CLL phenotyping with BD Rhapsody™ Targeted Panels and BD® AbSeq Assays

Background

- Chronic lymphocytic leukemia (CLL) is the most common form of leukemia in adults and is characterized by a progressive accumulation of functionally incompetent lymphocytes in blood and bone marrow.
- Immunophenotyping is the standard diagnostic tool for CLL.
- Recent bulk RNA sequencing studies suggest that there is heterogeneity within CLL samples, demonstrating that single cell RNA sequencing is necessary to characterize the underlying transcriptional profile.
- Here, we demonstrate the use of the BD Rhapsody™ Single-Cell Analysis System to simultaneously analyze gene and protein expression in single cells in clinical CLL samples.

Experimental design and workflow

Healthy Sample
(4 samples)

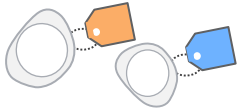
PBMCs

+

CLL Sample
(4 samples)

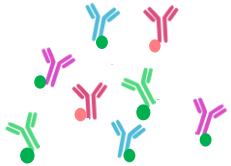
PBMCs

BD® Single-Cell Multiplexing Kit
(8 tags)



BD® AbSeq Assays
(32 markers)

Fluorescent Antibodies
(7 markers)



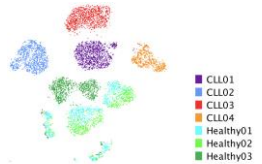
BD FACSMelody™ Cell Sorter
(sorting for B cells)

BD Rhapsody™ Single-Cell Analysis System

Post cDNA synthesis, the BD Rhapsody beads can be stored until ready to proceed to library prep.

BD Rhapsody™ Targeted Panel
(Human Immune Response Panel + Supplemental Panel)

NGS Sequencing



SeqGeq™ Software

Experimental design and workflow

Healthy Sample
(4 samples)

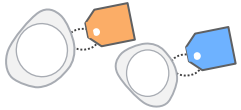
PBMCs

+

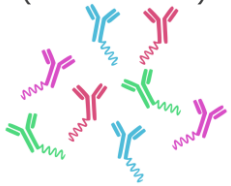
CLL Sample
(4 samples)

PBMCs

BD® Single-Cell Multiplexing Kit
(8 tags)



BD® AbSeq Assays
(32 markers)

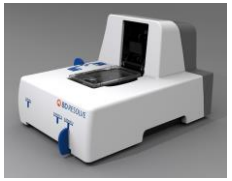


Fluorescent Antibodies
(7 markers)

Co-staining of PBMCS from each sample

BD FACSMelody™ Cell Sorter
(sorting for B cells)

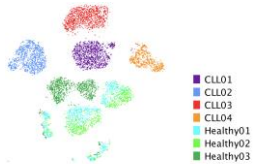
BD Rhapsody™ Single-Cell Analysis System



Post cDNA synthesis, the BD Rhapsody beads can be stored until ready to proceed to library prep.

BD Rhapsody™ Targeted Panel
(Human Immune Response Panel + Supplemental Panel)

NGS Sequencing



SeqGeq™ Software

Panel design

B-cell Sorting	
Marker	**Fluorochrome**
CD3	APC
CD14	APC
CD16	APC
CD19	PE
CD41a	APC
CD56	APC
CD235a	APC

32-Plex AbSeq Panel			
CD5	CD24	CD69	CD127
CD7	CD27	CD80	CD133
CD8	CD33	CD81	CD184
CD9	CD34	CD86	CD275
CD10	CD38	CD90	CD279
CD11c	CD40	CD103	CD294
CD19	CD45	CD117	IgD
CD20	CD45RA	CD123	IgG

- The BD AbSeq panel contains 32 proteins, including B-cell markers, CLL specific markers, and other markers that characterize B cells at various stages of differentiation.
- The table on the left shows the fluorescent antibodies used for the B-cell sorting.
- The scRNA-Seq panel includes ~500 genes involved in immune responses.

Experimental design and workflow

Healthy Sample
(4 samples)

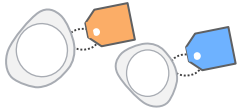
PBMCs

+

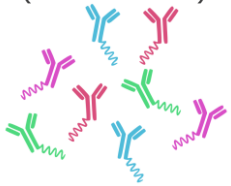
CLL Sample
(4 samples)

PBMCs

BD® Single-Cell Multiplexing Kit
(8 tags)



BD® AbSeq Assays
(32 markers)



Fluorescent Antibodies
(7 markers)

BD FACSMelody™ Cell Sorter
(sorting for B cells)

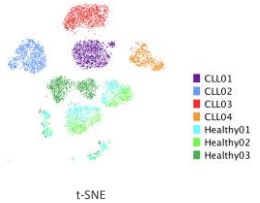
Sort and purity check

BD Rhapsody™ Single-Cell Analysis System

Post cDNA synthesis, the BD Rhapsody beads can be stored until ready to proceed to library prep.

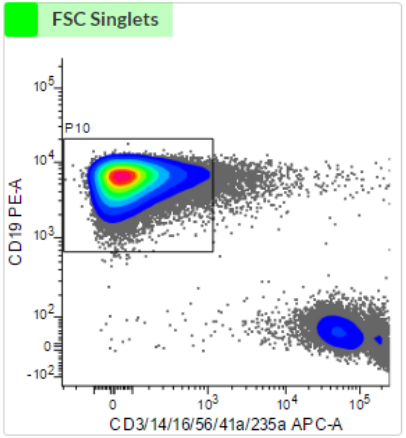
BD Rhapsody™ Targeted Panel
(Human Immune Response Panel + Supplemental panel)

NGS Sequencing

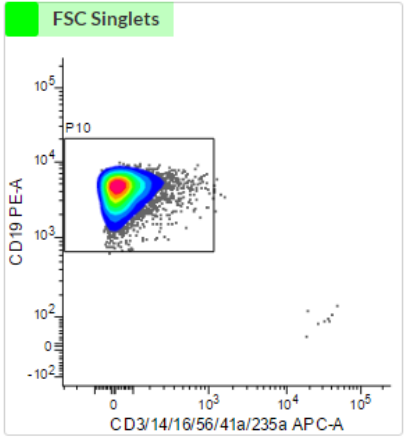


SeqGeq™ Software

Confirming the purity of the sorted cells



Sort Gate of CD19 Cells



Post Sort Purity Check

- Purity of the sorted CD19$^+$ B-cells were confirmed using a BD FACSMelody cell sorter.
- On the left, the gate was set to sort on CD19$^+$ cells. The chart on the right shows that the majority of the sorted cells are in fact in the desired CD19$^+$ gate, showing purity of over 99% for each sample.

Experimental design and workflow

Healthy Sample
(4 samples)

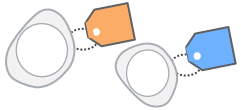
PBMCs

+

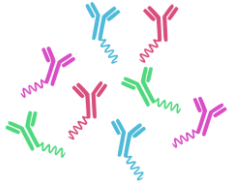
CLL Sample
(4 samples)

PBMCs

BD® Single-Cell Multiplexing Kit
(8 tags)



BD® AbSeq Assays
(32 markers)



Fluorescent Antibodies
(7 markers)

BD FACSMelody™ Cell Sorter
(sorting for B cells)

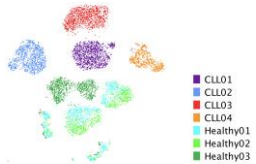
BD Rhapsody™ Single-Cell Analysis System

QC tool

Post cDNA synthesis, the BD Rhapsody beads can be stored until ready to proceed to library prep.

