Chemomab
THERAPEUTICS

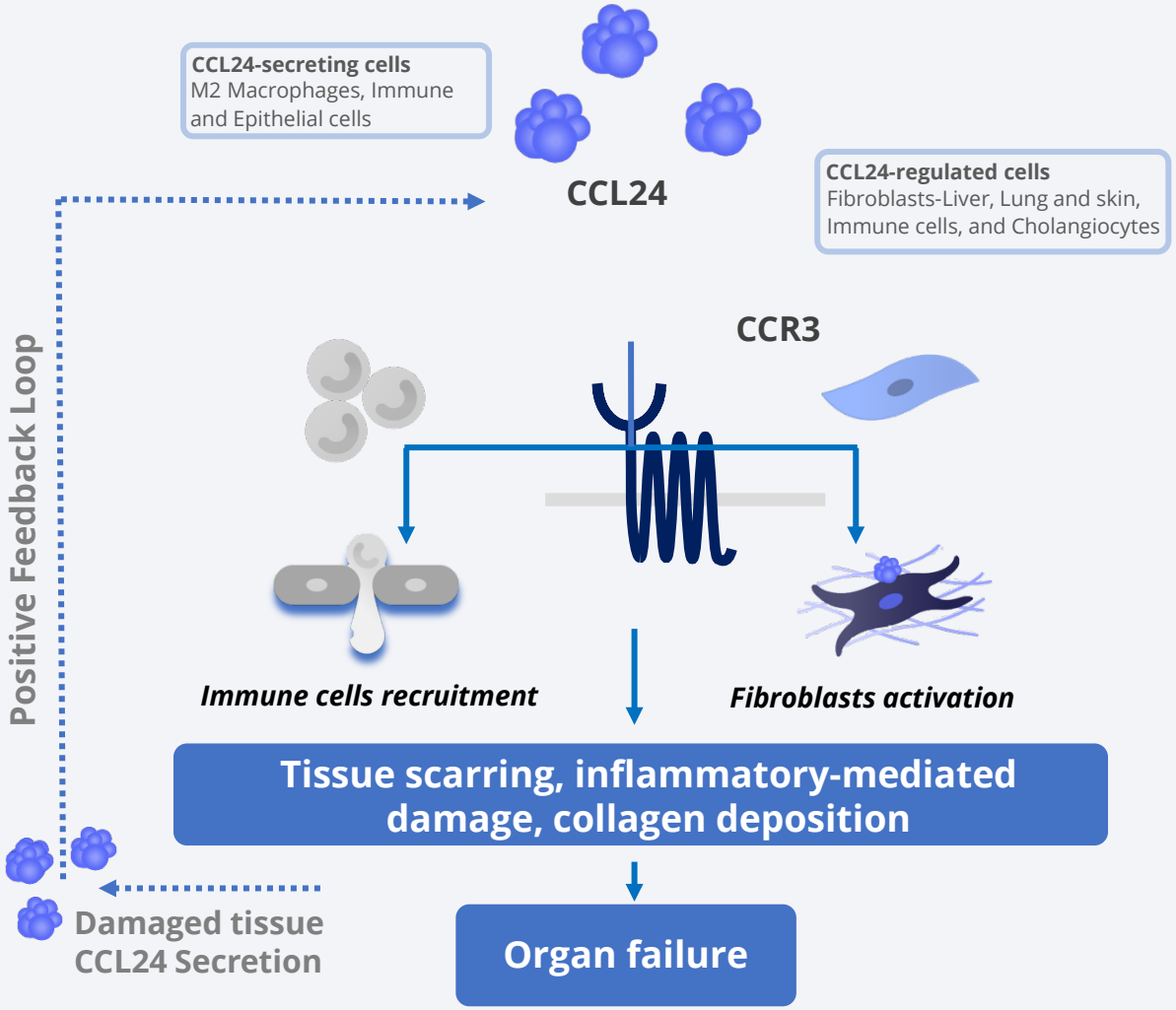
Combination of Whole Liver Single Cell RNA Sequencing and Spatial Transcriptomics Reveals Specific Cell Sub-Populations and Pathways Regulated by CCL24

June 2022

EASL™
The Home of Hepatology

CCL24 is a Novel Therapeutic Target for Fibrosis

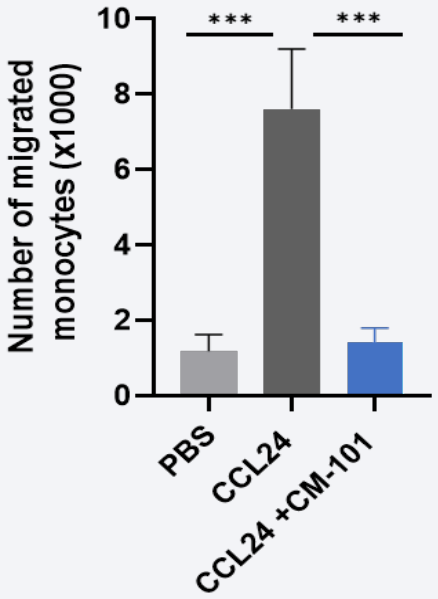
Critical Mediator Promoting Inflammation and Fibrosis

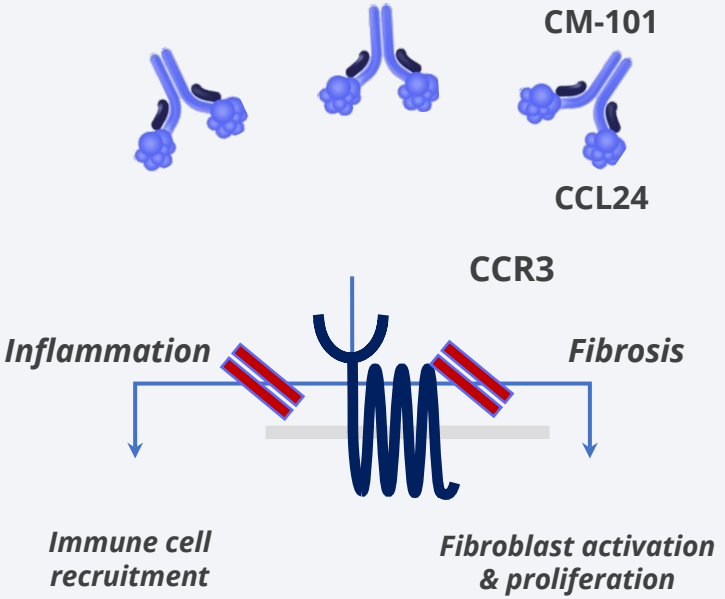


- ✓ **Dual role in promoting fibrosis**
  - directly activates fibroblasts
  - enhances local immune cell recruitment
- ✓ **Unique and differentiated activity**
  - ex vivo and in vivo data confirms unique role vs other CCLs
  - correlates with disease outcome and fibrotic biomarkers
- ✓ **Minor expression in healthy tissue**
  - significantly elevated in liver, skin, lung fibrotic tissue
  - wide therapeutic margin
- ✓ **Positive feedback loop potentiates tissue damage**
  - responsible for initiation and perpetuation of fibrosis

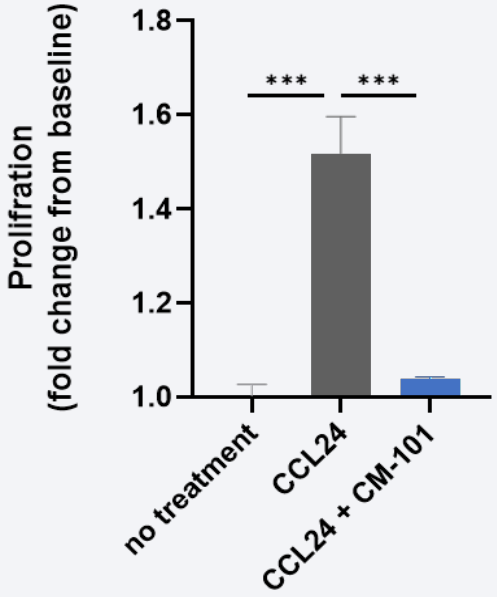
CM-101: First-in-Class mAb Blocking CCL24

CM-101 Reduces *In vivo* Monocyte Recruitment





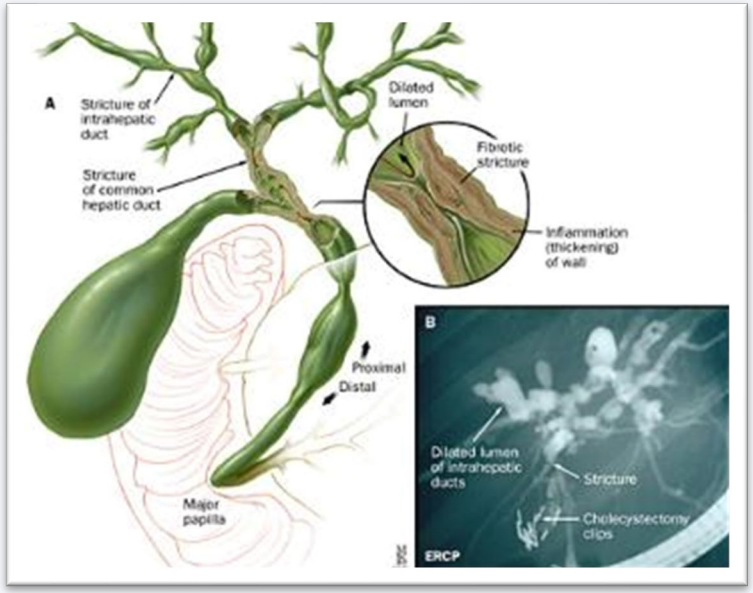
CM-101 Inhibits Primary Hepatic Fibroblast Proliferation



