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

June 2022
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 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)

**Graph:**
- Scatter plot showing the correlation between PSC (ALP>200 IU/L) and ELF
- Formula: ELF = 0.02 * CCL24 pg/ml + 7.6
- Data points for n = 12
- Correlation coefficient: R = 0.62

**Histopathology Images:**
- CCL24 staining in BEC and immune cells
- CCR3 staining in BEC, immune cells, and HSC

**Legend:**
- PanCK – BEC
- Iba1 – macrophages
- αSMA – 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
Studying CCL24 Pathways Using Two Complementary High-Throughput Methods

Single cell RNA sequencing (scRNA-seq)

- Tissue dissociation
- Cytometry or single-cell RNA-sequencing
- Single-cell data

Spatial transcriptomics

- Tissue sectioning
- Multiplexed imaging or spatial transcriptomics
- Spatially-resolved data

Adopted from de Vries et al, Front Oncol 2020
scRNA-seq of Mdr2^-/- Liver Identifies Five Populations of Mononuclear Phagocytes

- Aif1 (Iba1)
- CD68
- Adgre1 (F4/80)
Spatial Transcriptomics of the Damaged Periductal Area of Mdr2\(^{-/-}\) Mice

**Stain**

- **PanCK / F4/80**

**Segment periductal area**

- Cholangiocytes (PanCK+)
- Non-cholangiocytes (PanCK-)

**Collect oligos**

**Count**

- NanoString Technologies

PanCK – BEC
F4/80 - macrophages

N = 12 ROIs, 4 mice
Combination of scRNA-seq and Spatial Transcriptomics Identifies Kupffer Cells in the Injured Peribiliary Area

**scRNA-seq**

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

**Spatial Transcriptomics**

Cluster specific gene expression

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

NanoString cell-type gene signature, based on ImmGen classification

Centrocyte (Ccr7)
Clec9a (cDC1)
Clec10a (cDC2)
CM-101 Reduces Macrophage and Monocyte Recruitment to the Peribiliary Area

scRNA-seq

Spatial Transcriptomics (cell deconvolution)

Monocytes

Macrophages

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
- Keratinization
- RAC3 GTPase cycle
- Collagen modifying enzymes
- RAC1 GTPase cycle
- Fatty acid metabolism
- Phase II
- Cytochrome P450
- Metabolism of steroids
- Peroxisomal protein import
- Electron transport
- Uncoupling proteins
- TCA cycle
- Lipoprotein metabolism
- Phase I
- Biological oxidants
- Amino acid metabolism

Normalized enrichment score (NES)
CM-101 Treatment Reduces Cholangiocyte Senescence and Proliferation

Differentially Expressed Genes

Gene Set Enrichment Analysis

Senescence-Associated Secretory Phenotype
Telomere Stress Induced Senescence
cellular senescence
M phase
Heat stress response
G2/M transition
AURKA Activation by TPX2
PTEN regulation
Telomere maintenance
G2-G2/M phases
Cell Cycle, Mitotic
Insulin-like Growth Factor response
Amino acid metabolism
Metabolism of steroids
Cytochrome P450
Lipoprotein metabolism
Biological oxidants

Normalized enrichment score (NES)

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

**CCL2**

**ALP**

**Col1A2**

**Col4A1**

**Col6A3**
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
Acknowledgements

Chemomab Ltd.
Adi Mor
Michal Segal-Salto
Neta Barashi
Avi Katav
Omer Levi

NanoString Technologies
David Scoville
Anthony Zucca
Yan Liang
Massimo Cerfeda

Hadassah Medical Center, Jerusalem
Amnon Peled
Inbal Mishalian
Devorah Olam
Ophir Hay
Bioinformatics unit (scRNA-seq)
Sharona Elgavish
Yuval Nevo
Thank you