BD Rhapsody™ Single-Cell Multi-Omics
Resolving the elusive and controversial identity of innate lymphoid cells

Hasi Patel

BD Biosciences

Snr Single Cell Solutions Architect

CASE STUDY
Single cell Multiomic analysis for the resolution of ILC heterogeneity

Background

- Important effector cells of innate Immunity, play a role in tissue homeostasis and inflammation.

- 3 Major groups ILC1, ILC2, ILC3

- Broadly defined based on cytokine output & expression of specific transcription factors

- Potential target for immunotherapy approaches

The elusive and controversial identity of ILCs

Immunology
British Society for Immunology

IMMUNOLOGY REVIEW ARTICLE
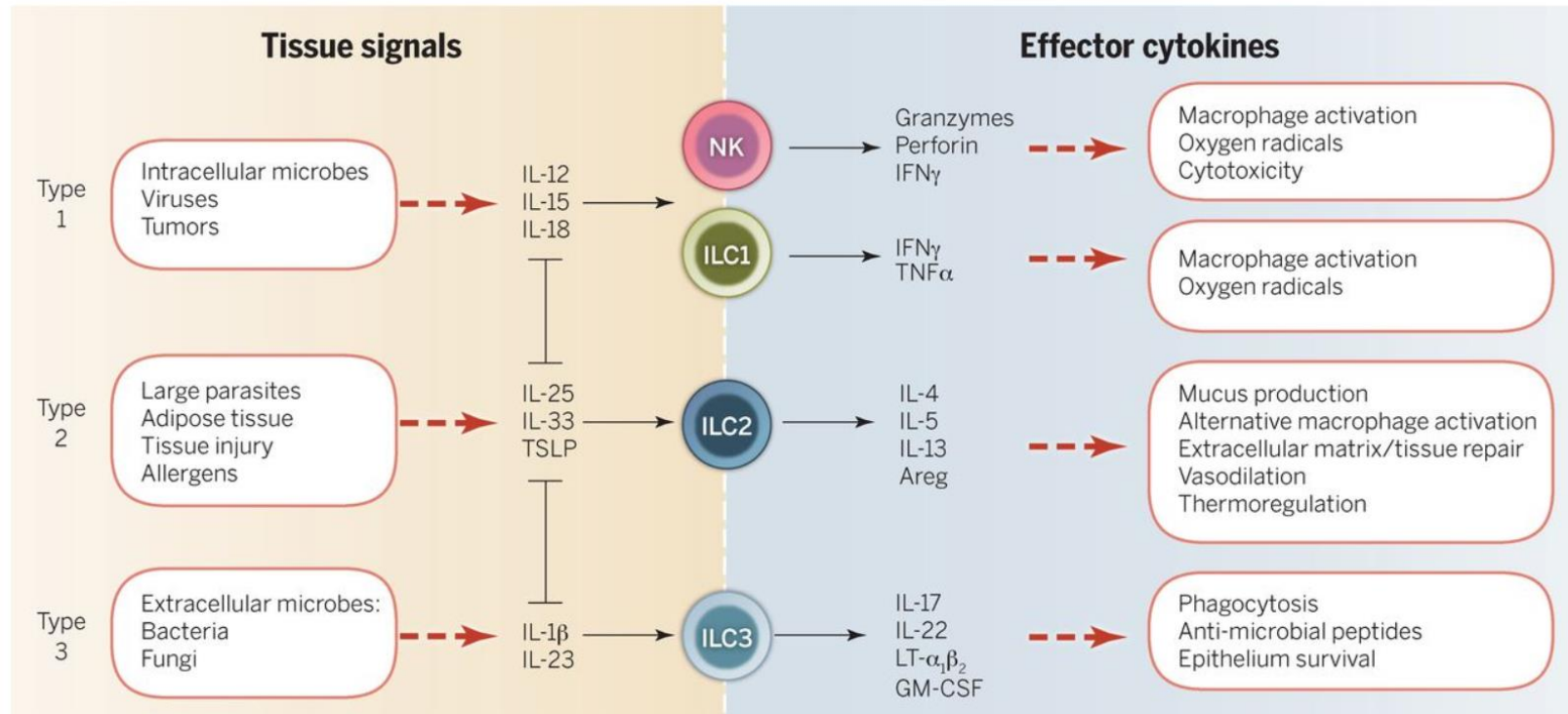
Dissecting human ILC heterogeneity: more than just three subsets

Simoni, Y. and Newell, E.W. (2018),. Immunology.

Resource | Published: 15 February 2016

The heterogeneity of human CD127$^+$ innate lymphoid cells revealed by single-cell RNA sequencing

Åsa K Björklund, Marianne Forkel, Simone Picelli, Viktoria Konya, Jakob Theorell, Danielle Friberg, Rickard Sandberg ✉ & Jenny Mjösberg ✉

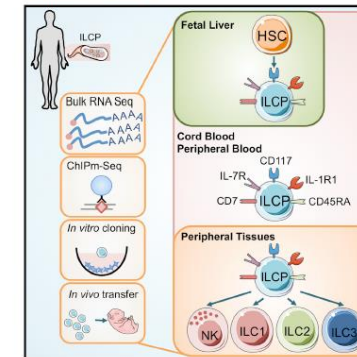*Nature Immunology* **17**, 451–460 (2016) | Download Citation ⬇

- Highlight ILC variability amongst tissue
- Demonstrate that ILCs In circulation more progenitor like

Article

Cell

Systemic Human ILC Precursors Provide a Substrate for Tissue ILC Differentiation

Graphical Abstract

Authors
Ai Ing Lim, Yan Li, Silvia Lopez-Lastra, ..., Ido Amit, Hans Yssel, James P. Di Santo

Correspondence
james.di-santo@pasteur.fr

In Brief
Human innate lymphoid cell progenitors circulate systemically, differentiating into diverse subtypes in specific tissues in response to localized cues.

