Anti-HER2 CAR monocytes demonstrate targeted anti-tumor activity and enable a single day cell manufacturing process

#1530

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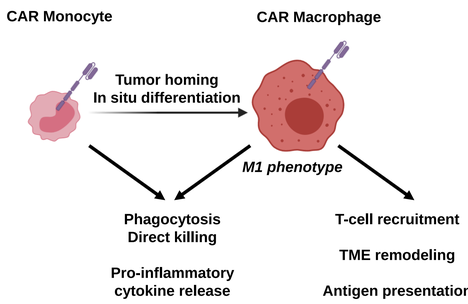
Introduction

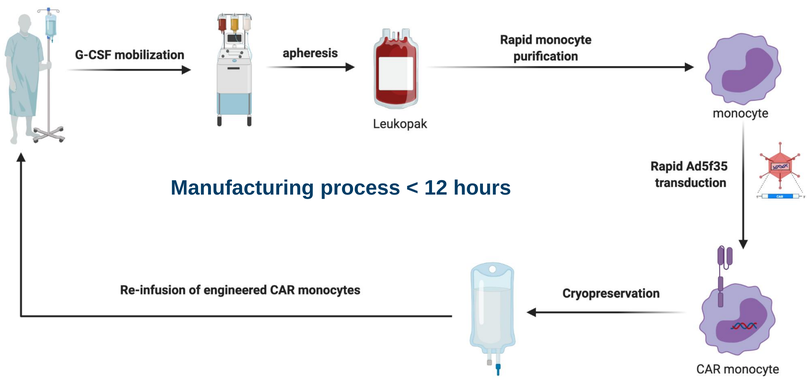
Recent advances in cell therapy have led to significant efficacy in hematologic malignancies, but solid tumors remain an intractable challenge. We have previously developed a CAR Macrophage (CAR-M) adoptive cell therapy platform and demonstrated potent anti-tumor activity in pre-clinical models. CAR-M overcome several of the barriers to efficacy in the solid tumor setting – trafficking, immunosuppression in the tumor microenvironment, lymphocyte exclusion, and antigen heterogeneity[2].

Currently, CAR-M are generated via *ex vivo* differentiation of peripheral blood monocytes into macrophages prior to genetic manipulation. In order to streamline cell manufacturing into a single day process, we sought to evaluate the feasibility of directly engineering CD14+ CAR monocytes (CAR Mono).

Mechanism of action of CAR Monocytes



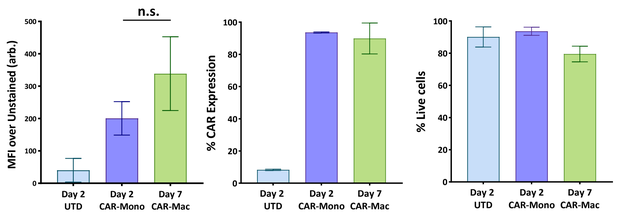
Single day CAR Mono manufacturing process



Manufacturing process < 12 hours

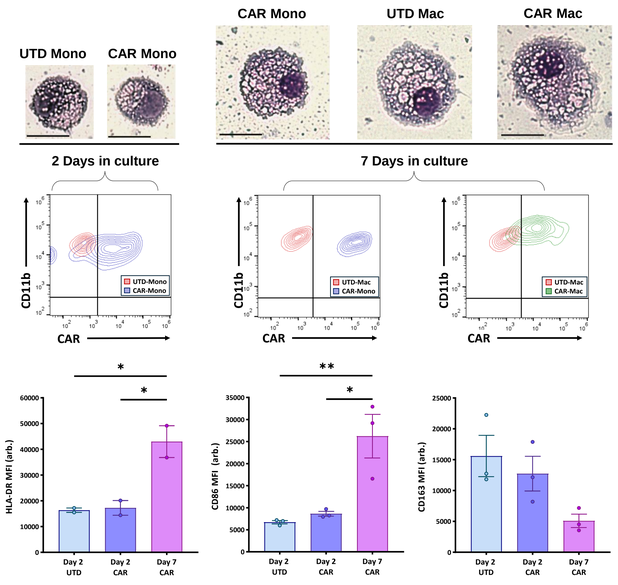
Cell manufacturing time: < 12 hours
Cell recovery: >80%
Viability: >95%
CAR expression: >80%

CAR expression and viability



Human CAR Mono achieve robust CAR expression after transduction with Ad5f35. CAR expression and viability are comparable to standard CAR-M.

