Macrophages expressing synthetic cytokine receptors reverse immunosuppressive signals in solid tumors

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Introduction

Cytokines mediate immunosuppression in solid tumors

Immunosuppression
Resistance to cancer therapies

Cytokines regulate pro- and anti-inflammatory signals.

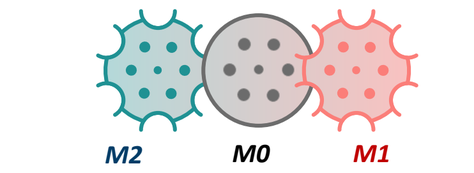
Dysregulated cytokines in the TME induce pathogenic immunosuppression that supports tumor growth.

Rebalancing inflammation locally offers a generalizable approach to treat many solid tumors, but systemic cytokine blockade carries risks such as increased risk of infection.

Macrophage cell therapies for modulating inflammation

Cell therapies offer a localized solution to rebalance inflammation.

Macrophages are capable of initiating (M1) and resolving (M2) inflammation.

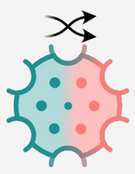


Engineered macrophages have demonstrated promising ability to target tumor cells using CARs [1-2].

Objectives

Develop a macrophage-based platform to:

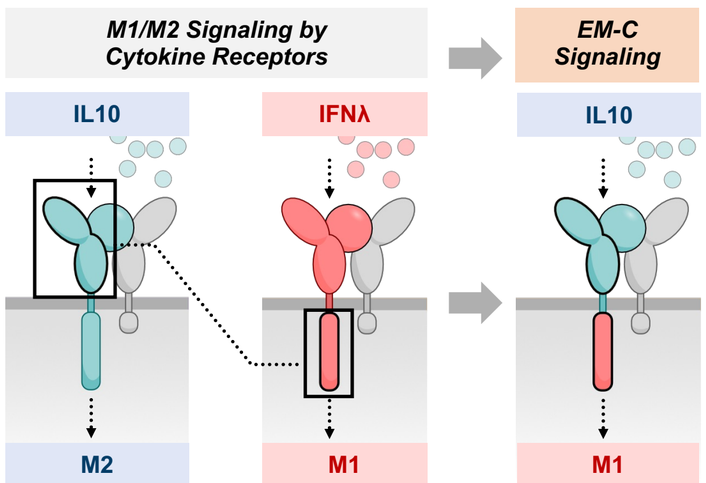
1. Convert immunosuppressive cytokines (IL10, TGFβ) into pro-inflammatory signals
2. Boost the inflammatory profile of solid tumors
3. Promote an anti-tumor response



Engineered Microenvironment Converters (EM-C)
Cytokine Switch Receptor
+ Myeloid Cell

Materials and Methods

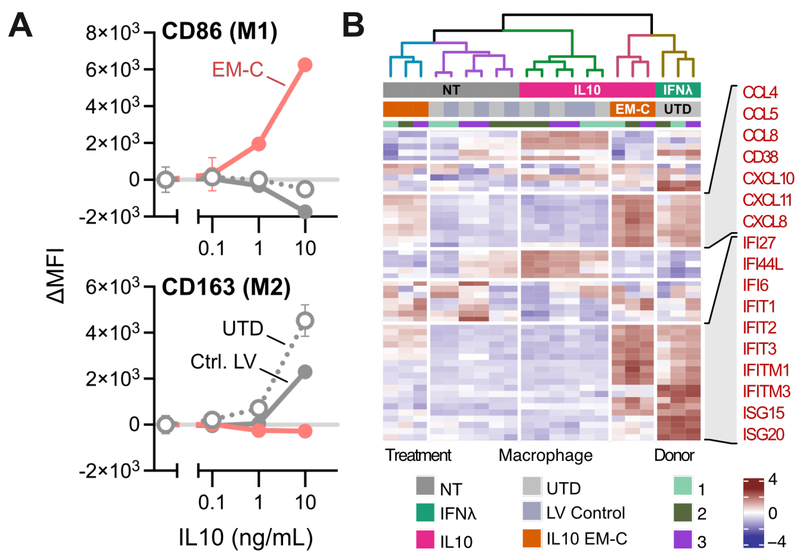
Switch Receptors (SR) are chimeric proteins consisting of the ligand binding domain from one cytokine receptor, paired with a compatible cytosolic domain from a second receptor. SR design enables EM-C to convert anti-inflammatory M2 cytokines into M1 signals, or *vice versa*.