CM-101 attenuates inflammation and fibrosis by inhibiting immune cell recruitment and fibroblast activation

Primary Sclerosing Cholangitis (PSC)

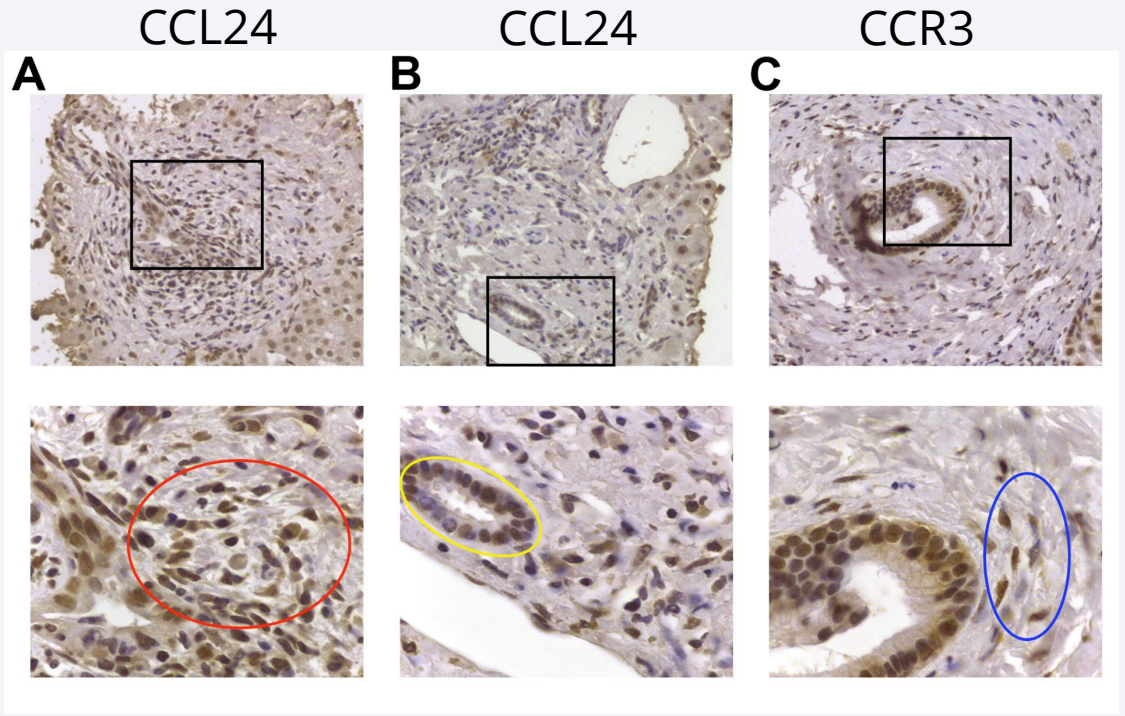
- Chronic **bile duct inflammatory and fibrotic disease** leading to end-stage liver disease and cirrhosis
- 70% of PSC patients have concomitant inflammatory bowel disease
- No FDA approved drug
- **Median Survival of 10-12 years** with no intervention
- ~**70K PSC** patients in 7 major markets
- **Orphan Drug Designation** granted for PSC by FDA and EMA

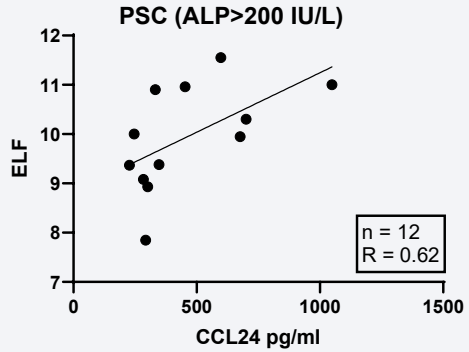


https://www.hopkinsmedicine.org/health/conditions-and-diseases/primary-sclerosing-cholangitis

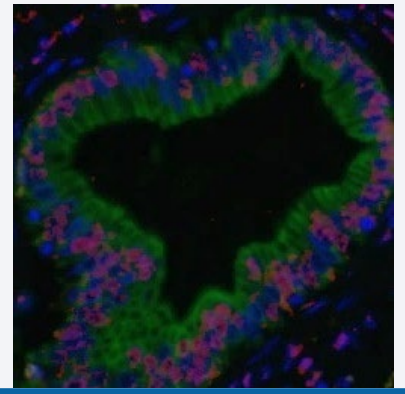
CCL24 and CCR3 are Highly Expressed in PSC Patients' Liver Biopsies

- High CCL24 expression in biliary epithelial cells (BEC) and immune cells (including macrophages)
- High CCR3 expression in BEC, immune cells and hepatic stellate cells (HSC)
- High serum CCL24 levels correlate with fibrosis stage (ELF)

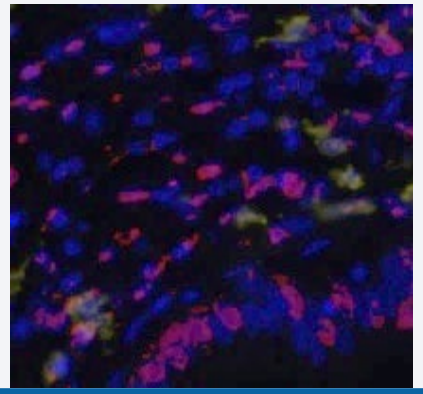




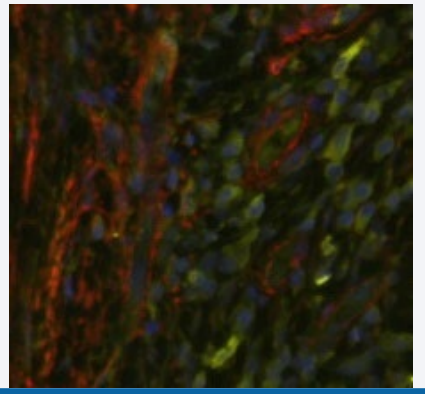
CCL24 / PanCK



CCL24 / Iba1



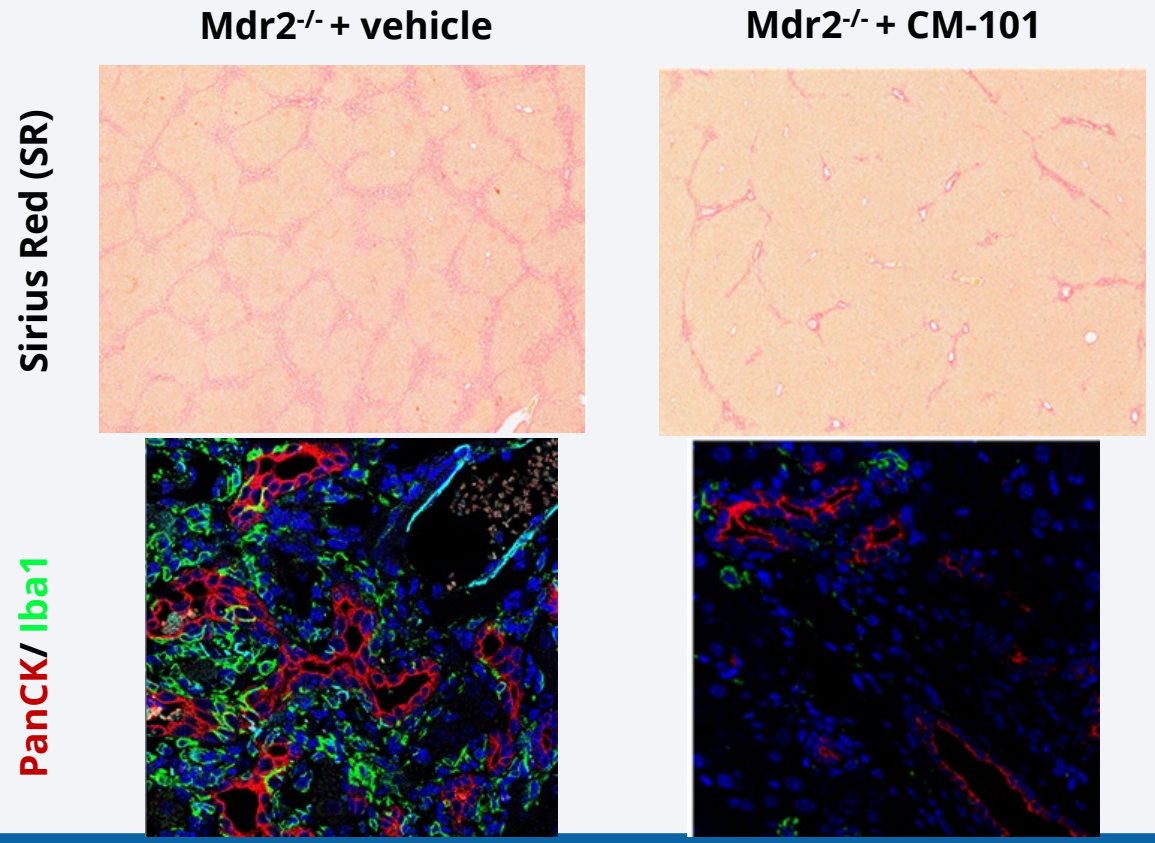
αSMA / CCR3

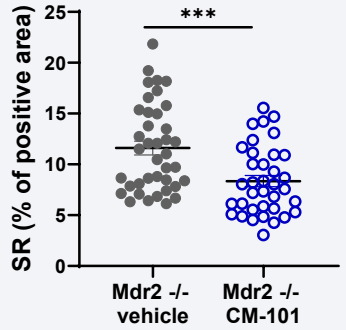


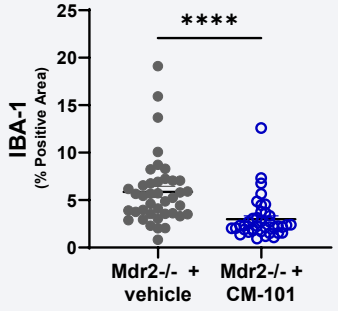
PanCK – BEC
Iba1 – macrophages
aSMA – fibroblasts

