CM-101, a Novel CCL24 Blocking Monoclonal Antibody, Attenuates HSC Activation and Reduces Fibrosis in the TAA Murine Model

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INTRODUCTION

CCL24 (C-C chemokine ligand 24, Eotaxin-2) is a chemokine that regulates inflammatory and fibrotic activities through its receptor, CCR3. This chemokine was found to be highly expressed in the livers of Nonalcoholic Steatohepatitis (NASH) and Primary Sclerosing Cholangitis (PSC) patients. Reduction of CCL24 was associated with decreased liver damage, most significantly by reducing fibrosis, in several experimental murine models[1]. CM-101 is a first in class humanized IgG1 monoclonal antibody targeting human CCL24 that is currently in clinical development for the treatment of NASH and PSC. The aim of this pre-clinical work was to study the role of the CCL24-CCR3 axis in liver fibrotic processes, both in vivo and in-vitro, and to evaluate the anti-fibrotic activity of CCL24 blockade using CM-101.

AIM

- To study the role of the CCL24 in liver fibrosis and hepatic stellate cell activation.
- Explore the anti-fibrotic activity of CCL24 blockade using CM-101.

MATERIAL & METHODS

Activation of hepatic stellate cells (HSC) by CCL24 was evaluated in the human LX2 cell line using the scratch motility assay, quantification of the fibrotic marker α-SMA and production of pro Collagen 1. Preincubation with CM-101 was used to block CCL24 induced activity of LX2 cells.
To evaluated the in-vivo effect of CM-101 on development of fibrosis we used the murine model of thioacetamide (TAA)-induced liver injury[2]. Male BALB/C mice (6-8 weeks) received IP injections of TAA for 12 weeks twice weekly and either vehicle control (PBS), or CM-101 (D8) 2.5 mg/kg (CM-101 murine surrogate) concurrently by SC injections. Fibrosis was evaluated by histopathological analysis of H&E stained liver sections and quantification of collagen deposition in Sirius red stained slides. Gene expression of α-SMA, TIMP-1 and Col3a1 were tested against GAPDH normalization by Real-time PCR using TaqMan probes.

REFERENCES

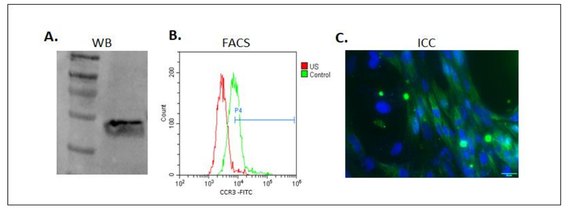
[1] **Segal-Salto M et. al.** *A Blocking Monoclonal Antibody to CCL24 Alleviates Liver Fibrosis and Inflammation in Experimental Models for Liver Damage (2019, Submitted)*
[2] **Wallace MC et. al.** *Standard operating procedures in experimental liver research: thioacetamide model in mice and rats (2015)*

RESULTS

CCL24 induced activation of LX2 hepatic stellate cells

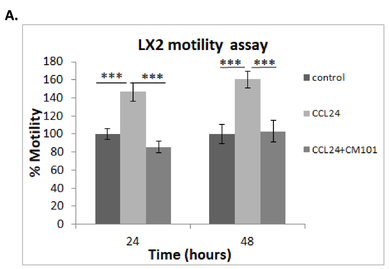
Human hepatic stellate cell line, LX2, express the CCL24 receptor, CCR3

Expression of CCR3 on LX2 cells was evaluated by (A) western blot (B) FACS staining and (C) Immunocytochemistry staining.