BD Rhapsody™ Targeted Panel
(Human Immune Response Panel + Supplemental panel)

NGS Sequencing



SeqGeq™ Software

BD Rhapsody Scanner as a QC tool

- The viability and concentration of the sorted cells were calculated on the BD Rhapsody™ Scanner after cells were stained with viability dyes, Calcein and DRAQ7™.
- One of the healthy samples had poor viability and a low cell concentration and was excluded from the rest of the workflow.
- The BD Rhapsody Scanner also reports the number of captured cells, allowing users to exclude any samples with poor capture rate for the downstream sequencing steps.

Experimental design and workflow

Healthy Sample
(4 samples)

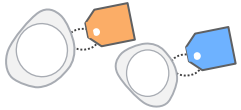
PBMCs

+

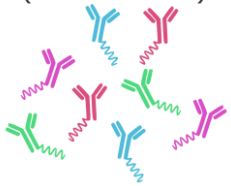
CLL Sample
(4 samples)

PBMCs

BD® Single-Cell Multiplexing Kit
(8 tags)



BD® AbSeq Assays
(32 markers)



Fluorescent Antibodies
(7 markers)

BD FACSMelody™ Cell Sorter
(sorting for B cells)

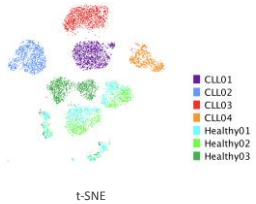
BD Rhapsody™ Single-Cell Analysis System

Post cDNA synthesis, the BD Rhapsody beads can be stored until ready to proceed to library prep.

Stopping point

BD Rhapsody™ Targeted Panel
(Human Immune Response Panel + Supplemental panel)

NGS Sequencing



SeqGeq™ Software

Flexible workflow

- Post cDNA synthesis, the cell capture beads can be stored up to 12 weeks before proceeding to library preparation workflow.
- In our case, this gave us the flexibility of waiting for more clinical research samples to arrive for single-cell capture on BD Rhapsody beads so that we could prepare all libraries simultaneously, thereby reducing batch effects from preparing libraries on different days.

Experimental design and workflow

Healthy Sample
(4 samples)

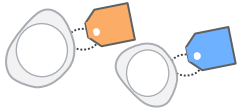
PBMCs

+

CLL Sample
(4 samples)

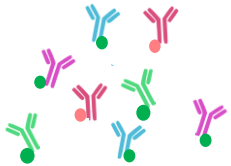
PBMCs

BD® Single-Cell Multiplexing Kit
(8 tags)



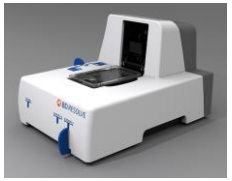
BD® AbSeq Assays
(32 markers)

Fluorescent Antibodies
(7 markers)



BD FACSMelody™ Cell Sorter
(sorting for B cells)

BD Rhapsody™ Single-Cell Analysis System

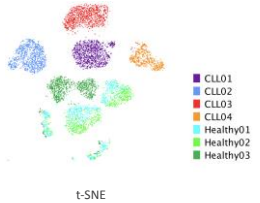


Post cDNA synthesis, the BD Rhapsody beads can be stored until ready to proceed to library prep.

Stopping point

BD Rhapsody™ Targeted Panel
(Human Immune Response Panel + Supplemental panel)

NGS Sequencing



SeqGeq™ Software

Targeted NGS library preparation

- Following cDNA synthesis, we used a targeted approach for library preparation.
- BD Rhapsody™ Immune Response Targeted Panel (Human) and a custom, CLL-specific, BD Rhapsody™ Supplemental Panel were used.

Experimental design and workflow

Healthy Sample
(4 samples)

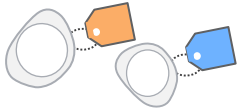
PBMCs

+

CLL Sample
(4 samples)

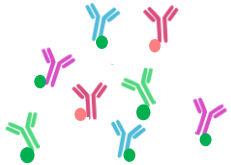
PBMCs

BD® Single-Cell Multiplexing Kit
(8 tags)



BD® AbSeq Assays
(32 markers)

Fluorescent Antibodies
(7 markers)



BD FACSMelody™ Cell Sorter
(sorting for B cells)

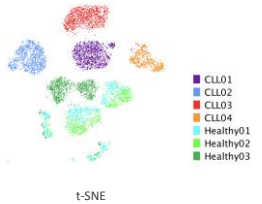
BD Rhapsody™ Single-Cell Analysis System

Post cDNA synthesis, the BD Rhapsody beads can be stored until ready to proceed to library prep.

Stopping point

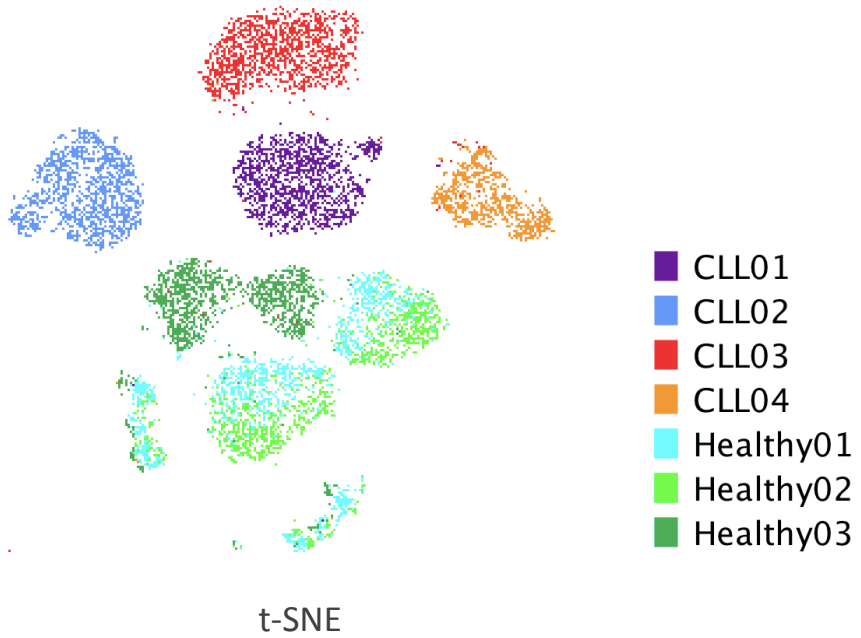
BD Rhapsody™ Targeted Panel
(Human Immune Response Panel + Supplemental panel)

