Development of an M1-Polarized, Non-Viral CAR Macrophage (CAR-M) Platform for Cancer Immunotherapy

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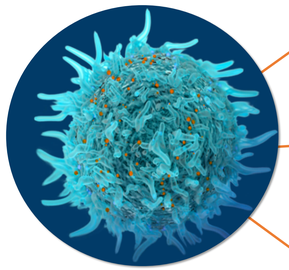
INTRODUCTION

CAR-M cellular immunotherapy for solid tumors

We have previously developed CAR-M as a novel cell therapy approach for the treatment of solid tumors[1]. CAR-M have the potential to overcome key challenges that cell therapies face in the solid tumor setting.



Tumor Trafficking and Penetration

Monocytes and macrophages are actively recruited to solid tumors and metastatic sites

Immunosuppressive TME

CAR-M are polarized toward an anti-tumor M1 phenotype, and upregulate genes that enhance the recruitment/activation of immune cells

Target antigen heterogeneity

Macrophages are professional antigen presenting cells that prime T cells, induce epitope spreading and overcome target antigen heterogeneity

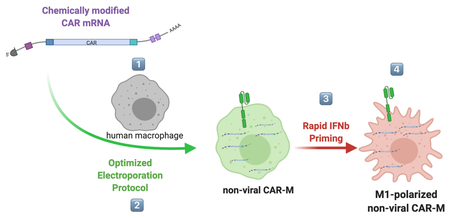
Non-viral CAR delivery

Although macrophages transduced with the adenoviral vector Ad5f35 (Ad CAR-M) traffic to tumors, provide robust anti-tumor activity, and recruit and activate T cells, we sought to identify a robust non-viral method of macrophage engineering and a companion method of inducing a durable pro-inflammatory (M1) phenotype.

METHODS

1. Human CD14+ monocytes were cultured for 7 days with GM-CSF to generate macrophages.
2. Anti-HER2 CAR mRNA comprising multiplexed chemical modifications was electroporated using an optimized protocol.
3. Electroporated macrophages were treated with IFNs for 4 hours after electroporation. Macrophage viability, CAR expression, and phenotype were evaluated using FACS at various time points.
4. Phagocytosis and killing of target tumor cells AU-565 and SKOV-3 were detected with via live-imaging with the Incucyte System. Macrophage phenotype was assessed using RNA-Seq.

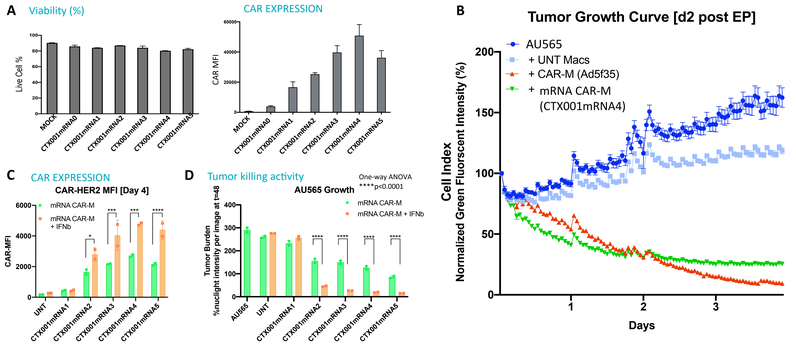
Schema of M1-primed non-viral CAR-M production



CONCLUSION

- We have established a novel, optimized non-viral M1 CAR-M platform based on chemically modified mRNA and IFN-β priming.
- IFN-β priming induced a durable **M1 phenotype**, **improved CAR expression**, **CAR persistence**, led to **enhanced anti-tumor function**, and rendered resistance to immunosuppressive factors.
- This novel platform is amenable to scale-up, GMP manufacturing, and represents a significant advance in the development of non-viral CAR-M.

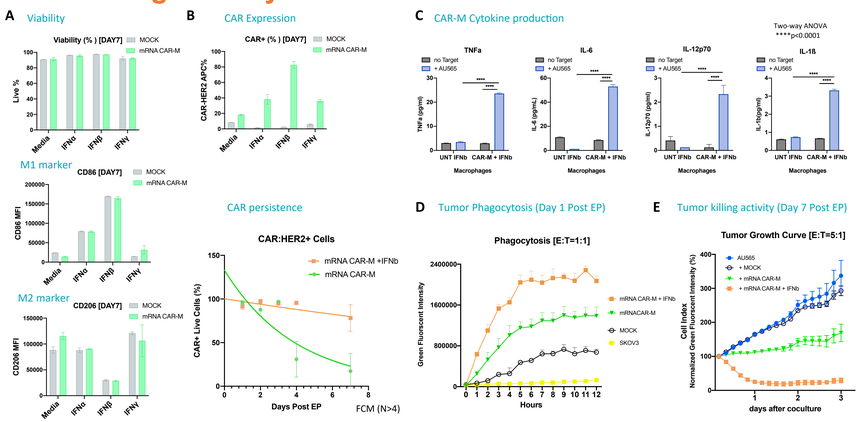
Optimization of mRNA Chemistry for Human Macrophage Engineering



Evaluating CAR mRNA with Chemical Modifications on CAR-M Viability, CAR Expression and Function

(A) Chemically modified CAR mRNA (variant 0-5) was electroporated (EP) into human macrophages. CAR-M viability and CAR expression is shown 2 days after CAR mRNA EP. **(B)** Chemically modified CAR mRNA 4 as well as Ad5f35 CAR-M led to robust target killing activity 2 days after mRNA EP. AU565 (HER2+ breast cancer) growth was monitored via live fluorescent imaging on Incucyte. **(C)** Chemically modified CAR mRNA (variant 0-5) was EP'd into macrophages followed by treatment with IFNβ for 4h post EP. CAR expression on day 4 was significantly enhanced by a pulse of IFNβ. One-way ANOVA *p<0.05 ***p<0.001, ****p<0.0001 **(D)** IFNβ treated mRNA CAR-M enhanced target killing activity 2 days after mRNA EP. The graph shows target cell (AU565) growth 48 hours after co-culture started.

