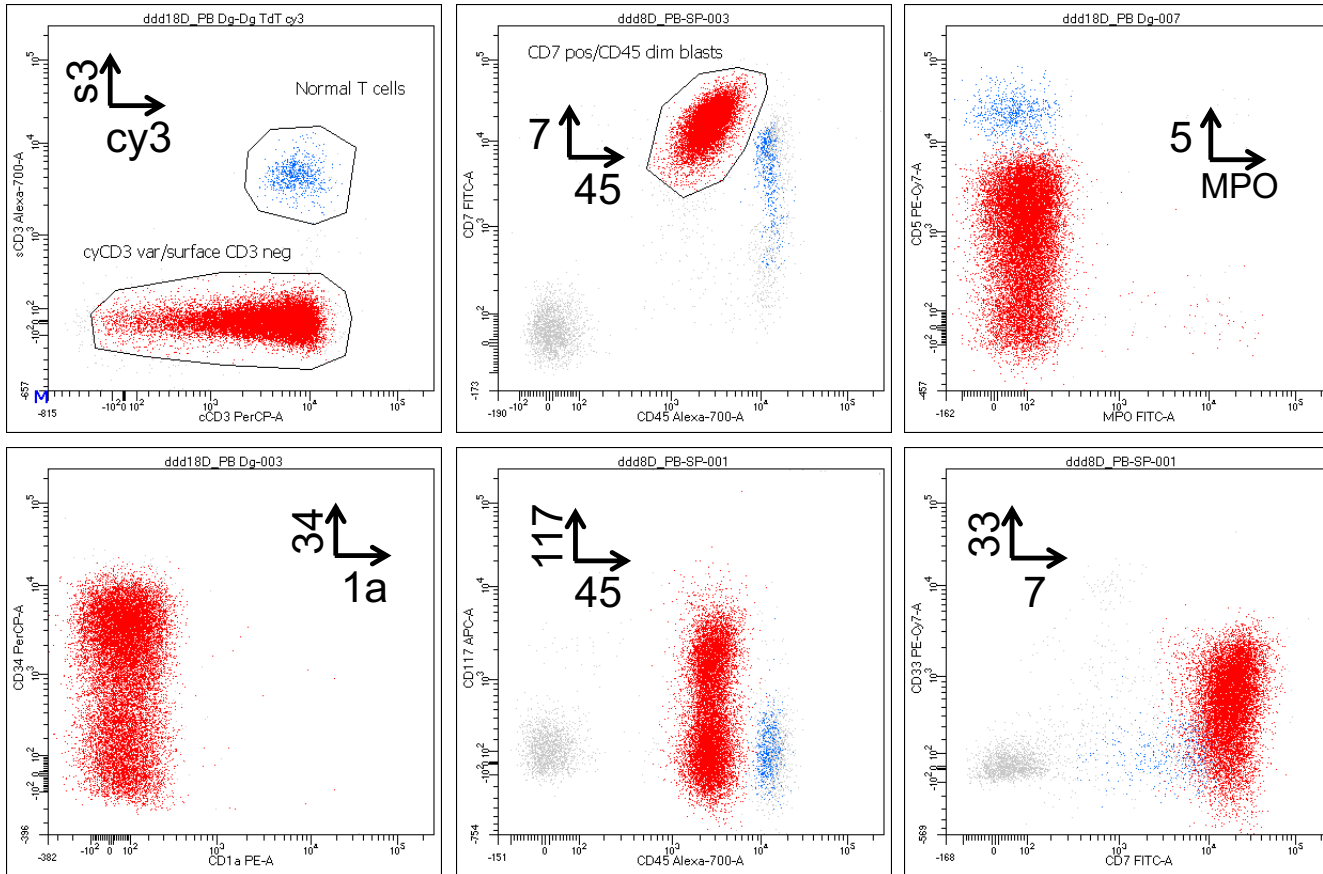
Tube: Dg TdT cy3

Population	#Events	%Parent	%Total
All Events	20,000	###	100.0
Scatter	18,236	96.2	96.2
Single cells	18,794	97.7	94.0
cyCD3 var/surface CD3 neg	17,971	95.6	89.9
CD19/CD33 neg/HLA-DR neg	4,757	26.5	23.8
Normal T cells	686	3.7	3.4

Blasts are cyCD3var, sCD3-, CD45dim, TdT negative, CD19 or CD33 var, and HLA-DR negative, CD4 negative, CD5 variable, CD7 bright

Need more information

An interesting case



Patient 7 Diagnostic (PB)

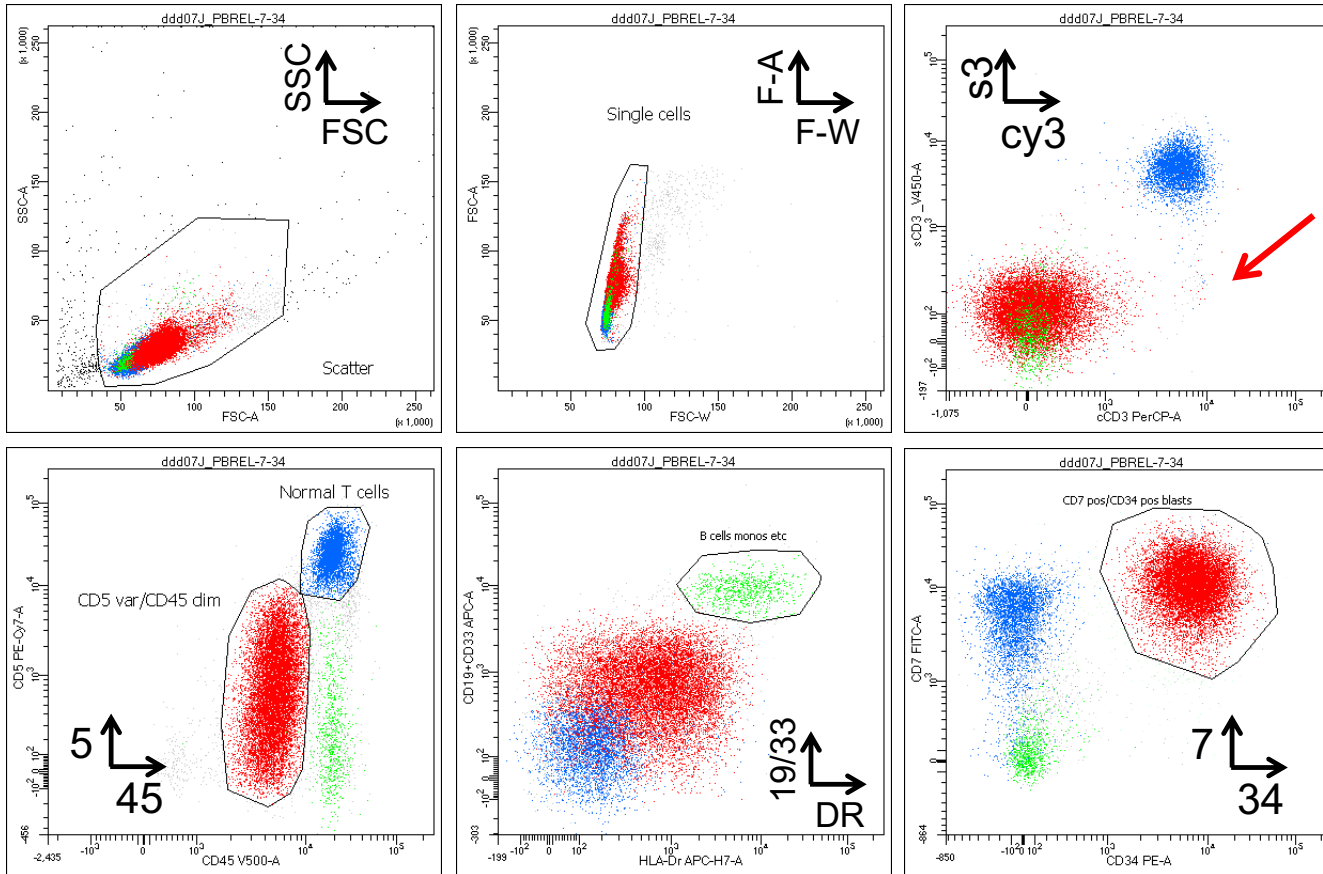
Tube: Dg TdT cy3			
Population	#Events	%Parent	%Total
■ All Events	20,000	###	100.0
□ Scatter	18,236	96.2	96.2
□ Single cells	18,794	97.7	94.0
■ cyCD3 var/surface CD3 neg	17,971	95.6	89.9
■ CD19/CD33 neg/HLA-DR neg	4,757	26.5	23.8
■ Normal T cells	686	3.7	3.4

Blasts are cyCD3var, sCD3-, CD45dim, TdT-, **CD33 dim**, HLA-DR-, CD4-, CD5 variable, CD7 bright, MPO negative, CD34 var, CD117 var, CD11b var, CD15 var

Early T cell precursor = ETP

Early T cell precursor = ETP

Patient 7 Relapse (PB)

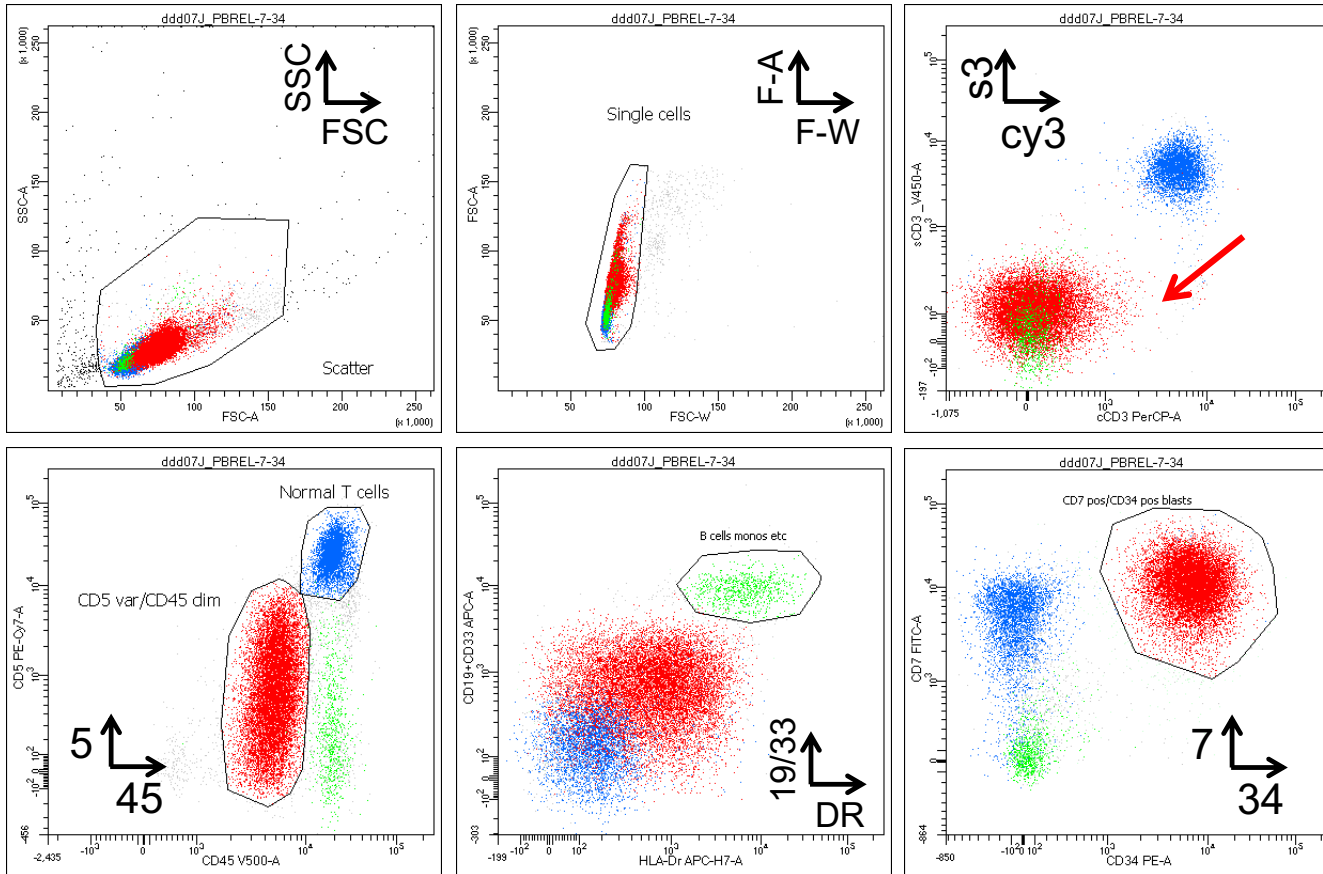


Tube: 7-34

Population	#Events	%Parent	%Total
All Events	20,000	###	100.0
Scatter	19,538	97.7	97.7
Single cells	19,117	97.8	95.6
Normal T cells	4,387	22.9	21.9
CD5 var/CD45 dim	12,952	67.8	64.8
CD7 pos/CD34 pos blasts	12,651	97.7	63.3
B cells monos etc	789	4.1	3.9

Upon relapse, blasts are now sCD3-, cyCD3 **negative**, CD45dim, CD34 uniformly positive, CD7 bright, CD33 var, HLA-DR dim

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
4	CD7	CD34	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



Patient 7 Relapse (PB)

Upon relapse, blasts are now sCD3-, cyCD3 negative, CD45dim, CD34 uniformly positive, CD7 bright, CD33 var, HLA-DR dim

For this relapse, we can follow with **CD7/CD34** tube

Early T cell precursor ALL = ETP-ALL

ETP blasts typically express:

CD2, CD7, cyCD3 and may express CD4

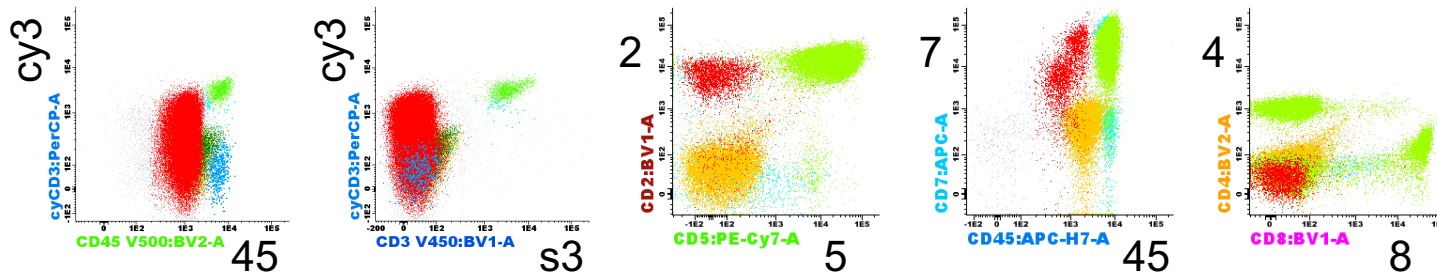
Positive for one or more stem cell and/or myeloid antigens:

CD117, CD34, HLA-DR, CD13, CD33, CD11b, and/or CD65

Negative for:

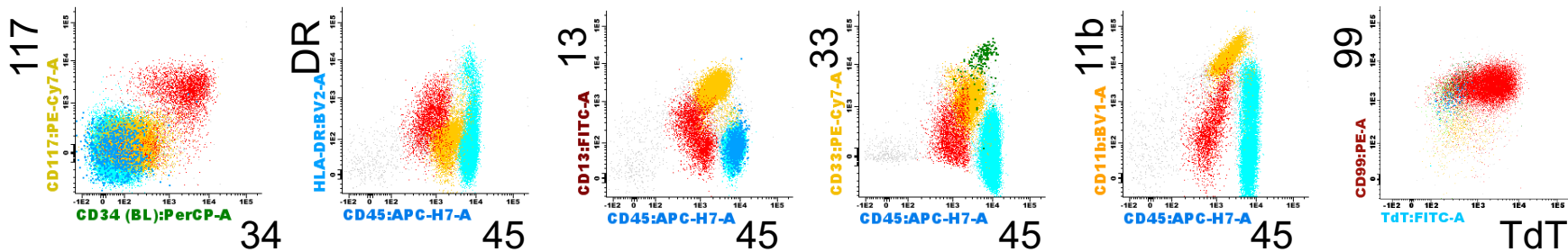
CD1a-, CD8-, CD5-/weak

ETP blasts typically express: **CD2**, **CD7**, **cyCD3** and may express **CD4**

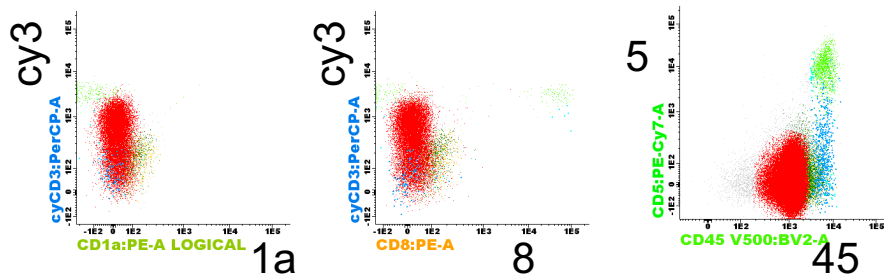


ETP-ALL

Positive for one or more stem cell and/or myeloid antigens: **CD117**, **CD34**, **HLA-DR**, **CD13**, **CD33**, **CD11b**



Negative for: **CD1a-**, **CD8-**, **CD5-/weak**



Gating Strategies - Examples

Start with four simple cases that are easy to follow at later treatment stages

Move on to progressively more difficult cases

- loss of aberrant marker expression with treatment
- aberrantly low expression on blast population becomes more like normal with treatment

Note 3: ETP can be very challenging to follow using standard T-ALL approach – at SJCRH we use the AML MRD strategy to follow ETP cases

At SJCRH, observed that CD7 was the most reliable marker for ETP (more than cyCD3), this was the major driver in our switch to the AML panel for all ETP MRDs.

Patient 8 CD7+/CD19+

Another interesting case ...

Patient 8

Patient presented with mediastinal mass ...

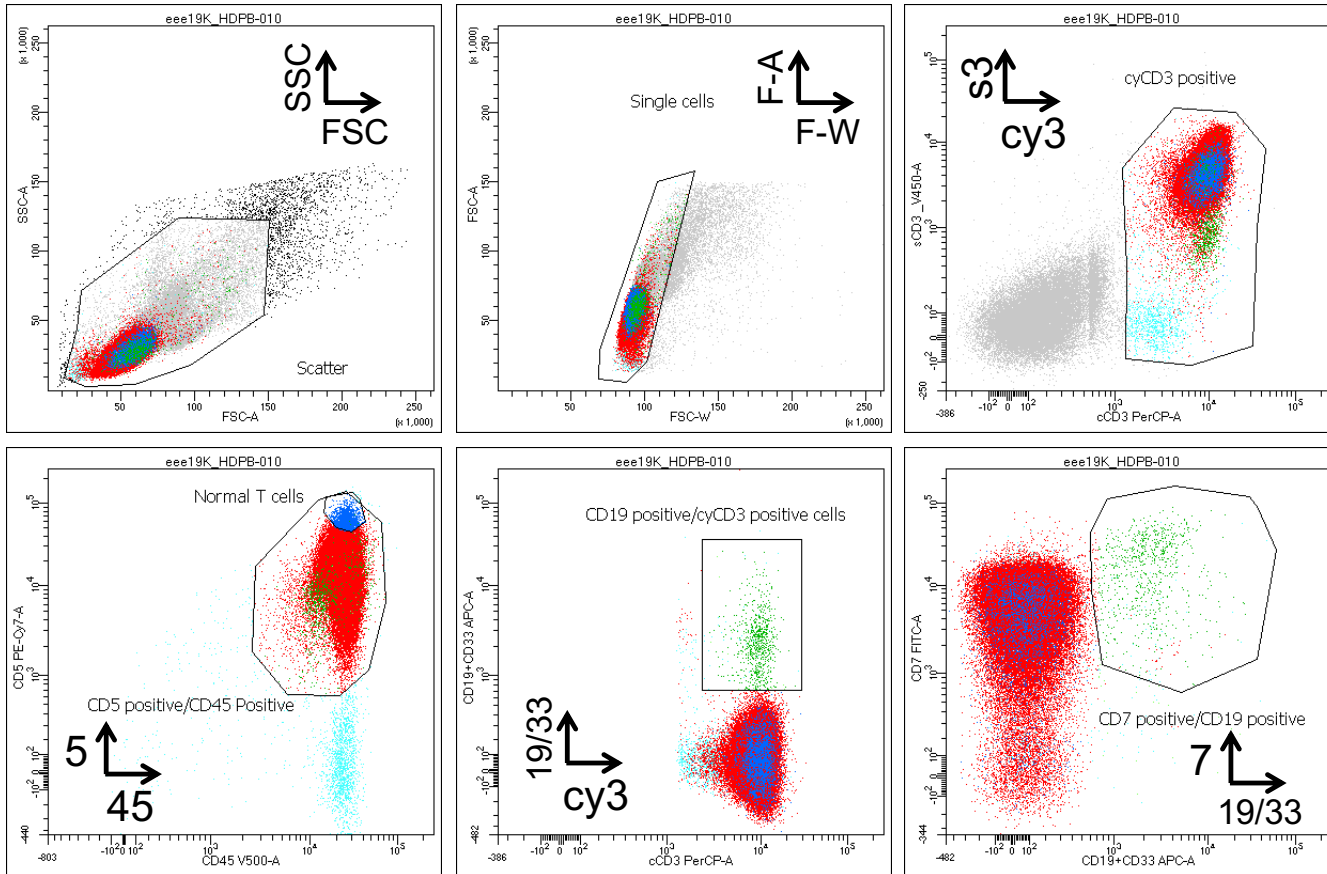
... immunophenotyping showed complex phenotype of blasts in periphery

blasts were present at less than <1% in BM and PB at diagnosis

cyCD3+, sCD3+, CD45+, CD5+, CD7+, CD8+, CD99+, and **CD19+**

... flow cytometry using the T-ALL MRD panel confirmed the IP data ...

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



Patient 8 Diagnostic (PB)

Tube: 010			
Population	#Events	%Parent	%Total
All Events	113,313	###	100.0
Scatter	111,454	98.4	98.4
Single cells	106,269	95.3	93.8
cyCD3 positive	75,951	71.5	67.0
CD5 positive/CD45 Positive	74,105	97.6	65.4
CD19 positive/cyCD3 positive cells	752	1.0	0.7
CD7 positive/CD19 positive	726	96.5	0.6
Normal T cells	2,559	3.5	2.3

Blasts are cyCD3+, sCD3+,
CD45 positive, CD7 positive,
CD1a negative,
CD5 **positive**, CD19 **positive**
MRD positive 0.256%

Another interesting case ...

Patient 8

Patient presented with mediastinal mass ...

... immunophenotyping showed complex phenotype of blasts in periphery
blasts were present at less than <1% in BM and PB at diagnosis
cyCD3+, sCD3+, CD45+, CD5+, CD7+, CD8+, CD99+, and **CD19+**

... flow cytometry using the T-ALL MRD panel confirmed the IP data ...

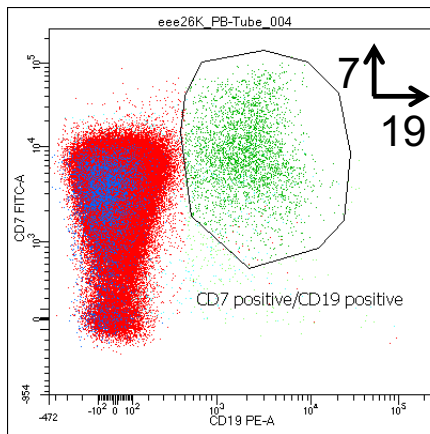
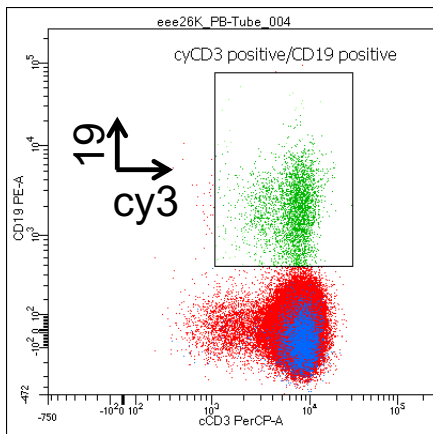
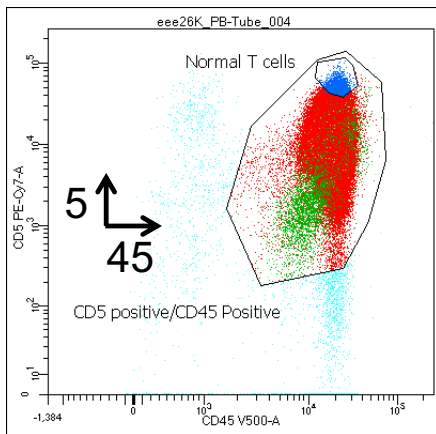
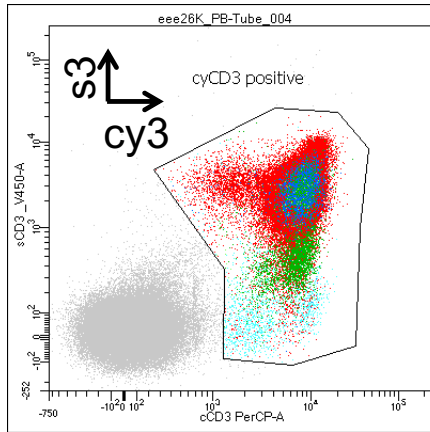
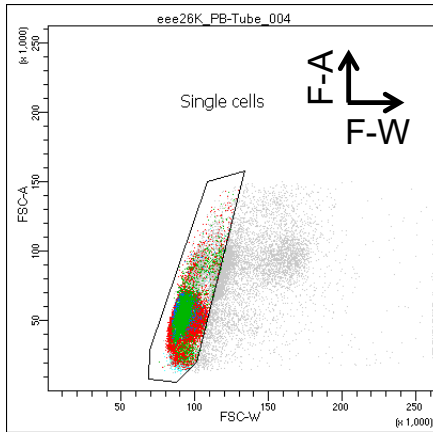
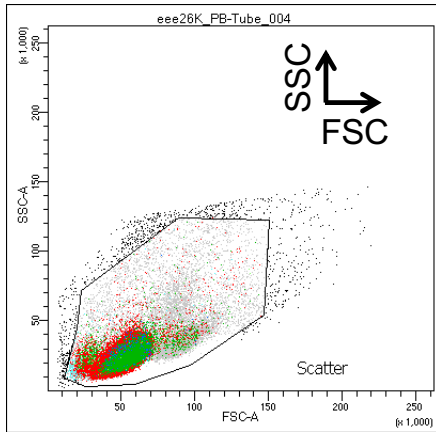
... at Day 8, changed the panel and backbone ...

