

Single Cell Genomics: finer lenses into human immunity

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Massachusetts General Hospital

Understanding the Tumor Microenvironment
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Washington, November 9th 2018



Disclosure

Consultant, AstraZeneca

How do we define and classify cell types?

molecular markers
morphology
spatial localization
physical properties
functions
developmental origins
transcription factor dependency
growth factor dependency
chromatin states
biochemical states
...

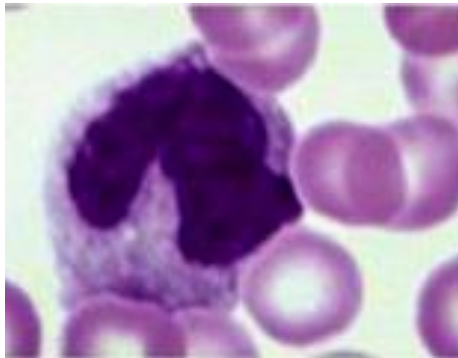
Limitations of current cell type/state definitions

- **Purity:** Defined cell types may not be pure using the historically defined markers
- **Species:** The more well-defined mouse cell types may not directly translate to human
- **Variations:** An immune response induces new and unexpected states
 - Do existing 'standard' set of surface markers truly define distinct immune cell types?
 - Are there more cell subsets that are not currently appreciated?

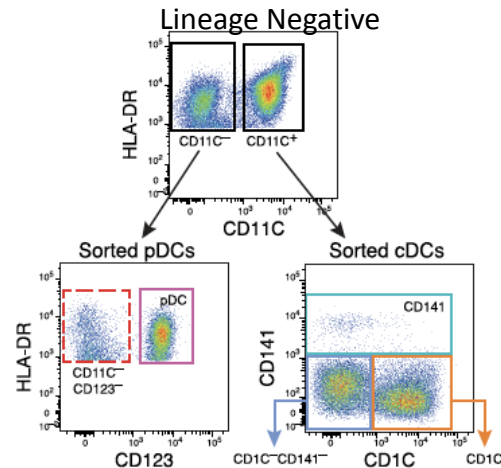
Embracing the revolution in single cell approaches to define immune cell identity

Over the years, the blood myeloid cell population was defined from 1 to 10 cell populations

**1 dimension
(1860s)**



**1-20 dimensions
(1953)**



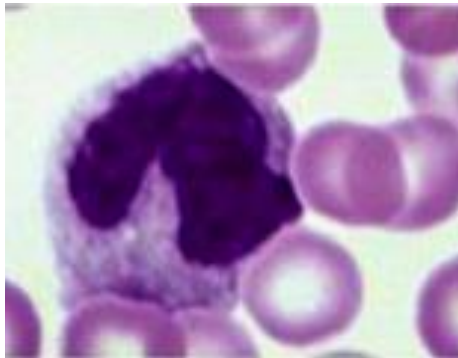
1 population defined through morphology H&E staining **5 populations** defined through flow cytometry analysis

How can we revisit our classification of human cell types?

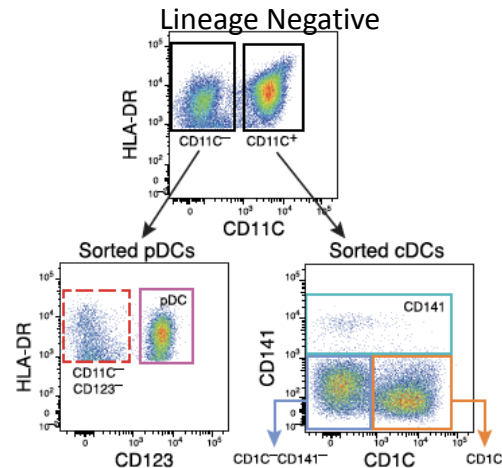
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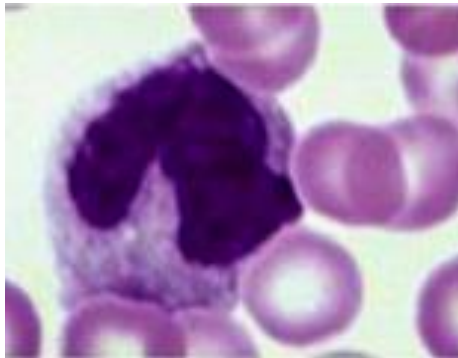
1 population defined through morphology H&E staining **5 populations** defined through flow cytometry analysis

Solution: Generating detailed map through systematic single-cell profiling to enable data-driven molecular definition of cell types

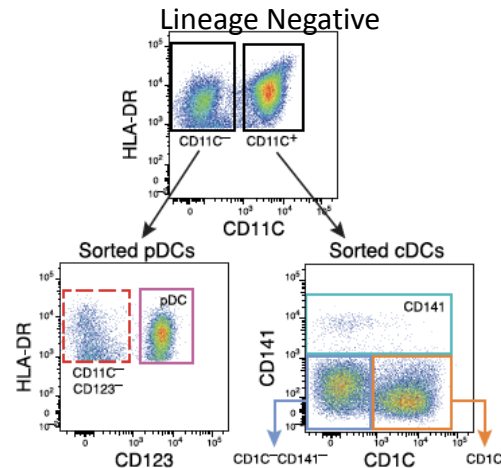
Embracing the revolution in single cell approaches to define immune cell identity

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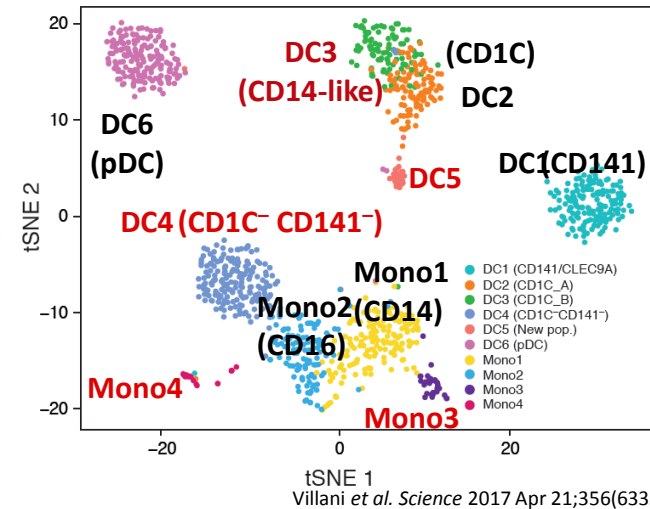
**1 dimension
(1860s)**



**1-20 dimensions
(1953)**



**1000's of dimensions
(2017)**



1 population defined through morphology H&E staining

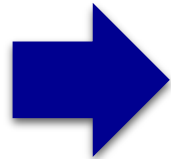
5 populations defined through flow cytometry analysis

10 populations defined through single cell genomics analysis



Revolution in genomics from bulk to single cell analyses

Complex Mixture



Composition Analysis



Fruit bowl
Complex cellular composition

Fruit smoothie
**Blending information from all cells and
derive an average**

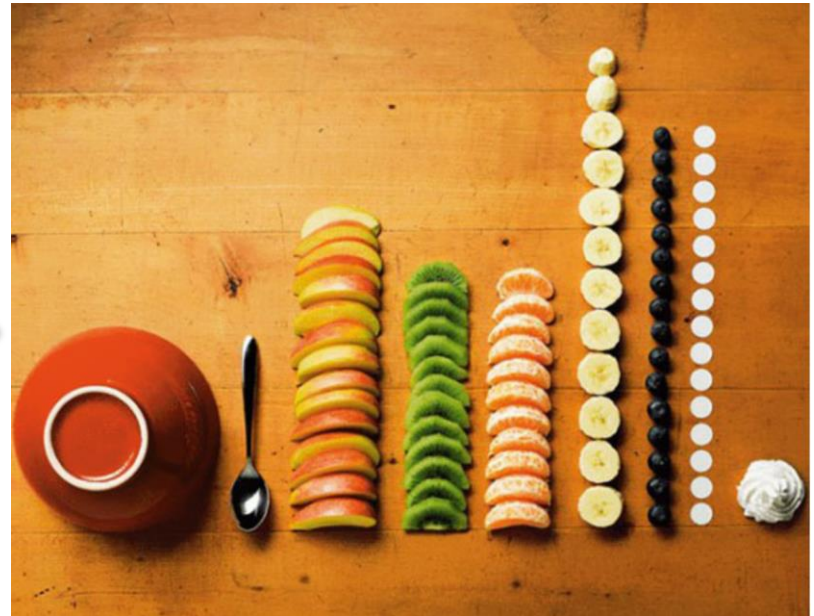
Revolution in genomics from bulk to single cell analyses

Complex Mixture



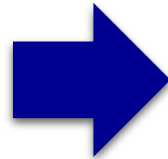
Fruit bowl
Complex cellular composition

Composition Analysis



Fruit bowl deconvolution
Identifying all cells present through single cell genomics analyses

Even specific families of cells have clear distinctions (e.g. T Cells vs B Cells)



Braeburn



Cameo



Cox



Fuji



Golden Delicious



Granny Smith



Jazz



Pink Crisp



Red Delicious



Royal Gala

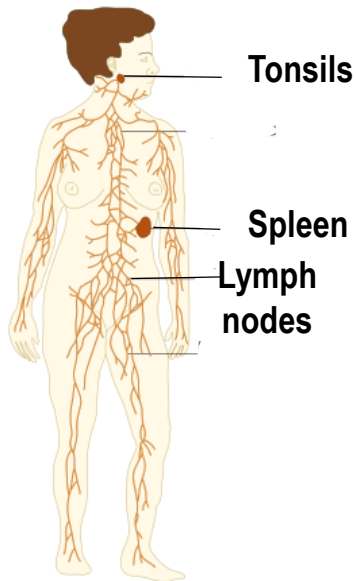
**Even specific families of cells
have clear distinctions (e.g. T Cells vs B Cells)**



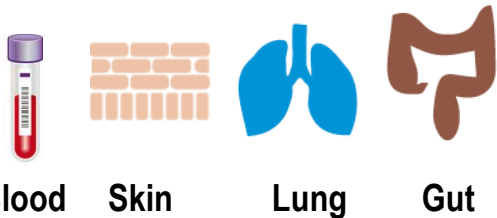
Next-Generation Microscope: Single Cell Genomics Strategies

Tissues to be profiled

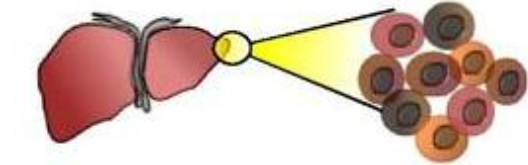
Lymphoid Organs



Non-Lymphoid "Barrier" Organs



Sample
dissociation,

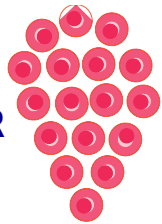


Solid Tissue

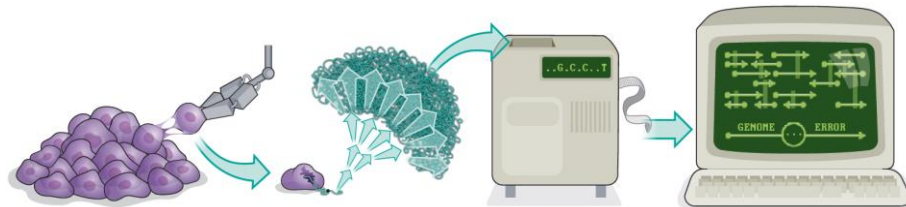
Dissociated Tissue



OR

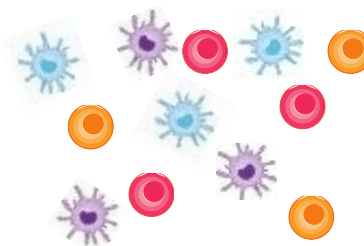


Single cell isolation



Single cell
RNA-Sequencing

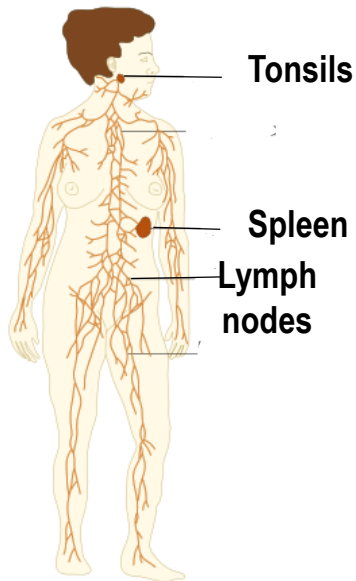
Cell subsets map



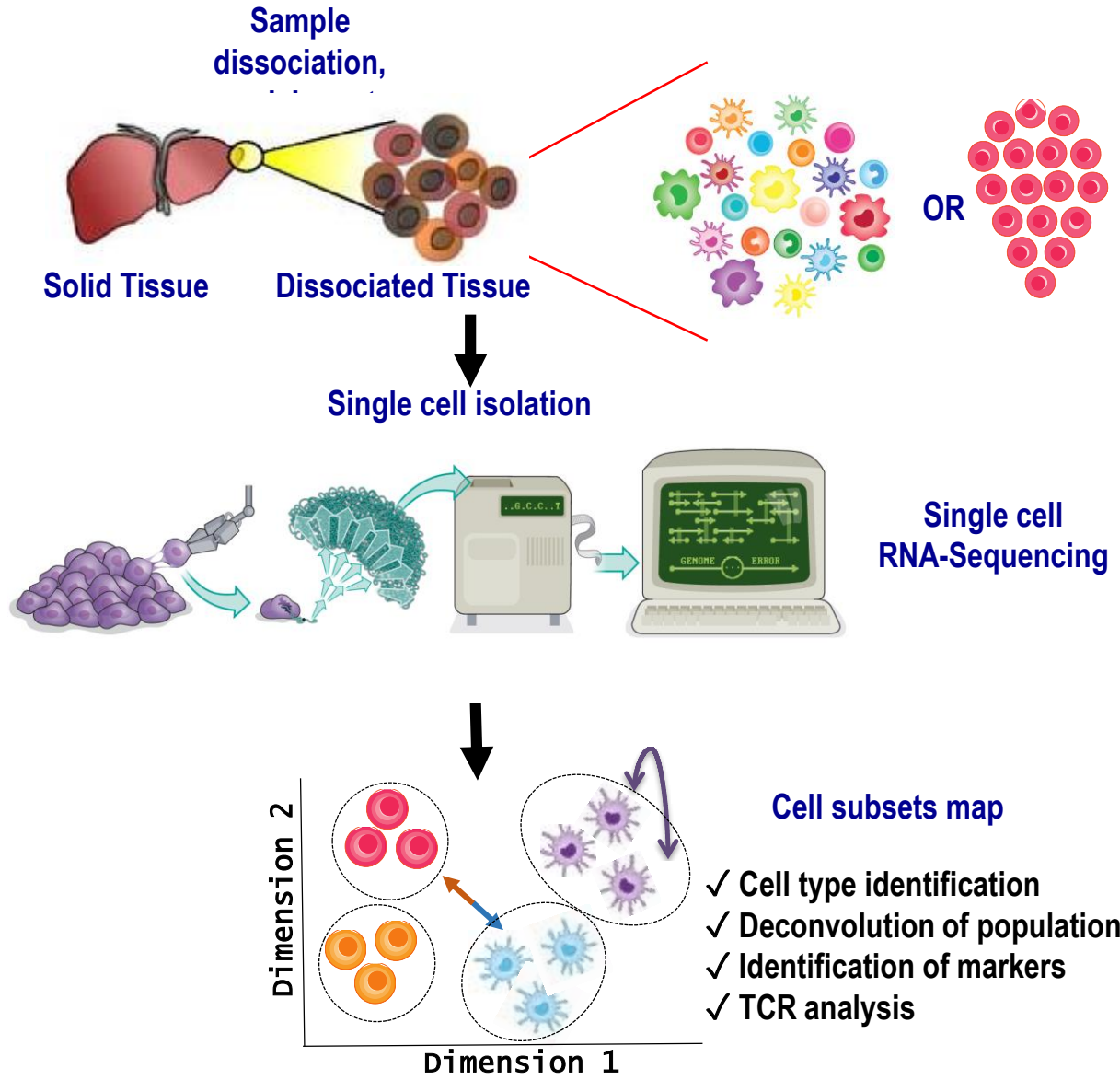
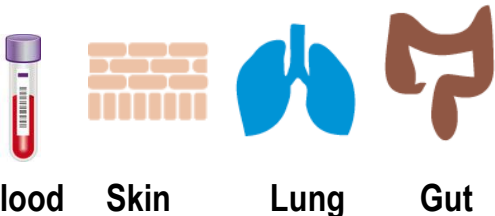
Next-Generation Microscope: Single Cell Genomics Strategies

Tissues to be profiled

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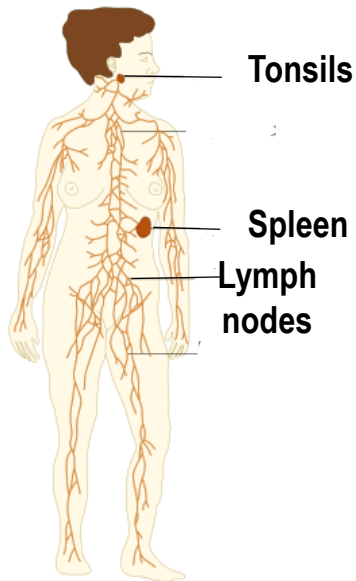
Non-Lymphoid "Barrier" Organs



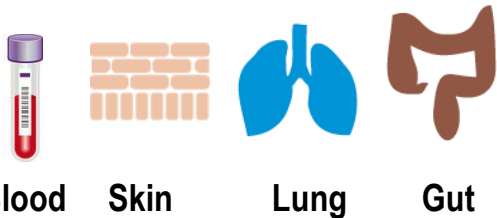
Next-Generation Microscope: Single Cell Genomics Strategies

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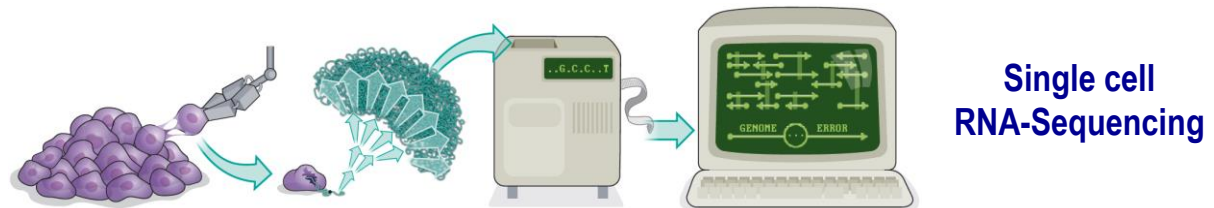
Lymphoid Organs



Non-Lymphoid "Barrier" Organs

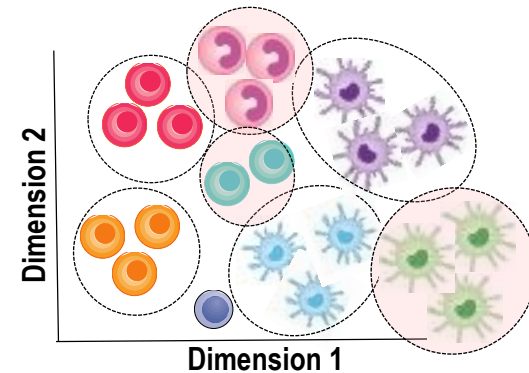
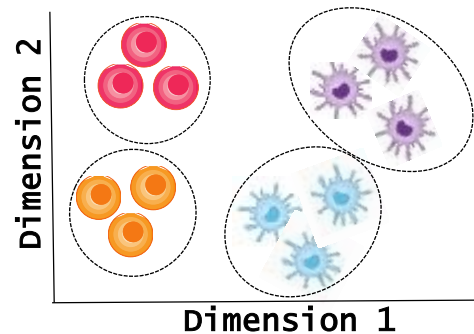


