Pre-clinical development of CAR Monocytes (CAR Mono) for solid tumor immunotherapy

Daniel Blumenthal, Linara Gabitova, Brett Menchel, Patricia Reyes-Uribe, Rehman Qureshi, Sabrina Ceeraz DeLong, Sascha Abramson, Thomas Condamine, Michael Klichinsky

Carisma Therapeutics, Philadelphia PA USA

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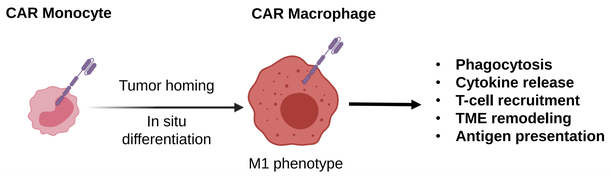
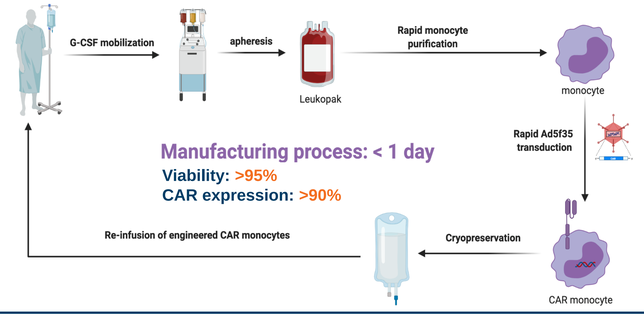
Introduction

Engineered cell therapies have demonstrated significant clinical activity against hematologic malignancies, but responses have been rare in solid tumors. Our previously developed human chimeric antigen receptor macrophage (CAR-M) platform has shown potent anti-tumor activity in pre-clinical solid tumor models1, and the anti-HER2 CAR-M CT-0508 is currently being evaluated in a Phase I trial. The use of myeloid cells as a platform for cell therapy provides the tools to overcome critical solid tumor challenges such as 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, specificity, and anti-tumor activity of human CD14+ CAR monocytes.

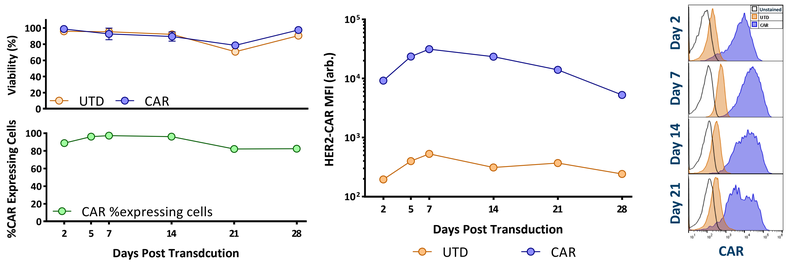
Mechanism of action mediated by CAR Monocytes (CAR Mono)



Single Day Process

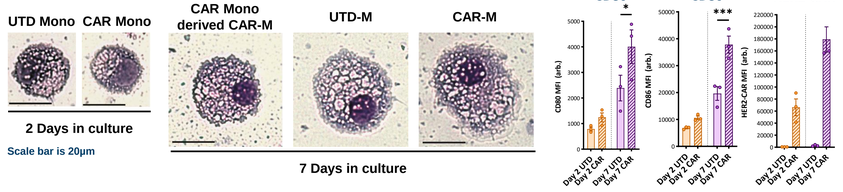


Ad5f35 Transduced Monocytes Show High Sustained CAR Expression and Viability *In Vitro*



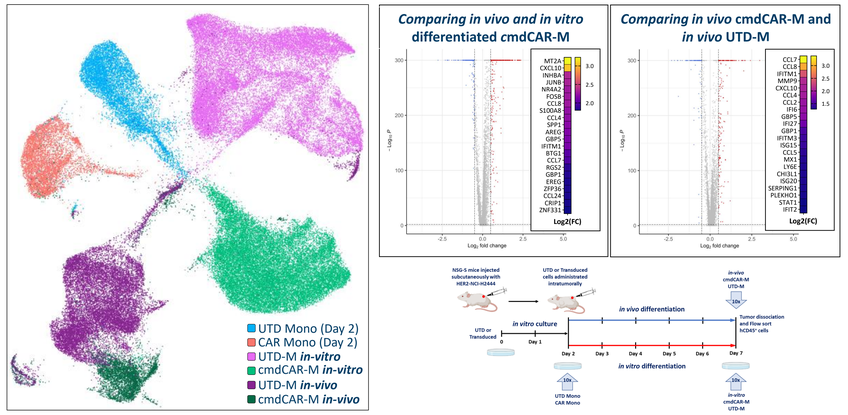
CAR Mono show robust CAR expression and Persistence
CAR expression is high following Ad5f35 HER2-CAR transduction. Both CAR expression and cell viability remain high for at least 28 days *in vitro*.

