Macrophages Engineered with Cytokine Switch Receptors: Development of a Modular Platform for Rebalancing Inflammation in Microenvironments

Chris Sloas, Yuhao Huangfu, Rehman Qureshi, Michael Ball, Thomas Condamine, Michael Klichinsky, Yumi Ohtani

Carisma Therapeutics, Philadelphia, PA

#4054

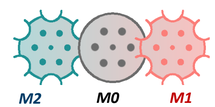
Introduction

Balancing pro- and anti-inflammatory cytokines in disease

- Cytokines regulate pro- and anti-inflammatory signals. Dysregulated cytokines can cause pathogenic immunosuppression or inflammation
- Rebalancing inflammation or immunosuppression offers a generalizable approach to treating many diseases, but systemic cytokine blockade carries risks such as increased risk of infection

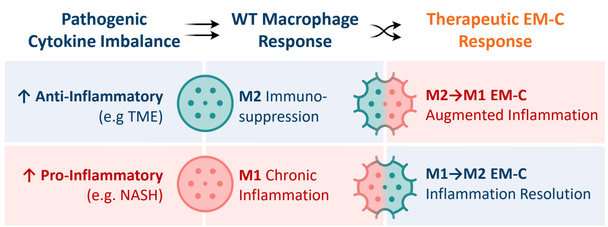
Macrophage cell therapies for rebalancing inflammation

- Cell therapies offer a localized solution to rebalance inflammation. Macrophages are proficient at both initiating and resolving inflammation
- Engineered macrophages have demonstrated promising ability to target tumor cells using CARs [1-2]



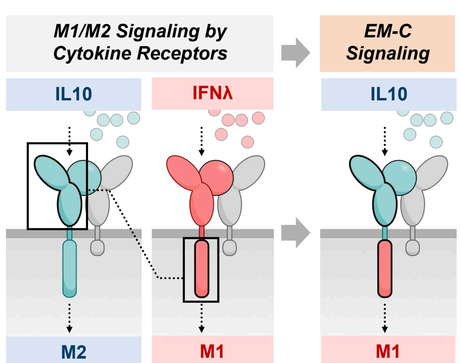
Objectives

Goal: Develop Engineered Microenvironment Converters (EM-C) as a platform technology to regulate inflammation in disease



Materials and Methods

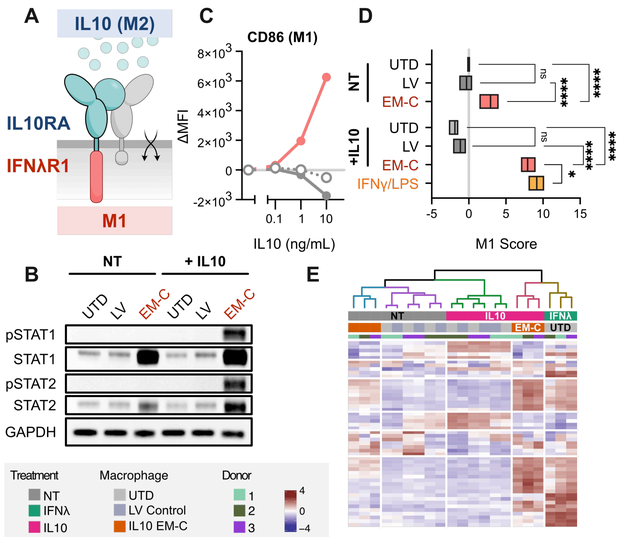
- EM-C are generated by expressing Switch Receptors (SR) in primary human macrophages or monocytes
- SR are delivered using VPX-Lentiviral particles
- For M2→M1 signal conversion, SR are generated to target IL10 or TGFβ
- For M1→M2 signal conversion, SR are generated to target IFNγ or IL17A
- All data shown are representative of at least three independent donors and/or experiments
- Measurements are reported as mean ± SD