Single cell isolation

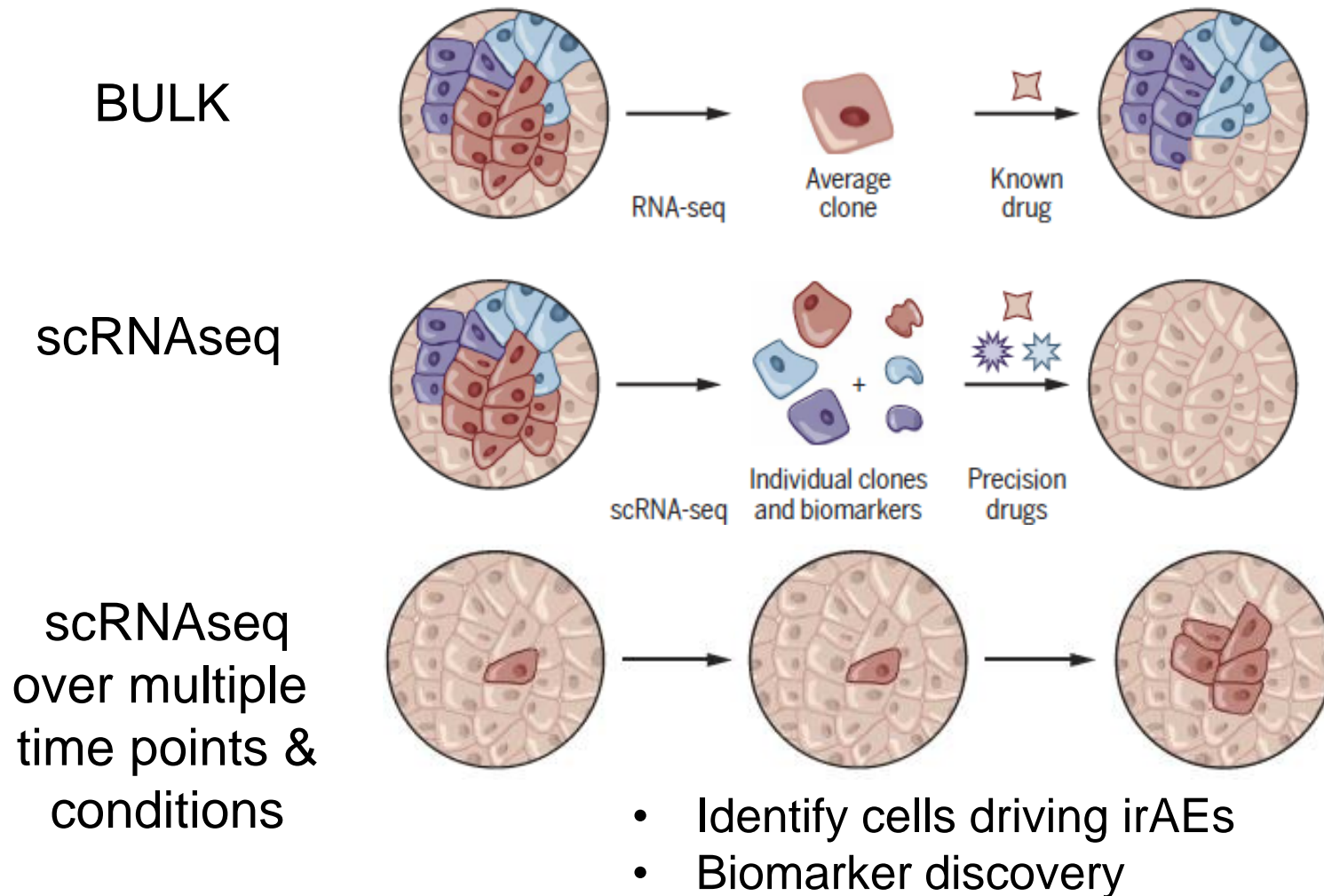


"Healthy" State

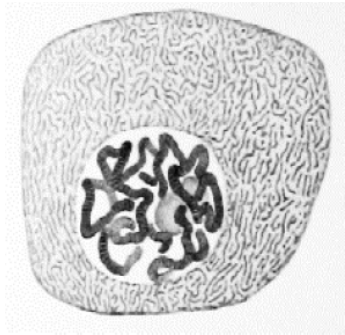
Disease State



Strategies – working towards precision medicine



Analysis at single cell level is an old concept

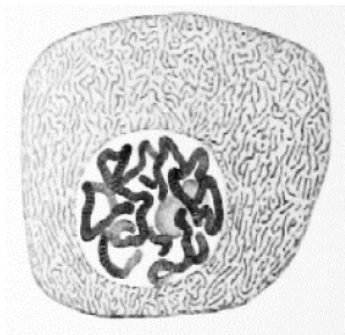


A single-cell genome image of polytene
chromosomes from insects
from 1882 monograph by Flemming

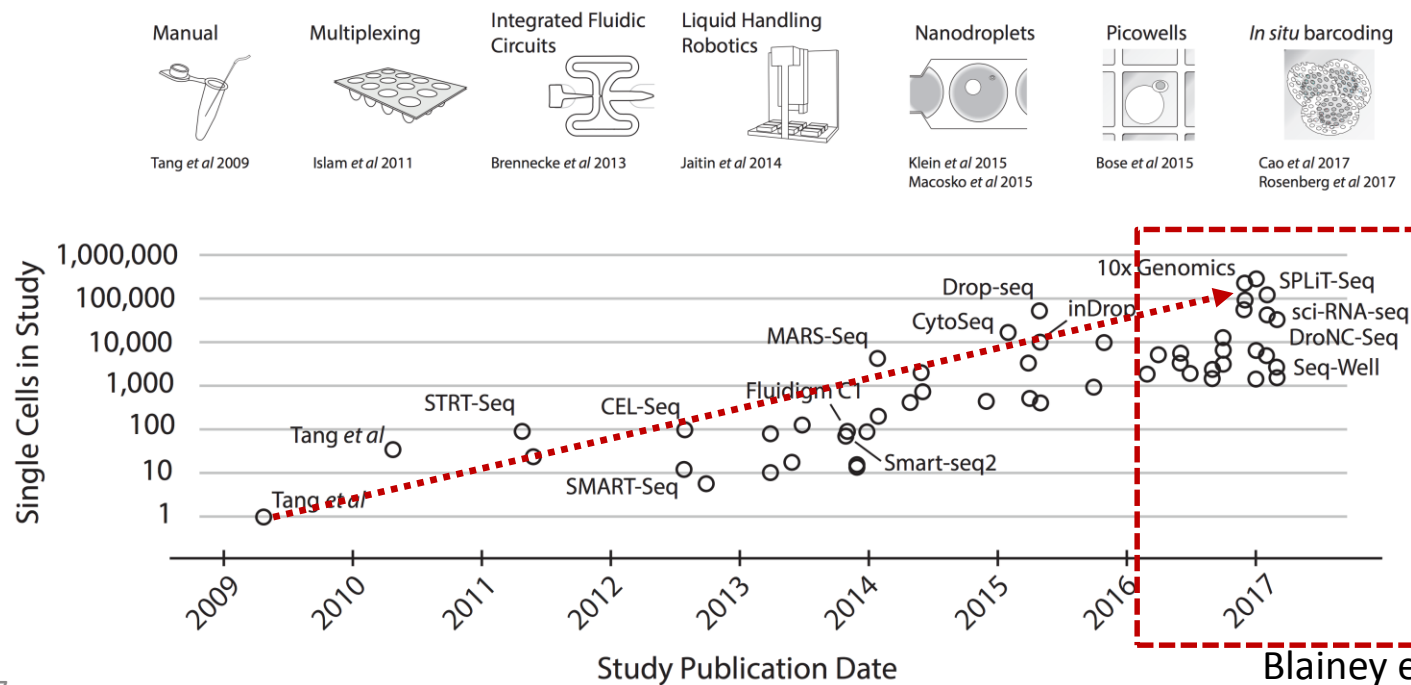
But technology is now allowing us to do this at scale

Millions of single-cells can be analyzed by flow cytometry or mass cytometry, but **the challenge remain that parameters to be measured have to be pre-determined**

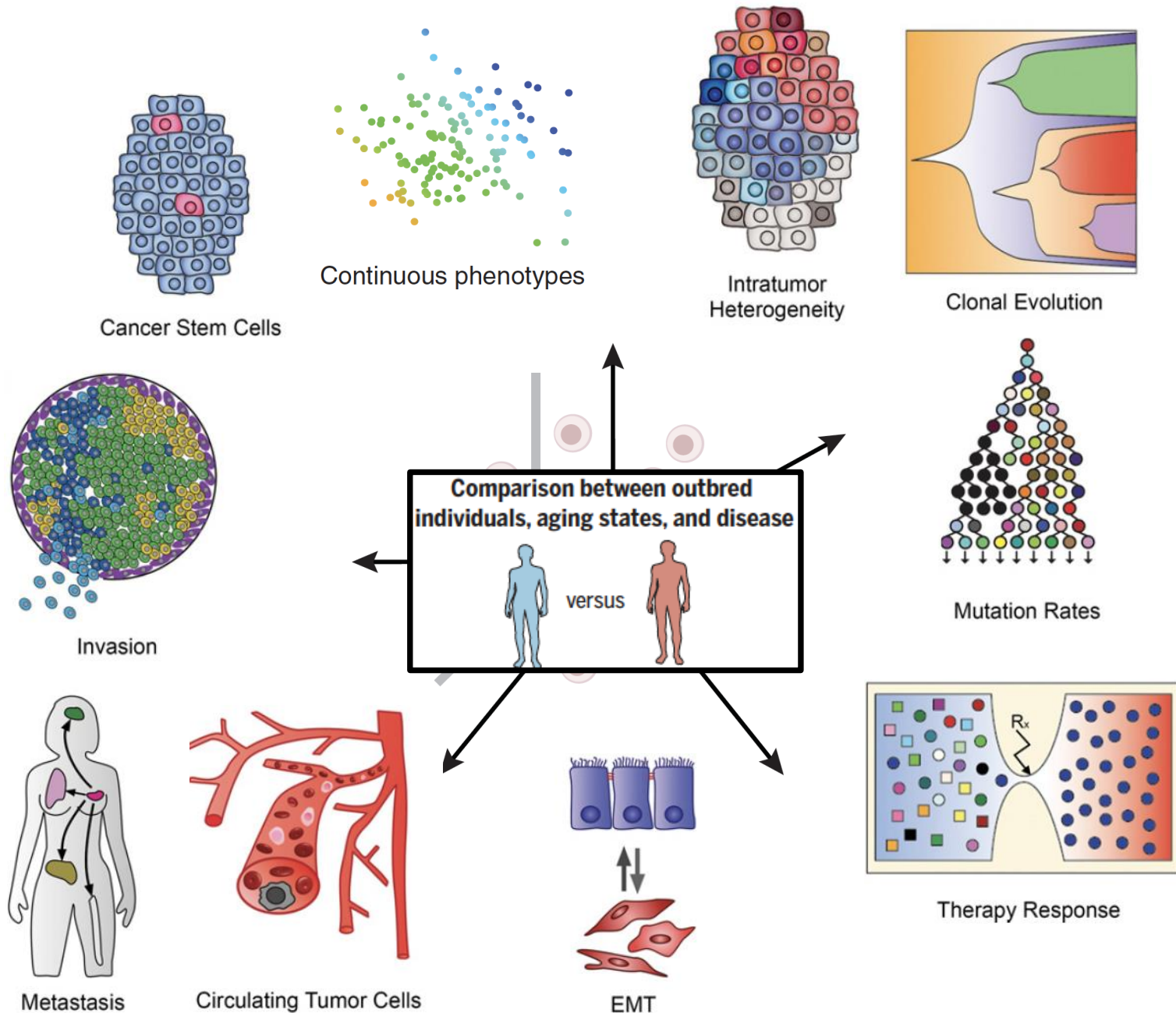
Analysis at single cell level is an old concept but it is scalable today!



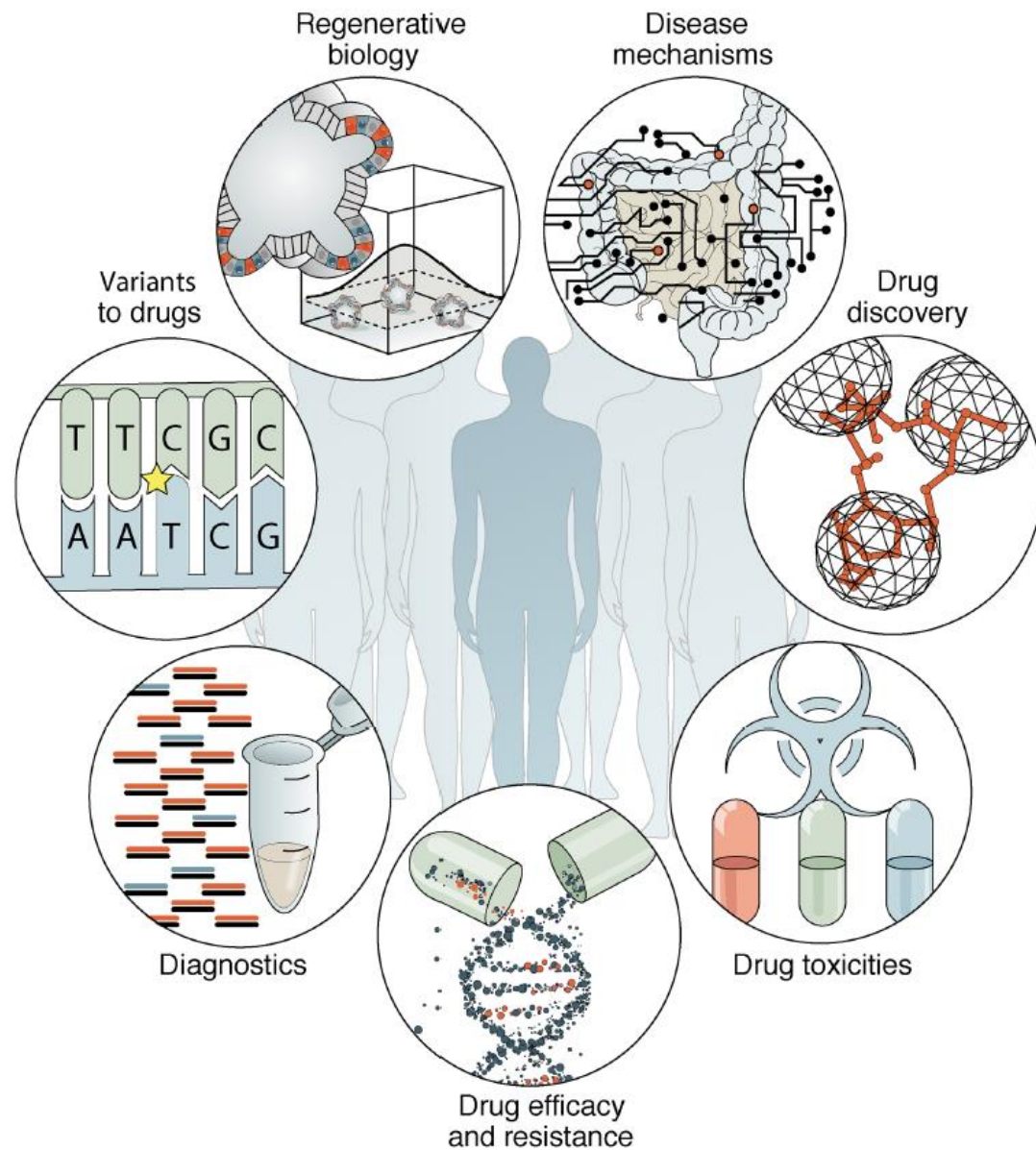
A single-cell genome image of polytene
chromosomes from insects
from 1882 monograph by Flemming



Inference – from single-cell data to cancer biology



Redefining the human system at single cell resolution has tremendous potential for biology & medicine



Vignette #1: Unraveling drug mechanisms of action *in vivo* at unprecedented resolution

Work in collaboration with AstraZeneca



Patricia McCoon



Amy Yang Xu

Biological question: Can we improve the outcome of ICI inhibitor therapy by leveraging innate immunity?



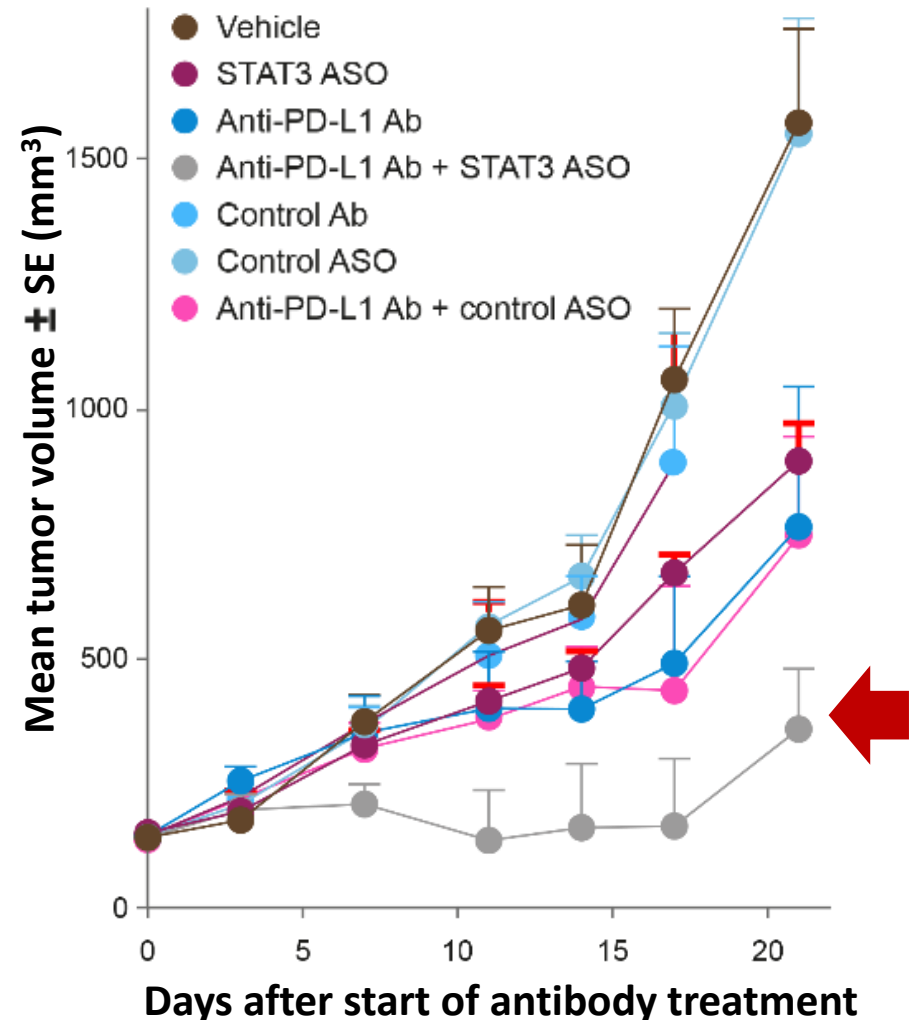
Danvatirsen, a STAT3-targeting therapeutic ASO drug, enhances responses to PDL1-targeting therapy