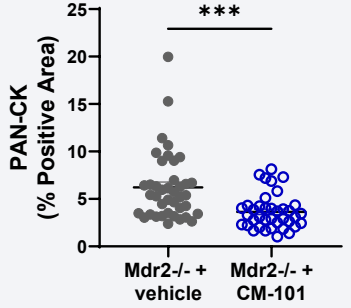
CM-101 Decreases Liver Fibrosis and Biliary Mass in the Mdr2$^{-/-}$ PSC Model

- Mdr2$^{-/-}$ mice develop similar PSC features in terms of cholangitis, severe ductular reaction and fibrosis
- CM-101 (D8) treatment (10 mg/kg) reduces serum levels of ALP, bile acids and ALT
- CM-101 (D8) treatment (10 mg/kg) reduces liver levels of collagen, Timp1, biliary mass and liver macrophages





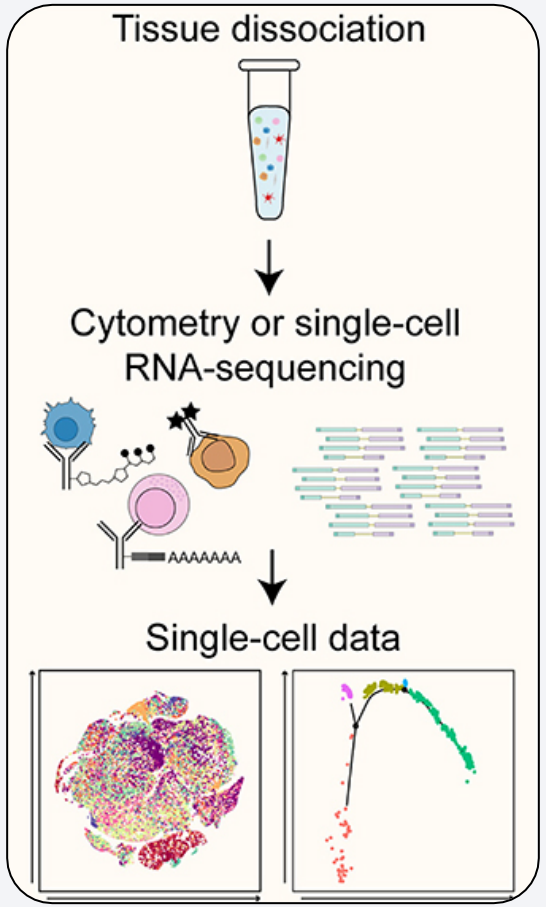


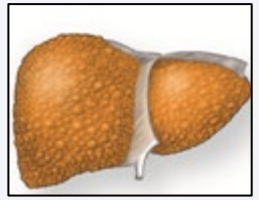


PanCK – BEC
Iba1 - macrophages

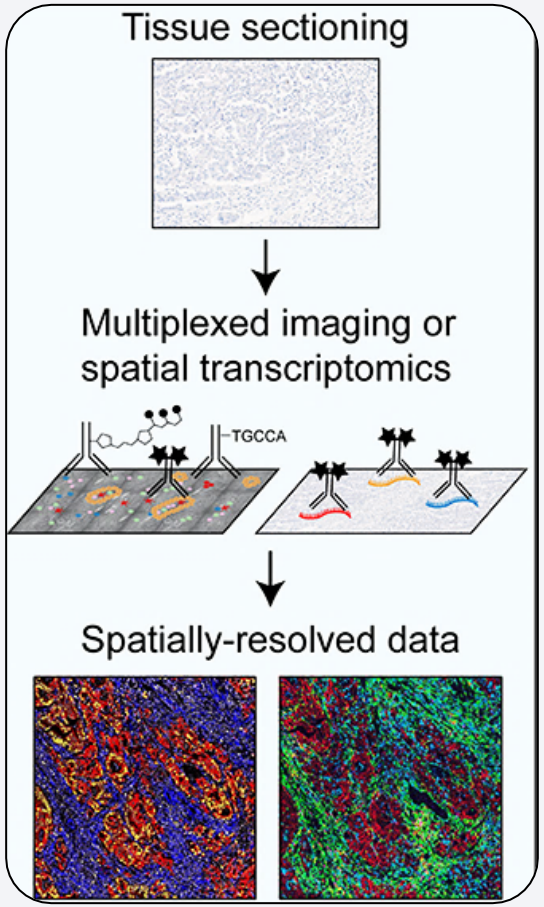
Studying CCL24 Pathways Using Two Complementary High-Throughput Methods

Single cell RNA sequencing (scRNA-seq)



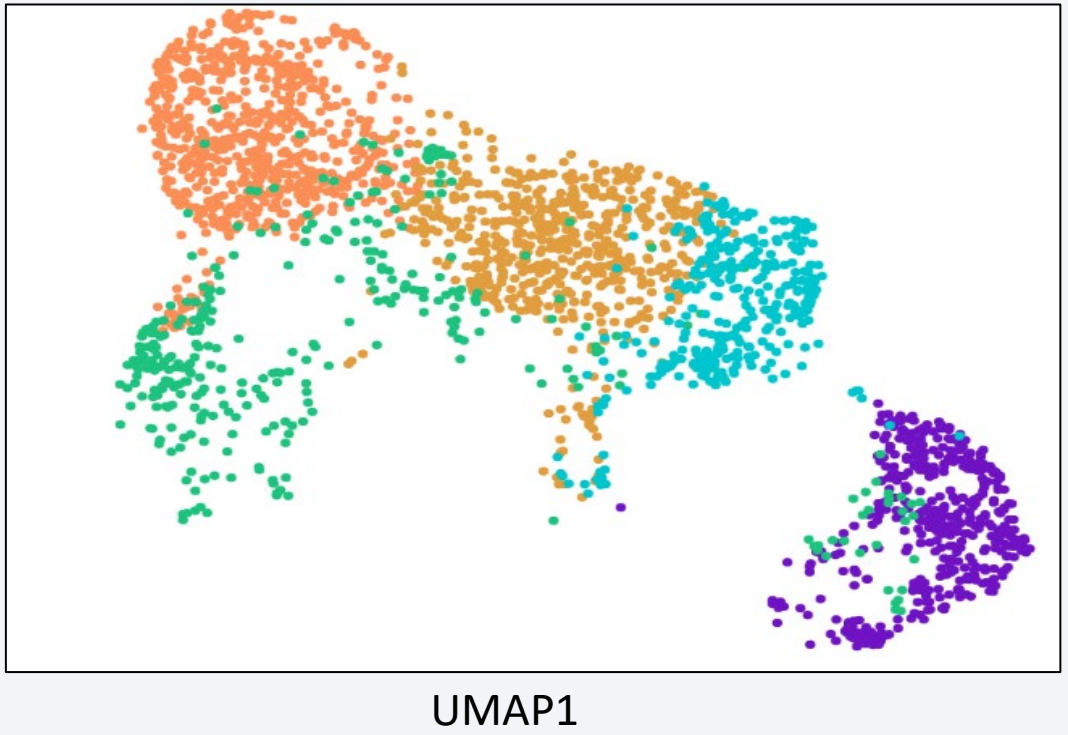


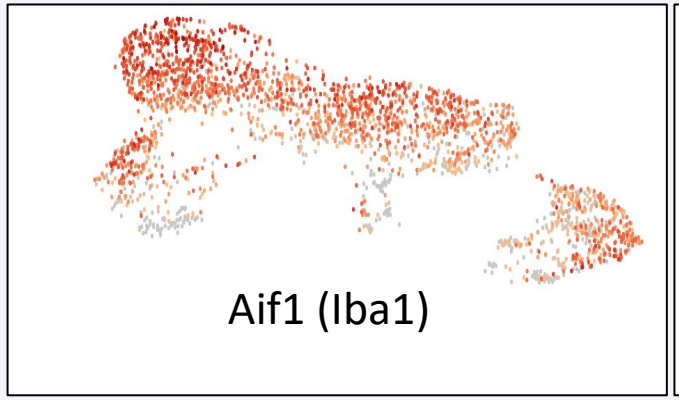
Spatial transcriptomics

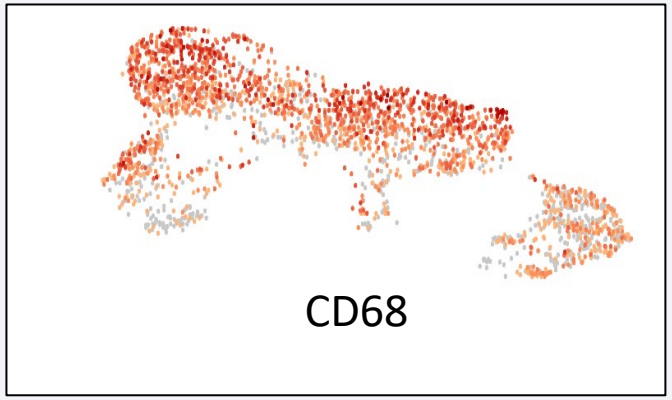


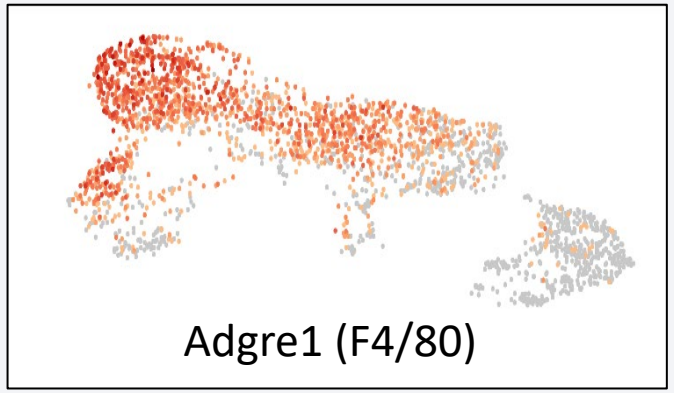
Adopted from de Vries et al, Front Oncol 2020