NGS Sequencing

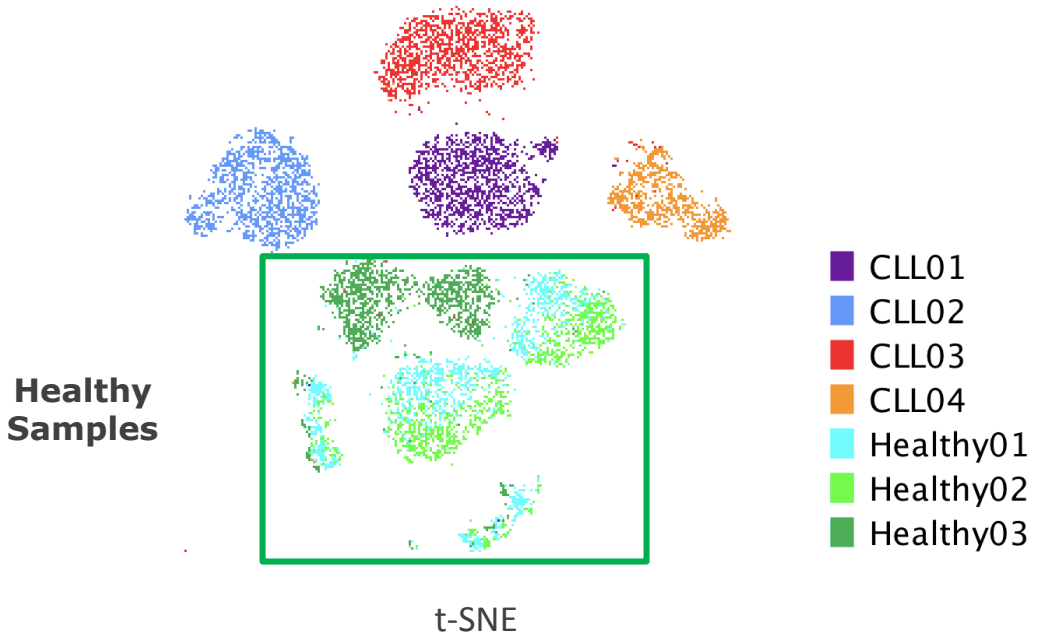


SeqGeq™ Software

Clusters are well defined using mRNA and protein analysis

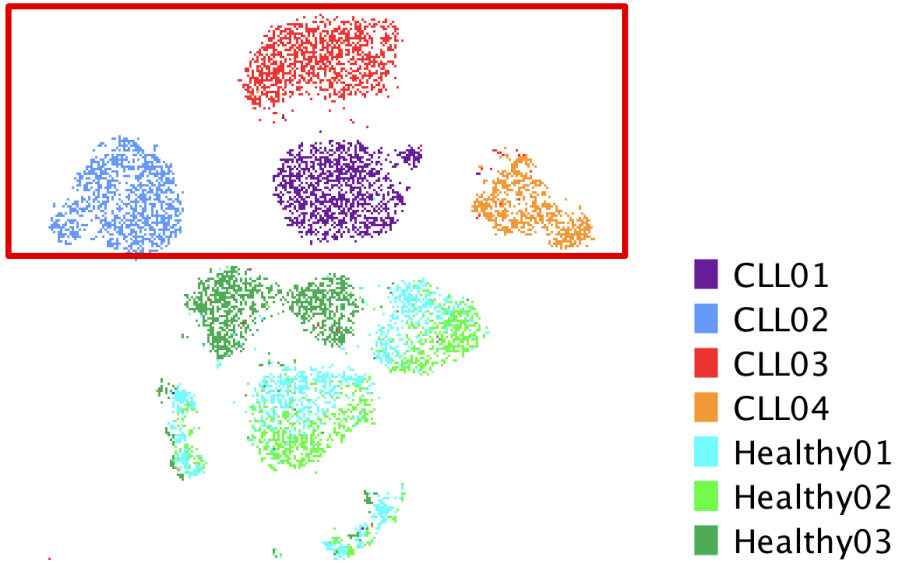


Samples from healthy donors cluster similarly

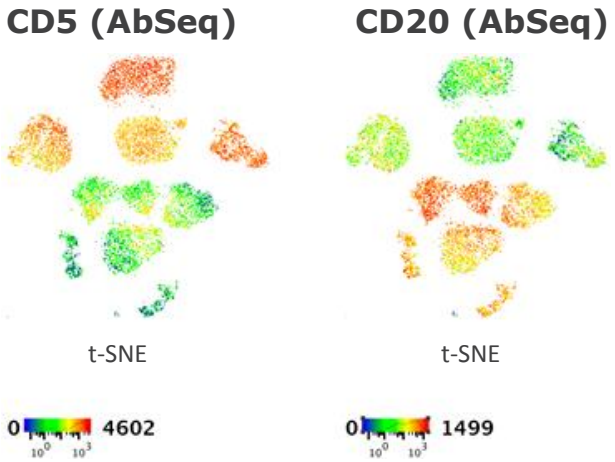


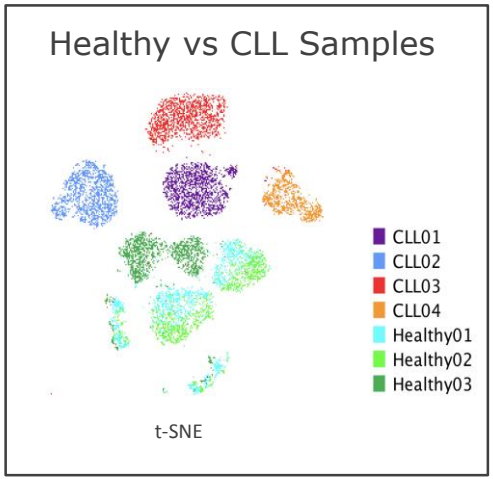
CLL samples cluster separately and uniquely

CLL Samples



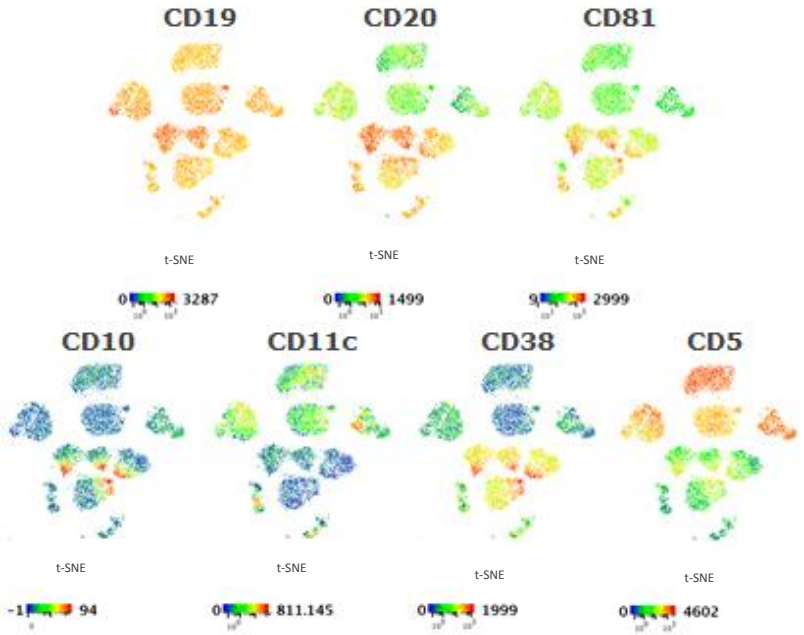
Expression of CD5 (AbSeq) and CD20 (AbSeq) correlates with disease-state





- Using BD AbSeq assays, the expression profile of CD5 is higher in the CLL samples while the expression profile of CD20 is higher in the healthy samples, consistent with the literature.