STAT3

- Ubiquitously expressed TF known to regulate immune suppression in TME

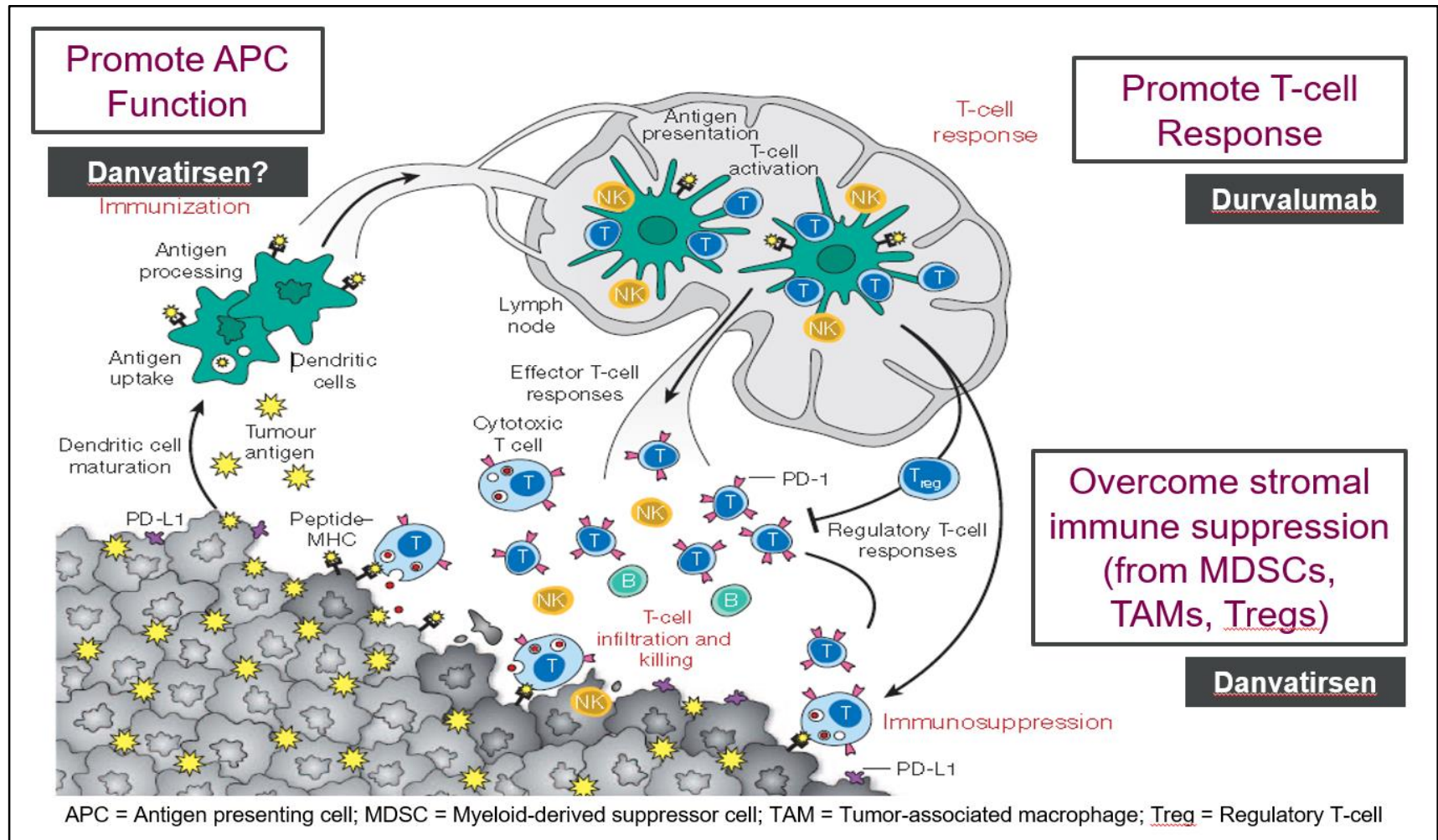
Danvatirsen

- Inhibits STAT3 levels in stromal and immune cells
- Safety and efficacy demonstrated as monotherapy & in combination with anti-PDL1 durvalumab in Phase 1/2 clinical studies
- How does it work?**



Working hypothesis:

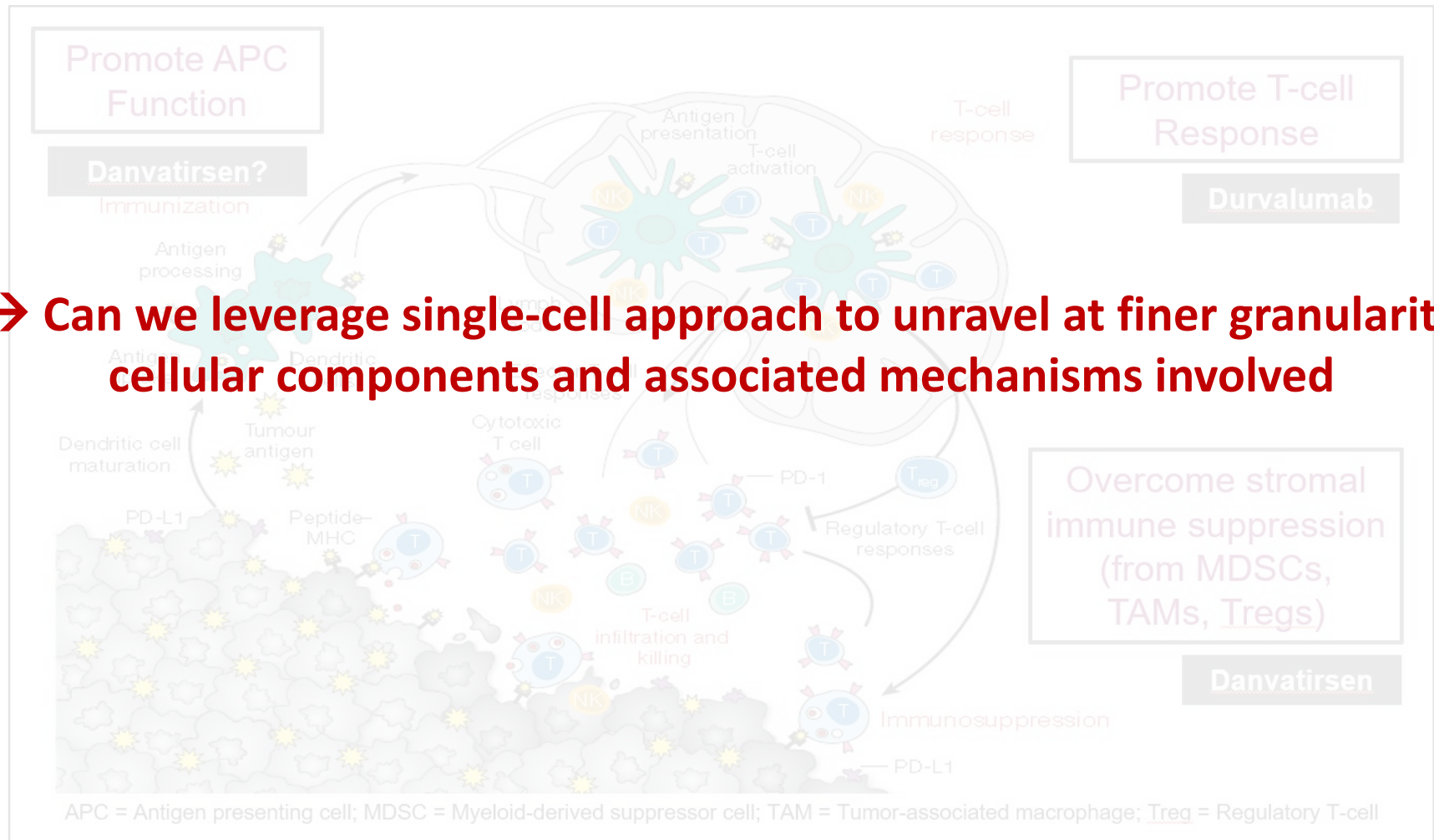
→ Preclinical and clinical immunophenotyping & “bulk” gene expression results suggest that Danvatirsen reduces immunosuppression in the TME



Working hypothesis:

→ Preclinical and clinical immunophenotyping & “bulk” gene expression results suggest that Danvatirsen reduces immunosuppression in the TME

→ Can we leverage single-cell approach to unravel at finer granularity cellular components and associated mechanisms involved

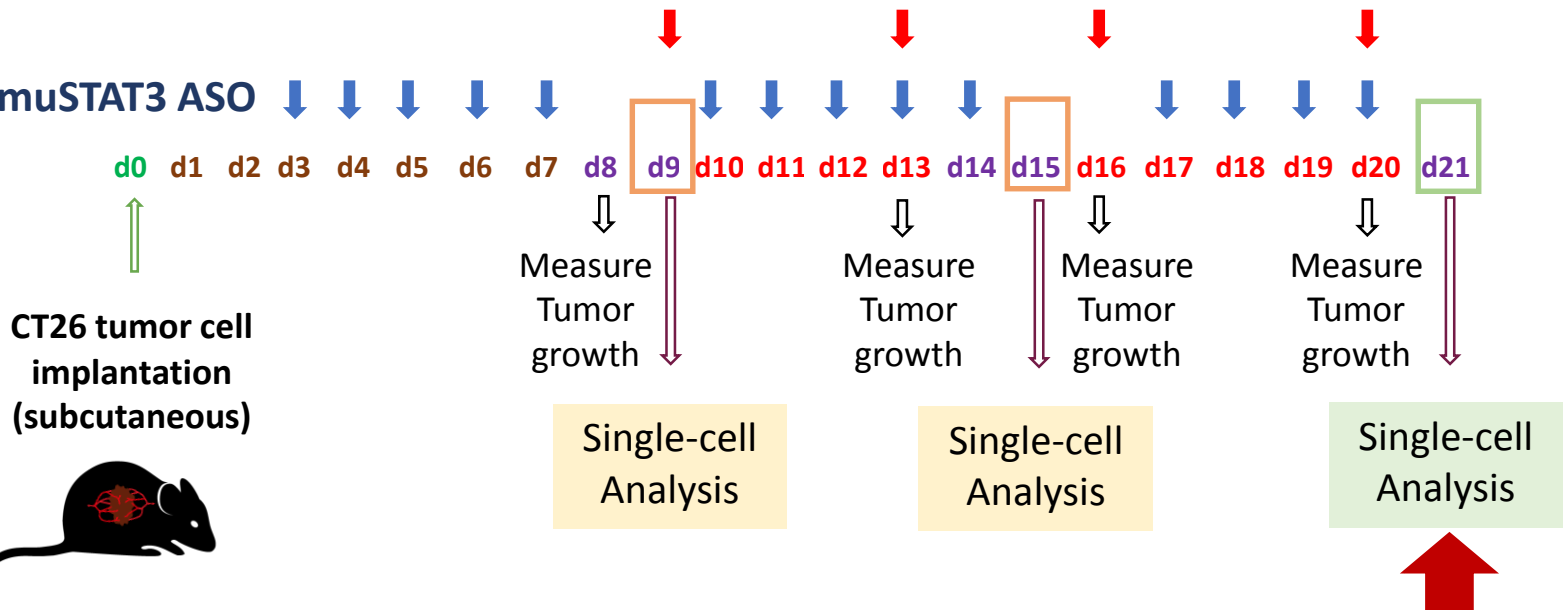


Experimental design

Anti-PD-L1

Control or muSTAT3 ASO

Day



CT26 tumor cell
implantation
(subcutaneous)



Treatment groups

1. Vehicle
2. Control ASO
3. Anti-PDL1
4. STAT3 ASO
5. Anti-PDL1+STAT3 ASO
(Combination)

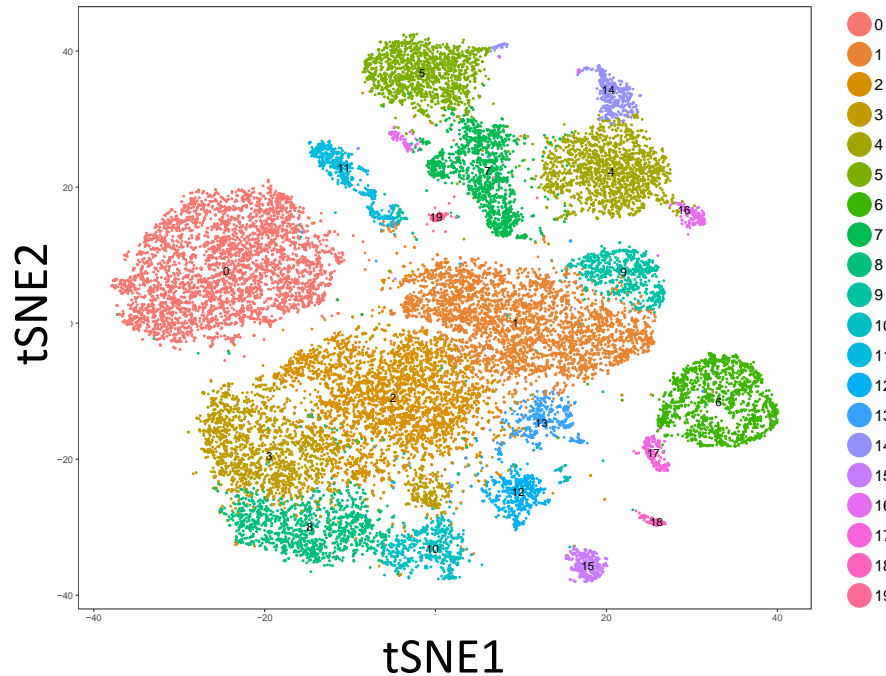
Using scRNAseq analysis, can we define:

1. cellular ecosystem
2. cell state spectrum
3. associated regulatory programs

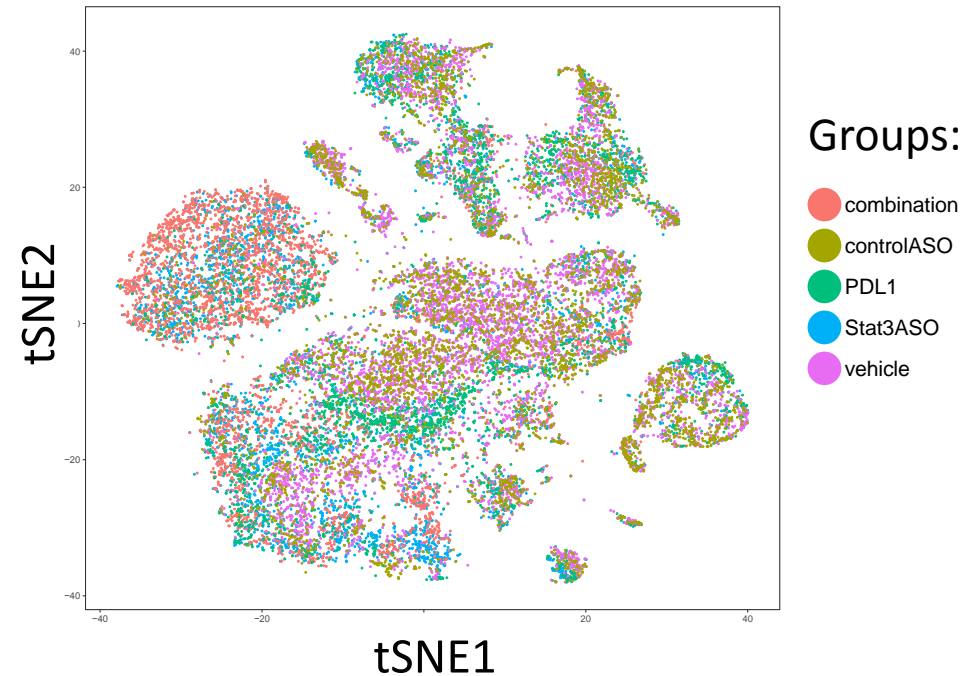
→ Analyze ≈ 5,000 cells/treatment group

Single-cell RNA sequencing predicts 19 cell populations in TME

Single cells colored by predicted cell population
(all treatment groups together)



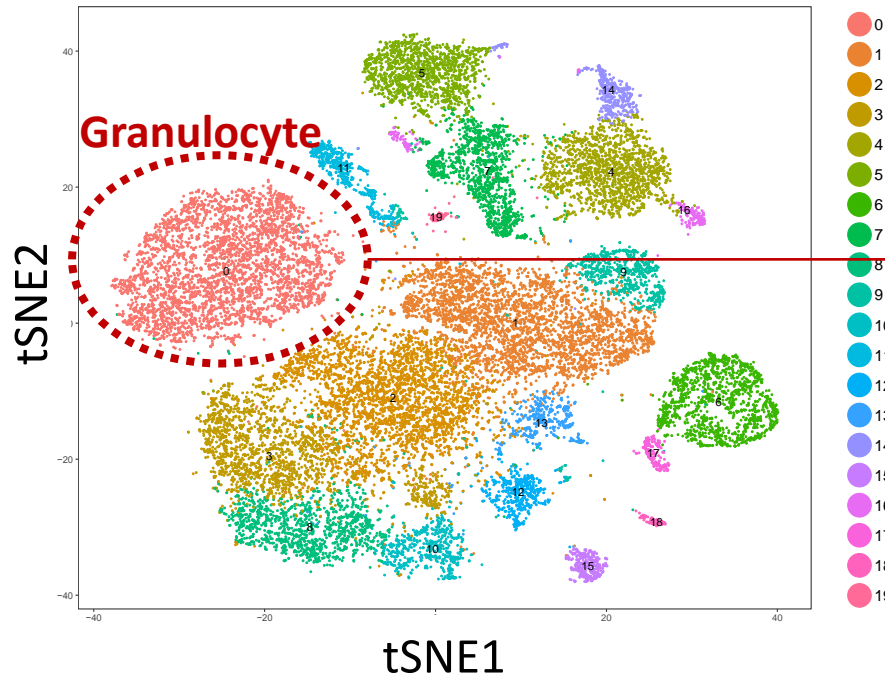
Single cells colored by
treatment group



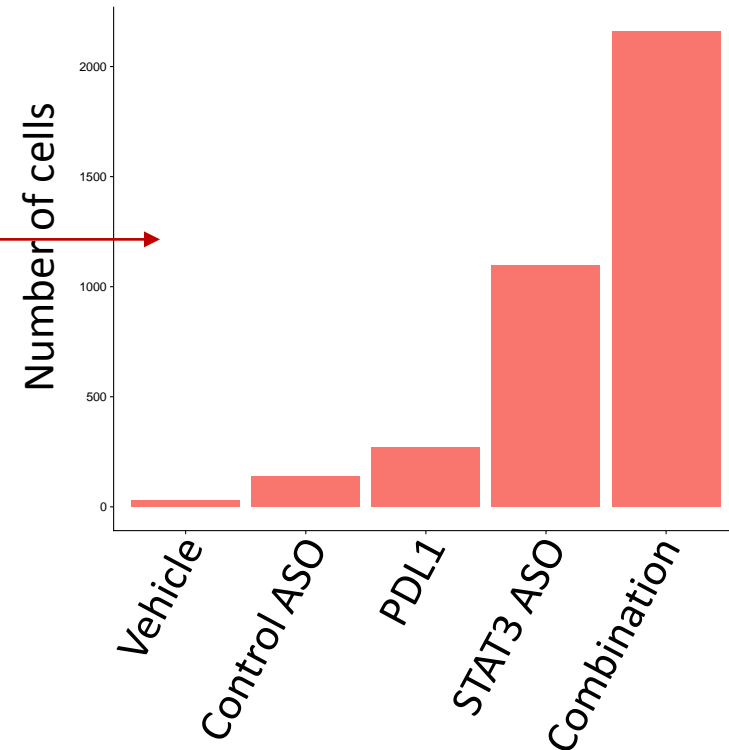
→ Are there clusters enriched with cells from a particular treatment group?

Granulocyte cluster is enriched with cells from STAT3 treatment group

Single cells colored by predicted cell population
(all treatment groups together)



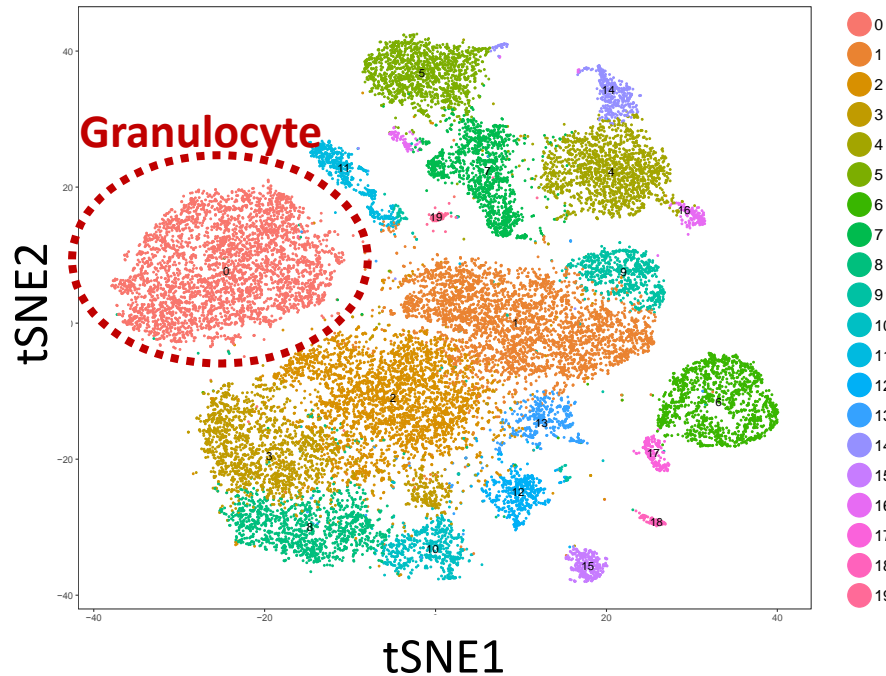
Single cell distribution in the
granulocyte cluster across treatment groups



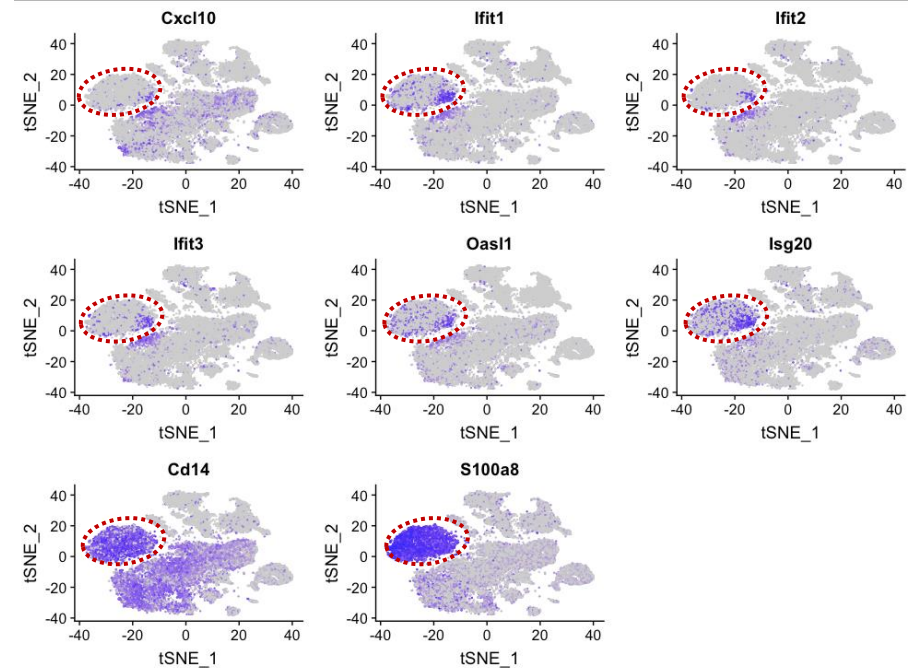
→ Are there particular gene modules that could explain better immunity?