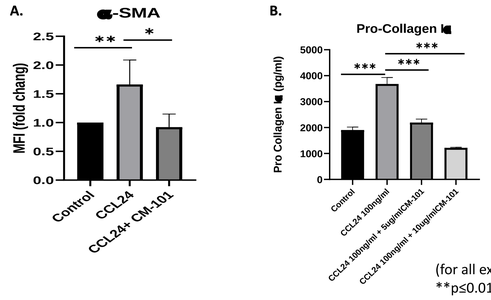
CM-101 significantly reduces LX2 cell motility induced by CCL24

LX2 cells motility was increased by 46% ± 9 and 60 ± 9% following treatment with CCL24 (25ng/ml) for 24 and 48 hours, respectively. This increased motility was inhibited by the addition of 5ug/ml CM-101, reducing scratch closure at both 24h and 48h time points (n=6).



CM-101 significantly reduces CCL24 induced LX2 activation

CCL24 induced LX2 cell activation increasing α-SMA (alpha- smooth muscle actin) expression and secretion of Pro-Collagen 1. Pre-incubation of CCL24 with CM-101, markedly attenuated HSC activation, resulting in significant reduction of both α-SMA expression and Pro-Collagen 1 secretion. (A) FACS staining for α-SMA in LX2 cells (B) evaluation of pro-collagen secretion by ELISA.

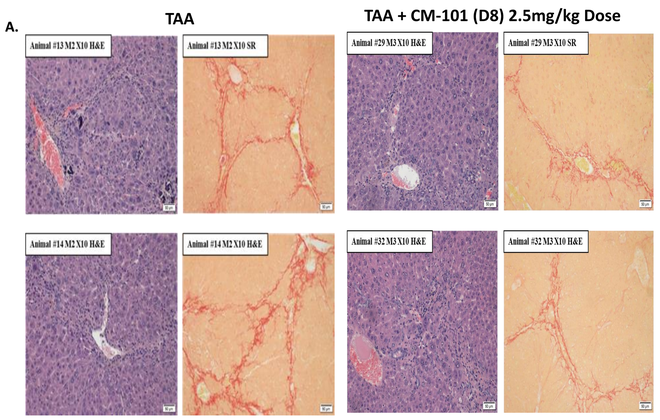


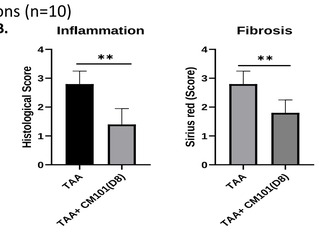
(for all experiments-*p≤0.05; **p≤0.01; ***p≤0.001)

CM-101 attenuates Inflammation and Fibrosis in TAA- induced liver damage

CM-101 (D8) significantly reduces development of liver fibrosis and attenuates TAA induced liver damage

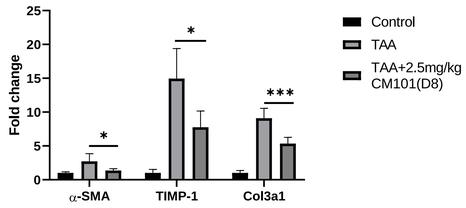
TAA injections induced severe liver fibrosis and inflammation. Liver sections stain with H&E and Sirius-Red (for collagen deposition) revealed significant attenuation of liver damage with a 35% reduction in inflammation and a 50% reduction in fibrosis in animals treated with CM-101 (D8). Representative images (A) and histological quantification (B) of H&E and Sirius-Red staining in liver sections (n=10)





Treatment with CM-101 reduced expression of pro-fibrotic genes in the liver of TAA mice

Expression of α-SMA, TIMP-1, and Col3a1, that are associated with increased ECM formation, were all significantly upregulated in TAA mice. In accordance with histological analysis, CM-101(D8) treatment significantly reduced the expression of these genes in the liver. (n=5)



CONCLUSION

CCL24 is a potent activator of HSC, affecting their motility and fibrotic gene expression. CCL24 blockade using CM-101, a specific anti-CCL24 monoclonal antibody, reversed HSC activation in-vitro and reduced fibrosis development in-vivo in the TAA-murine model.

These findings further support the role of CCL24 as a therapeutic target in liver fibrotic diseases and the anti-fibrotic activity of CM-101.

Two phase IIa studies testing CM-101 in NASH and PSC are planned during 2020.

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