scRNA-seq of Mdr2$^{-/-}$ Liver Identifies Five Populations of Mononuclear Phagocytes





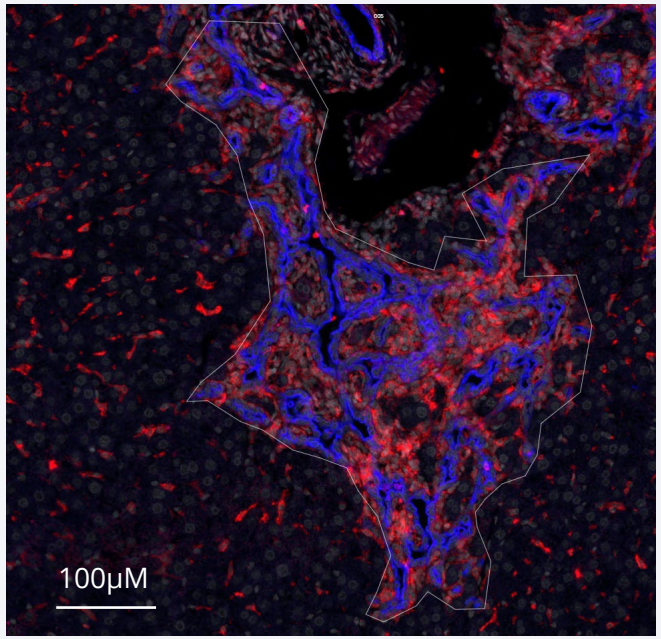




Spatial Transcriptomics of the Damaged Periductal Area of Mdr2$^{-/-}$ Mice

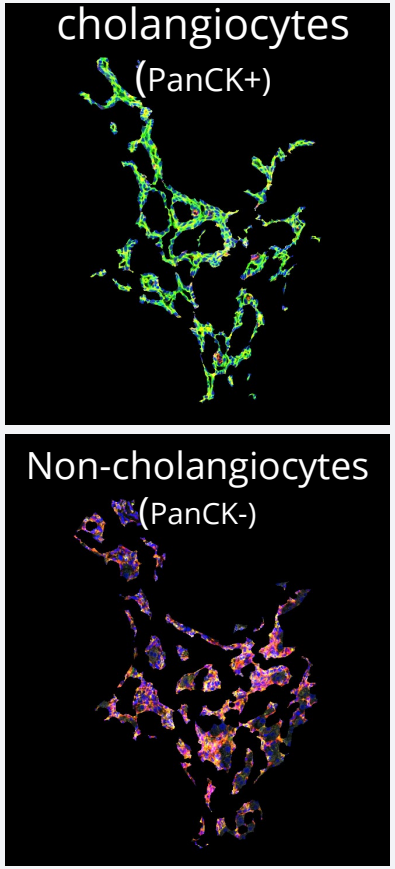
Stain

PanCK / F4/80



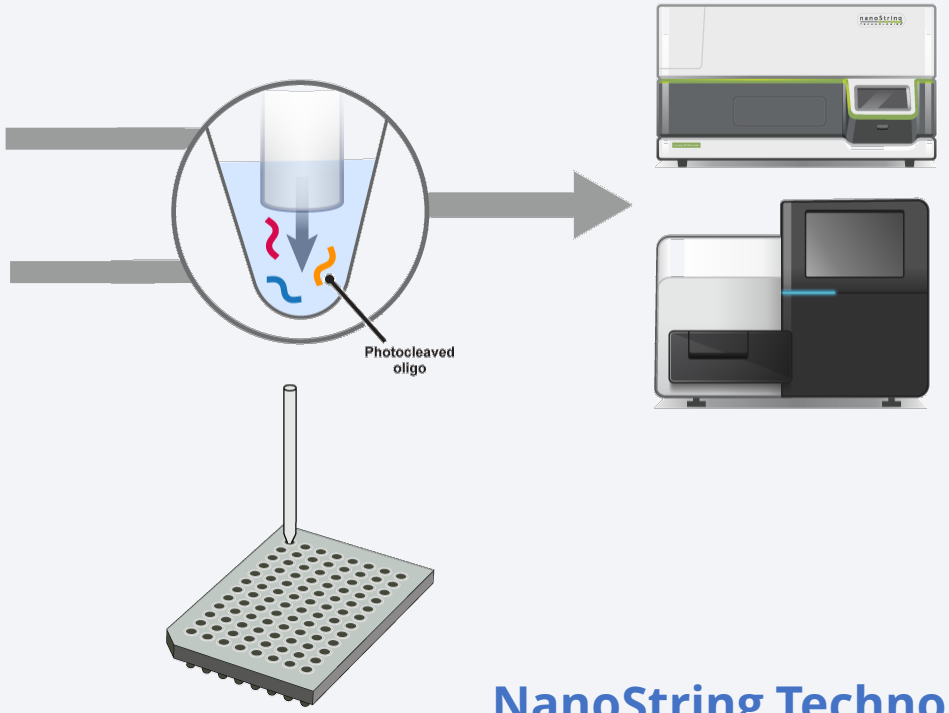
PanCK – BEC
F4/80 - macrophages

Segment periductal area



Collect oligos

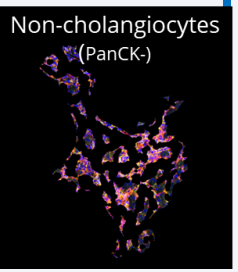
Count



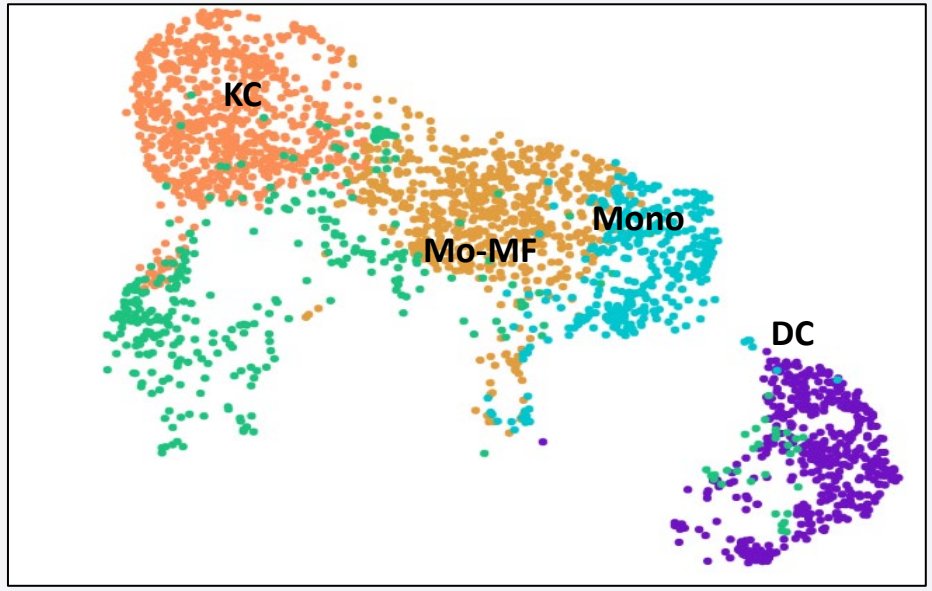
N = 12 ROIs, 4 mice

NanoString Technologies

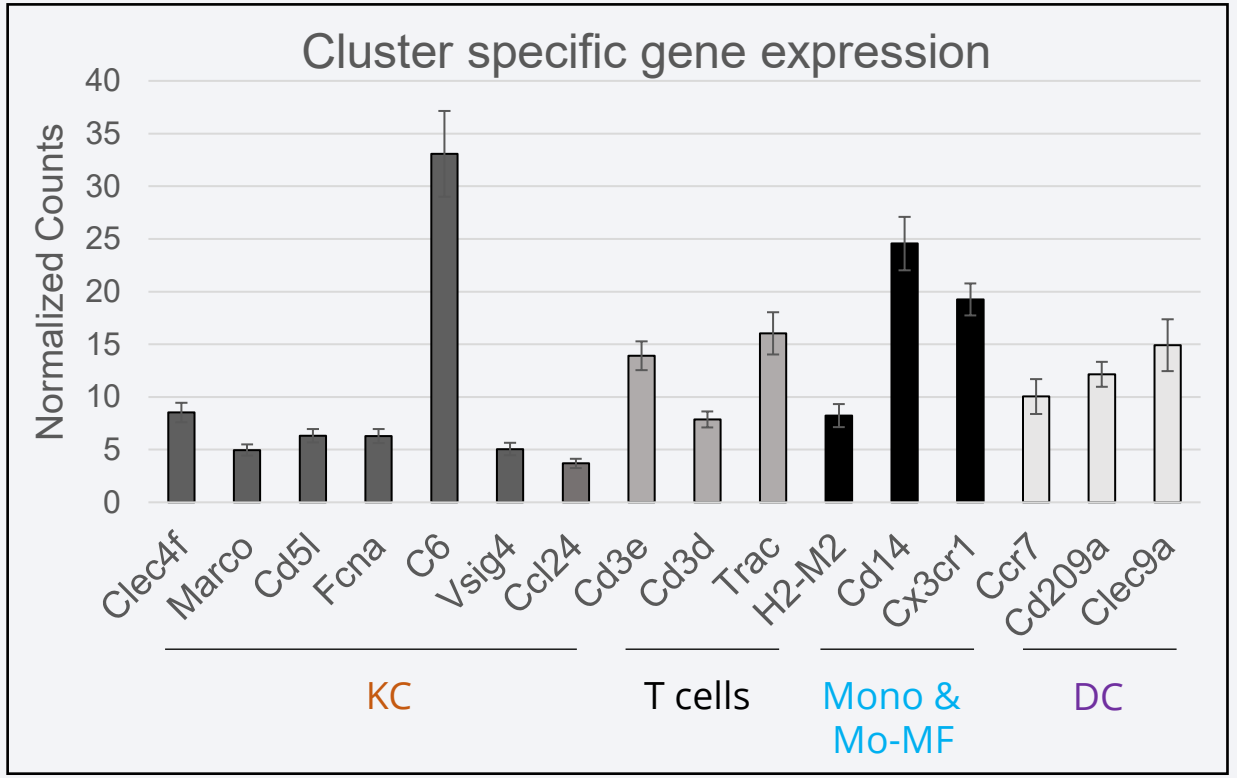
