

# New Science: The Future of Liver Atlasing in Health and Disease

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CRC Tier 2 in Liver Immunobiology

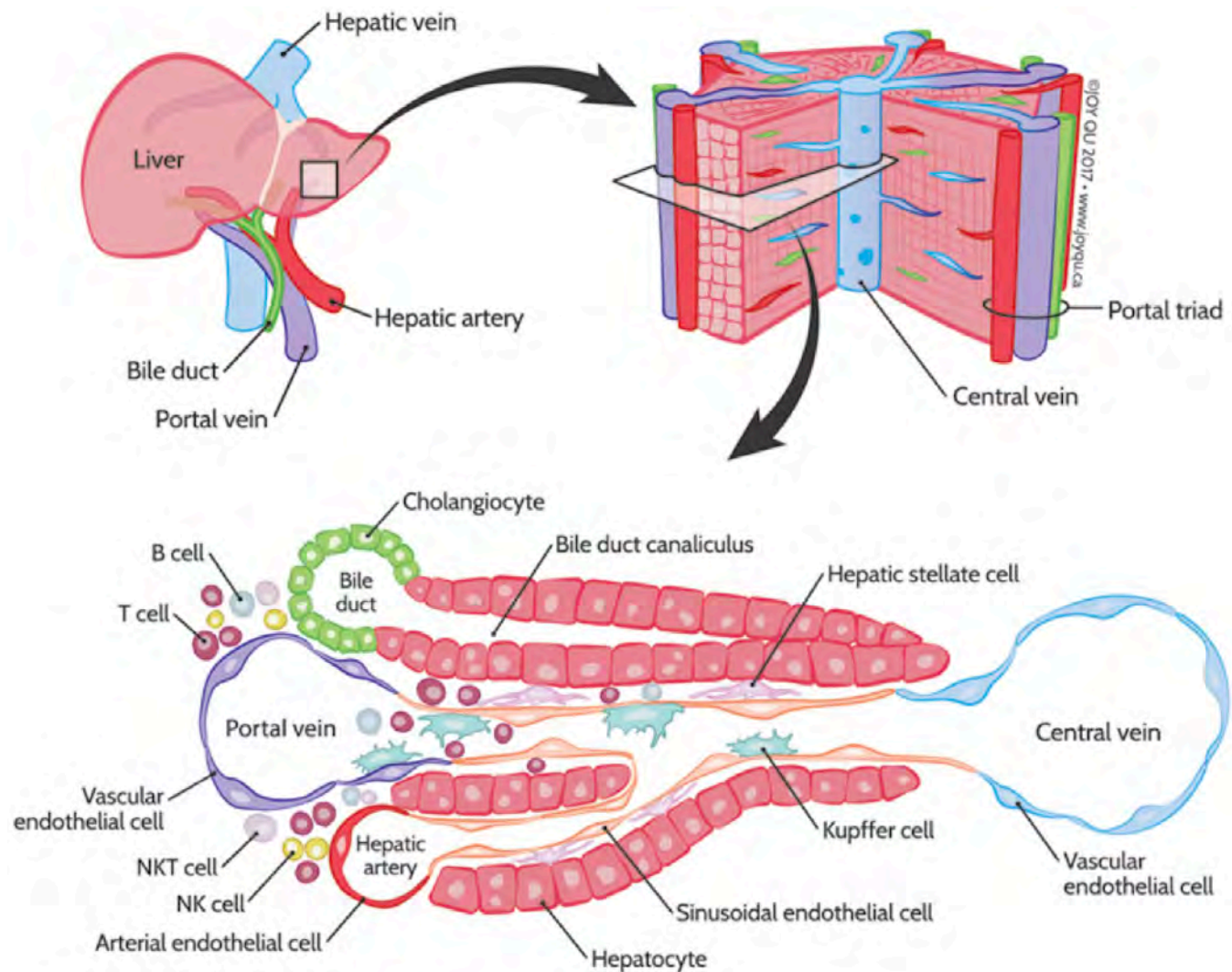
Diana Nakib  
PhD Candidate | University of Toronto  
Ajmera Transplant Centre

PSC Forum  
Nov 8th, 2022

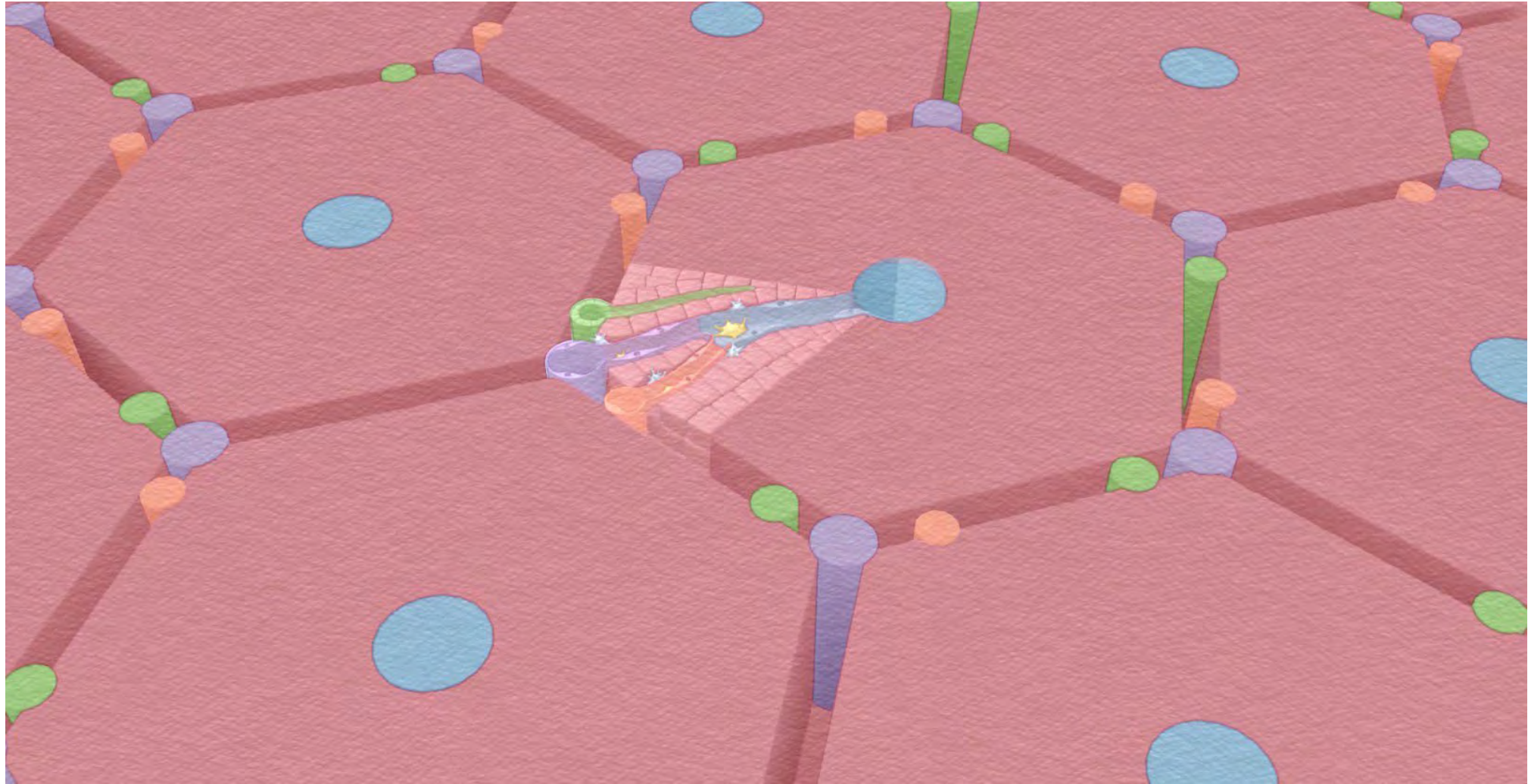
# Disclosures

- None.

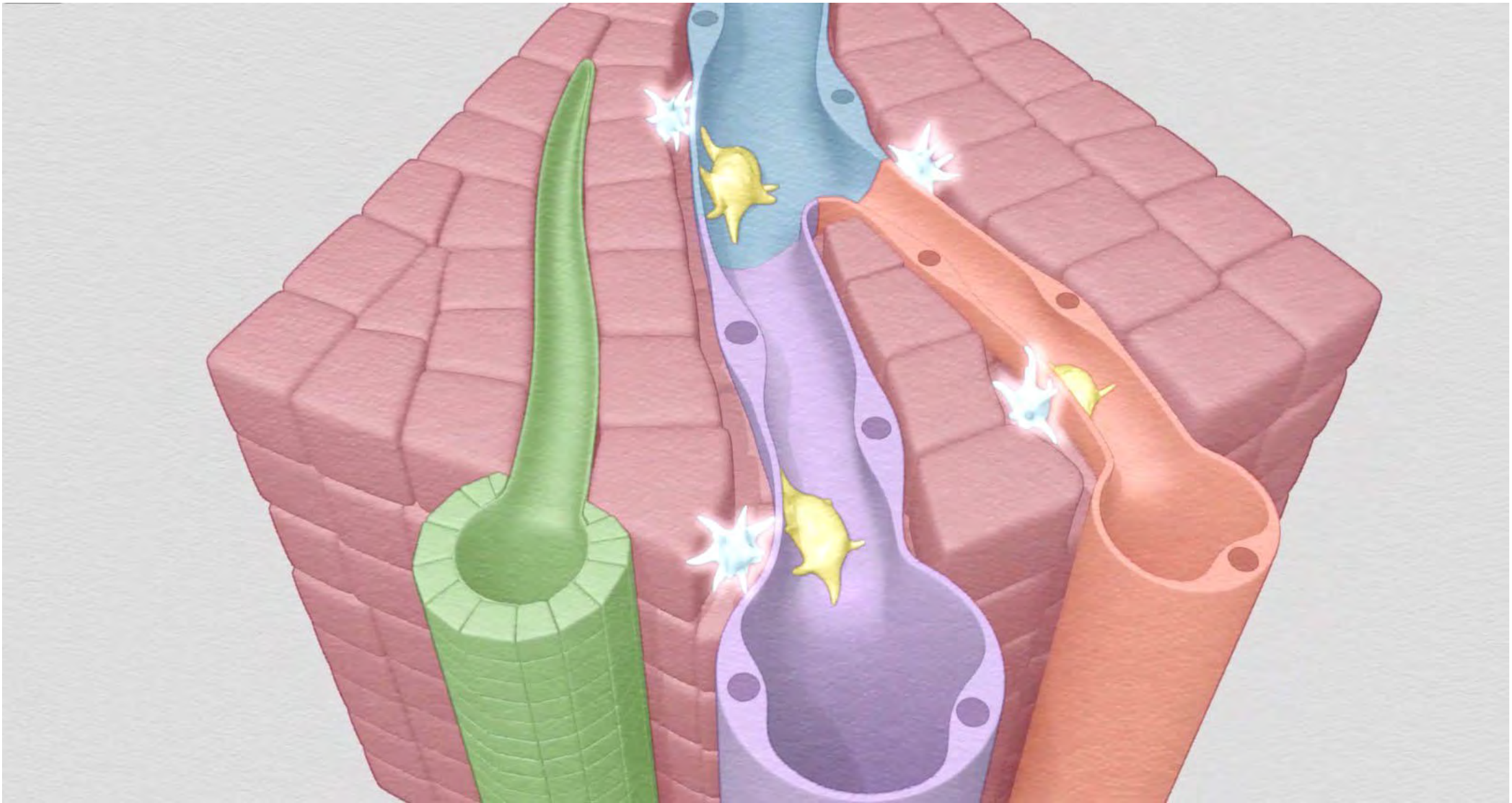
# The Human Liver



- **Metabolic factory**
  - 500 functions
  - e.g. drug breakdown
- **Immune center**
  - 80% of tissue macrophages
- **Regenerative ability**
  - Up to 80% of liver
- **Associated diseases**
  - Non-alcoholic fatty liver disease 1.7B
  - Viral hepatitis 500M