- Rarity and sensitivity to cell manipulation/isolation processes

- Highly heterogeneous

- Phenotype shown to be variable across different donors and as different tissues
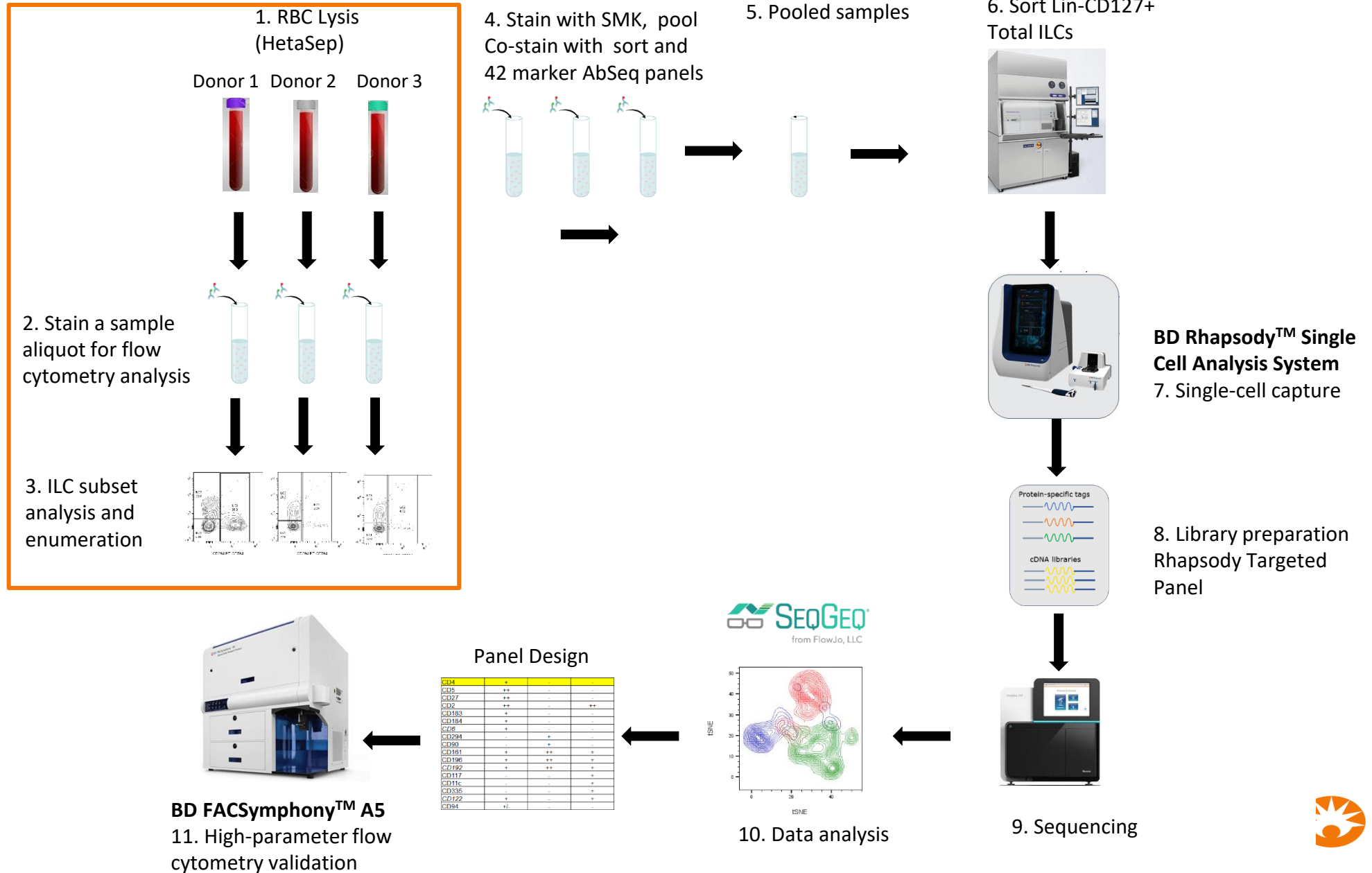
Challenges of ILC characterization

- Purpose of study is to address these challenges and elucidate the identity of these cells

- Simultaneous analysis of 42 surface markers and 399 genes at the single cell level   .

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Workflow solution

Workflow

1. RBC Lysis (HetaSep)

Donor 1 Donor 2 Donor 3

2. Stain a sample aliquot for flow cytometry analysis

3. ILC subset analysis and enumeration

4. Stain with SMK, pool Co-stain with sort and 42 marker AbSeq panels

5. Pooled samples

BD FACS Aria Fusion™
6. Sort Lin-CD127+ Total ILCs

BD Rhapsody™ Single Cell Analysis System
7. Single-cell capture

8. Library preparation Rhapsody Targeted Panel

9. Sequencing

10. Data analysis

Panel Design

BD FACSymphony™ A5
11. High-parameter flow cytometry validation

Panels

Flow Cytometry analysis

Marker	Fluorochrome
Lineage	BV510
CD45	FITC
CD3	APC-H7
CD56	PE-Cy7
CD127	BV421
CD161	APC
CD117	PE
CD294	PE-C594

Used to define the 3 major subset of ILCS

Cell sorting Panel

Marker	Fluorochrome
Lineage	BV510
CD45	FITC
CD3*	APC-H7
CD56*	PE-Cy7
CD127	BV421

-co-stained with the AbSeq panel
-lineage depletion
-*major contaminants>
unique flurophore>elimnation

42-Plex AbSeq panel

Ab-Oligos		
CD103	CD90	CD98
CD161	CD62L	CD294
CD11b	CD16	LAG3
CD69	CD184	B7-H1
CD278	CD117	B7-H2
CD25	CD314	TIM3
CD183	CD335	PD-1
CD4	CD226	CTLA-4
CD196	CD94	CD49d
CD7	CD57	CD336
CD11c	CD28	CD45RA
CD8	CD34	CD27
CD3	CD2	CD19
CD14	CD5	CD56

Included lineage markers in AbSeq panel to confirm or further eliminate contaminants in downstream AbSeq analysis

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Pre- and post-sort ILC subset analysis

