CCL24 INHIBITION BY CM-101 ATTENUATES EXTRACELLULAR MATRIX AND FIBROTIC BIOMARKERS IN BOTH PATIENTS AND EXPERIMENTAL MURINE MODELS

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Introduction

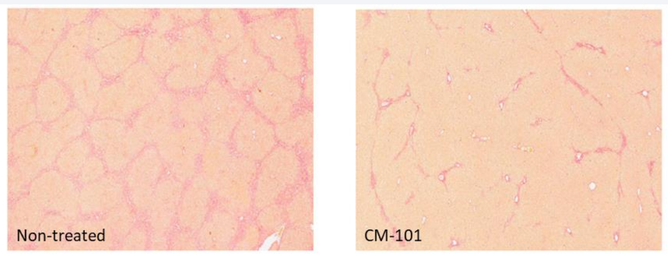
CCL24 is a chemokine that was shown to be involved in the development of liver fibrosis. Blocking CCL24 attenuated liver fibroblast activation and significantly reduced liver fibrosis in multiple primary sclerosing cholangitis (PSC) and NASH animal models. This activity supports CCL24's potential role as a therapeutic target for liver fibrotic diseases. CM-101 is a fully humanized CCL24 blocking monoclonal antibody that showed a favorable safety profile in a single dose study in healthy volunteers and a Phase 1b repeat administration study in NAFLD patients. Here we present preclinical and clinical data, supporting CM-101's remodeling of extracellular matrix key proteins.

Results

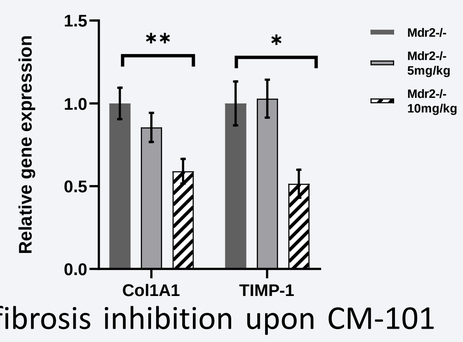
Blocking CCL24 using CM-101 in an exploratory sclerosing cholangitis model (Mdr2-/-) ameliorated disease state, demonstrated by reduced cholestatic inflammatory and fibrotic liver injury. Peribiliary collagen deposition and ECM alternations derived by cholangiocytes were shown. Treatment with CM-101 significantly reduced expression of several collagens, including Col4A1, Col1a2, Col4a2, Col6a3, Col13a, Col9A3 and Elastin. The effect of CCL24 inhibition on Col4a1 and Col9a3 was further tested using human primary cholangiocytes. In NAFLD patients, CM-101 treatment led to reductions of fibrogenesis and fibrolysis circulating biomarkers including Pro-C3, Pro-C4 and C3M.

Fibrosis inhibition and ECM modulation in Mdr2-/- mice model

Sirius red staining



RT-qPCR



Sirius red staining (left panel) shows substantial fibrosis inhibition upon CM-101 treatment. RT-qPCR analysis of RNA extracted from liver samples of Mdr2-/- further support the above and demonstrate significant Col1A1 and TIMP-1 downregulation upon CM-101 administration.

Methods

Mdr2-/- mice mode

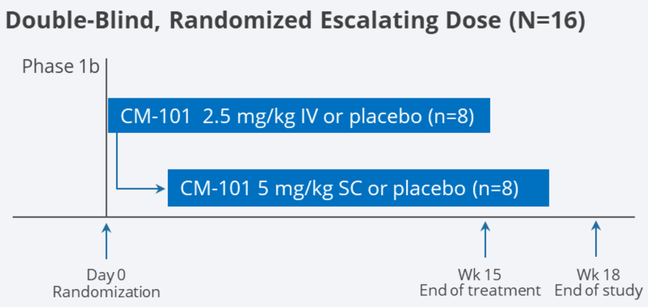
Mdr2-/- mice develop features that are very similar to PSC in terms of cholangitis, severe ductular reaction and fibrosis. We conducted Sirius red staining and RT-qPCR analysis on whole livers. We analyzed bile duct injured areas from Mdr2-/- mice treated with CM-101 from week 6 until week 13, as compared to non treated mice. GeoMx Nanostring was used to assess spatial ECM related gene expression from PanCK positive segment (bile epithelial cells, cholangiocytes).

Human primary cholangiocytes (bile epithelial cells)

Human bile epithelial cells were treated with CM-101 or left untreated. RT-qPCR was used to determine Col4a1 and Col9a3 expression levels.