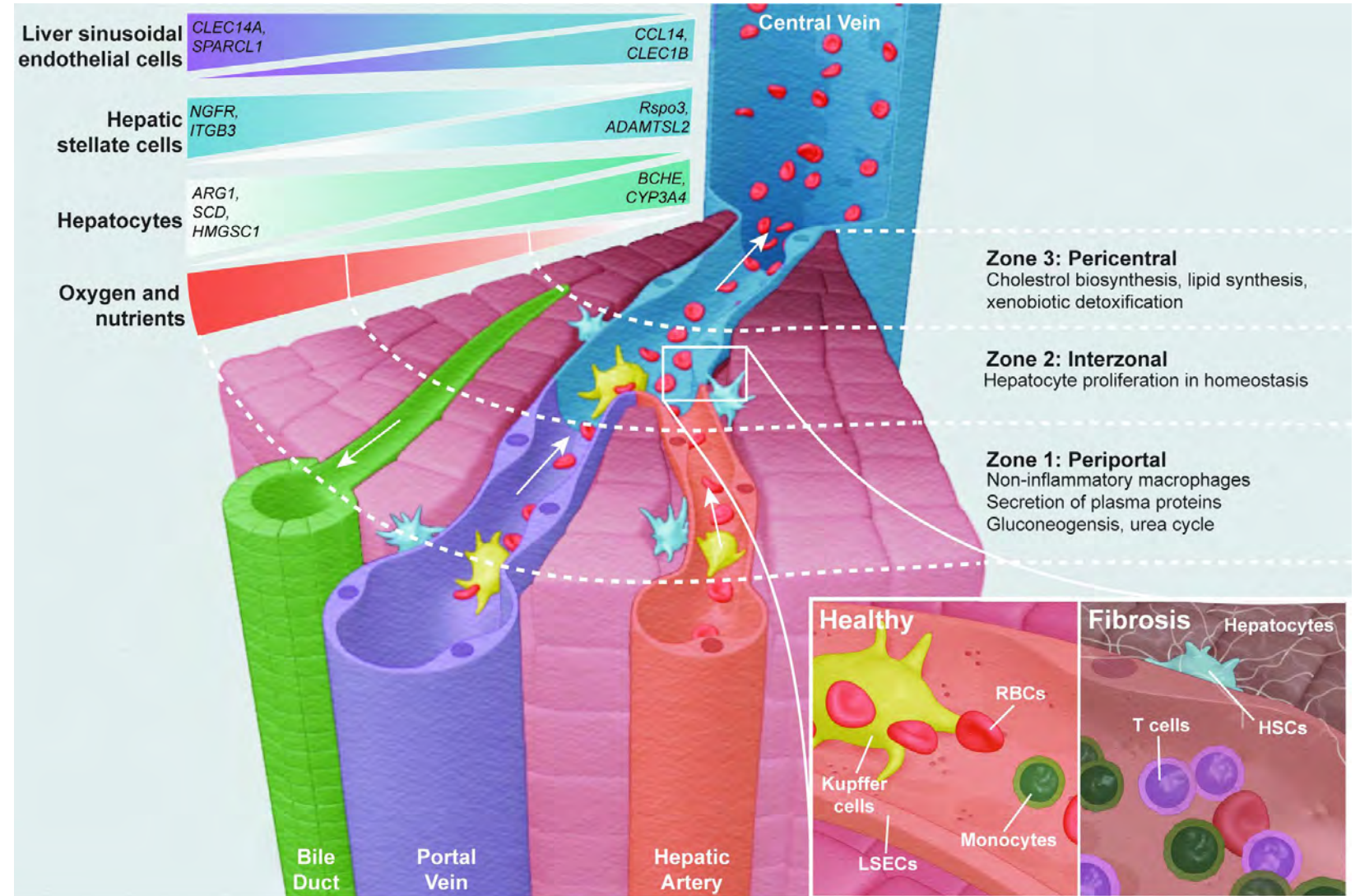
*Illustration by Farah Hamade, Msc Candidate*



*Illustration by Farah Hamade, Msc Candidate*

# Mapping the cellular microenvironment of the human liver.

- Define and target cellular drivers of processes central to liver disease



# Limitations of our current understanding of liver cellular biology..



Distinct populations of cells within the liver

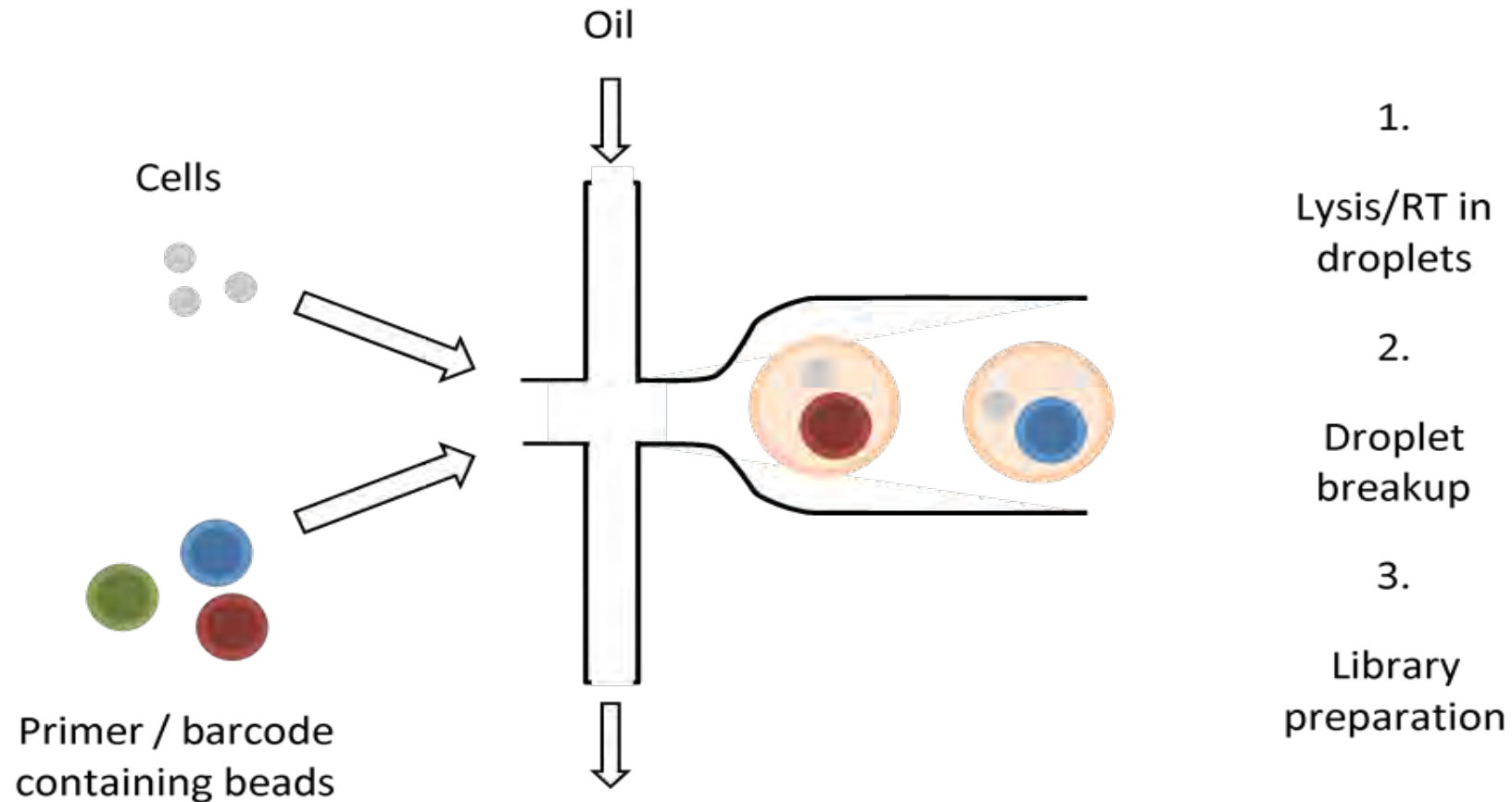
These cells respond to environmental cues based on what is taking place in the liver

Cellular signatures within the liver are associated with responses to antiviral therapy

**However..**

-We really need knowledge of the roles of individual populations in the liver.

# Single cell RNA sequencing



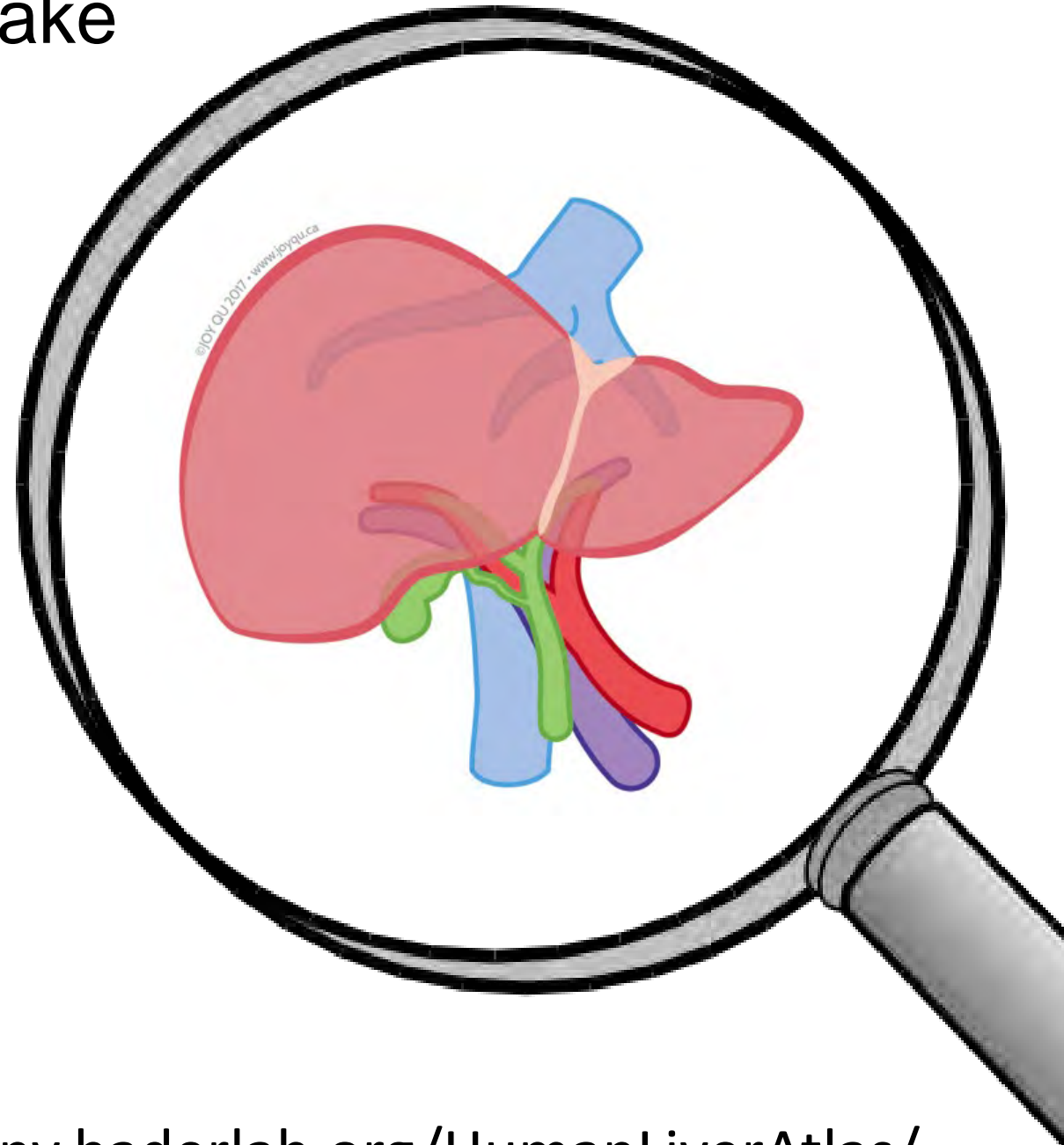
Every cell is associated with a bead- each bead has a cell barcode, then each cDNA fragment also has a barcode that links fragment to original cell



**Question:** What is the cellular make up of the healthy human liver?

## Objectives

- To generate a comprehensive cellular map of the human liver
- Foundation for single cell liver disease studies

