Engineered Macrophages Expressing Fibrosis-Modifying Transgenes Ameliorate Liver Fibrosis in Preclinical Models

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Introduction

Unmet need for liver fibrosis therapies

Approximately 25% of US adults have nonalcoholic fatty liver disease (NAFLD) and 5% have advanced liver fibrosis [1].

Liver fibrosis results from inflammatory and pro-fibrotic pathways that promote collagen deposition and organ dysfunction. Liver fibrosis is a central late-stage pathway in multiple liver diseases, including MASH, ALI, and PSC.

Treatment options remain few for severe (F3/F4) MASH patients. Currently available therapies have minimal direct impact on fibrosis while primarily targeting steatosis.

Macrophages (Mϕ) as anti-fibrotic cell therapies

Mϕ 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 Mϕ in the treatment of MASH [2].

Phase I clinical studies have demonstrated the safety and feasibility of using engineered Mϕ cell therapies in disease areas outside of fibrosis [3].

Objectives

1. Generate and characterize engineered Mϕ that overexpress anti-fibrotic, anti-inflammatory, and pro-efferocytosis constructs
2. Evaluate engineered Mϕ for efficacy in pre-clinical models of liver fibrosis

Strategies to engineer anti-fibrotic Mϕ:

Reverse fibrosis
ECM degradation
Block TGFβ signaling axis

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

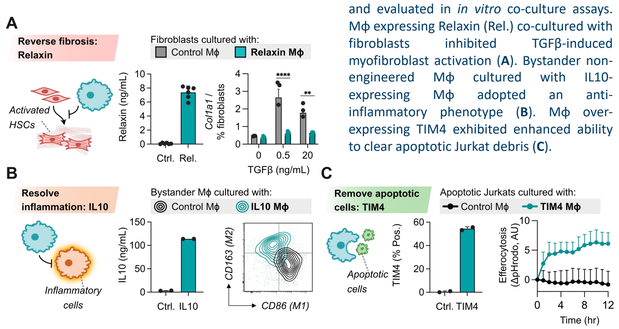
Silently remove apoptotic cells
↑ Efferocytosis
↑ Pro-resolving mediators

Materials and Methods

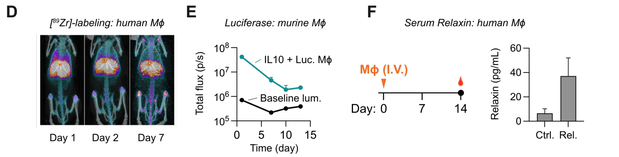
- **Murine Mϕ**: primary hematopoietic stem cells (HSCs) are isolated from bone marrow of BL6 mice. HSCs are transduced using retrovirus encoding a construct of interest, then differentiated for 5 days into Mϕ using M-CSF.
- **Construct expression** is evaluated using ELISA or flow cytometry ≥ 5 days after transduction.
- **Human Mϕ**: primary CD14+ monocytes are isolated from leukopaks. Cells are transduced using VPX-lentiviral particles then differentiated into Mϕ using GM-CSF or M-CSF.
- ***In vitro* functional assays**: human Mϕ are co-cultured either with fibroblasts for 3d to evaluate fibroblast activation via qPCR, with apoptotic target cells for 12hr to evaluate efferocytosis via flow cytometry, or with GM-CSF-differentiated Mϕ for 3d to evaluate bystander polarization.
- **CCl_4-induced liver fibrosis**: BL6 mice (n = 9 per group) were dosed with CCl_4 2x per week. Mϕ were administered IV after 4 weeks. Livers were harvested 2 weeks after treatment, and efficacy was evaluated histologically.
- **Westernized diet (WD) with low-dose CCl_4 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. Mϕ were administered IV after 21, 23, and 25 weeks. Livers were harvested at week 26 for evaluation.
- **Choline-deficient, L-amino acid-defined high-fat diet (CDAHFD) fibrosis:** BL6 mice (n= 5-12 per group) were fed CDAHFD for 6-7 weeks. In one instance, Mϕ were administered IV after 4, 5, and 6 weeks, and livers were harvested at week 7. In a second instance, Mϕ were administered IV after 4 weeks, and livers were harvested at week 6.