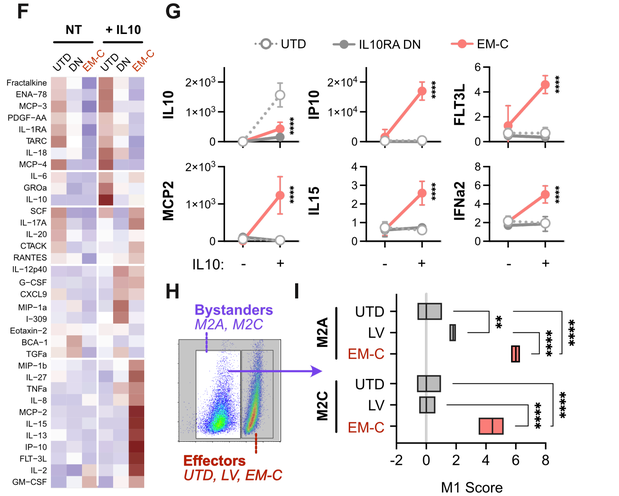
Conceptual design of Switch Receptors for EM-C signaling. Switch Receptors (SR) are chimeric proteins consisting of the ligand binding domain from one cytokine receptor, paired with a compatible cytosolic domain from a second receptor. Successful SR design enables EM-C to convert anti-inflammatory M2 cytokines into M1 signals, or *vice versa*.

IL10 EM-C exhibit a pro-inflammatory response to IL-10

IL10 EM-C express a SR that converts IL10, a common immunosuppressive factor in solid tumors, into a pro-inflammatory (M1) signal (**A**). Primary human EM-C interpret IL10 as a STAT1/2-activating signaling (**B**). IL10-treated EM-C resemble interferon-treated macrophages by surface markers (**C-D**) and gene expression (**E**).

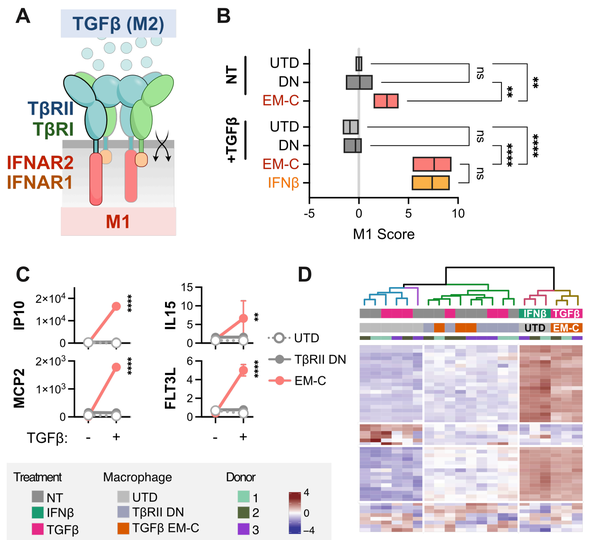


IL10 EM-C augment their microenvironment with a repertoire of pro-inflammatory factors in response to IL10 (**F-G**). TAM-like M2 macrophages cultured with EM-C are skewed towards an M1 phenotype, demonstrating that EM-C can repolarize surrounding immune cells (**H-I**).

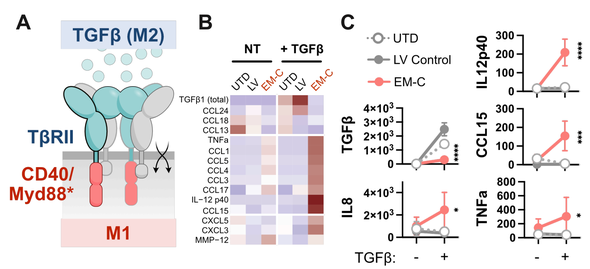


Expanding pro-inflammatory EM-C to target TGFβ

The EM-C platform is broadened to target TGFβ, an additional immunosuppressive factor prevalent in solid tumors (**A**). TGFβ EM-C respond to TGFβ by upregulating pro-inflammatory markers (**B**), cytokines/chemokines (**C**), and genes that mirror direct interferon treatment (**D**).