Combination of scRNA-seq and Spatial Transcriptomics Identifies Kupffer Cells in the Injured Peribiliary Area



scRNA-seq

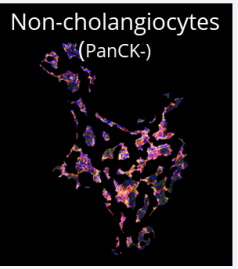


Spatial Transcriptomics



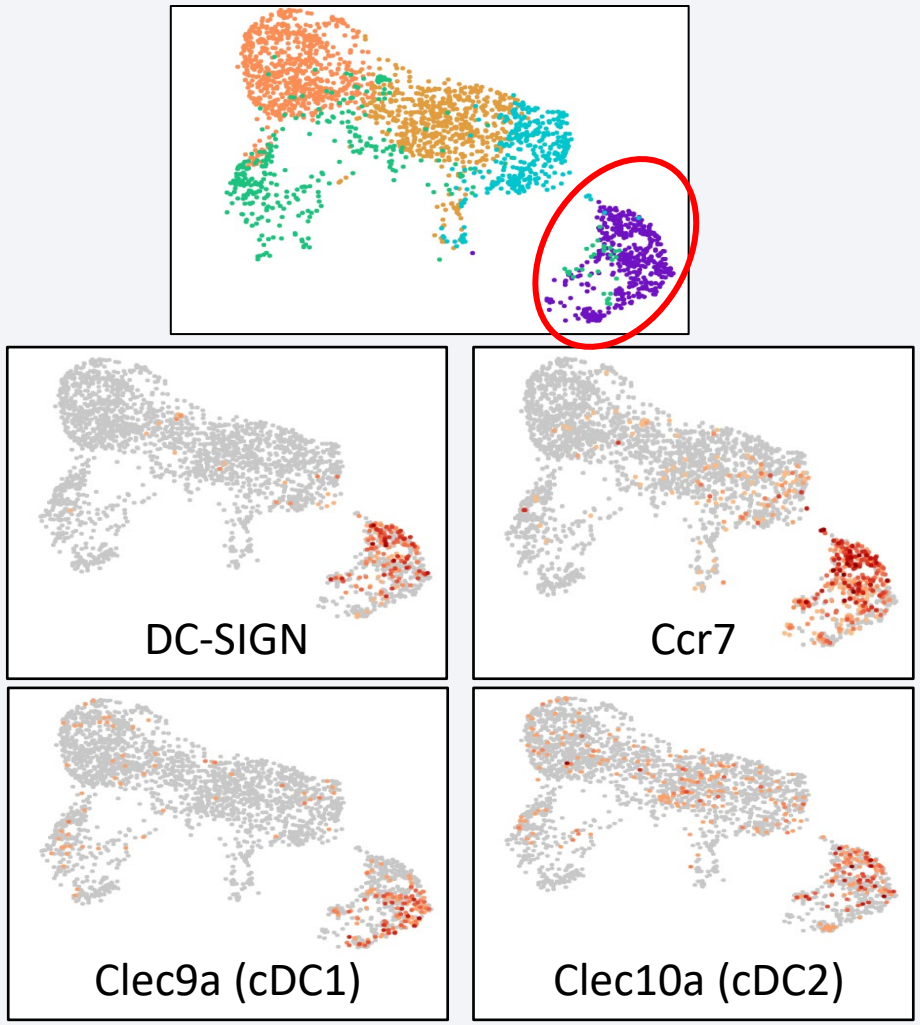
KC – Kupffer cells
Mono – monocytes
Mo-MF – monocytes-derived macrophages
DC – dendritic cells

CM-101 Reduces Type 2 Dendritic Cells in the Peribiliary Area

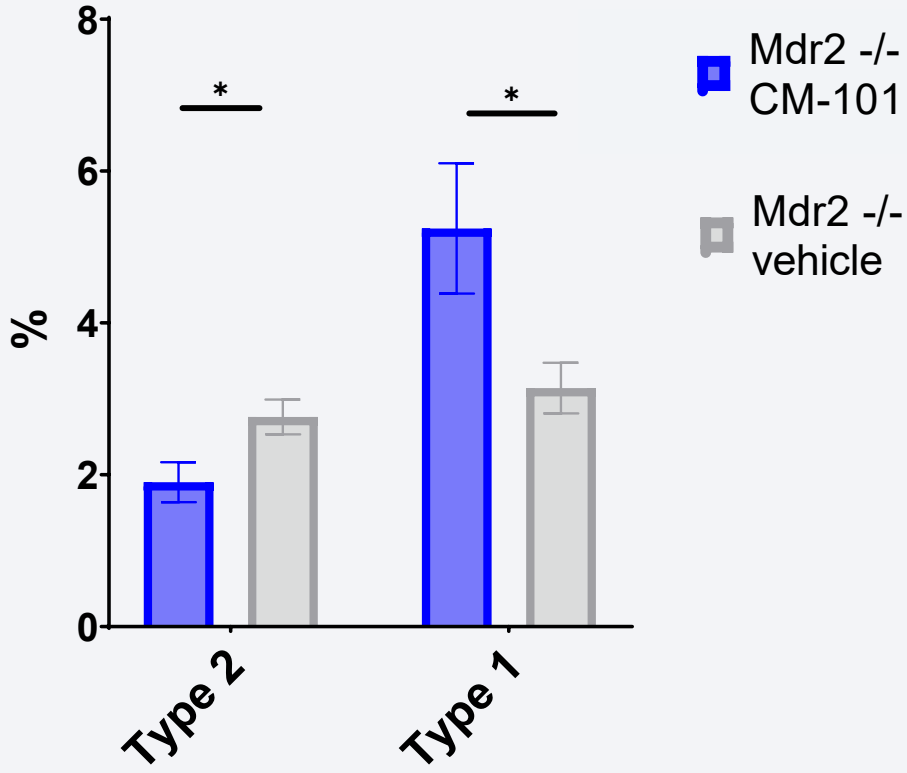


cDC1 – classical type 1 dendritic cells
cDC2 – classical type 2 dendritic cells

scRNA-seq

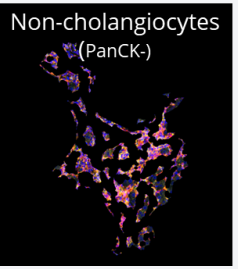


Spatial Transcriptomics (cell deconvolution)