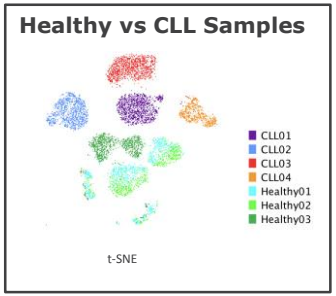
Expression of CLL markers is similar when determined using BD® AbSeq reagents and fluorochrome-conjugated antibodies



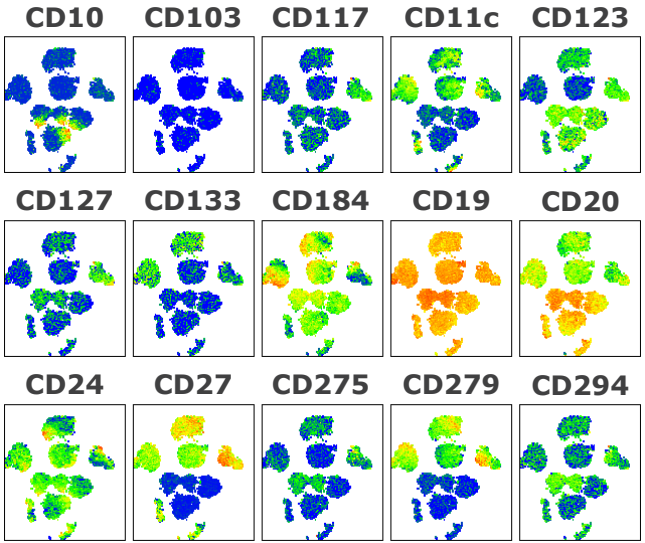
	Healthy	CLL
CD19	++	++
CD20	++	+
CD81	++	-
CD10	+	-
CD11c	-	+/-*
CD38	+	+/-*
CD5	-	++

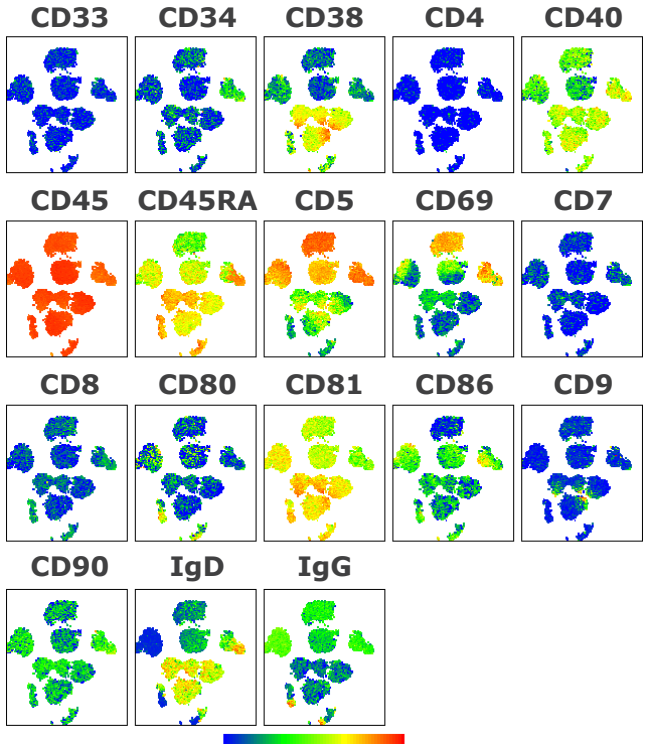
High expression: ++
Expression: +
No expression: –