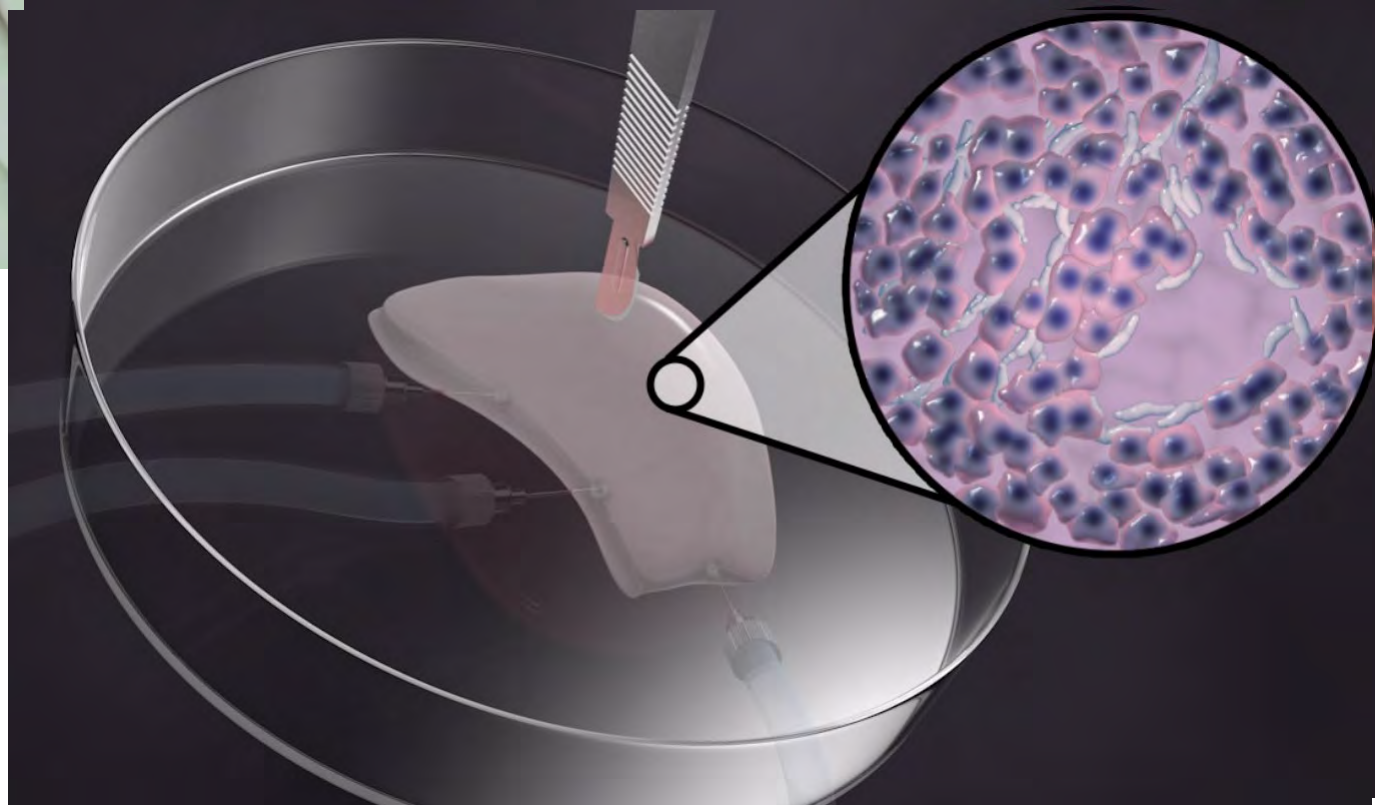




Ian McGilvray

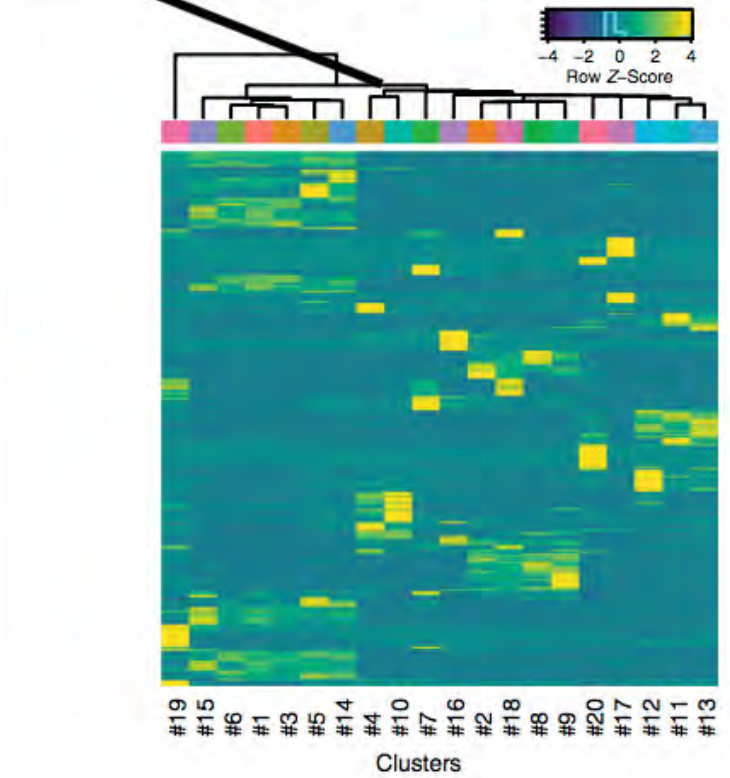
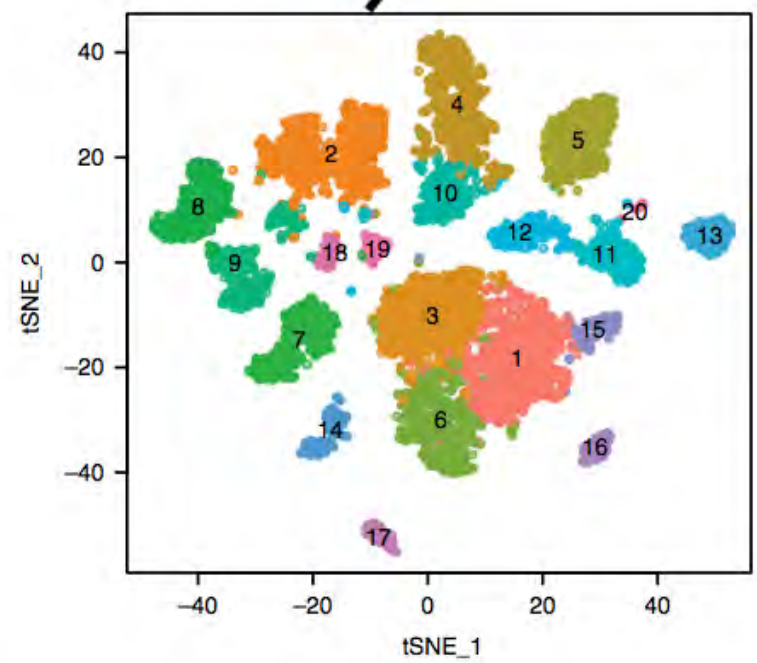
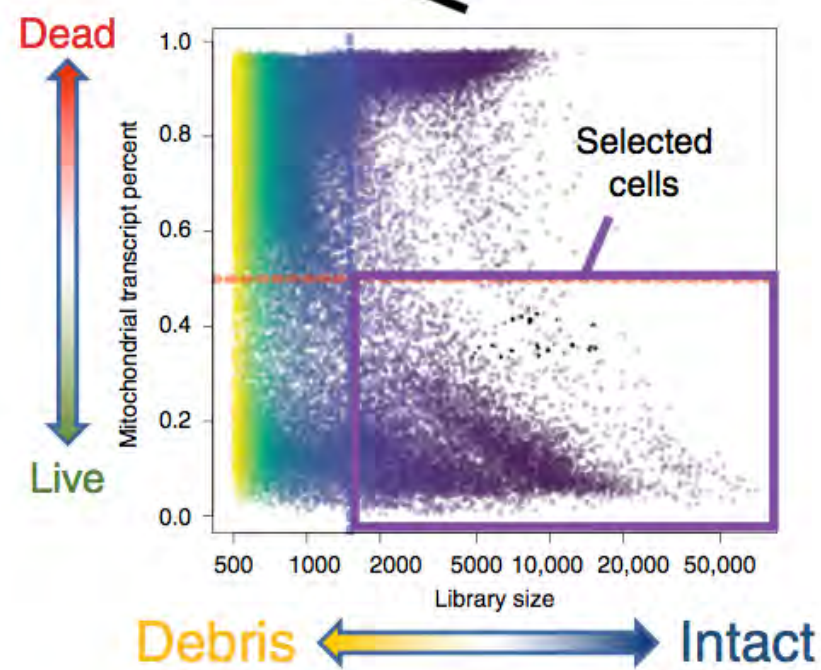
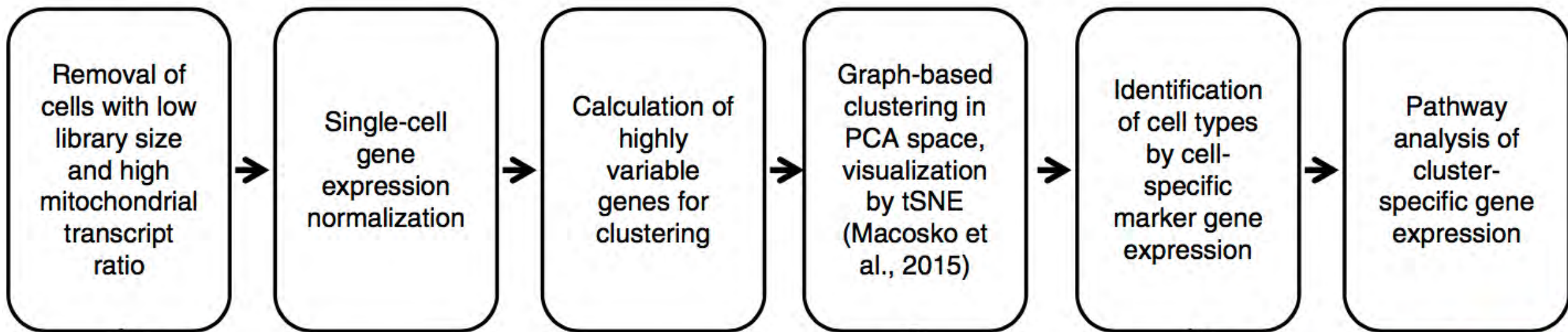
Gary Bader

# Dissociating Liver Tissue



**Gentle dissociation allows efficient capture of hepatocytes!**

# Analysis workflow



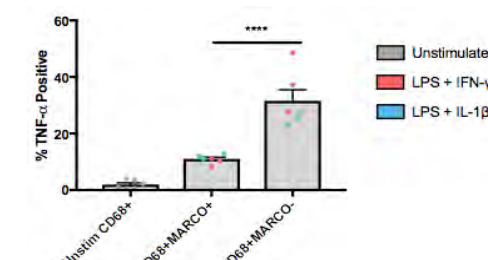
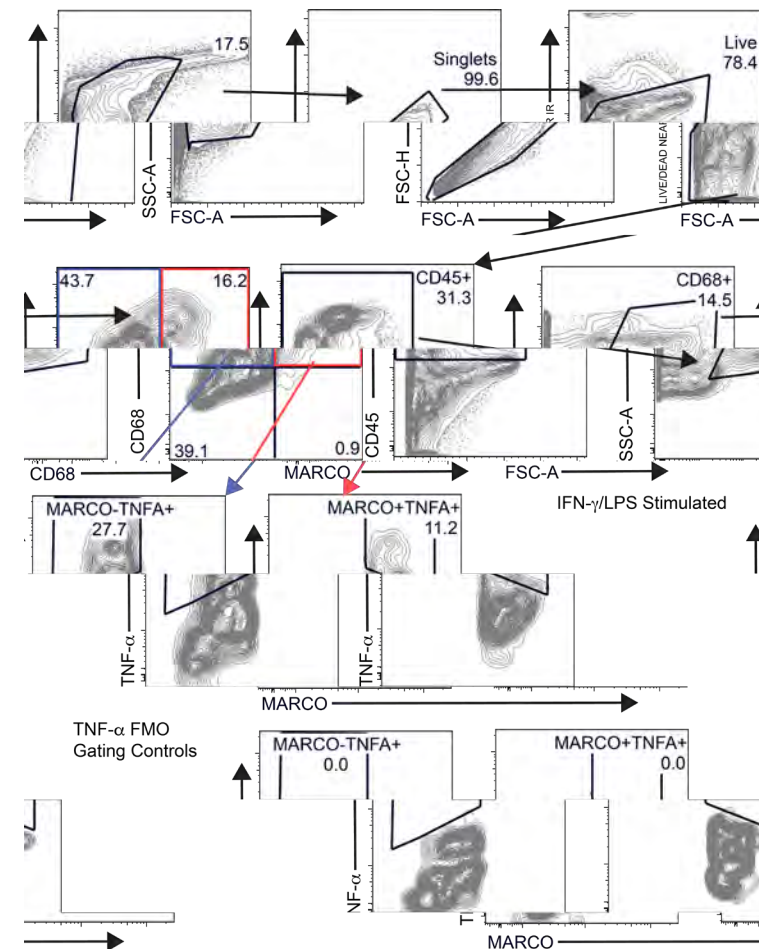
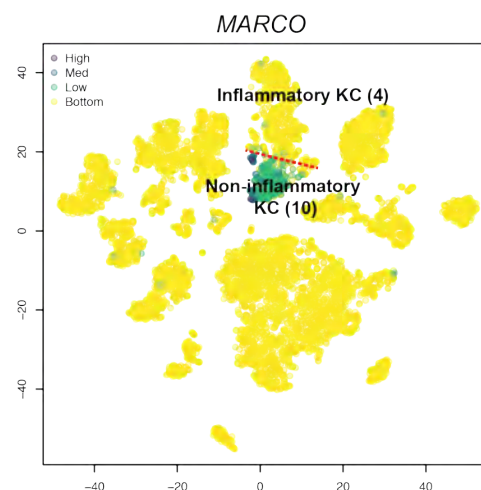
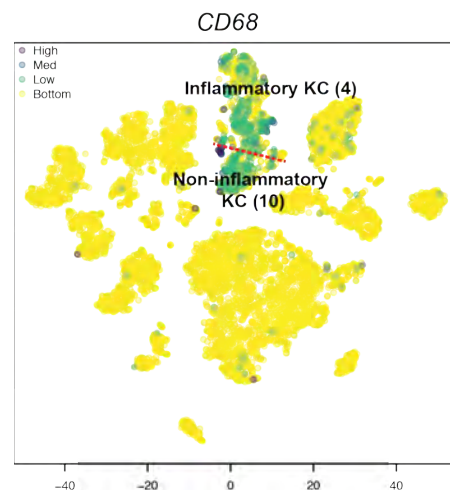
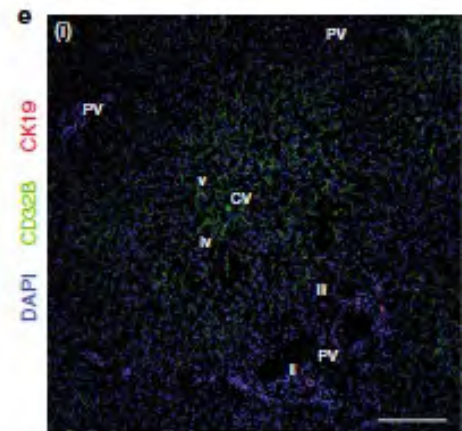
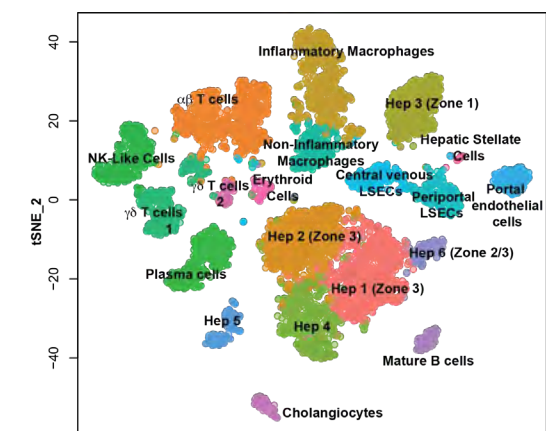
Article | [Open Access](#) | [Published: 22 October 2018](#)

