Genetically Engineered Macrophage Cell Therapy Reverses Liver and Lung Fibrosis in Preclinical Models

Chris Sloas, Yumi Ohtani, Angela Mosebarger, Ben Johnson, Nancy Carson, Julia Smith, MacKenzie Villano, James Montgomery, Lauren Shaw, Joanna Schmidt, Sascha Abramson, Thomas Condamine, Michael Klichinsky, Bradley Zinker

Carisma Therapeutics Inc., Philadelphia, PA

Introduction

Unmet need for liver and lung fibrosis therapies

Fibrosis development is an interplay of inflammatory and pro-fibrotic pathways that promote collagen deposition and organ dysfunction.

Metabolic dysfunction-associated steatohepatitis (MASH) prevalence is expected to increase in the US and worldwide, paralleling the increase of obesity and type 2 diabetes. There is a high unmet need for safe and effective MASH treatments that correct both steatosis and fibrosis.

Idiopathic pulmonary fibrosis (IPF) has a median survival < 5 years from diagnosis. Currently approved therapies have adverse side effects and cannot correct established lung injury.

Fibrosis of the liver and lung can lead to organ failure. However, currently available therapies are unable to reverse established, advanced fibrosis.

Macrophages as anti-fibrotic cell therapies

Macrophages play a central role in the development and resolution of fibrosis.

Pre-clinical studies have shown feasibility, safety, and potential efficacy of non-genetically engineered macrophages in the treatment of MASH [1].

Phase I clinical studies have demonstrated the safety and feasibility of using engineered macrophage cell therapies in disease areas outside of fibrosis [2].

Objectives

1. Generate and characterize macrophages that overexpress anti-fibrotic and anti-inflammatory "payloads"
2. Evaluate efficacy of engineered macrophages in pre-clinical models of liver and pulmonary fibrosis

Resolve inflammation
Directly send anti-inflammatory signals
↑activity by Treg and "M2" Mϕ

Reverse fibrotic tissue
ECM degradation
Block TGFβ signaling axis

Silently remove cellular debris
↑ Efferocytosis
↑ Pro-resolving mediators

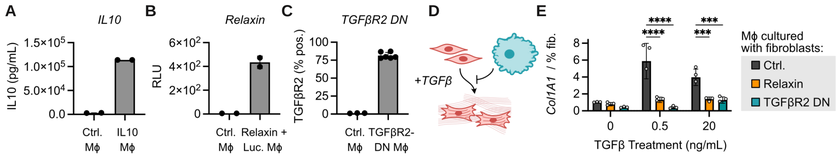
Restore tissue/metabolic health
Promote hepatocyte regeneration
Improve insulin sensitivity

Materials and Methods

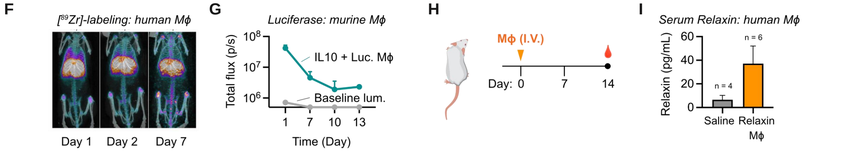
- **Murine macrophages**: primary hematopoietic stem cells (HSCs) are isolated from bone marrow of BL6 mice. HSCs are transduced using retrovirus encoding a payload of interest, then differentiated into bone marrow derived macrophages (BMDM) using M-CSF.
- **Payload expression** is evaluated using ELISA, flow cytometry, or Western blot ≥ 5 days after transduction.
- **Human macrophages**: primary CD14+ monocytes are isolated from leukopaks. Cells are transduced using VPX-lentiviral particles then differentiated into macrophages using GM-CSF or M-CSF.
- ***In vitro* functional assays**: human macrophages are co-cultured either with fibroblasts for 3 days to evaluate fibroblast activation via qPCR, or with apoptotic target cells for 4 hours to evaluate efferocytosis via flow cytometry.
- **CCl_4-induced liver fibrosis**: BL6 mice (n = 9 per group) were dosed with CCl_4 2x per week. BMDM were administered IV after 4 weeks. Livers were harvested 2 weeks after treatment, and efficacy was evaluated histologically.
- **Diet-induced liver fibrosis:** BL6 mice (n = 9-12 per group) were fed a modified HFD for 26 weeks, supplemented with weekly low-dose CCl_4 beginning at week 11. BMDM were administered IV after 21, 23, and 25 weeks. Livers were harvested at week 26 for evaluation.
- **BLM-induced IPF**: BL6 mice (n = 10-15 per group) were dosed once with bleomycin. BMDM were administered IV after 24 hours. Lungs were harvested 3 weeks after BLM dosing and efficacy was evaluated via hydroxyproline (HYP) biochemical analysis.

Engineered macrophages exhibit anti-fibrotic function and localize to the liver

Human macrophages can be engineered to express diverse anti-fibrotic payloads such as IL10 (**A**), Relaxin (**B**), or a dominant-negative TGFβR2 (TGFβR2 DN) (**C**). In an *in vitro* co-culture, engineered human macrophages inhibited TGFβ-induced myofibroblast activation (**D-E**).

