Ex-vivo translational assay of hepatic stellate cells using patient-derived serum characterizes the anti-fibrotic activity of CM-101

EASL CONGRESS 2024 Milan, Italy 5–8 June

Chemomab THERAPEUTICS

Contact information
Revital.Aricha@Chemomab.com

J. Amer[1], A. Salhab[1], R. Greenman[2], A. Katav2, T. Snir[2], R. Aricha[2], M. Frankel[2], J. Lawler[2], F. Saffioti[3,4], D. Thorburn[3,4], M. Pinzani[3,4], I. Vaknin[2], A. Mor[2] and R. Safadi[1]

[1] Hadassah Medical Hospital, Israel, [2] Chemomab Therapeutics, Israel & United States, [3] Royal Free London NHS Foundation Trust, United Kingdom, [4] UCL Institute of Immunity & Transplantation, United Kingdom

Hadassah Medical Hospital Liver Institute

Introduction

CCL24, a chemokine that regulates inflammatory and fibrotic activities, was found to be highly expressed in livers of patients with liver fibrosis including those with primary sclerosing cholangitis (PSC)[1] and metabolic dysfunction-associated steatohepatitis (MASH)[2]. Treatment with CM-101, a first-in-class humanized antibody targeting CCL24, has impacted consequential biomarkers of liver fibrosis in patients with MASH (NCT05824156) and in multiple PSC preclinical models[1,3].

Aim

This study aimed to better characterize CM-101's anti-fibrotic activity in patients using an ex-vivo hepatic stellate cells (HSC) activation model.

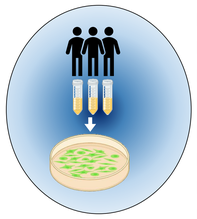
Methods

- **Activation of HSC** [human LX2 cell line]: Cells were activated by 24-hour incubation with either recombinant proteins or sera from MASH patients (NCT05824156) before or after 16 weeks of exposure to either placebo (vehicle) or CM-101 (5 mg/kg subcutaneous every 3 weeks). Activators were added on top of 10% FCS in the media. Quantification of the fibrotic marker αSMA (smooth muscle actin) was assessed by flow-cytometry.
- **The secretome of CCL24-stimulated LX2** was examined by RayBio L-507 protein array.
- **Serum proteomic analysis**: Sera of PSC patients (Royal Free Hospital bio-bank and baseline sera of patients enrolled in the SPRING study, NCT04595825) and healthy controls (NCT06025851) were analyzed using Olink proximity extension assay.

Results

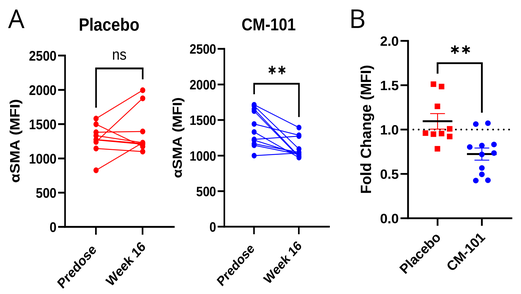
CM-101 treated patients display reduced serum-based HSC activation

MASH Serum-based HSC activation



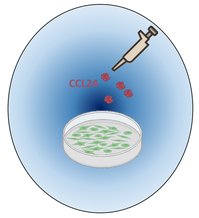
	CM-101 (N=11)	Placebo (N=9)
Age [y]	53.8 (11.4)	46.6 (16.8)
Female gender (%)	6 (42.9%)	6 (66.7%)
Alanine Aminotransferase [U/L]	43.7 (13.0)	29.5 (6.5)
Aspartate Aminotransferase [U/L]	45.8 (25.8)	45.6 (23.1)
Triglycerides [mg/dL]	142.8 (52.1)	157.6 (67.9)
LDL-Cholesterol [mg/dL]	93.2 (30.5)	112.1 (32.7)
Type 2 Diabetes Mellitus	10 (76.9%)	3 (33.3%)
NAFLD Activity Score [NAS]	4.6 (1.5)	4.8 (0.8)
FAST Score	0.53 (0.23)	0.34 (0.19)
Liver Disease Severity		
Fibrosis Stage 1a	0 (0%)	1 (11.1%)
Fibrosis Stage 1c	6 (42.9%)	5 (55.6%)
Fibrosis Stage 2	3 (21.4%)	3 (33.3%)
Fibrosis Stage 3	5 (35.7%)	0 (0%)
MELD Score	7.7 (2.6)	6.8 (2.0)
FibroScan [kPa]	11.5 (5.2)	8.4 (1.1)
MRI-PDFF (%)	19.0 (7.0)	18.7 (6.3)
ELF score	9.8 (0.9)	8.6 (0.3)
Pro-C3 [ng/mL]	40.0 (8.9)	31.3 (5.8)
FIB4 score	1.5 (1.2)	0.7 (0.3)
CRP [mg/dL]	1.2 (1.0)	0.6 (0.6)
Serum CCL24 [pg/ml]	1101 (852)	880 (883)

Demographics and baseline characteristics of SPLASH study in MASH patients.
Data presented as n (%) or mean (SD) unless otherwise stated. NAFLD = Nonalcoholic fatty liver disease; FAST = FibroScan-AST, ELF = Enhanced Liver Fibrosis.

