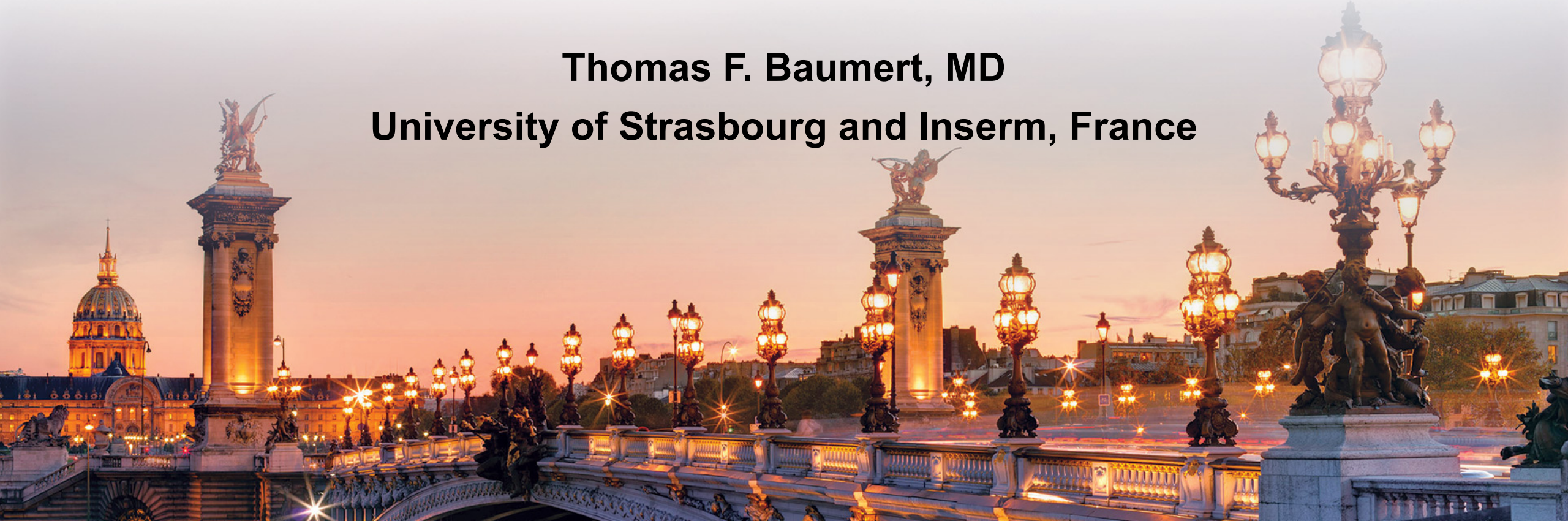
Paris NASH Meeting
International Think Tank

October 22 & 23, 2020
Digital Edition
6th edition

Application of single cell RNA-seq in understanding of NASH and liver fibrosis

Thomas F. Baumert, MD

University of Strasbourg and Inserm, France



Paris NASH Meeting
International Think Tank

Conflict of interest disclosure

- Inventor on patent applications on liver disease drug and target discovery filed by U Strasbourg, Strasbourg U Hospitals, IHU and Inserm
- Founder and advisor Alentis Therapeutics Basel and Strasbourg
- Consultant Gilead, BioMedPartners

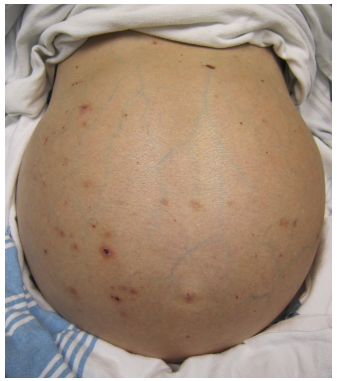
Outline

- Introduction: concepts and methods of scRNA-seq
- The human liver cell atlas: a reference for the study of liver disease and cancer
- Single cell RNA-seq to understand disease biology in advanced liver fibrosis and NASH
- Conclusions and Perspectives

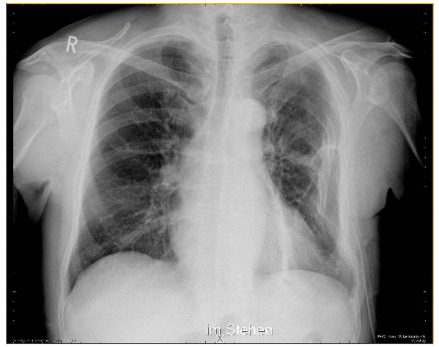
Understanding liver disease from the past to presence