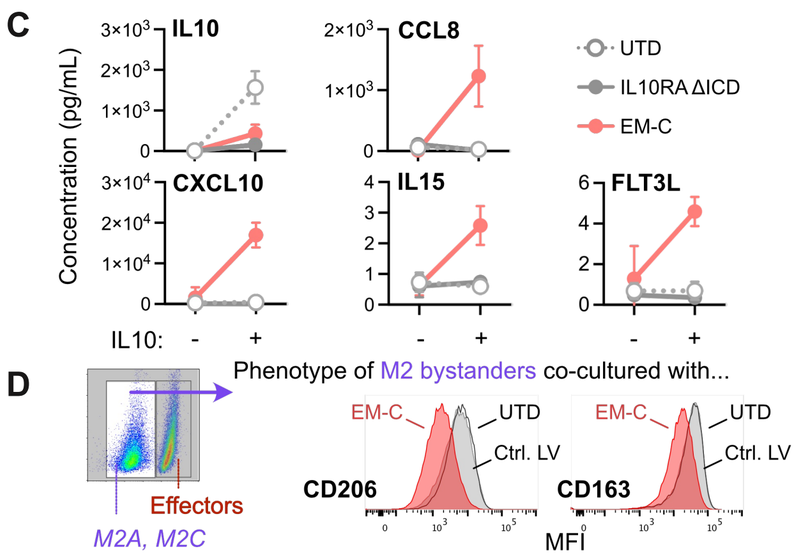
- EM-C are generated by expressing Switch Receptors (SR) in primary human macrophages, human monocytes, or murine macrophages
- SR are delivered using VPX-Lentiviral particles (for *in vitro* human studies) or adenoviral particles (for *in vivo* murine studies)
- For M2→M1 signal conversion, SR are generated to target IL10 or TGFβ
- *In vivo* tumor models are performed in Balb/c mice with syngeneic tumors
- All *in vitro* data shown are representative of at least three independent donors and/or experiments
- Measurements are reported as mean ± SD

EM-C macrophages convert IL10 into a pro-inflammatory signal

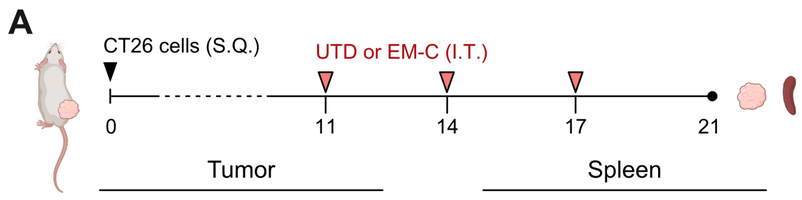
Primary human EM-C interpret IL10, a common immunosuppressive factor in solid tumors, as a pro-inflammatory (M1) signal (**A**). IL10-treated EM-C resemble interferon-treated macrophages by gene expression (**B**).

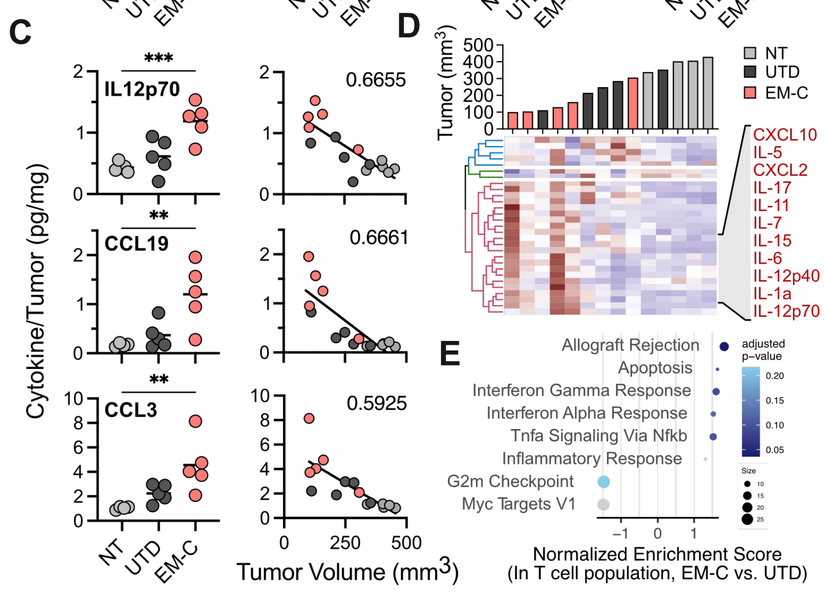


EM-C augment their microenvironment with pro-inflammatory soluble factors (**C**). TAM-like macrophages cultured with EM-C are skewed away from an M2 phenotype, demonstrating that EM-C can repolarize surrounding immune cells (**D**).