CAR Mono Differentiate into M1 CAR Macrophages



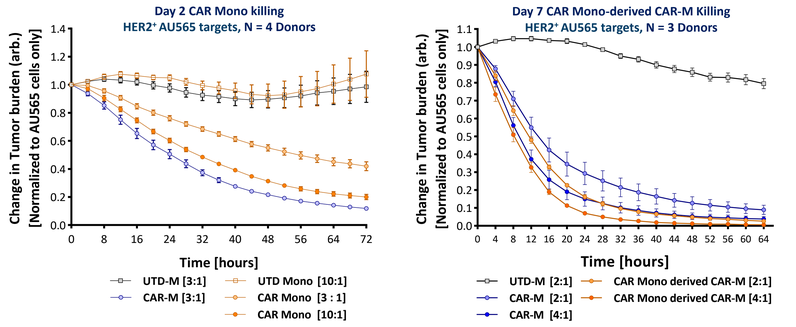
Ad5f35 transduced CAR Mono differentiate into M1-like macrophages. CAR Mono show a progressively increasing M1 phenotype and CAR expression. CAR mono derived CAR-M show Morphology and phenotype similar to our current CAR-M product

CAR Mono Differentiate into M1 CAR-M in tumors



CAR Mono-derived CAR-M show strong pro-inflammatory profile enhanced by *intratumoral* differentiation in the presence of HER2 expressing cancer cells. scRNA-Seq of UTD and CAR mono cultured in vitro or derived from solid tumors. N = 3 donors

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

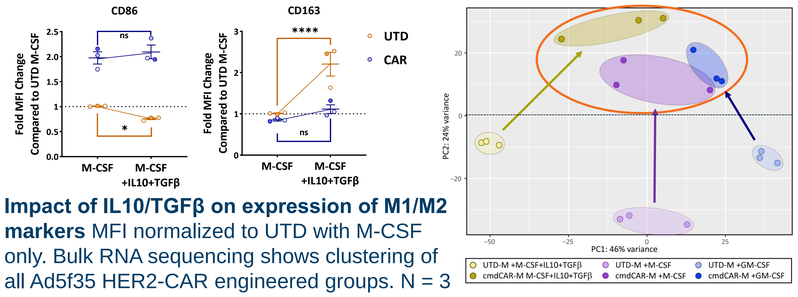


CAR Mono show killing ability 48hr post transduction. CAR-M are shown as reference.

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

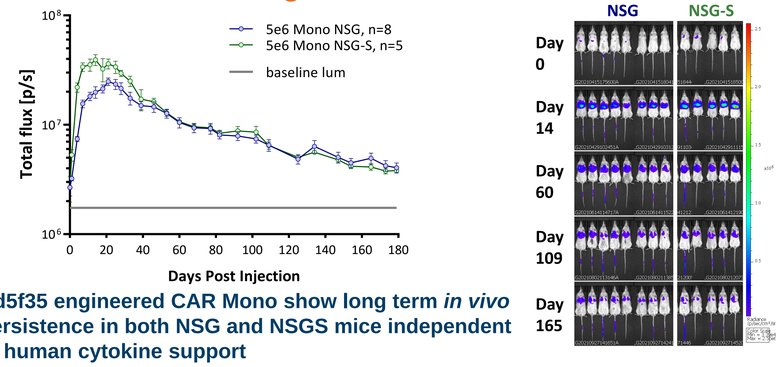
In vitro Incucyte based real-time killing assays of HER2+ AU565 breast cancer cells.

CAR Mono Are Protected Against M2 Skewing



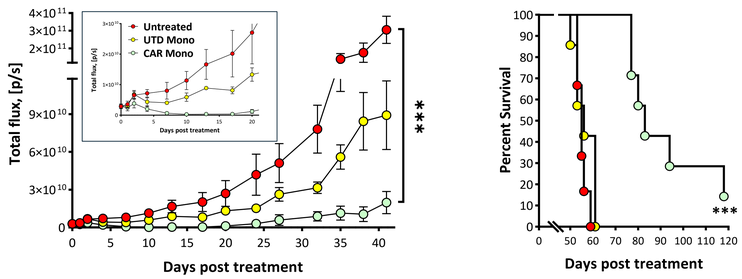
Impact of IL10/TGFβ on expression of M1/M2 markers MFI normalized to UTD with M-CSF only. Bulk RNA sequencing shows clustering of all Ad5f35 HER2-CAR engineered groups. N = 3

CAR Mono Show Long Term Persistence *in vivo*



Ad5f35 engineered CAR Mono show long term *in vivo* persistence in both NSG and NSGS mice independent of human cytokine support

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 on day 0. 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

* Contact: michael.klichinsky@carismatx.com

UTD – untransduced
CAR – chimeric antigen receptor
cmdCAR-M – CAR Mono derived CAR Macrophages

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