Engineered macrophages exhibit anti-fibrotic functions

Macrophages can be engineered to express diverse anti-fibrotic payloads that are tailored to target specific features of fibrosis pathology. Primary human Mϕ were equipped with anti-fibrotic payloads and evaluated in *in vitro* co-culture assays. Mϕ expressing Relaxin (Rel.) co-cultured with fibroblasts inhibited TGFβ-induced myofibroblast activation (**A**). Bystander non-engineered Mϕ cultured with IL10-expressing Mϕ adopted an anti-inflammatory phenotype (**B**). Mϕ over-expressing TIM4 exhibited enhanced ability to clear apoptotic Jurkat debris (**C**).

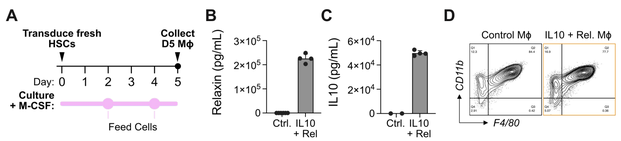


Engineered Mϕ exhibit liver-homing properties and express payloads *in vivo*. IV-administered cells homed to the liver (**D**) and persisted ≥ 2 weeks (**E**). Elevated levels of human Relaxin could be detected in the serum of NSG mice 2 weeks after macrophage dosing (**F**).



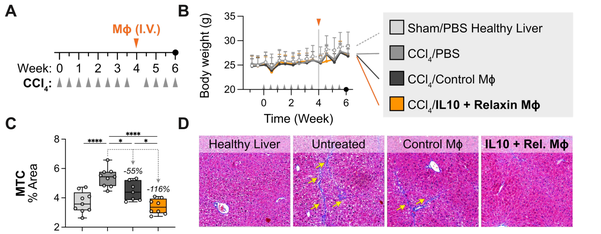
Generation of macrophages co-expressing Relaxin and IL10

Anti-fibrotic murine Mϕ were engineered to co-express Relaxin and IL10 and be used as adoptive cell therapies in *in vivo* studies. Freshly isolated hematopoietic stem cells (HSCs) were transduced with retrovirus and differentiated in M-CSF for 5 days (**A**). Mϕ were collected, characterized, and used as therapeutics in subsequent mouse studies. Engineered Mϕ expressed high levels of Relaxin and IL10 (**B-C**). Engineering did not alter differentiation, as compared to control Mϕ (**D**).

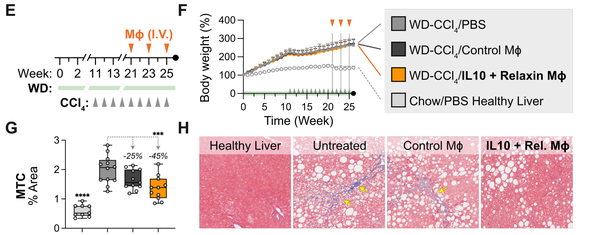


Macrophages expressing Relaxin and IL10 improve liver fibrosis in multiple models

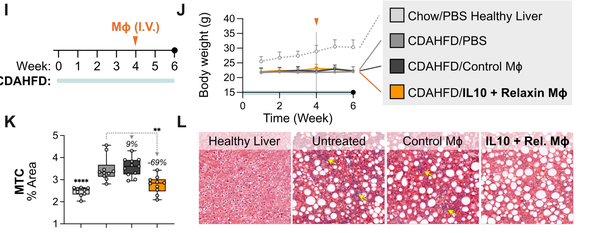
Engineered Mϕ co-expressing IL10 and Relaxin were evaluated in preclinical models of liver fibrosis induced by CCl_4 (**A-D**), WD with low-dose CCl_4 (**E-H**), and CDAHFD (**I-L**). IV-administered Mϕ were well-tolerated and did not induce changes in bodyweight (**B**, **F**, **J**). Fibrosis was evaluated by quantifying collagen content via MTC staining. In CCl_4-induced disease, fibrotic collagen was reduced by control Mϕ and further reduced to baseline by engineered Mϕ (**C-D**).