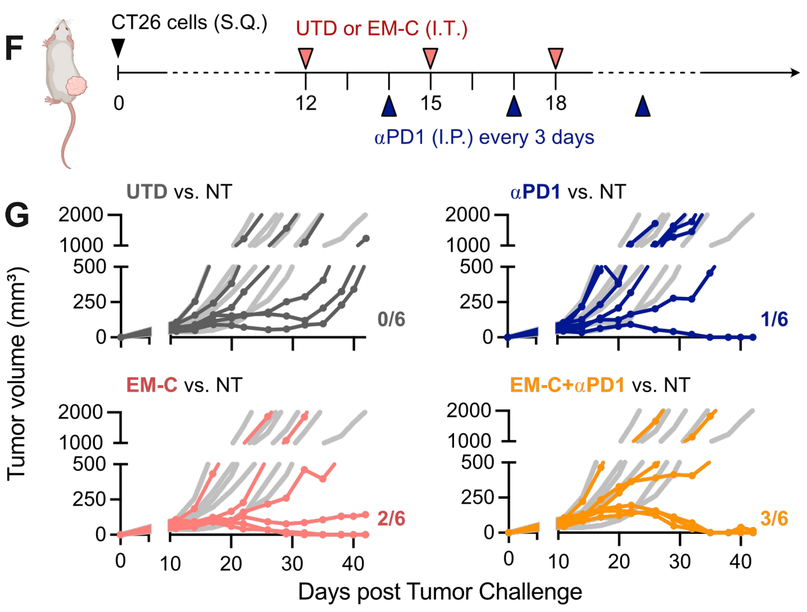
IL10 EM-C boost inflammation and promote an anti-tumor response *in vivo*





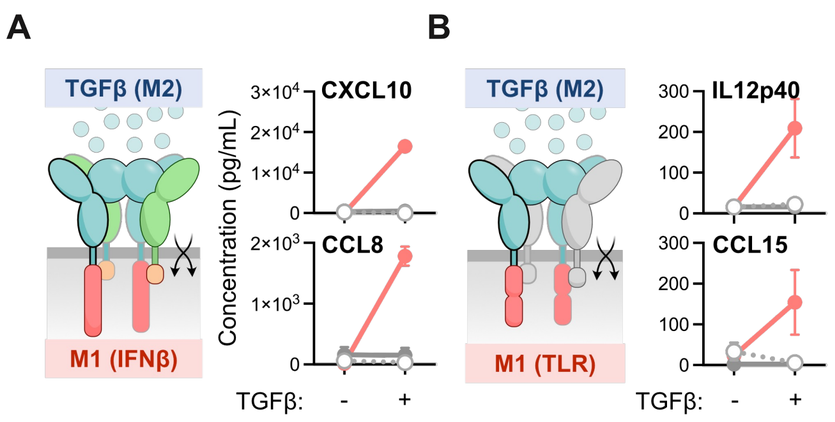
Left: The ability of IL10 EM-C to reprogram an immunosuppressive TME was evaluated using a SQ/IT CT26 model (**A**). EM-C remodeled the immune compartment locally within the tumor (↑CD8, ↓Treg) and systemically in the spleen (↑Effectors, ↓Naïve) (**B**). EM-C promoted a pro-inflammatory profile of cytokines and chemokines, which correlated with overall anti-tumor response (**C-D**). scRNAseq on CD45+ cells within the tumor confirmed remodeling of the immune compartment and activation of T cells by EM-C (**E**).

Below: Anti-tumor response was evaluated, ± checkpoint blockade (**F**). EM-C alone delayed tumor growth in an antigen-independent manner (**G**). Addition of PD1 blockade synergized for tumor control greater than either monotherapy.



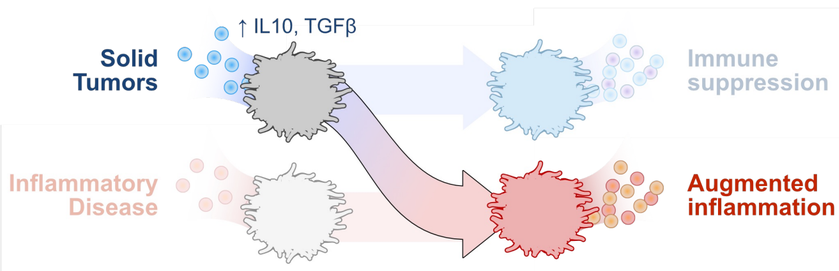
Expanding EM-C to target TGFβ

The modular EM-C platform is broadened to target TGFβ. EM-C can interpret TGFβ as an interferon (**A**) or TLR-like (**B**) signal to upregulate distinct repertoires of pro-inflammatory cytokines and chemokines.



Conclusions

EM-C is an immunotherapy platform that uses myeloid cells as 'living converters' to locally modulate inflammation



Robust control of inflammatory phenotype.

Sequester IL10 or TGFβ and heat up the microenvironment.

Boost antigen-agnostic anti-tumor immune responses *in vivo*.

[1] Klichinsky, M. *et al.* Human chimeric antigen receptor macrophages for cancer immunotherapy. *Nat Biotechnol* 1–7 (2020).
[2] Anderson, N. R., Minutolo, N. G., Gill, S. & Klichinsky, M. Macrophage-Based Approaches for Cancer Immunotherapy. *Cancer Res* **81**, 1201–1208 (2021).
Schematics of mouse experimental timelines created using Biorender.com

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