Standard panel	Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
	7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45

Modified panel	Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
	8	CD7	CD19	cyCD3	CD33	CD5	HLA-DR	sCD3	CD45



Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
8	CD7	CD19	cyCD3	CD33	CD5	HLA-DR	sCD3	CD45



Patient 8 Day 8 (PB)

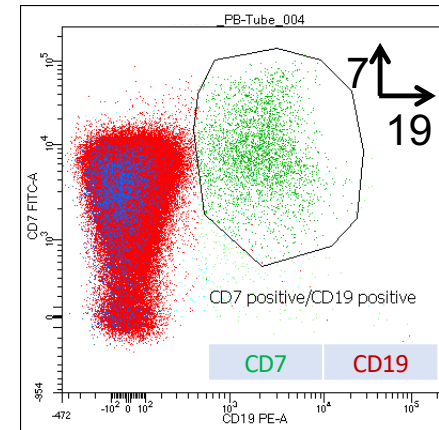
Tube: Tube_004			
Population	#Events	%Parent	%Total
■ All Events	277,690	###	100.0
■ Scatter	276,791	99.7	99.7
■ Single cells	270,368	97.7	97.4
■ cyCD3 positive	191,147	70.7	68.8
■ CD5 positive/CD45 Positive	188,551	98.6	67.9
■ cyCD3 positive/CD19 positive	3,251	1.7	1.2
■ CD7 positive/CD19 positive	3,010	92.6	1.1
■ Normal T cells	3,576	1.9	1.3

Blasts are cyCD3+, sCD3var,
CD45 positive, CD7 positive,
CD1a negative,
CD5 **positive**, CD19 **positive**

MRD calculation

Day 8 (PB)

Run many events



Tube: Tube_004

Population	#Events	%Parent	%Total
■ All Events	277,690	###	100.0
■ Scatter	276,791	99.7	99.7
■ Single cells	270,368	97.7	97.4
■ cyCD3 positive	191,147	70.7	68.8
■ CD5 positive/CD45 Positive	188,551	98.6	67.9
■ cyCD3 positive/CD19 positive	3,251	1.7	1.2
■ CD7 positive/CD19 positive	3,010	92.6	1.1
■ Normal T cells	3,976	1.9	1.3

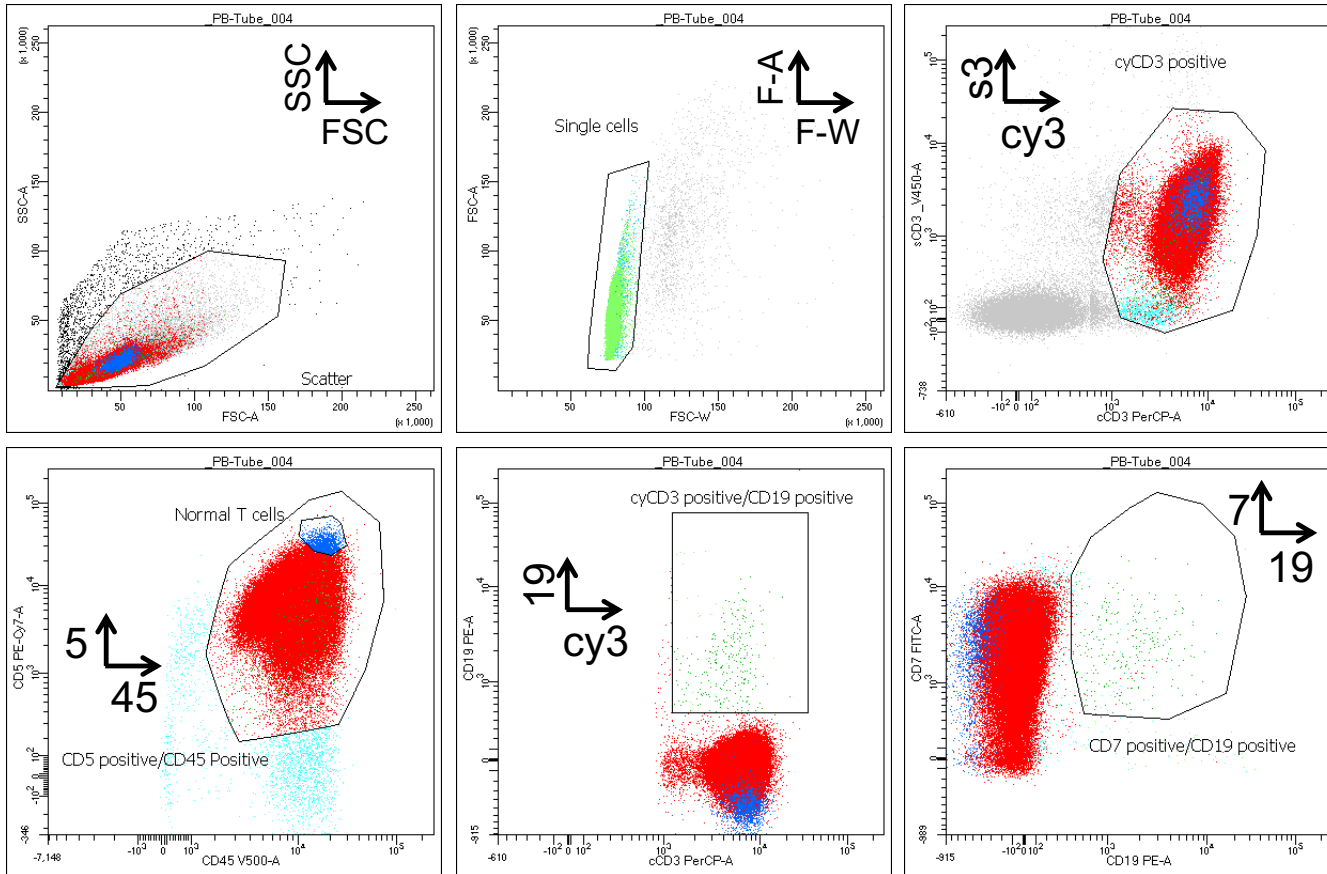
Minimal Residual Disease = Corrected number of Blasts/Total number of Events

$$= ((3,010/445,874) - (344/316,862)) \times CF \times 100/1$$

$$= 0.00566 \times 1.079 \times 100$$

$$= 0.611\% \text{ MRD positive}$$

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
8	CD7	CD19	cyCD3	CD33	CD5	HLA-DR	sCD3	CD45



Patient 8 Day 22 (PB)

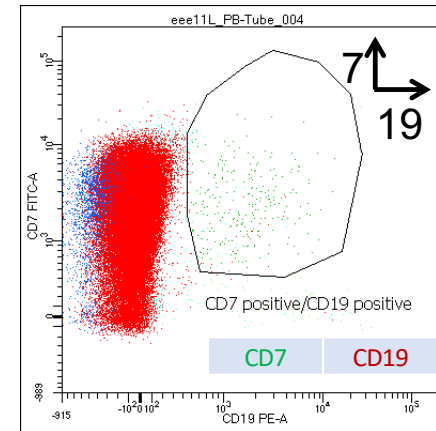
Tube: Tube_004			
Population	#Events	%Parent	%Total
■ All Events	125,284	###	100.0
□ Scatter	123,949	98.9	98.9
□ Single cells	120,190	97.0	95.9
□ cyCD3 positive	94,274	78.4	75.2
■ CD5 positive/CD45 Positive	92,210	97.8	73.6
■ cyCD3 positive/CD19 positive	303	0.3	0.2
■ CD7 positive/CD19 positive	255	84.2	0.2
■ Normal T cells	1,370	1.5	1.1

Blasts are cyCD3+, sCD3var,
CD45 positive, CD7 positive,
CD1a negative,
CD5 **positive**, CD19 **positive**

MRD calculation

Day 22 (PB)

Run many events



Tube: Tube_004

Population	#Events	%Parent	%Total
All Events	125,284	###	100.0
Scatter	123,949	98.9	98.9
Single cells	120,190	97.0	95.9
CD3 positive	94,274	78.4	75.2
CD5 positive/CD45 Positive	92,210	97.8	73.6
CD3 positive/CD19 positive	202	0.3	0.2
CD7 positive/CD19 positive	255	84.2	0.2
Normal T cells	1,370	1.5	1.1

$$\begin{aligned}
 \text{Minimal Residual Disease} &= \text{Corrected number of Blasts/Total number of Events} \\
 &= ((255/628,654) - (30/280,228)) \times \text{CF} \times 100/1 \\
 &= 0.0002986 \times 1.105 \times 100 \\
 &= 0.033\% \text{ MRD positive}
 \end{aligned}$$

Gating Strategies - Examples

Start with four simple cases that are easy to follow at later treatment stages

Move on to progressively more difficult cases

- loss of aberrant marker expression with treatment
- aberrantly low expression on blast population becomes more like normal with treatment

Note 4: Adapt when possible to use the best combination(s) for identifying blasts. In this case, the cyCD3+/CD19+ is clearly aberrant and represents a robust way to gate the blast population = modify the tube for this patient to make use of this unusual feature.

Patient 9 CD7/CD1a

Another interesting case ... a cautionary tale

Patient 9

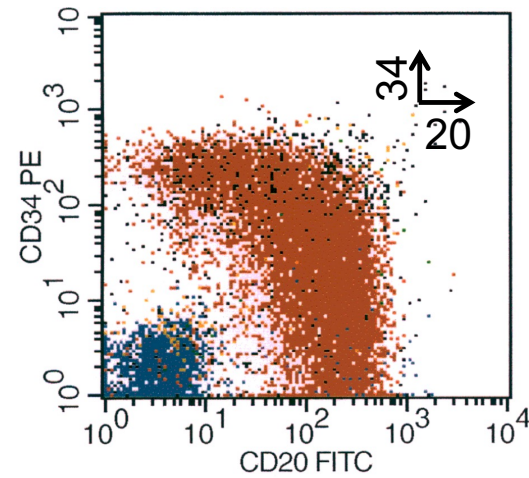
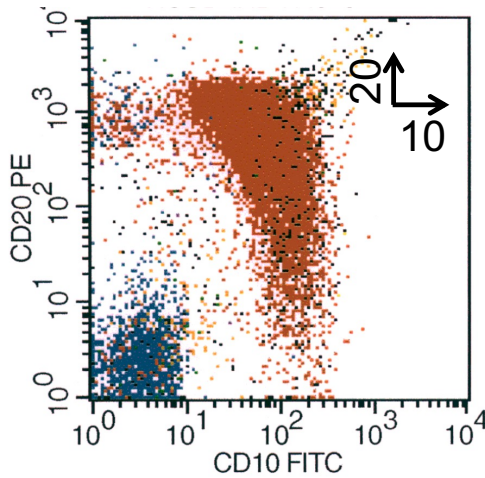
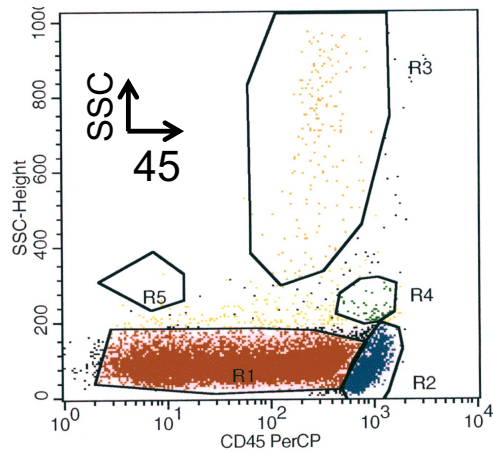
Patient presented with B-ALL ...

Flow cytometry of PB and BM showed ...

Another interesting case ...

Patient 9

Patient presented with B-ALL



CD45dim, CD19+, CD20+, CD22+, CD24+, CD10+, CD34+

Another interesting case ...

Patient 9

Patient presented with B-ALL ...

Flow cytometry of PB and BM showed ...

Cytogenetics/molecular studies showed ...

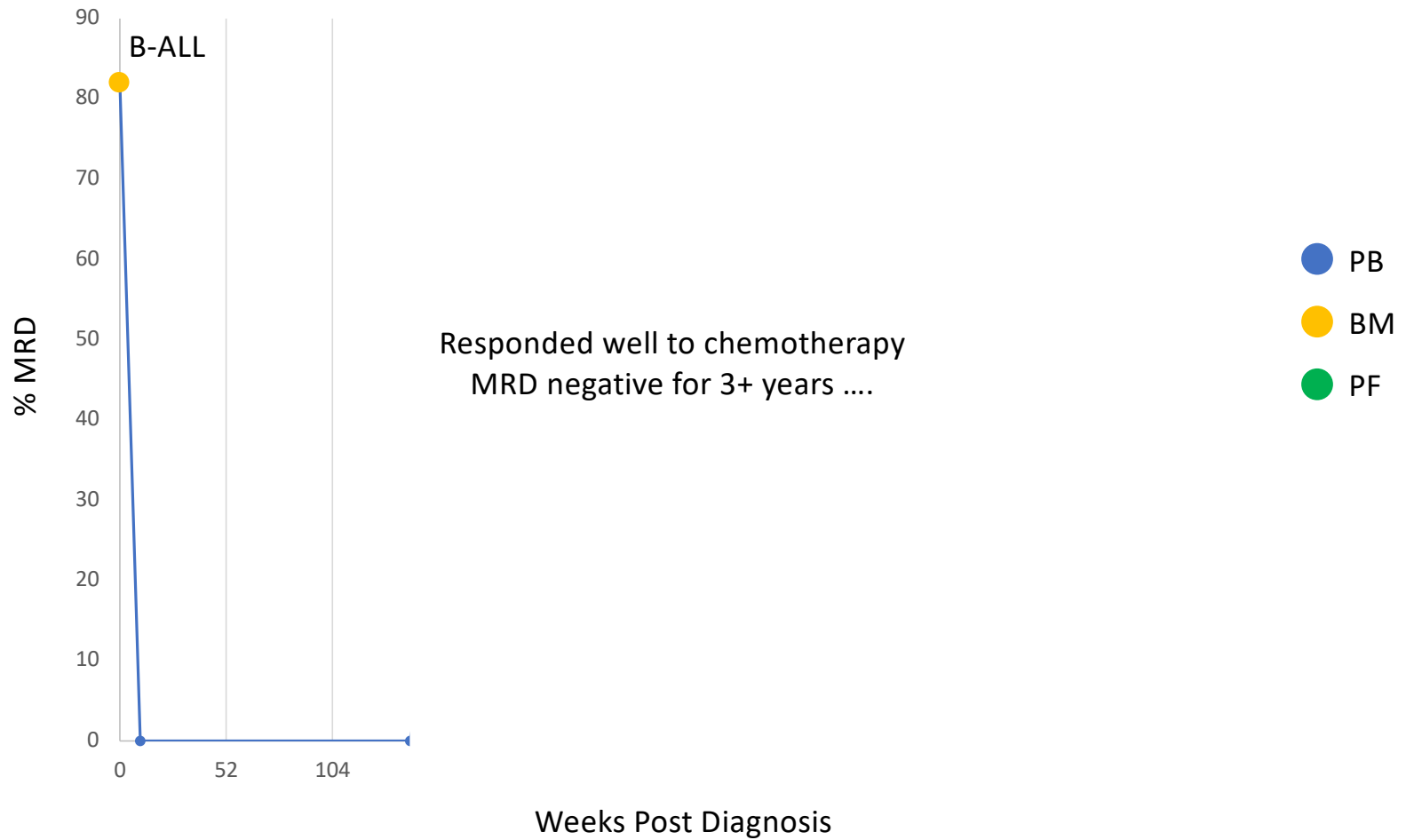
Hyperdiploid blasts: 53,XX,+X,+4,+14,+17,+18,+21,+21

RT-PCR MOLECULAR DIAGNOSTICS

ALL	PRIMERS	PROBE	RESULTS
t(9;22) CML	5'(9;22)A/3'(9;22)A	Abl-P	NEGATIVE
t(9;22) ALL	5'(9;22)B/3'(9;22)A	Abl-P	NEGATIVE
t(1;19)	5'(1;19)/3'(1;19)B	(1;19)-P	NEGATIVE
t(4;11) 4;11e	5'/4;11e3'	411-P	NEGATIVE
t(12;21)	5'ex3tel/3'AML-1 der(21)	AML-P	NEGATIVE

Another interesting case ...

Patient 9



Another interesting case ...

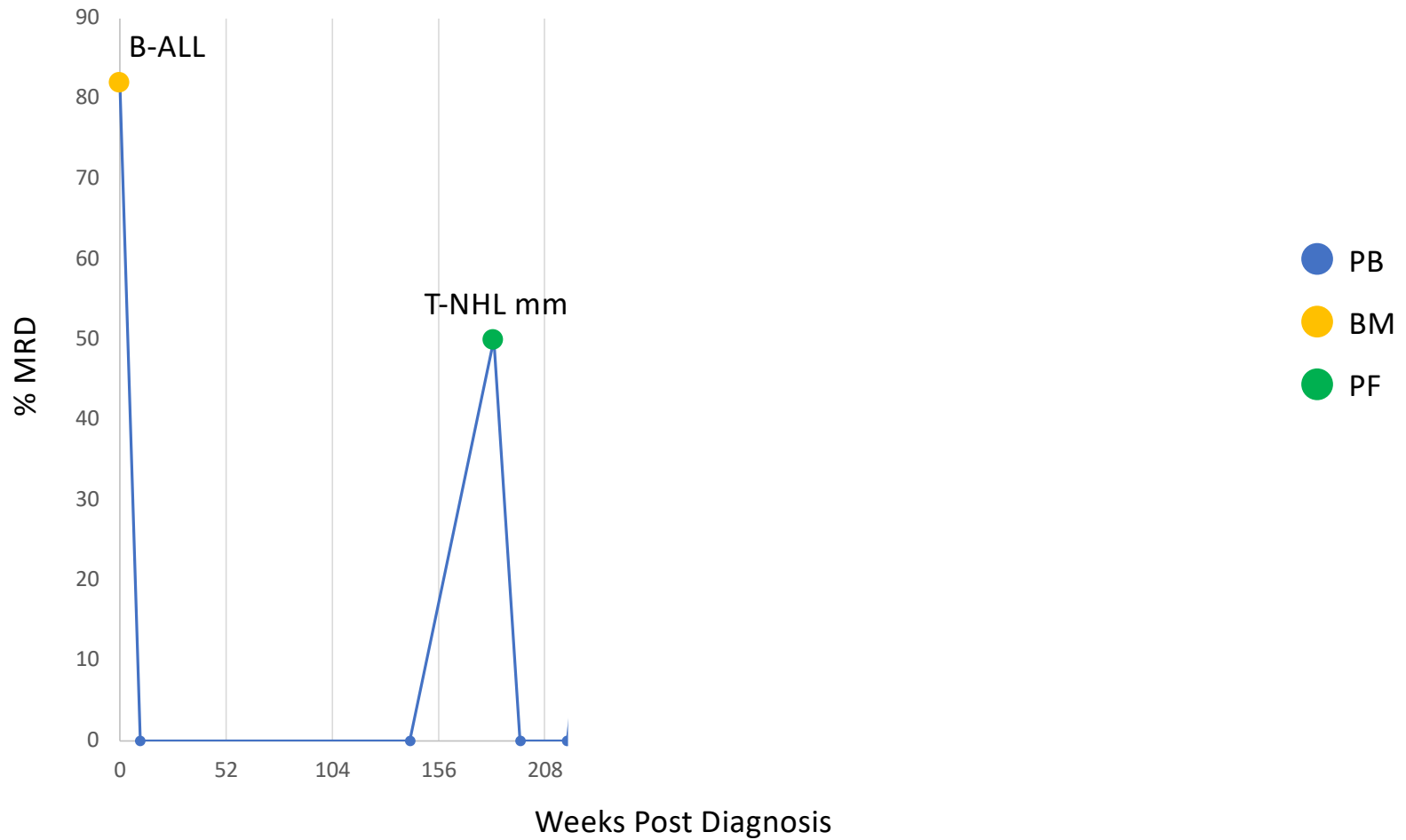
Patient 9

Patient presented with B-ALL ...

... 42 months later developed mediastinal mass with onset of respiratory failure ...

Another interesting case ...

Patient 9



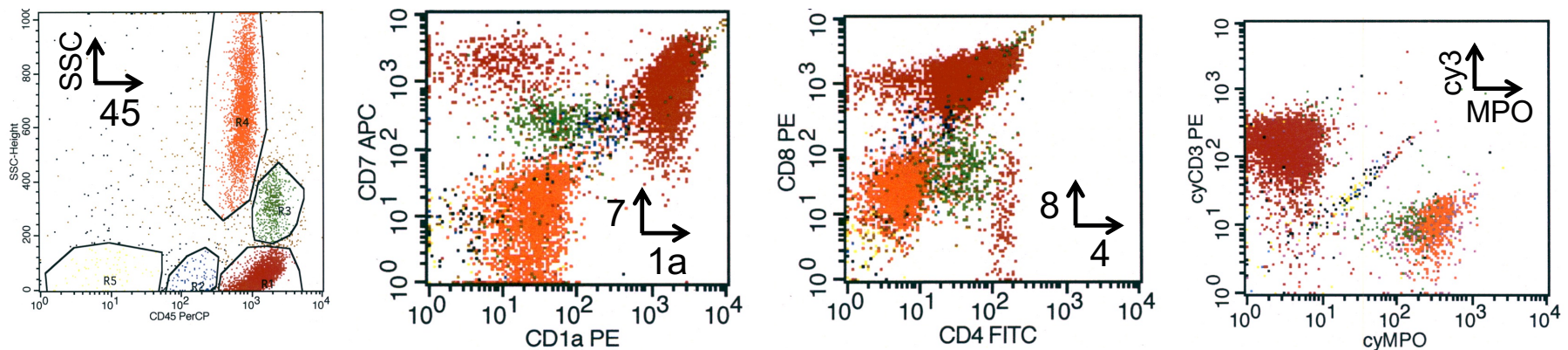
Another interesting case ...

Patient 9

Patient presented with B-ALL ...

... 42 months later developed mediastinal mass with onset of respiratory failure ...

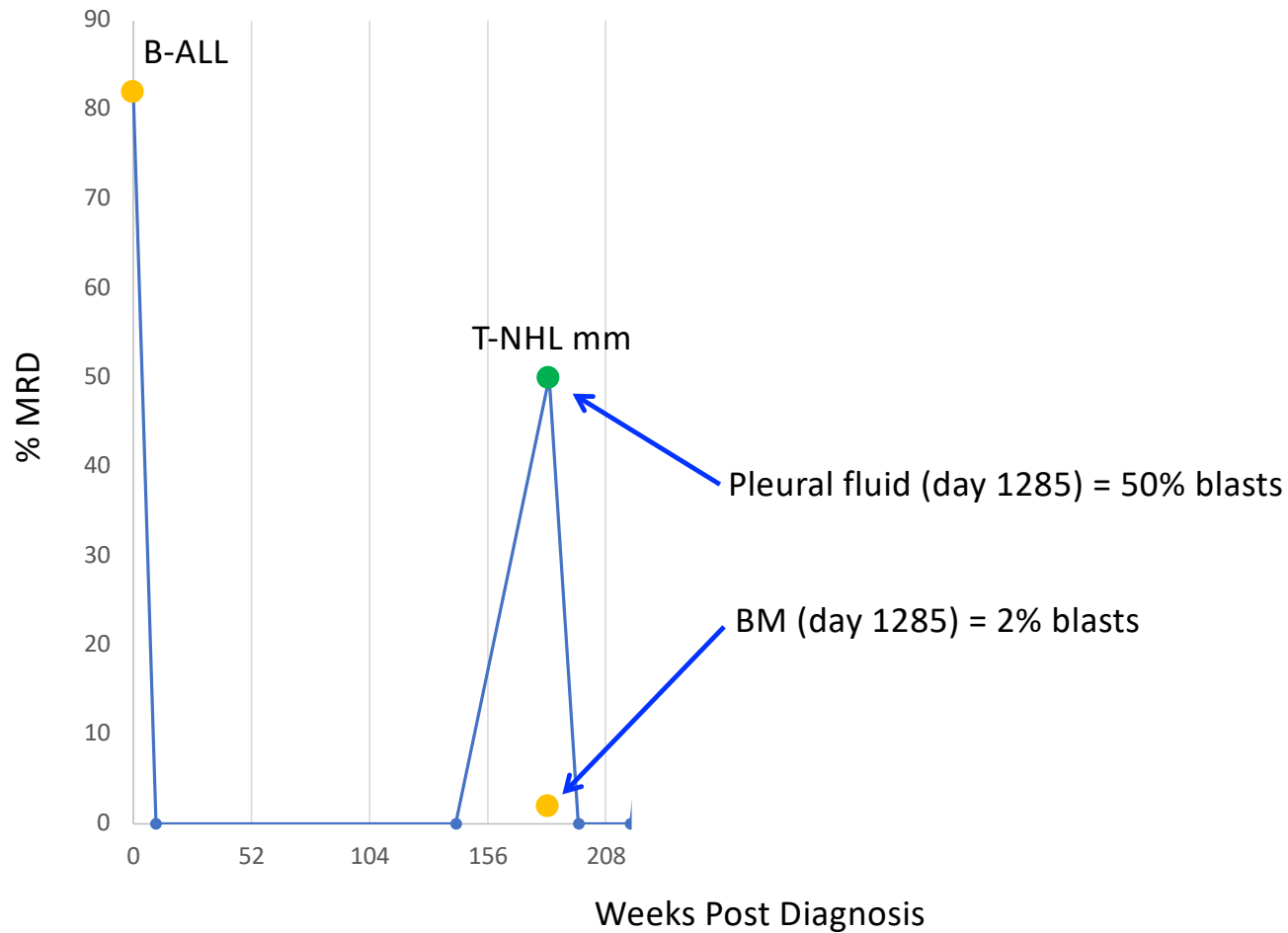
Flow cytometry of pleural fluid (Day 1285) ...