Pre-Sort (Flow Cytometry)
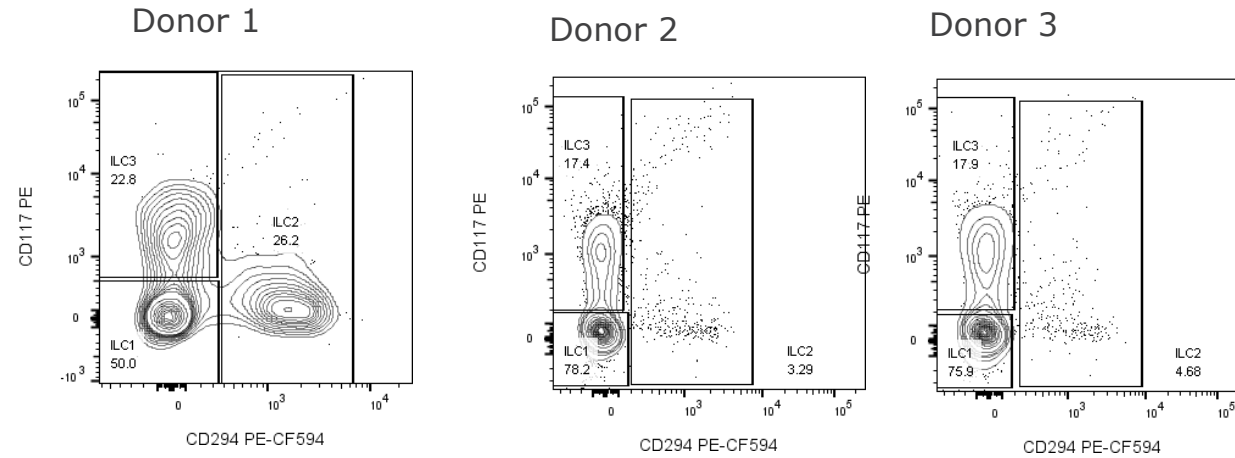
Post-sort (AbSeq)
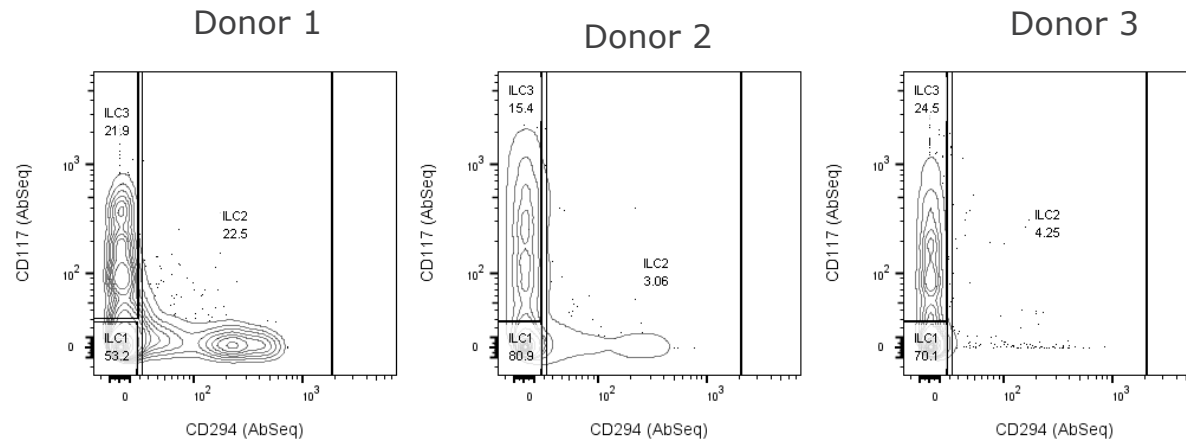
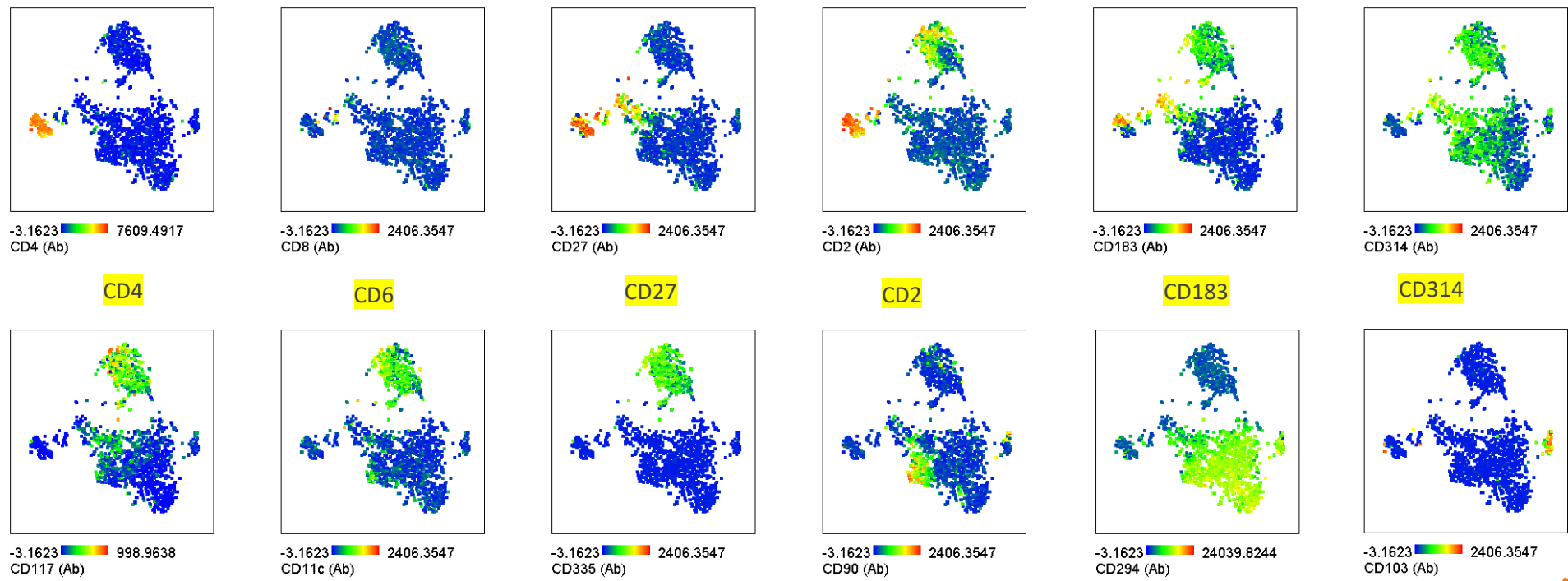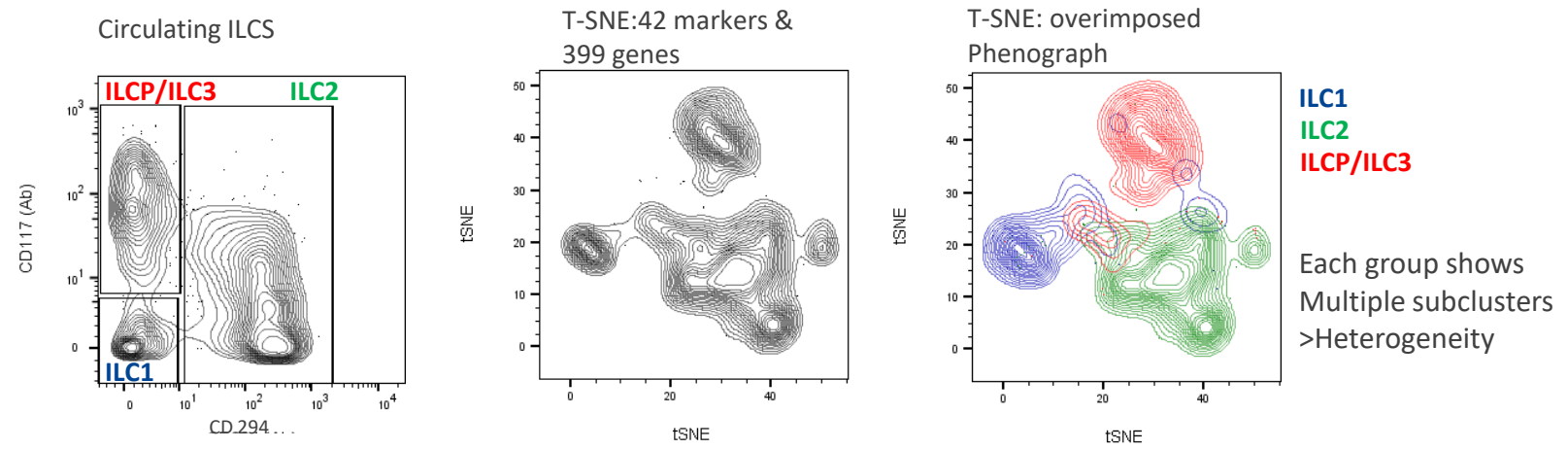3 donors demonstrate the variability in the distribution of these subgroups Across the different donors