Imaging

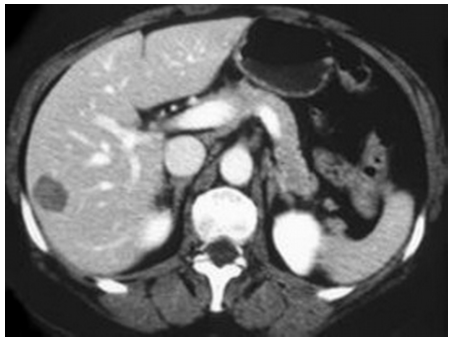
Early



Last century



Recent decades

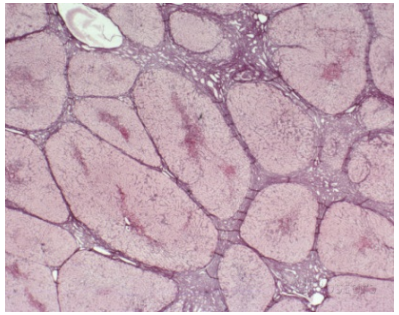


Pathology

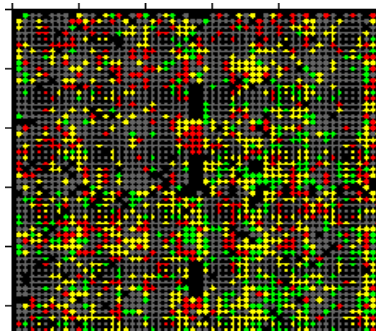
Early

Rembrandt van Rijn,1632

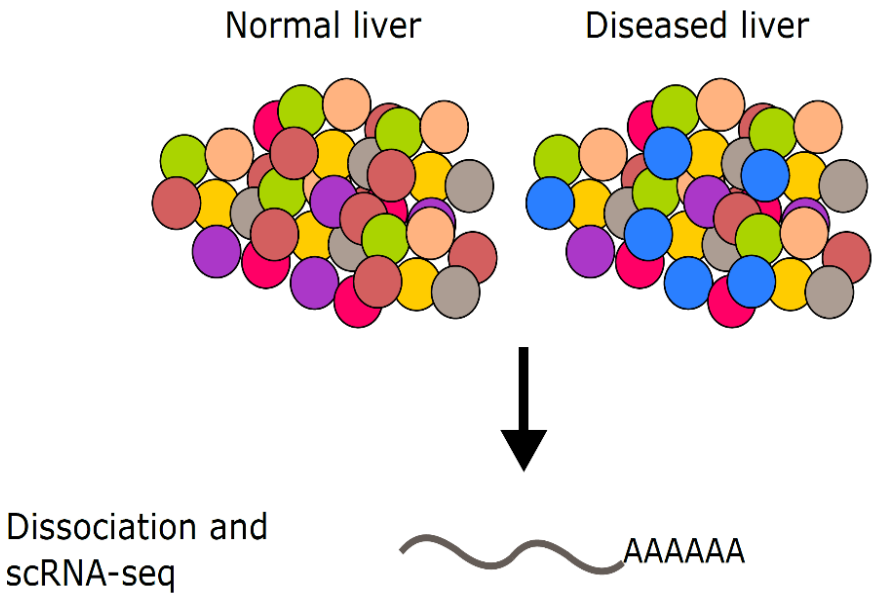
Last century

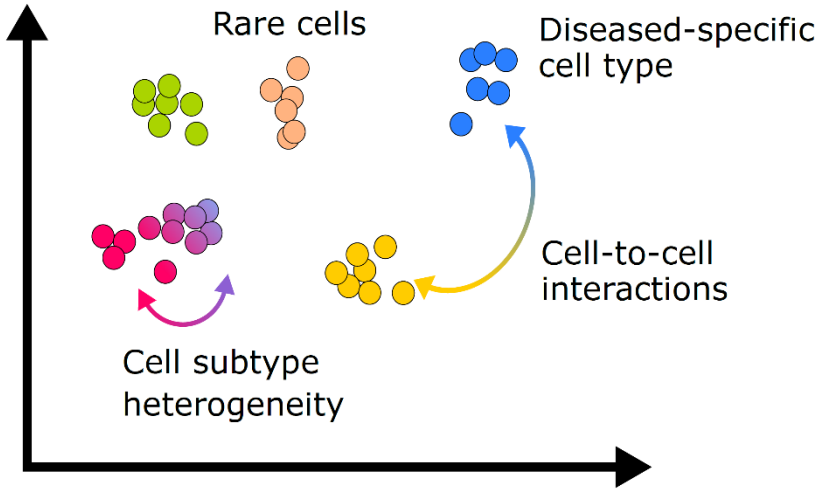


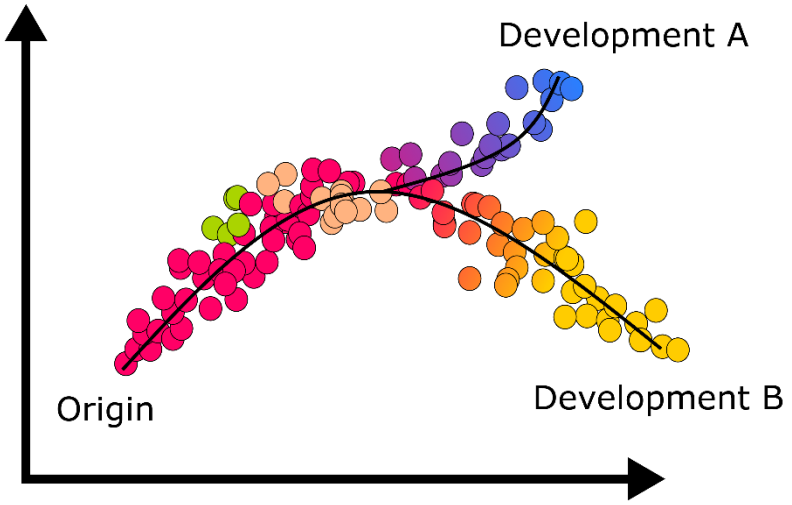
Recent decades



Unraveling liver disease biology on the single cell level



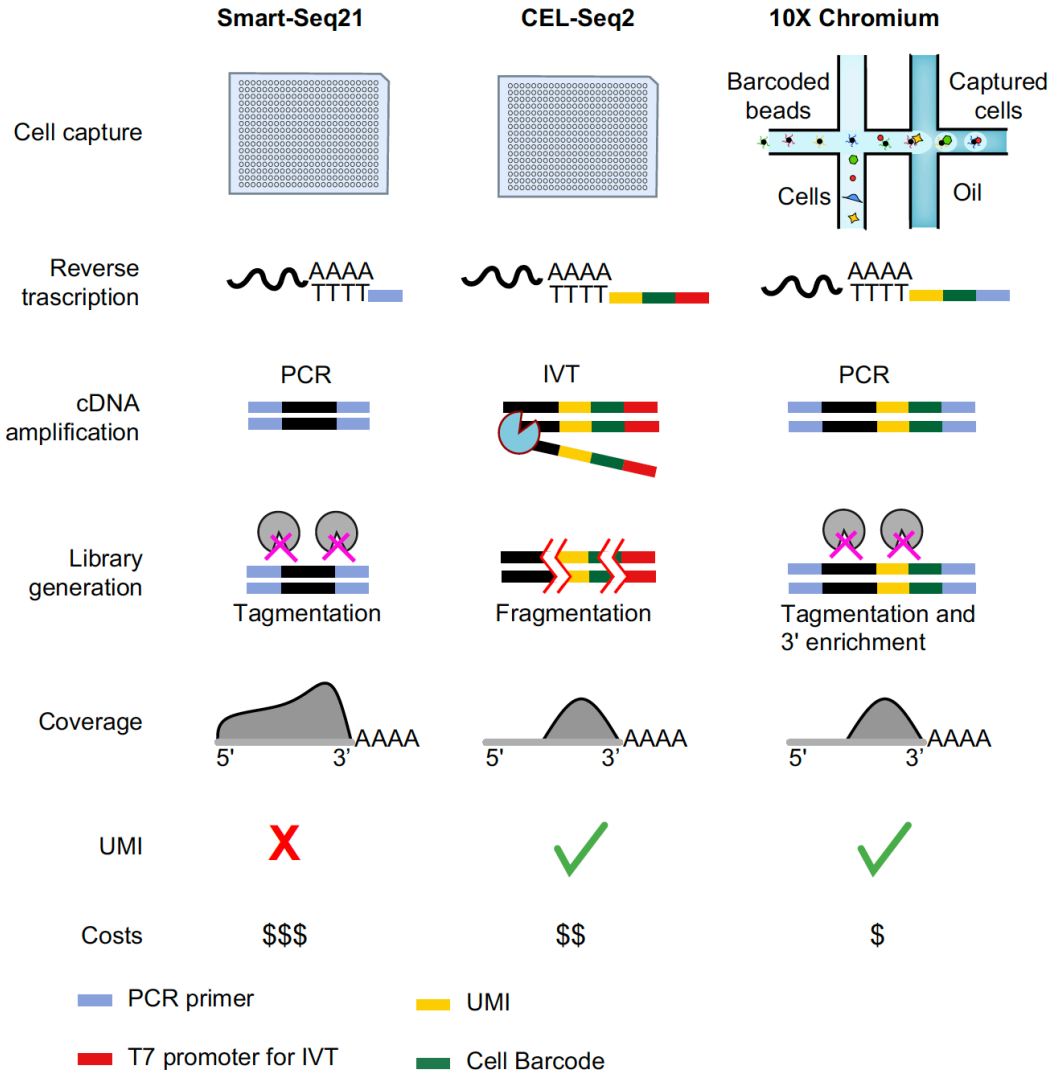




- ✓ **New opportunities to understand disease biology**
- ✓ **Drug and target discovery for liver disease**
- ✓ **Novel diagnostic and prognostic tool**