CD45⁺, CD5^{dim to mod}, CD1a^{bright}, CD7⁺, CD8^{bright}, CD4^{dim}, sCD3⁻, cyCD3⁺

Another interesting case ...

Patient 9



Another interesting case ...

Patient 9

Patient presented with B-ALL ...

... 42 months later developed mediastinal mass with onset of respiratory failure ...

Flow cytometry of pleural fluid (day 1285) ...

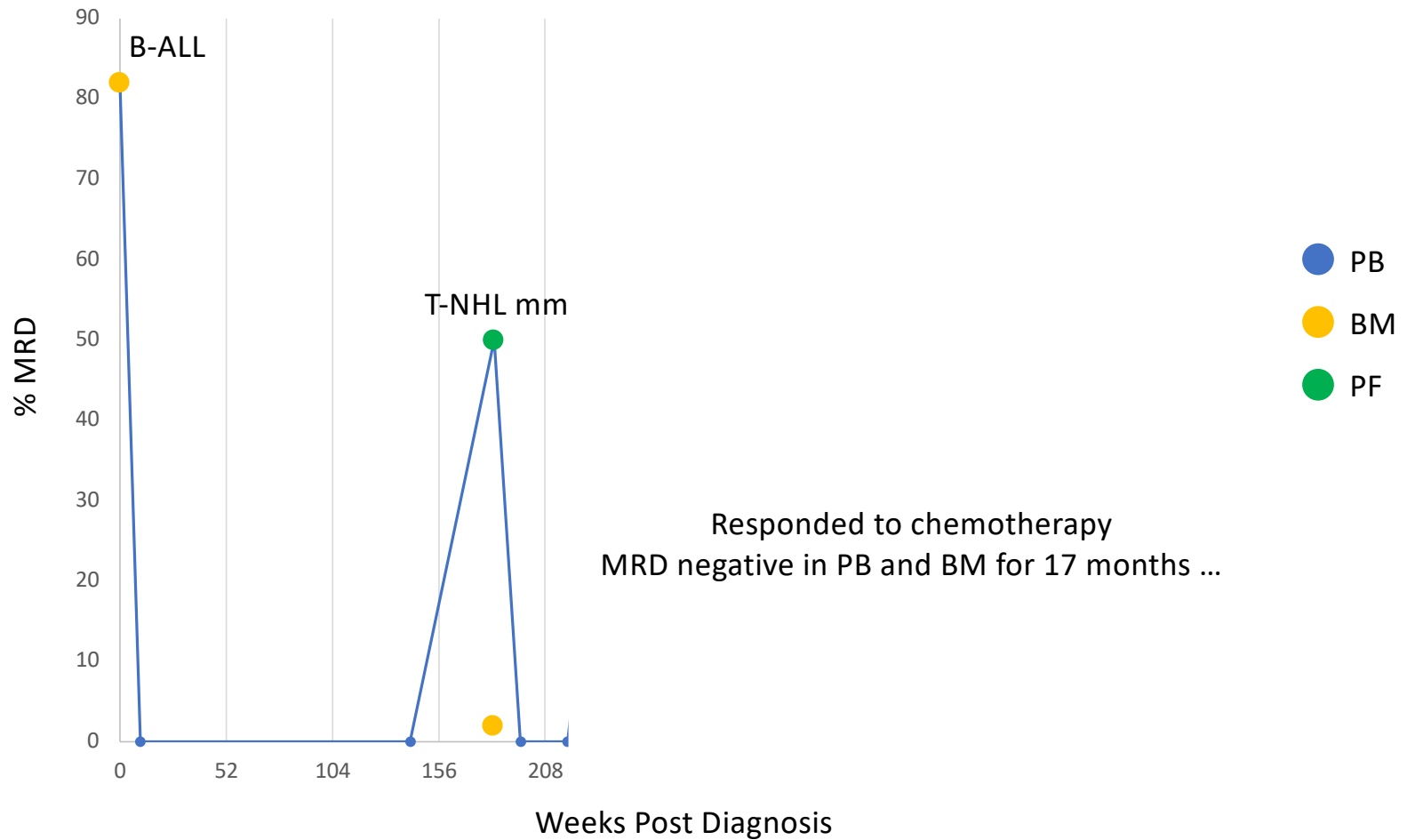
Cytogenetics/molecular (day 1285) studies showed ...

BM: all metaphases were apparently normal: 46,XX

PF: FISH with BAP Probe MLL: Locus 11q23 showed MLL rearrangement

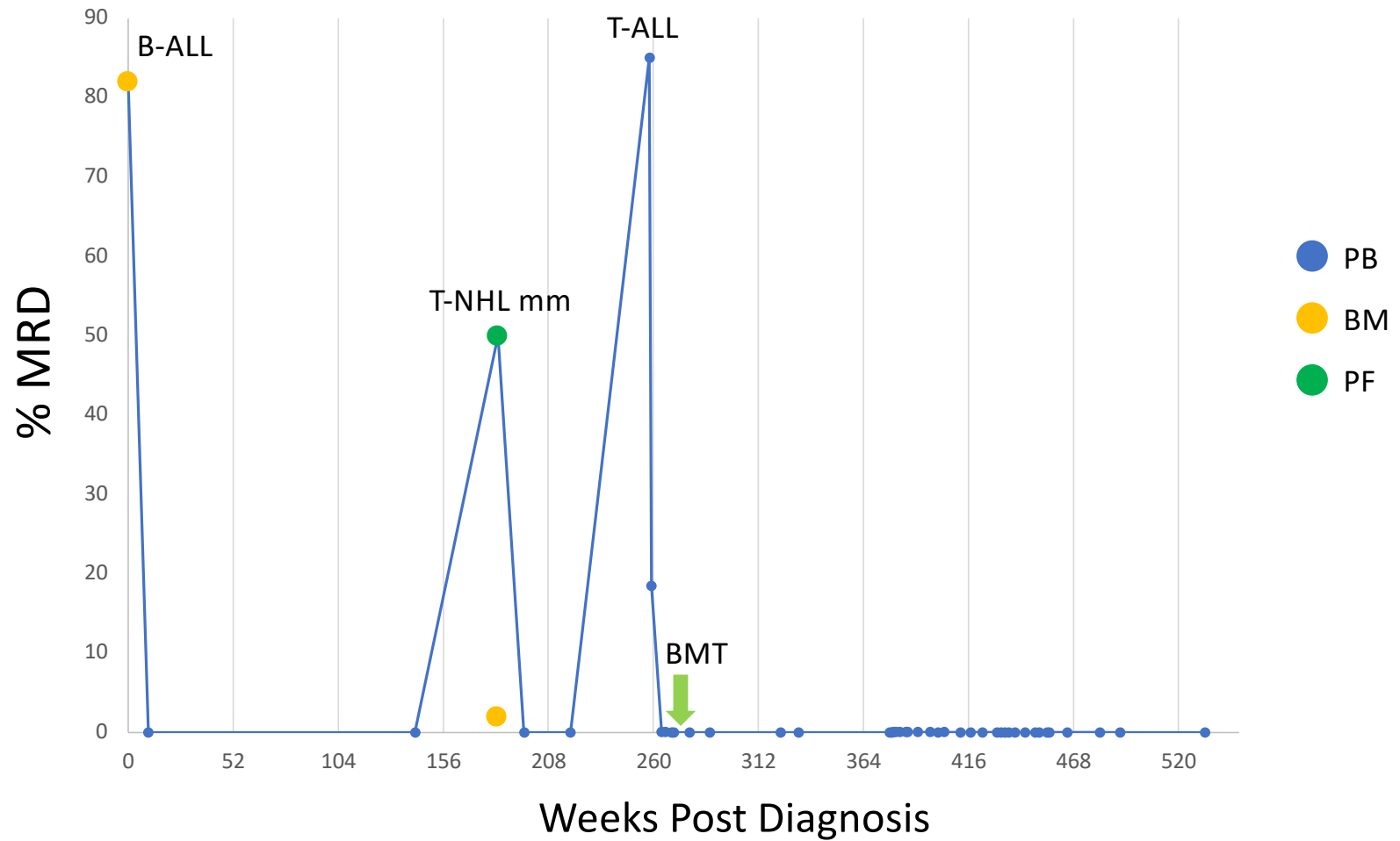
Another interesting case ...

Patient 9



Another interesting case ...

Patient 9



Another interesting case ...

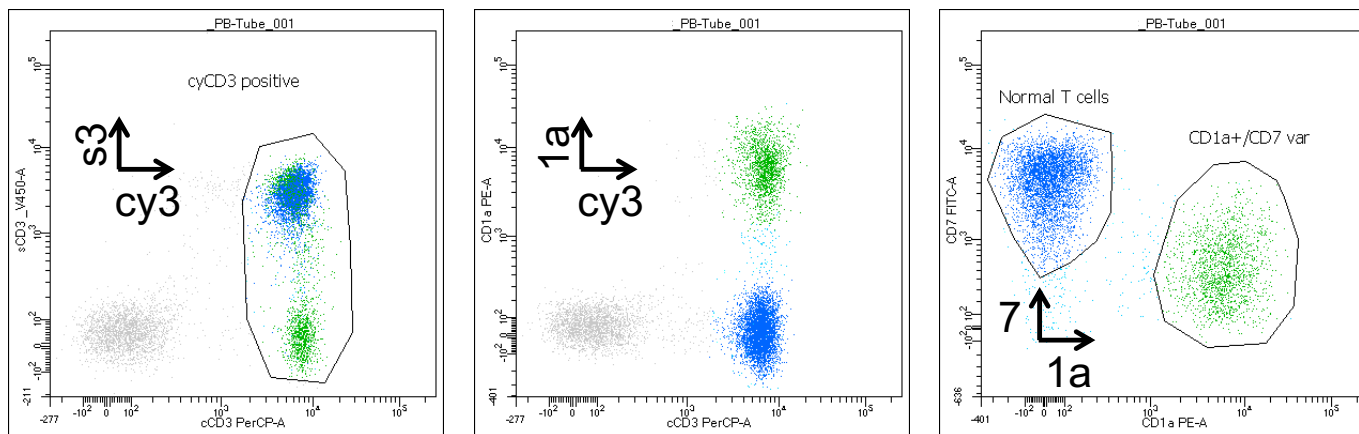
Patient 9

Patient presented with B-ALL ...

... 42 months later developed mediastinal mass with onset of respiratory failure ...

... 17 months later developed T-ALL ...

Flow cytometry of PB and BM showed ...

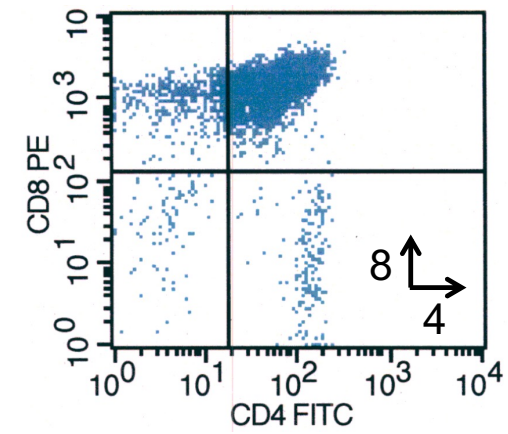
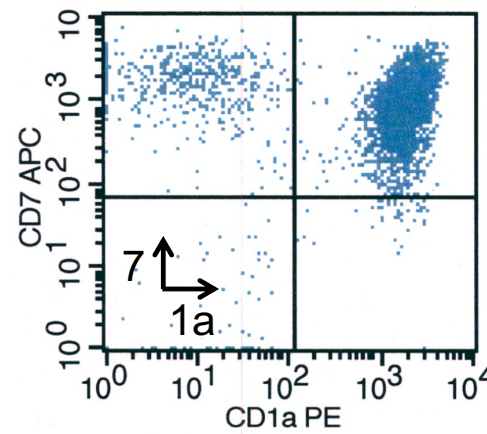
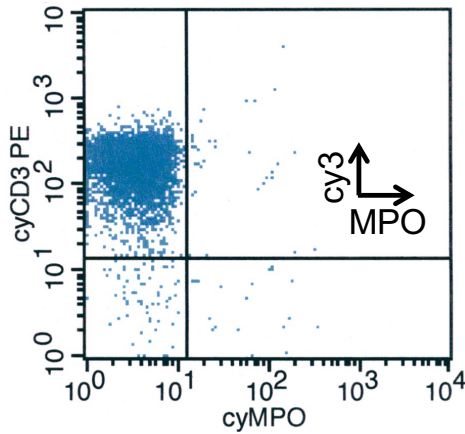


CD45^{bright}, CD5^{mod}, CD1a^{bright}, CD7+, CD8^{bright}, CD4^{dim}, TdT^{var}, sCD3^{var}, cyCD3+

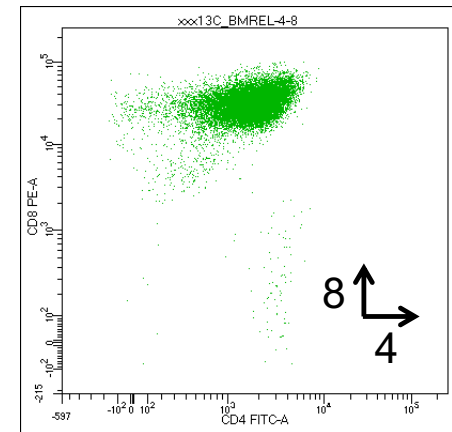
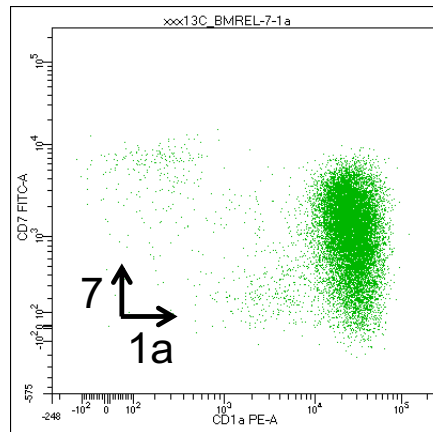
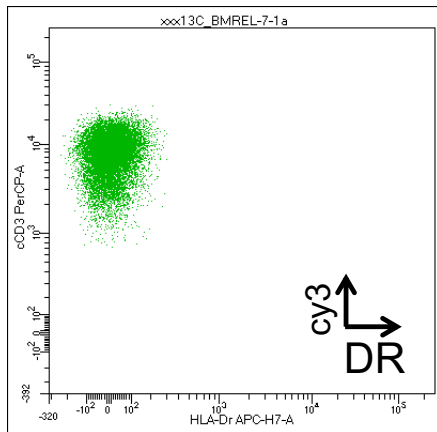
Another interesting case ...

Patient 9

T-NHL mm
Pleural Fluid
Day 1285



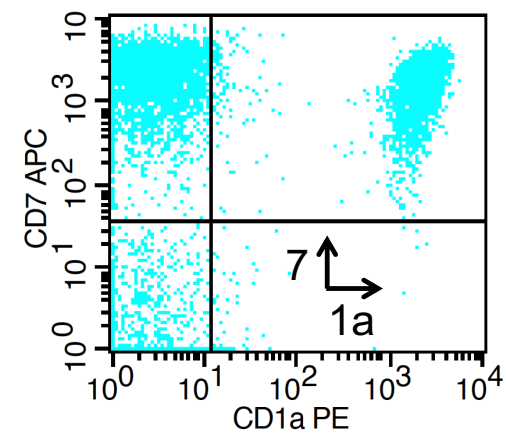
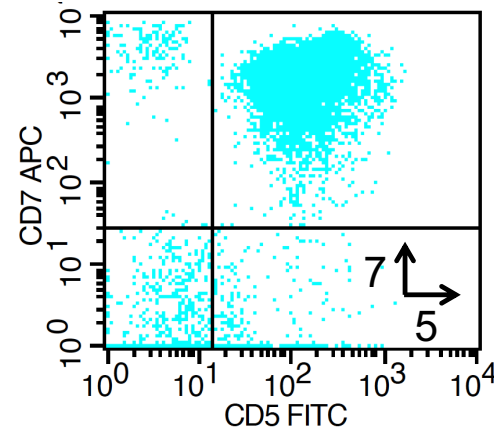
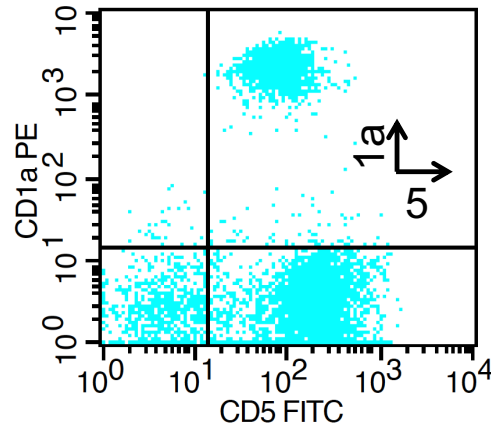
T-ALL relapse
BM
Day 1809



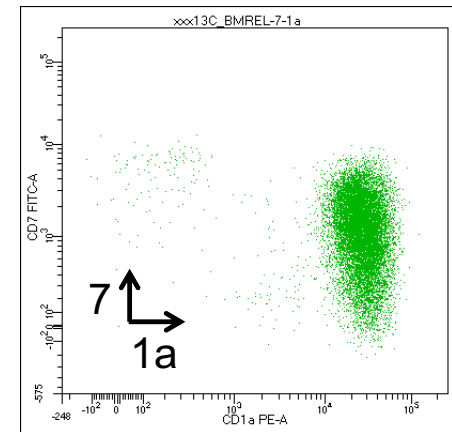
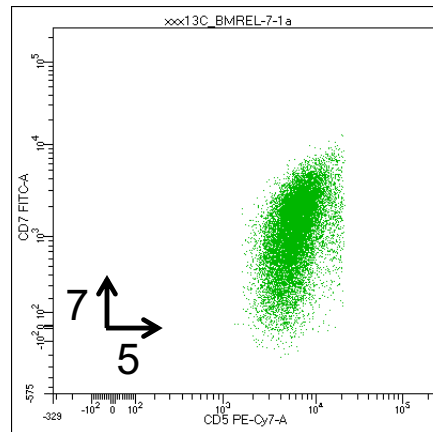
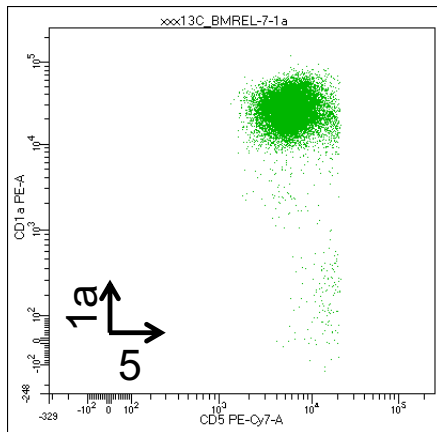
Another interesting case ...

Patient 9

T-NHL mm
BM
Day 1285



T-ALL relapse
BM
Day 1809



Another interesting case ...

Patient 9

Patient presented with B-ALL ...

... 42 months later developed mediastinal mass with onset of respiratory failure ...

... 17 months later developed T-ALL ...

Flow cytometry of PB and BM showed ...

Cytogenetics/molecular studies showed ...

46,XX,inv(11)(q21q23)

= MLL rearrangement

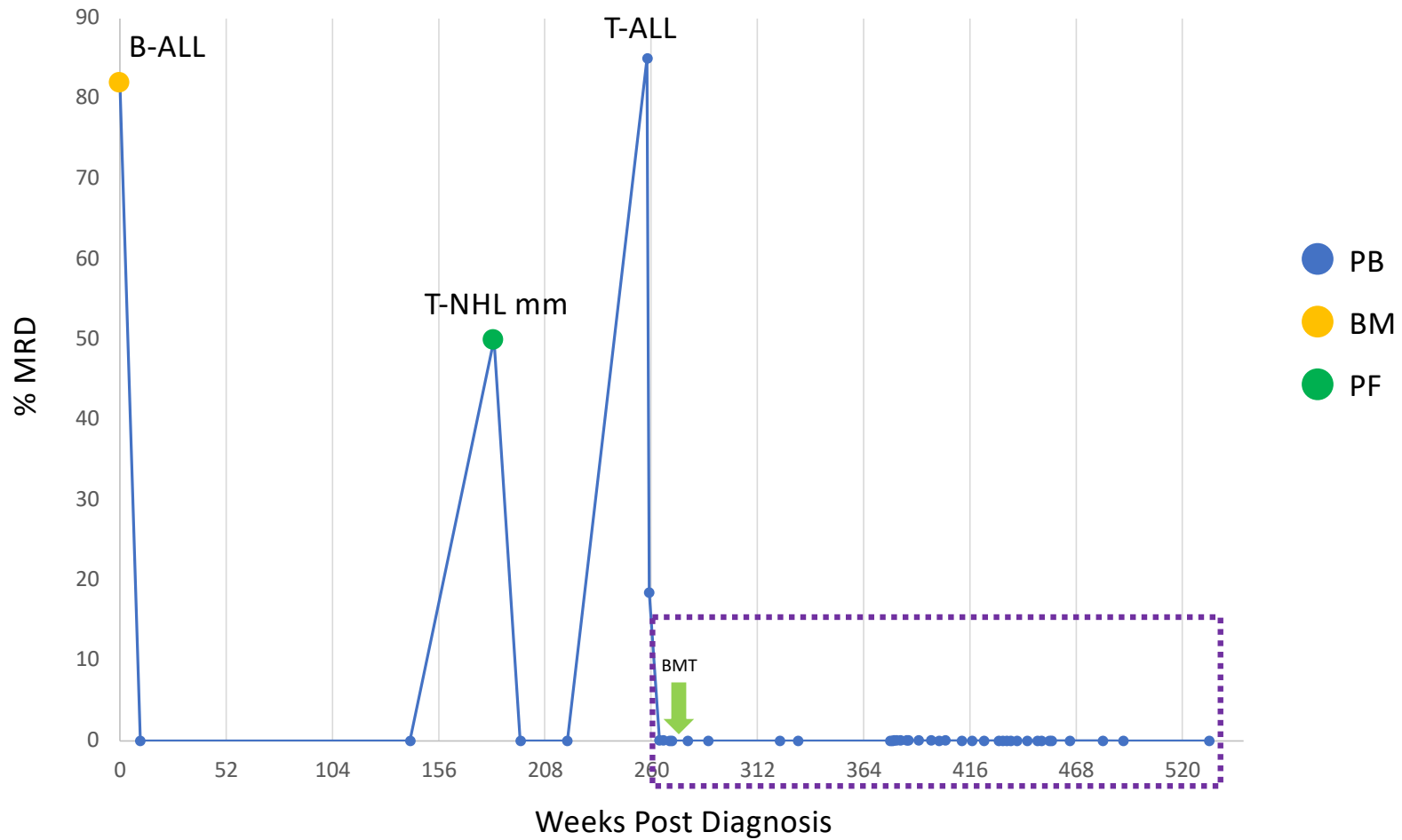
has been seen with therapy-related ALL (including T-ALL)

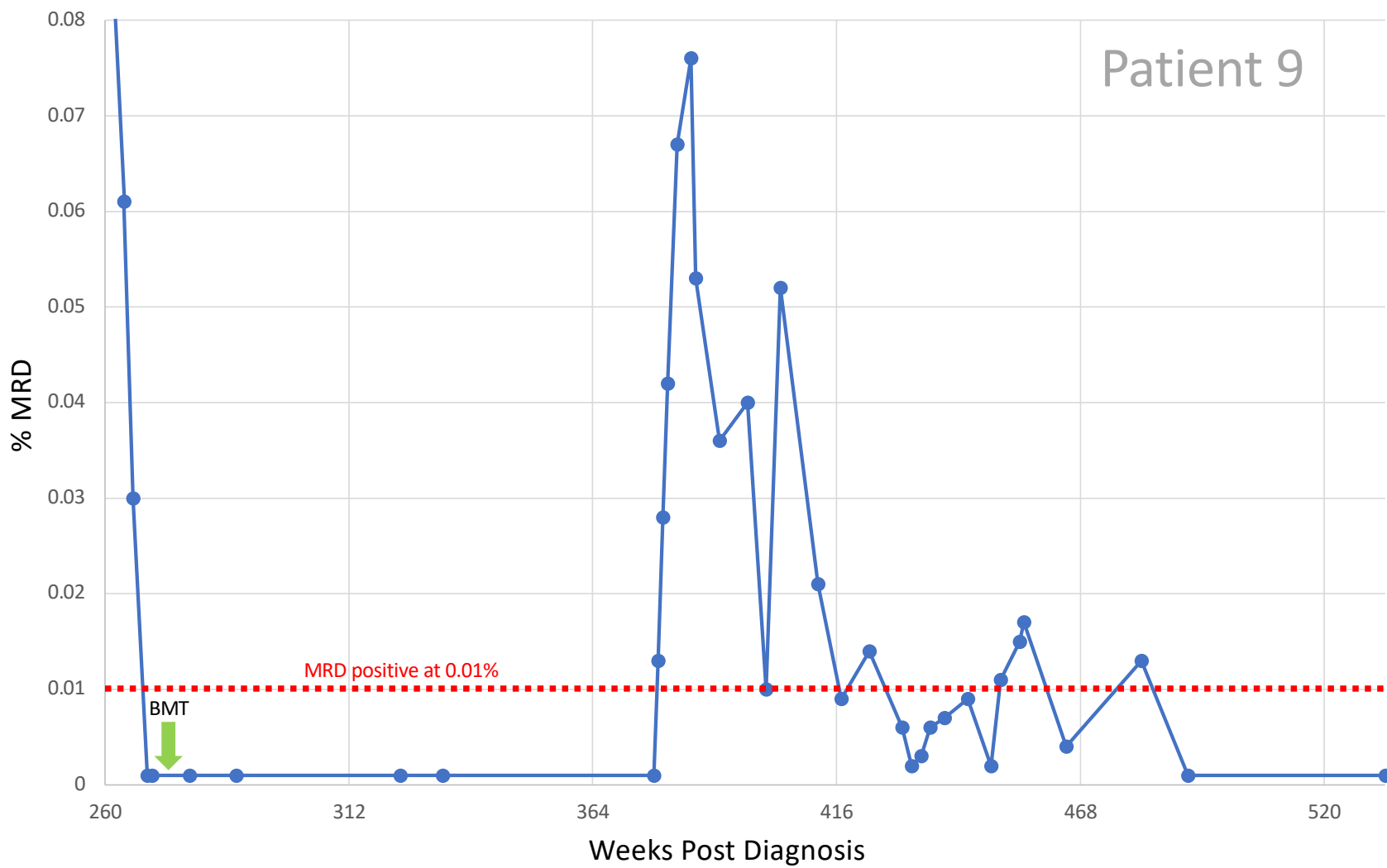
Responded to therapy → BMT (MUD) 15 weeks after relapse (week 273).