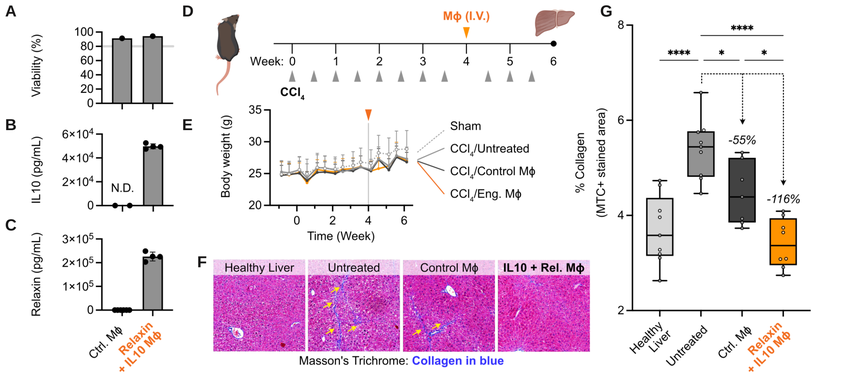


Engineered macrophages exhibit liver-homing properties and express payloads *in vivo*. IV-administered cells homed to the liver (**F**) and persist ≥ 2 weeks (**G**). Elevated levels of human Relaxin could be detected in the serum of NSG mice 2 weeks after macrophage dosing (**H-I**)



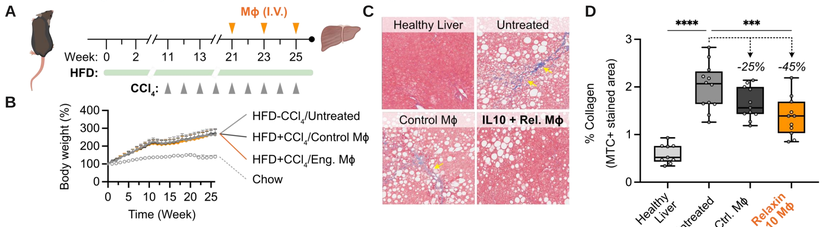
Engineered macrophages producing Relaxin and IL10 reverse CCl_4-induced liver fibrosis

Engineered murine BMDM were generated to treat CCl_4-induced liver fibrosis. BMDM were viable (**A**) and released high levels of murine IL10 (**B**) and Relaxin (**C**) *in vitro*. Liver fibrosis was induced by dosing CCl_4 for 6 weeks, and at week 4 mice were dosed with BMDM (**D**). There was no change in bodyweight, and treatment was well-tolerated (**E**). Fibrosis was evaluated by quantifying collagen content via MTC staining (**F**). Fibrotic collagen was reduced by control macrophages, and further reduced to baseline by engineered macrophages (**G**).



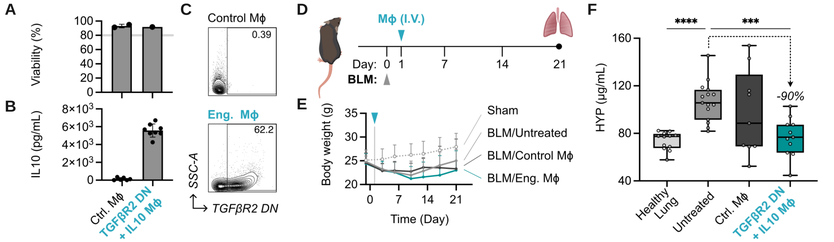
Engineered macrophages reduce liver fibrosis in a high fat diet-induced model

Engineered macrophages co-expressing IL10 and Relaxin were additionally tested in a MASH model comprising a modified high-fat diet (HFD) with low-dose CCl_4 (**A-B**). Fibrosis was evaluated by quantifying collagen content via MTC staining, with collagen shown in blue (**C**). Fibrotic collagen was significantly lower in mice treated with engineered macrophages (**D**).



Engineered macrophages prevent bleomycin-induced pulmonary fibrosis

Engineered BMDM were next generated for BLM-induced pulmonary fibrosis. BMDM were viable (**A**), and engineered cells co-expressed high levels of IL10 (**B**) and TGFβR2 DN (**C**). Pulmonary fibrosis was induced by a single BLM administration, followed by treatment 24 hr later. Lungs were collected for analysis after 21 days (**D**). There was no change in bodyweight, and treatment was well-tolerated (**E**). BLM-treated mice that received engineered cells had lower levels of lung collagen, quantified by biochemical measurement of HYP content (**F**).

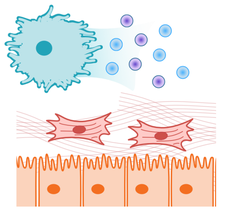


Conclusions

Macrophage biology can be engineered to target multiple aspects of fibrotic disease.

Systemic administration of Relaxin-IL10 co-expressing macrophages significantly improved established liver fibrosis.

A single systemic dose of TGFβR2 DN-IL10 co-expressing macrophages prevented bleomycin induced pulmonary fibrosis.



Engineered macrophages provide a durable reservoir of therapeutic signals
- Anti-inflammatory cytokines
- Anti-fibrotic factors
- Regenerative factors

Directly counteract drivers of fibrotic disease
- Chronic inflammation
- Matrix deposition
- Hepatic injury

[1] Moroni, F. et al. Safety profile of autologous macrophage therapy for liver cirrhosis. *Nat Med*. 25, 1560–1565 (2019).
[2] Anderson, N. R., Minutolo, N. G., Gill, S. & Klichinsky, M. Macrophage-Based Approaches for Cancer Immunotherapy. *Cancer Res* **81**, 1201–1208 (2021).

BLM: Bleomycin
TGFβR2 DN: Dominant negative TGFβ receptor II
IPF: Idiopathic pulmonary fibrosis
MTC: Masson's Trichrome
BMDM: Bone marrow-derived macrophage
HYP: Hydroxyproline
MASH: Metabolic dysfunction-associated steatohepatitis
Mϕ: Macrophage

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