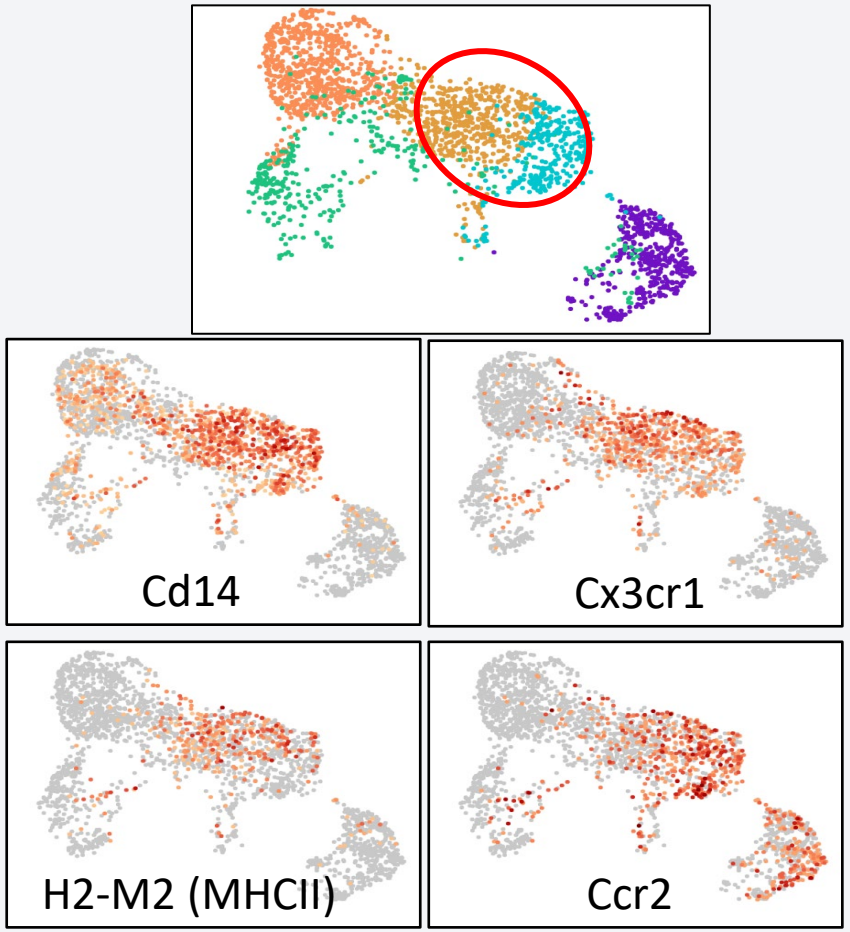


NanoString cell-type gene signature, based on ImmGen classification

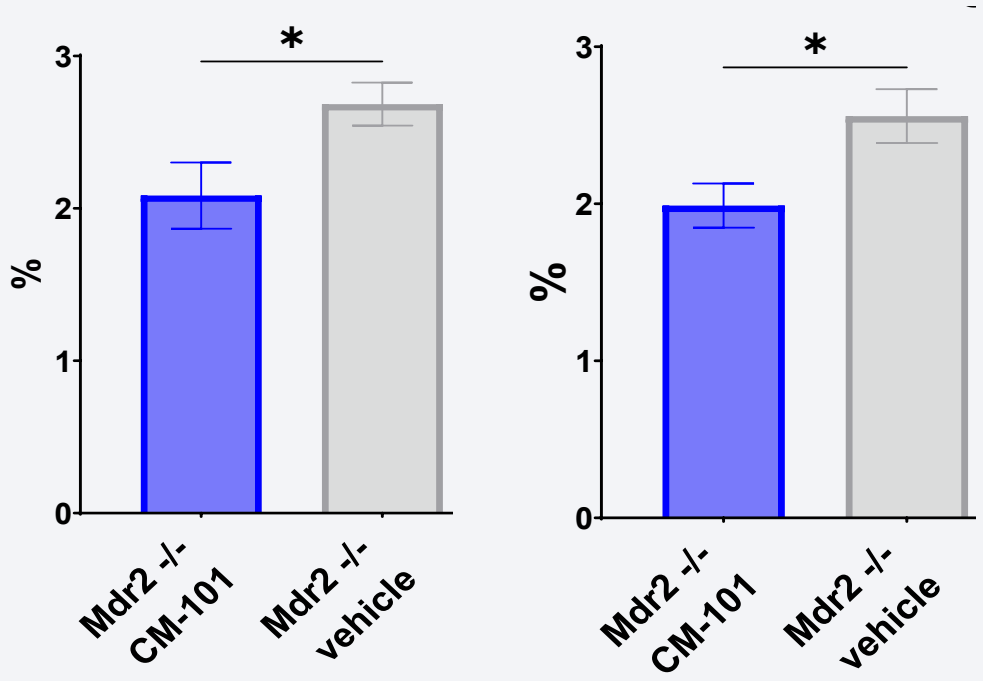
CM-101 Reduces Macrophage and Monocyte Recruitment to the Peribiliary Area



scRNA-seq

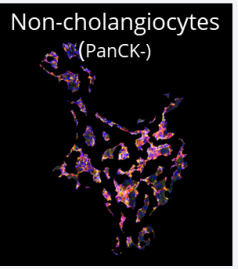


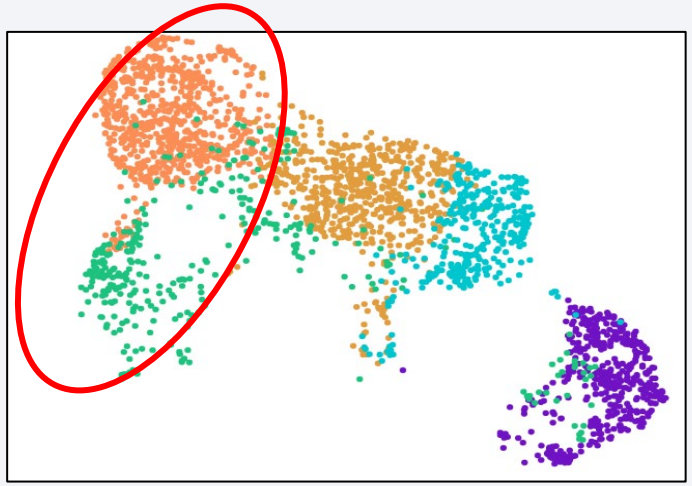
Spatial Transcriptomics (cell deconvolution)

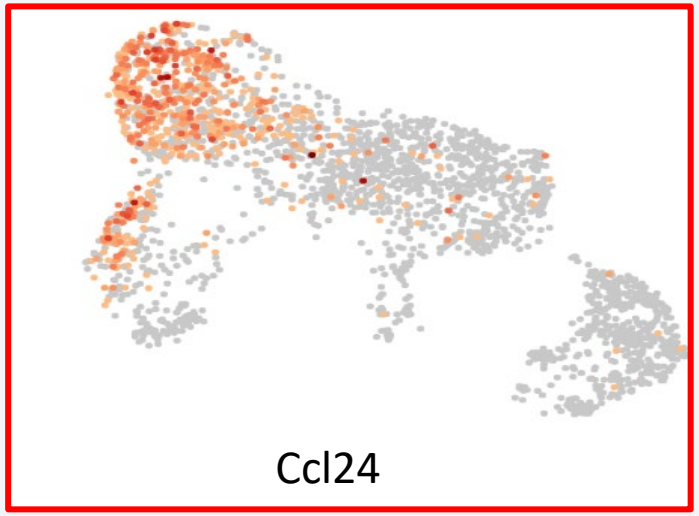


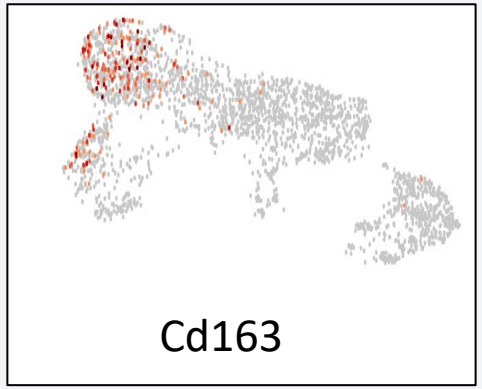
NanoString cell-type gene signature, based on ImmGen classification

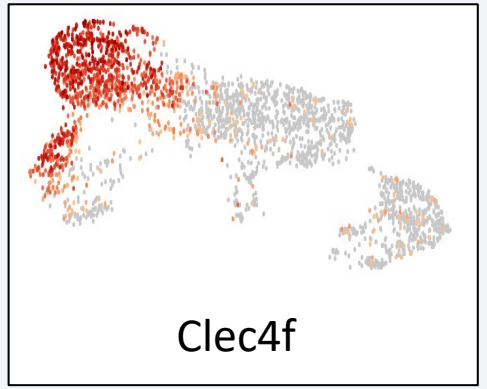
CCL24 is Expressed by Two Resident Liver Macrophage Populations