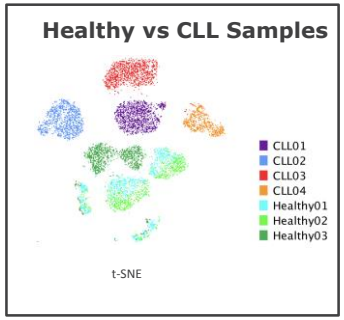
* Indicates heterogeneous population



Results using the complete AbSeq panel

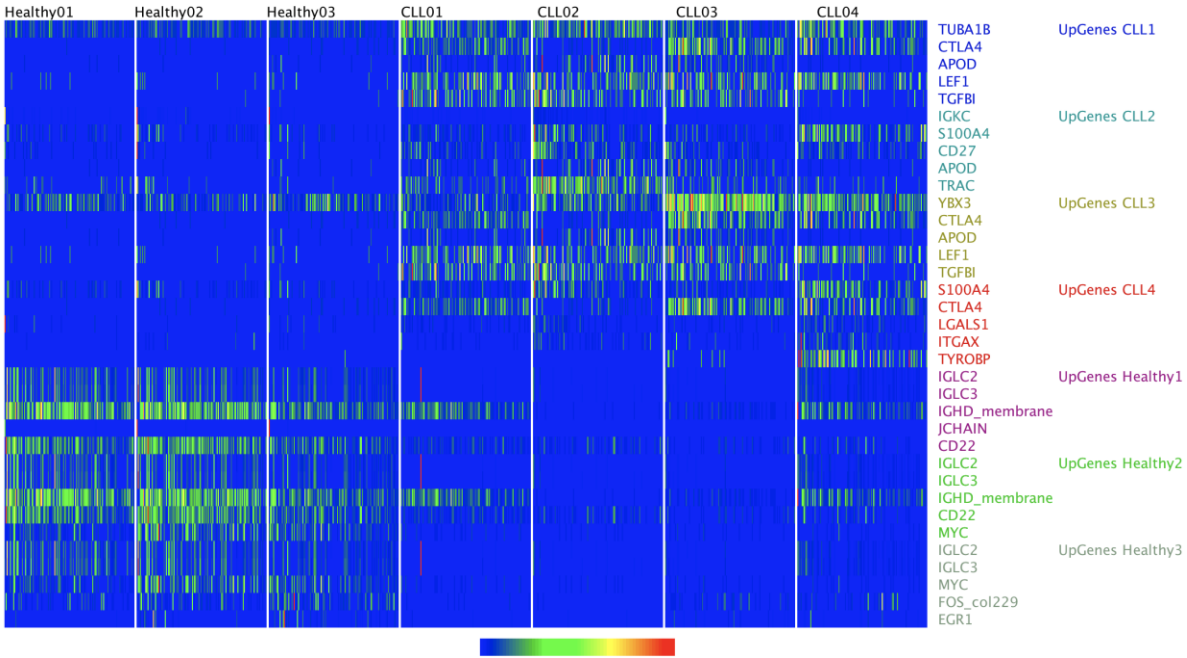






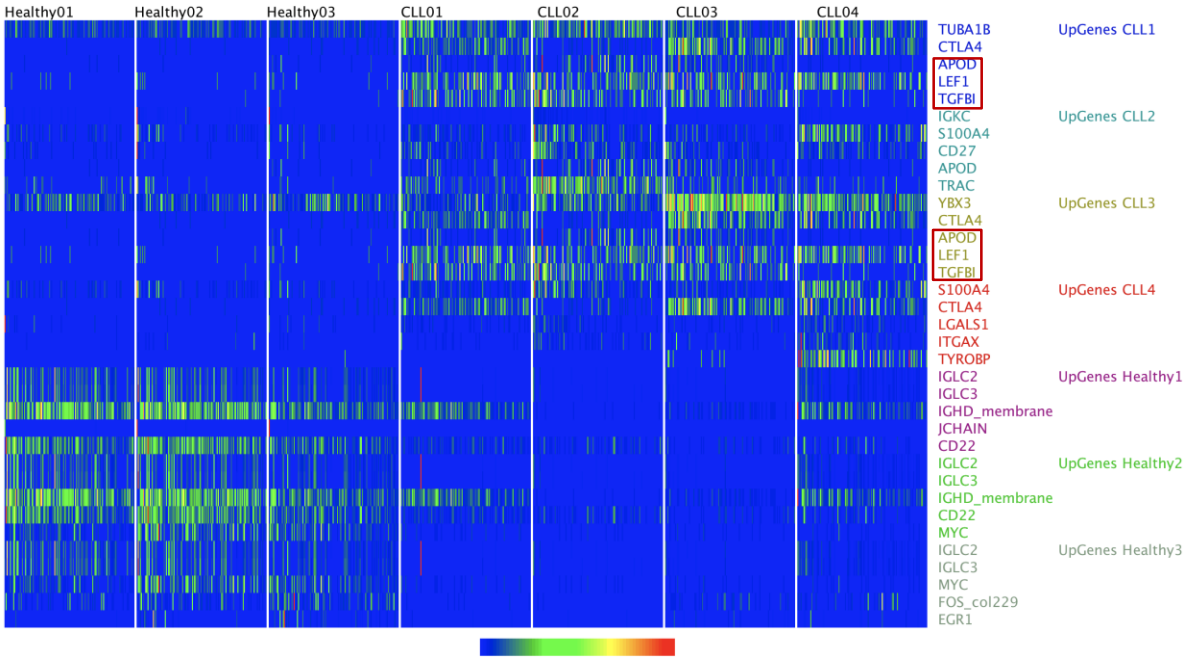
Evaluation of the top 5 highly differentially expressed genes across donors

The heat map shows the top 5 highly expressed genes in each sample



Evaluation of the top 5 highly differentially expressed genes (DEGs) across donors

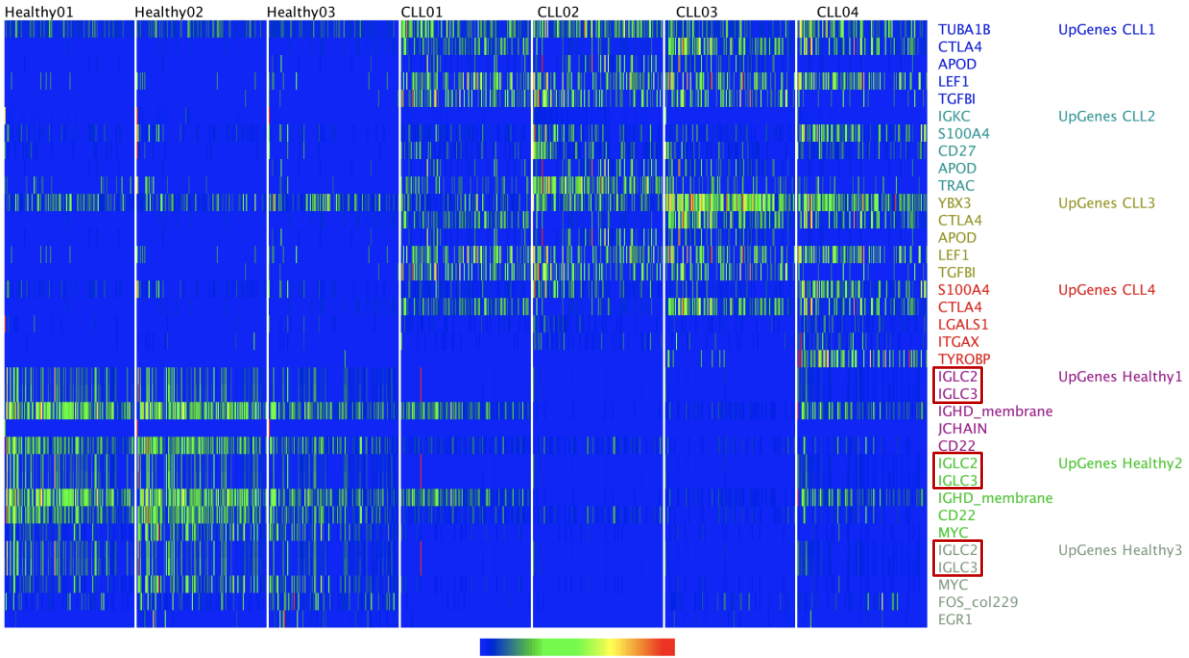
The heat map shows the top 5 highly expressed genes in each sample



DEGs between CLL01 and CLL03 are similar.

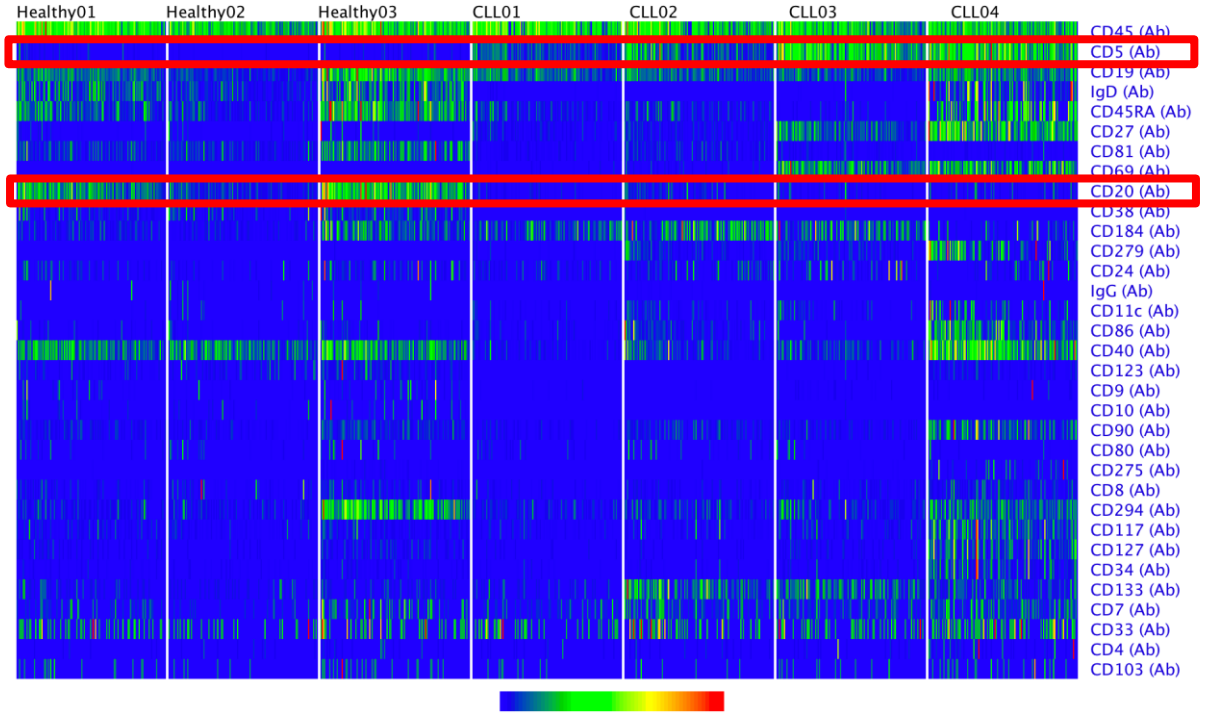
Evaluation of top 5 highly differentially expressed genes across donors