Saviano A, Henderson NC, Baumert TF, *J Hepatol* 2020

Novel sequencing methods enable to profile the transcriptomes of individual cells

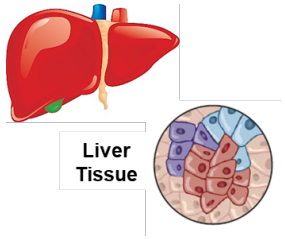


Saviano A, Henderson NC, Baumert TF, *J Hepatol* 2020

- **Methods of scRNA-seq comprise multiple technologies which are complementary**
- **Array based versus microdroplet**
- **Choice of platform depends on the biological question (high resolution in less cells or high number of cells with lower resolution).**
- **Smart-seq2 is preferred when analyzing splicing, transcriptome annotations or genome integrations**
- **High-throughput microdroplet-based microfluidic technologies are preferred for broader cell coverage at shallower sequencing read depths**
- **Microdroplet technologies in particular useful for immune cells**

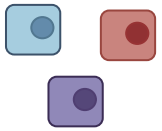
Building a tissue liver scRNASeq pipeline to establish a human liver cell atlas

Liver resection of diseased and non diseased tissues

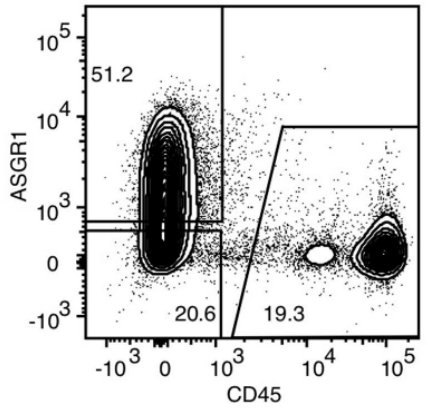


Strasbourg University Hospitals

Tissue dissociation and single cell suspension



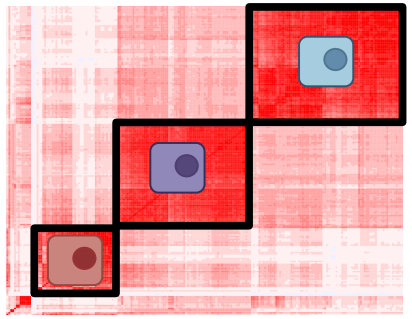
Single cell sorting by FACS in 384-well plate



Sequencing (mCEL-Seq2 and Smart-Seq2)

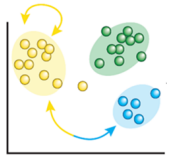
ACATGACGCCCTAGTTGC
GATCCACATGACGCTGCT
ACGCCCTAGACATGATGG
CGGTACGGACATGATGTC
ACAGGGTTCGATCGTCGC
ACGCCCTAGACATGATGG
CGGTACGGACATGATGTC
ACAGGGTTCGATCGTCGC
ACGCCCTAGACATGATGG

Filter and cluster data (RaceID3, SC3, Seurat)

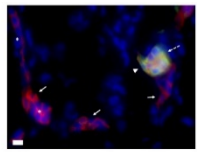


Data analysis and validation studies

Gene expression (tsne maps)



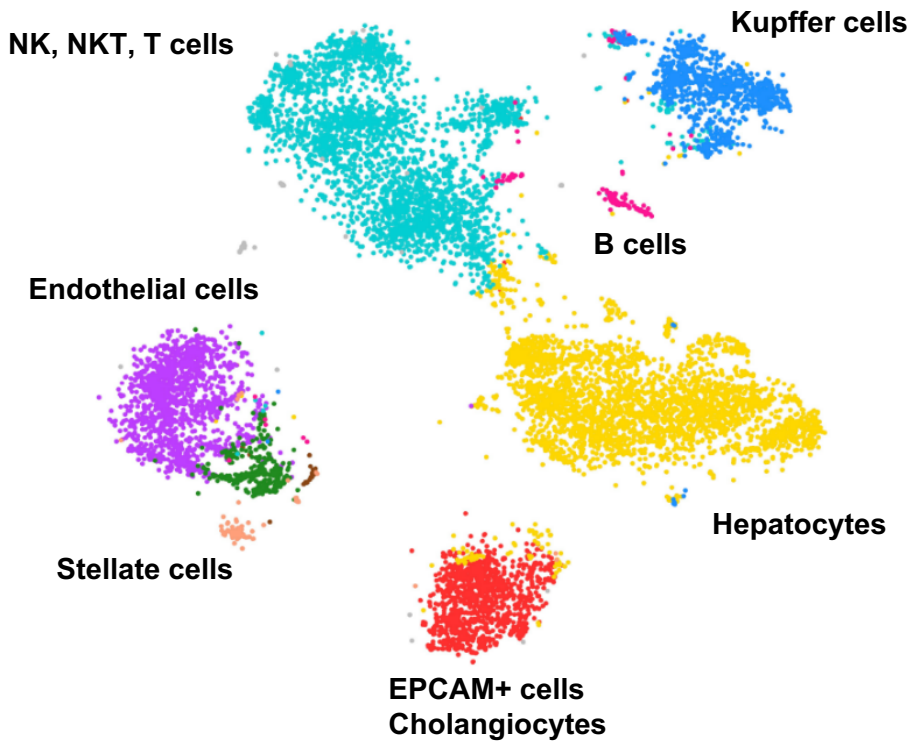
Validation studies



- Collaboration with Broad Institute of Harvard and MIT (N. Pochet, A. Shalek, A. Regev) and Max-Planck Institute Freiburg (D. Grün)
- Single cells from healthy liver and HCC resections (P. Pessaux, Inserm U1110 NHC Strasbourg) are isolated and sorted by FACS
- Library preparation, barcode labeling and RNA-sequencing by mCEL-seq2 (MPI) or SMARTSeq2 (Broad Institute) protocol
- Clones inferred by RACE ID3 algorithm for cell origin prediction, single cells and clusters are analyzed and compared with reference cells