MRD negative in PB and BM since week 270.

Another interesting case ...

Patient 9





Another interesting case ...

Patient 9

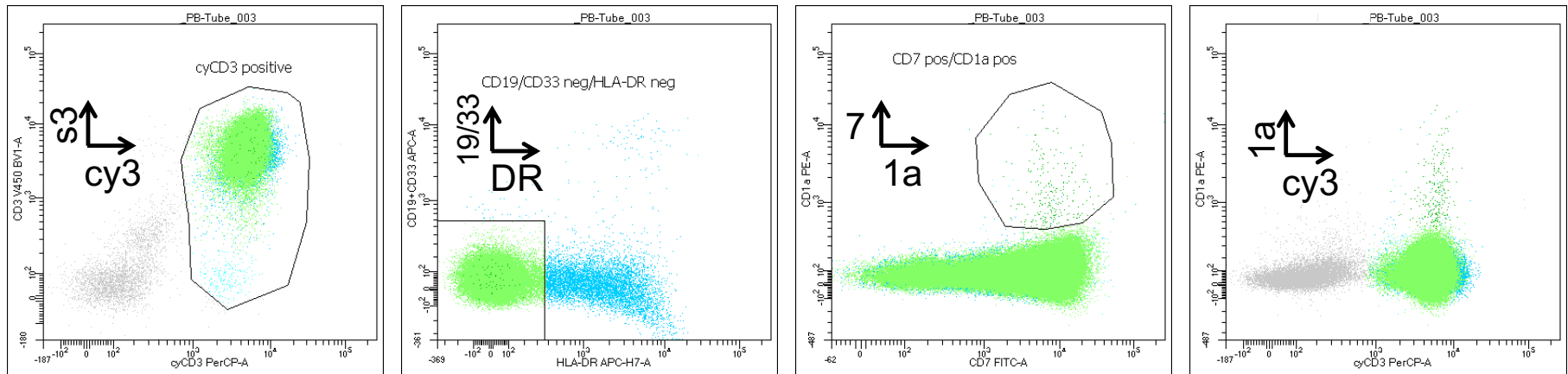
Patient presented with B-ALL ...

... 42 months later developed mediastinal mass with onset of respiratory failure ...

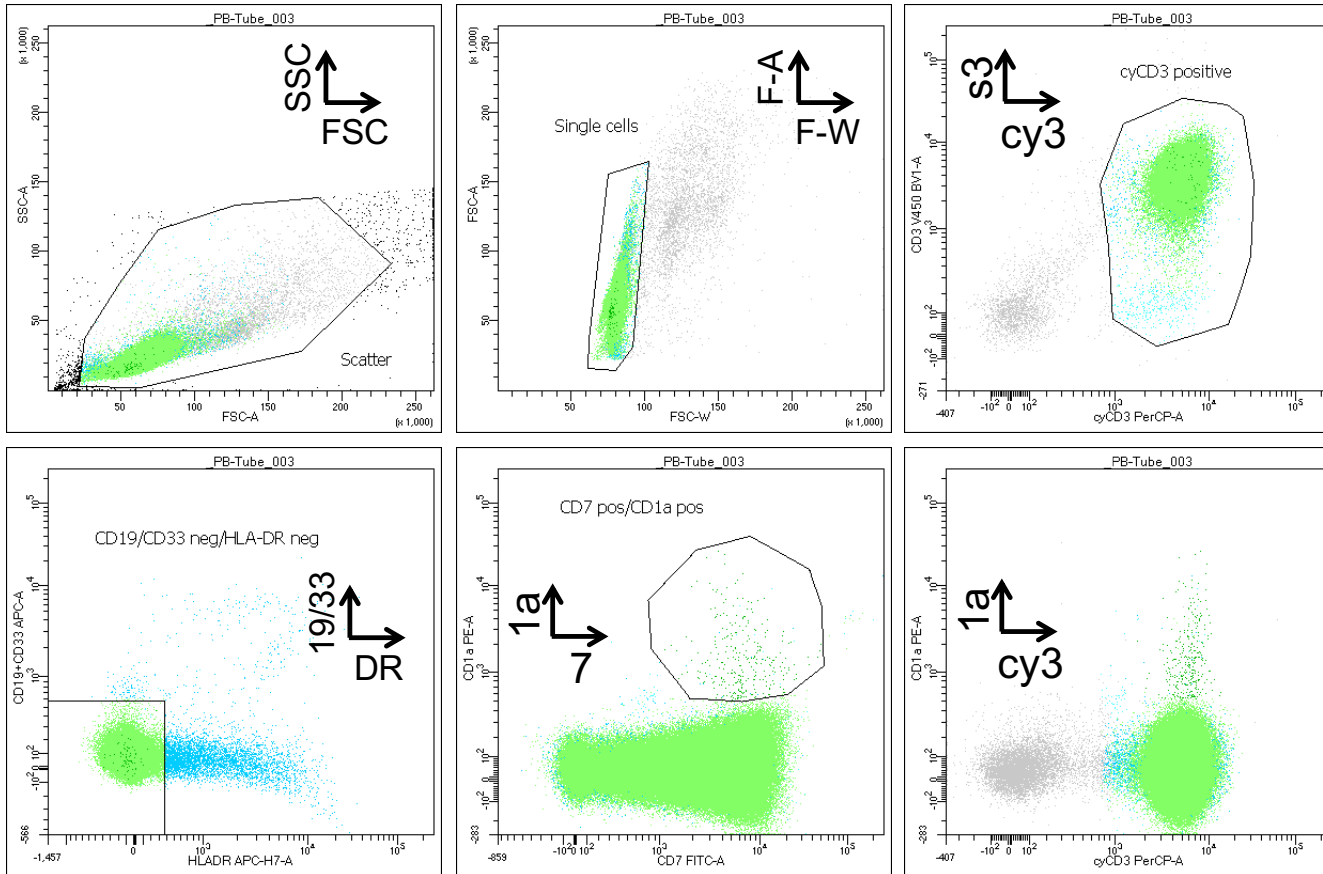
... 17 months later developed T-ALL ...

... 3 months later BMT ...

... 25 months later T-ALL relapsed ...



Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



Patient 9

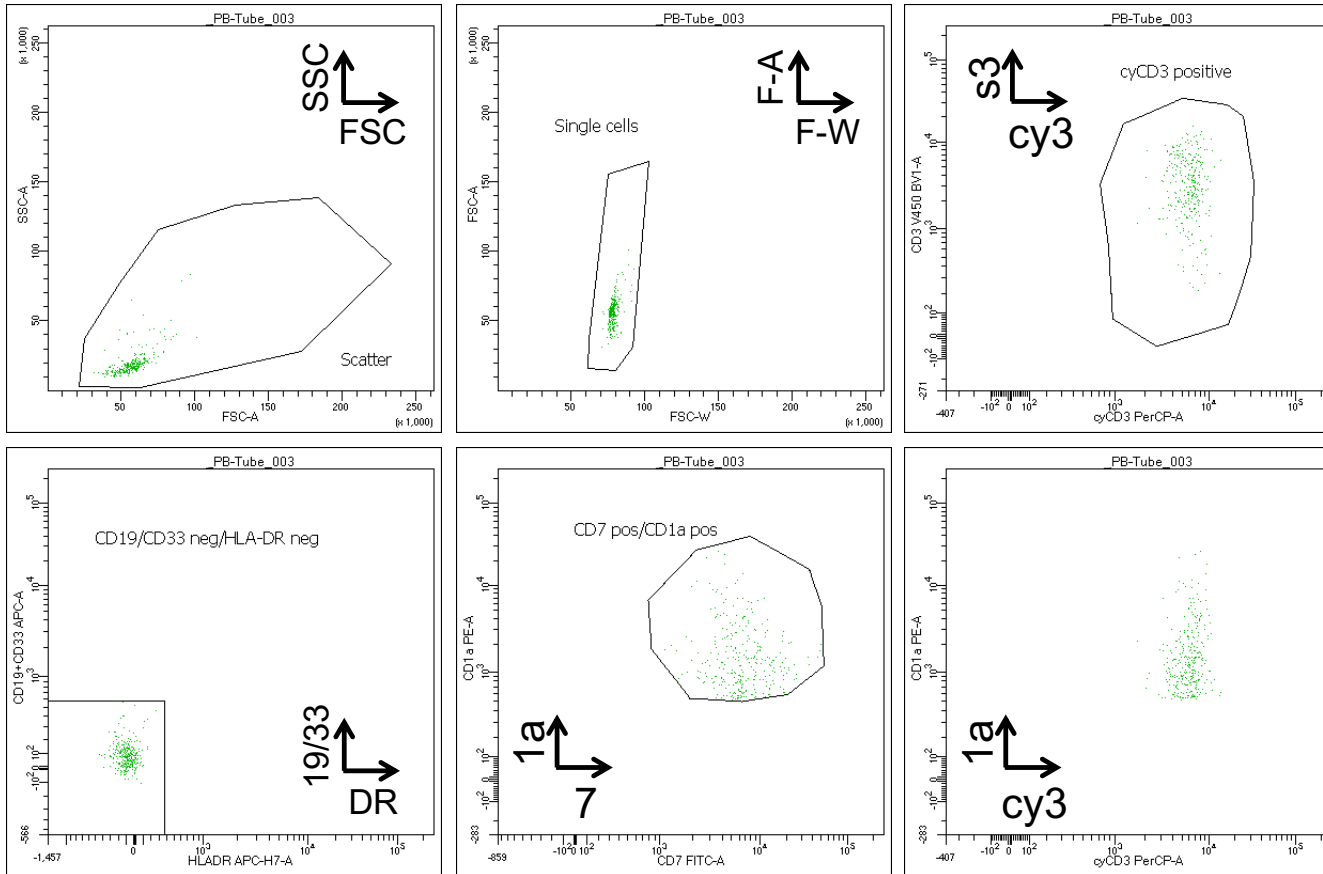
Relapse (PB) week 382

Tube: Tube_003			
Population	#Events	%Parent	%Total
■ All Events	301,712	###	100.0
■ Scatter	298,309	98.9	98.9
■ Single cells	283,350	95.0	93.9
■ cyCD3 positive	275,075	97.1	91.2
■ CD5 pos/CD3 var	272,410	99.0	90.3
■ CD19/CD33 neg/HLA-DR neg	253,313	93.0	84.0
■ CD7 pos/CD1a pos	299	0.1	0.1

Upon relapse, blasts are now sCD3var, cyCD3 pos, CD45 pos, CD7 bright and CD1a positive

For this relapse, we can follow with **CD7/CD1a** tube

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
7	CD7	CD1a	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



Patient 9

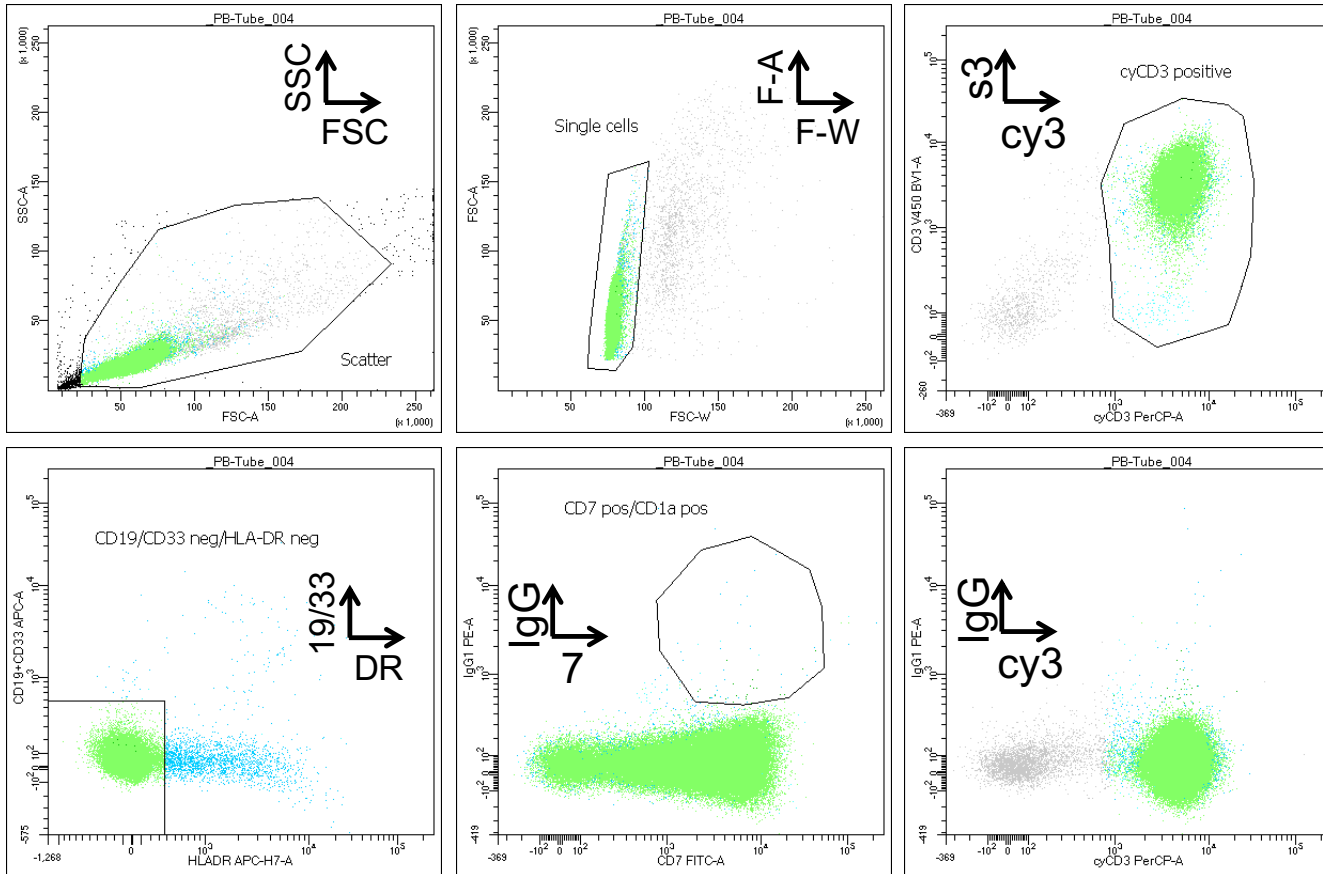
Relapse (PB) week 382

Tube: Tube_003			
Population	#Events	%Parent	%Total
■ All Events	301,712	###	100.0
■ Scatter	298,309	98.9	98.9
■ Single cells	283,350	95.0	93.9
■ cyCD3 positive	275,075	97.1	91.2
■ CD5 pos/CD3 var	272,410	99.0	90.3
■ CD19/CD33 neg/HLA-DR neg	253,313	93.0	84.0
■ CD7 pos/CD1a pos	299	0.1	0.1

Upon relapse, blasts are now sCD3var, cyCD3 pos, CD45 pos, CD7 bright and CD1a positive

Back gating!

Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
3	CD7	IgG1	cyCD3	CD19 + CD33	CD5	HLA-DR	sCD3	CD45



Patient 9

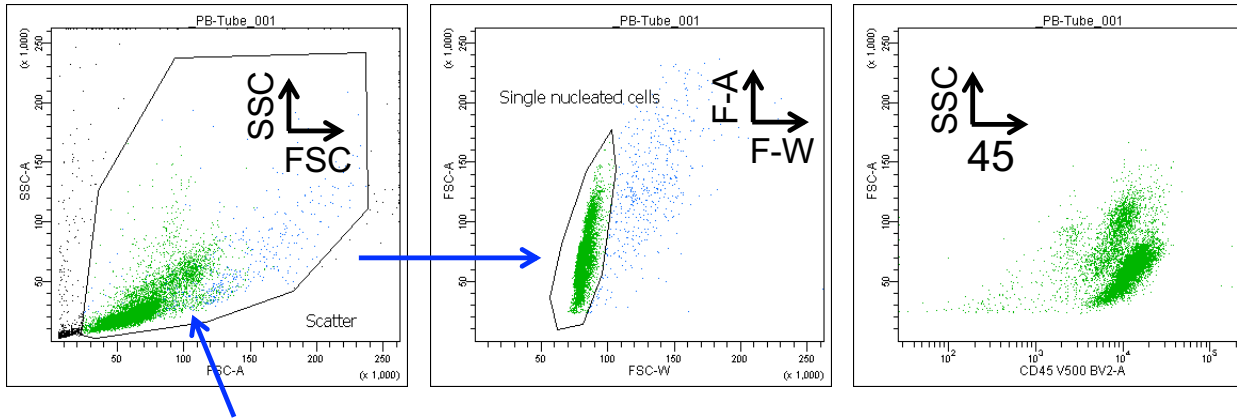
Relapse (PB) week 382

Tube: Tube_004			
Population	#Events	%Parent	%Total
All Events	134,697	###	100.0
Scatter	130,949	97.2	97.2
Single cells	124,541	95.1	92.5
cyCD3 positive	120,944	97.1	89.8
CD5 pos/CD3 var	119,928	99.2	89.0
CD19/CD33 neg/HLA-DR neg	111,647	93.1	82.9
CD7 pos/CD1a pos	24	0.0	0.0

Upon relapse, blasts are now sCD3var, cyCD3 pos, CD45 pos, CD7 bright and CD1a positive

Isotype control

Correction Factor calculation



Day 2680 (PB)
Run 10,000 ungated events

Tube: Tube_001			
Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ Scatter	9,295	93.0	93.0
■ Single nucleated cells	8,817	94.9	88.2

Draw scatter gate to eliminate dead cells and debris.

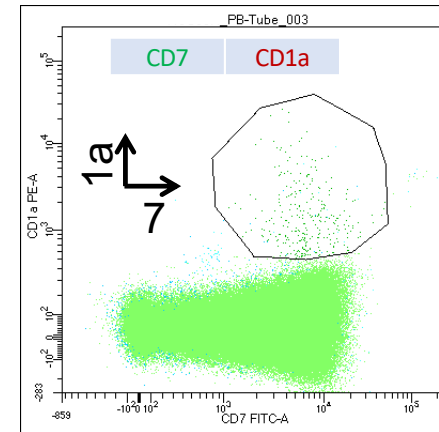
Use FSC-A vs FSC-W plot to eliminate doublets = approximates single nucleated cells.

$$\begin{aligned}
 \text{Correction Factor (CF)} &= 10,000 / \text{Single nucleated cells (P2)} \\
 &= 10,000 / 8,817 \\
 &= 1.134
 \end{aligned}$$

MRD calculation

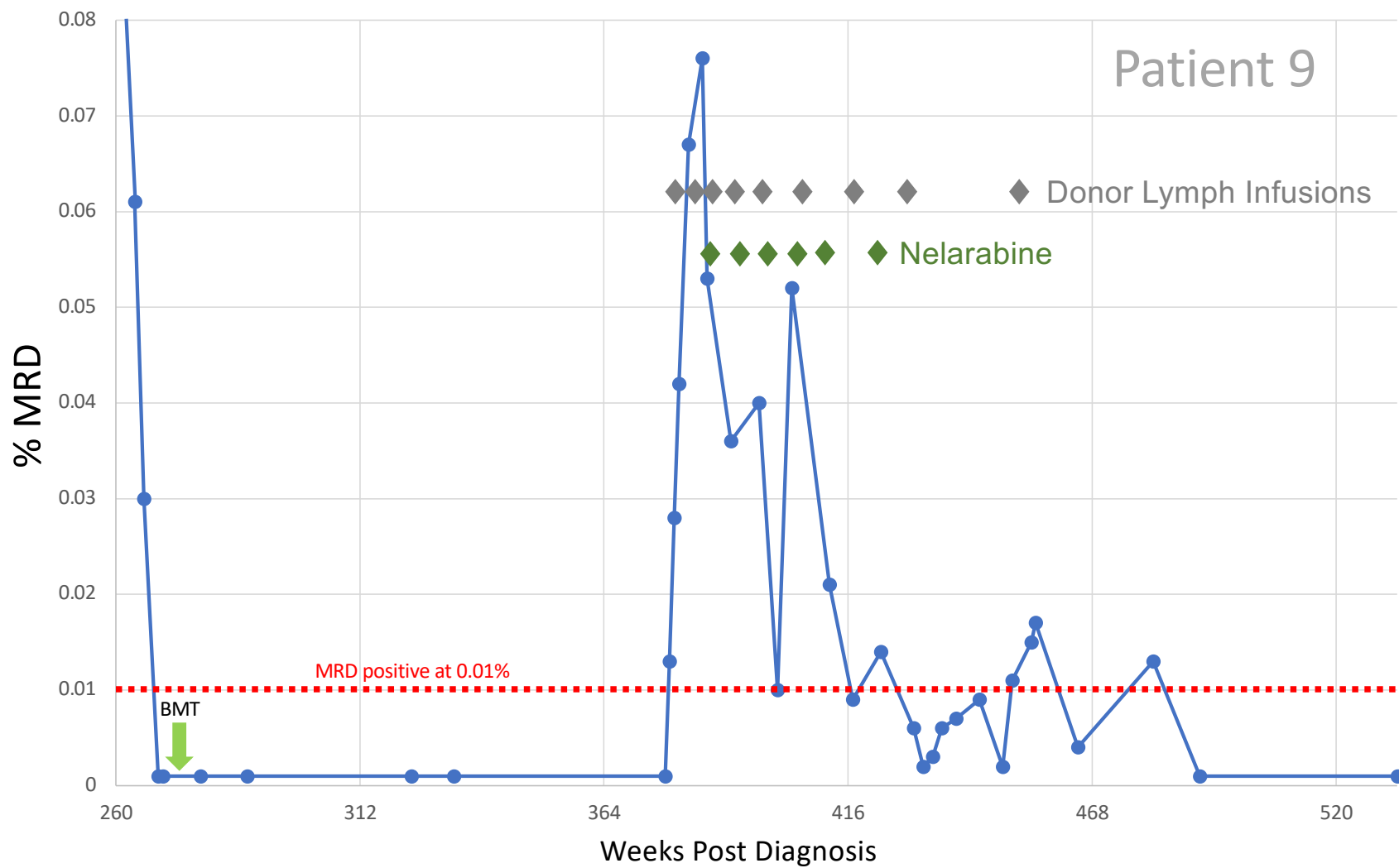
Day 2680 (PB)

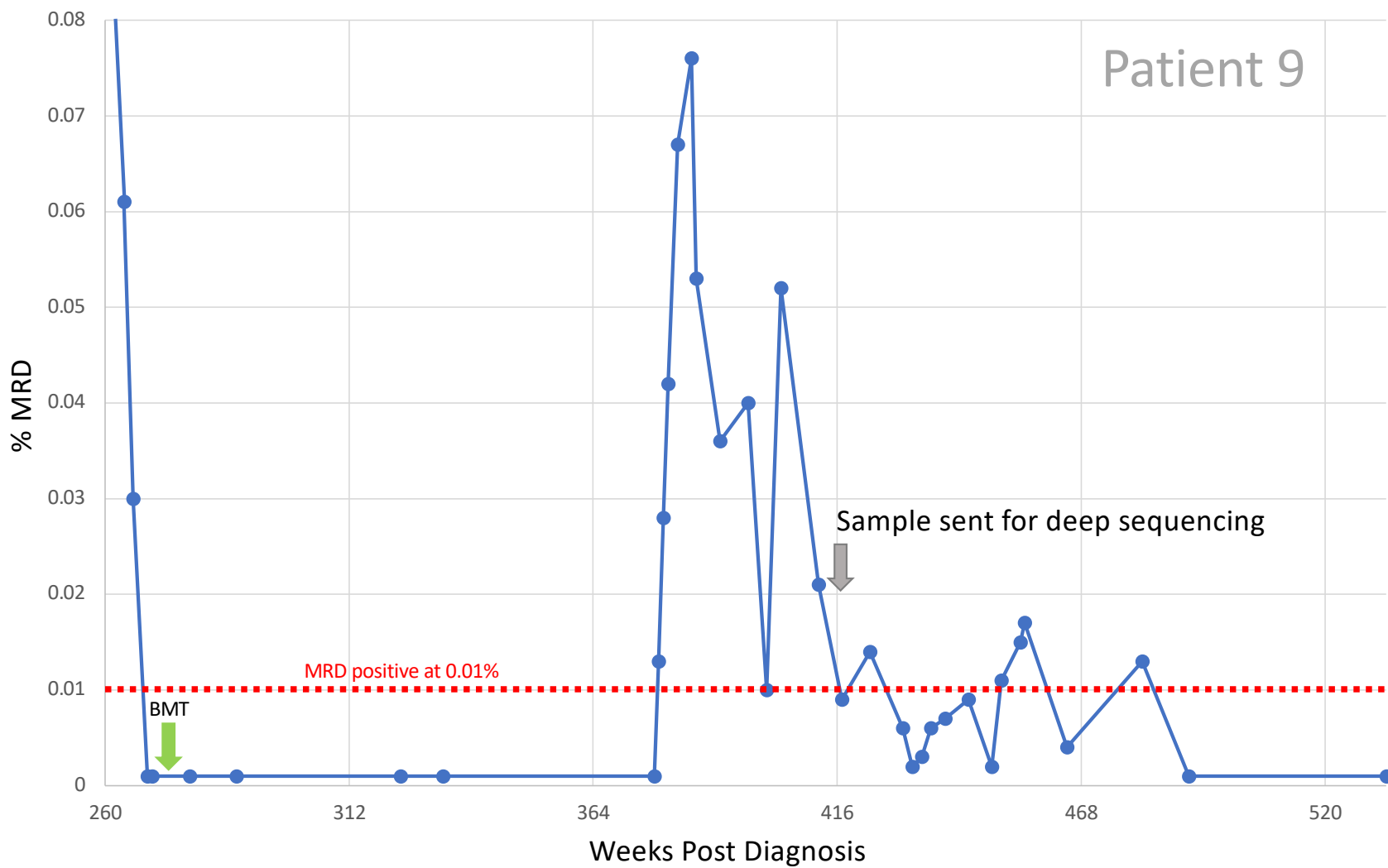
Run many events



Tube: Tube_003			
Population	#Events	%Parent	%Total
■ All Events	301,712	###	100.0
□ Scatter	298,309	98.9	98.9
□ Single cells	283,350	95.0	93.9
■ CD3 positive	275,075	97.1	91.2
■ CD5 pos/CD3 var	272,410	99.0	90.3
■ CD19/CD33 neg/HLA-DR neg	253,712	93.0	84.0
■ CD7 pos/CD1a pos	299	0.1	0.1

$$\begin{aligned}
 \text{Minimal Residual Disease} &= \text{Corrected number of Blasts/Total number of Events} \\
 &= ((299/481,196)-(24/214,955)) \times \text{CF} \times 100/1 \\
 &= 0.00050972 \times 1.134 \times 100 \\
 &= 0.057\% \text{ MRD positive}
 \end{aligned}$$





Gating Strategies - Examples

Start with four simple cases that are easy to follow at later treatment stages

Move on to progressively more difficult cases

- loss of aberrant marker expression with treatment
- aberrantly low expression on blast population becomes more like normal with treatment

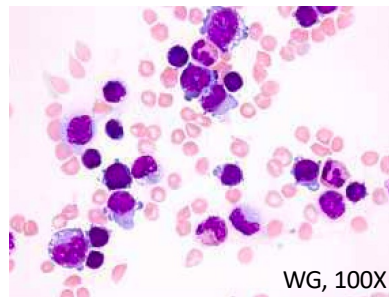
Note 5: Relapse can be subtle, not necessarily large numbers of blasts. Use care when assessing low-level MRD positive or near-positive samples. Where possible use more than one tube to confirm presence of blasts.