# Single cell RNA sequencing of human liver reveals distinct intrahepatic macrophage populations

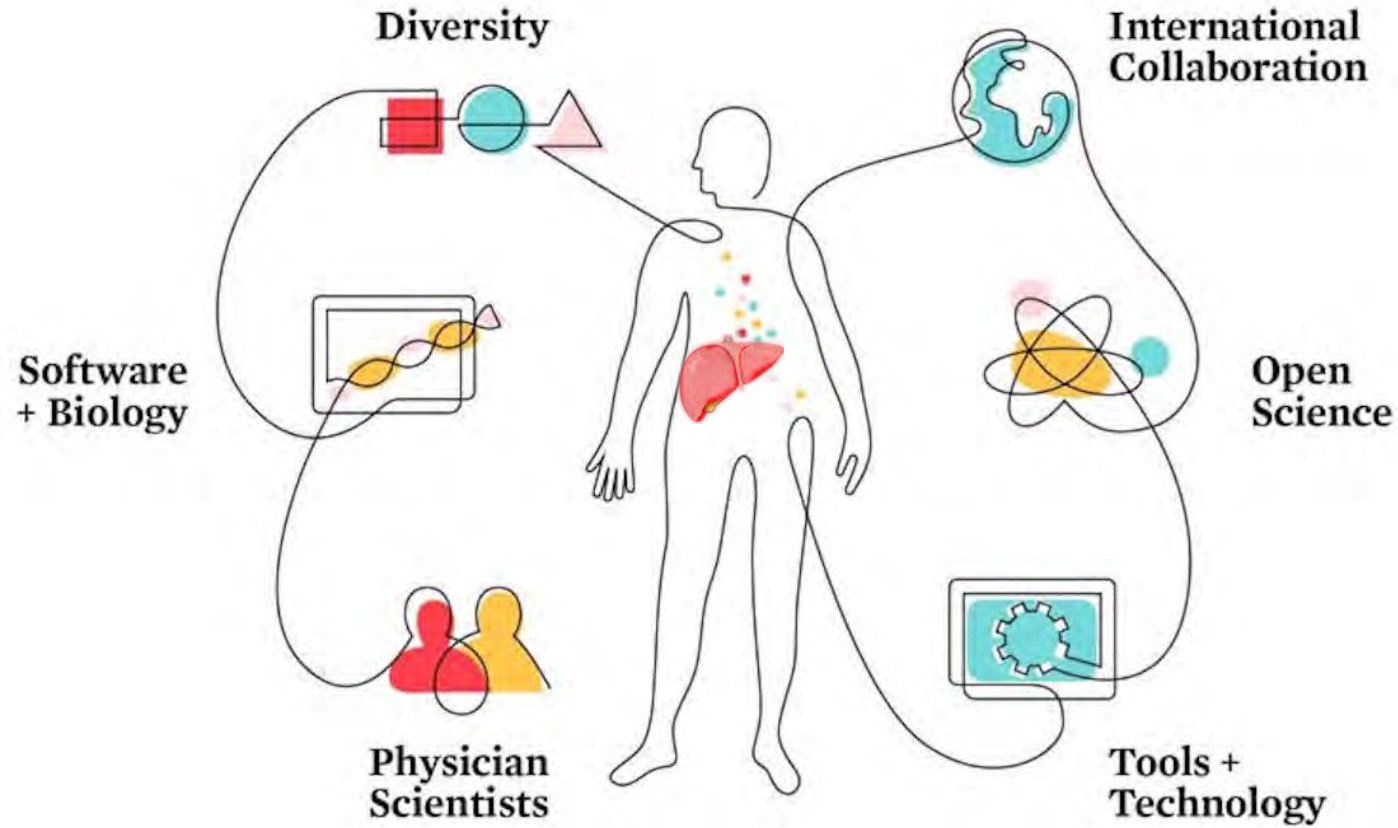
Sonya A. MacParland , Jeff C. Liu, Xue-Zhong Ma, Brendan T. Innes, Agata M. Bartczak, Blair K. Gage, Justin Manuel, Nicholas Khuu, Juan Echeverri, Ivan Linares, Rahul Gupta, Michael L. Cheng, Lewis Y. Liu, Damra Camat, Sai W. Chung, Rebecca K. Seliga, Zigong Shao, Elizabeth Lee, Shinichiro Ogawa, Mina Ogawa, Michael D. Wilson, Jason E. Fish, Markus Selzner, Anand Ghanekar, ... Ian D. McGilvray  + Show authors

[Nature Communications](#) **9**, Article number: 4383 (2018) | [Cite this article](#)

98k Accesses | 516 Citations | 200 Altmetric | [Metrics](#)



# Liver



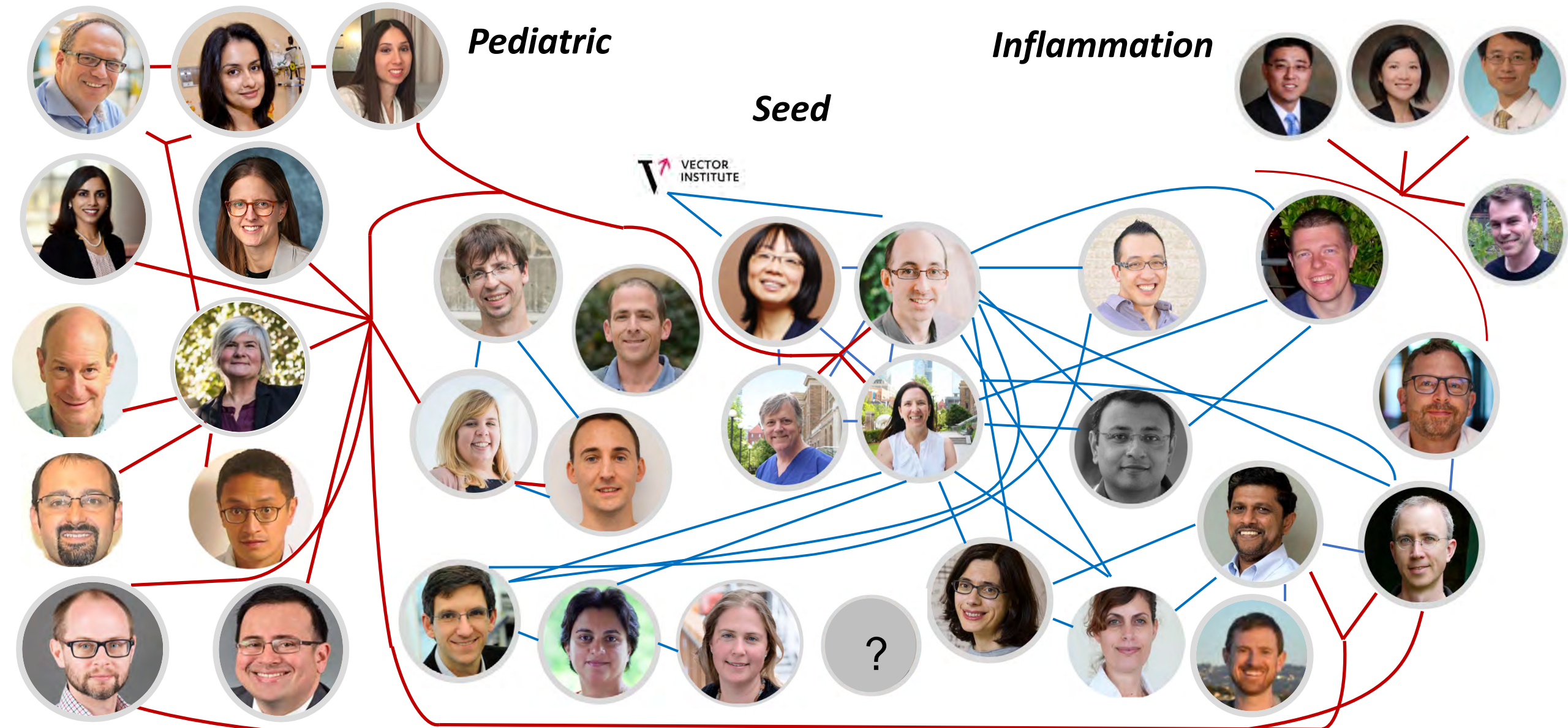
## CZI Seed Networks

Supporting the Human Cell Atlas

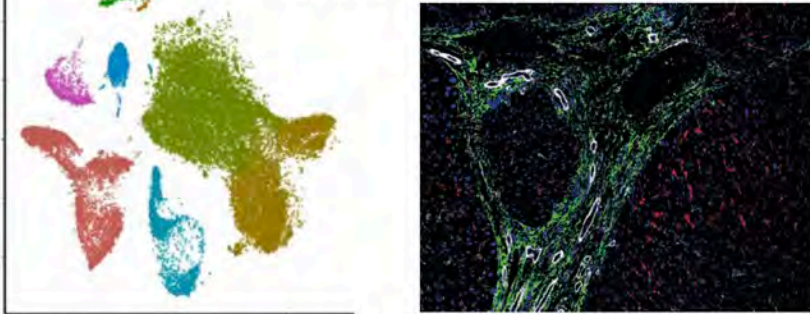


# Liver collaborations

(supported by CZI and HCA Seed, Inflammation and Pediatric Networks)

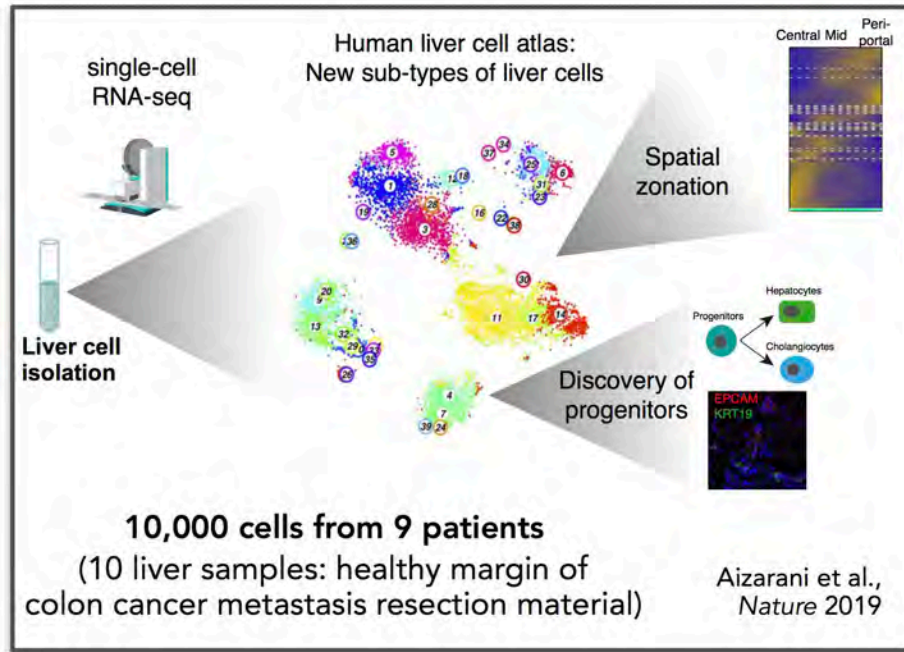


### Resolving the fibrotic niche of human liver cirrhosis at single cell level



**33,628 cells from 5 healthy human livers**  
 11 healthy samples (multiple lineages including leucocytes, endothelial and mesenchymal cells)

Ramachandran *et al. Nature* 2019



### Fetal liver

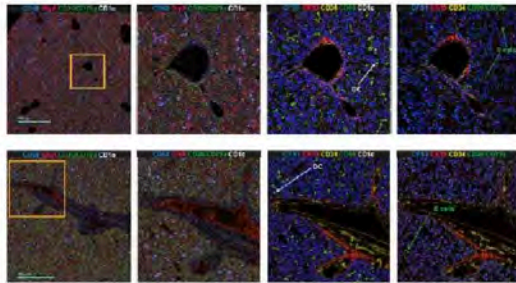
Haniffa, HDBR, Vallier, Teichmann, Behjati

### Single cell transcriptome map of fetal liver

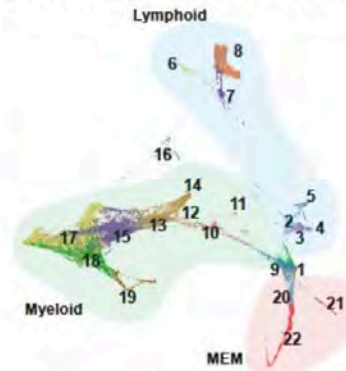


### Multi-modal and spatial validation of cell types

pseudo-coloured Hyperion image



### Fetal liver and NLT hematopoiesis

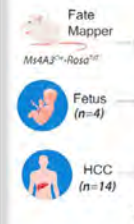


Popescu *et al., Nature* 2019

### A single-cell atlas of human liver from development to disease

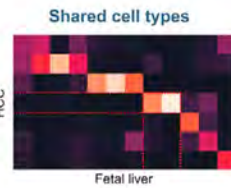
#### scRNA-seq

~212,000 cells



Mouse liver 79,000 cells  
 Fetal liver 60,000 cells  
 Tumor 57,250 cells  
 Adj. Normal 16,350 cells