Exploring biology associated with granulocytes cluster:

Single cells colored by predicted cell population
(all treatment groups together)



Single cells colored according to
gene expression levels



→ Interferon regulated genes more specifically
expressed in granulocyte cluster

Planning follow-up experiments

Anti-PD-L1

Control or muSTAT3 ASO

Day

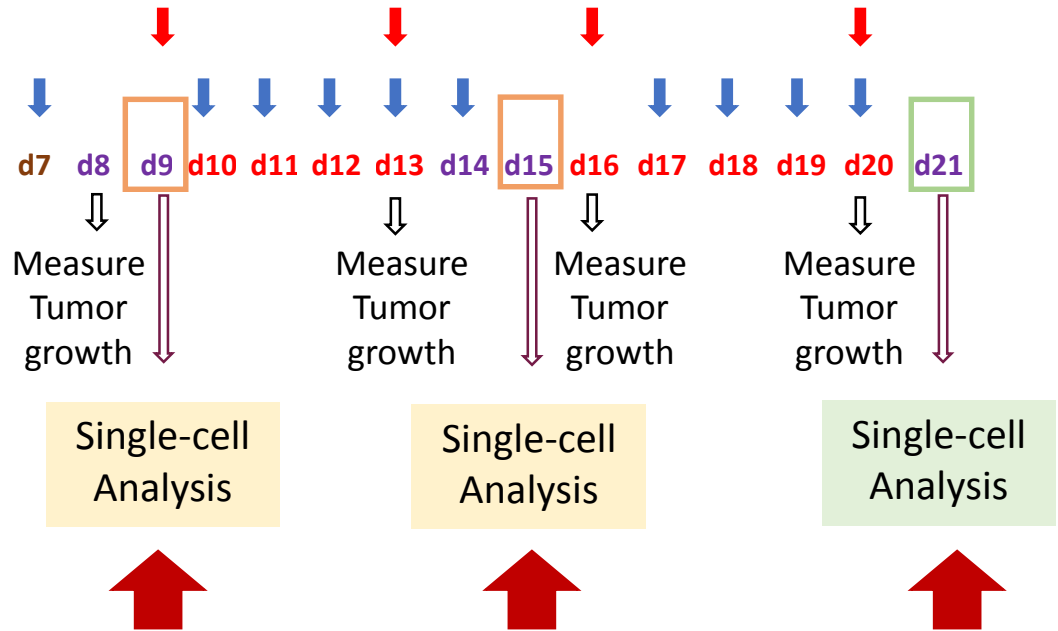
d0 d1 d2 d3 d4 d5 d6 d7 d8 d9 d10 d11 d12 d13 d14 d15 d16 d17 d18 d19 d20 d21

CT266 tumor cell
implantation
(subcutaneous)



Treatment groups

1. Vehicle
2. Control ASO
3. Anti-PDL1
4. STAT3 ASO
5. Anti-PDL1+STAT3 ASO (Combination)



**Single-cell temporal analyses of TME cellular ecosystem
to dynamically map populations and mechanisms
involved in promoting anti-tumor immunity**

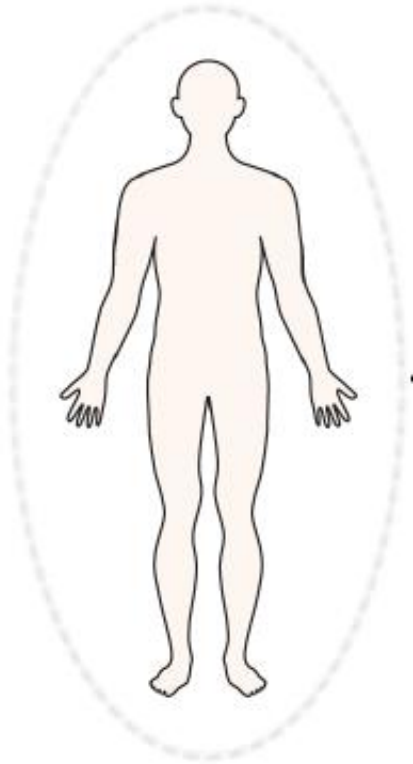
Vignette #2:

Empower future translational efforts through human cell atlas initiatives

**Biological question: Do we know all the cells
defining the ‘healthy’ human system?**



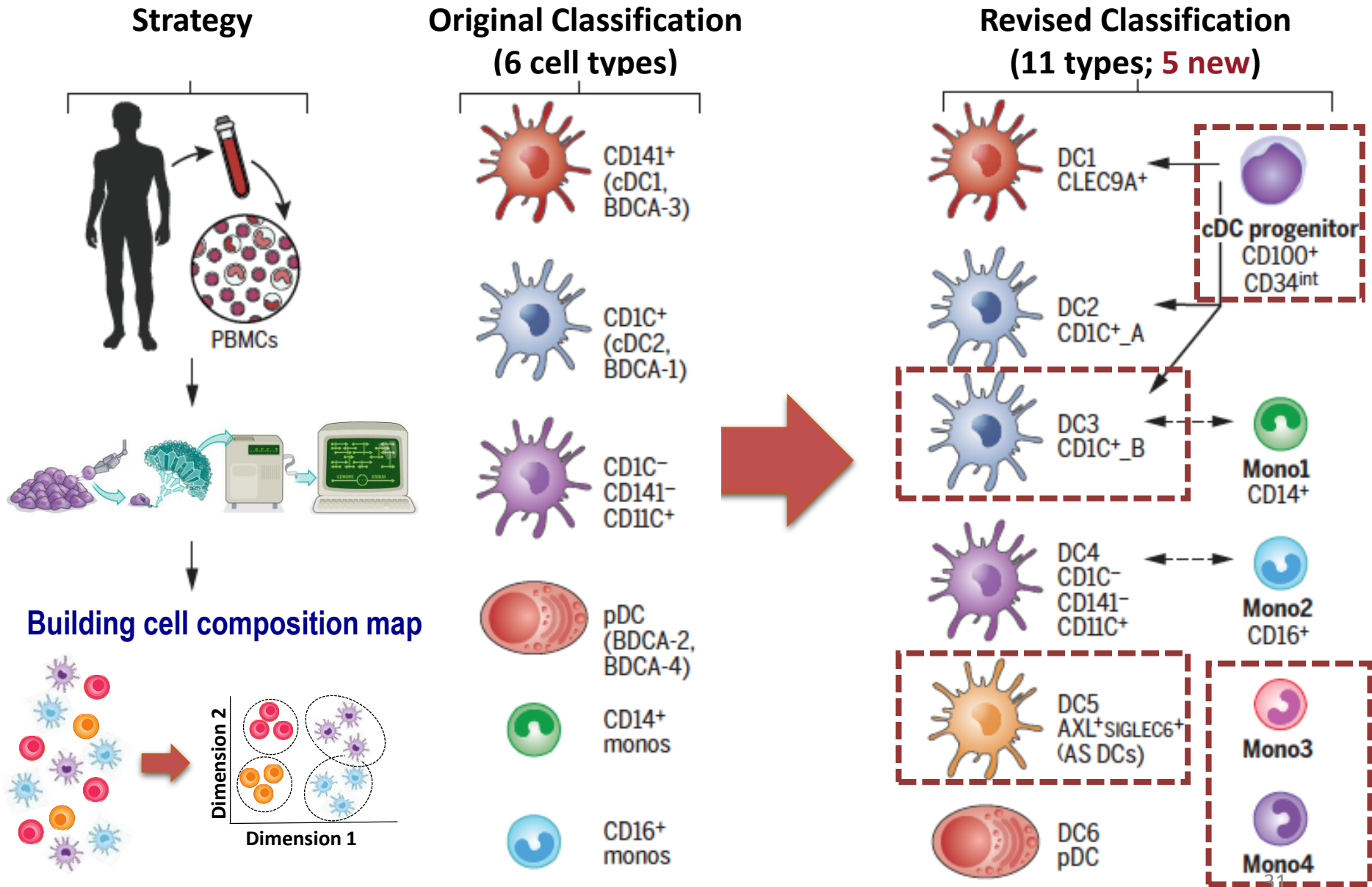
The quest of finding the “guilty” cells driving diseases:



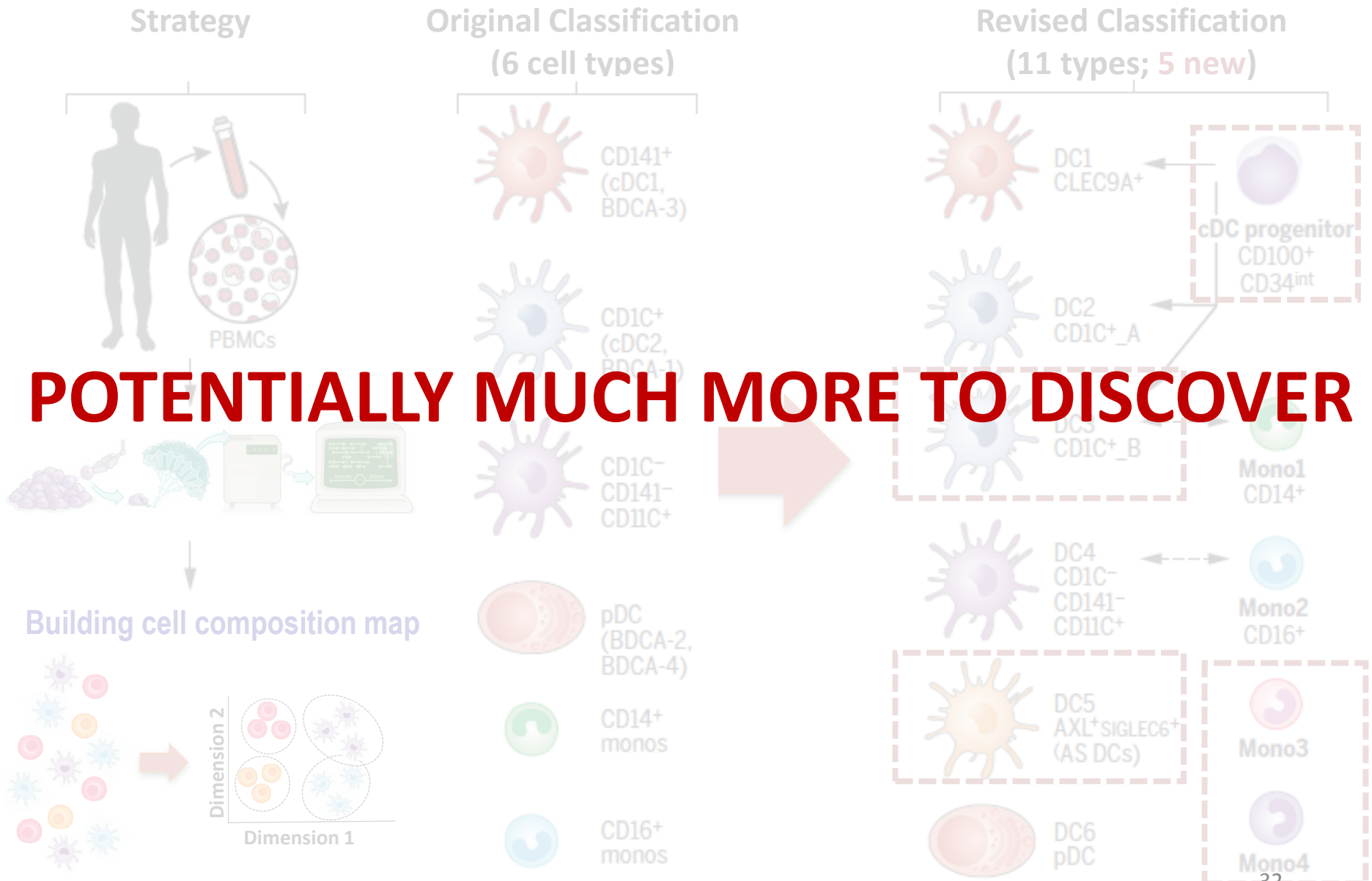
- ~ 30 trillion cells
- Text book → ~ 300 ‘major’ cell types?
- Science → ~ 100 subtypes of immune cells!

Do we really know cells defining the human system?

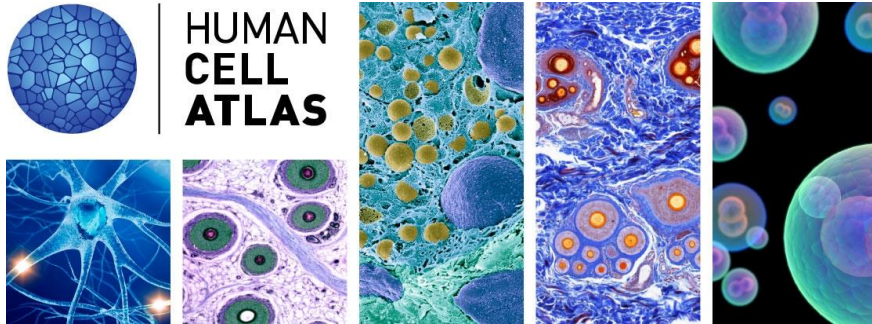
Single cell strategies identified NEW blood DC and monocyte populations



Single cell strategies identified NEW blood DC and monocyte populations



Scaling-up the effort: working towards Immune Cell Atlas



Mission: To create comprehensive reference maps of all human cells—the fundamental units of life—as a basis for both understanding human health and diagnosing, monitoring, and treating disease

<https://www.humancellatlas.org>

VILLANNA, C. ET AL. SCIENCE 356, 644-653 (2017)

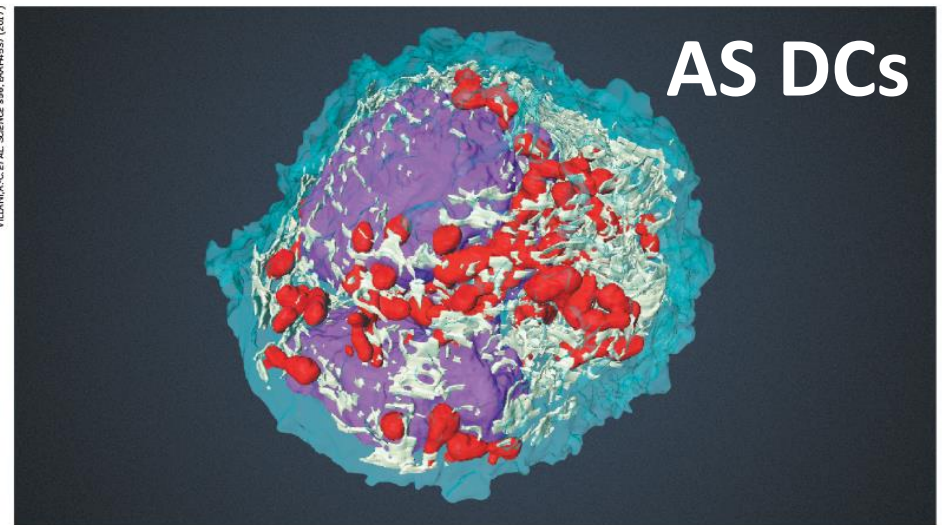
COMMENT

DESCRIPTION Three lines of text go in here go in here until its time for p.xxx

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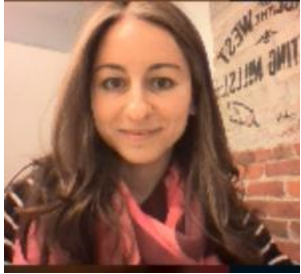
DESCRIPTION Three lines of text go in here go in here until its time for p.xxx



A new type of human dendritic cell recently discovered using single cell RNA sequencing.