The human liver cell atlas – a reference for the human liver in health and disease

10,372 cells from 9 patients without liver disease

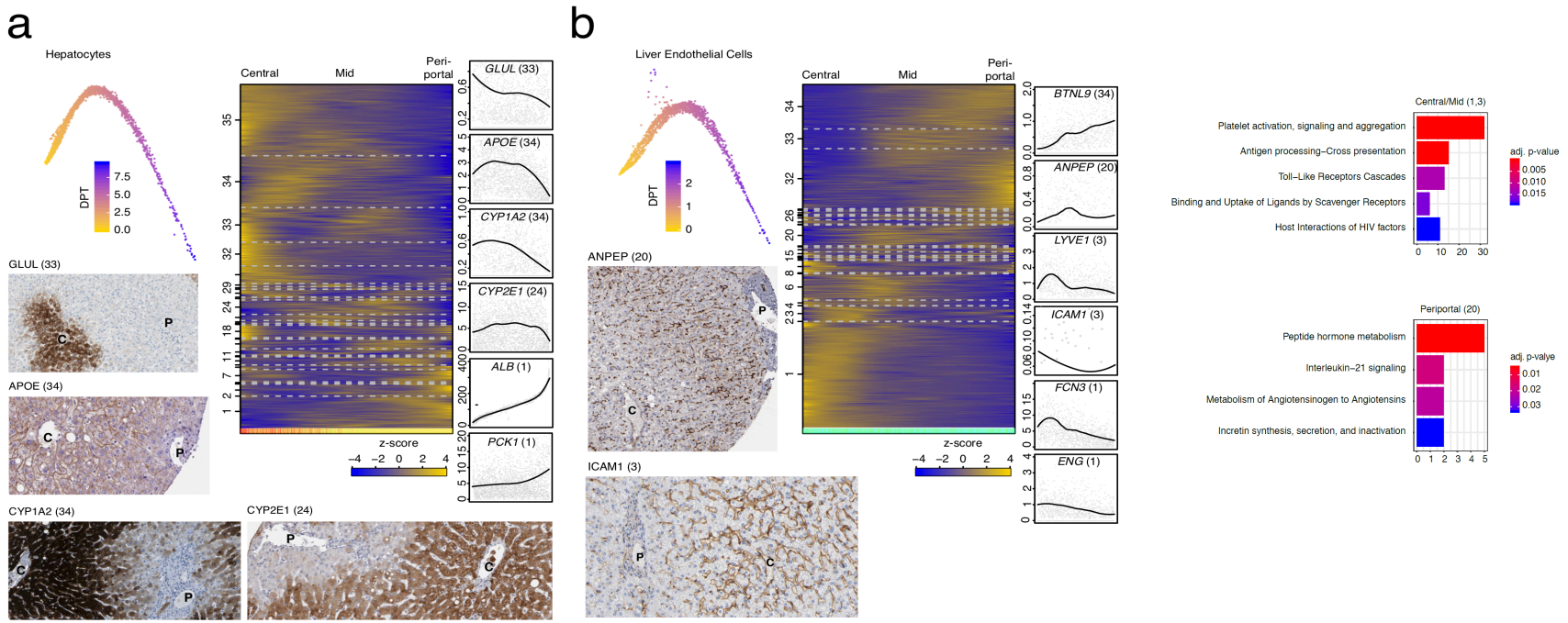


- ✓ ***t-SNE*** **map (t-distributed stochastic neighbor embedding)**
- ✓ **High dimensional information compressed in a 2D space**
- ✓ **Each dot represents a single cell**
- ✓ **The distance between cells is a function of their transcriptional similarity («stochastic neighbor embedding»)**
- ✓ **Marker genes enable identication of known and novel cell types**

Aizarani N, Saviano A et al. *Nature 2019*

Heterogeneity and zonation of hepatocytes and nonparenchymal cells

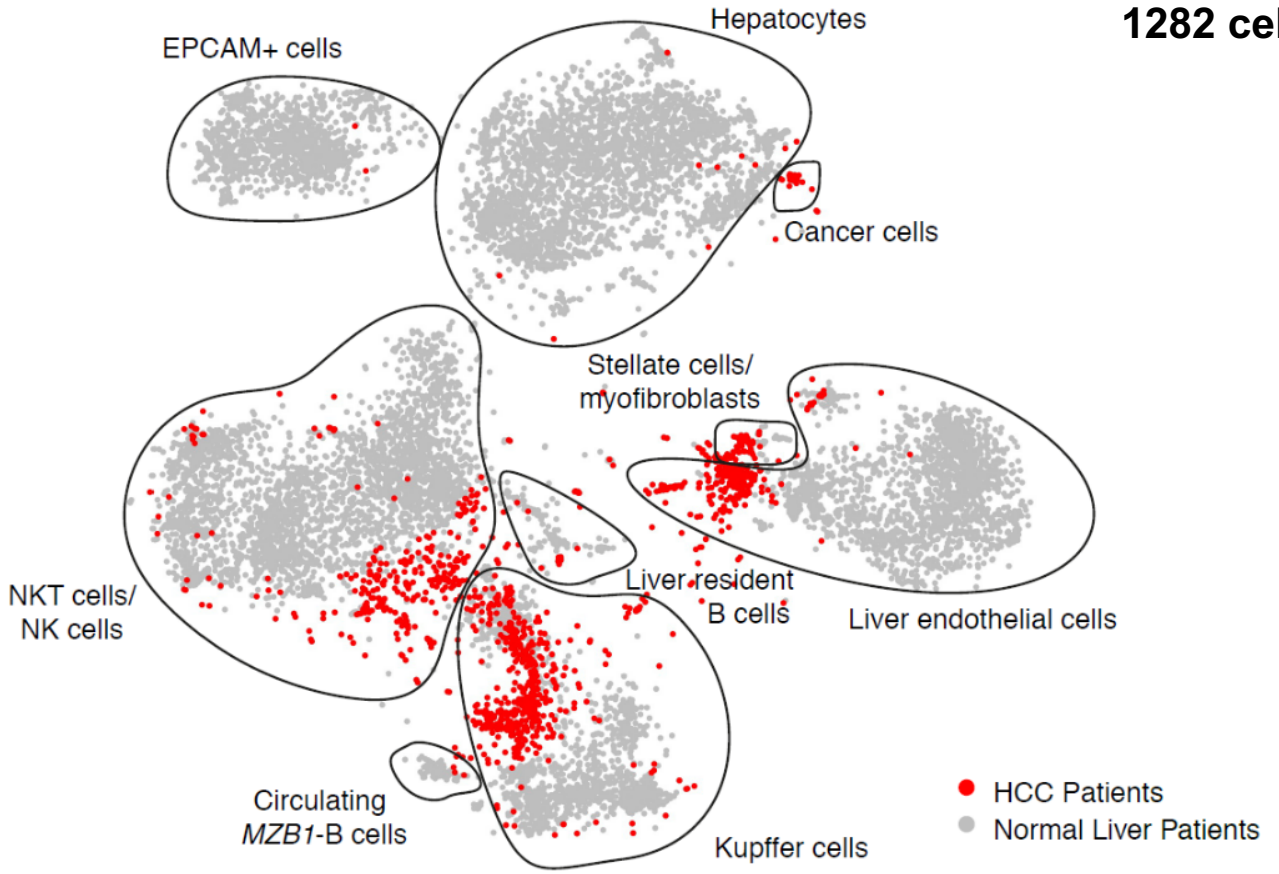
Diffusion maps (left) and self-organizing maps (SOM, middle) of single-cell transcriptome-derived zonation profiles for hepatocytes (n=2,534) and endothelial cells (n=1,361)