Patient 10 Anaplastic Large Cell Lymphoma

Another interesting case ... ALCL Patient 10

16 mo patient presented with hyperleukocytosis (WBC = 259.1) due to acute leukemia ...

PB smear showed ...



... immunophenotyping by flow cytometry showed ...

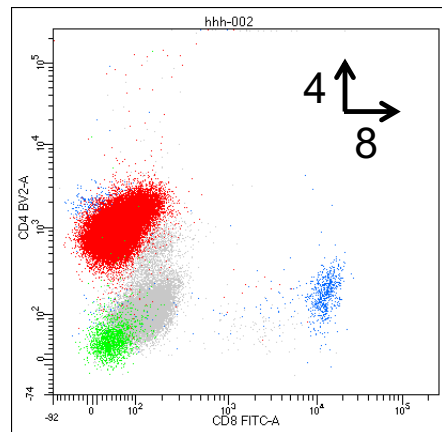
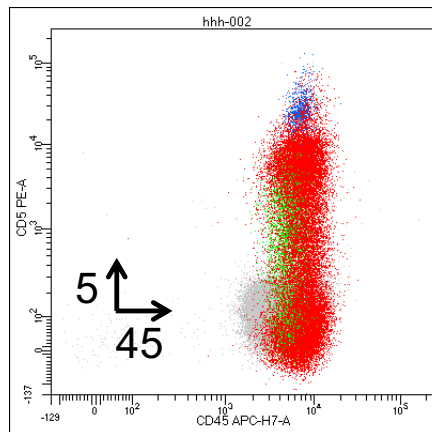
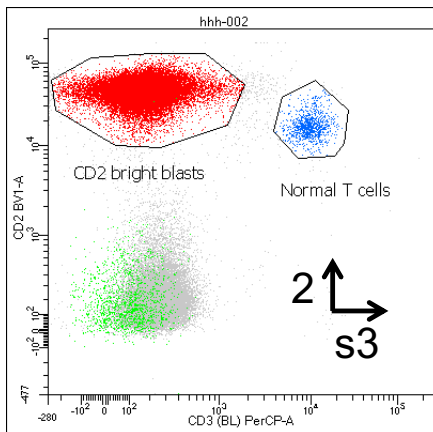
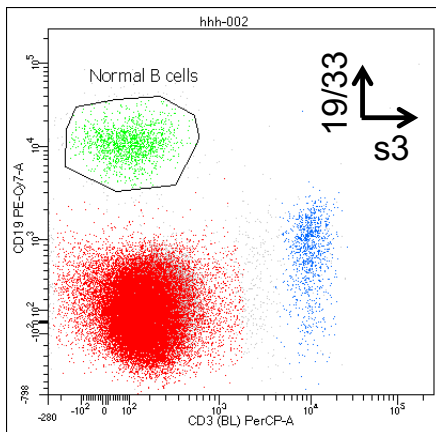
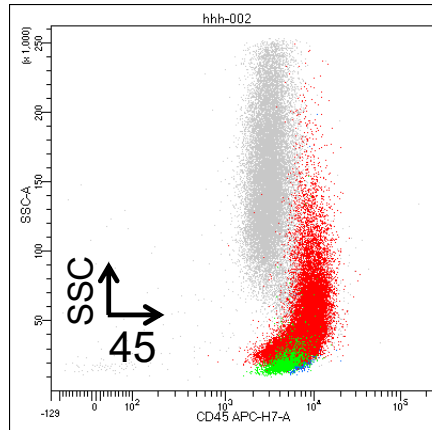
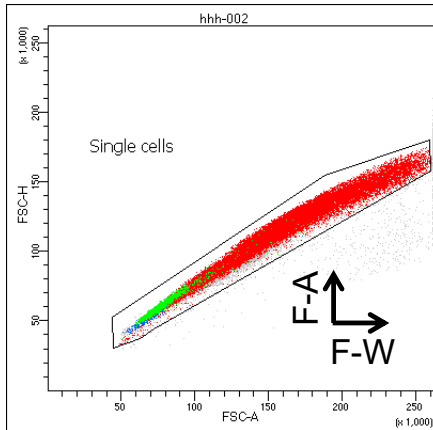
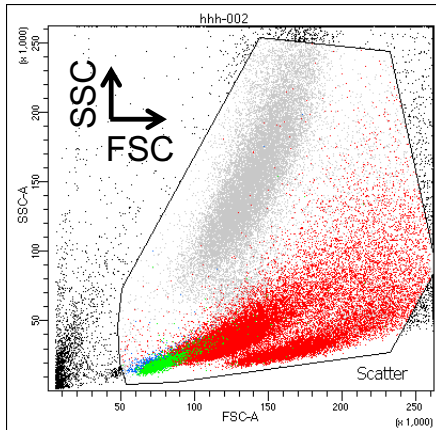
Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
IP S2	CD8	CD5	CD3	CD7	CD19	CD45	CD2	CD4

Patient 10

Diagnostic PB (Day 0)

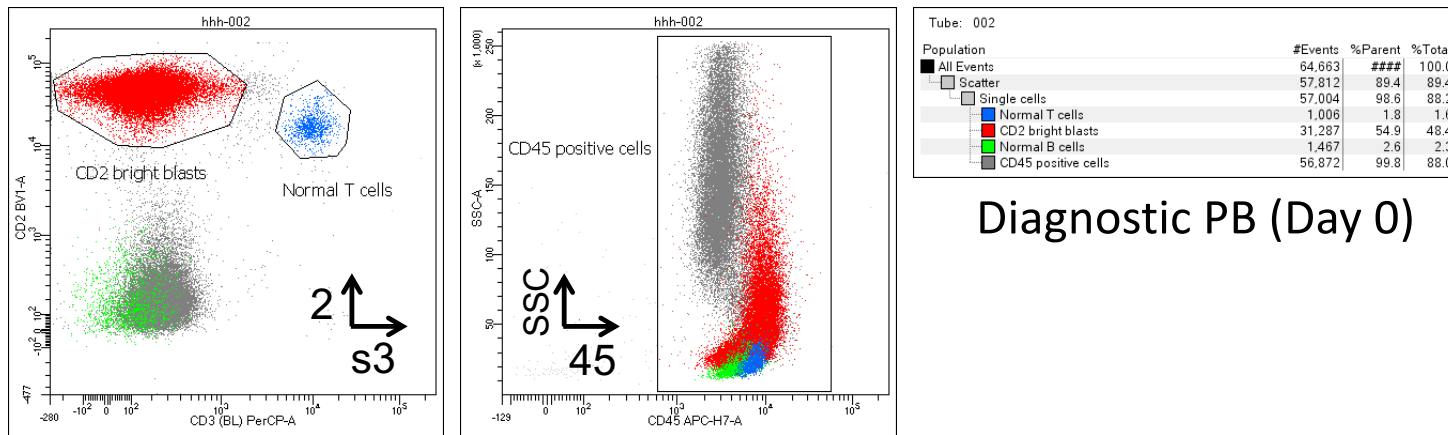
Tube: 002			
Population	#Events	%Parent	%Total
■ All Events	64,663	###	100.0
■ Scatter	57,812	89.4	89.4
■ Single cells	57,004	98.6	88.2
■ Normal T cells	1,006	1.8	1.6
■ CD2 bright blasts	31,287	54.9	48.4
■ Normal B cells	1,467	2.6	2.3

Blasts are sCD3-, cyCD3 var,
CD45 pos, CD2 bright,
CD5var, CD4 pos, CD8 neg



Another interesting case ...

Patient 10



Minimal Residual Disease = Number of CD2+ Blasts/Total CD45+ cells

$$= (31,287/56,872) \times 100/1$$

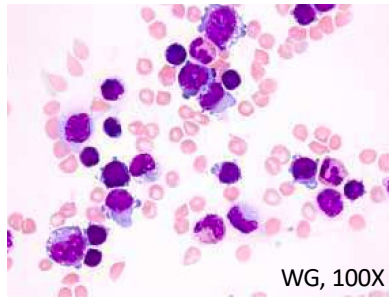
$$= 0.5501 \times 100$$

$$= 55.01\% \text{ MRD positive}$$

Another interesting case ... ALCL Patient 10

16 mo patient presented with hyperleukocytosis (WBC = 259.1) due to acute leukemia ...

PB smear showed ...



ALCL, small cell variant

... immunophenotyping by flow cytometry showed ...

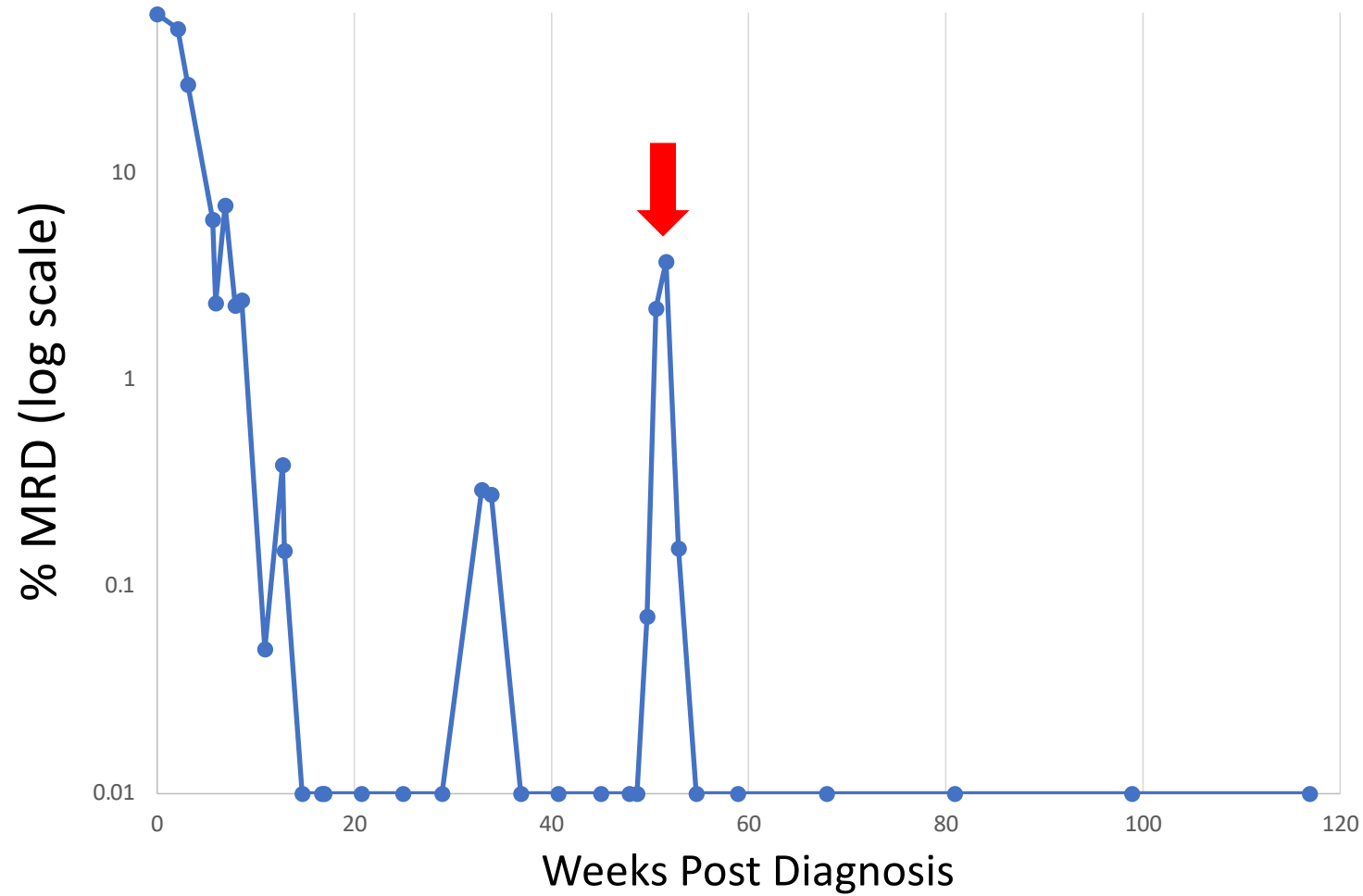
... IHC, Cytogenetics, WGS, WES, and RNAseq showed ...

CD30+, ALK+, t(2;8)(p23. 2;q22.3) *PABPC1-ALK* fusion

... diagnosis Anaplastic Large Cell Lymphoma (ALCL) ...

Another interesting case ... ALCL

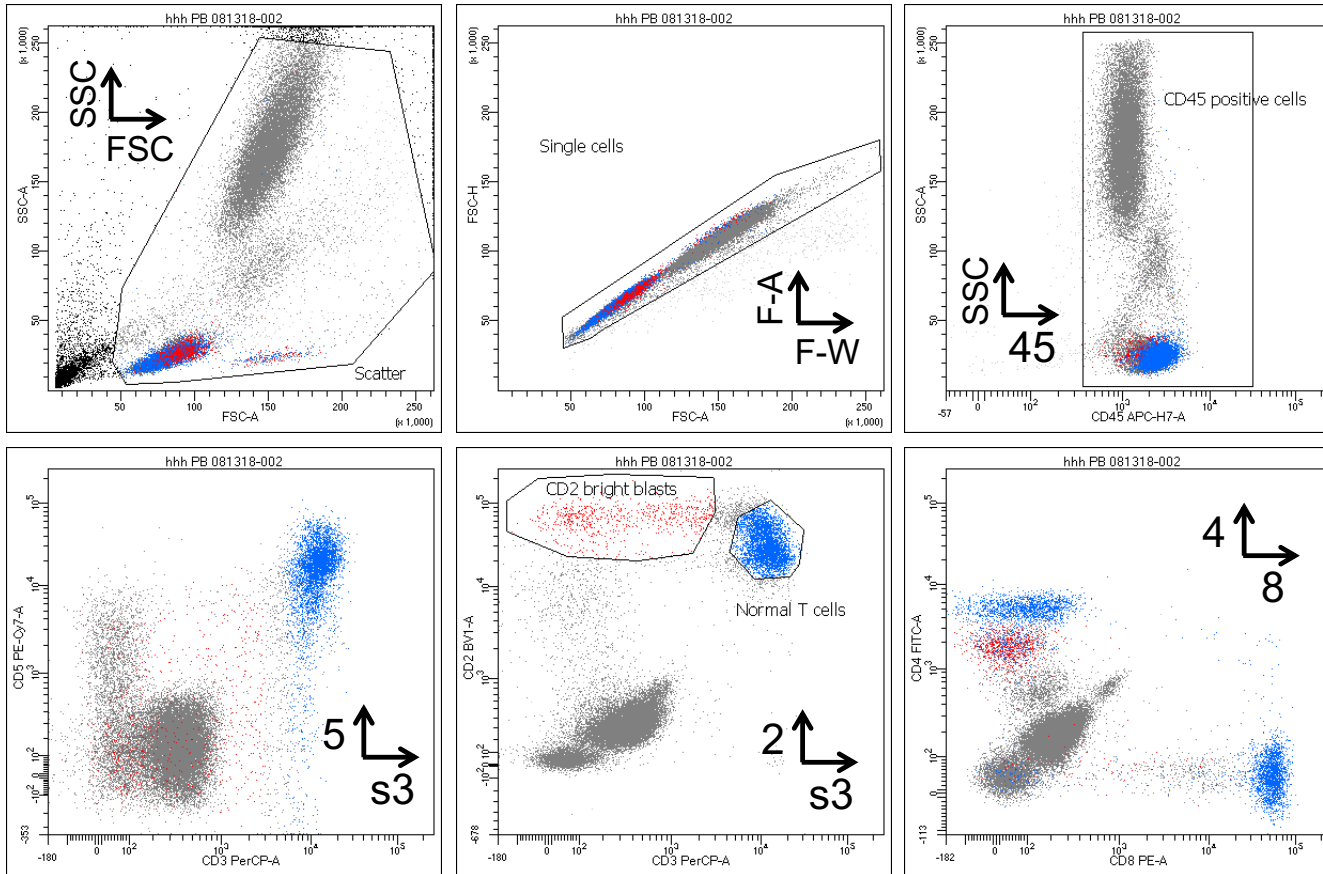
Patient 10



Tube	FITC	PE	PerCP	APC	PE-Cy7	APC-H7	V450	V500
IP S2n	CD4	CD8	CD3	CD7	CD5	CD45	CD2	CD56

Patient 10

PB (Day 361)



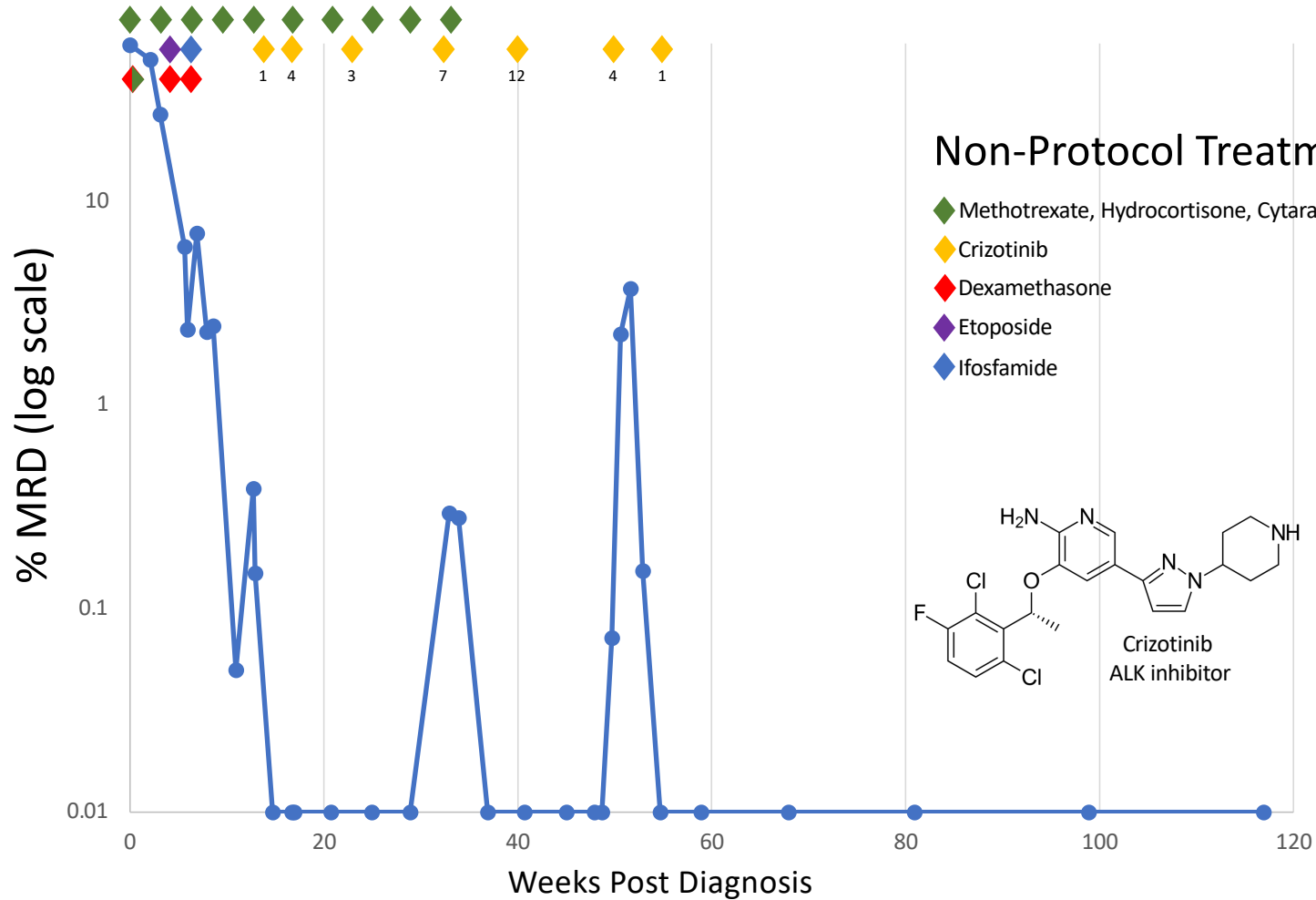
Tube: 002			
Population	#Events	%Parent	%Total
■ All Events	34,965	###	100.0
□ Scatter	28,811	82.4	82.4
□ Single cells	28,301	98.2	80.9
■ Normal T cells	4,041	14.3	11.6
■ CD2 bright blasts	707	2.5	2.0
■ CD45 positive cells	28,130	99.4	80.5

Blasts are sCD3-, cyCD3 var, CD45 pos, CD2 bright, CD5var, CD4 pos, CD8 neg

MRD positive (3.74%)

Another interesting case ... ALCL

Patient 10



Choi's exceptions to the rules ...

- Some patients have rare normal T cells with an unusual immunophenotype ...
 - CD1a **positive**, CD45 bright, CD5 bright
 - CD2 **negative**/TCR $\gamma\delta$ **positive**, CD45 bright, CD5 bright
 - CD45 bright, CD5 bright, CD4/CD8 double **positive**
 - CD45 bright, CD5 bright, CD4/CD8 double **negative**
 - sCD3 **negative**, cyCD3 **positive**, CD45 moderate, CD5 moderate



John Choi, MD, PhD

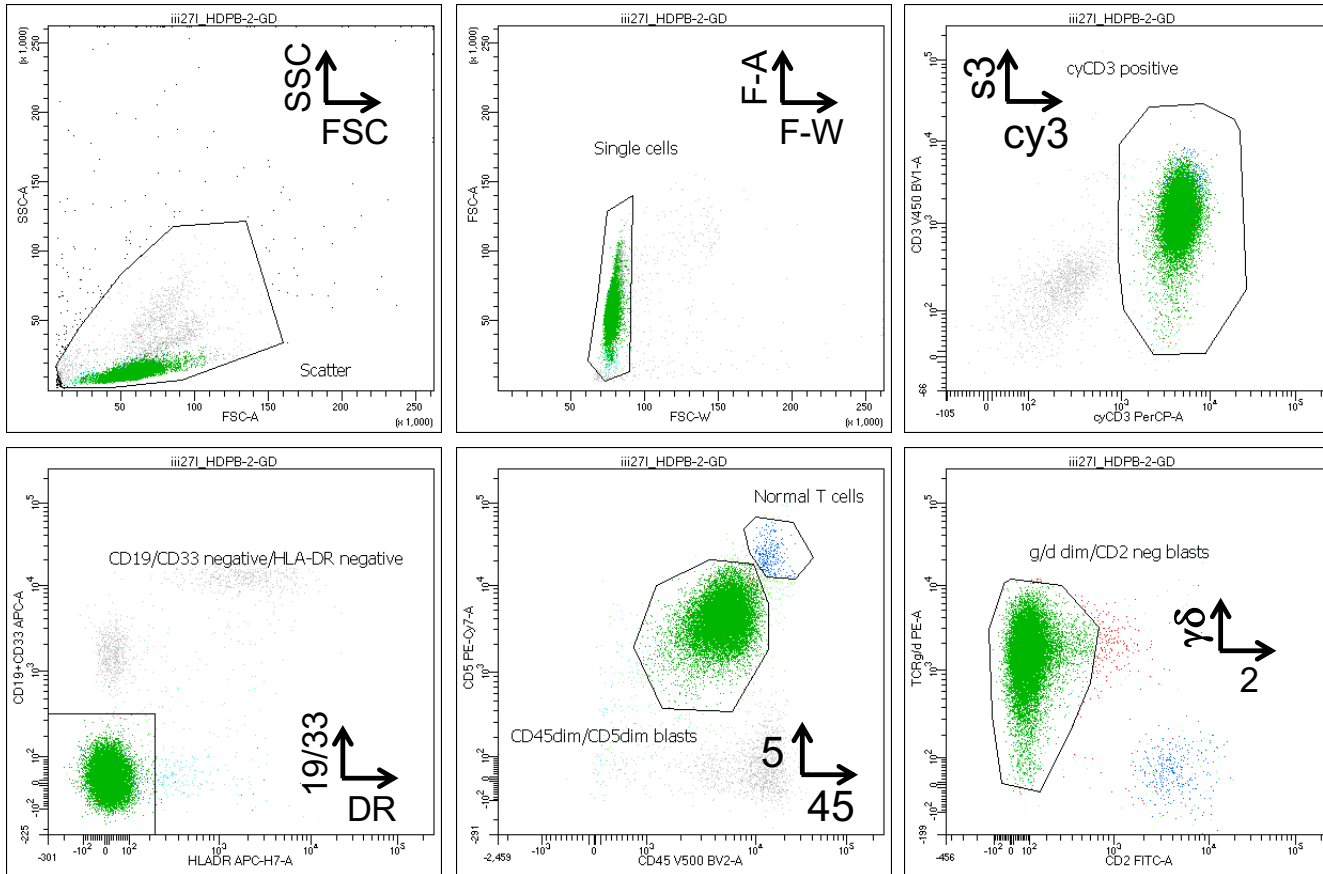
Choi's exceptions to the rules ...