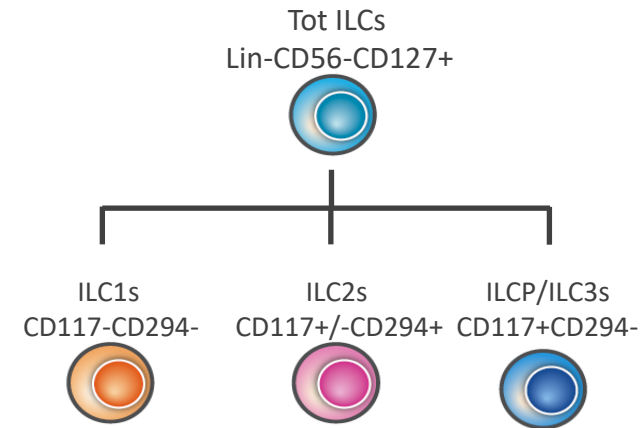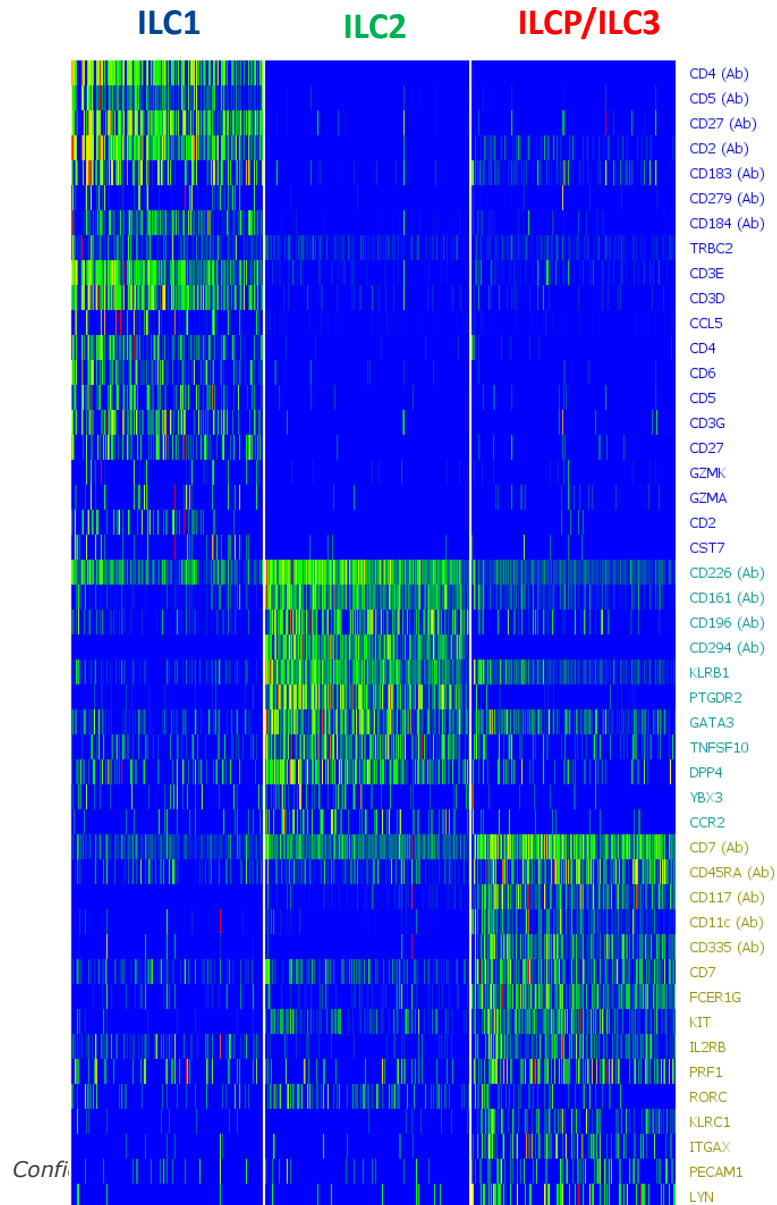Concordant resolution and distribution to flow

BD Rhapsody™ Single-cell multiomic analysis

Heterogeneity of major ILC subsets

Circulating ILCS

T-SNE:42 markers & 399 genes

T-SNE: overimposed Phenograph

ILC1
ILC2
ILCP/ILC3

Each group shows
Multiple subclusters
>Heterogeneity

SeqGeq™ Software

Identify unique signatures defining distinct ILC subsets based on combined gene and protein expression analysis

	ILC1s CD117-CD294-	ILC2s CD117+/-CD294+	ILCP/ILC3s CD117+CD294-
CD4	+	-	-
CD5	++	-	-
CD27	++	-	-
CD2	++	-	++
CD183	+	-	-
CD184	+	-	-
CD6	+	-	-
CD294	-	+	-
CD90	-	+	-
CD161	+	++	+
CD196	+	++	+
CD192	+	++	+
CD117	-	-	+
CD11c	-	-	+
CD335	-	-	+
CD122	+	-	+
CD94	+/-	-	-

ILC1 Subsets heterogeneity

CD4$^+$ Group 1 Innate Lymphoid Cells (ILC) Form a Functionally Distinct ILC Subset That Is Increased in Systemic Sclerosis

Florence Roan, Thomas A. Stoklasek, Elizabeth Whalen, Jerry A. Molitor, Jeffrey A. Bluestone, Jane H. Buckner and Steven F. Ziegler
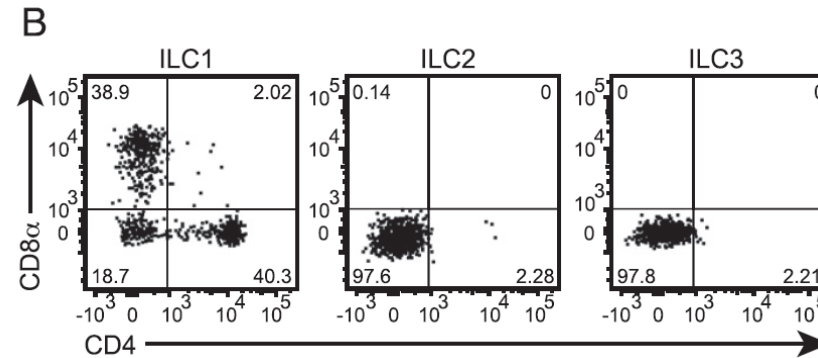
- Study confirmed the existence of a subset of circulating ILC1 cells expressing T-cell markers CD4, CD8, CD5, intracellular CD3e but not surface CD3e and TCR.
- These cell may represent more than just a T-cell contaminant and have a role in disease and homeostasis, therefore further analysis warranted to establish whether bona fide ILCs or not

ILC1 heterogeneity
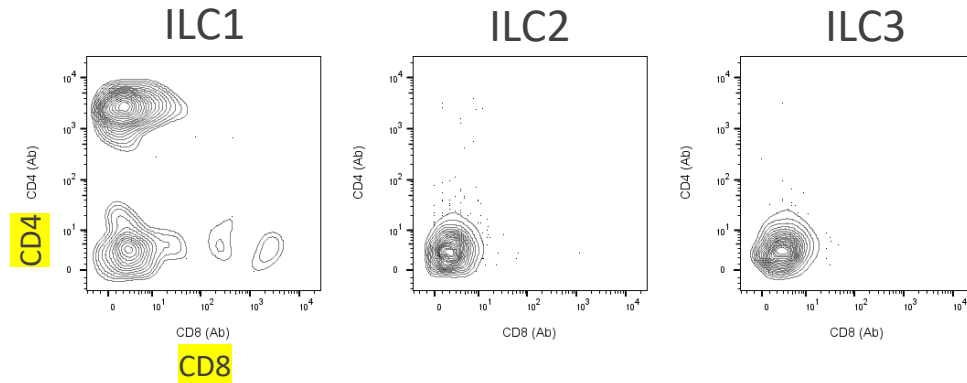
- As previously reported, CD4+ and CD8+ cells are observed within CD3- ILC1, but not ILC2 and ILC3.