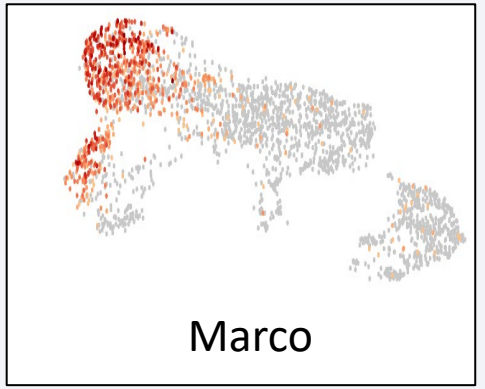


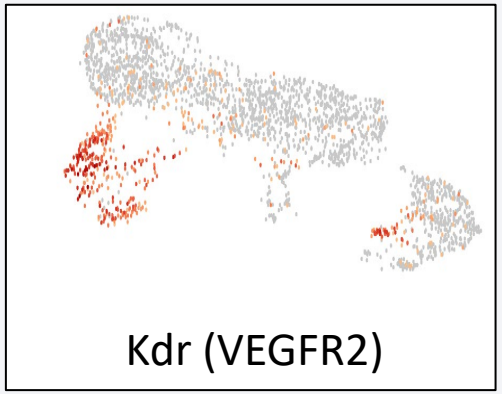


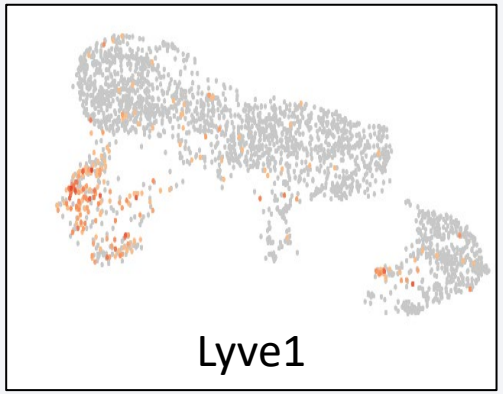




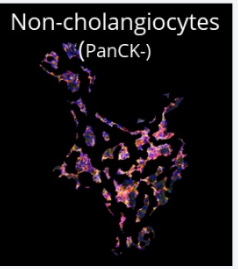




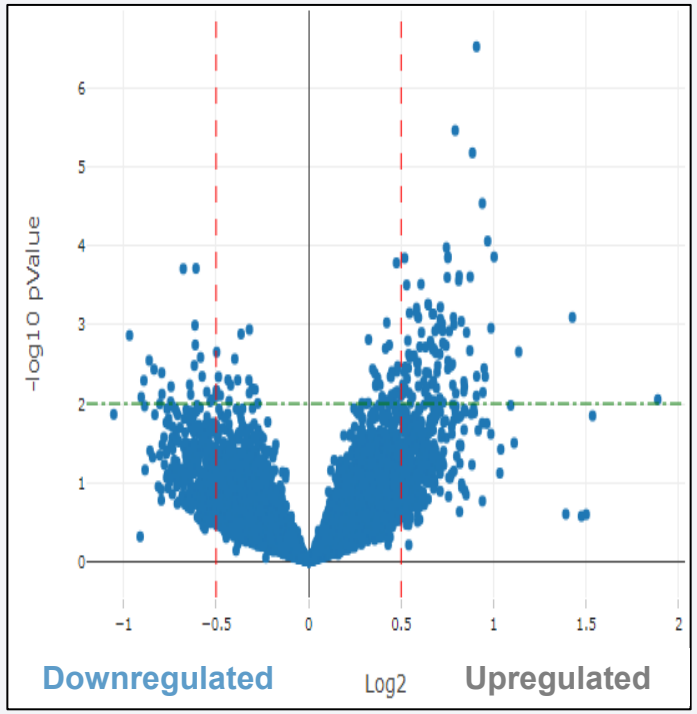




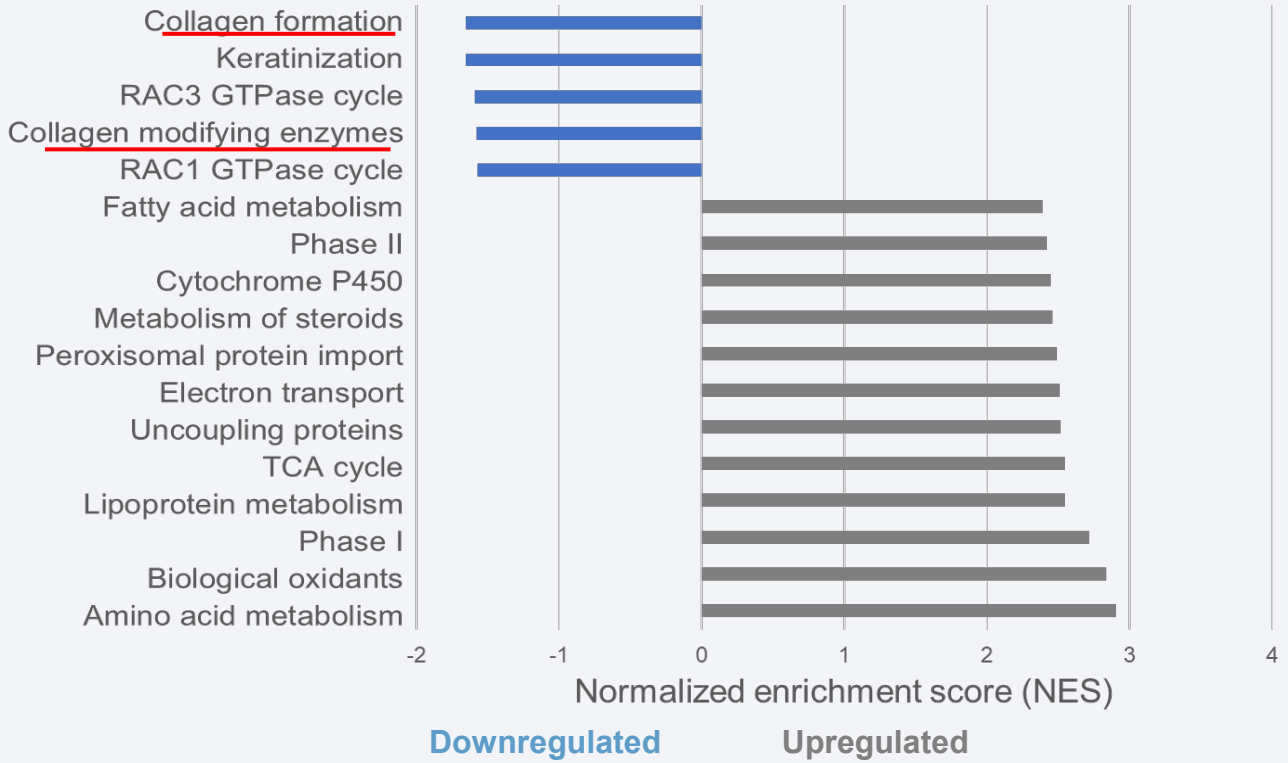
CM-101 Treatment Reduces ECM Formation Pathways in Peribiliary PanCK- Populations