- Some patients have rare normal T cells with an unusual immunophenotype ...
 - CD1a **positive**, CD45 bright, CD5 bright
 - CD2 **negative**/TCR $\gamma\delta$ **positive**, CD45 bright, CD5 bright
 - CD45 bright, CD5 bright, CD4/CD8 double **positive**
 - CD45 bright, CD5 bright, CD4/CD8 double **negative**
 - sCD3 **negative**, cyCD3 **positive**, CD45 moderate, CD5 moderate

Patient 11 Choi's exceptions to the rules

8 yo M with no known significant PMH presented with 2-3 wk hx of Rt inguinal bulge and lymphadenopathy

Choi's exceptions to the rules ...



Patient 11 Diagnostic (PB)

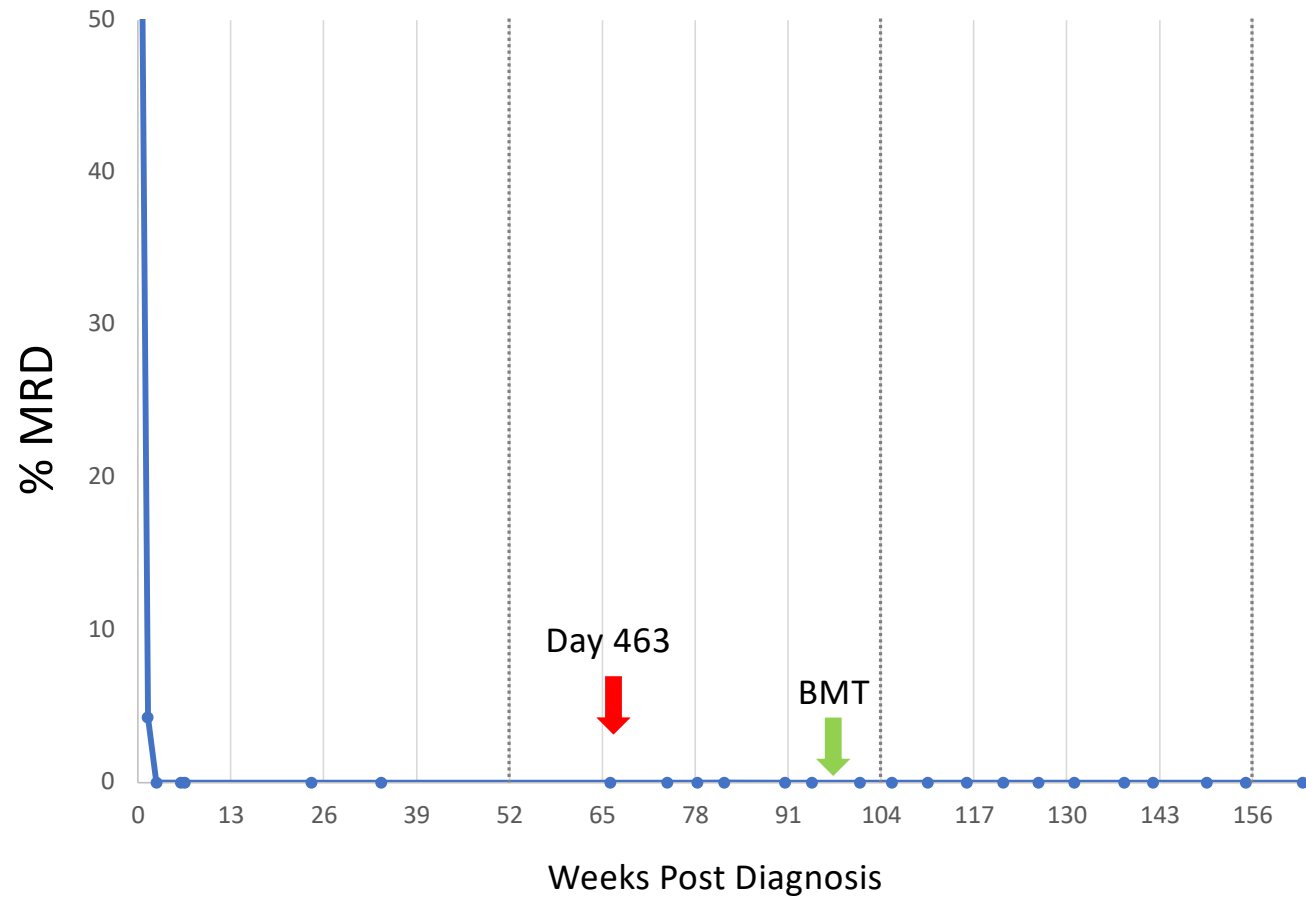
Tube: 2-GD			
Population	#Events	%Parent	%Total
All Events	20,000	###	100.0
Scatter	19,723	98.6	98.6
Single cells	19,363	98.2	96.8
cyCD3 positive	18,052	93.2	90.3
CD19/CD33 negative/HLA-DR negative	17,885	99.1	89.4
CD45dim/CD5dim blasts	17,391	97.2	87.0
g/d dim/CD2 neg blasts	17,183	98.8	85.9
Normal T cells	281	1.6	1.4

Blasts are cyCD3+, sCD3var,
CD45dim, CD5dim, CD2 neg,
and TCR γ/δ **positive**

MRD positive = 89%

Choi's exceptions to the rules ...

Patient 11

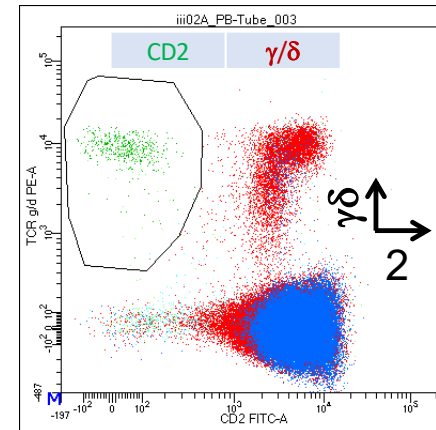


MRD calculation

Day 463 (PB)

Run many events

Patient 11



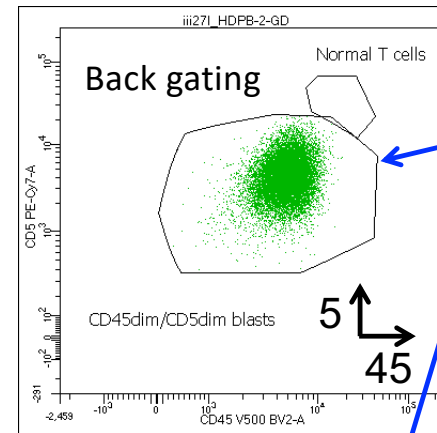
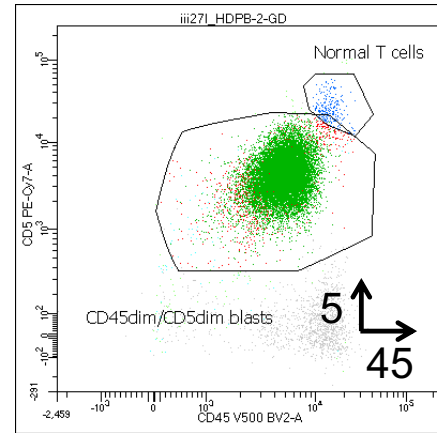
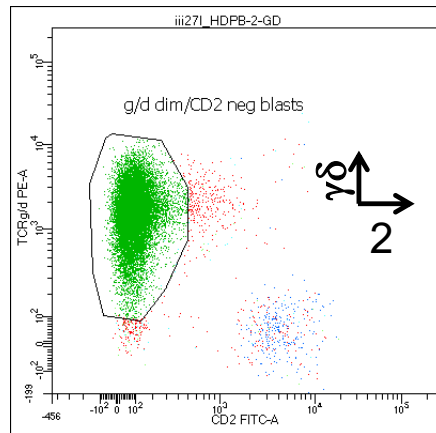
Tube: Tube_003			
Population	#Events	%Parent	%Total
■ All Events	164,152	###	100.0
■ Scatter	163,184	99.4	99.4
■ Single cells	160,432	98.3	97.7
■ γCD3 positive	145,799	90.9	88.8
■ CD19/CD33 negative/HLA-DR negative	141,561	97.1	86.2
■ CD45dim/CD5dim blasts	120,045	84.8	73.1
■ g/d dim/CD2 neg blasts	405	0.3	0.2
■ Normal T cells	52,880	36.3	32.2

$$\begin{aligned}
 \text{Minimal Residual Disease} &= \text{Corrected number of Blasts} / \text{Total number of Events} \\
 &= ((405/211,640) - (5/84,656)) \times \text{CF} \times 100/1 \\
 &= 0.001855 \times 1.065 \times 100 \\
 &= 0.196\% \text{ MRD positive}
 \end{aligned}$$

Something looks a bit odd about this result ...

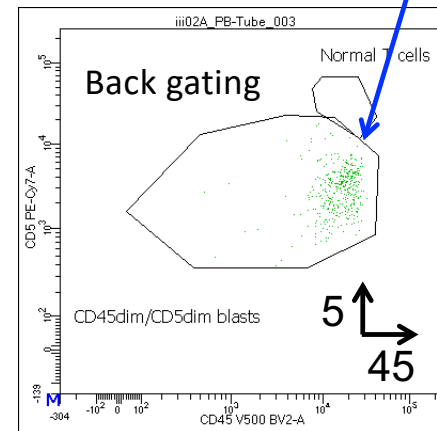
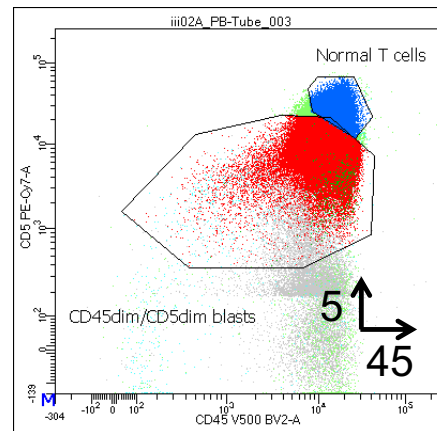
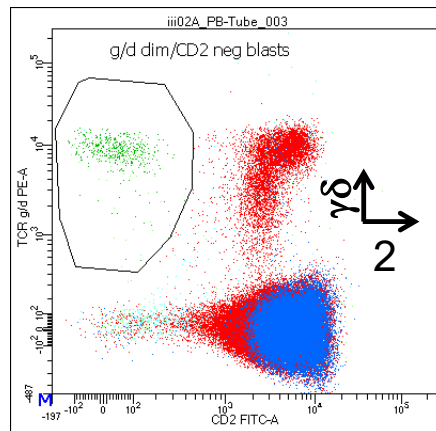
Patient 11

Diagnosis (PB)



CD2 **neg**/γ/δ **pos** population has increased CD45 expression at 'relapse' compared to the diagnosis sample

Day 463 (PB)



Deep Sequencing analysis of Day 0 and Day 463 samples

Something looks a bit odd about this result ...

Patient 11

Deep Sequencing (NGS) of TCRG locus (ClonoSeq) of diagnostic sample (Day 0) ...

... Two dominant clones (A & B) from a total of 5,250 unique sequences from 488,118 total sequences from 61,957 cells. Clonality = 0.98

... at Day 463 ...

Deep Sequencing showed ...

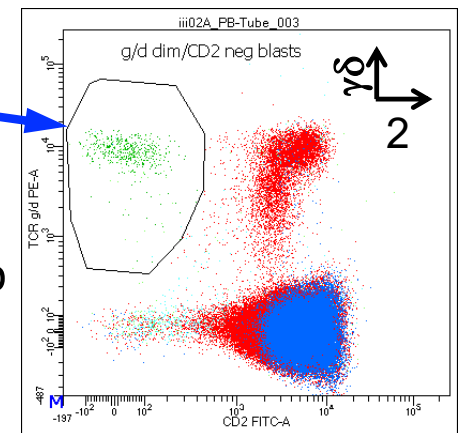
Clone A = undetectable (0 reads per 1×10^6 cells)

Clone B = 46 reads per 1×10^6 cells = 0.0046% MRD (**negative**)

This unusual γ/δ **positive**/CD2 **negative**/CD45 **bright** population (0.195%) identified at Day 463 is not part of the leukemic clone identified at diagnosis ...

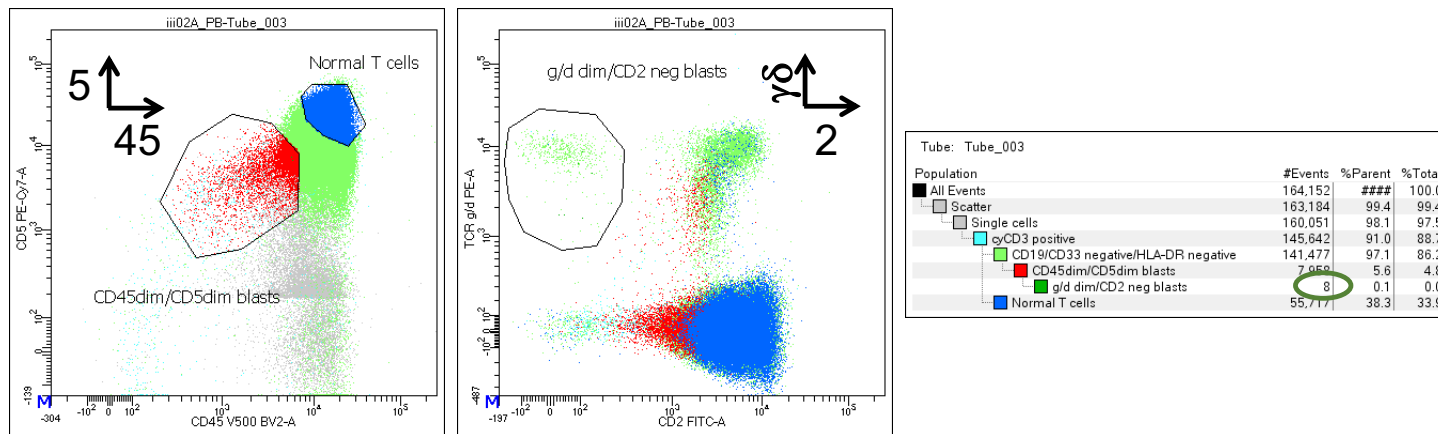
= most likely represents a rare (unusual) 'normal' T cell pop

= becomes one of "Choi's Exceptions to the Rules"



Something looks a bit odd about this result ... Patient 11

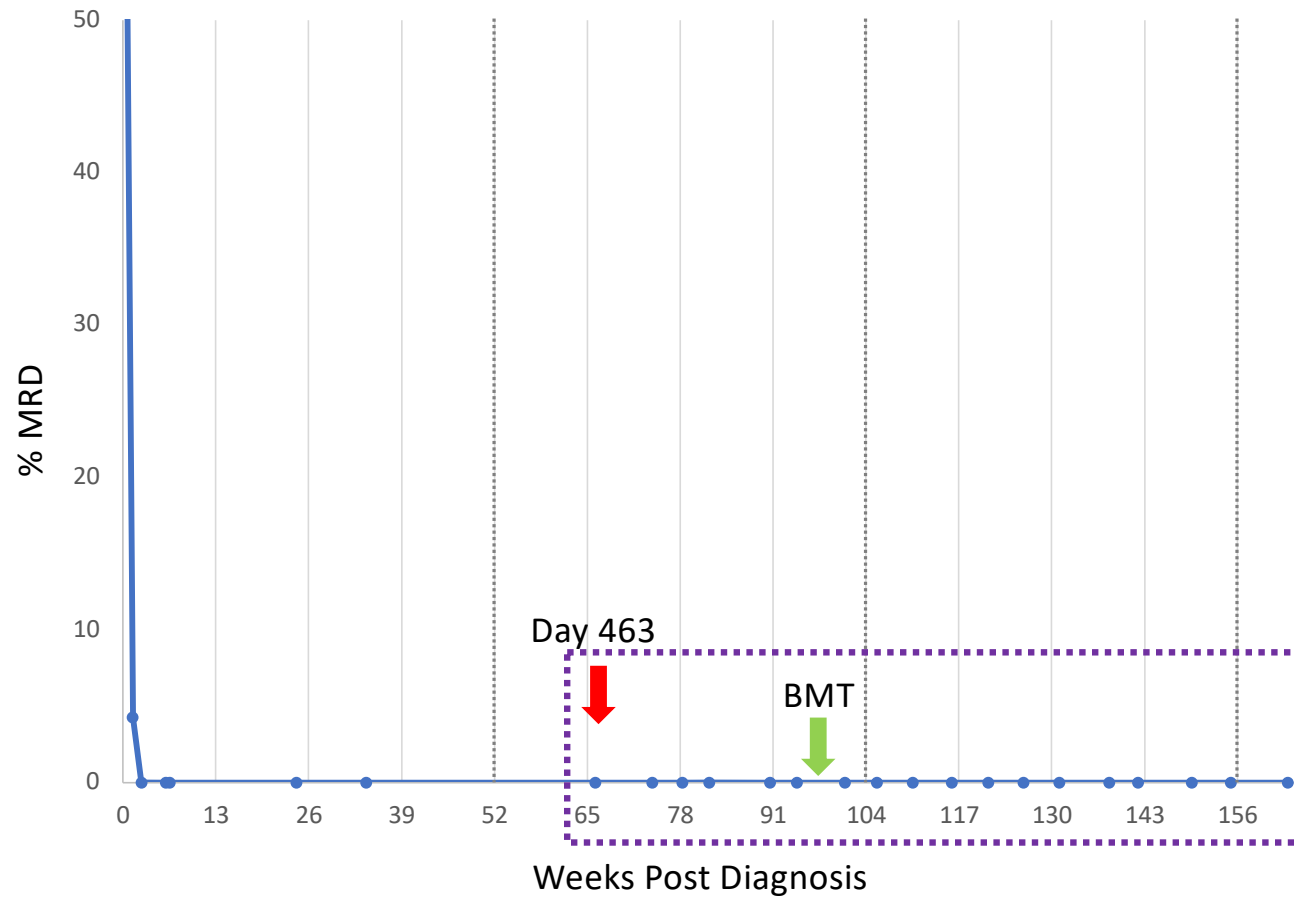
Re-gate the γ/δ **positive**/CD2 **negative** population to exclude the CD45 bright cells ...



Minimal Residual Disease = Corrected number of Blasts/Total number of Events
 = $((8/211,640) - (0/84,656)) \times CF \times 100/1$
 = $0.0000378 \times 1.065 \times 100$
 = 0.004% **MRD negative**
 = same as that determined by NGS = 0.0046% **MRD negative**

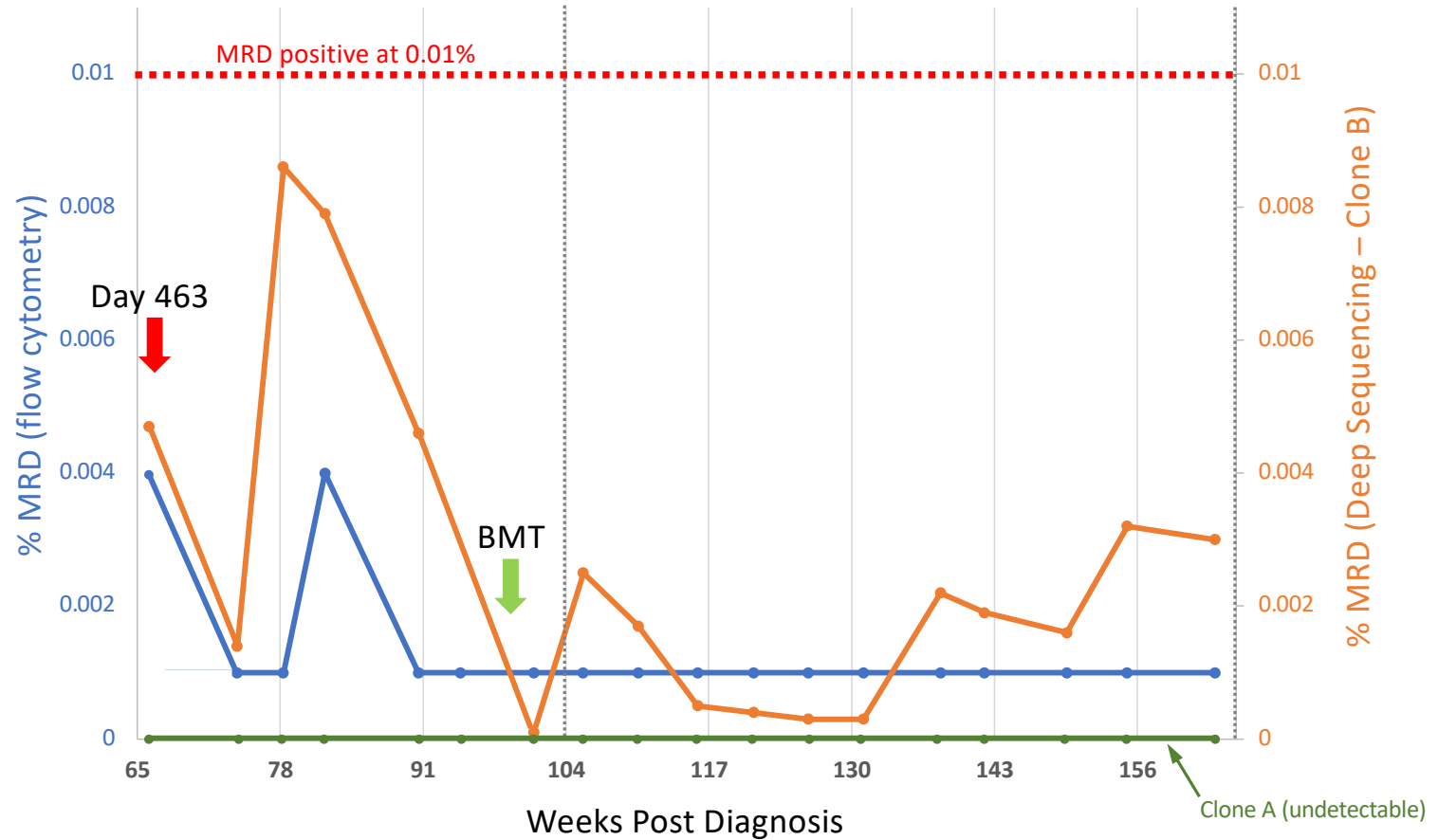
Choi's exceptions to the rules ...

Patient 11



Choi's exceptions to the rules ...

Patient 11



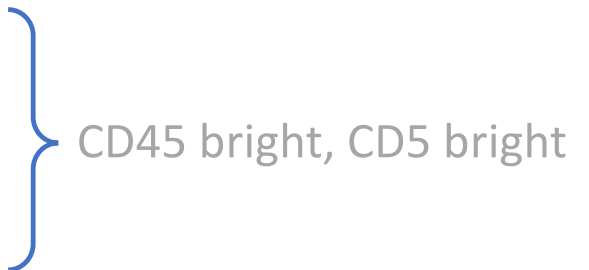
Choi's exceptions to the rules ...

- Some patients have rare normal T cells with an unusual immunophenotype ...
 - CD1a **positive**, CD45 bright, CD5 bright
 - CD2 **negative**/TCR $\gamma\delta$ **positive**, CD45 bright, CD5 bright
 - CD45 bright, CD5 bright, CD4/CD8 double **positive**
 - CD45 bright, CD5 bright, CD4/CD8 double **negative**
- } CD45 bright, CD5 bright

... must look at the CD45 and CD5 expression of blasts at diagnosis (or relapse). If both CD45 and CD5 are bright (overlap normal T cells) from outset, then these leukemias may be difficult to follow at later stages when MRD levels are low ...

Choi's exceptions to the rules ...

- Some patients have rare normal T cells with an unusual immunophenotype ...

