Development and Characterization of Chimeric Antigen Receptor Monocytes (CAR Mono), a Novel Cell Therapy Platform for Solid Tumor Immunotherapy

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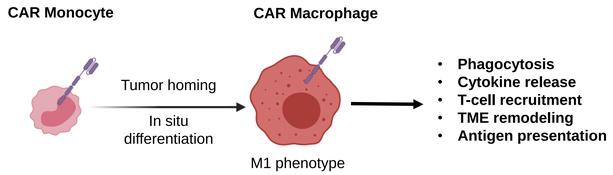
#104

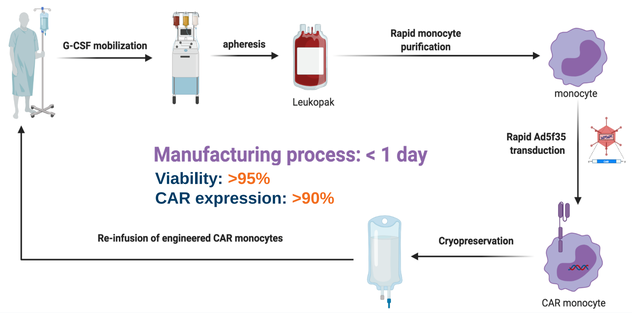
Introduction

Engineered cell therapies have demonstrated significant clinical activity against hematologic malignancies, but solid tumors remain an intractable challenge. We have previously developed a human chimeric antigen receptor macrophage (CAR-M) platform for adoptive cell therapy and shown potent anti-tumor activity in pre-clinical solid tumor models[1]. CAR-M overcome critical solid tumor challenges such as tumor infiltration, immunosuppression within the tumor microenvironment, lymphocyte exclusion, and target antigen heterogeneity. Currently, CAR-M are generated in a week-long ex-vivo process in which peripheral blood monocytes are differentiated into macrophages prior to genetic manipulation. Here, we demonstrate the production feasibility, phenotype, pharmacokinetics, cellular fate, antigen specificity, and anti-tumor activity of human CD14+ CAR monocytes.

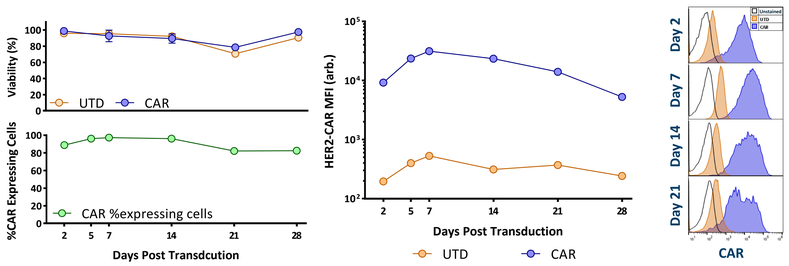
Mechanism of action mediated by CAR Monocytes (CAR Mono)



Single Day Manufacturing Process

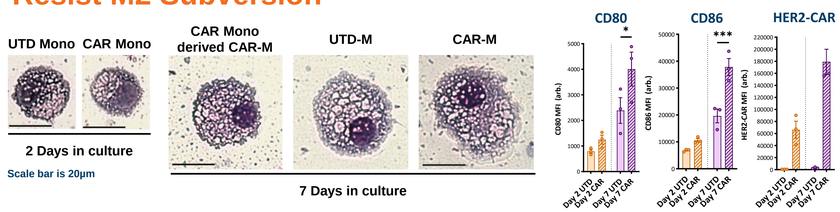


Ad5f35 Transduced Monocytes Show High Sustained CAR Expression and Viability *In Vitro*

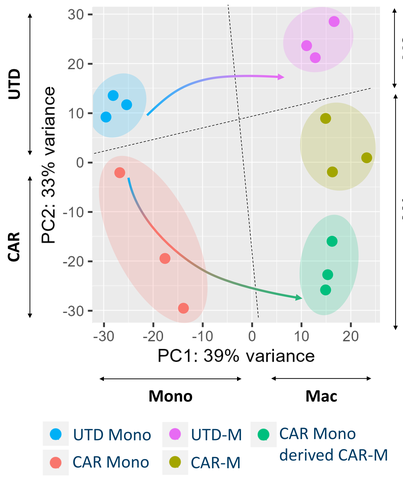


CAR Mono show robust CAR expression and Persistence
CAR expression is high following Ad5f35 HER2-CAR transduction. Both CAR expression and cell viability remain high for at least 28 days *in vitro*.

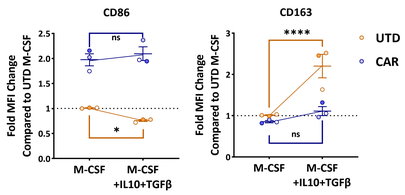
CAR Mono Differentiate into M1 CAR Macrophages that Resist M2 Subversion



Ad5f35 transduced CAR Mono differentiate into M1-like macrophages
CAR Mono show a progressively increasing M1 phenotype and increased CAR expression. Differentiated CAR Mono show morphology and phenotype similar to CAR-M.