Immunostaining from the Human Protein Atlas

- **Zonation not only for hepatocytes but other liver resident cells: endothelial cells and NPCs**
- **Co-zonated genes and functions across hepatocytes and endothelial cells**
- **Comparison between mouse and human revealed only limited evolutionary conservation of gene expression zonation**

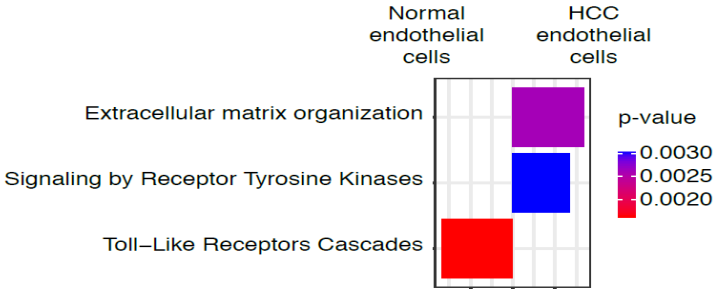
The human liver cell atlas as a reference to study HCC

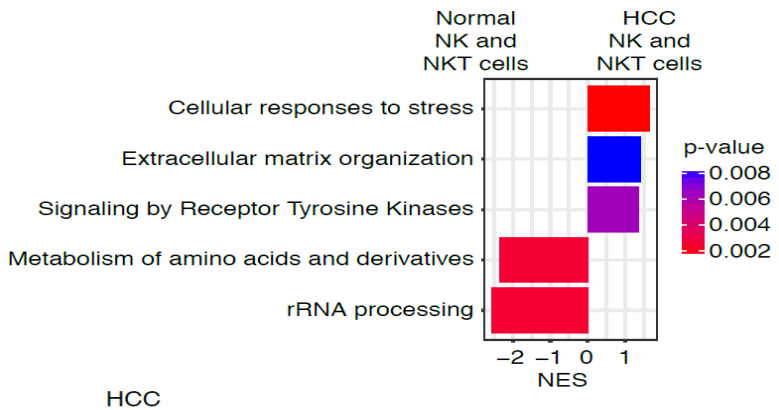


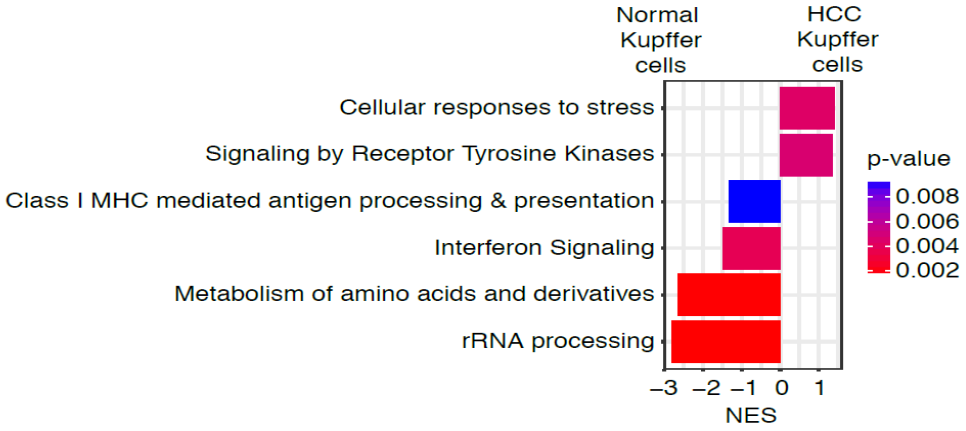
1282 cells from 3 HCCs with ALD

Aizarani N, Saviano A et al. *Nature 2019*

scRNA-seq of patient tumors unravels unique features of the HCC microenvironment





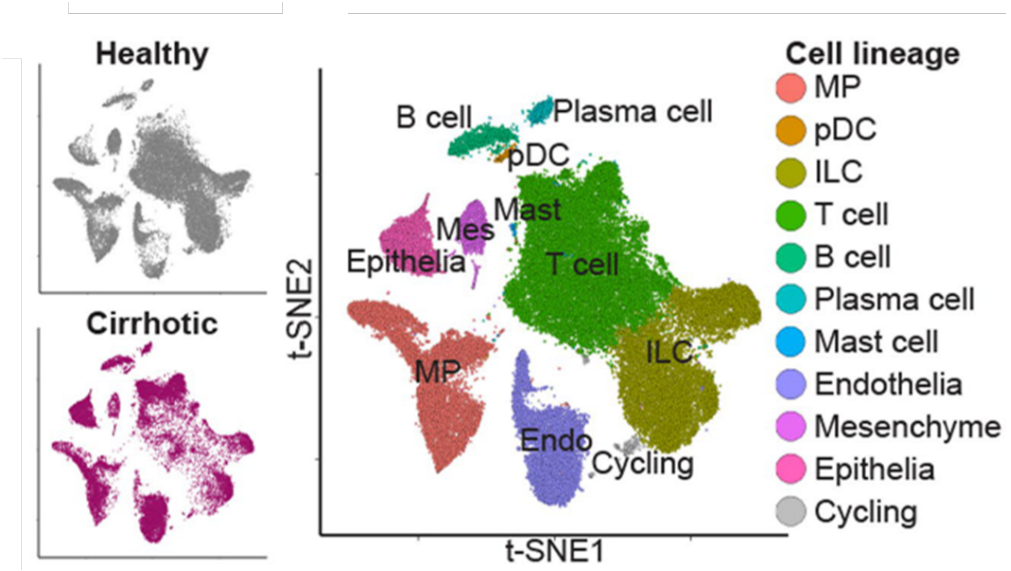


- ✓ **tumor-associated macrophages** (CD16⁺CD14⁺, VSIG4⁺ and CD68⁺ compartments)
- ✓ **cancer-associated fibroblasts** (aSMA⁺, PDGFRB⁺, GFAP⁺, Desmin⁺, CYGB⁺, CRBP-1⁺, FAP⁺ and SYN⁺ compartments)
- ✓ **endothelial cells** (PECAM⁺, AQP1⁺, CLEC4G⁺, CLEC4M⁺ compartments