- CD1a positive, CD45 bright, CD5 bright
 - CD2 negative/TCR $\gamma\delta$ positive, CD45 bright, CD5 bright
 - CD45 bright, CD5 bright, CD4/CD8 double positive
 - CD45 bright, CD5 bright, CD4/CD8 double negative
 - sCD3 **negative**, cyCD3 **positive**, CD45 moderate, CD5 moderate ...
- 
- CD45 bright, CD5 bright

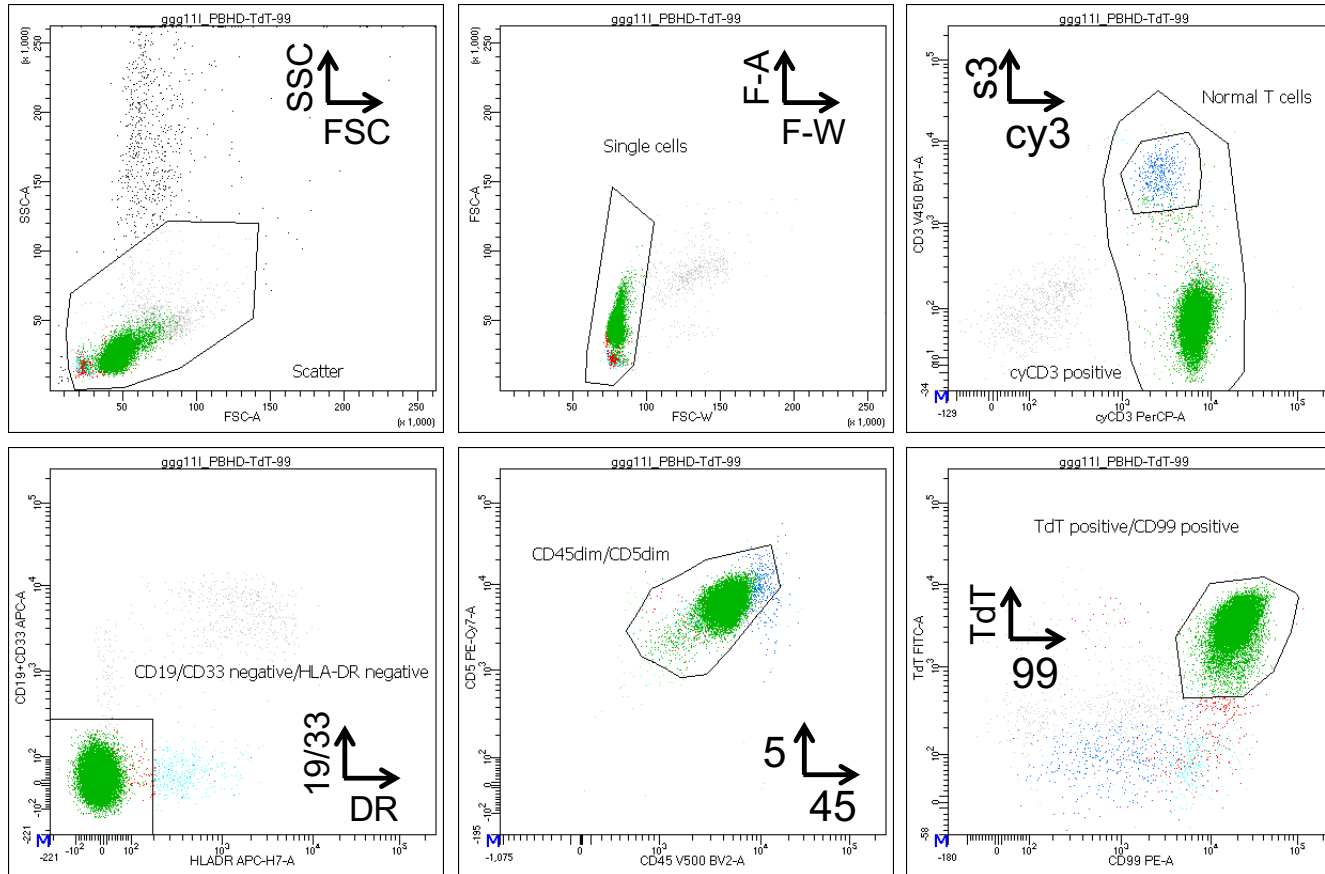
... that could be nearly any T-ALL ...

... must use an ancillary marker to identify blasts ...

... can not rely on backbone markers alone!

Patient 12 Subset populations can help

Gating example

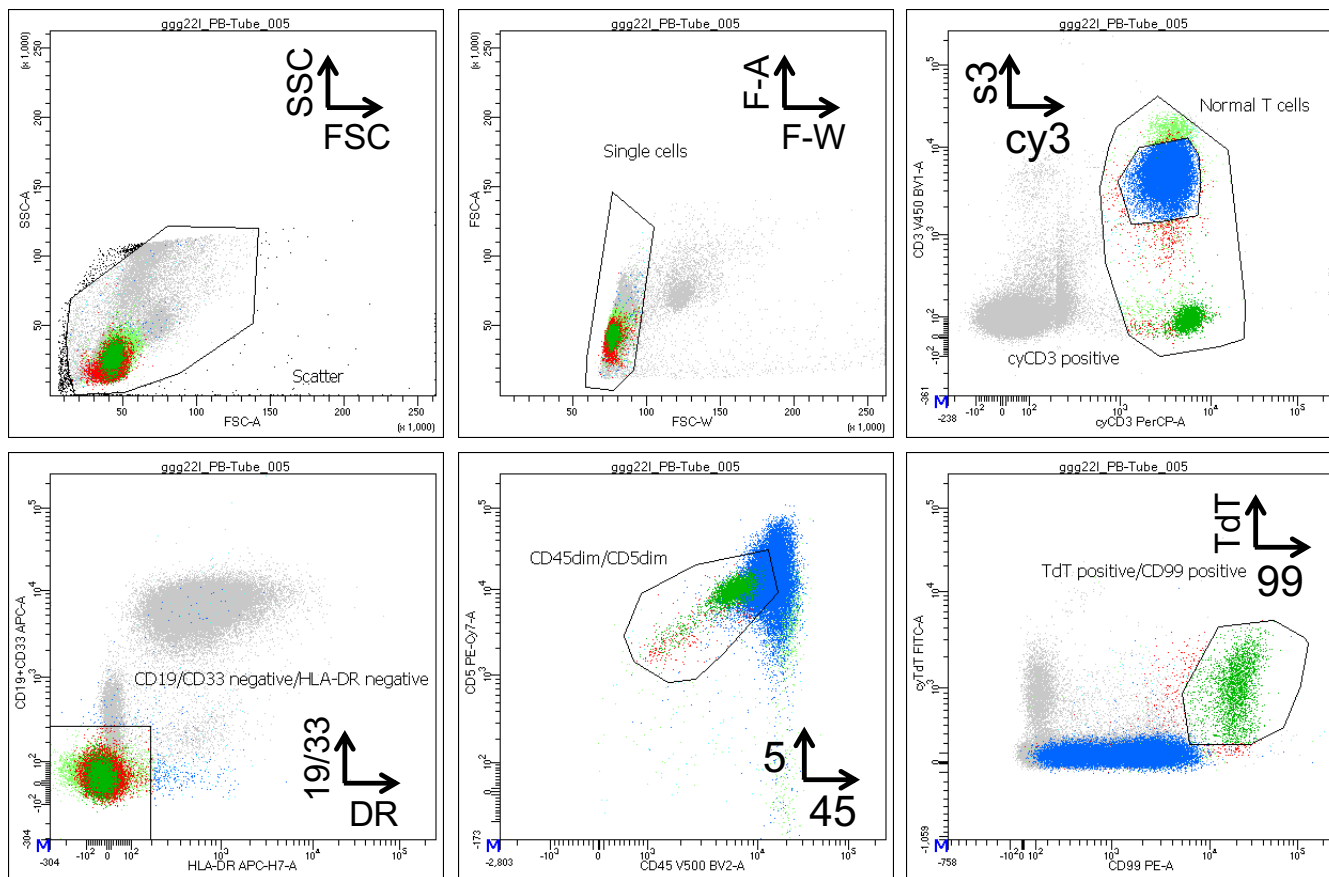


Patient 12 Diagnostic (PB)

Tube: TdT-99			
Population	#Events	%Parent	%Total
■ All Events	20,000	###	100.0
■ Scatter	19,301	96.5	96.5
■ Single cells	18,783	97.3	93.9
■ cyCD3 positive	18,099	96.4	90.5
■ Normal T cells	410	2.3	2.1
■ CD19/CD33 negative/HLA-DR negative	17,758	98.1	88.8
■ CD45dim/CD5dim	17,631	99.3	88.2
■ TdT positive/CD99 positive	17,055	96.7	85.3

Blasts are cyCD3var, sCD3-, CD45dim, CD5dim, CD99 positive, and TdT positive

Gating example



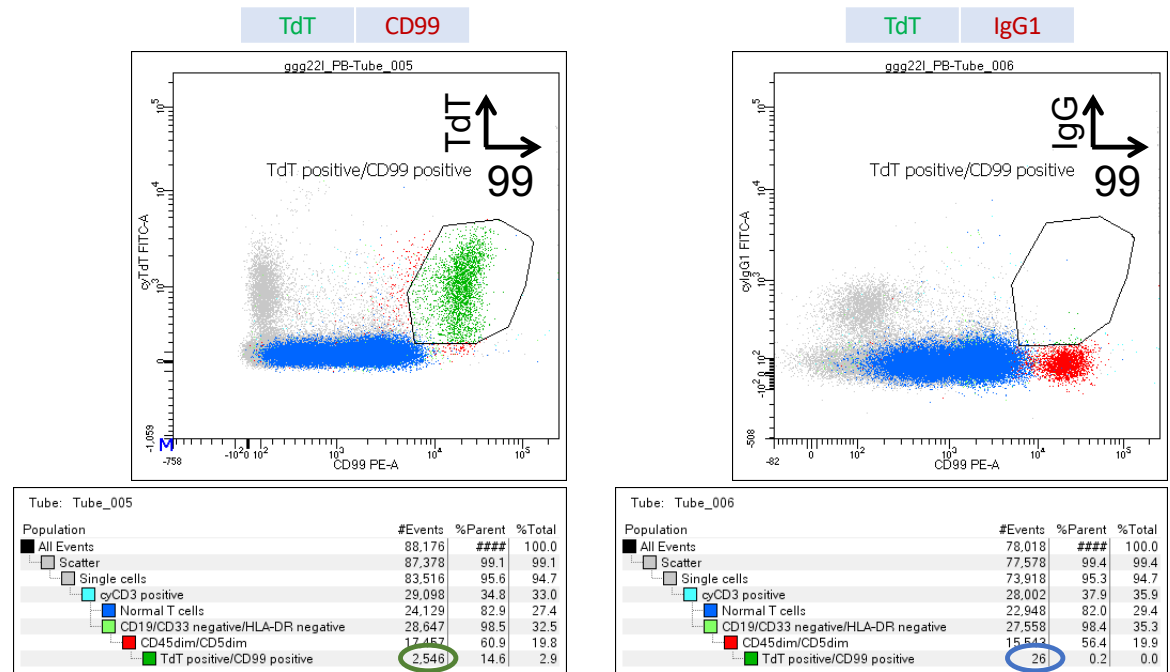
Patient 12
Day 8 (PB)

Tube: Tube_005			
Population	#Events	%Parent	%Total
■ All Events	88,176	###	100.0
■ Scatter	87,378	99.1	99.1
■ Single cells	83,516	95.6	94.7
■ cyCD3 positive	29,098	34.8	33.0
■ Normal T cells	24,129	82.9	27.4
■ CD19/CD33 negative/HLA-DR negative	28,647	98.5	32.5
■ CD45dim/CD5dim	17,457	60.9	19.8
■ TdT positive/CD99 positive	2,546	14.6	2.9

Blasts are cyCD3var, sCD3-,
CD45dim, CD5dim, CD99
positive, and TdT positive

MRD calculation

Day 8 (PB)



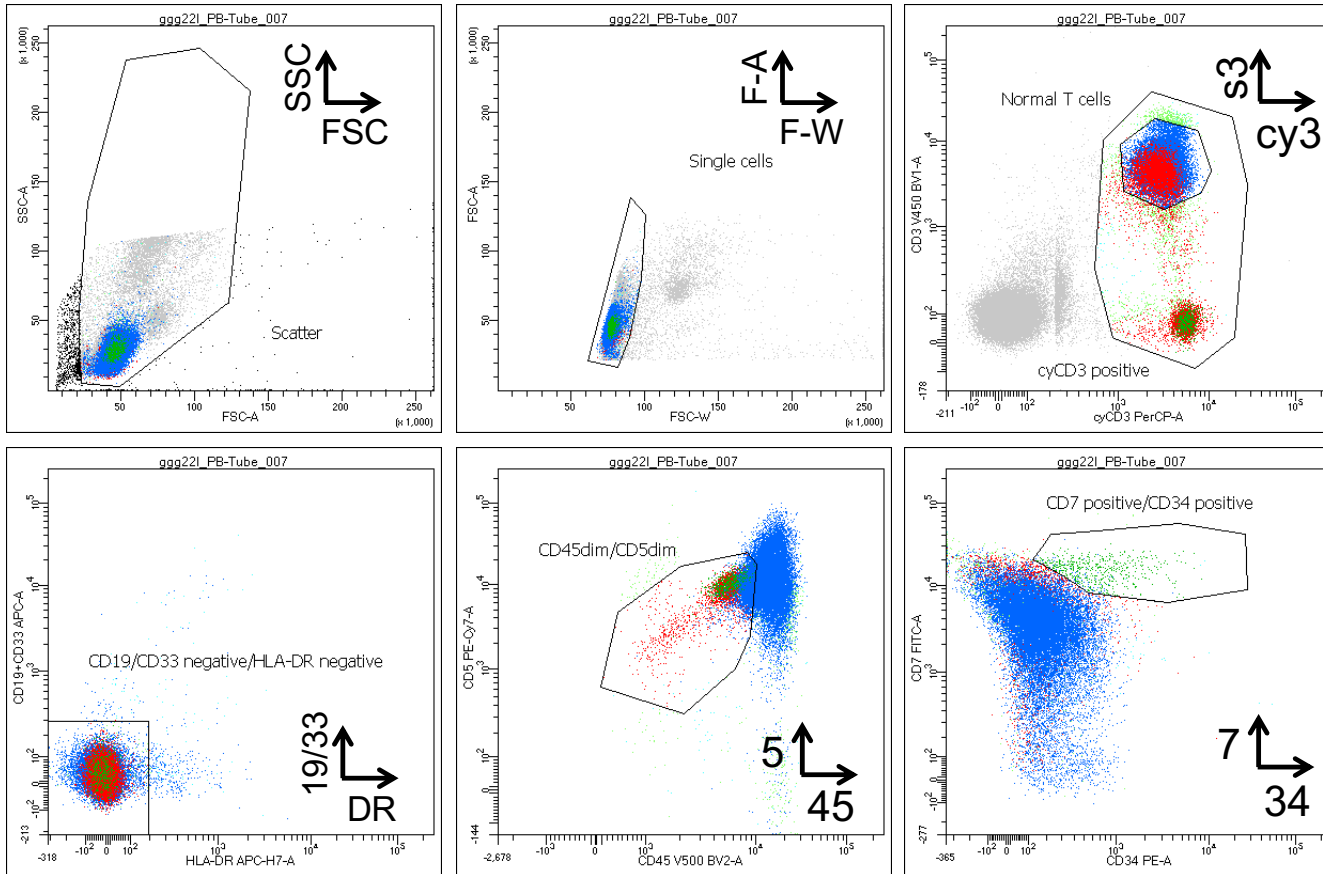
Minimal Residual Disease = Corrected number of Blasts/Total number of Events

$$= ((2,546/768,576) - (26/688,197)) \times CF \times 100/1$$

$$= 0.003275 \times 1.135 \times 100$$

$$= 0.372\% \text{ MRD positive}$$

Gating example



Tube: Tube_007

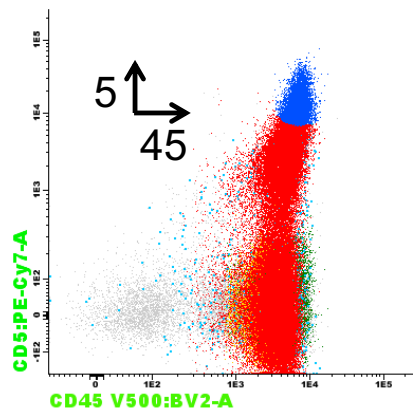
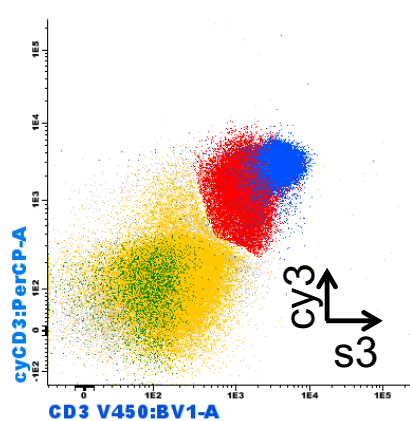
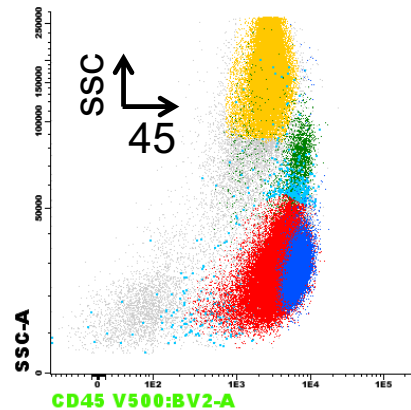
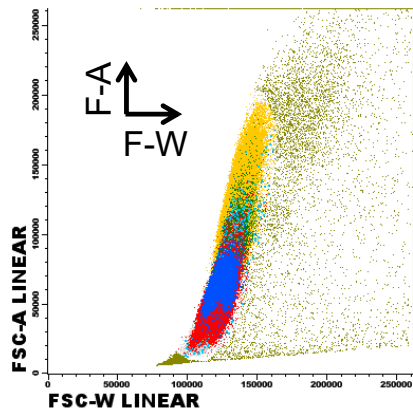
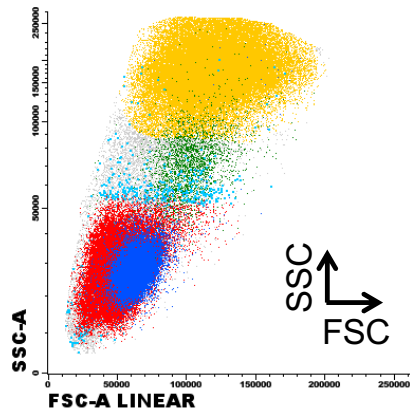
Population	#Events	%Parent	%Total
All Events	76,892	###	100.0
Scatter	75,672	98.4	98.4
Single cells	72,815	96.2	94.7
cyCD3 positive	28,986	39.8	37.7
Normal T cells	24,112	83.2	31.4
CD19/CD33 negative/HLA-DR negative	28,549	98.5	37.1
CD45dim/CD5dim	7,447	26.1	9.7
CD7 positive/CD34 positive	488	6.6	0.6

Patient 12
Day 8 (PB)

Blasts are cyCD3var, sCD3-, CD45dim, CD5dim, CD99 positive, TdT positive, and CD7 positive, CD34 subset+

Can use this subset CD34+ population to verify presence of blasts, use TdT/CD99 tube for numbers.

Patient 13 No useful 'Add on' Markers



Population	Review	Events	Frequency	Total %
EVENTS		160000		
Other EVENTS		11300	NA	7.063
DEBRIS/DOUBLETS		9558	NA	5.974
Scatter		139142	100.000	86.964
Other Scatter		11362	8.166	7.101
Granulocytes		59244	42.578	37.028
Cytoplasmic CD3 positive		65960	47.405	41.225
Other Cytoplasmic CD3 positive		1051	0.755	0.657
Normal T cells		11701	8.409	7.313
Blasts		53208	38.240	33.255
CD5 dim/CD45dim blasts		0	0.000	0
Normal lymphocytes		0	0.000	0
Other Normal lymphocytes		0	0.000	0
B cells		0	0.000	0
Monocytes		2576	1.851	1.610

Patient 13 Diagnostic (PB)

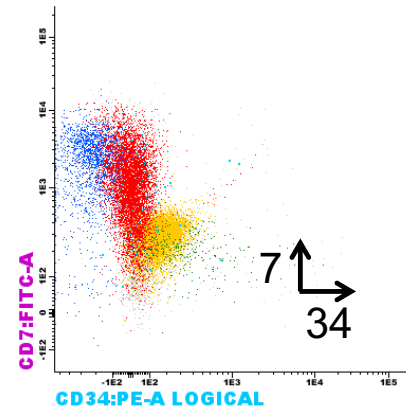
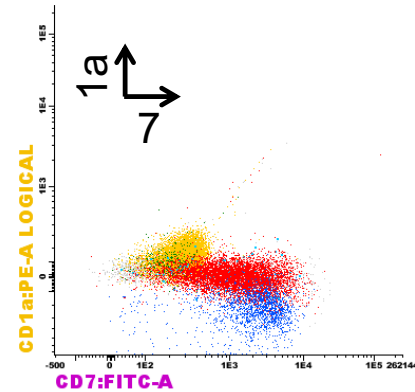
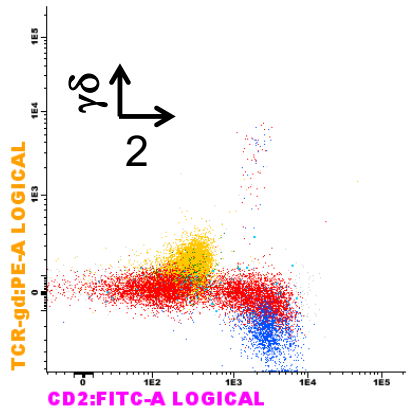
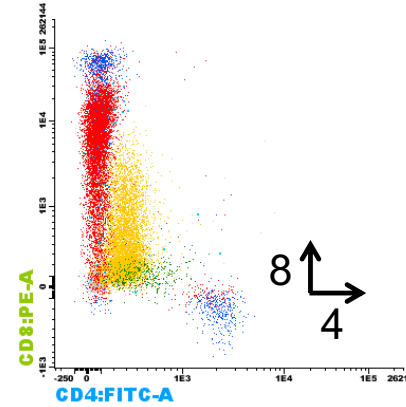
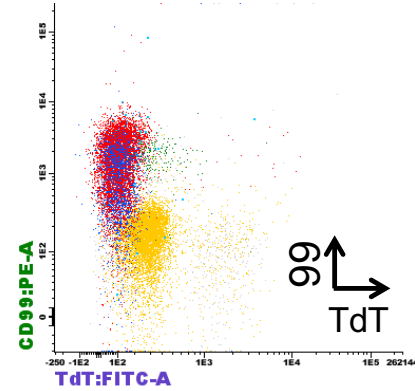
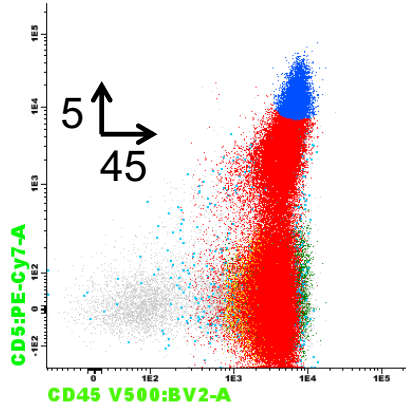
Backbone markers



Patient 13 Diagnostic (PB)

No useful 'Add on' markers

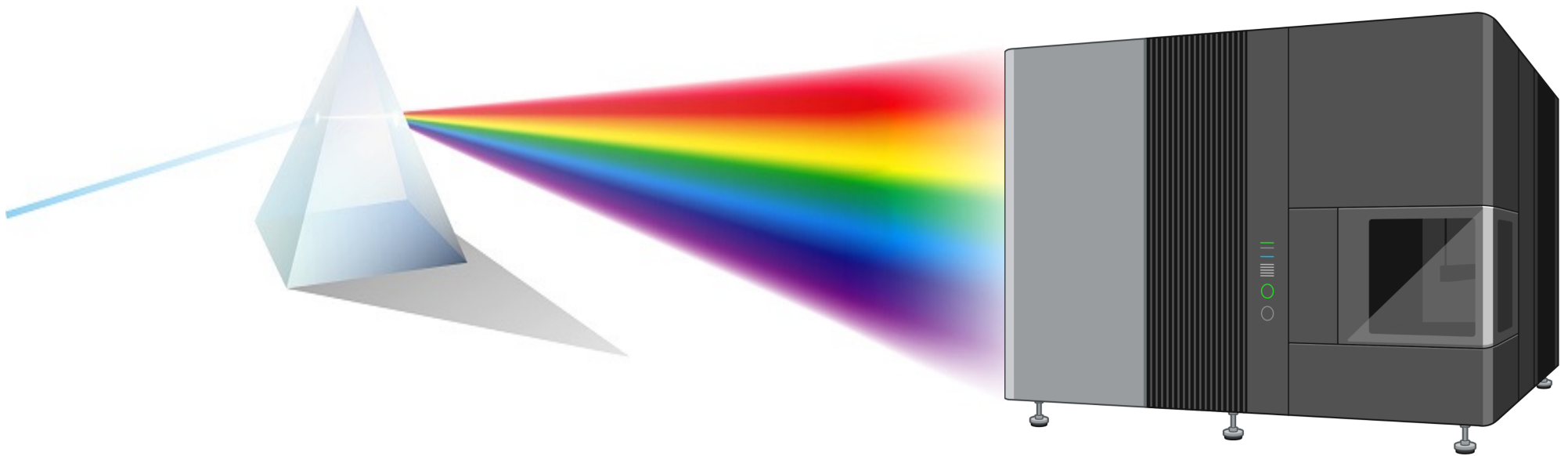
Can not follow by flow ...