Differentially Expressed Genes

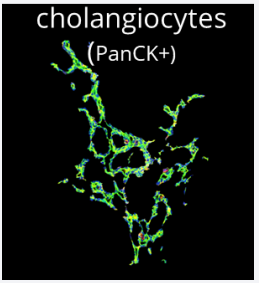


Gene Set Enrichment Analysis

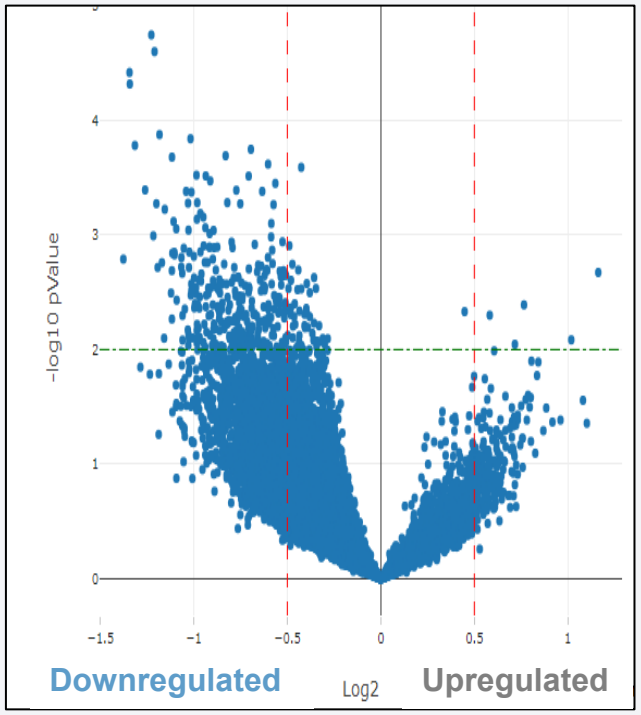


Collagen formation pathways

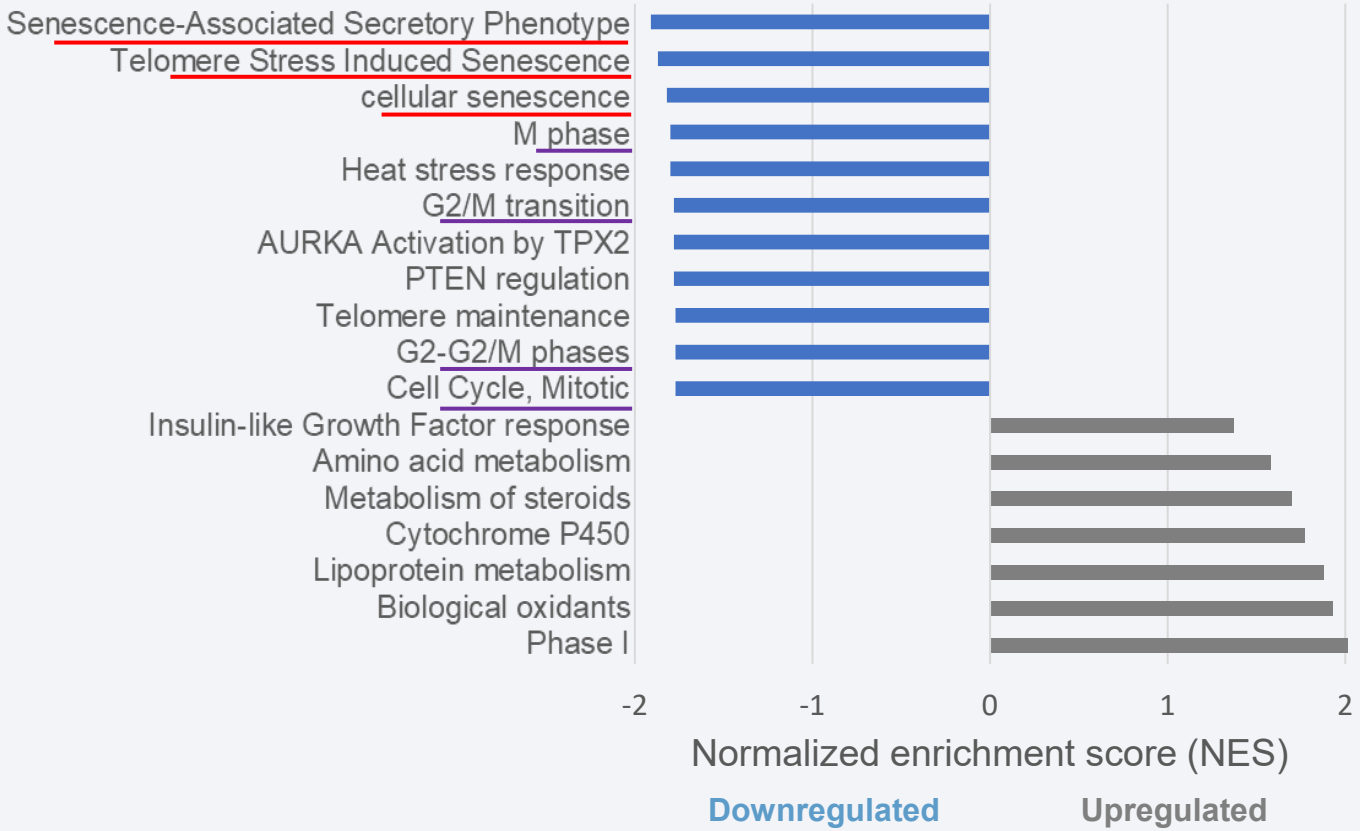
CM-101 Treatment Reduces Cholangiocyte Senescence and Proliferation



Differentially Expressed Genes



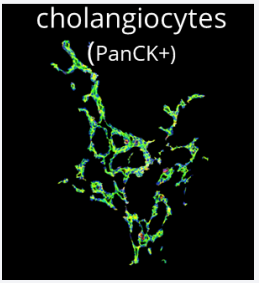
Gene Set Enrichment Analysis

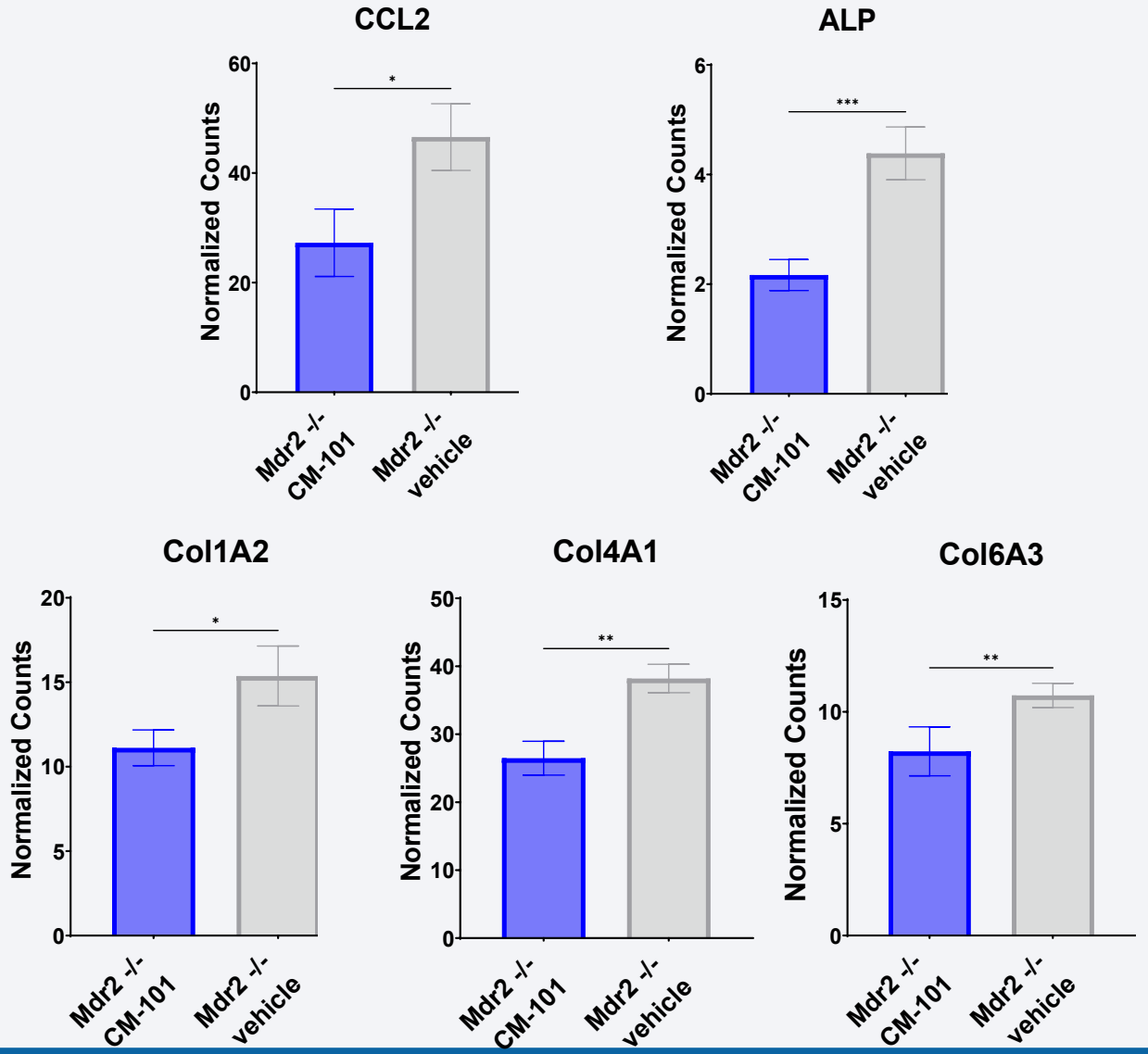


Senescence pathways

Mitosis pathways

CM-101 Treatment Reduces Expression of Inflammatory and ECM Genes in Cholangiocytes



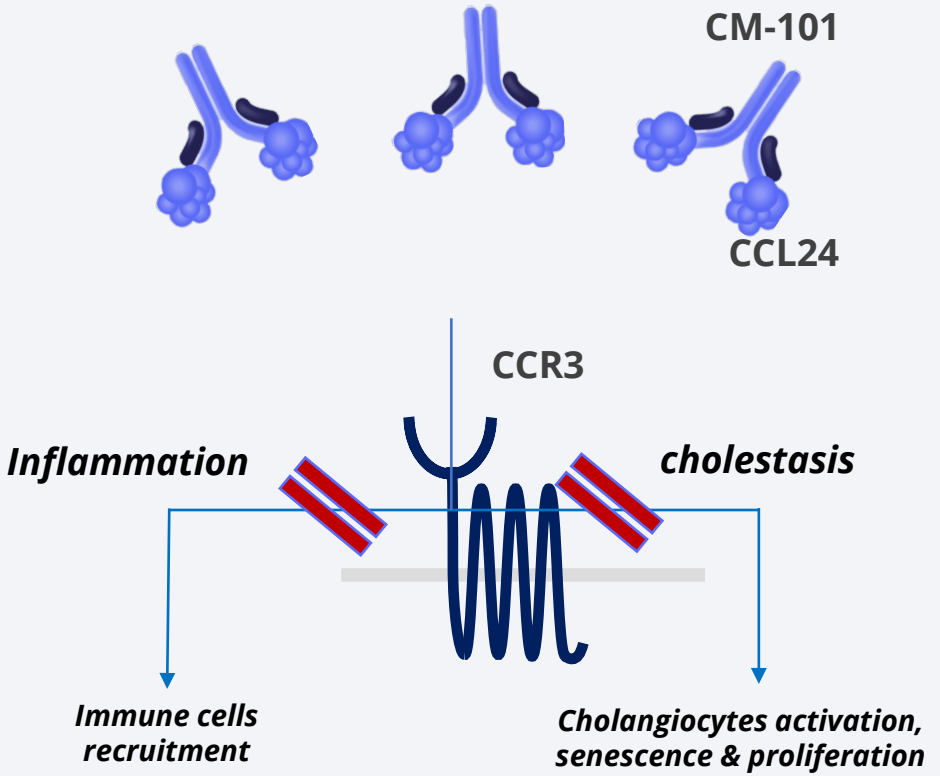


Mdr2 -/- CM-101

Mdr2 -/- vehicle

Summary

- Combination of single-cell and spatial transcriptomics methods enabled in depth analysis of relevant sub-populations and pathways
- Kupffer-cells and a novel population of resident-like macrophages identified as CCL24 main secreting cells
- Resident macrophages were found in injured peribiliary area
- CM-101 reduced monocyte and macrophage presence
- CM-101 decreased cholangiocyte senescence and proliferation
- CM-101 inhibited induction of inflammation and fibrosis by cholangiocytes
- CM-101 interferes with the core pathways of sclerosing cholangitis in experimental model
- A CM-101 Phase 2 trial in PSC patients is currently ongoing



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Amnon Peled
Inbal Mishalian
Devorah Olam
Ophir Hay

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Yuval Nevo

Thank you

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