The heat map shows the top 5 highly expressed genes in each sample

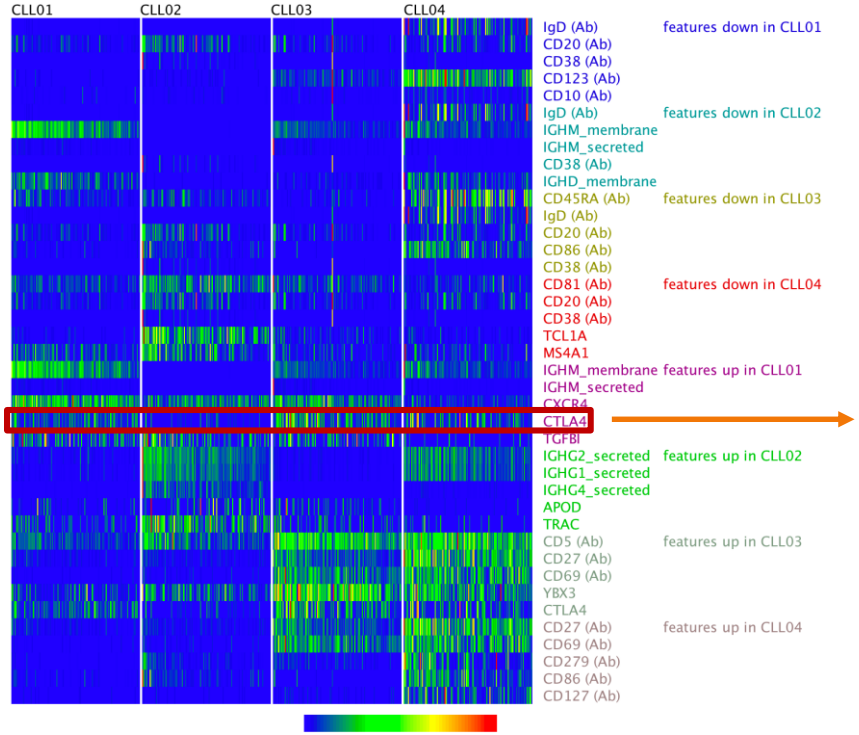


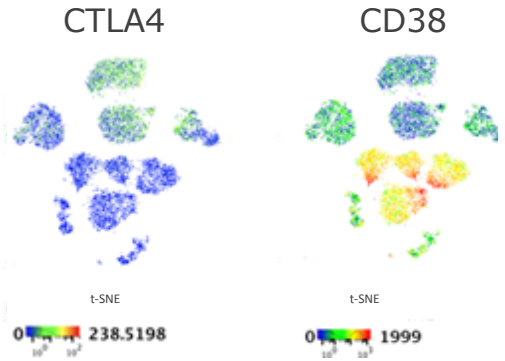
IGLC2 and IGLC3 expression is higher in healthy donors.

Evaluation of unique protein expression across donors



Evaluation of the top 5 differentially expressed features across CLL samples

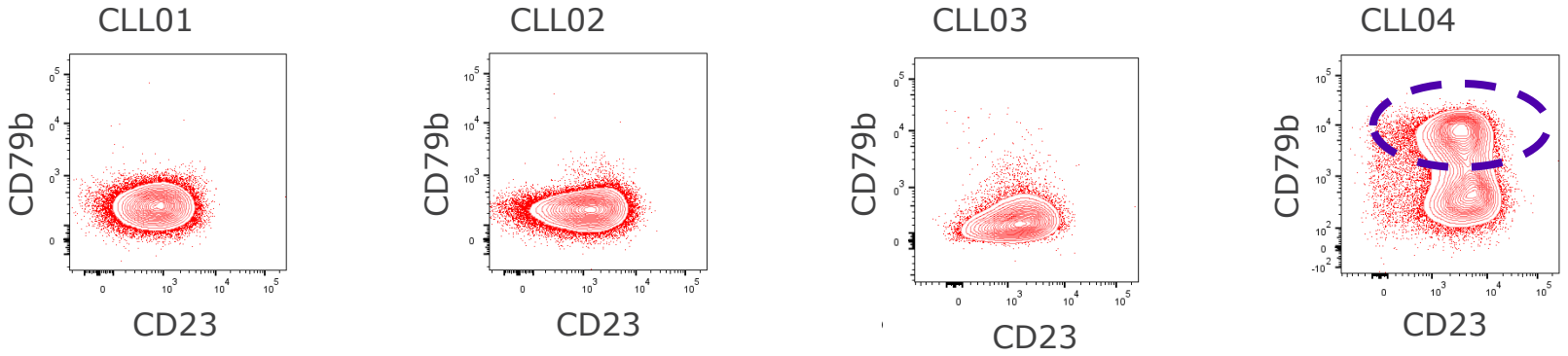




CTLA4:

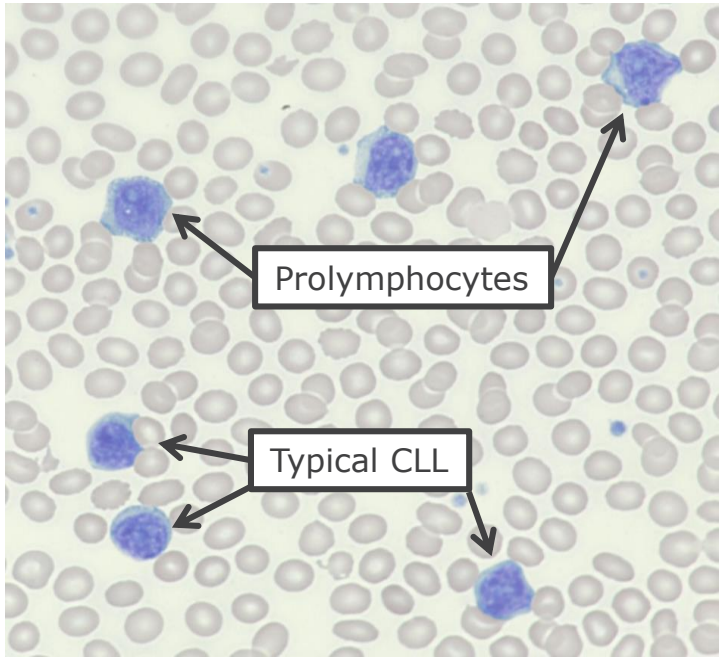
- Associated with a higher number of cells in G0-G1 phase; may delay cell cycle progression
- Potential useful immunotherapy strategy for patients with cancer
- Important marker for determining treatment

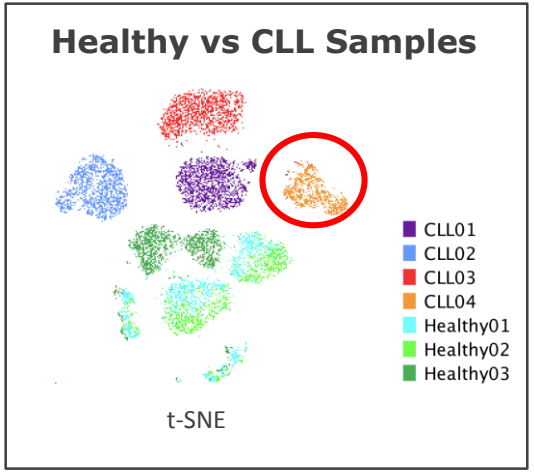
CLL04 has a CD79^{+} population detected by flow analysis