- Differential gene and protein expression analysis defines signature of each ILC1 subset.
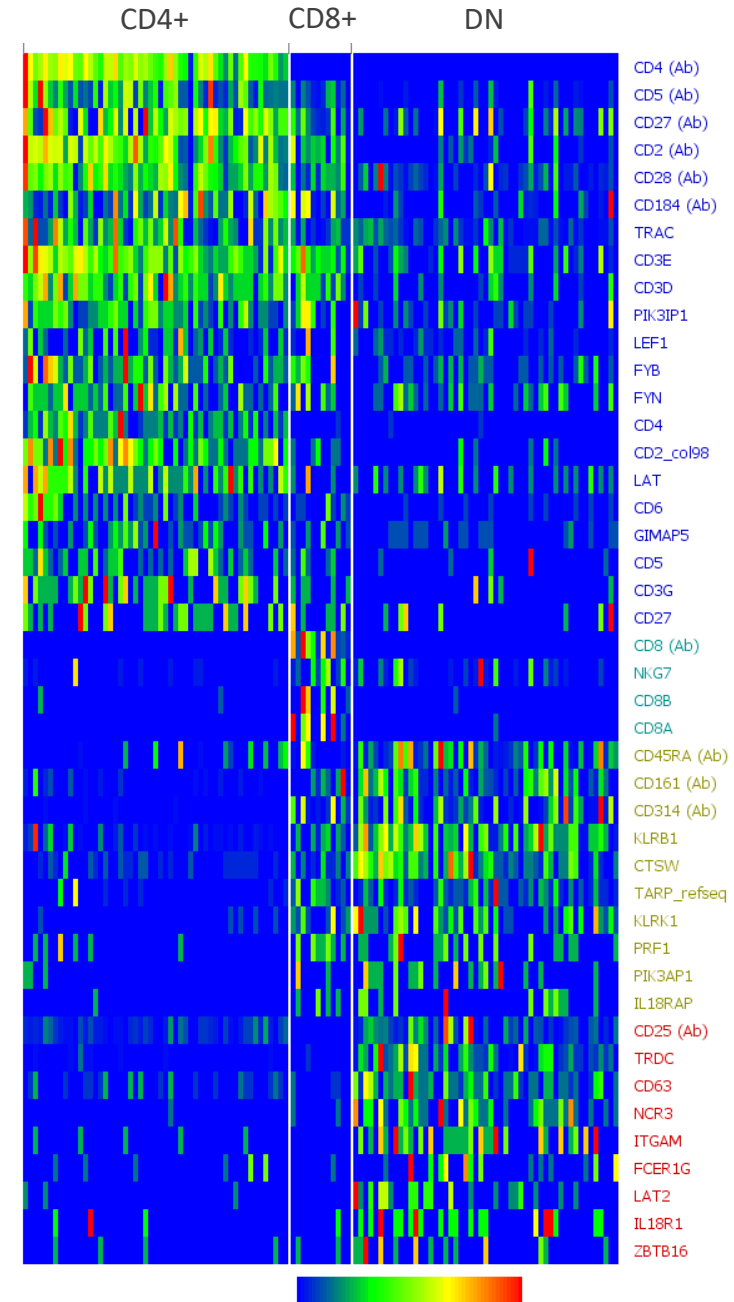
Flow cytometry validation

High-parameter flow cytometry panel for deep characterization of ILC 1 Subset
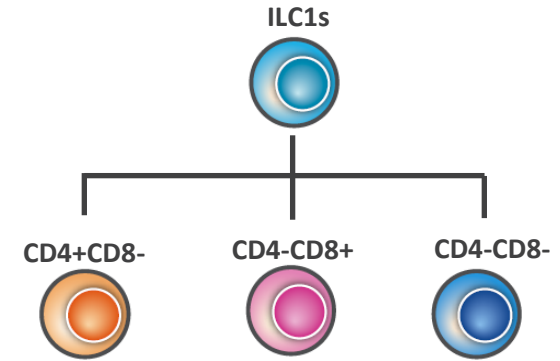
- 13 surface markers differentially expressed between the three subsets of ILC1.
- one differentially expressed gene (CD63) coding for the surface protein CD63.
- 21-color flow cytometry panel that included lineage markers, for the gating of the three main ILC groups created
- Relative antigen density and co-expression data facilitates panel design

	CD4+CD8-	CD4-CD8+	CD4-CD8-	
Lineage	-	-	-	PerCP-Cy5./BB700/7AAD
CD3	-	-	-	BUV661
CD56	-	-	-	BUV563
CD94	+/-	+/-	+/-	BV786
CD117	-	-	-	BV711
CD294	-	-	-	BV750
CD127	+	+	+	BV421
CD4	+	-	-	APC-H7
CD8	-	+	-	BUV496
CD5	+	+	-	BUV805
CD27	+	-	+	APC
CD2	+	+	-	BV605
CD184	+	-	-	PE
CD62L	+/-	+/-	+/-	BB515
CD45RA	+	-	+	BUV395
CD25	-	-	+	BV480
CD28	+	+	+/-	PE-CF594
CD161	-	+	+	BUV737
CD63	-	-	+	PE-Cy7
CD196	+/-	+	+/-	APC-R700
CD314	-	+/-	+	BV650