The Human Cell Atlas: from vision to reality

Human Immune Cell Atlas Project at the Broad



**Sisi
Sarkizova**



**Bo
Li**



**Orr
Ashenberg**



**Marcin
Tabaka**



**Timothy
Tickle**



**Monika
Kowalczyk**



**Molly
Fisher**



**Danielle
Dionne**



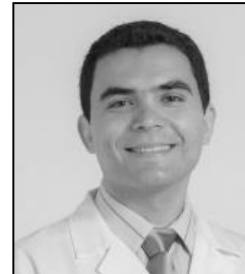
**Lan
Nguyen**



**Patricia
Rogers**



**Will
Ge**



**Mazen
Nasrallah**



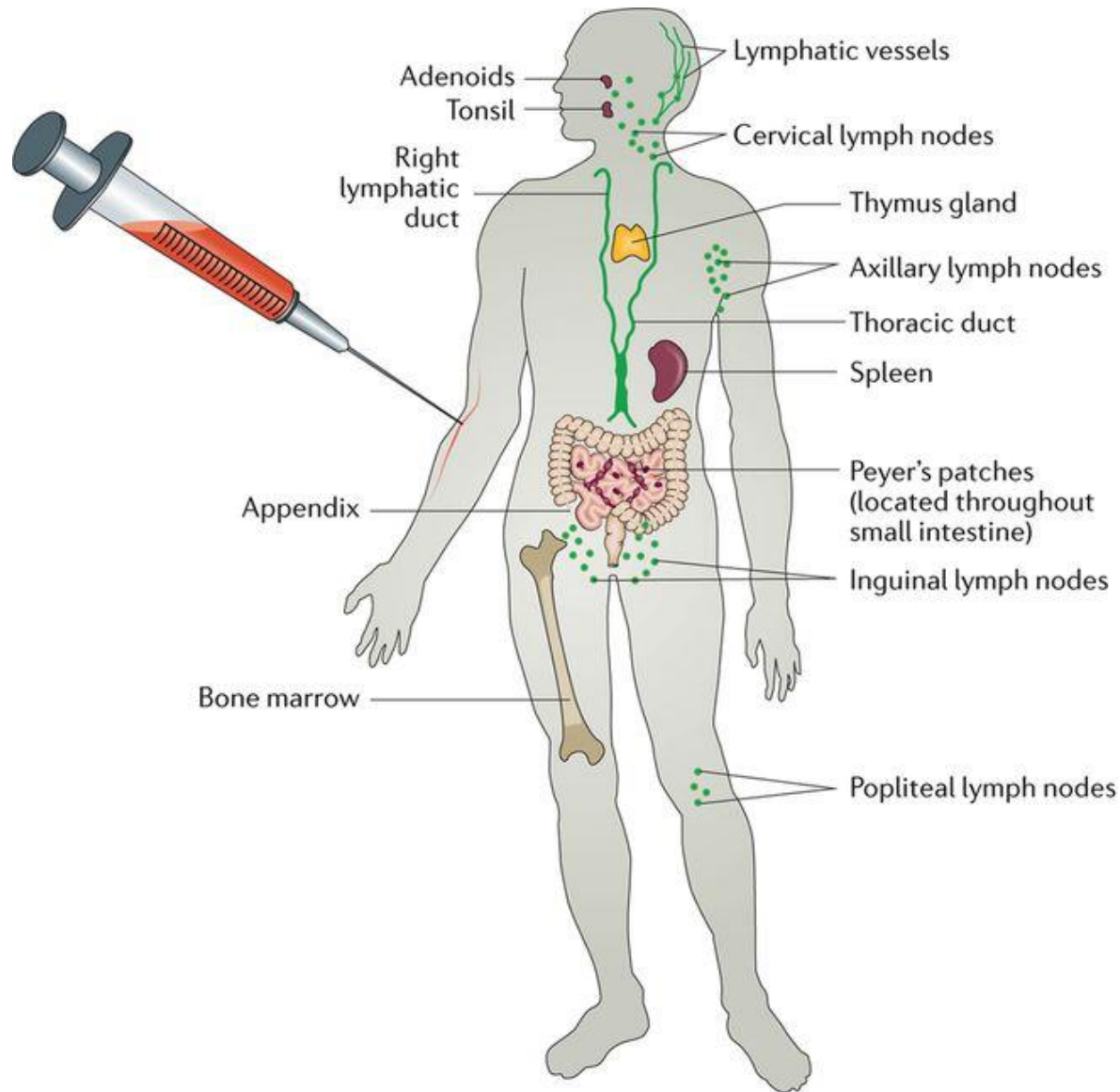
**Jane
Lee**



**Orit
Rozenblatt-
Rosen**

Supported by Manton Foundation

The blood as a window for global immune system analysis: most commonly accessible sample in the clinic



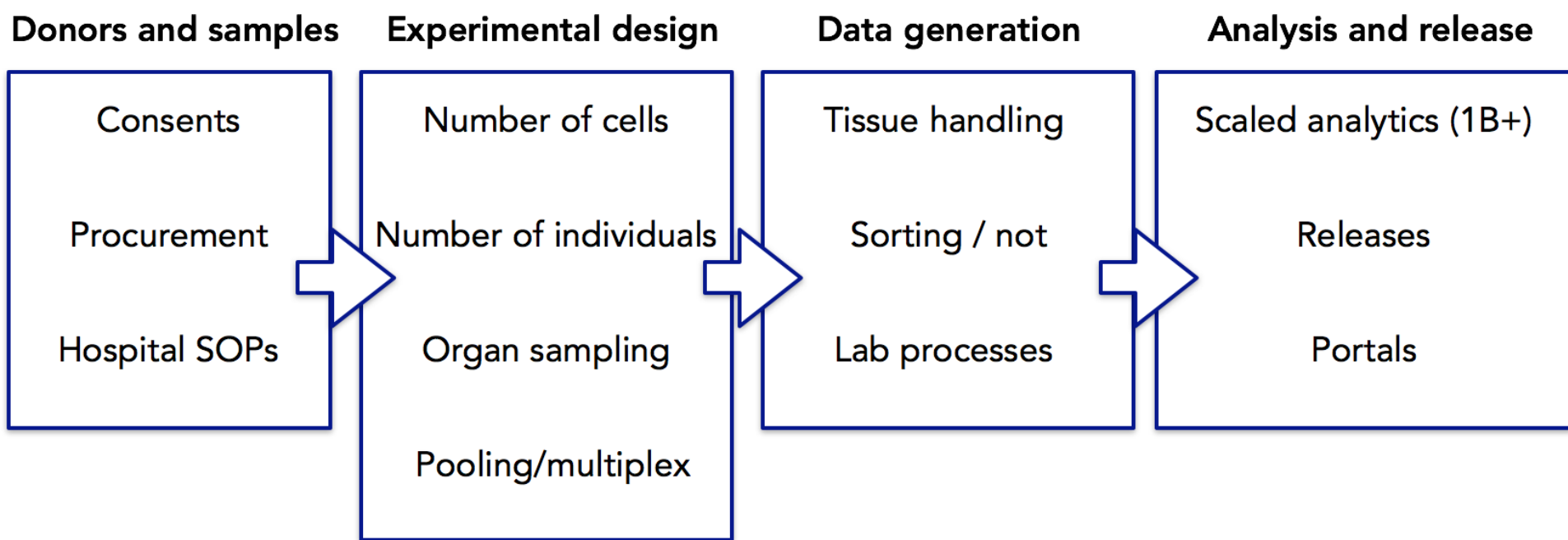
Objectives of the human immune cell atlas

- Define & benchmark experimental procedures and computational algorithms to empower other atlas efforts
- Identifying unknown rare cells and better define cell state spectrum
 - Mapping all existing cell types at frequency of at least $\sim 0.1\%$
- Developing better tools for Immunology Community:
 - Defining minimal set of discriminatory markers for each populations
 - Establish “healthy” reference set to study disease
- Developing better tools for Clinical Community:
 - Apply new knowledge to revisit clinical tools (e.g. CBC 2.0)

How to build a cell atlas?

→ Developing and benchmarking experimental & analytical frameworks that will empower the Community to undertake translational studies across a wide-range of diseases

Key considerations in cell atlas design



Summary of considerations for human blood profiling

1. No differences across anticoagulants
2. No difference across cell isolation procedures (e.g. Ficoll, lymphoprep, RBC lysis)
3. No difference between sorting and bead enrichment*
(* given specific sorting parameters that will be shared)
4. No major differences between fresh and frozen*, important consideration given biobanking efforts (*excluding PMNs)
5. Enrichment strategy (by FACS or bead) can empower more cost-effective single cell analyses of rarer cell population

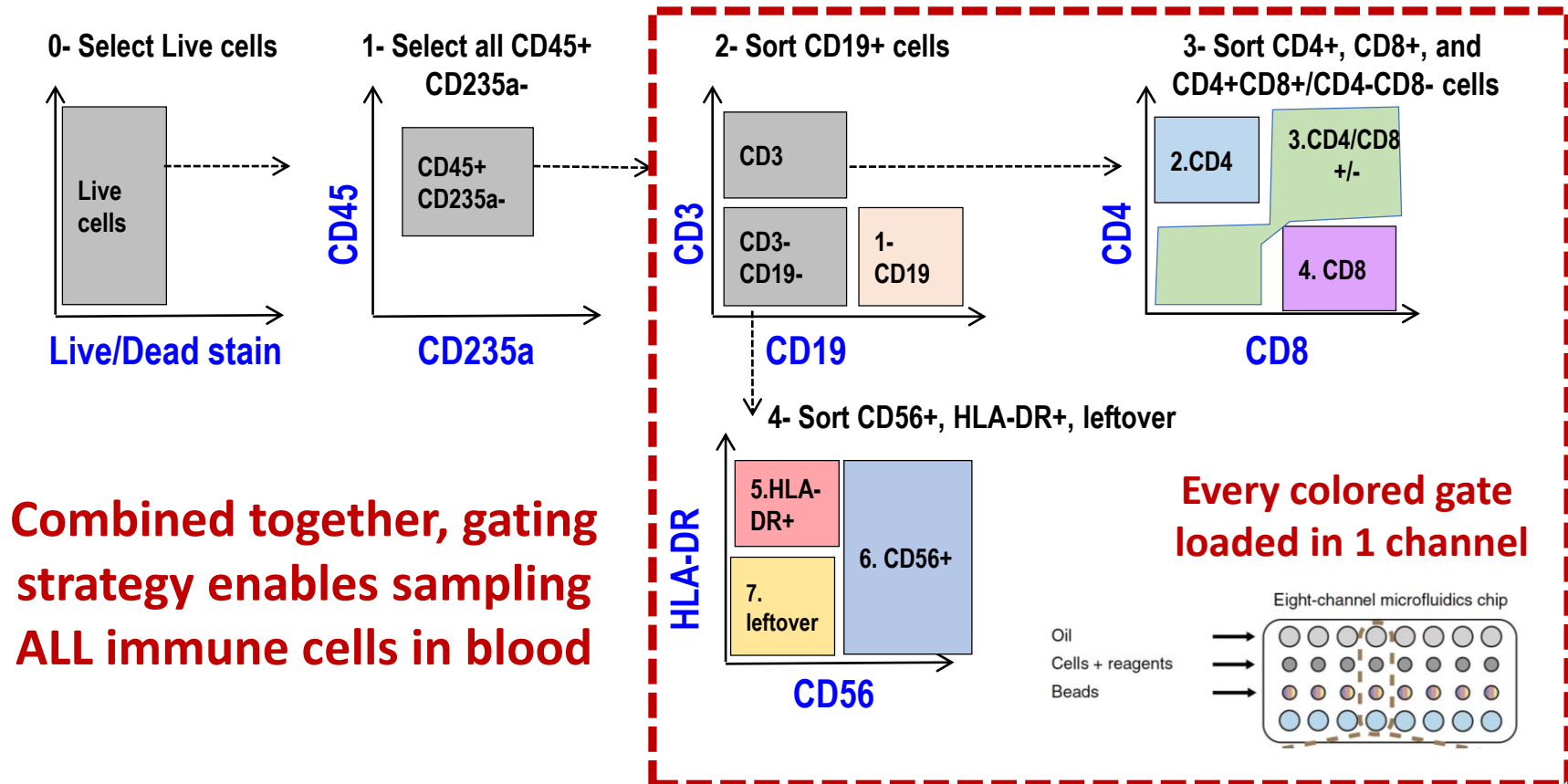
<https://www.protocols.io/groups/hca>

Sampling strategy of human blood

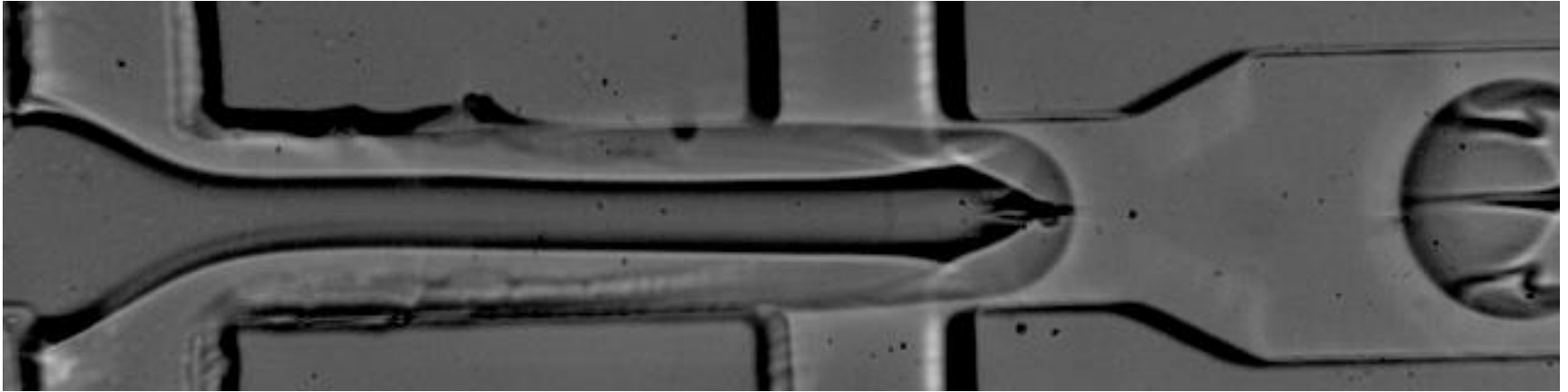
A- For 8 donors: loaded 8 channels of 10X of frozen PBMCs (~32K/indiv.)

B- For 9 donors:

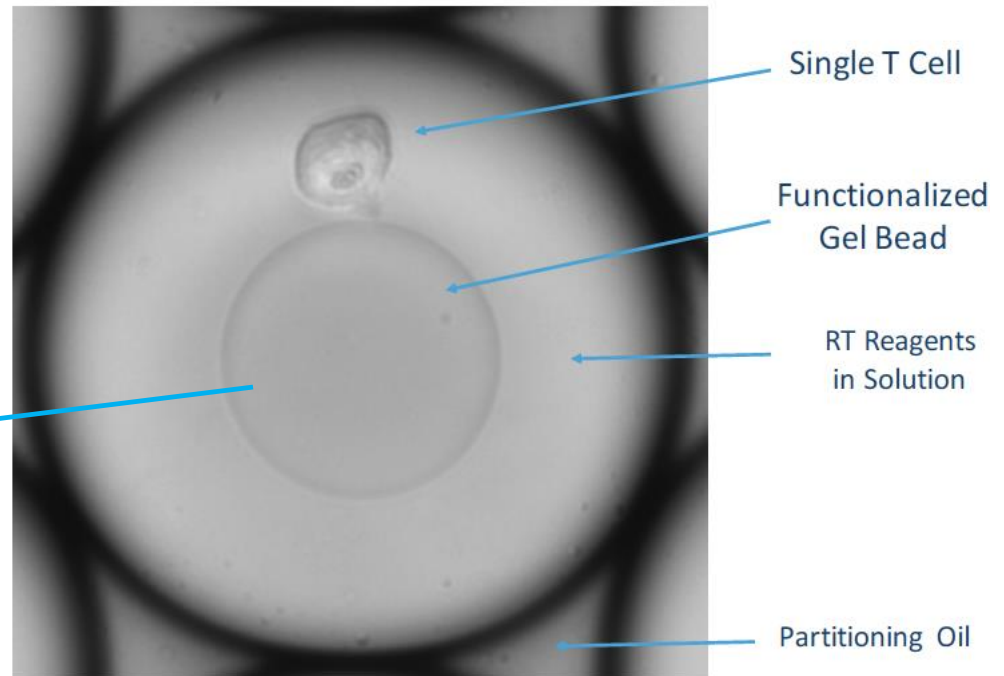
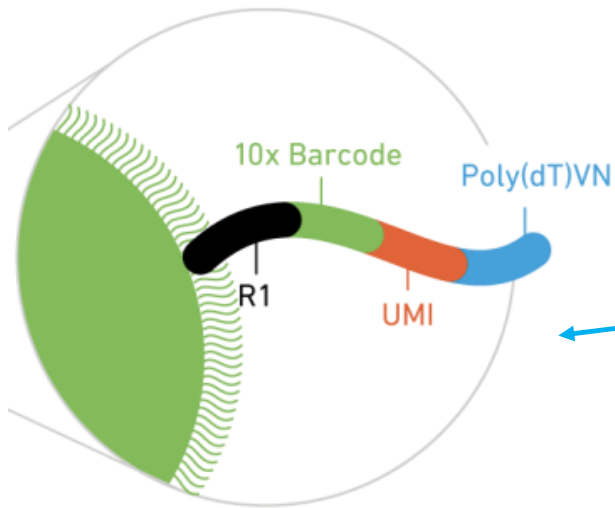
1. Whole blood (only depleting RBCs): 1 channel (~4K cells)
2. PBMCs (depleting RBCs + depleting granulocytes): 1 channel (~4K cells)
3. 8-bucket approach to enrich for rarer cells: 8 channels



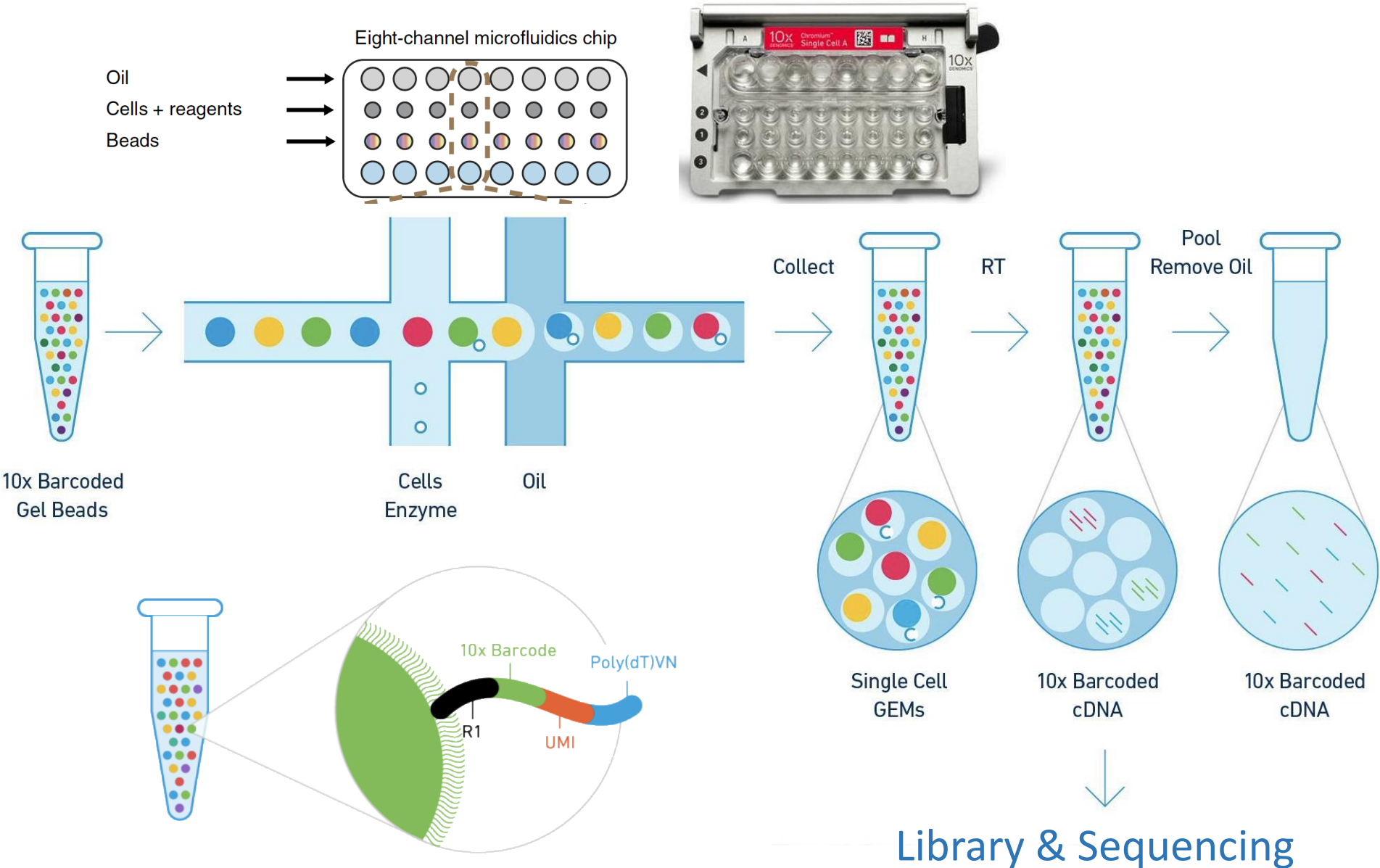
Droplet-based approach (10X Genomics) overview