Method: GSEA applied on specific single cell populations

- ➢ **HCC associated endothelial cells are enriched for ECM and tyrosine kinase gene expression, while TLR gene expression was suppressed**
- ➢ **HCC NK cells showed an upregulation of ECM and TK gene expression as well as a downregulation of rRNA processing and aminoacids metabolism**
- ➢ **Macrophages showed downregulation of gene expression for antigen processing and presentation**

scRNA-seq of advanced fibrotic liver disease including NAFLD



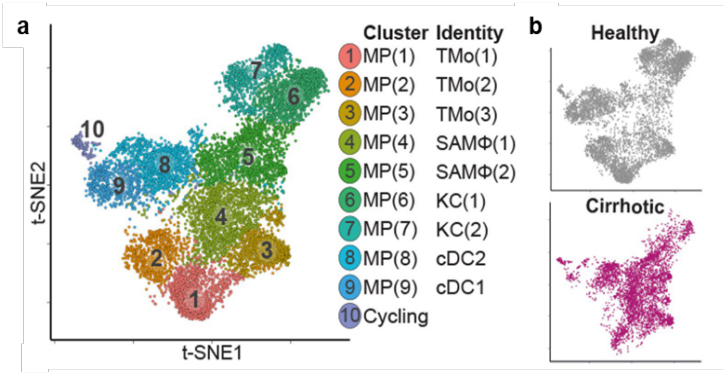
	Healthy liver (n=5)	Cirrhotic liver (n=5)	Blood (n=4)
Age (yrs)	57.4±7.9	56.6±5.8	63.2±3.8
Gender (M:F)	4:1	3:2	3:1
Aetiology of liver disease	N/A	2xNAFLD 2xALD 1xPBC	3xNAFLD 1xHH
Haemoglobin (g/l)	145±14	106±17	131±2.1
White Cell Count ($x10^9$/l)	8.2±2.2	5.9±1.7	3.7±1.5
Platelets ($x10^9$/l)	300±91	137±56	73±38
Prothrombin Time (s)	11.6±1.1	19.6±3.8	16.0±3.6
Creatinine (µmol/l)	76.4±14.5	96.8±28.6	74.5±9.7
Na^+ (mmol/l)	141±2.6	131±7.0	139±2.1
Bilirubin (µmol/l)	10±5.2	79.6±83.5	36.3±20.0
ALT (IU/l)	27.8±19.3	77.8±80.7	96.2±121.0
ALP (IU/l)	122±47	140±80	203±153
MELD Score	6.6±0.5	17.3±4.5	11.7±4.3

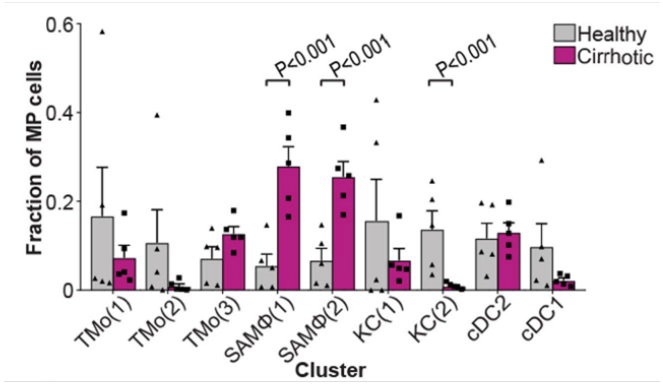
NAFLD:Non-alcoholic fatty liver disease; ALD:Alcohol-related liver dsease; PBC:Primary biliary cholangitis; HH:Hereditary haemochromatosis; ALT:Alanine transaminase; ALP:Alkaline Phosphatase; MELD:Model for End-Stage Liver Disease

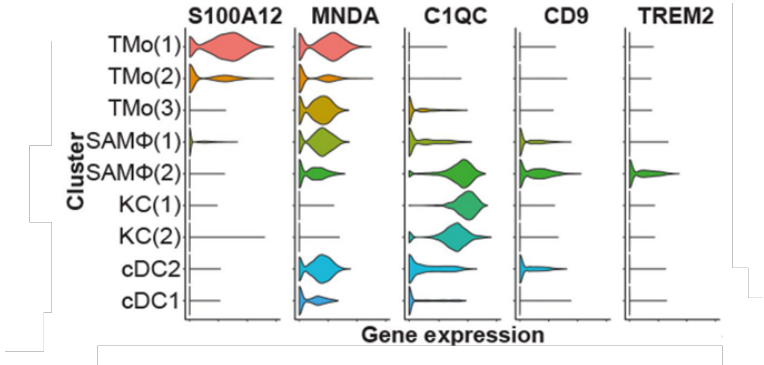
- **ScRNA-seq of 66,135 liver-resident cells from 10 livers (n = 5 healthy and n = 5 cirrhotic)**
- **Analysis of the fibrotic niche of human liver cirrhosis, identifying scar-associated cell types:**
  - **TREM2+CD9+ macrophages**
  - **ACKR1+ and PLVAP+ endothelial cells**
  - **PDGFRα+ collagen-producing myofibroblasts**