In a MASH model comprising a modified WD and low-dose CCl_4, fibrotic collagen was significantly lower in mice treated with engineered Mϕ co-expressing IL10 and Relaxin (**G-H**).

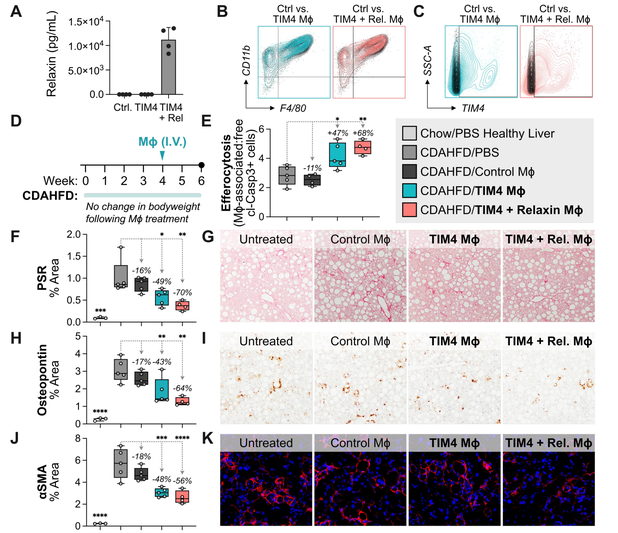


In a third liver fibrosis model induced by CDAHFD, fibrotic collagen was again significantly lower in mice treated with engineered Mϕ co-expressing IL10 and Relaxin (**K-L**).



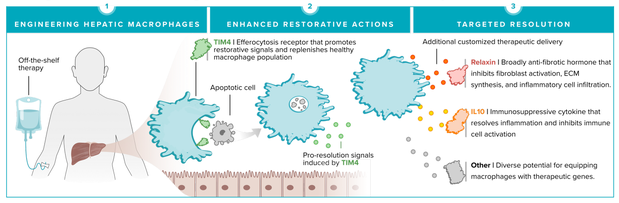
Engineered efferocytic macrophages expressing TIM4 improve fibrosis in a CDAHFD model

Reduced efferocytosis is a novel pathway in MASH that remains unaddressed. TIM4 is an efferocytosis receptor that is expressed by healthy Kupffer Cells but is lost during MASH [4]. Mϕ were engineered to express TIM4 ± Relaxin (**A-C**) and were evaluated in a CDAHFD liver fibrosis model (**D**) TIM4-expressing Mϕ significantly increased histologically measured efferocytosis while reducing markers of fibrosis and inflammation (**E-K**).



Conclusions

Engineered Mϕ strategies are novel off-the-shelf therapies for liver fibrosis



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[3] Anderson, N. et al. Macrophage-Based Approaches for Cancer Immunotherapy. *Cancer Res* **81**, 1201–1208 (2021).
[4] Shi, H. et al. Loss of TIM4-Dependent Efferocytosis in Kupffer Cells Promotes Liver Fibrosis in Nonalcoholic Steatohepatitis. bioRxiv (2024).

ALI: Acute liver injury
ECM: Extracellular matrix
Mϕ: Macrophage
PSC: Primary sclerosing cholangitis
WD: Westernized diet
CDAHFD: Choline-deficient, L-amino acid-defined, high-fat diet
MASH: Metabolic dysfunction-associated steatohepatitis
NAFLD: Nonalcoholic fatty liver disease
PSR: Picrosirius red
αSMA: Alpha smooth muscle actin
Ctrl: Control
MTC: Masson's trichrome
PBS: Phosphate-buffered saline
Rel.: Relaxin

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