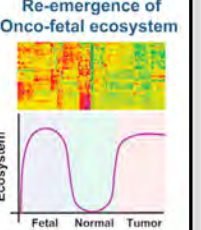
#### Comparative single cell transcriptomics



#### Cross-species comparison



#### Spatial transcriptomics

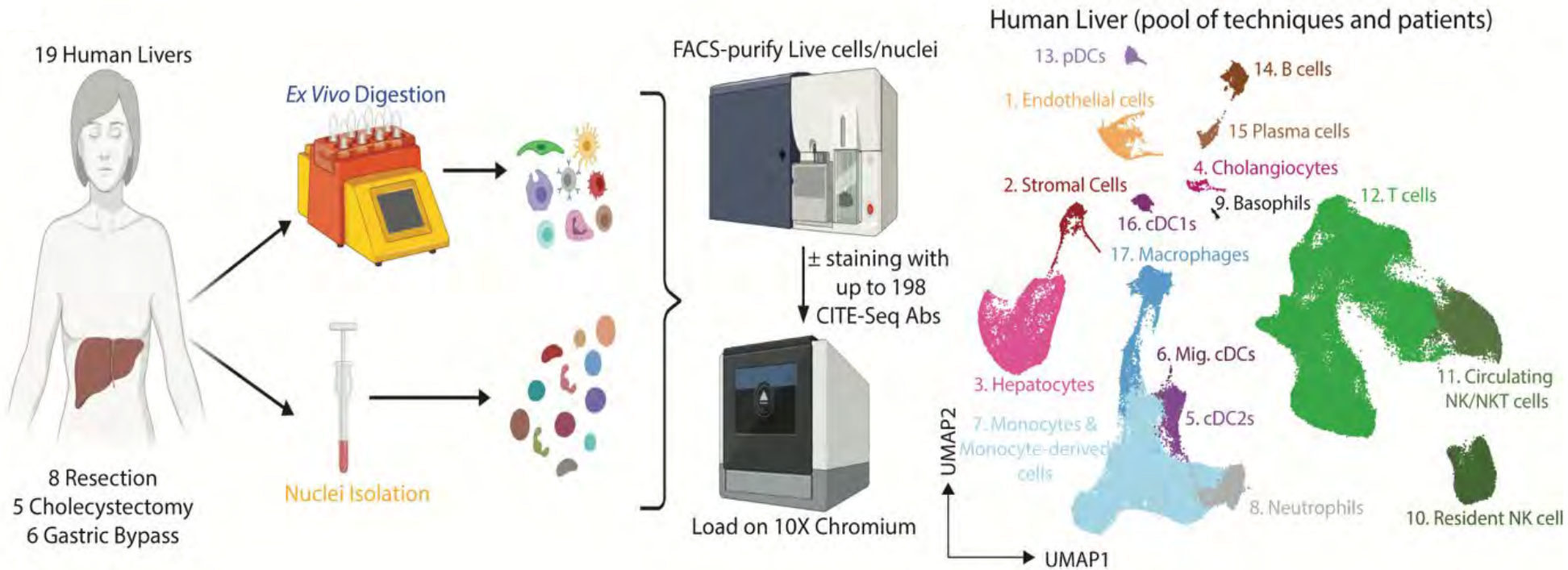


**~134,000 human liver cells** from 14 HCC samples (Adjacent normal and tumor tissue), 4 human fetal livers

Sharma *et al., Cell* 2020



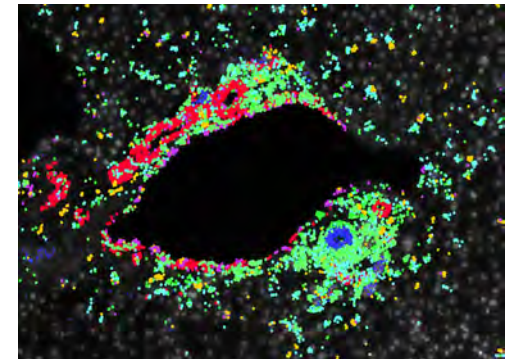
# Spatial Proteogenomic Atlas of Human Liver



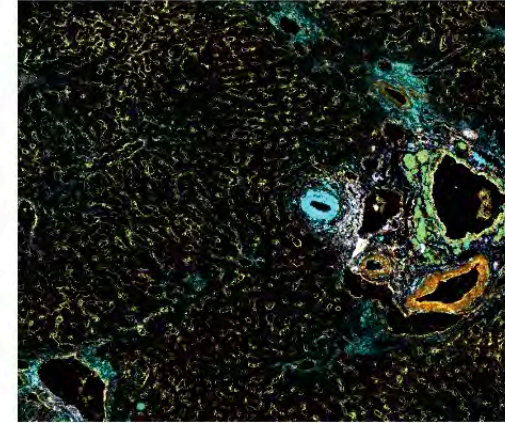
10X Visium



100-plex mRNA (Resolve)



100-plex protein (MACSima)

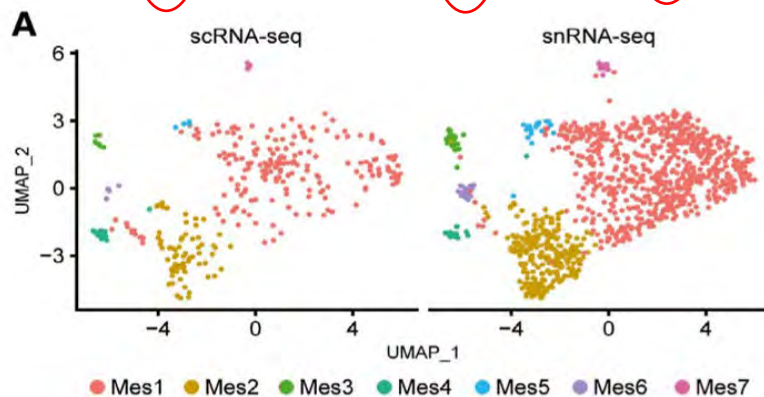
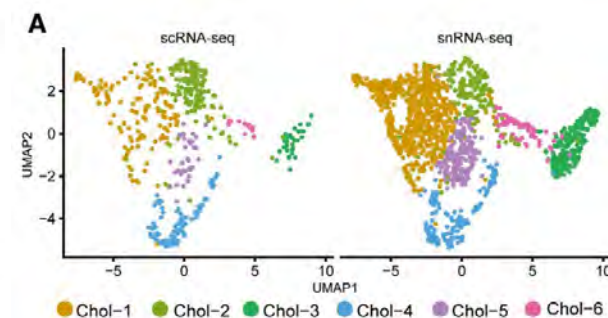
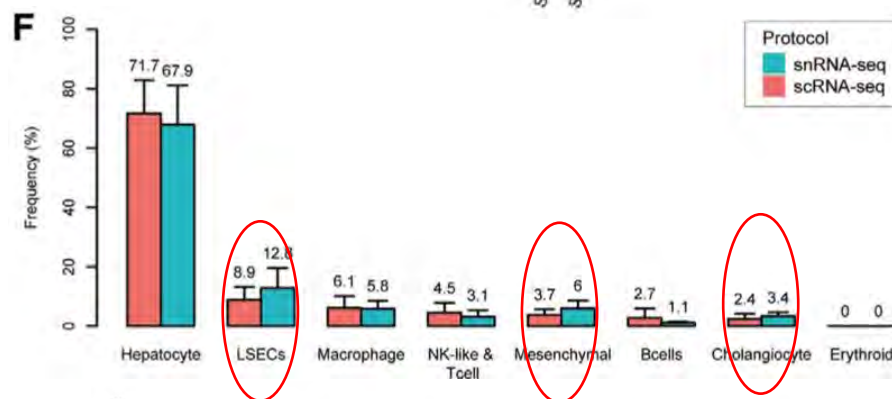
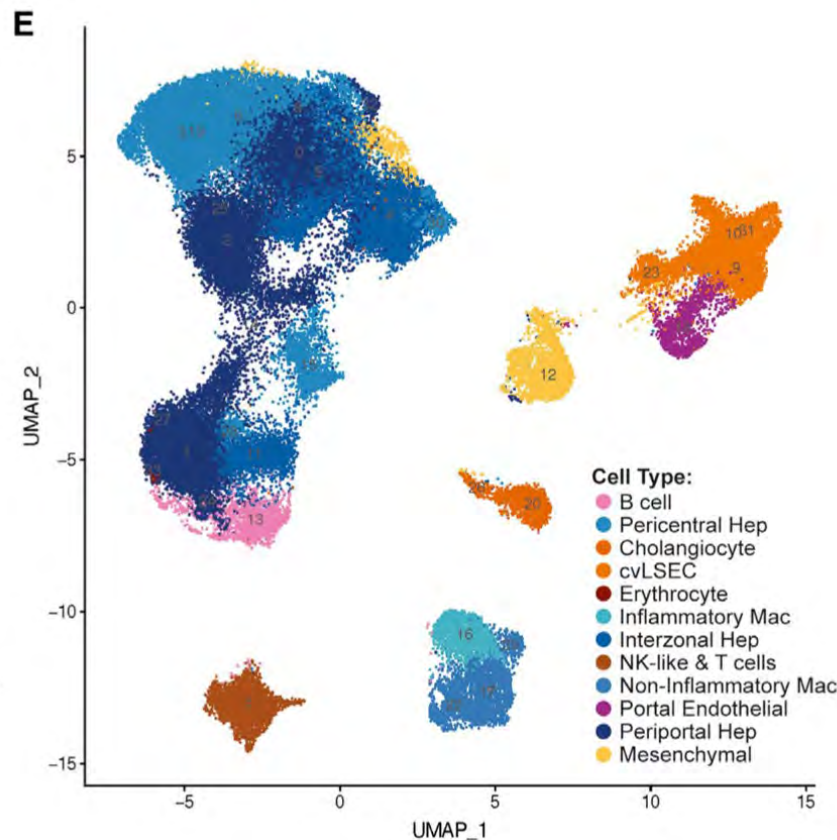
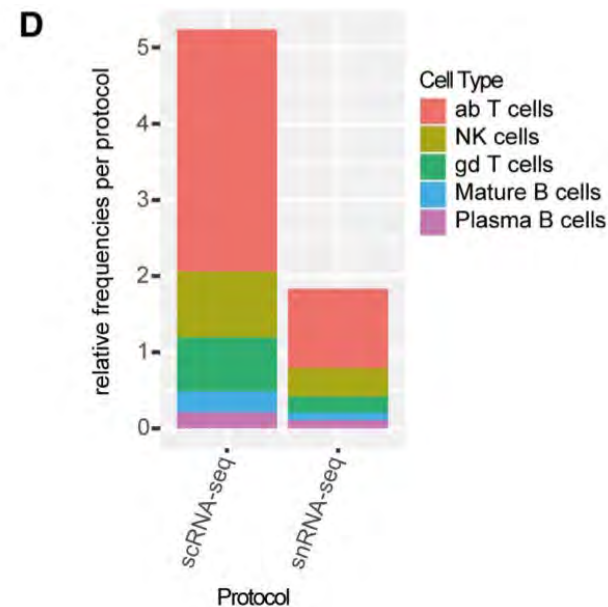
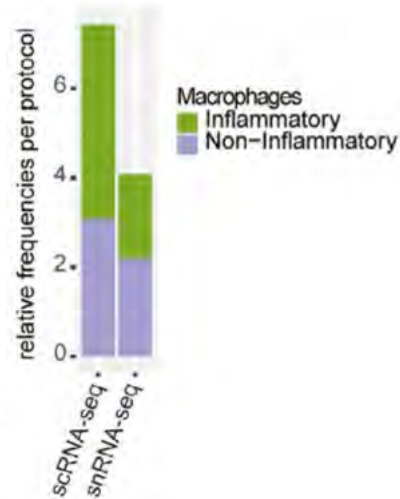


# Questions asked..

- Which cell populations are better captured by each technology
- How well are hepatic biological pathways captured by each technology?