CAR Mono differentiate into M1 CAR Mac

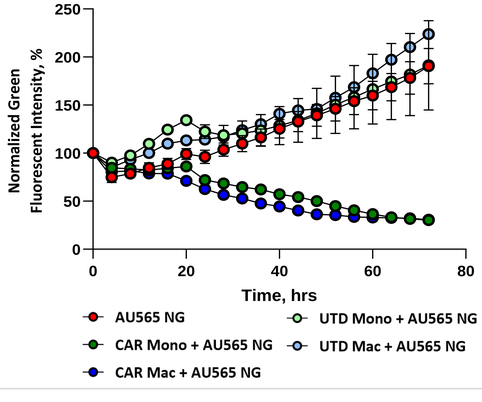


In response to *ex vivo* GM-CSF treatment, CAR Mono differentiate into CAR-M. Differentiated CAR Mono present a morphology and phenotypic profile that is similar to standard CAR-M.

Scale bar is 20µm

CAR Mono differentiate into CAR-M in response to GM-CSF and maintain M1 polarization post-differentiation.

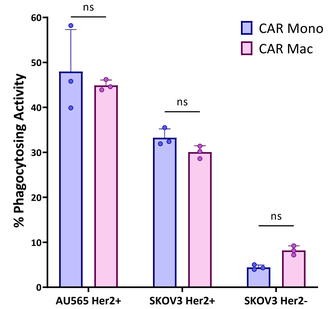
CAR Mono directly kill tumor cells *in vitro*



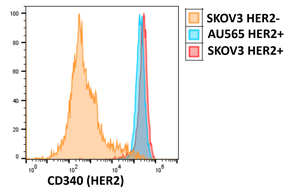
Human anti-HER2 CAR Mono efficiently eradicate HER2+ AU565 breast cancer cells in an *in vitro* killing assay.

AU565 growth was tracked in real time using an IncuCyte live imaging system. CAR Mono show similar killing kinetics to CAR-M.

CAR Mono phagocytose tumor cells *in vitro*

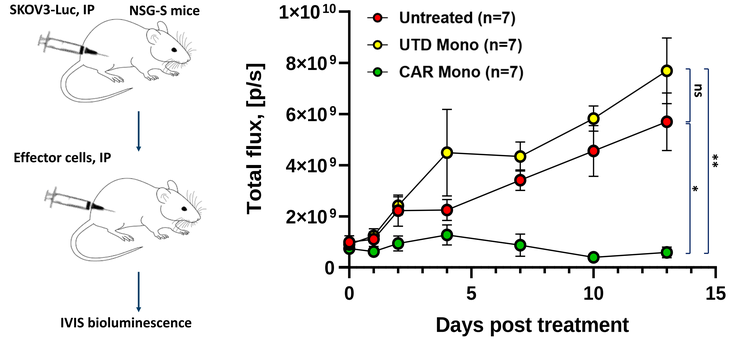


CAR Mono show similar ability to phagocytose HER2+ SKOV3 and AU565 cells. Both CAR Mono and Mac show minimal phagocytosis of HER2- SKOV3 cells.



HER2 expression on target cancer cell lines. The SKOV3 HER2- cells were generated with CRISPR/Cas9 and show ~10% HER2 expression, corresponding to low levels of phagocytosis by effector cells.

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.

Conclusion

- Primary human CAR Monocytes can be successfully generated with high efficiency and viability in a single day manufacturing process using Ad5f35.
- CAR Mono differentiate into macrophages and maintain M1 polarization.
- CAR Mono phagocytose and eradicate HER2+ ovarian and breast cancer cells *in vitro*, showing comparable function to CAR-M.
- CAR Mono suppress tumor growth *in vivo* a HER2+ ovarian cancer model
- These data support further development of CAR Mono for the cellular immunotherapy of solid tumors.

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

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UTD – untransduced control cells
CAR – chimeric antigen receptor

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