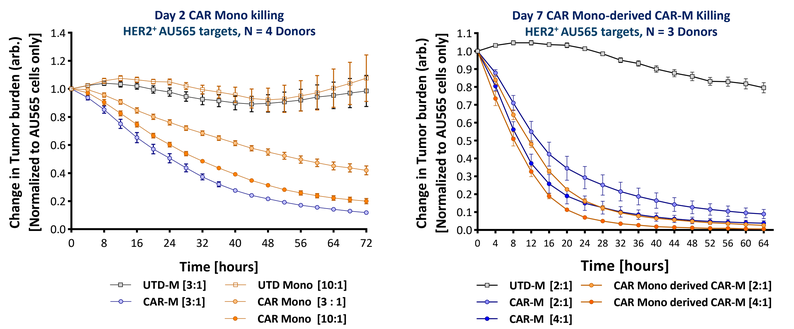


CAR Mono-derived CAR-M and CAR-M share a similar transcriptomic profile
RNA-seq comparison of UTD vs CAR Mono and CAR-M, N = 3 matched donors



CAR Mono are Protected against M2 Skewing with IL10/TGFβ
Monocytes cultured with M-CSF± IL10 and TGFβ. Data normalized to UTD cells cultured with M-CSF only, N = 3 donors (shading correspond to different donors)

CAR Mono Show Significant Killing Ability 48hr Post Transduction That Increases With Differentiation

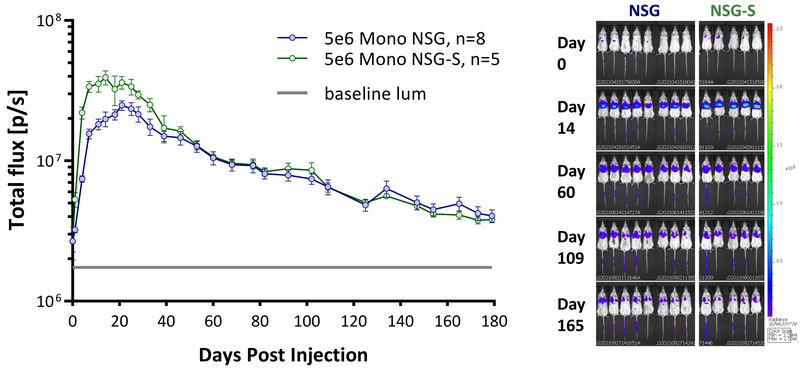


CAR Mono show killing ability 48hr post transduction. CAR-M are shown as reference.

Fully differentiated CAR Mono-derived CAR-M show efficient eradication of tumor cells.

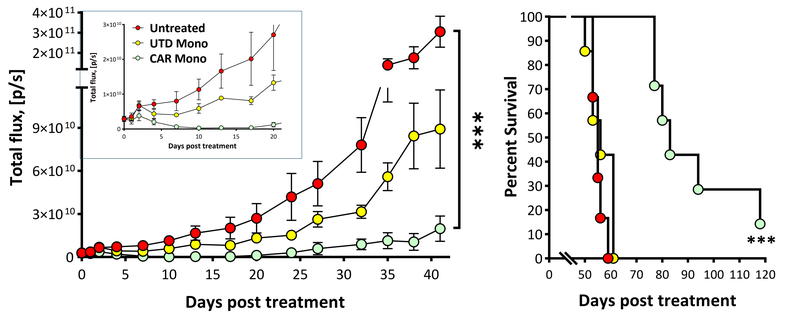
In vitro Incucyte based real-time killing assays of HER2+ AU565 breast cancer cells.

CAR Mono Show Long Term Persistence *In Vivo*



Human CAR Mono show long term persistence in an *in vivo* xenograft mouse model independent of human GM-CSF.
CAR Mono $t_{1/2}$ is ~45 days and were still detected at last follow up Day 174.

CAR Mono Suppress Tumor Growth *In Vivo*



Human anti-HER2 CAR Mono suppress the growth of SKOV3 tumors in a xenograft mouse model of ovarian cancer intraperitoneal carcinomatosis
Inset shows the first 20 days of the experiment following a single dose of treatment.
Median Survival: Untreated/UTD = 55 days, CAR Mono = 83 days

Conclusion

- **Primary human CAR Mono were successfully generated with high efficiency and viability in a single day manufacturing process using Ad5f35.**
- **CAR Mono differentiated into macrophages, maintain M1 polarization and resist M2 subversion.**
- **CAR Mono and CAR Mono-derived CAR-M eradicated HER2+ cancer cells.**
- **CAR Mono persisted long term in vivo ($t_{1/2}$ = 45 days) and mediated anti-tumor activity, improving overall survival.**

[1] Klichinsky M, et al. Human chimeric antigen receptor macrophages for cancer immunotherapy. Nature Biotechnology. 2020

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UTD – untransduced
CAR – chimeric antigen receptor

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