# Single-Cell, Single-Nucleus, and Spatial RNA Sequencing of the Human Liver Identifies Cholangiocyte and Mesenchymal Heterogeneity

Tallulah S. Andrews,<sup>1\*</sup> Jawairia Atif,<sup>1,2\*</sup> Jeff C. Liu,<sup>3,4\*</sup> Catia T. Perciani,<sup>1,2,5</sup> Xue-Zhong Ma,<sup>1</sup> Cornelia Thoeni,<sup>5</sup> Michal Slyper,<sup>6</sup> Gökçen Eraslan,<sup>6</sup> Asa Segerstolpe,<sup>6</sup> Justin Manuel,<sup>1</sup> Sai Chung,<sup>1</sup> Erin Winter,<sup>1</sup> Iulia Cirlan,<sup>7</sup> Nicholas Khuu,<sup>7</sup> Sandra Fischer,<sup>5</sup> Orit Rozenblatt-Rosen,<sup>6#</sup> Aviv Regev,<sup>6,8,9,#</sup> Ian D. McGilvray,<sup>1</sup> Gary D. Bader,<sup>3,4</sup> and Sonya A. MacParland<sup>1,2,5</sup>



Tallulah Andrews



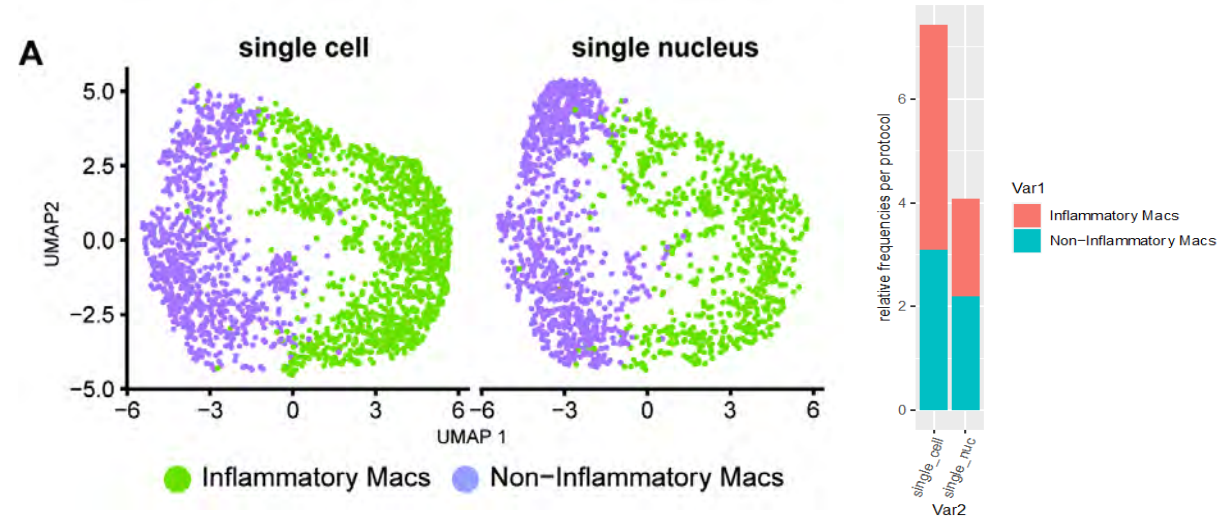
Jawairia Atif



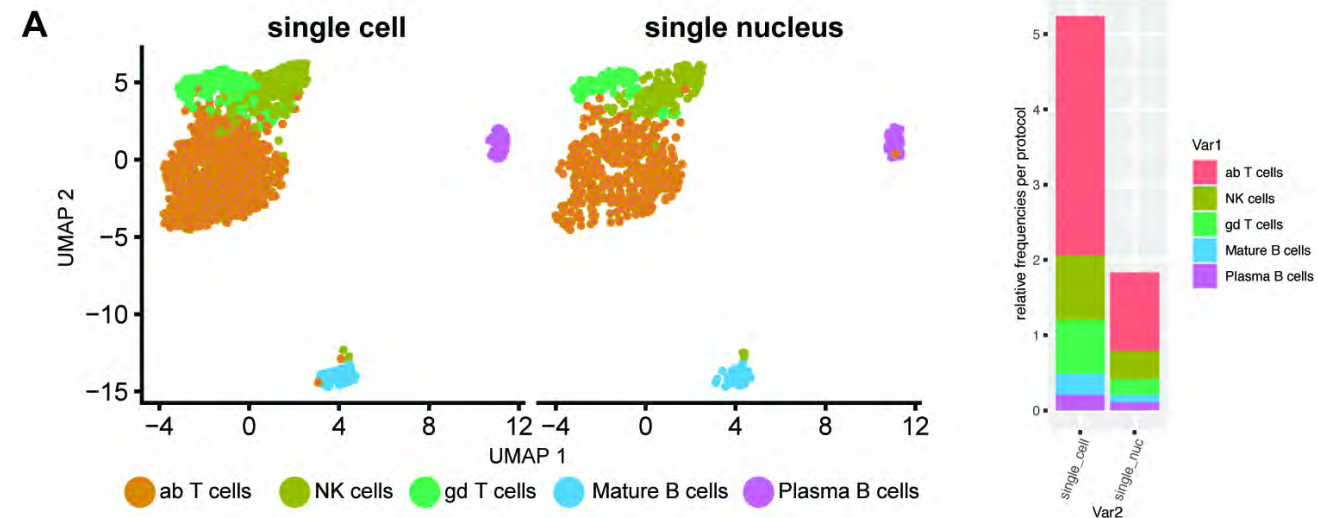
Jeff Liu

# All Immune cells are best captured by scRNA-seq

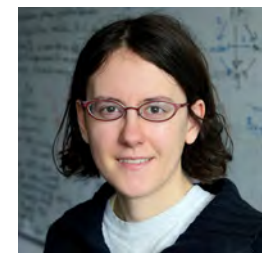
Macrophages:



Lymphocytes:



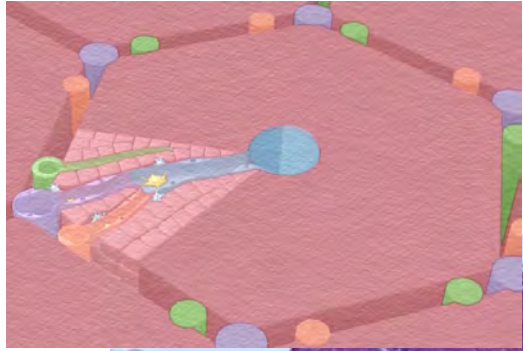
# Spatial Transcriptomics to confirm Human Hepatic Zonation



Tallulah Andrews



Catia Perciani

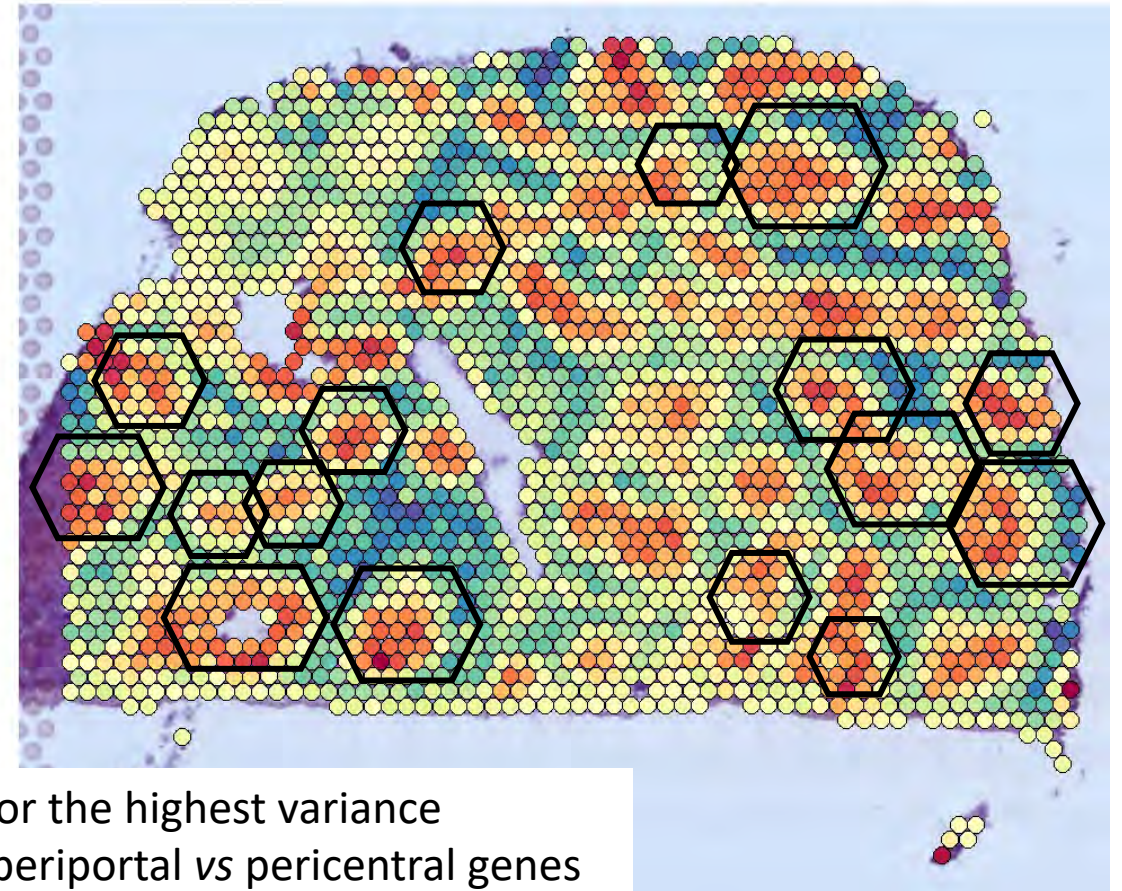
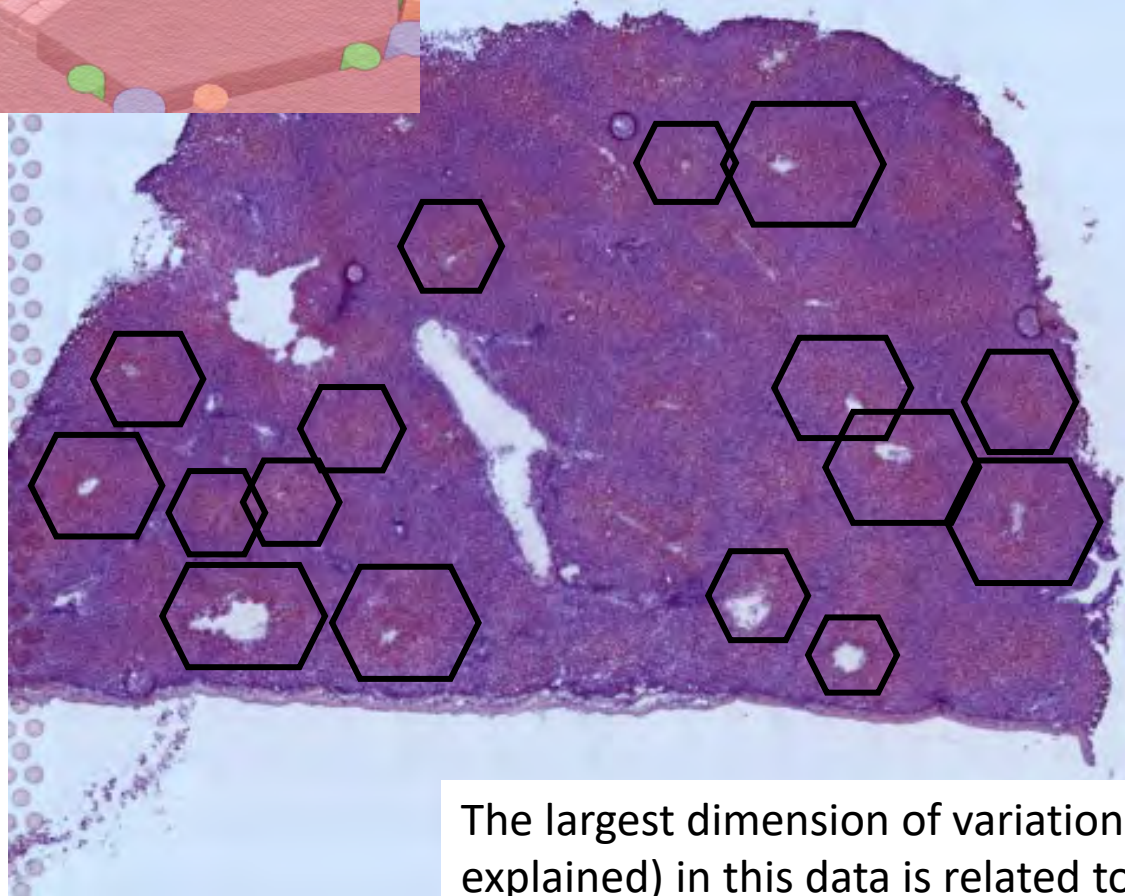


**PeriCentral**  
CYP1A2, CYP2E1,  
CYP3A4, GLUL, DCXR,  
FTL, GPX2, GSTA1

VS

**PeriPortal**  
CYP2A7, FABP1, HAL,  
AGT, ALDOB,  
HSD17B13, GLS2,  
SDS

RotPC\_1



The largest dimension of variation (or the highest variance explained) in this data is related to periportal vs pericentral genes

# Examining Liver Disease: What are the challenges?



- Diseased tissue can be more difficult to dissociate to a single cell level because of fibrosis
- Disease itself can be quite patchy so a biopsy might not catch the most diseased areas



# *Defining and Targeting Autoimmune Liver disease*

Sonya MacParland, Ian McGilvray, Jordan Feld, TGHRI, University of Toronto  
Gideon Hirschfield, TGH, Aliya Gulamhussein, TGH, University of Toronto; Adam Gehring, TGHRI,  
University of Toronto; Bettina Hansen, TGHRI, University of Toronto; Binita Kamath, Sick Kids,  
University of Toronto; Dianne Chadwick, TGH; Gary Bader, University of Toronto; University of  
Toronto, Sandra Fischer, TGH; Yaron, Avitzur, Sick Kids, TGH, Amanda Riccuito, Sick Kids

## **Collaborators:**

**PSC partners Canada, Dr. Aviv Regev, Dr. Selena Sagan, Dr. Trevor McKee, Dr. Kathleen Bingham**

## **Knowledge Translation:**

**PSC Partners Canada, Mary Pressley Vyas, Albert Fung, Dr. Frank Bialystok, Rachel Gomel**

# PSC Collaborative Autoimmune Network (PSC-CAN)

## PSC-CAN Team



Patient Partners  
Clinicians  
Surgeons  
Bioinformaticians  
Basic Scientists  
Clinical Research  
Coordinators

## Patient Recruitment



With end-stage,  
early-stage, or  
prospective PSC  
or alternative  
cholestatic liver  
disease



**SickKids**



## Sample Collection

Diagnostic and  
Research Liver  
Biopsies



PSC Explanted  
Liver Tissue



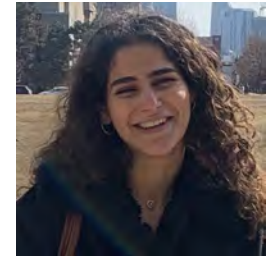
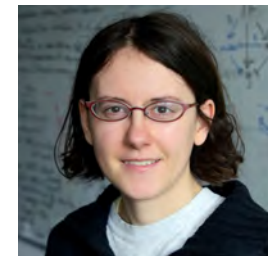
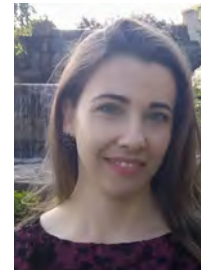
Blood  
(whole, serum,  
PBMCs)





# Defining and Targeting Autoimmune Liver Disease

**GOAL.** To generate cellular maps of the livers of patients with primary sclerosing cholangitis and to identify **active pathways** and **immune mediators** involved in the underlying immune dysfunction of PSC both in **pediatric** and **adult** patients by comparing the PSC maps to healthy human liver maps



Catia Perciani Tallulah Andrews Diana Nakib Lewis Liu



**PSC Partners Canada** @PSCPPartnersCa · Feb 29, 2020

#RareDisease patient advocates and rare disease researchers together on #RareDiseaseDay at #CLM2020. Wrapping up a long and productive day with @PSCPPartners and @MacparlandSonya talking research networks and @cziscience. #RareAsOne



1

7

24



**Integrated Knowledge translation:** We will engage end users in the planning and execution of this work so that we can gain end user input as we refine our research questions. This approach should produce research findings that are more likely to be directly relevant to and used by knowledge users.