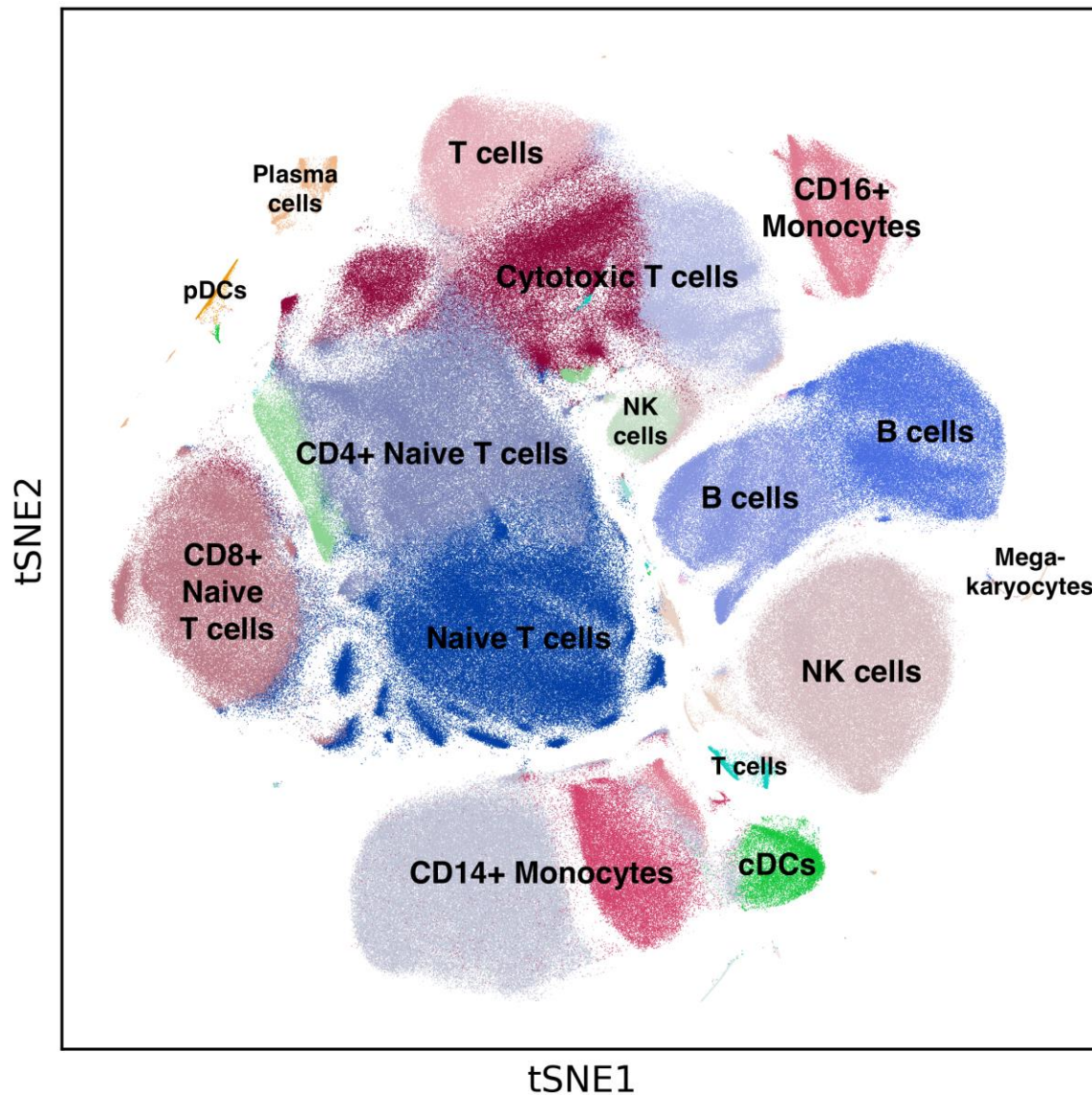
Functionalized Gel Bead



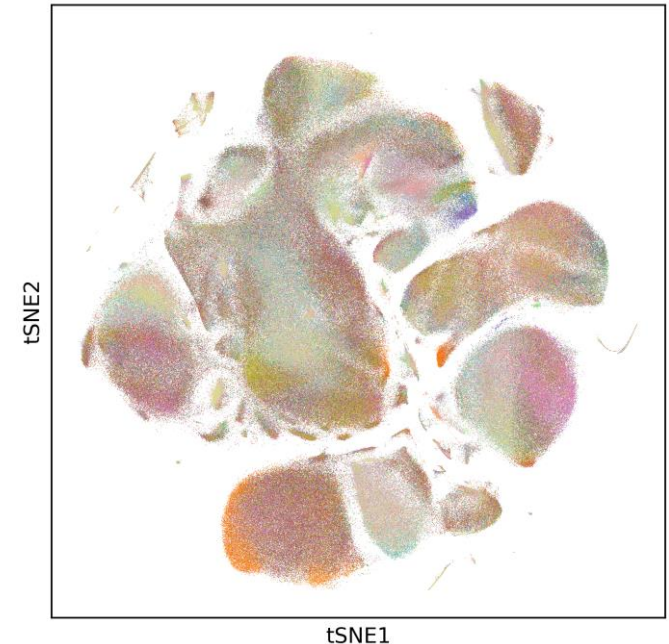
Emulsion-based 10X genomics Approaches



906,536 single cells from 17 individuals



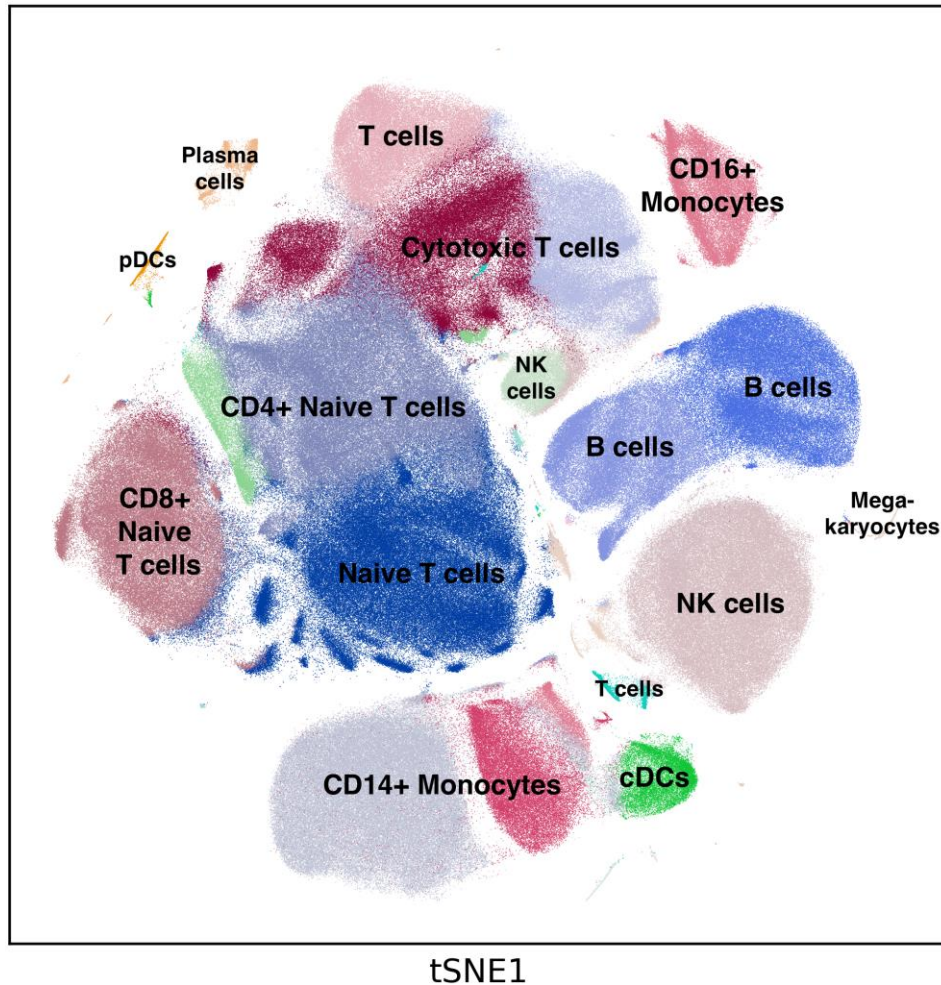
Colored by individual donor



AMI = 0.0582

0 **AMI** 1
Mix Separated

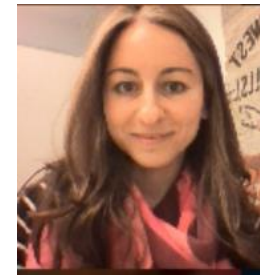
Ongoing analyses



- Sub-clustering analyses
- Defining markers
- Defining cell spectrum



Bo
Li



Sisi
Sarkizova



Orr
Ashenberg

Human Cell Atlas Preview Datasets

The first single-cell sequencing datasets from the Human Cell Atlas are now available to the research community.

Census of Immune Cells¹

Profiling of immunocytes by single cell RNA-seq for understanding human health and disease.

Species	Homo sapiens
Organ	Umbilical cord blood and bone marrow
Method	10x
Cell count	~530,000 cells
File size	1.3 TB

DOWNLOAD DATASET
with script

Additional instructions below

[Download metadata only](#)

Ischaemic Sensitivity of Human Tissue²

Assessment of ischaemic sensitivity of human spleen tissue by single cell RNA-seq.

Species	Homo sapiens
Organ	Spleen
Method	10x
Cell count	~2,000 cells
File size	14 GB

DOWNLOAD DATASET
with script

Additional instructions below

[Download metadata only](#)

Melanoma Infiltration of Stromal and Immune Cells³

Single cell RNA-seq of CD45+ and CD45- cells isolated from tumour and lymph nodes of a mouse model of melanoma.

Species	Mus musculus
Organ	Lymph node
Method	Smart-seq2
Cell count	6,639 cells
File size	380 GB

DOWNLOAD DATASET
with script

Additional instructions below

[Download metadata only](#)

These include primary datasets (fastq and metadata). Additional analysis (alignment and quantification) produced from a standardized analysis pipeline will be made available this summer.

open access, pre-publication; 1st pre-release: 4/4/18

Empowering downstream analyses:

- Query genes
- Cell type
- Trajectory

<https://preview.data.humancellatlas.org>

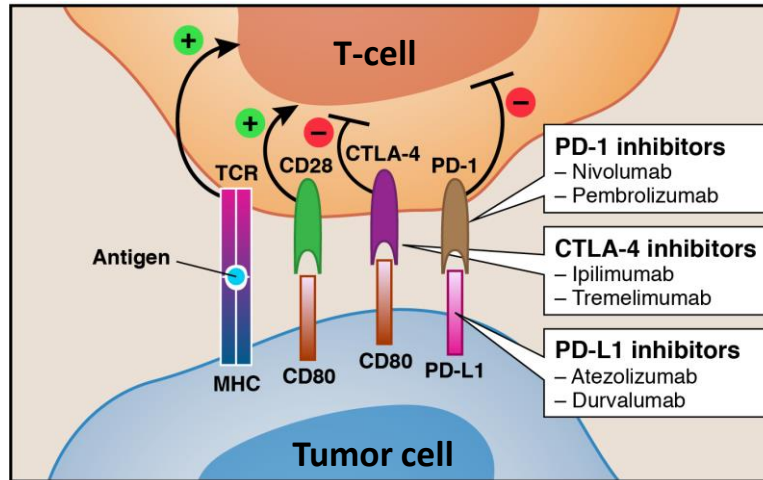
Vignette #3: Bench-to-bedside translation effort example
Improving ICI efficacy through better management of irAEs

**Translational question: Can we map comprehensively
the underpinnings of ICI-related irAEs**

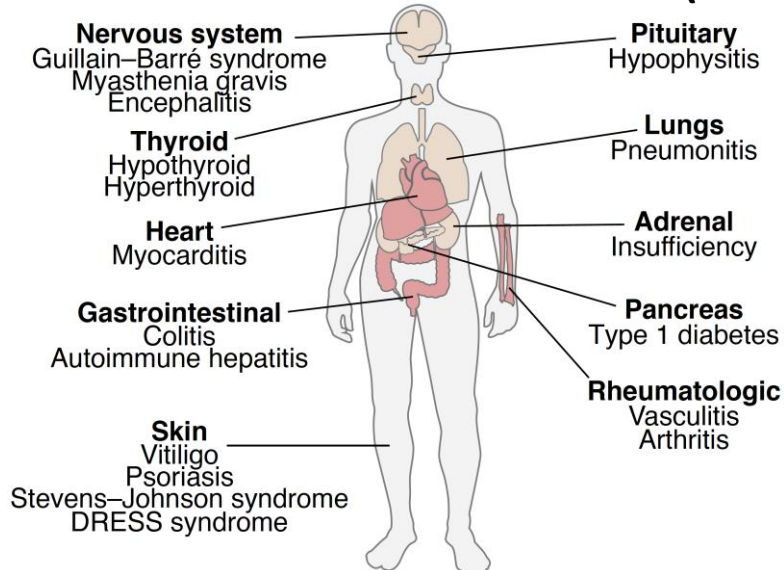


Lifesaving potential ICI therapy is severely limited by irAEs

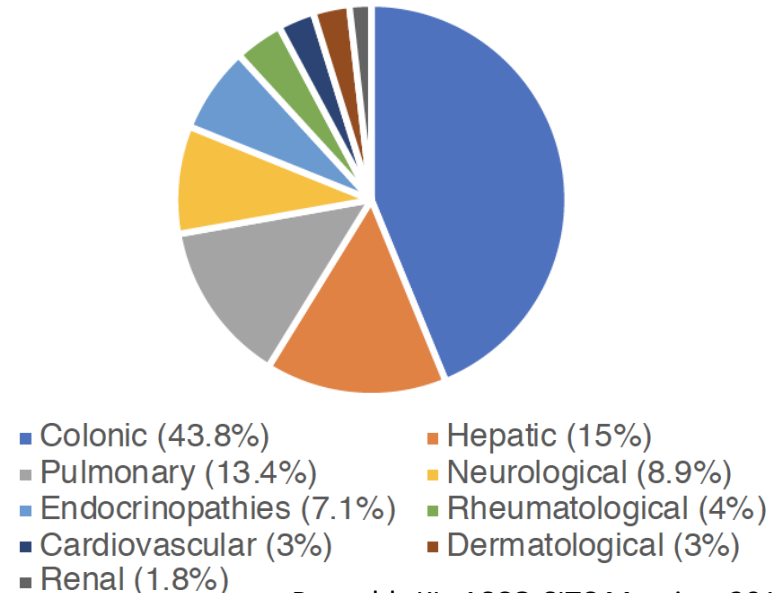
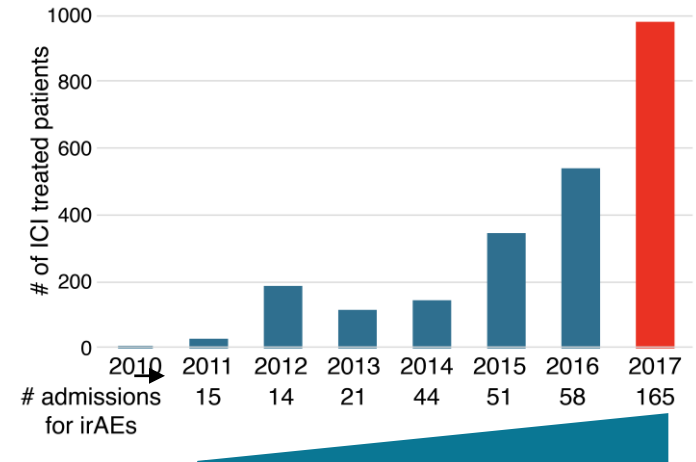
Background: Immune checkpoint inhibitor therapy and associated complications



immune related adverse events (irAEs)



MGH experience: # of ICI treated patients and distribution of irAEs,



Reynolds KL, ASCO-SITC Meeting, 2018

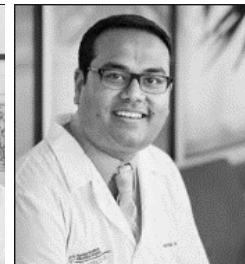
MGH Vision: Becoming Center of Excellence in this Novel Arena

1. Develop expertise in the clinical recognition of these atypical presentations and the management of toxicity
2. Coordinate oncology and interdisciplinary care
3. Develop multi-disciplinary and cross-cutting translational research program

Severe Immunotherapy Complications (SIC) Service

Attendings:

- Kerry Reynolds
- Ryan Sullivan
- Don Lawrence
- Justine Cohen
- Aparna Parikh
- Dejan Juric
- Aditya Bardia
- Jong Park
- Meghan Mooradian
- Justin Gainor
- Howard Kaufman
- Xin Gao
- Oladapu Yeku





**Represents Clinical
Research**

Immunotherapy Toxicity Service



**Translational
Research**





Represents Clinical
Research



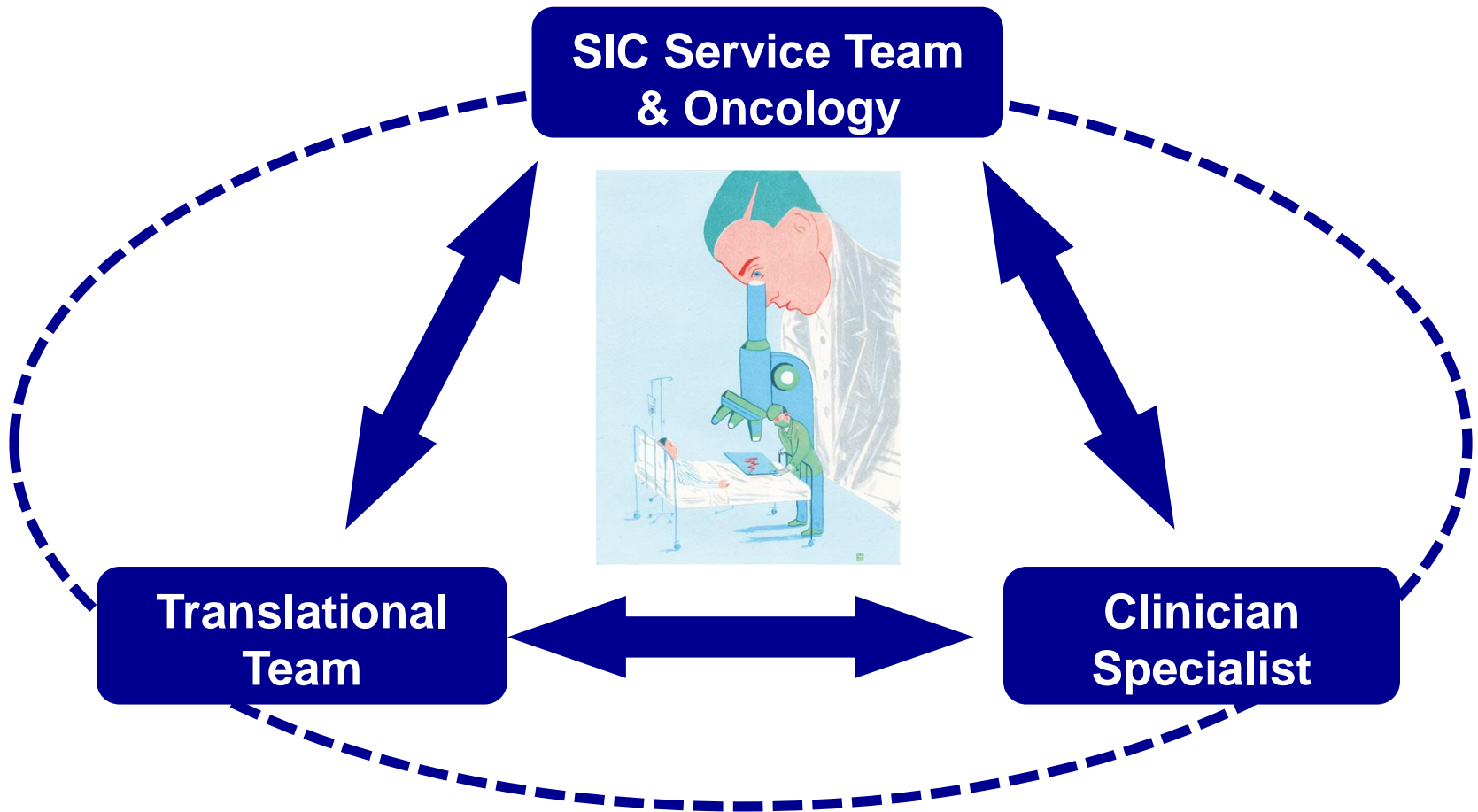
Translational
Research

Immunotherapy Toxicity Service



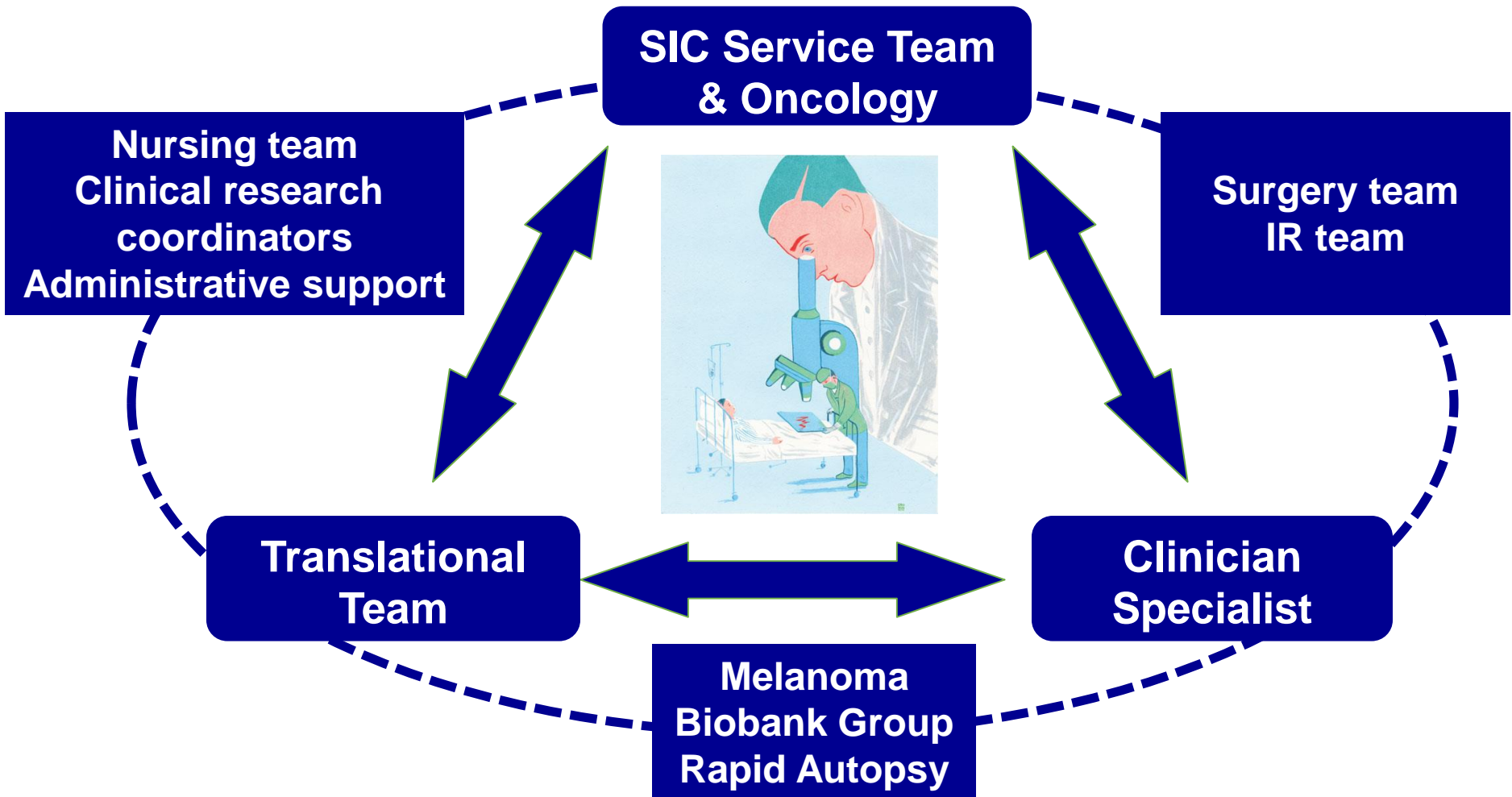
Overview of SIC Service Translational Effort