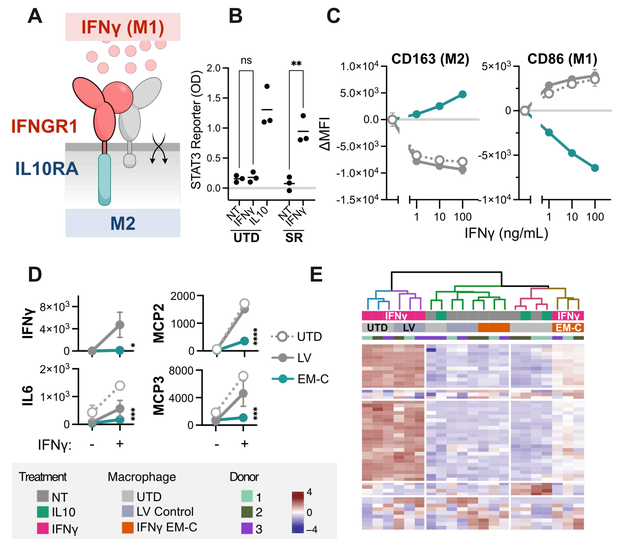


Signaling outputs in the EM-C platform are modular. Below, an additional SR targeting TGFβ instead activates NF-κB pathways (**A**). TGFβ is sequestered, and pro-inflammatory cytokines/chemokines are upregulated (**B-C**).

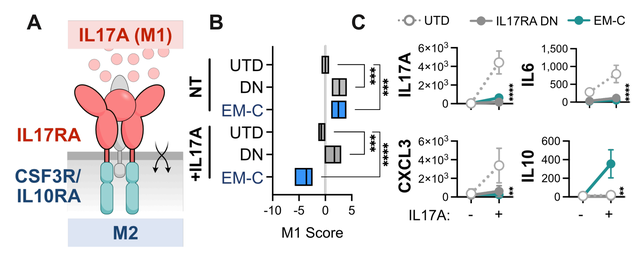


EM-C targeting IFNγ and IL17A for inflammatory disease

The EM-C approach can be "inverted" to target inflammatory cytokines for resolving inflammation. An IFNγ SR is designed to target IFNγ (**A**) and convert into an M2 signal via STAT3 activation (**B**). IFNγ-treated EM-C augment their M2 phenotype (**C**) and resist expression of inflammatory factors (**D-E**).



Anti-inflammatory EM-C are expanded to target IL17A, a commonly upregulated factor in chronic inflammation and autoimmunity (**A**). IL17A-treated EM-C express M2 markers (**B**), sequester IL17A, and produce anti-inflammatory IL10 (**C**).



Takeaways: Pro-Inflammatory EM-C for Solid Tumors

Robust control of inflammatory phenotype in myeloid cells.

EM-C sequester IL10 or TGFβ and heat up the microenvironment.

Takeaways: Anti-inflammatory EM-C for Non-Oncology

Applicable for both oncology and inflammatory indications.

EM-C sequester IFNγ or IL17 and promote inflammation resolution.

The EM-C platform represents a novel, modular immunotherapy that harnesses macrophages as 'living converters' to locally regulate inflammation

[1] Klichinsky, M. *et al.* Human chimeric antigen receptor macrophages for cancer immunotherapy. *Nat Biotechnol* 1–7 (2020) doi:10.1038/s41587-020-0462-y.
[2] Anderson, N. R., Minutolo, N. G., Gill, S. & Klichinsky, M. Macrophage-Based Approaches for Cancer Immunotherapy. *Cancer Res* **81**, 1201–1208 (2021).

DN : Dominant Negative receptor
EM-C: Engineered Microenvironment Converter
LV: Lentivirus
NT: Nontreated/untreated
SR: Switch Receptor
UTD: Untransduced

Join us!

carisma THERAPEUTICS