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High parameter flow cytometry for extensive characterization of ILC 1 subsets
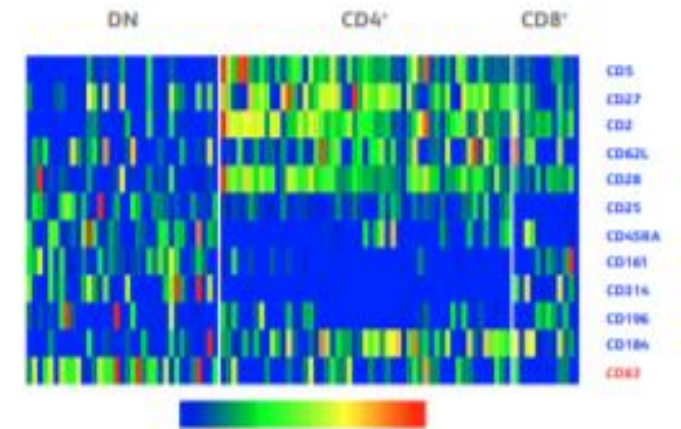
Gating strategy to define ILC1
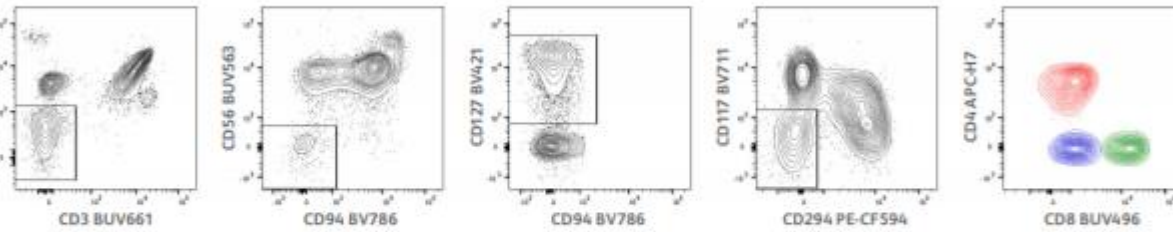
Three main ILC 1 subsets
CD4$^-$CD8$^-$ (blue),
CD4$^+$CD8$^-$ (red) CD4$^-$
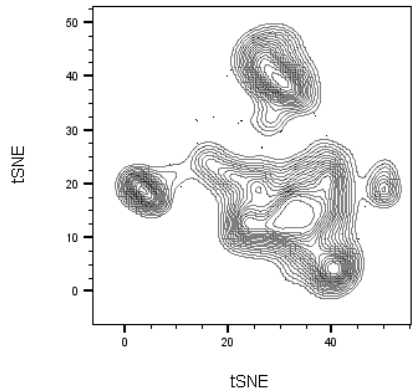CD8$^+$ (green)
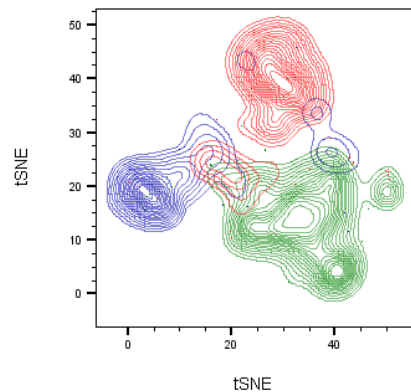
Figure 4B
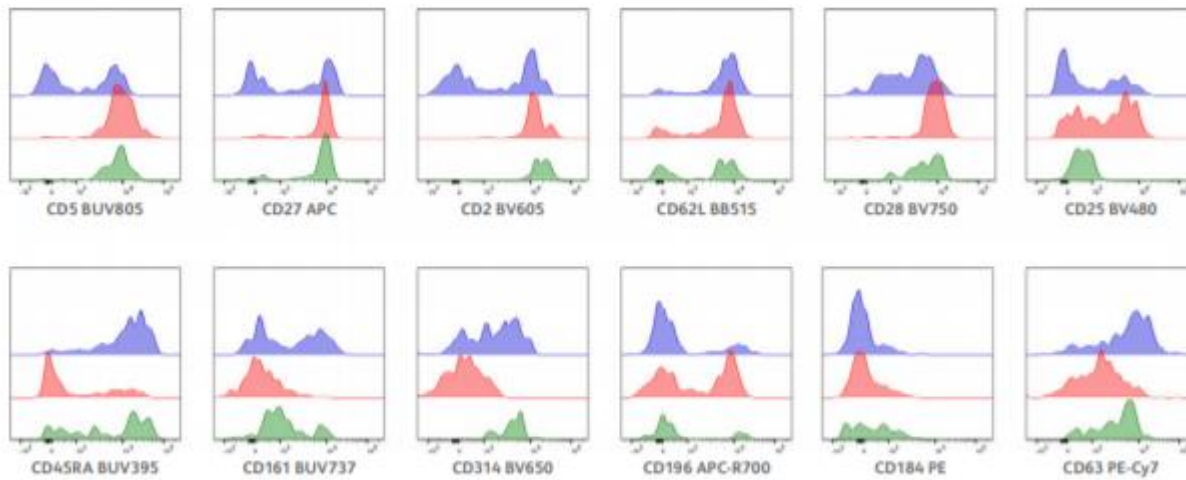
Expression signature from Multiomics workflow

The need for high dimensional data analysis

- Analysis of all 20 flow panel markers by use of bivariate plots is not adequate
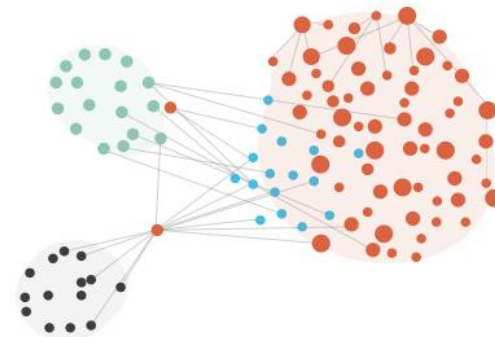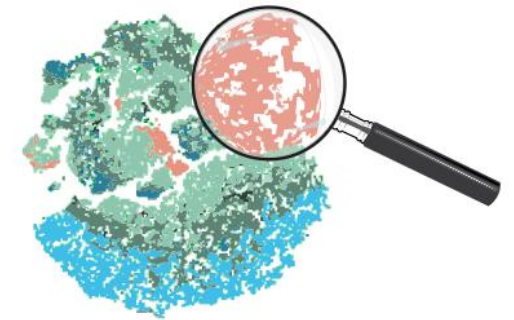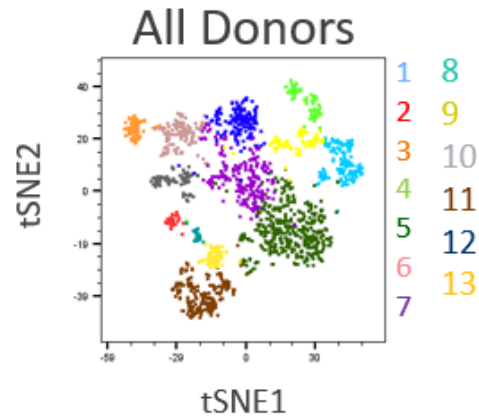
Solution: FLOWJO®

Dimensionality
Reduction
T-SNE

identify clusters and discriminate
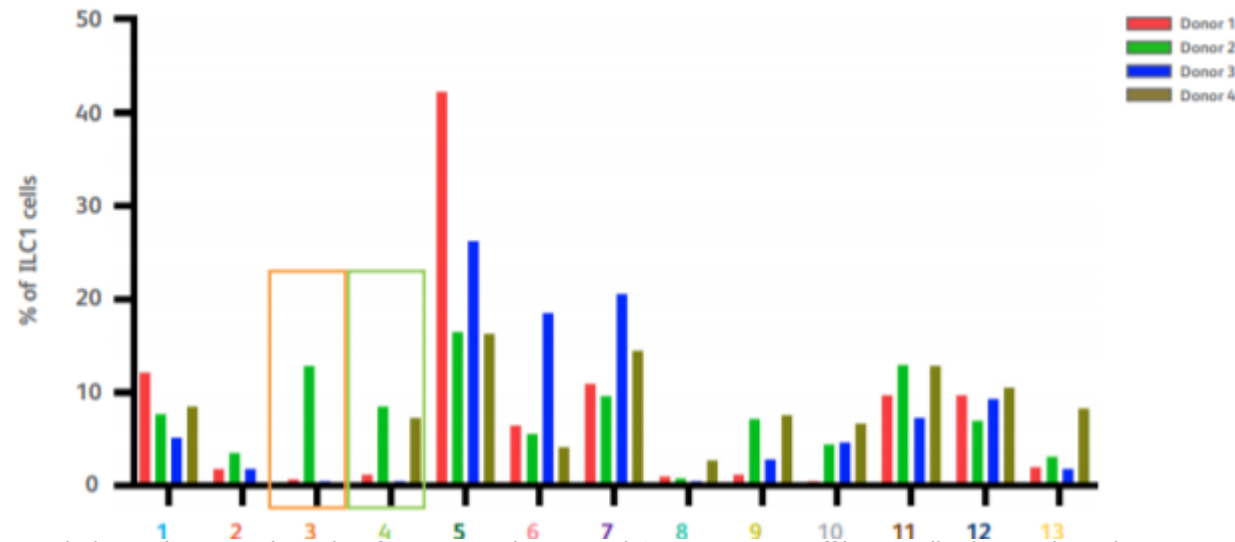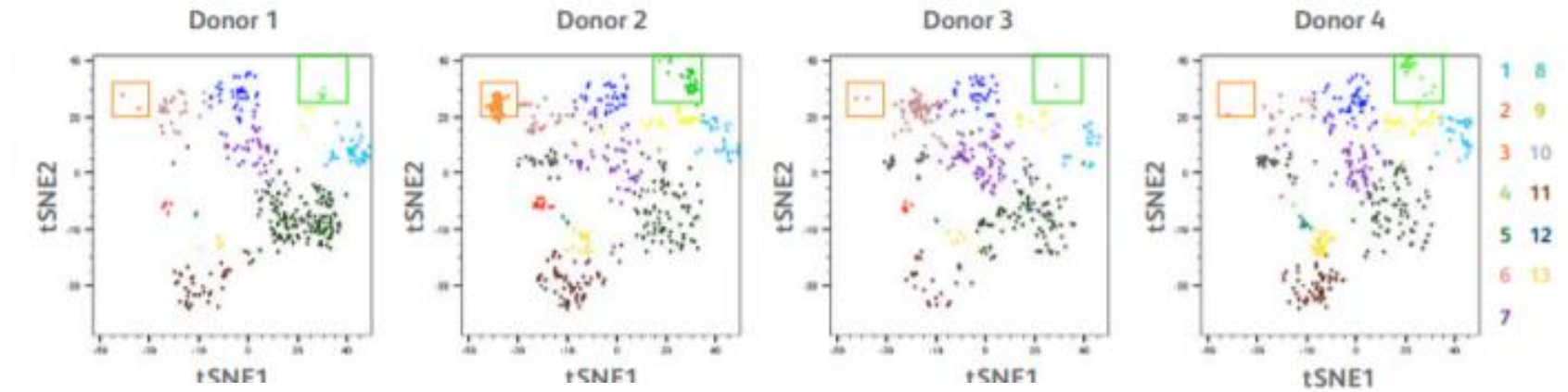Them based on differential
expression