Single-cell RNA Sequencing to Describe the  
Cellular Microenvironment of the Liver

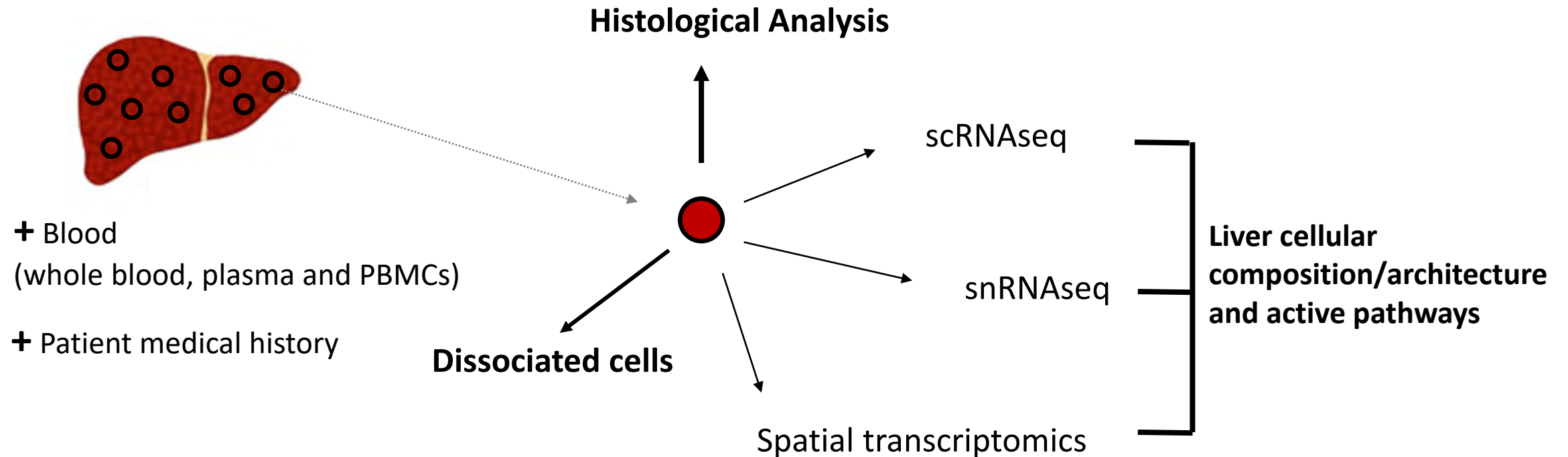
Sonya MacParland  
Scientist | Toronto General Hospital Research  
Institute  
Assistant Professor | University of Toronto  
Sept 29<sup>th</sup> 2019, PSC Partners Canada  
Community meet-up



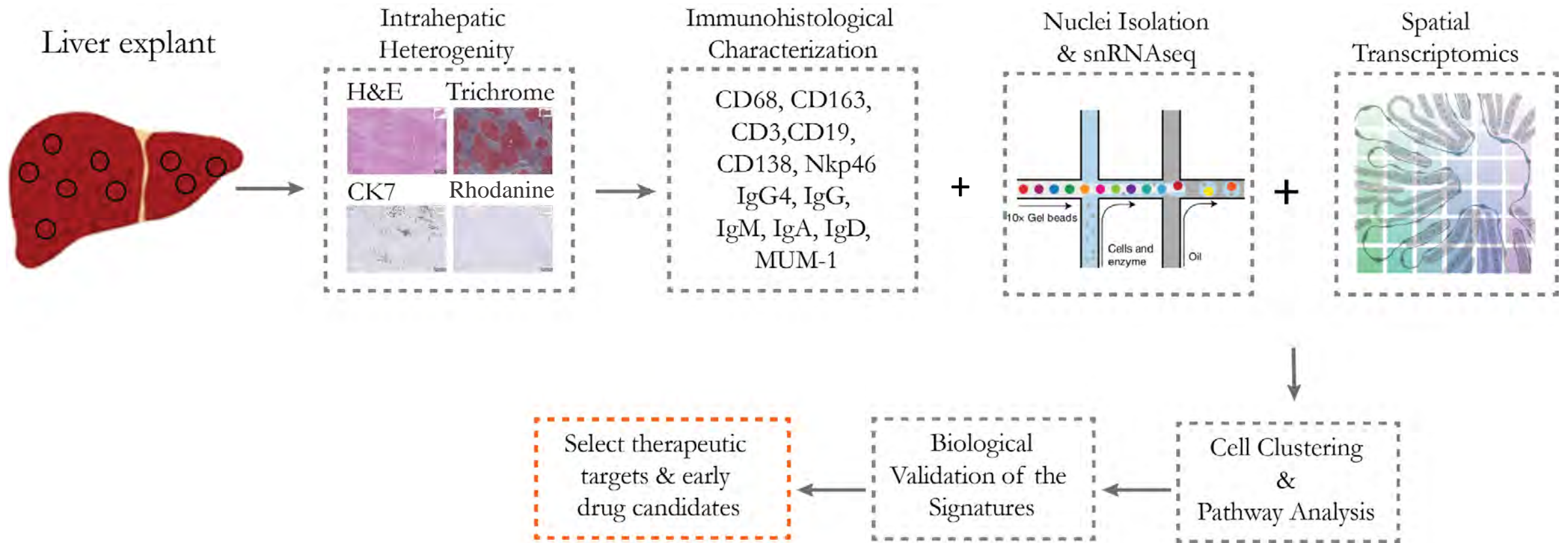
**UHN** Transplant  
Program



# PSC: Study Design

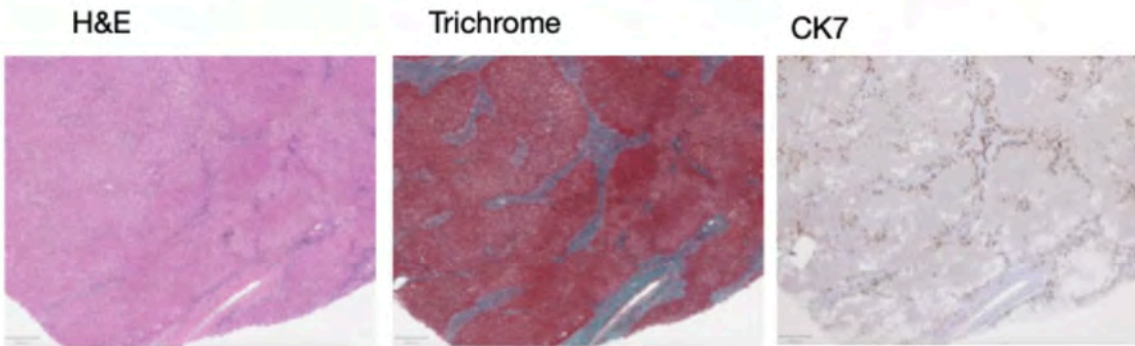


# PSC Study: Workflow

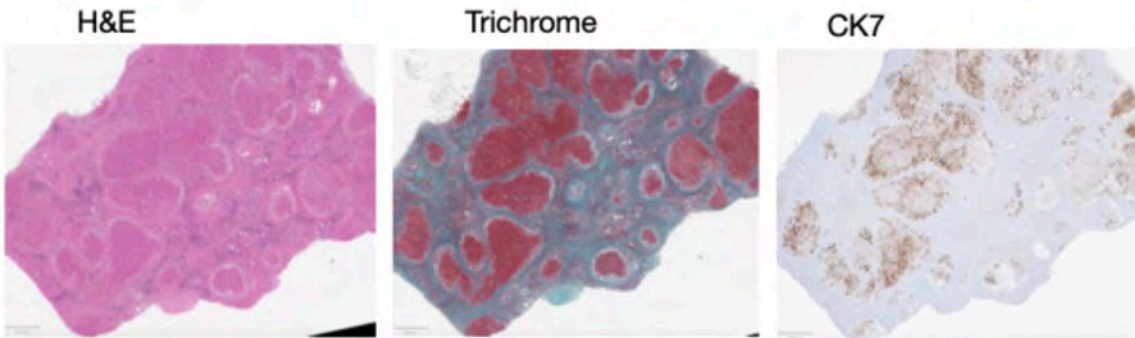


# Characterization of the intrahepatic heterogeneity of PSC liver

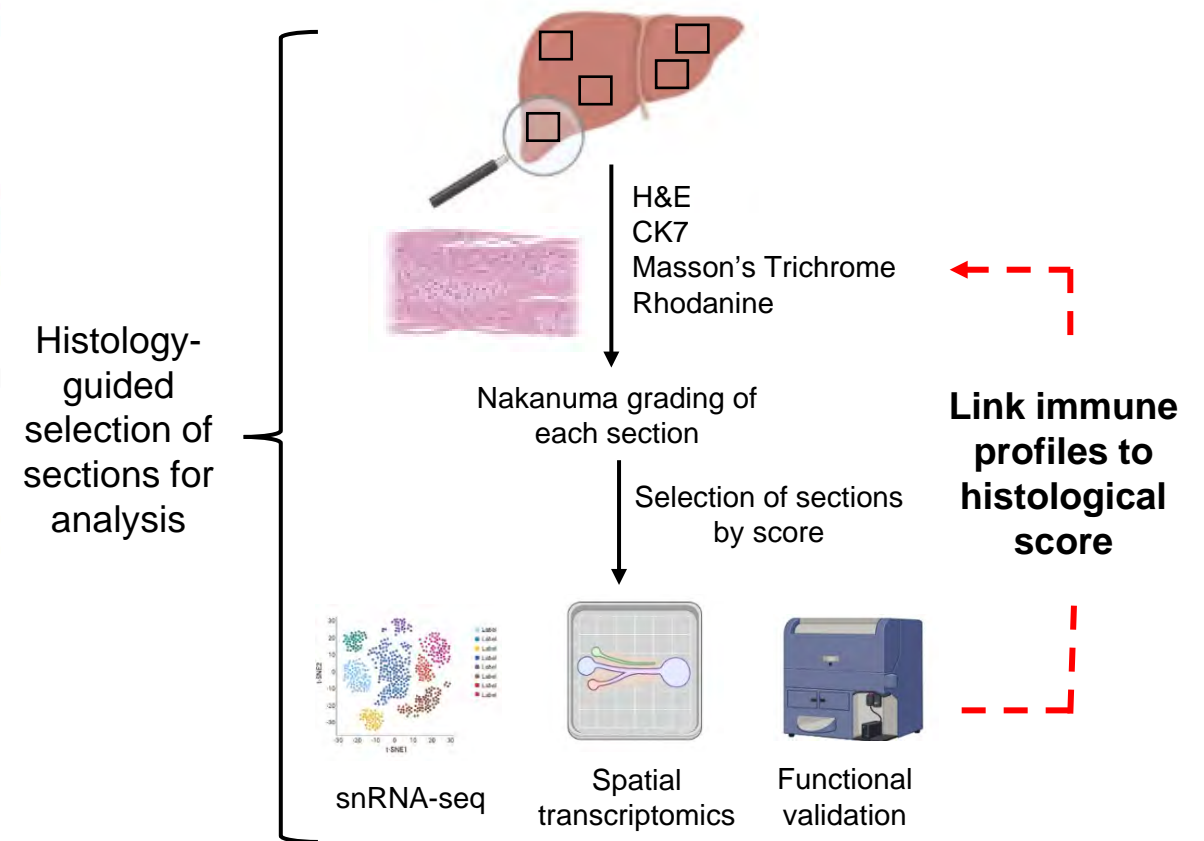
## Wedge 2



## Wedge 7



Histological heterogeneity is observed in different sections within a PSC liver



# Increased heterogeneity of disease in PSC shown by MRE

Article | [Open Access](#) | [Published: 10 May 2021](#)