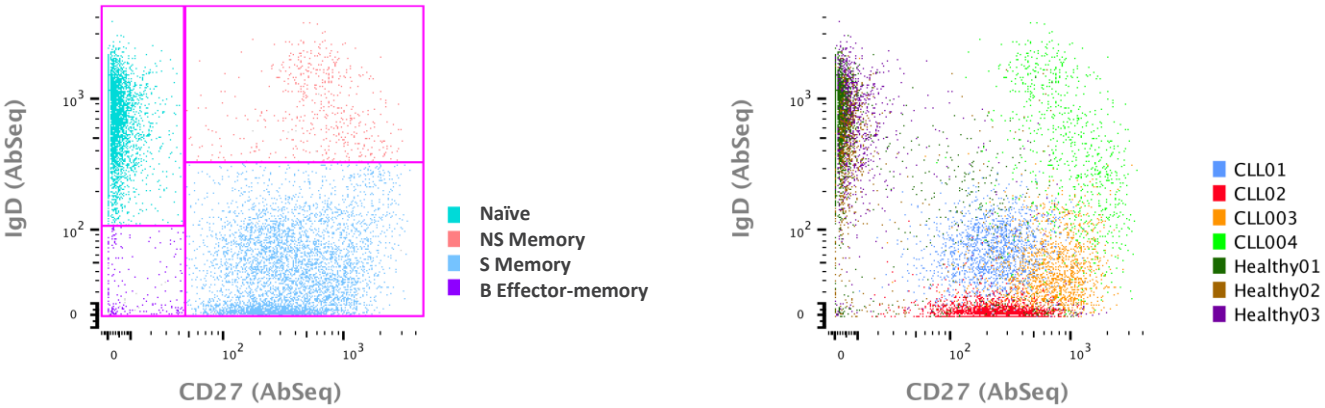
- CLL01, CLL02 and CLL03 are examples of the flow analysis profiles typical for CLL patients.
- CLL04 flow analysis reveals the presence of two populations. The lower population (CD23^{+}CD79b^{-}) shows the typical pattern observed in CLL patient samples. Interestingly, a second population (CD79b^{+}, circled in purple) is observed in addition.

CLL04 sample has 20% more prolymphocytes than a typical CLL sample





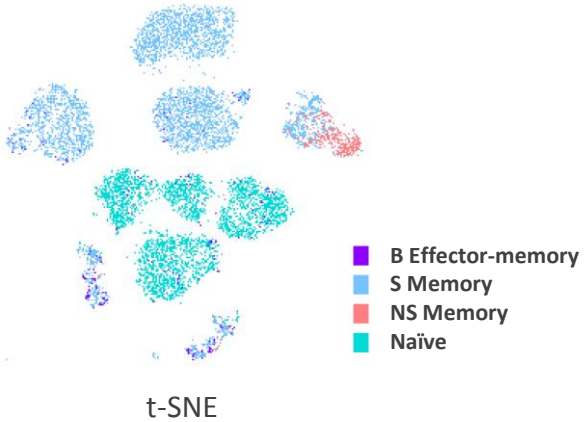
Further identification of sub-clusters across the different samples

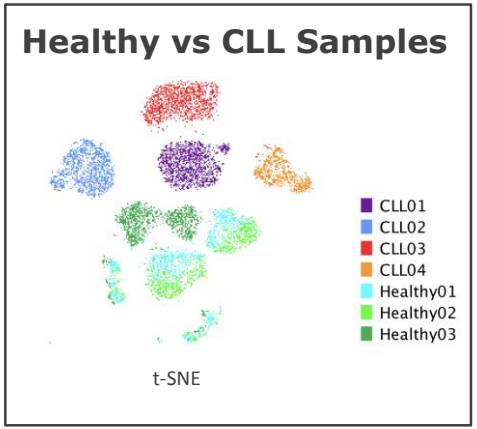


Switch (S) memory cells don't express IgD, instead they express IgG, IgA or IgE.

- B cells in seven samples were characterized using the BD AbSeq markers for CD27 and IgD.
- The samples cluster in four distinct quadrants: naïve cells ($C27^{-}IgD^{+}$), non-switched memory cells ($CD27^{+}IgD^{+}$), switched memory cells ($CD27^{+}IgD^{-}$) and B effector-memory cells ($CD27^{-}IgD^{-}$).

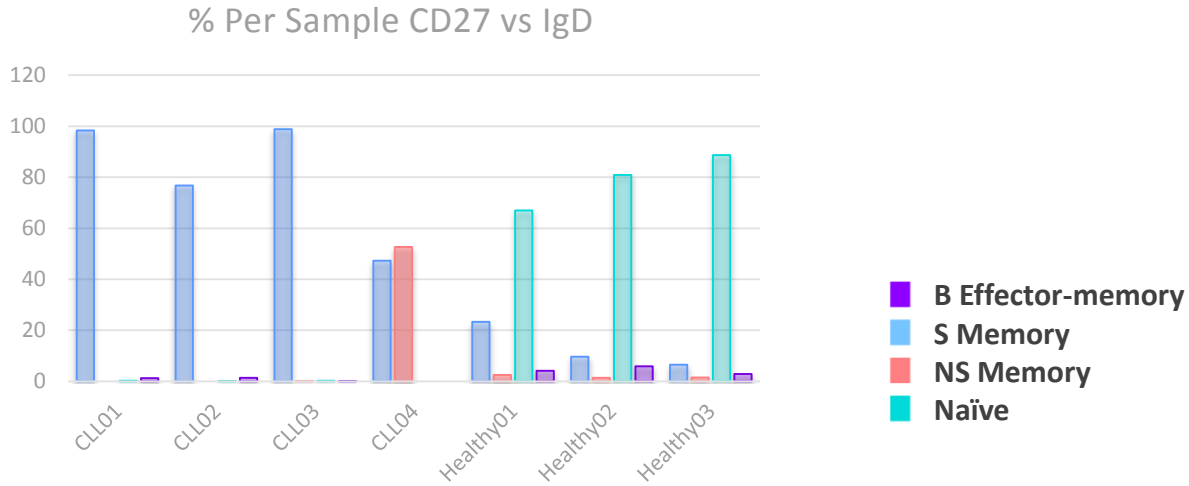
CLL04 sample contains S and NS memory cells





- The three samples from healthy donors are primarily naïve memory cells. The two small populations near the bottom of the t-SNE plots also contain switched memory cells.
- In contrast, CLL01, CLL02, and CLL03 samples are primarily switched memory cells. Interestingly, CLL04 seems to contain both switched and non-switched memory cells.
- The CLL04 sample contains similar proportions of switched memory cells and non-switched memory cells, as shown in the t-SNE.