Leveraging MGH unique multi-disciplinary environment to empower our bedside-bench-bedside SIC translational research program

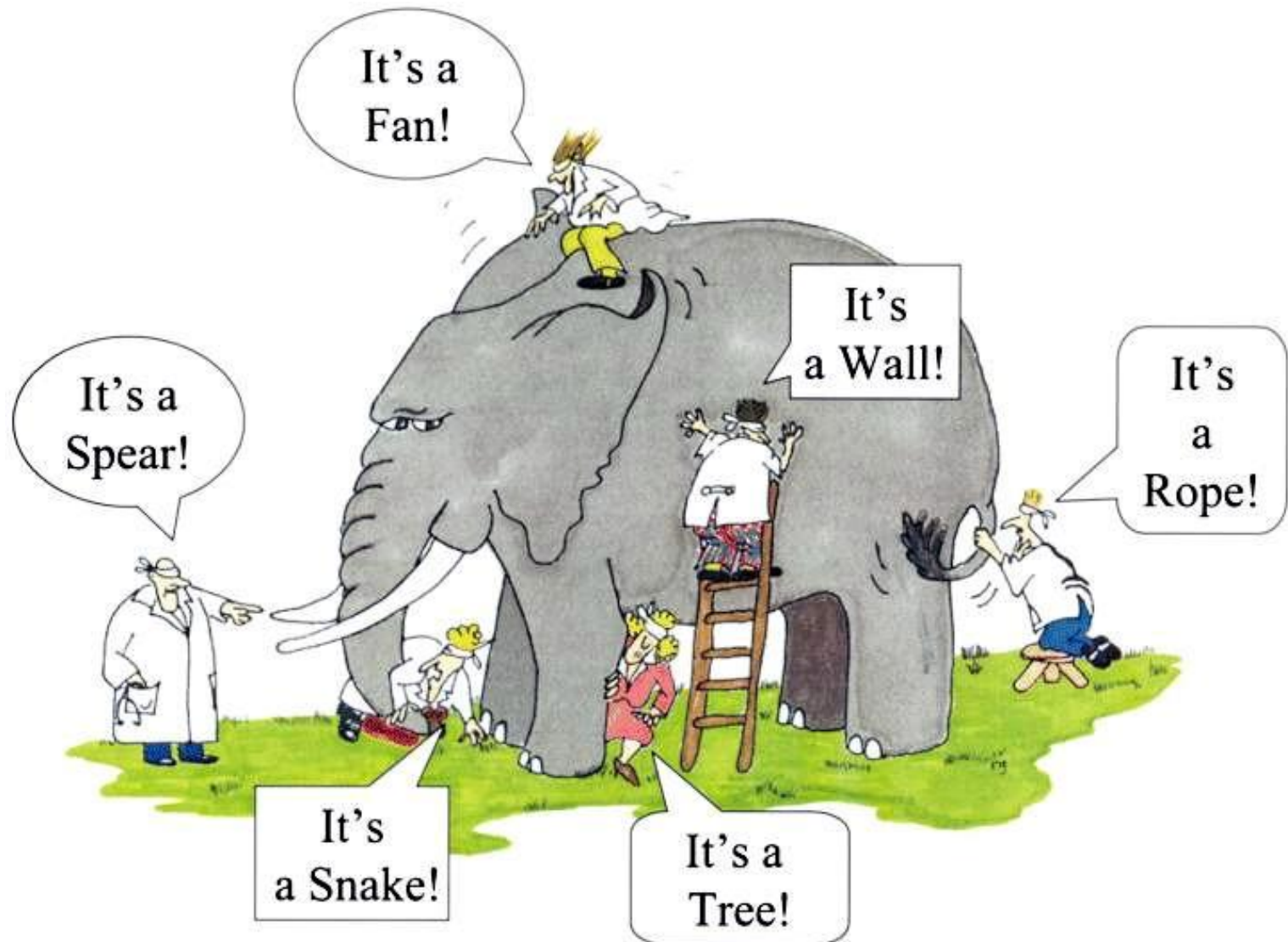


Overview of SIC Service Translational Effort

Leveraging MGH unique multi-disciplinary environment to empower our bedside-bench-bedside SIC translational research program

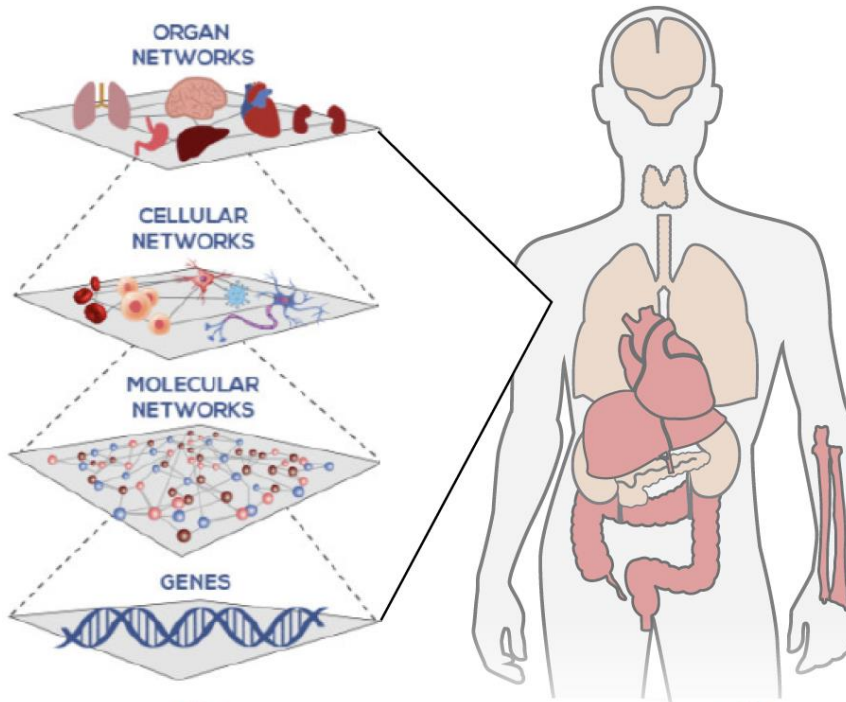


How Can We Generate a “Full Picture”?

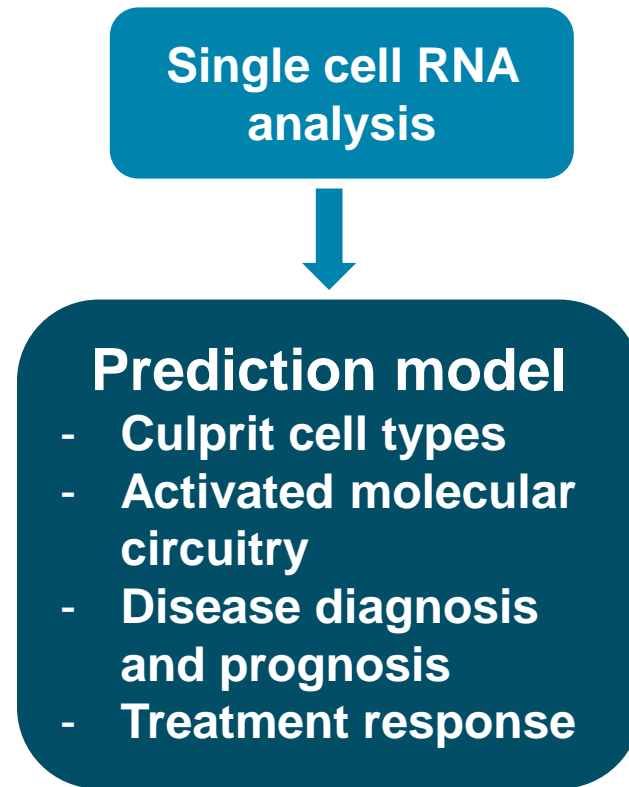


Translational effort: exploration of scale, time, and modalities

Different scales



Developing irAEs Prediction Models



Preliminary single cell data: colitis

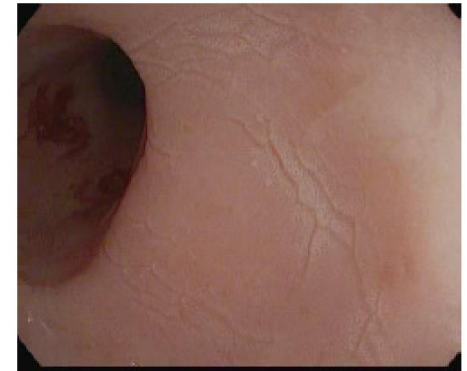
Control Case: Irritable Bowel

- 75 yo with metastatic melanoma who developed grade 1 diarrhea after 3 cycles of nivolumab
- Colonoscopy showed diverticulae and was otherwise normal
- Path: normal colon



Microscopic Colitis

- 61 yo with metastatic melanoma who developed grade 3 diarrhea after 12 cycles of nivolumab
- Colonoscopy showed edematous colon
- Path: Architecturally preserved active colitis with increased intraepithelial lymphocytes and surface epithelial damage



