Macrophages expressing synthetic cytokine receptors reverse IL10-mediated Immunosuppression within solid tumors and promote adaptive immunity

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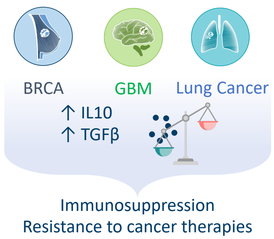
Abstract #5249

Introduction

Cytokines mediate immunosuppression in solid tumors



Cytokines regulate pro- and anti-inflammatory signals.

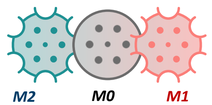
Dysregulated cytokines in the TME (tumor microenvironment) induce pathogenic immunosuppression that supports tumor growth.

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 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

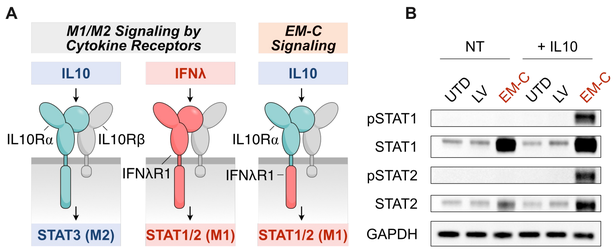
Engineered Microenvironment Converters (EM-C)
Cytokine Switch Receptor
+ Myeloid Cell

Materials and Methods

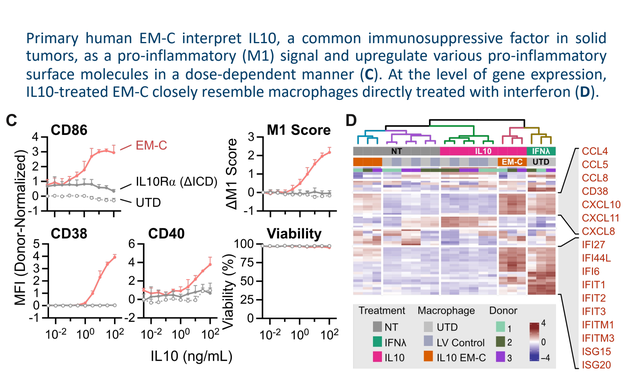
- EM-C are generated by expressing Switch Receptors (SR) in primary human macrophages, human monocytes, or murine macrophages
- SR are delivered using VPX-Lentiviral particles (for *in vitro* human studies) or adenoviral particles (for *in vivo* murine studies)
- For M2→M1 signal conversion, SR are generated 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 convert IL10 into a pro-inflammatory signal

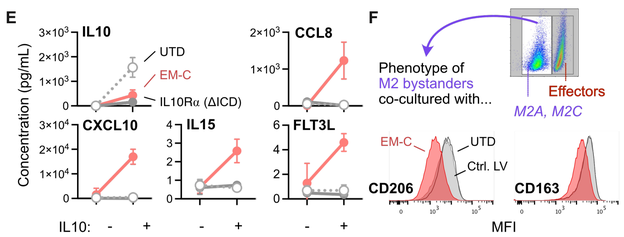
EM-C express Switch Receptors (SR), chimeric proteins consisting of the cytokine binding domain from one receptor and a signaling domain from a second receptor (**A**). SR enable EM-C to convert anti-inflammatory M2 cytokines into M1 signals, or *vice versa*. Here, IL10 induces STAT1/STAT2 phosphorylation only for EM-C (**B**).



Primary human EM-C interpret IL10, a common immunosuppressive factor in solid tumors, as a pro-inflammatory (M1) signal and upregulate various pro-inflammatory surface molecules in a dose-dependent manner (**C**). At the level of gene expression, IL10-treated EM-C closely resemble macrophages directly treated with interferon (**D**).