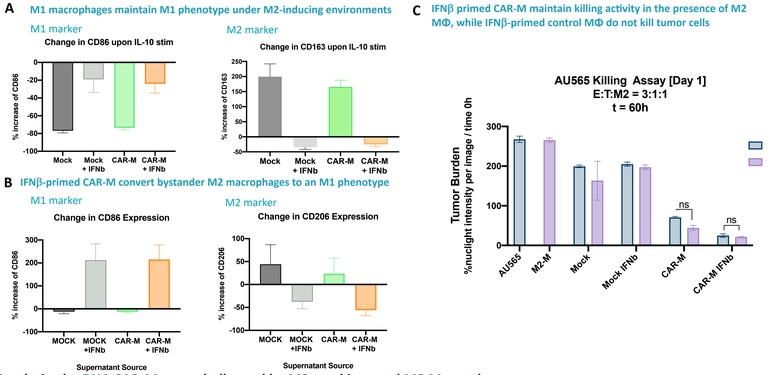
Generating Durably M1 Polarized Non-viral CAR-M



IFNβ stimulation skewed mRNA CAR-M to a durable M1 phenotype, improved CAR persistence, and enhanced anti-tumor function

(A) mRNA CAR-M were treated with either IFNα, IFNβ, or IFNγ for 4 hours after electroporation. CAR-M viability and phenotype are shown 7 days post EP and priming. **(B)** CAR expression and persistence were significantly enhanced with 4h of IFNβ treatment. The half-life of CAR expression increased from 4 days to 10 days with IFNβ treatment. **(C)** Cytokine production of CAR-M with or without stimulation by the HER2+ targets (AU565). Supernatant was collected 3 days after the coculture. **(D)** PhRodo-labeled SKOV3 phagocytosis was detected via Incucyte. IFNβ treatment significantly enhanced CAR-mediated phagocytosis. **(E)** IFNβ treated CAR-M maintains AU565 target cell killing activity 7 days after mRNA electroporation.

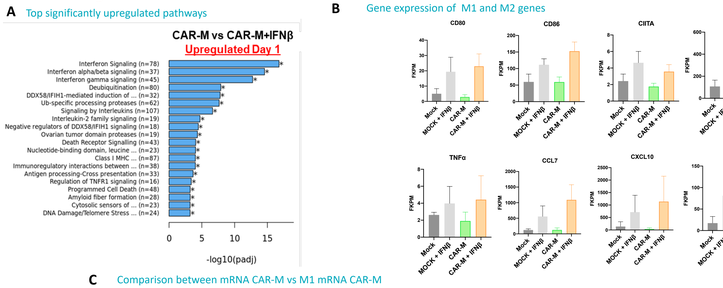
M1 Polarized mRNA CAR-M are Resistant to M2 Environments and Convert Bystander M2 Macrophage to M1



M1 polarized mRNA CAR-M were challenged by M2 cytokines and M2 Macrophages

(A) The percent change of M1/M2 surface markers on mock EP'd or CAR-M (+/- IFNβ) macrophages when treated with the M2 inducing cytokine IL-10 (20ng/ml for 24hrs). IL10 challenge was initiated 3 days post CAR mRNA EP/IFNβ priming, and surface marker expression was established through FACS on Day 4. **(B)** Percent change of M1/M2 surface marker expression on M2 macrophages after 24hr stimulation with Mock (+/-IFNβ) or CAR-M (+/-IFNβ) supernatant. **(C)** Incucyte-based killing assay of Mock (+/-IFNβ) or CAR-M (+/-IFNβ) against AU565 with or without addition of M2 macrophages at a ratio of 3:1:1 (E:T:M2) taken at 60 hours.

IFNβ-Primed Non-Viral CAR-M Upregulate Pro-inflammatory Pathways



C Comparison between mRNA CAR-M vs M1 mRNA CAR-M

Category	Genes	mRNA CAR-M	M1 mRNA CAR-M
Antigen Presentation	HLA-A, CIITA	-	✓
Adaptive Immunity	CD80, CD86, CD40, CD1D	-	✓✓
Cytokines	IL23,IL7, TNFa, IL6	-	✓
Chemokines	CXCl10, CCL8,CCL5,CCL7	-	✓✓
Transcription Factors	BATF3, IRF7, STAT1,STAT2	-	✓✓

Differentially expressed genes in CAR Macrophages treated with IFNβ from 3 independent donors.

(A) Top significantly upregulated pathways based on reactome analysis at Day 1. **(B)** Expression of select M1-associated genes. The cutoffs P(adj) < 0.001, fold change ≥1.0 log2 were used for determination of differentially expressed genes. **(C)** Chart showing comparison for key pathway categories induced by IFNβ priming of mRNA CAR-M.

1. Klichinsky M, et al. Human chimeric antigen receptor macrophages for cancer immunotherapy. Nat Biotechnol. 2020;38(8):947-953.

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