Ramachandran et al, *Nature 2019*

Identification of a novel scar-associated macrophage



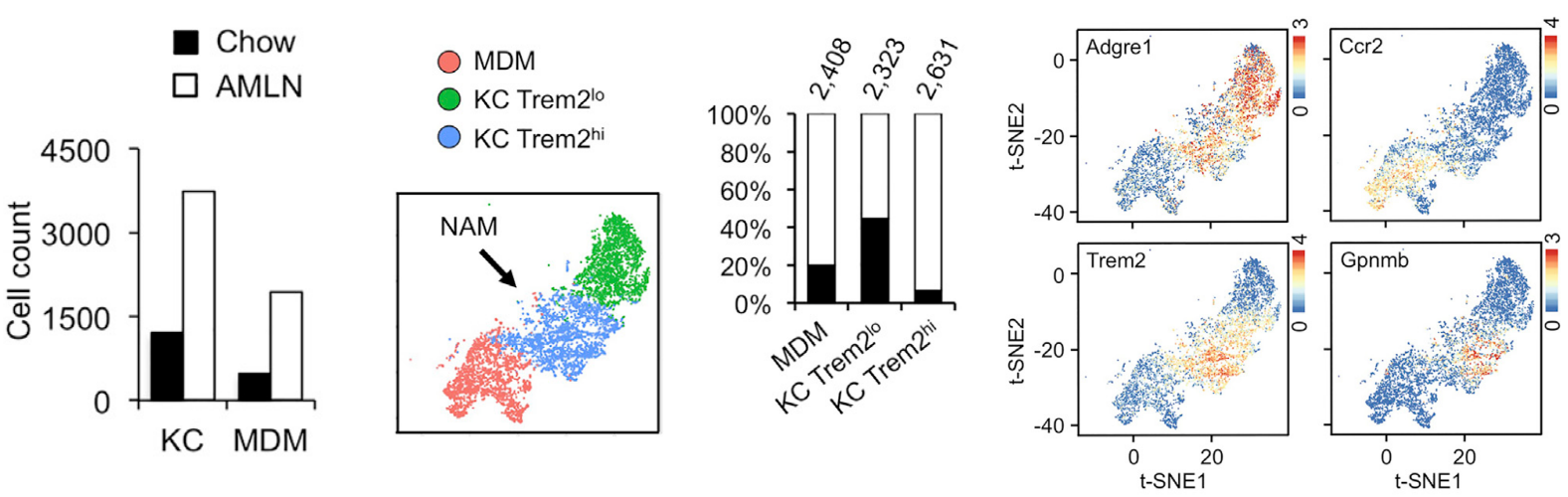




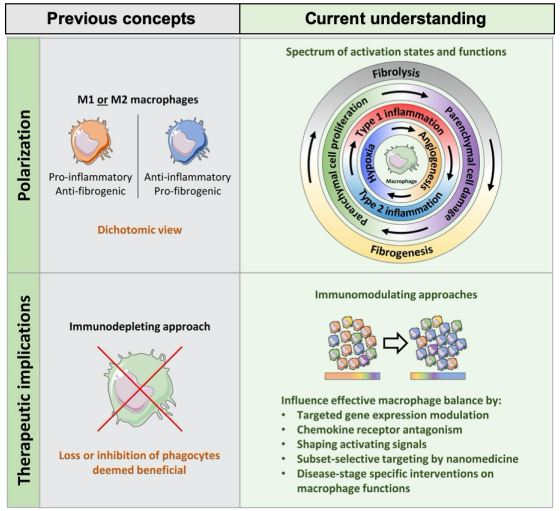
- **Scar-associated macrophages (SAMΦ) express the unique markers TREM2 and CD9 and displayed a hybrid phenotype, with features of both tissue monocytes (TMo) and KCs.**
- **TREM2 encodes for an innate immunity scavenger receptor implicated in phagocytosis and clearance of apoptotic cells**
- **Self-organizing maps and pseudotime analysis at single-cell level revealed that SAMΦ are derived from blood monocytes.**
- **The differentiation process towards SAMΦ fate involved the expression of genes related to antigen processing and presentation, phagocytosis, chemokines, angiogenesis, production of extracellular matrix and wound healing.**
- **SAMΦ interact with scar-associated endothelial cells and mesenchymal cells in the fibrotic niche**

Ramachandran et al, *Nature 2019*

ScRNA-Seq analyses in NASH diet mouse model supports pathogenic role of macrophages



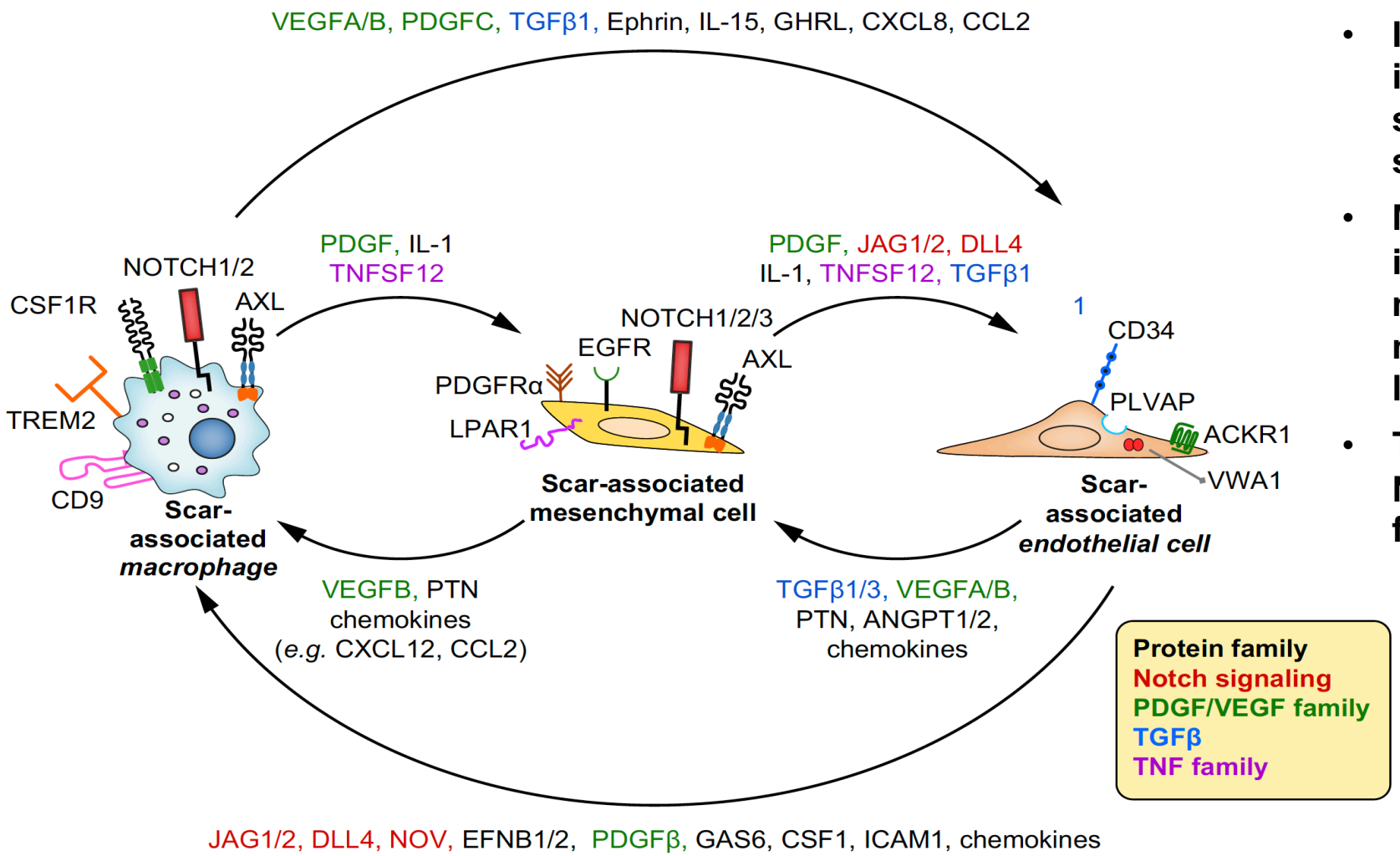
Xiong et al, *Mol Cell 2019*



Guillot and Tacke, *Hepatology Communications* 2019

- **In NASH AMLN diet mouse model, KC and monocyte-derived macrophages (MDM) are enriched**
- **A NASH-associated macrophage (NAM) TREM2-high, GPMB+ accounts for large fraction of liver macrophages in NASH mouse model.**
- **Liver *TREM2^{high}* macrophages were enriched in genes involved in antigen presentation, ECM remodeling, endocytosis and lysosomal degradation suggesting a role in NASH pathogenesis**
- **Supports evolving concept of macrophage plasticity and diversity and their impact in disease biology**