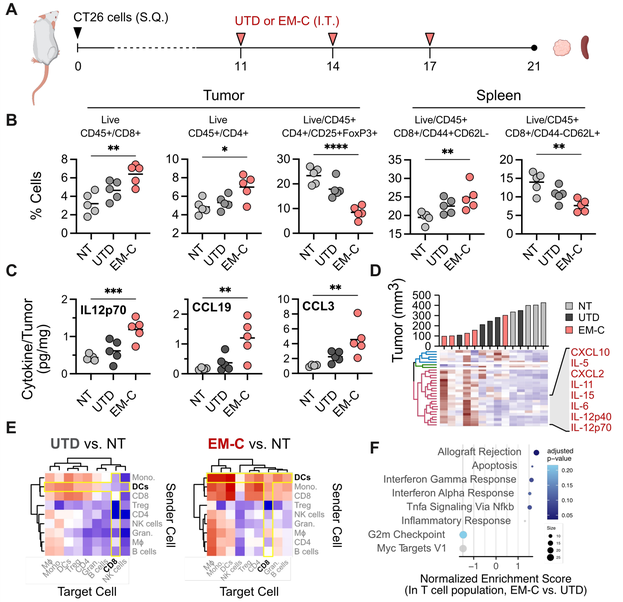


EM-C augment their microenvironment with pro-inflammatory soluble factors (**E**). TAM-like macrophages cultured with EM-C are skewed away from an M2 phenotype, demonstrating that EM-C can repolarize surrounding immune cells (**F**).

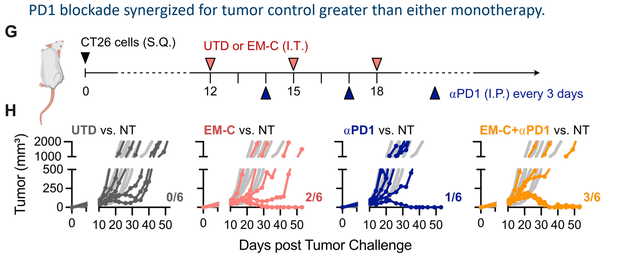


IL10 EM-C boost anti-tumor inflammation *in vivo*

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 elevated the presence pro-inflammatory soluble proteins, which correlated with anti-tumor response (**C-D**). scRNAseq revealed that EM-C promoted cell-cell communication between immune cells (**E**) and an activated phenotype in T cells (**F**).

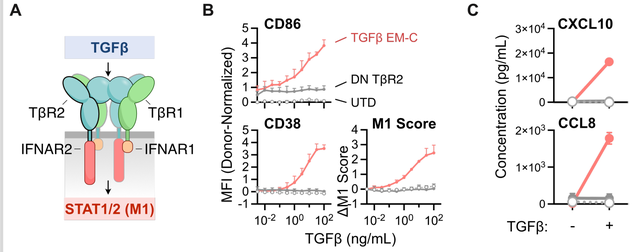


Anti-tumor response was evaluated, ± checkpoint blockade in a SQ/IT CT26 model (**G**). EM-C alone delayed tumor growth in an antigen-independent manner (**H**). Addition of PD1 blockade synergized for tumor control greater than either monotherapy.

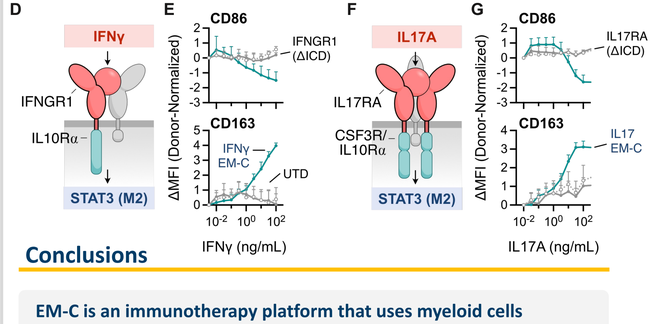


EM-C is a modular platform for targeting inflammation

The modular EM-C platform is broadened to target TGFβ (**A**). EM-C can interpret TGFβ as an interferon-based signal in a dose-dependent manner (**B**). EM-C targeting TGFβ release pro-inflammatory soluble factors following TGFβ treatment.

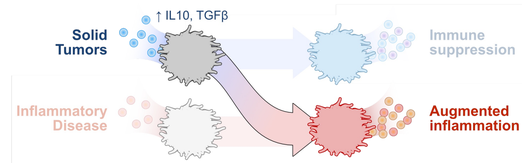


The EM-C approach can be "inverted" to target inflammatory cytokines for resolving inflammation. EM-C were designed to convert prototypical inflammatory cytokines IFNγ (**D-E**) and IL17A (**F-G**) into anti-inflammatory signals in a dose-dependent manner.



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) 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

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