Cell phenotyping

Distrubution of ILC1 subpopulations across donors

Phenograph reveals 13 clusters

- Clusters 3 & 4 variable between donors
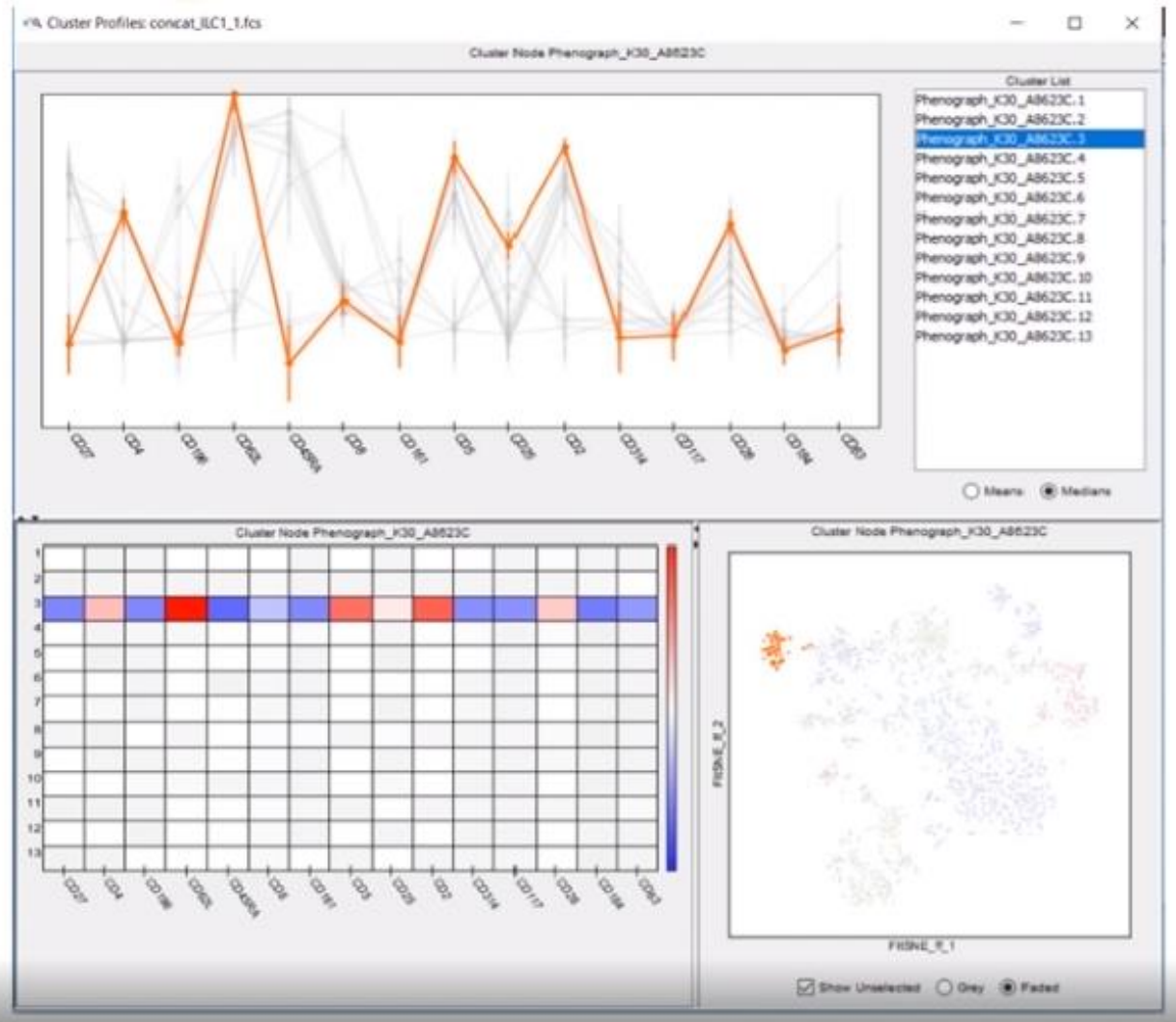- Determine phenotype with Cluster Explorer (next slide)