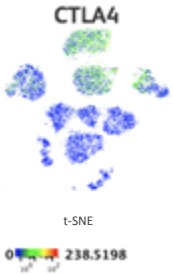
CLL04 has similar percentages of S and NS memory cells

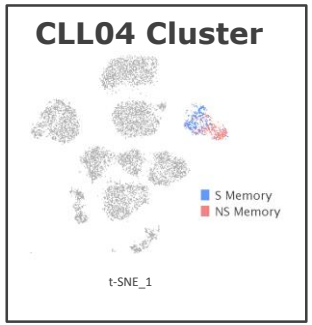


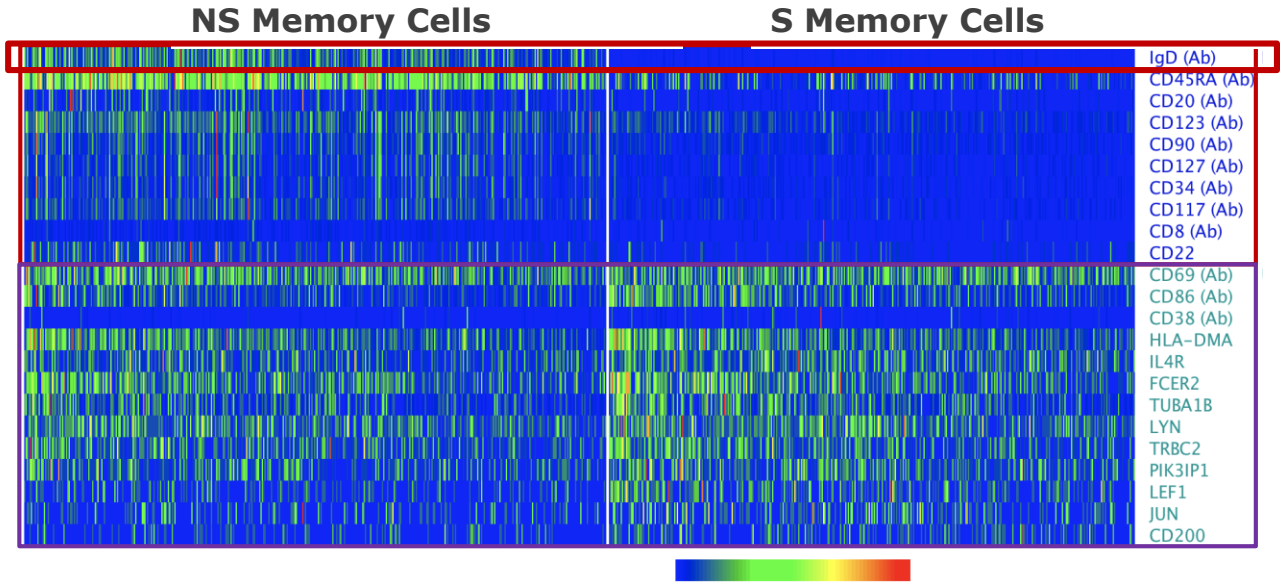
- The majority (>70%) of the populations for CLL01, CLL02, and CLL03, are switched memory cells.
- However, for CLL04, about 55% of the cells are non-switched memory cells and about 45% of the cells are switched memory cells.

Differential expression of analysis within the CLL04 cluster

IgD present in NS cells



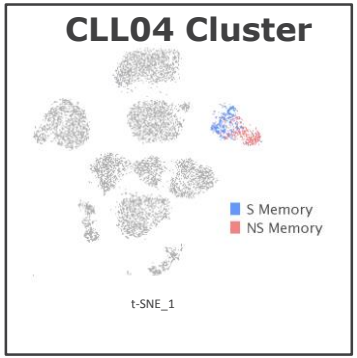


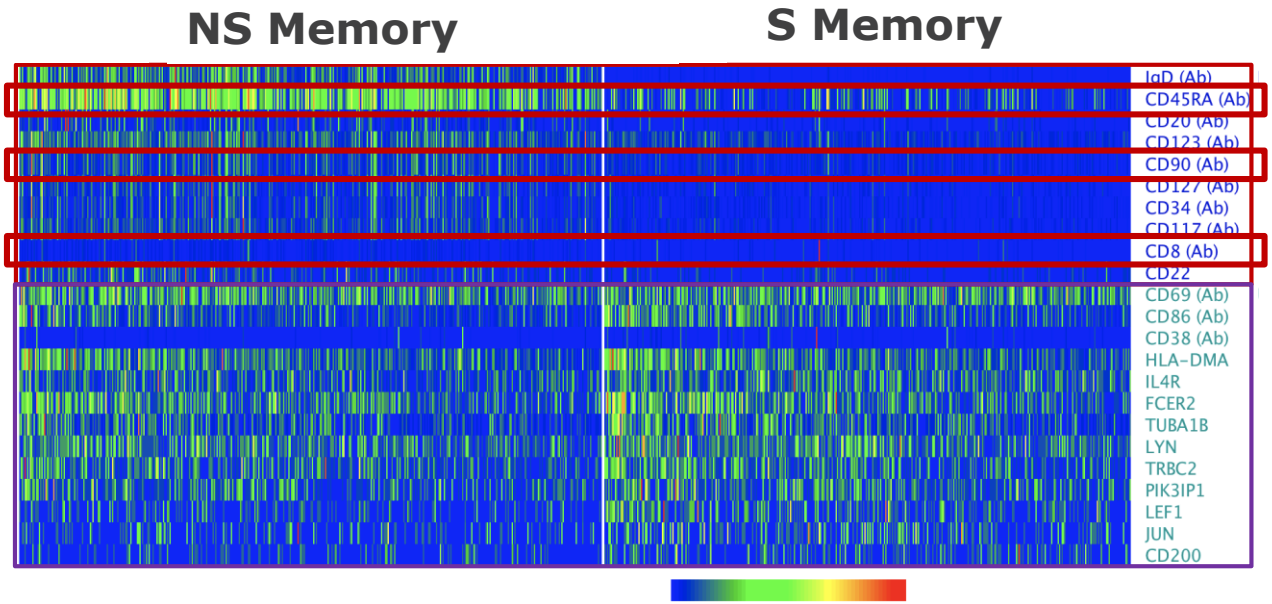


NS and S cells equally express genes involved in B-cell differentiation and maintenance

- **IL4R**: regulates IgE production; involved in the B-cell development pathways and cytokine signaling in the immune system
- **FCER2**: regulates IgE production; plays essential role in B-cell growth and differentiation
- **LYN**: regulates B-cell differentiation, proliferation, survival and apoptosis, and is important for immune self-tolerance
- **LEF1**: involved in the Wnt signaling pathway; this gene has been linked to some cancers
- **CD200**: plays an important role in immunosuppression and regulation of anti-tumor activity

Markers from T-cell lineage detected in higher levels





Summary

- Investigated differences between 4 CLL and 3 healthy samples with ~500 targeted genes and 32 BD AbSeq markers.
- Multiplexing samples using the BD Single-Cell Multiplexing Kit allowed the investigation of up to 4 different samples simultaneously.
- Significant heterogeneity was observed across CLL samples, particularly in CLL04.
- The three samples from healthy donors all showed elevated expression of the IgLC genes.
- Flow-cytometry analysis revealed two populations in the CLL04 sample. mRNA and protein expression data, measured using the BD Rhapsody Targeted Panel and BD AbSeq reagents, respectively, showed that the two populations contained both S and NS memory cells.
- We analyzed 32 proteins and 500 genes simultaneously in single cells to demonstrate that there are significant heterogeneity among CLL samples and uncover cell types that were not previously associated with the disease phenotype.

Thank you!

BD FACSMelody cell sorter is a Class 1 Laser Product.
For Research Use Only. Not for use in diagnostic or therapeutic procedures.