Michael Dougan

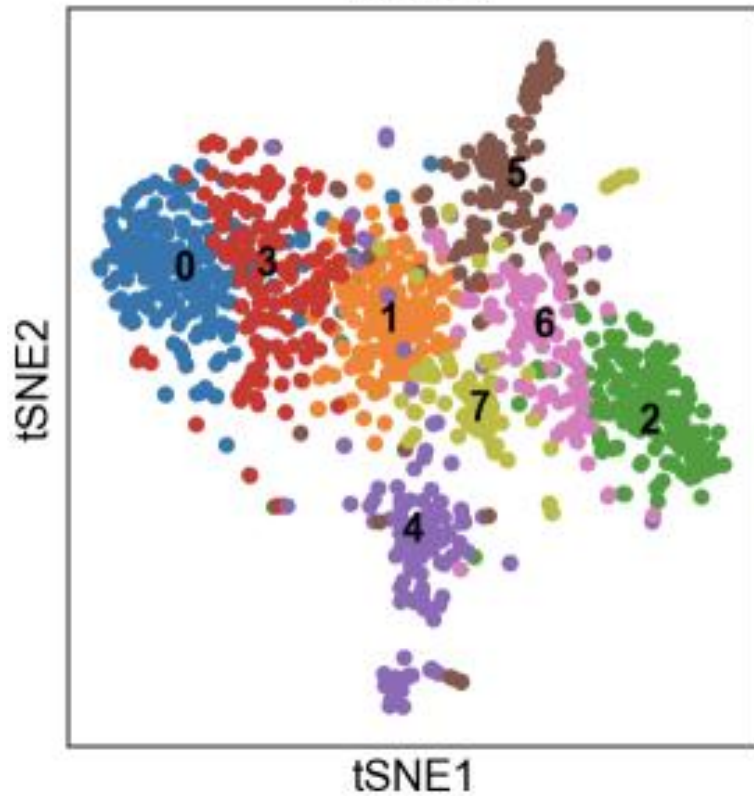


Molly Thomas

Preliminary single cell data: colitis

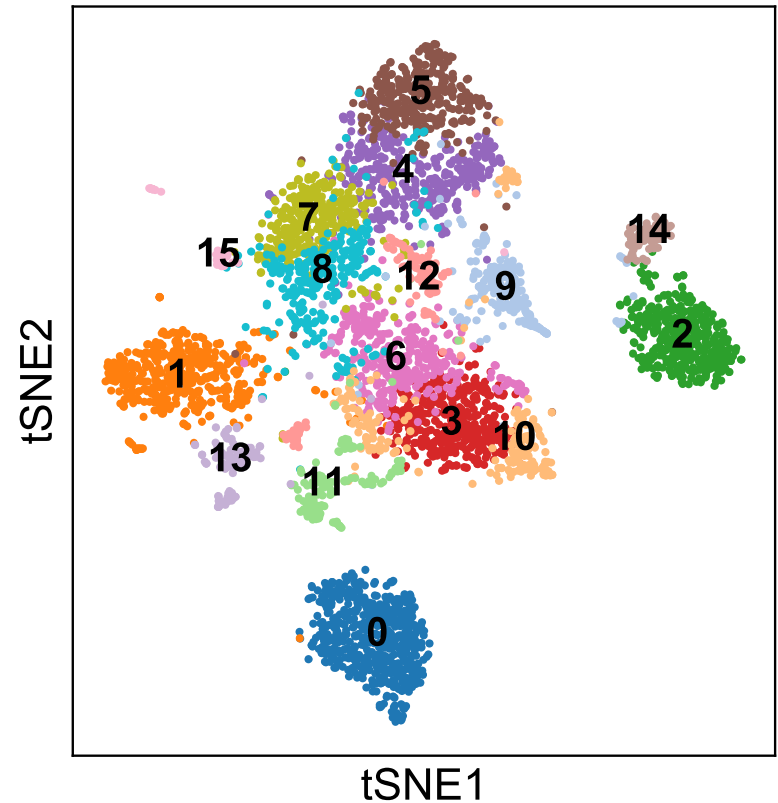
Control Case: Irritable
Bowel

louvain



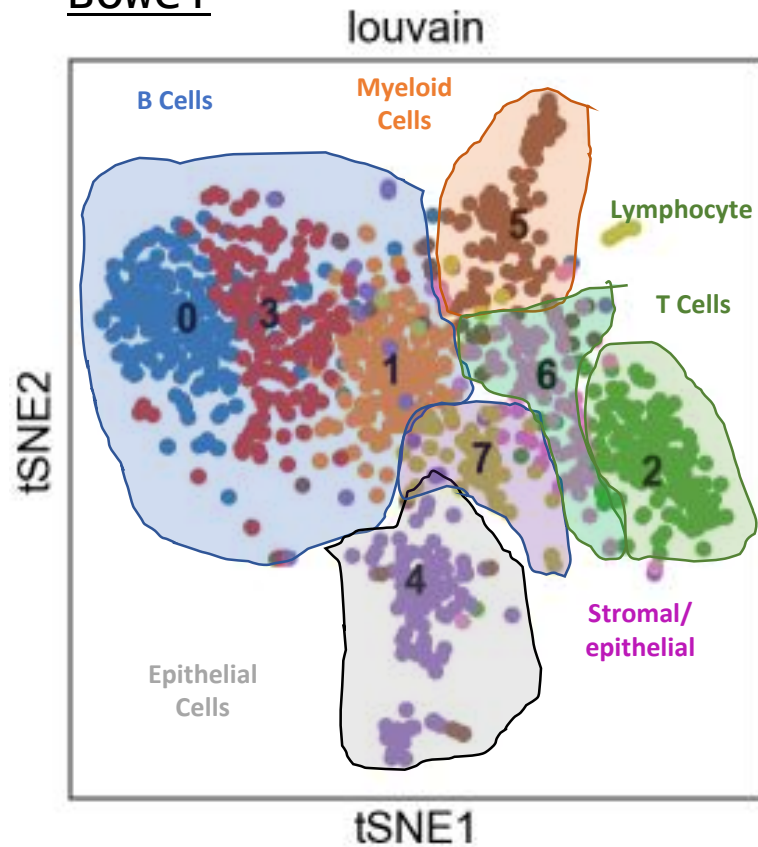
Microscopic

louvain



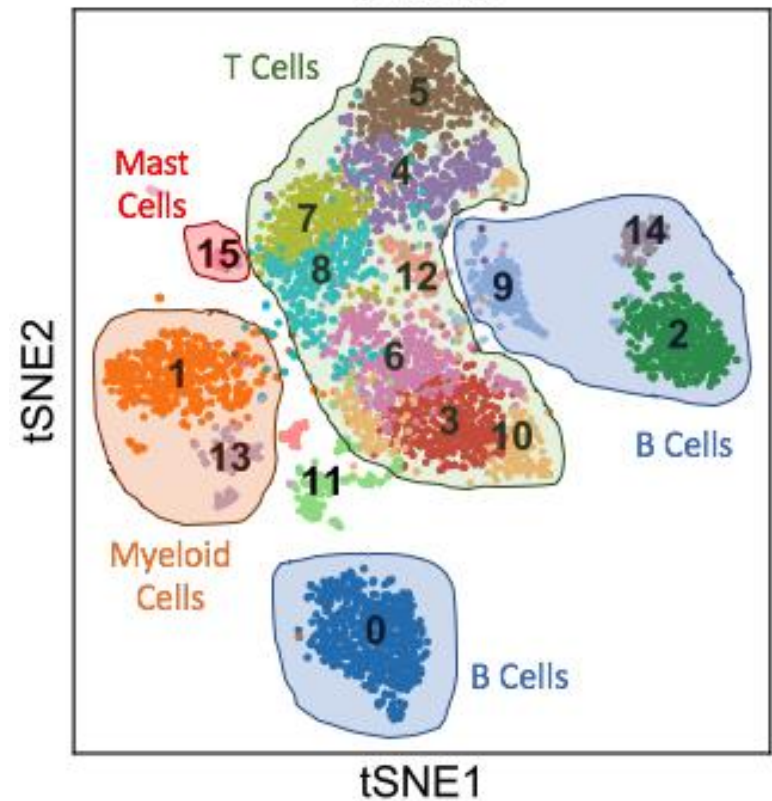
Preliminary single cell data: colitis

Control Case: Irritable Bowel



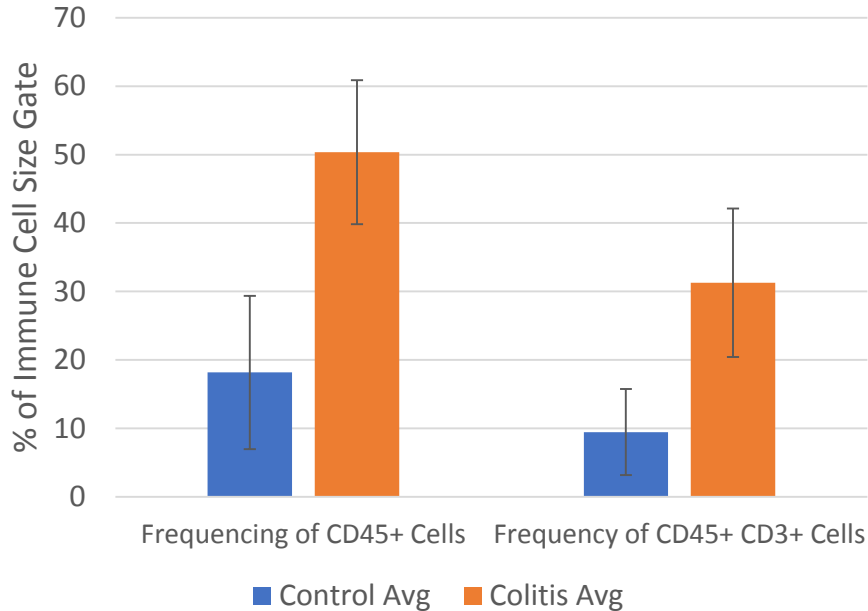
Microscopic Colitis

louvain

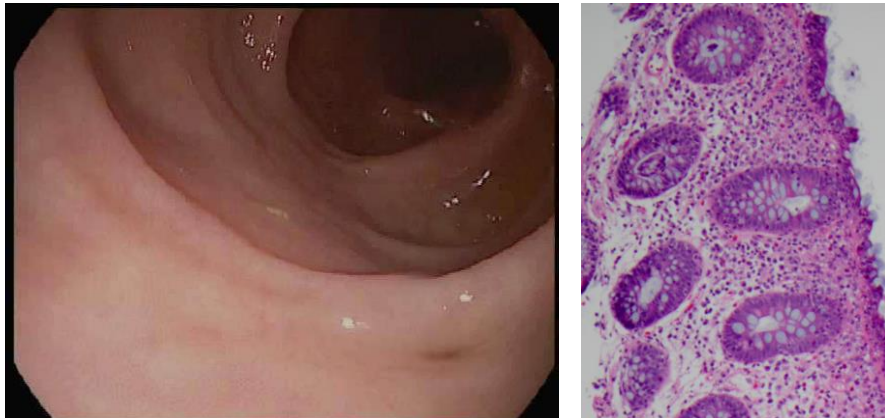


More comprehensive picture we added dimensionalities

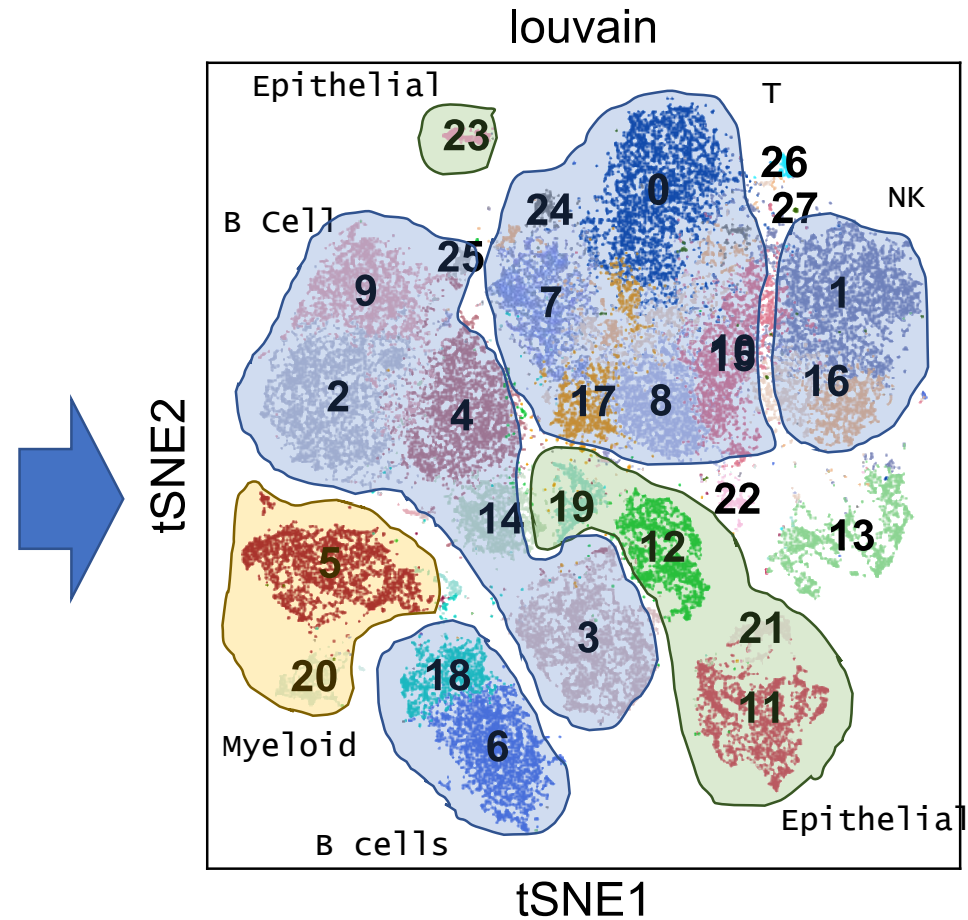
Proportion of CD45+ cells infiltrate in colon tissues



Histological features of microscopic colitis

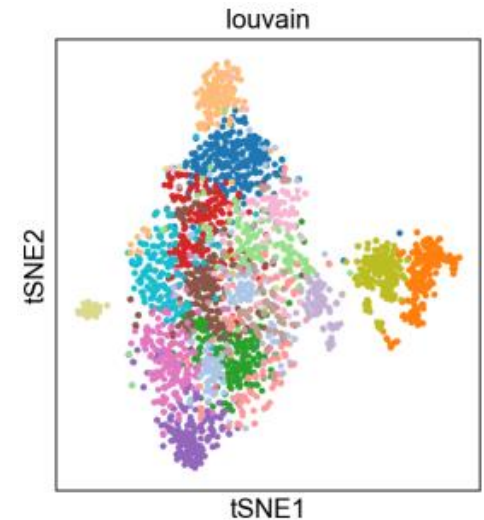
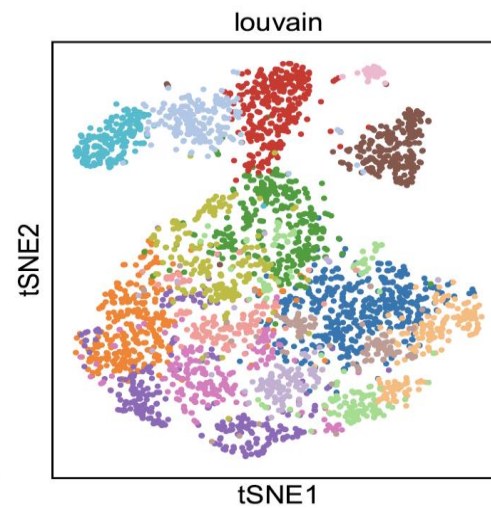
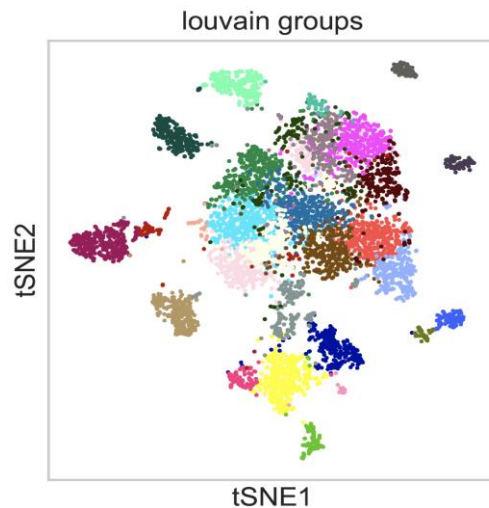


High-resolution cellular ecosystem of irAE colitis



Cellular composition of irAE arthritis synovial fluid is more complex than clinical differential

Clinical Readout	Patient 1	Patient 2	Patient 3
Total cell counts (cells/ul)	26	14940	11006
Neutrophil	2%	99%	6%
Lymphocyte	33%	1%	48%
Monocyte	17%	0%	6%
Eosinophil	1%	0%	
Macrophage/Lining Cell	47%	0%	40%



Minna Kohler

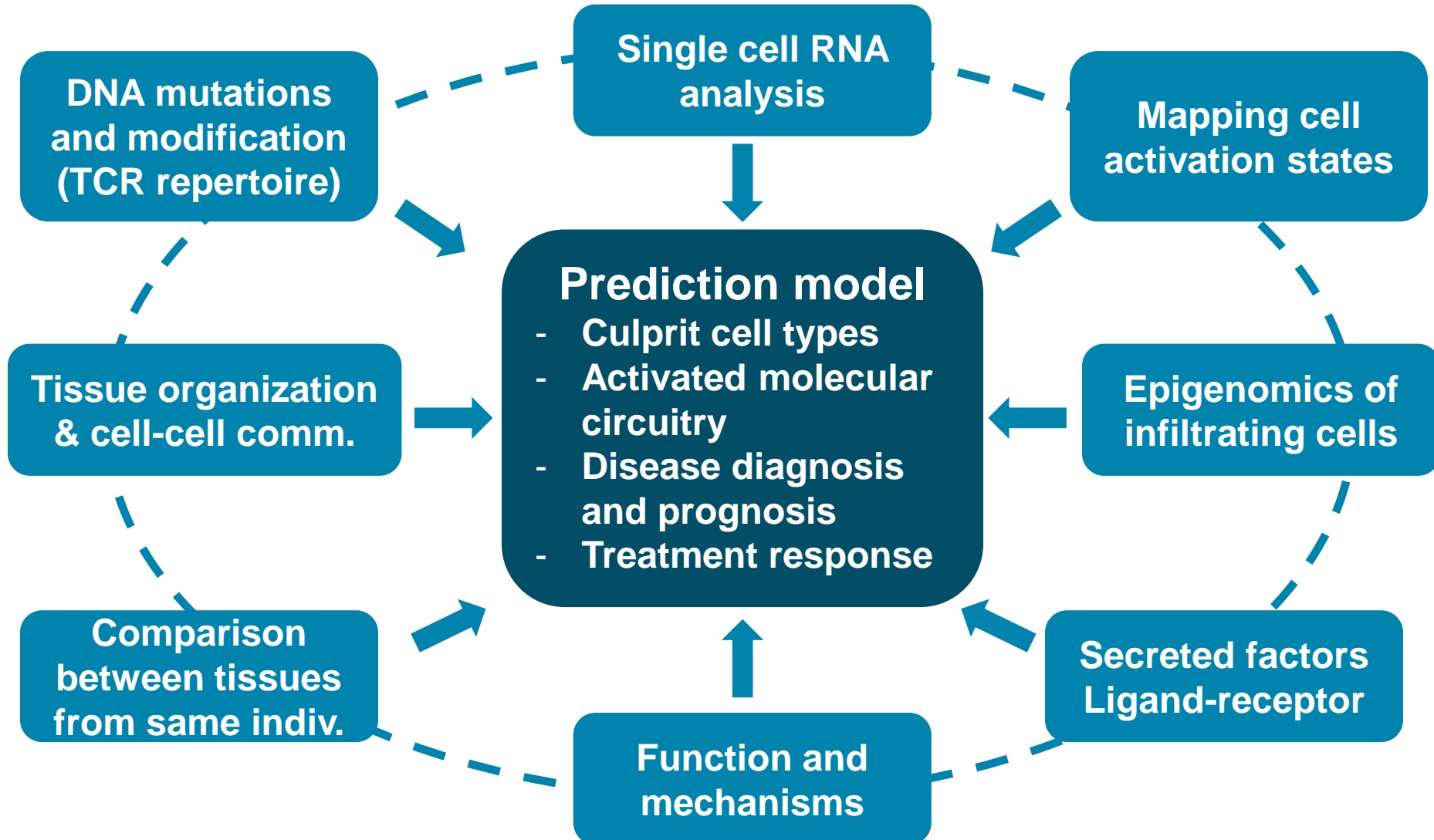


Sara Schoenfeld



Mazen Nasrallah

Developing irAEs Prediction Models

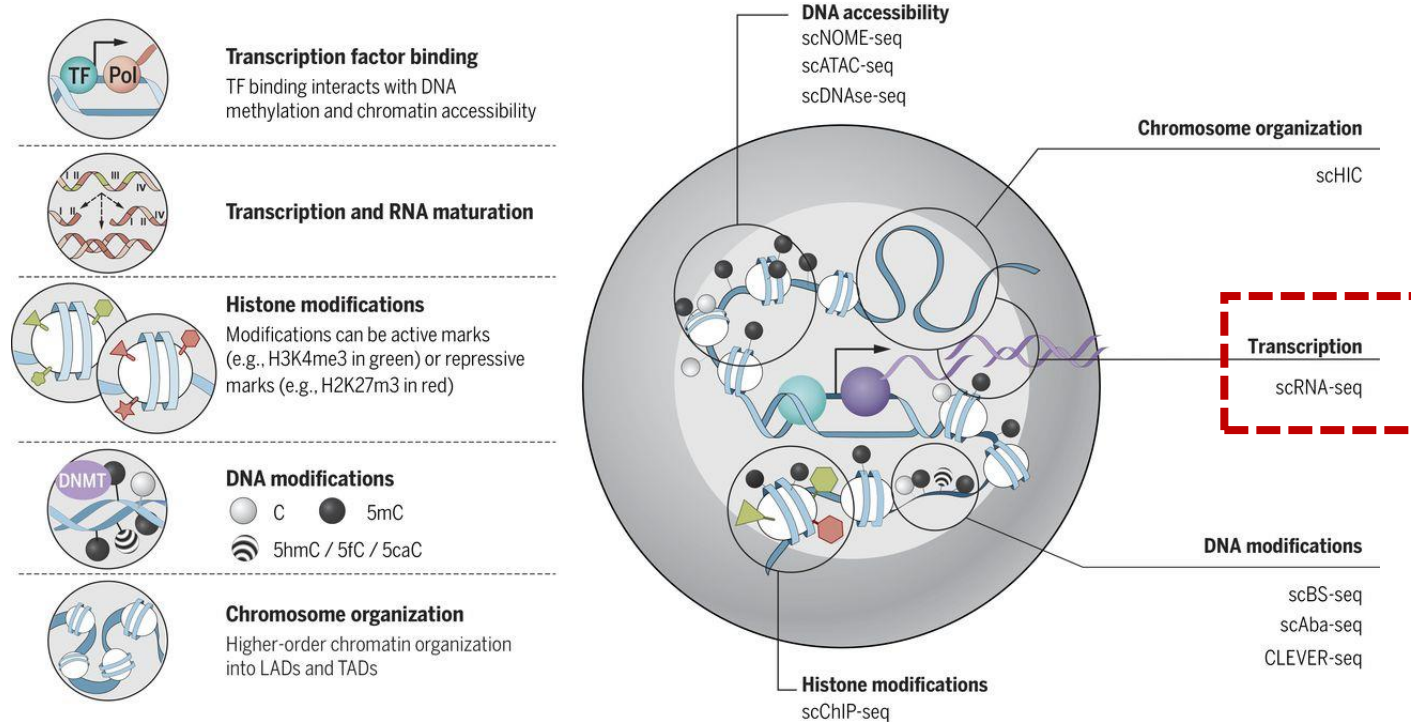


Envisioned outcomes of SIC Translational Research Program

1. Identify set of biomarkers to be implemented in clinic
2. Development of better therapy strategies to treat autoimmune-toxicities while maintaining anti-tumor immunity
3. Identifying novel druggable targets with immunosuppressive potential

Final thoughts ...

The future: integration of many single cell modalities



Spatial ‘Omics’ → integrating readout directly *in situ*

- Multiplex FISH (SeqFISH, MERFISH)
- *In situ* RNA-seq (e.g. FISSEQ)

Multi-omics

- DNA + RNA (G + T)
- RNA + protein (T + P)
- Epigenome + RNA



Tackling Big Data Challenge

- Billions of data points/experiment
- Need to innovate & develop new analytical frameworks!
- Interpretation: empowering bench-to-bedside translation of findings



STAT3 Project



Will Ge
Kasidet Manakongtreecheep
Mazen Nasrallah
Molly Thomas
Amelia Raymond
Oncology Bioinformatics
Translational Science Group

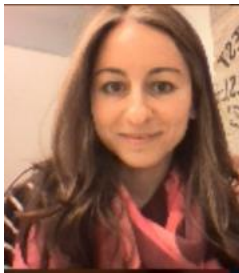
Supported by AstraZeneca

Questions: Patricia.McCoon@astrazeneca.com
avillani@mgh.harvard.edu

Human Immune Cell Atlas – a team effort



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Li**



**Sisi
Sarkizova**



**Orr
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**Michal
Slyper Biton**



**Danielle
Dionne**



**A.-Chloe
Villani**



**Lan
Nguyen**



**Patricia
Rogers**



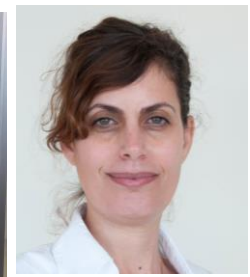
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**Timothy
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**Orit
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Rosen**



Nir Hacohen



Aviv Regev

Supported by
Manton Foundation



KLARMAN
CELL OBSERVATORY
AT BROAD INSTITUTE

Villani Lab & MGH SIC Translational Team



Mazen Nasrallah



Molly Fisher



Leyre Zubiri



Amy Xu



Tariq Daouda



Will Ge



Kas Manakongtreecheep

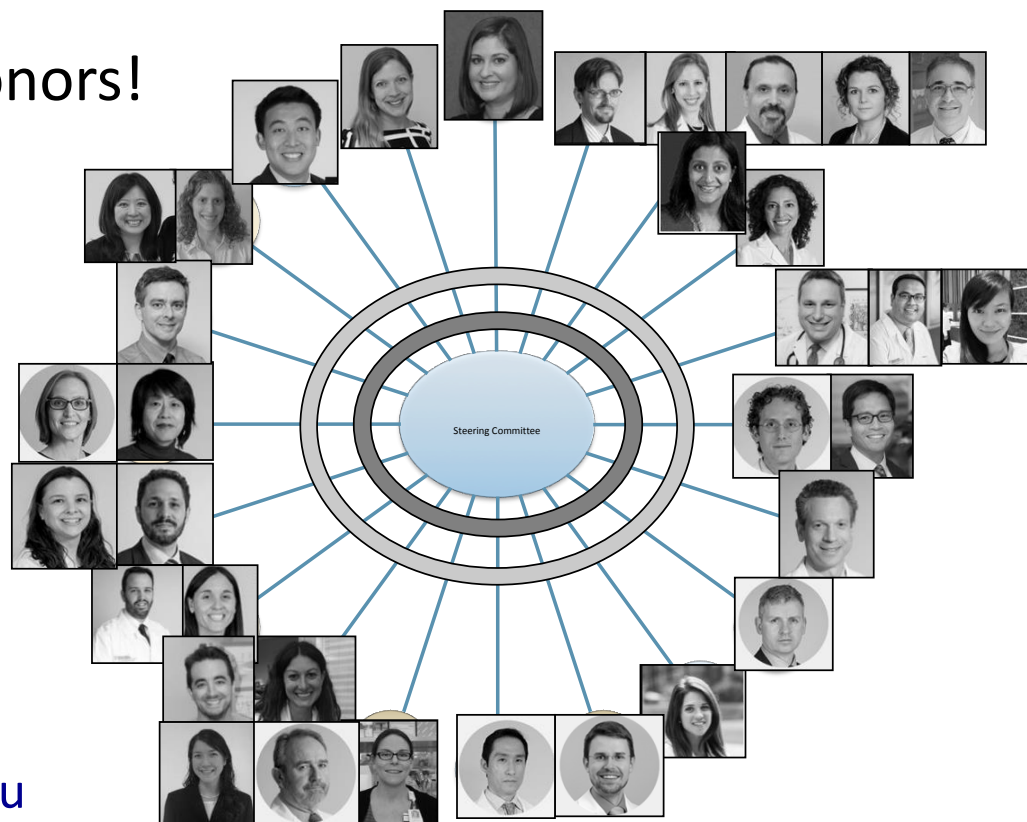


Michael Dougan



Kerry Reynolds

The Donors!



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MGH Transformative Scholar Award
Lawrence Summers Award
MGH Foundation

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