Cluster Explorer

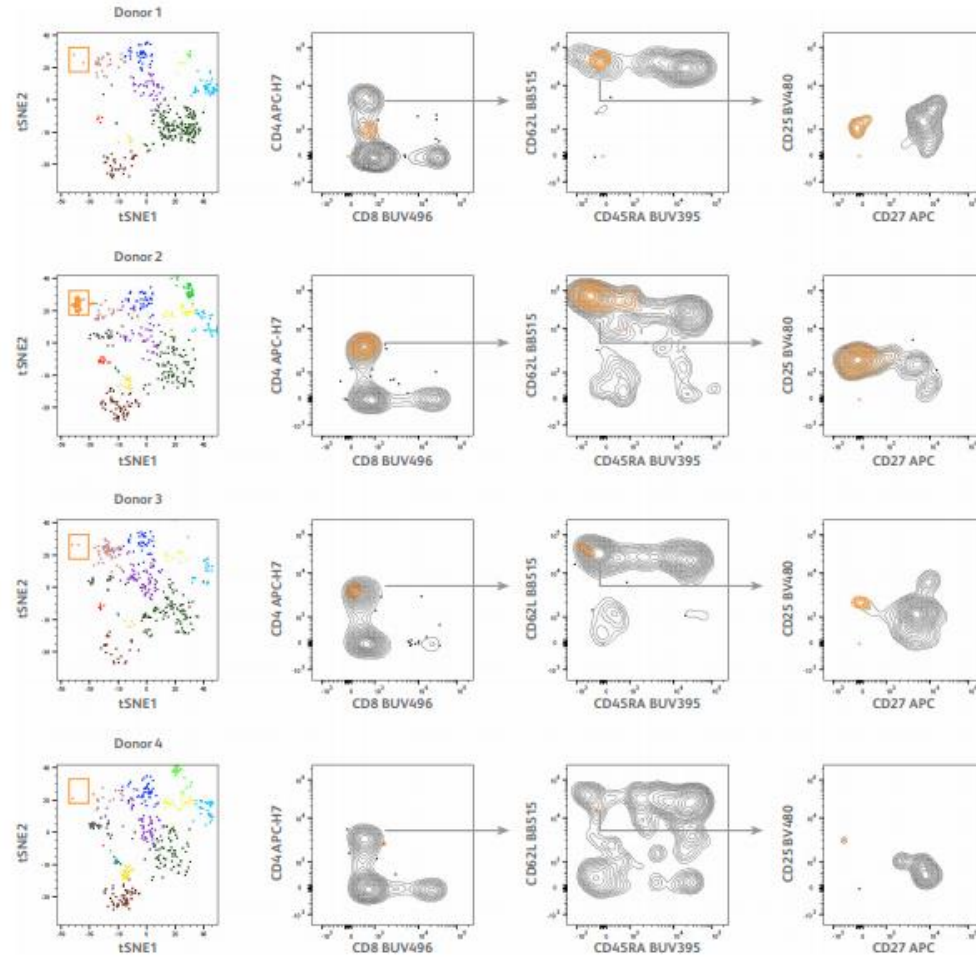Phenotype profile of all 13 clusters

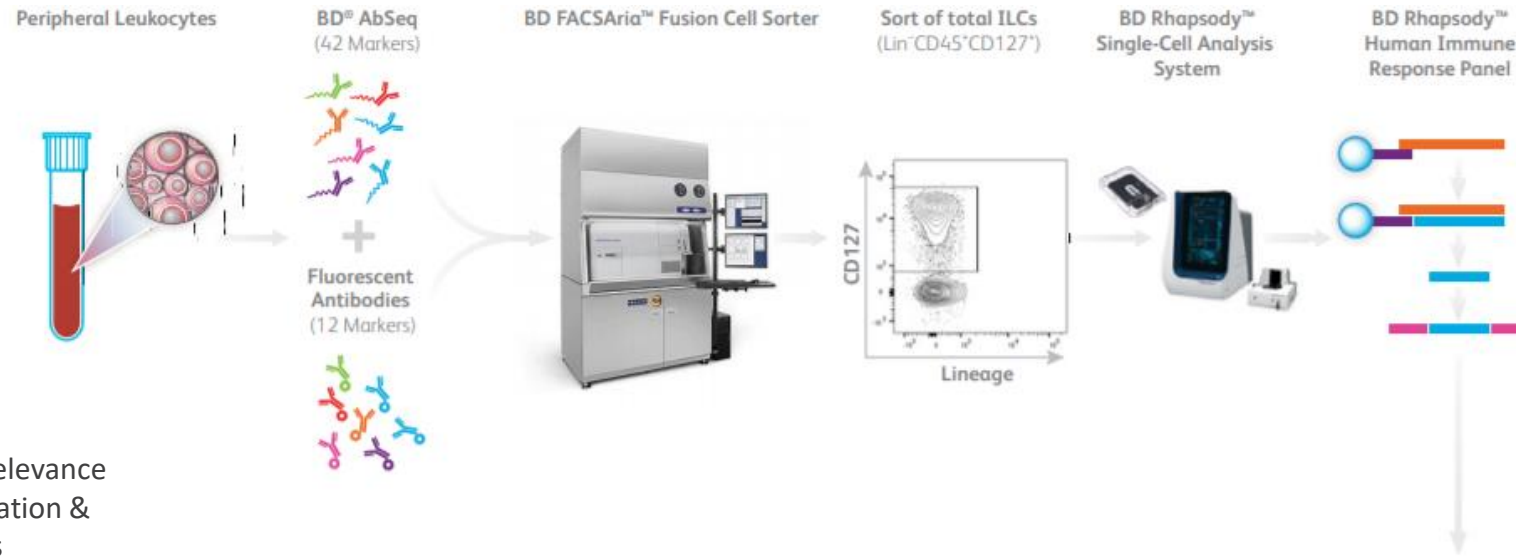# Profiling with cluster Explorer

Zoom in Cluster 3

Cluster profiling
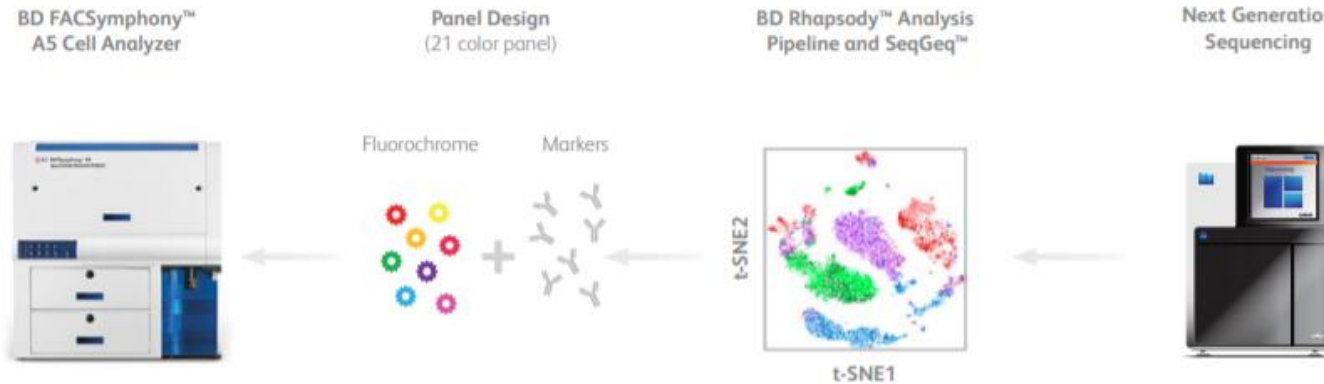# What is phenotype of these clusters (orange and green)

Donor 2. CD4+ cells, CD62L+ CD25+

Complete workflow solution for high parameter single cell characterization

Peripheral Leukocytes

BD® AbSeq (42 Markers)

Fluorescent Antibodies (12 Markers)

BD FACSAria™ Fusion Cell Sorter

Sort of total ILCs (Lin⁻CD45⁺CD127⁺)

BD Rhapsody™ Single-Cell Analysis System

BD Rhapsody™ Human Immune Response Panel

CD127 / Lineage

To determine biological relevance of cluster 3, purify population & perform functional assays

BD FACSymphony™ S6

BD FACSymphony™ A5 Cell Analyzer

Panel Design (21 color panel)

Fluorochrome   Markers

BD Rhapsody™ Analysis Pipeline and SeqGeq™

t-SNE2 / t-SNE1

Next Generation Sequencing

Thank you!

Class 1 Laser Product.
For Research Use Only. Not for use in diagnostic or therapeutic procedures.
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Questions ?

Contact us:
jens.dyekaer@bd.com
hasumati.patel@bd.com