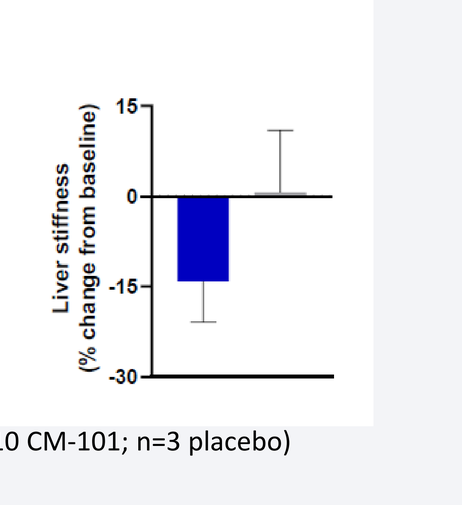
Phase 1b clinical study design in NAFLD

Either 2.5 mg/kg IV or 5 mg/kg SC CM-101 was administered to NAFLD patients for 5 repeated administrations once every 3 weeks. ECM and fibrotic circulating biomarkers were tested.

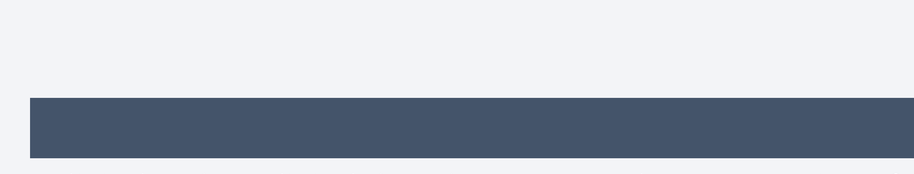


Human primary cholangiocytes

The effect of CCL24 inhibition on Col4a1 and Col9a3 was tested in human primary cholangiocytes. CM-101 downregulated the two fibrotic markers.



Spatial transcriptome analysis (NanoString)



Fold Change treated vs. non-treated

ECM target	Log_2 FC*
Col4a1	-0.58
Col1a2	-0.44
Col4a2	-0.46
Col6a3	-0.47
Col13a1	-0.75
Col9a3	-0.84
Eln	-0.71

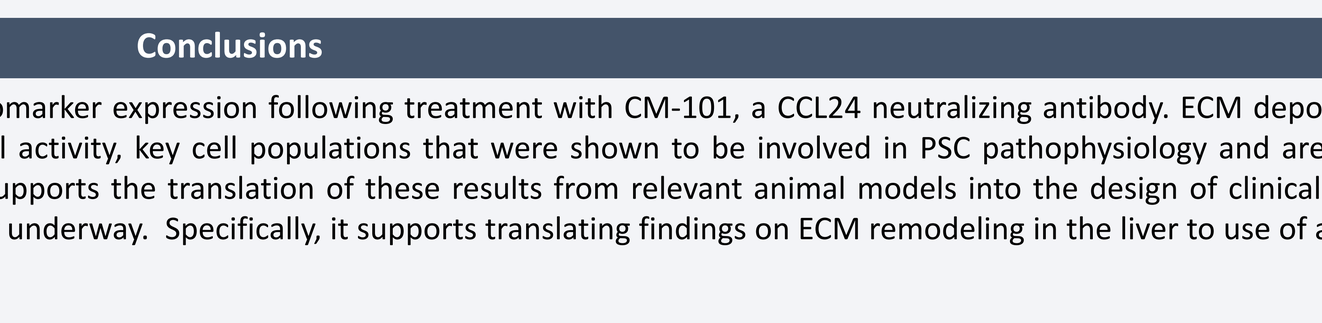
Spatial transcriptome analysis of cholangiocytes (PanCK positive cells, shown in green) revealed differential expression profile between CM-101 treated and non-treated Mdr2-/- mice (volcano plot). The table in the right panel shows downregulation of ECM-related genes following treatment. * p-value<0.05

Analysis of fibrotic biomarkers in NAFLD phase 1b trial

Collagen turnover markers

Serum fibrotic biomarkers

Elastography



*Patients that had baseline elastography (FibroScanTM) that was >4 kPA (n=10 CM-101; n=3 placebo)

Following CM-101 treatment, reduction in collagen turnover and fibrotic biomarkers was noted. For each marker, change from baseline (%) is presented as median value ± IQR. Pro-C3 is the N-terminal pro-peptide of Collagen type III (neo-peptide), Pro-C4 represent the internal epitope in 7S domain of type IV collagen; both are fibrogenesis markers. C3M is a neo-epitope of MMP-9 mediated degradation of type III collagen that reflect the liver inflammatory state. Tissue inhibitor of metalloproteinases-1 and 2 (TIMP1, TIMP2) and the growth factor PDGF-AA are known fibrosis markers. FibroScanTM data corroborates the effect on biomarkers with a mean reduction in liver stiffness.

Conclusions

Clinical and preclinical data demonstrate clear attenuation of ECM biomarker expression following treatment with CM-101, a CCL24 neutralizing antibody. ECM deposition is known to be affected by fibroblast activation as well as epithelial cell activity, key cell populations that were shown to be involved in PSC pathophysiology and are closely related to CM-101's mechanism of action. Importantly, this dataset supports the translation of these results from relevant animal models into the design of clinical studies assessing CM-101 as a potential treatment for PSC, which are currently underway. Specifically, it supports translating findings on ECM remodeling in the liver to use of a similar profile of serum biomarkers in patients.