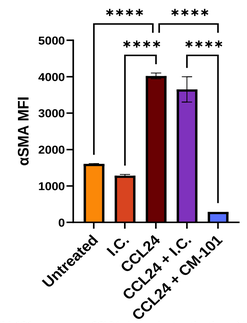


CM-101 treatment reduces serum-based ex-vivo activation of HSC.
HSC (LX2 cell line) were activated using 1% of serum from MASH patients, before first treatment (predose), or after 16 weeks of treatment (week 16). (A) α-SMA expression measured by FACS. (B) Fold change of α-SMA expression between predose and week 16.

Exogenous CCL24 HSC activation

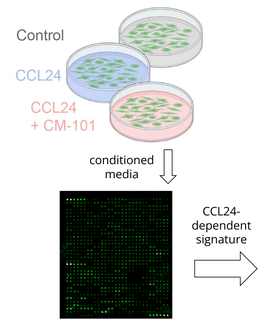


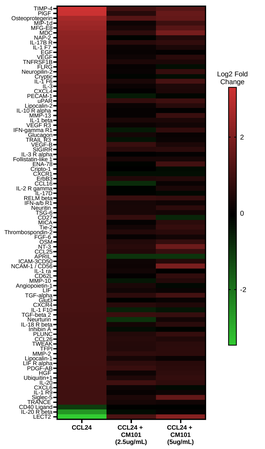
CCL24 directly activates HSCs



CM-101 suppresses CCL24-dependent activation of HSC. HSC (LX2 cell line) were activated using 50 ng/ml recombinant CCL24, with or without 10 µg/ml antibody (CM-101 or I.C.). I.C. isotype control.

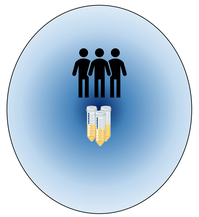
CCL24 regulated proteins in HSCs





Hepatic cell line CCL24-dependent secreted protein Signature
LX2 cells were incubated with either PBS, CCL24, or CCL24 with CM-101. Relative expression of secreted protein was determined by L507 protein array (RayBio). The CCL24-dependent protein signature was defined as protein that had a twofold change upon CCL24 treatment and return to baseline expression upon addition of CM-101.

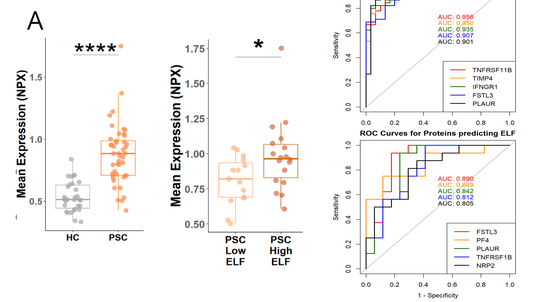
PSC CCL24 HSC signature in patients' sera



Differentiation of PSC severity by CCL24-dependent signature

	Healthy controls (N=30)	PSC (N=45)
Age [y]	23.5 (18-38)	45 (18-76)
Duration since diagnosis [y]	NA	4.9 (0-25.3)
Male, n (%)	30 (100)	26 (58)
IBD any, n (%)	0 (0)	30 (67)
ALP [U/L]	74 (42-106)	246 (52-1064)
ALT [U/L]	16.5 (7-45)	70 (10-796)
AST [U/L]	19 (12-34)	54 (15-919)
Bilirubin [mg/dL]	11 (7-18)	12 (3-41)
Fibroscan	NA	10.1 (5.0-17.3)
ELF score	NA	9.95 (7.85-12.84)

Demographics and baseline characteristics of PSC patients and healthy controls
Data presented as median (range) unless otherwise stated. IBD = inflammatory bowl disease, ELF = Enhanced Liver Fibrosis.



Hepatic cell line CCL24-dependent secreted proteins can differentiate individuals by PSC and its severity.
(A) The average expression of CCL24-dependent protein signatures is presented in serum of HC vs. patients with PSC (left panel) or in patients with PSC, stratified by ELF score of 9.8 (right panel). Boxes represent interquartile ranges with medians.
(B) The ROC curves for the five serum proteins with the highest AUC values for predicting disease presence (upper panel) or fibrosis severity (lower panel). HC = healthy controls.

Conclusions

- A serum-based ex-vivo HSC activation assay can help in characterizing anti-fibrotic drug effects.
- A serum-based assay derived from MASH patients treated with CM-101 restored HSC activation.
- A protein signature generated from CCL24-activated HSC predicted PSC disease and its severity.
- These findings support CM-101's mode of action in liver fibrosis.
- CM-101 is currently being tested in a phase 2 study in PSC patients.

References

1. Greenman, R, Snir, T, Katav, A, Aricha, R, Mishalian, I, et al. The Role of CCL24 in Primary Sclerosing Cholangitis: Bridging Patient Serum Proteomics to Preclinical Data. Cells 2024, 13, 209, doi:10.3390/cells13030209.
2. Segal-Salto M, Barashi N, Katav A, Edelshtein V, Aharon A, Hashmueli S, et al. A blocking monoclonal antibody to CCL24 alleviates liver fibrosis and inflammation in experimental models of liver damage. JHEP Reports. 2020;2(1).
3. Greenman R, Snir T, Katav A, Aricha R, Mishalian I, Hay O, et al. The Role of CCL24 in Primary Sclerosing Cholangitis: Bridging Patient Serum Proteomics to Preclinical Data. Cells. 2024 Jan;13(3):209.