Population	Review	Events	Frequency	Total %
EVENTS		160000		
Other EVENTS		11300	NA	7.063
DEBRIS/DOUBLETS		9558	NA	5.974
Scatter		139142	100.000	86.964
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Normal T cells		11701	8.409	7.313
Blasts		53208	38.240	33.255
CD5 dim/CD45dim blasts		0	0.000	0
Normal lymphocytes				
Other Normal lymphocytes				
B cells				
Monocytes				



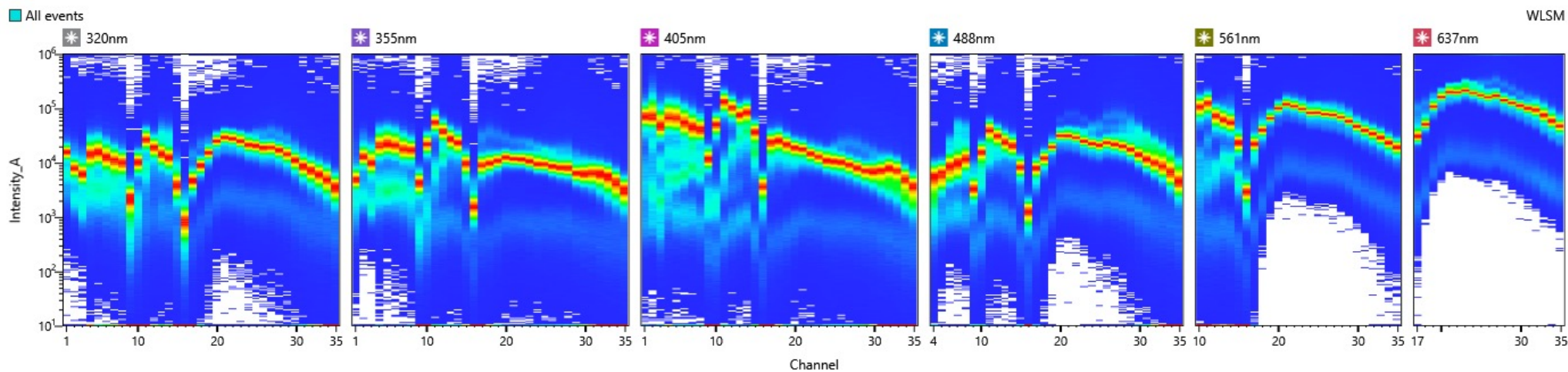
Spectral Flow Cytometry



Sony ID7000 Spectral Cytometer



6-laser 50+ colour Spectral cytometer



Scatter (Area)

FSC_A SSC_A

Fluorochrome (Area)

320nm

355nm

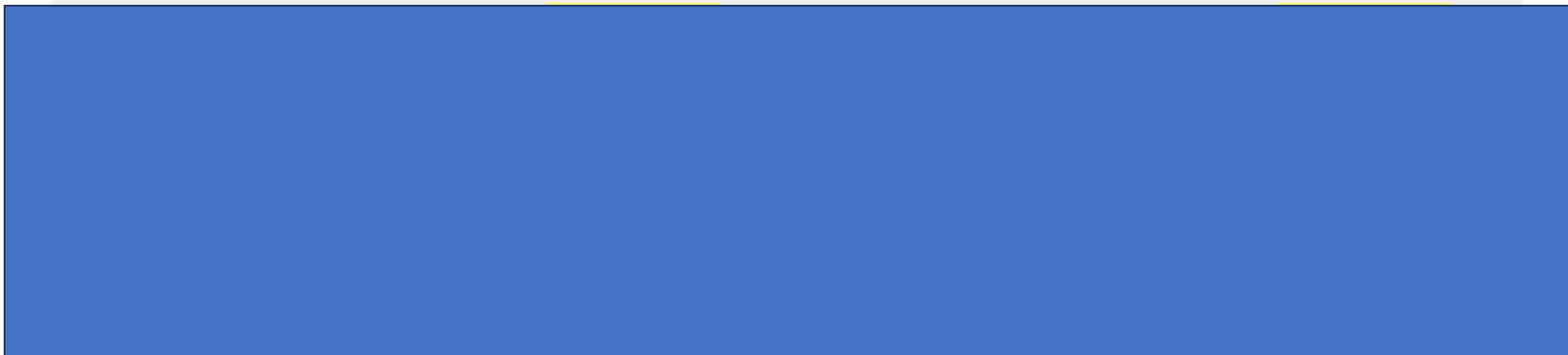
405nm

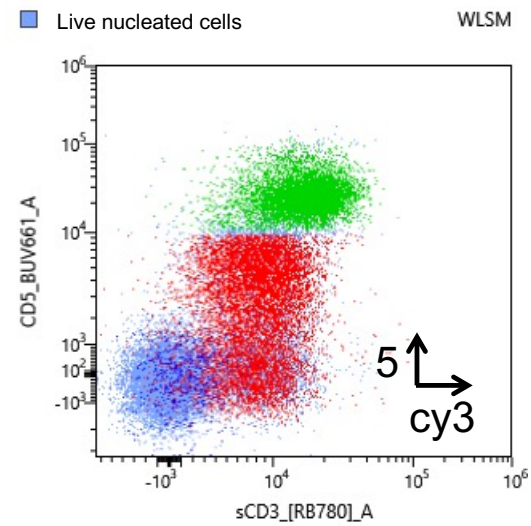
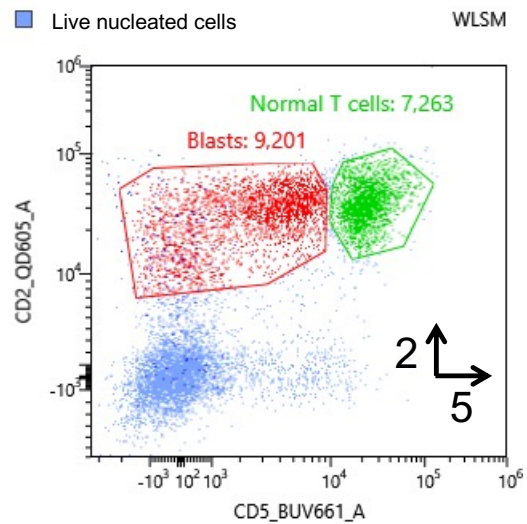
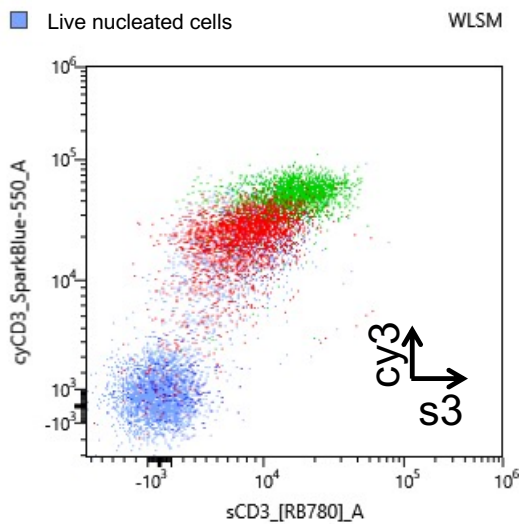
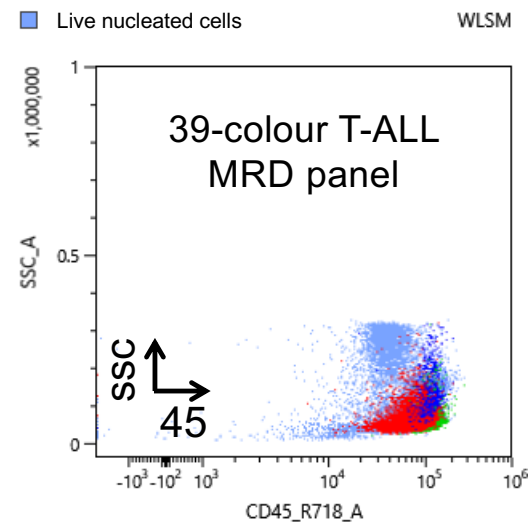
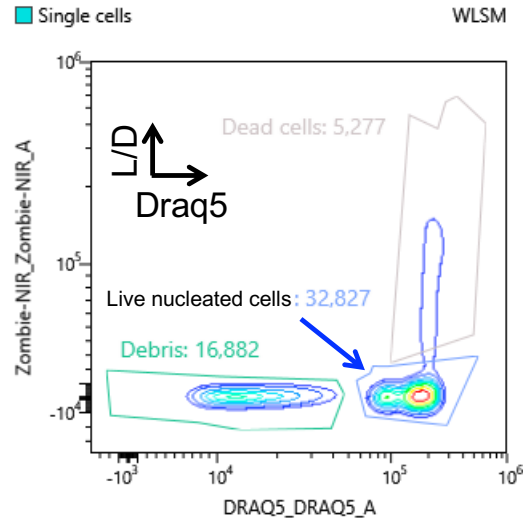
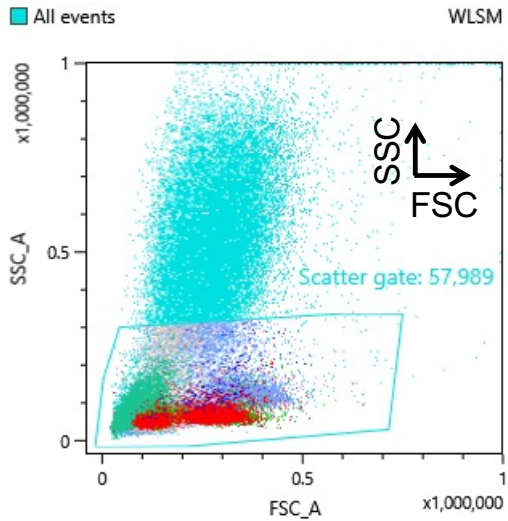
488nm

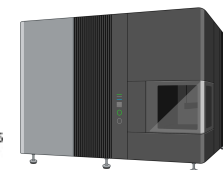
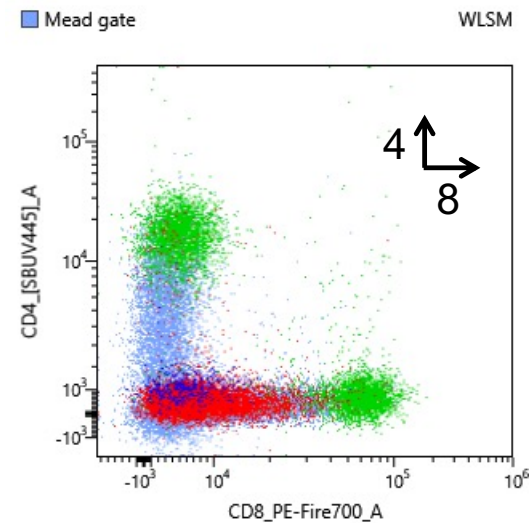
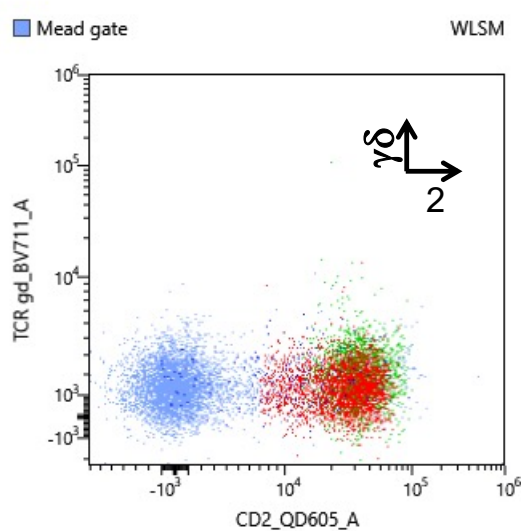
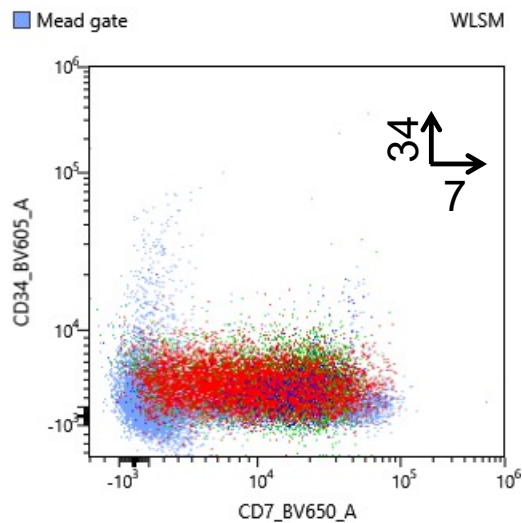
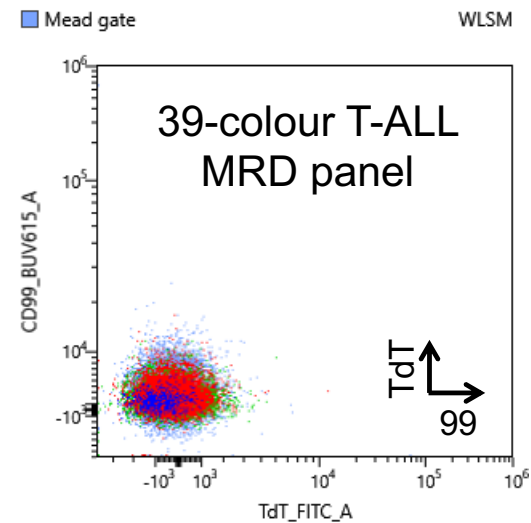
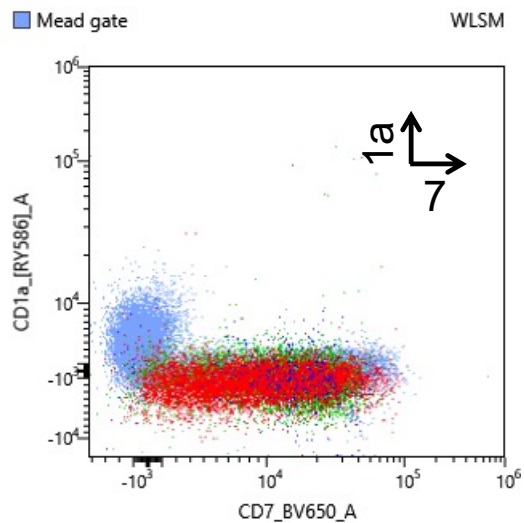
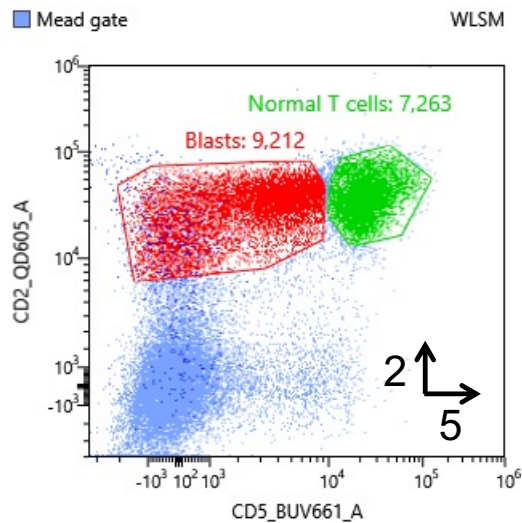
561nm

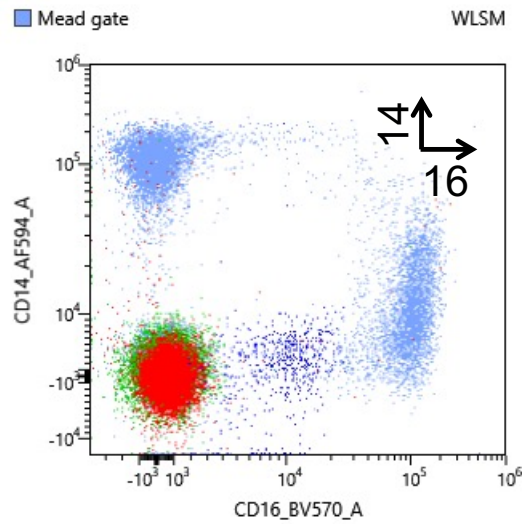
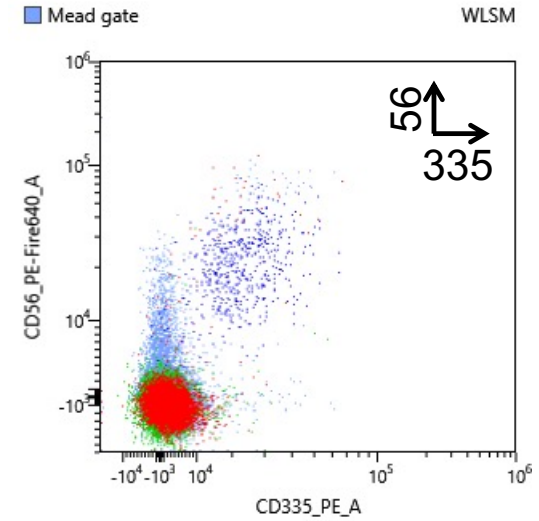
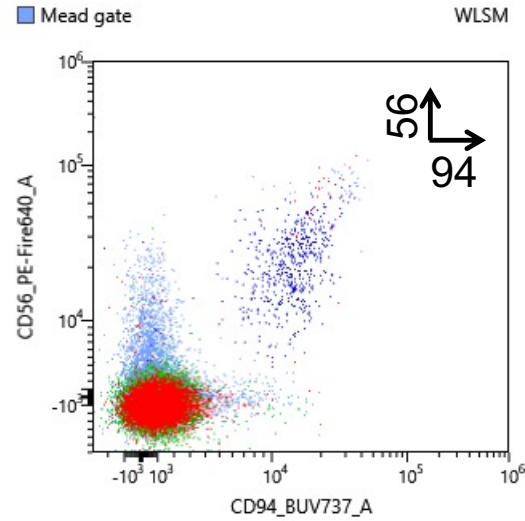
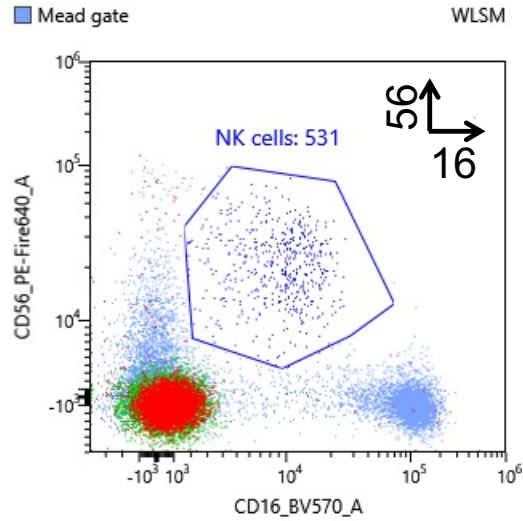
637nm

39-colour T-ALL MRD panel



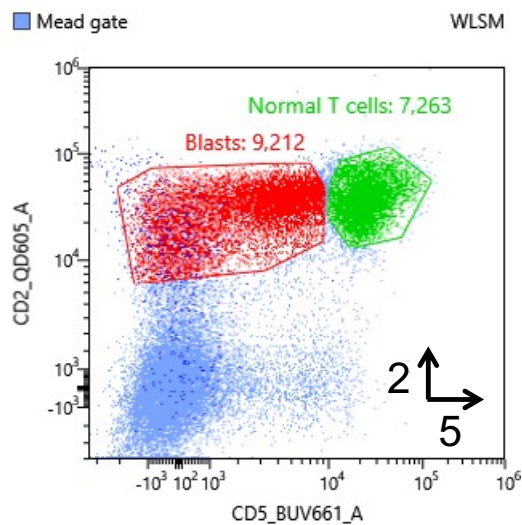




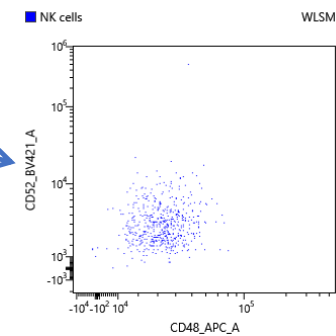
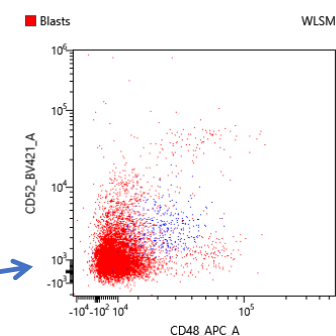
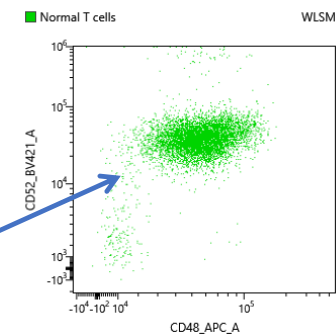
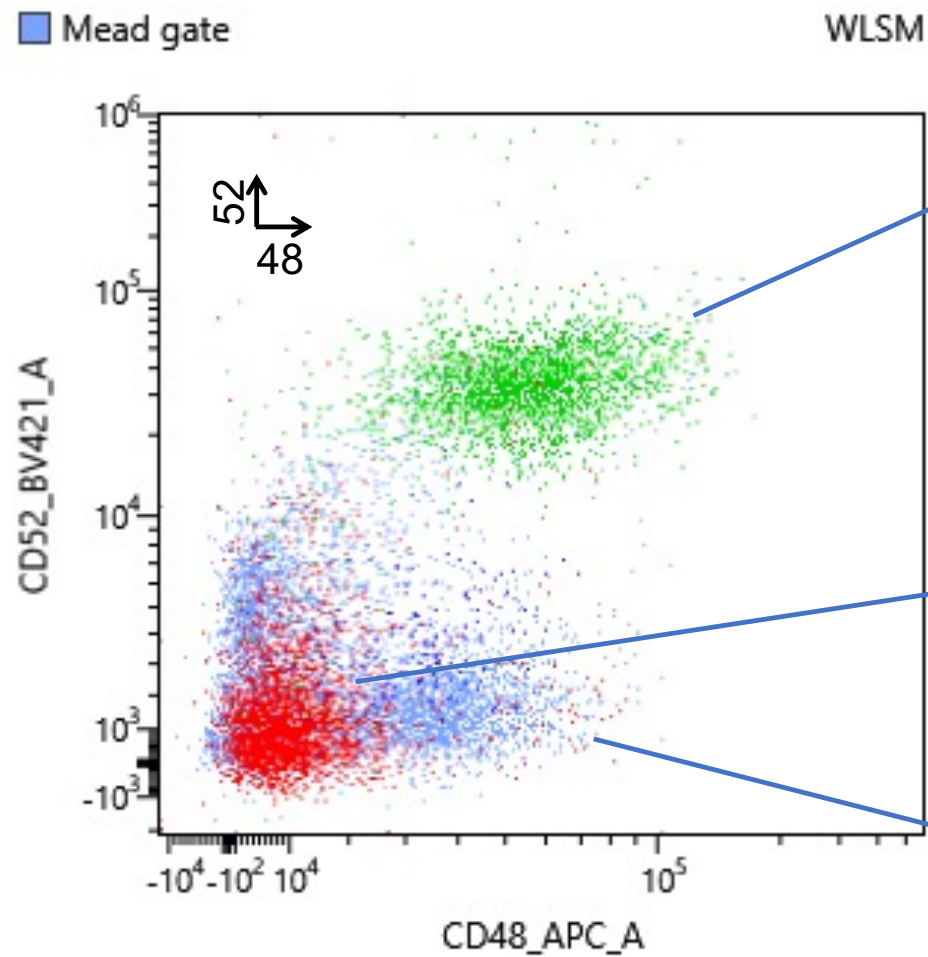


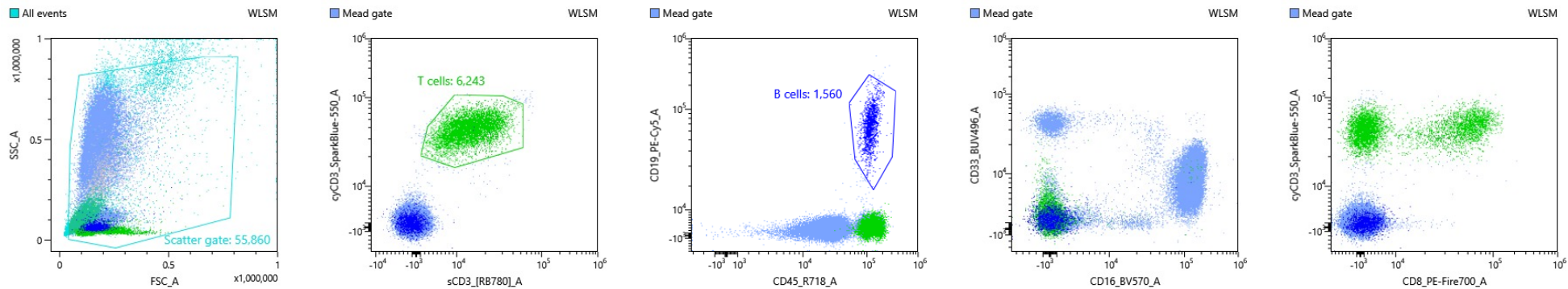
39-colour T-ALL
MRD panel
NK cell markers



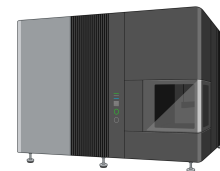
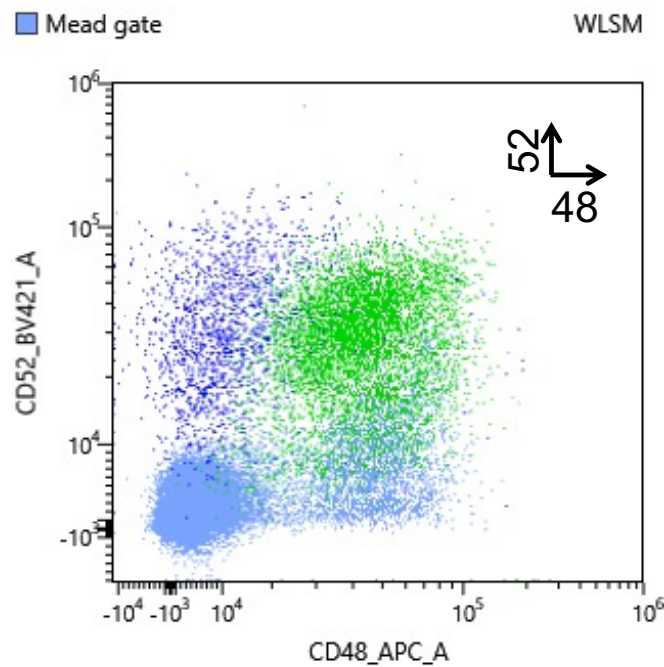


39-colour T-ALL MRD panel





39-colour T-ALL
MRD panel
Normal PB



Questions?

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