## **Spatial heterogeneity of hepatic fibrosis in primary sclerosing cholangitis vs. viral hepatitis assessed by MR elastography**

[Rolf Reiter](#) , [Mehrigan Shahryari](#), [Heiko Tzschätzsch](#), [Dieter Klatt](#), [Britta Siegmund](#), [Bernd Hamm](#), [Jürgen Braun](#), [Ingolf Sack](#) & [Patrick Asbach](#)

[Scientific Reports](#) **11**, Article number: 9820 (2021) | [Cite this article](#)

### **Magnetic resonance elastography:**

Non-invasive imaging method for measuring stiffness of the liver

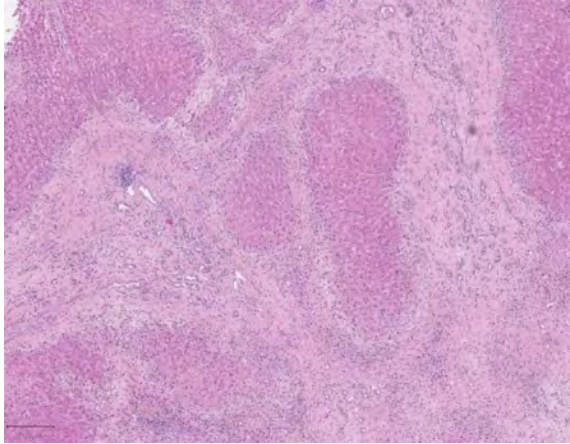
**PSC livers have increased fibrotic heterogeneity throughout in comparison to livers with viral hepatitis**

# Understanding the intrahepatic heterogeneity of PSC

## Histological and Immunohistochemical Stains

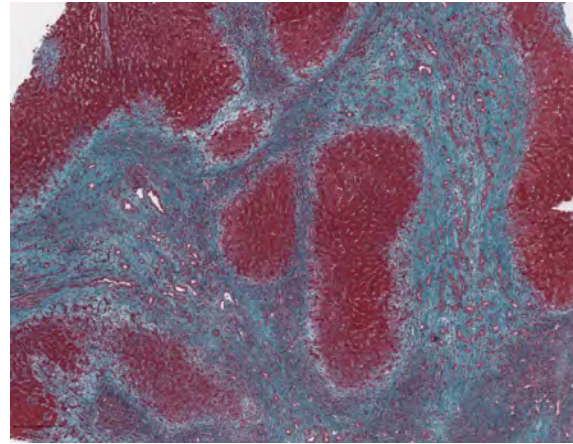
### Tissue Structure

H&E



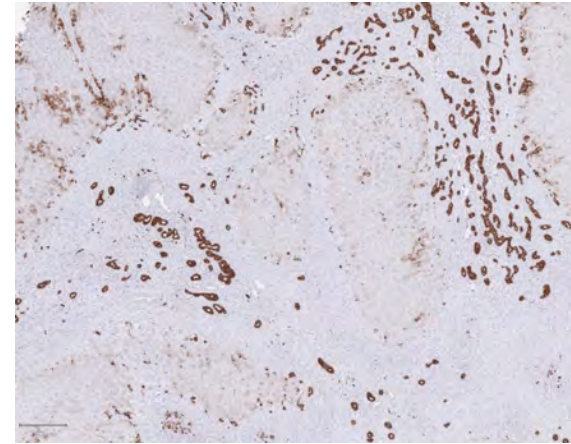
### Fibrosis

Trichrome



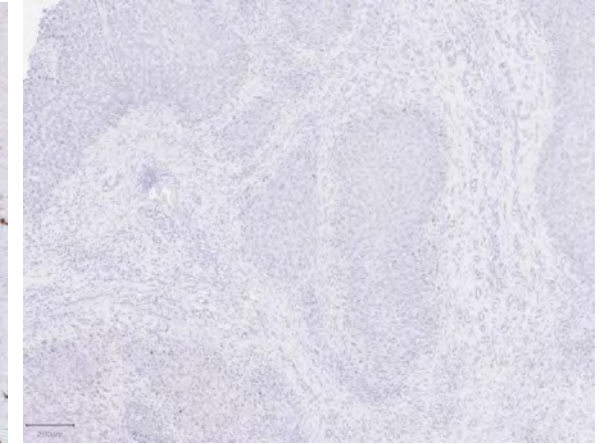
### Bile duct proliferation

CK7



### Copper excess

Rhodanine



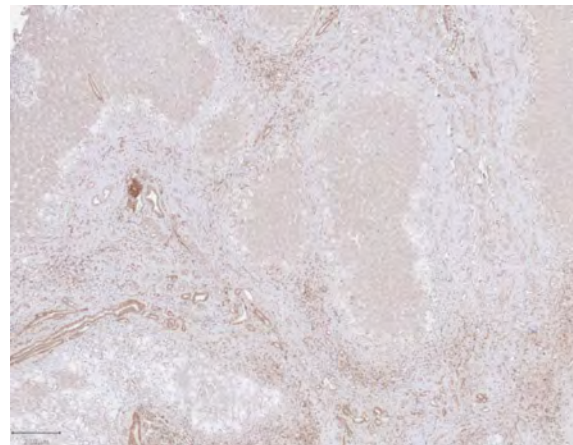
### T cells

CD3



### B cells

CD19



### Macrophages

CD68



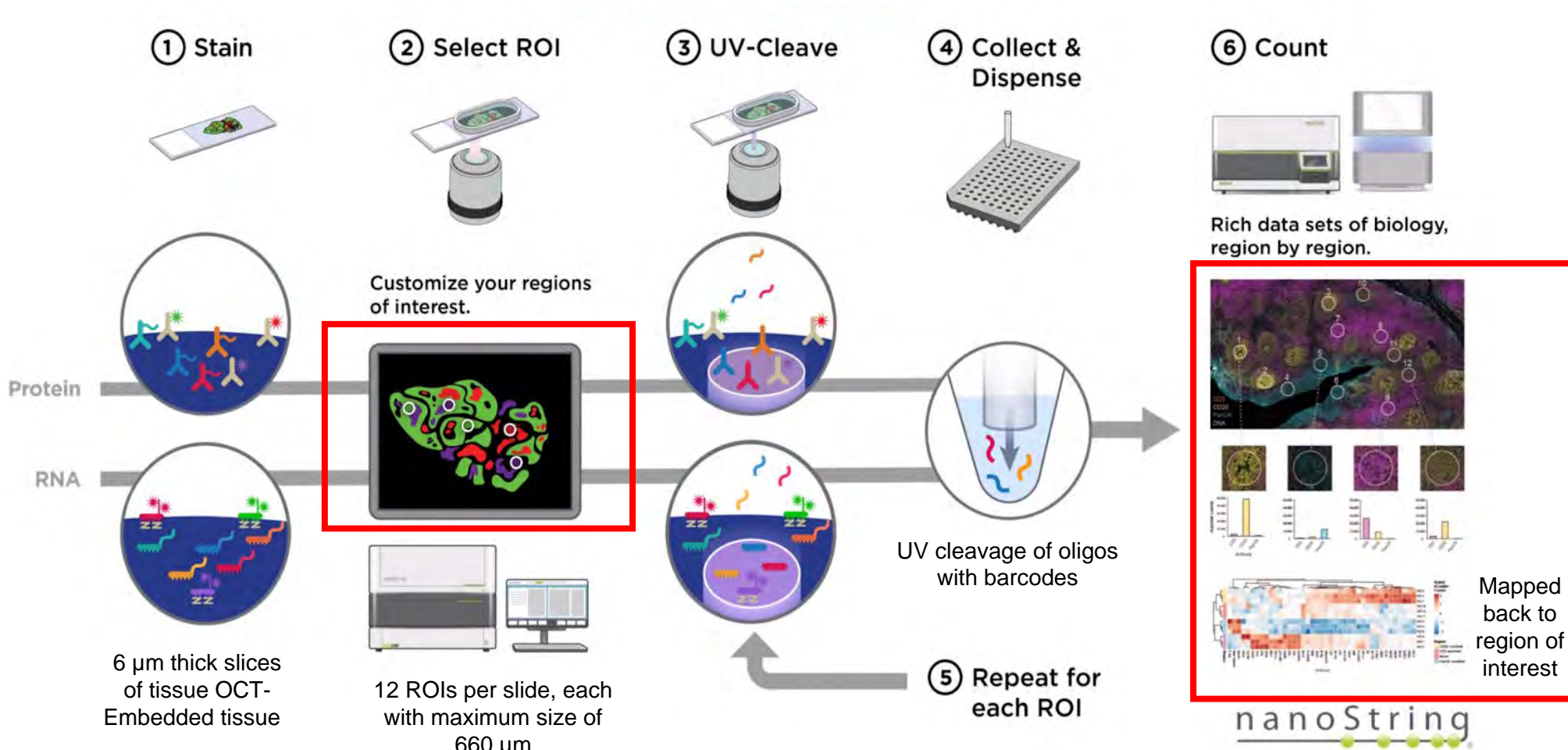
### Macrophages

CD163





# Application of NanoString GeoMx Spatial Profiling Platform to Mapping PSC Heterogeneity



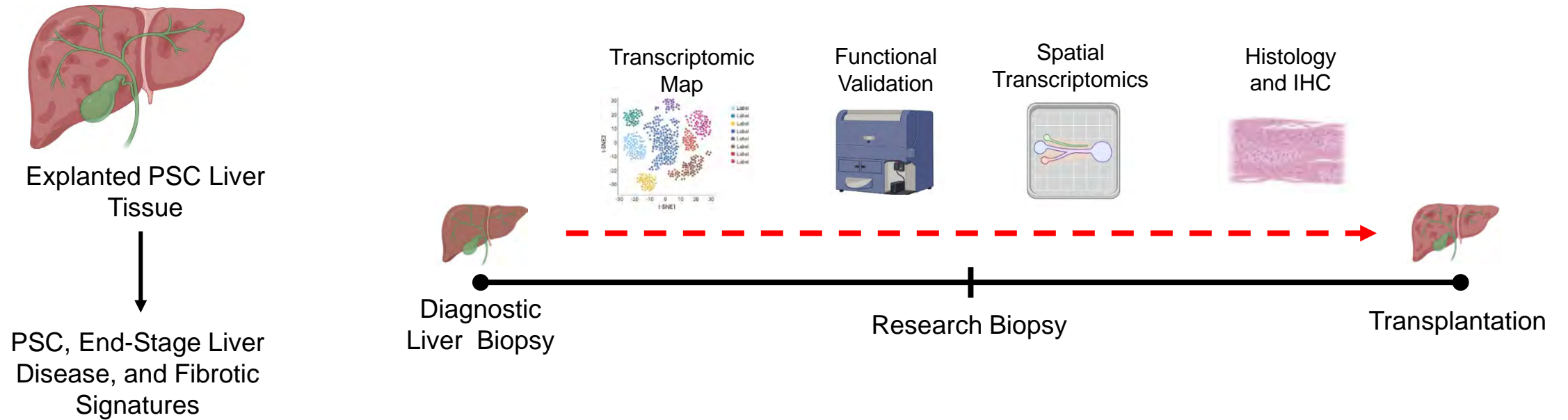
Visualize key morphological markers and perform bulk RNA-seq in ROIs

**Spatial deconvolution:** Identification of cell types present in the bulk RNA-seq of ROIs using a single-cell gene signatures

# Using this technology to uncover the heterogeneity of the PSC liver

- Apply Nanostring GeoMx DSP to different sections of the same PSC liver and characterize the relationship between:
  - Immune infiltration
  - Immune functionality
  - Spatial architecture
  - Histological score

# Characterize PSC at difference stages in progression



# Summary

- ScRNA-seq is a powerful tool to understand the cellular complexity of the healthy human liver
- A single cell, single nuclei, and spatial approach may be required to identify cellular drivers and the heterogeneity of liver disease
- Transcriptional profiling will uncover pathways to target to reprogram the livers of patients.

# Acknowledgements

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Kelly Liu

Felix Liu

Sabrina Tang

**UHN Liver Transplant**

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Dr. Markus Selzner

Dr. Anand Ghanekar

Dr. David Grant

Dr. Gonzalo Sapisochin

Dr. Paul Greig

**McGillvray Lab**

Dr. Max Ma

Justin Manuel

Dr. Agata Bartczak

Dr. Xue-Chung Chen

Dr. Hongtao Gu

**Bader Lab**

Brendan Innes

Jeff Liu

**Princess Margaret**

**Genomics Center**

**Neil Winegarden**

**Troy Ketala**

Nick Khuu

Zhibin Lu

Iulia Cirlan

Gurbaksh Basi

**Surgical Fellows**

Dr. Juan Echeverri

Dr. Dagmar Kollmann

Dr. Ivan Linaes

Dr. Nicholas Goldarecena

Dr. Michael Wilson (SKH)

Oyedele Adeyi (UMN)



HUMAN CELL ATLAS  
LIVER



Toronto General & Western  
Hospital Foundation  **UHN**

**KNOWLEDGE LIVES HERE.**

**TGHRI**

Dr. Gordon Keller

Dr. Blair Gage

Dr. Mina Ogawa

Dr. Shin Ogawa

Dr. Jason Fish

**The Broad Institute**

**Dr Aviv Regev**

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