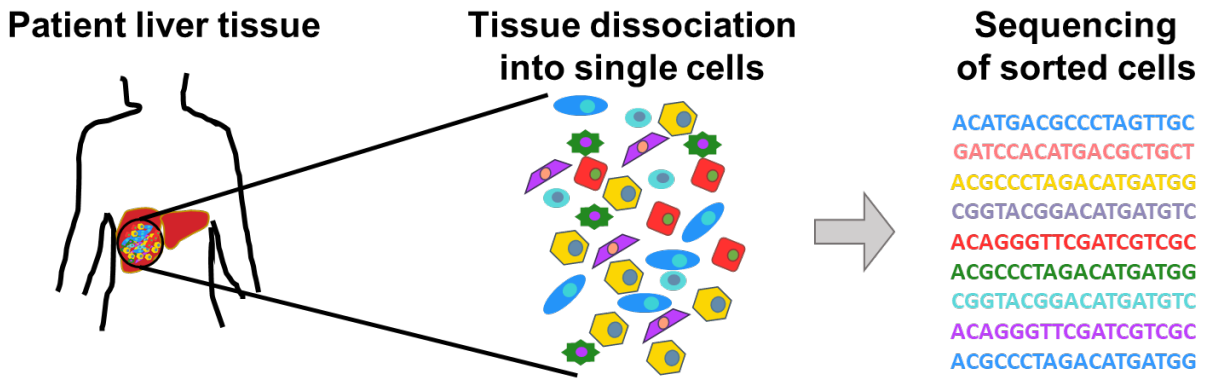
Modelling of cell--cell communication and signalling in the fibrotic niche

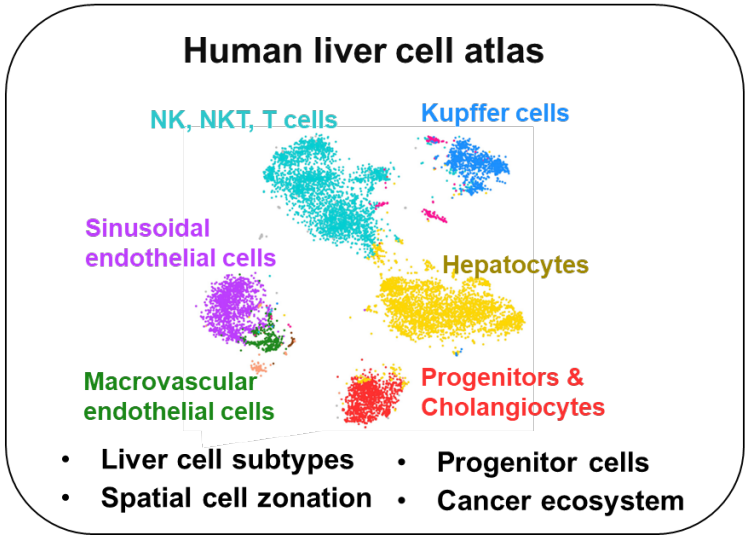


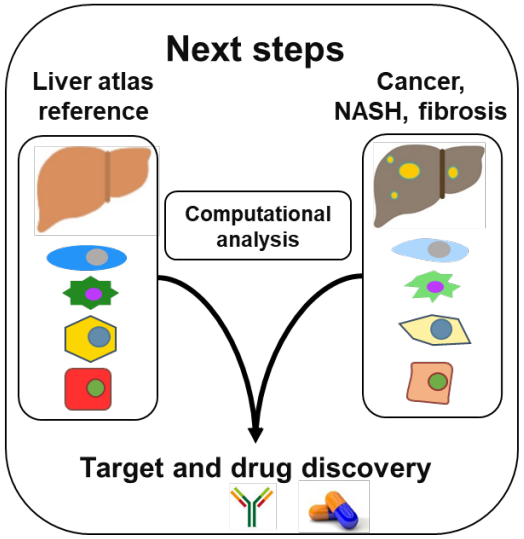
- **Intercellular ligand-receptor interactions in the human liver fibrotic niche based on scRNASeq combined with functional studies.**
- **Main receptors and ligands involved in interactions between scar-associated macrophages, scar-associated mesenchymal cells and scar-associated liver endothelia cells are presented.**
- **The most relevant molecules belong to Notch, PDGF, VEGF, TGFb and TNF families.**

Cartoon from: Saviano A, Henderson NC, Baumert TF, *J Hepatol 2020*

Conclusions







- ✓ **scRNA-seq has paved the way for the discovery of previously unknown cell types and subtypes in normal and diseased liver.**
- ✓ **The study of the phenotype and functional role of nonparenchymal cells (NPC) in chronic liver disease and cancer is transforming our knowledge of the liver microenvironment.**
- ✓ **scRNA-seq analyses of human liver tissues in advanced fibrosis and NAFLD have identified scar-associated macrophages as a mediator of liver disease biology.**
- ✓ **Functional studies unraveled novel mechanisms of NPC cell-cell communication.**
- ✓ **scRNA analyses of hepatocytes still pending**
- ✓ **scRNA-Seq combined with functional studies will enable the discovery of novel drugs and targets for NASH and liver fibrosis**

Acknowledgements

Inserm Institute of Viral and Liver Diseases, Inserm U1110, University of Strasbourg, France

Antonio Saviano
Frank Jühling
Emilie Crouchet
Laurent Mailly
Eloi Verrier
Sarah Durand
Christine Thumann
Emilie Crouchet
Andres Roca
Laura Heydmann
Charlotte Bach
Catherine Fauvelle
Hussein-El-Saghire
François Duong
Marine Oudet
Mirjam B. Zeisel
Joachim Lupberger
Catherine Schuster

Center of Digestive and Liver Diseases Strasbourg University Hospitals
Patrick Pessaux
Emanuele Felli

Broad Institute of MIT and Harvard, Cambridge
Nathalie Pochet
Alex Shalek
Aviv Regev

Max-Planck Institute for Immunobiology and Epigenetics Freiburg
Nadim Aizarani
Sagar
Dominic Grün

University of Edinburgh
Neil Henderson

Laboratory of Excellence HepSys

TheraHCC Consortium

EU H2020

1R21CA209940-01A1

ERC AdG HEPCIR, ERC PoC HEPCAN