PD-1 Blockade Protein in 4th Generation Armored CAR-T Cells Enhances Cytotoxicity Effect with in vitro Re-challenge System

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Abstract

Chimeric antigen receptor (CAR)-T cells are genetically engineered T cells that express a surface receptor to recognize tumor-associated antigens (TAA). CAR-T cell therapy have demonstrated remarkable success with hematological malignancies. However, limited headway has been made towards solid tumor, due to various challenges, including recognition of tumor-specific antigen trafficking and penetration, localization and survival within an immunosuppressive tumor microenvironment (TME) [1].

Overexpression of immunosuppressive cytokines and/or proteins, such as TGF-β and PD-L1, respectively, downregulates cytotoxic CD8+ T cells, reducing the efficacy of CAR-T cell therapy. Immune checkpoint inhibitors (ICIs), such as anti-PD-1/PD-L1 and anti-CTLA-4 antibody have gathered immense attention due to their efficacy across multiple solid malignancies [2]. As such, a combination of CAR-T and ICIs may prove to be a viable strategy to overcome an unfavorable TME.

Here, we have established a 4th generation, armored CAR-T coupled with a PD-1 blockade protein, to enhance the anti-exhaustion effect of CAR-T cells. Our results indicate that the addition of a PD-1 blockade protein reduces the amount of available PD-1 on the surface in both CAR-positive and -negative T cells, reduces exhaustion of CAR-T cells, and prolong cytotoxic killing effect of CAR-T cells in a 192 h *in vitro* re-challenge assay compared to 3rd generation CAR-T.

Methods

CAR-T cells were generated from primary human T cells, which were isolated from human PBMC using pan T cell isolation kit. Lentiviral particles containing MSLN CAR and/or anti-PD-1 transgene were generated with 293T cells. T cells were stimulated with CD3/CD28 beads for 2 days, followed by the addition of CAR lentiviral particles for an additional 24 hour, then further proliferate for 6 days. Cells were collected for surface marker expression analysis after second round of stimulation, enriched *via* bead-based method, then co-cultured with luciferase-transduced HCT-116 cells for re-challenge cytotoxicity assay.

Results

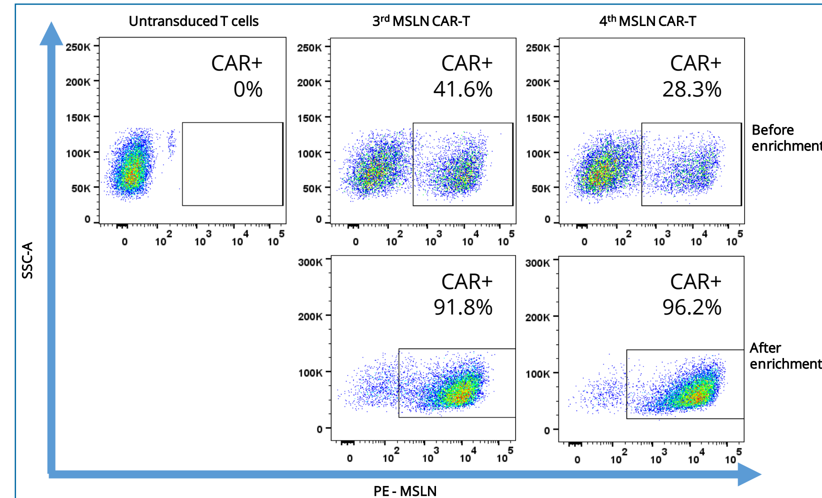

Figure 1: Transduction and enrichment of T cells with 3rd and 4th gen MSLN CAR construct. Lentiviral transduction of primary human T cells with MSLN CAR and/or anti-PD-1 transgene, and achieve an enrichment of 2.2 – 3.4 fold of CAR+ T cells with paramagnetic beads.

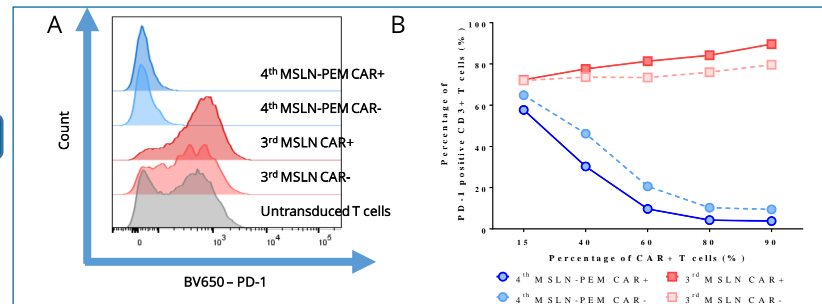

Figure 2: Surface PD-1 level of transduced T cells blocked by armored PD-1 blockade protein. (A) Surface PD-1 level in untransduced T cells, 3rd and 4th gen CAR-T cells were measured by FCM, (B) with the percentage of PD-1+ CD3+ T cells remain constant for 3rd gen CAR-T and a downward trend for 4th gen CAR-T armored with PD-1 blockade protein.

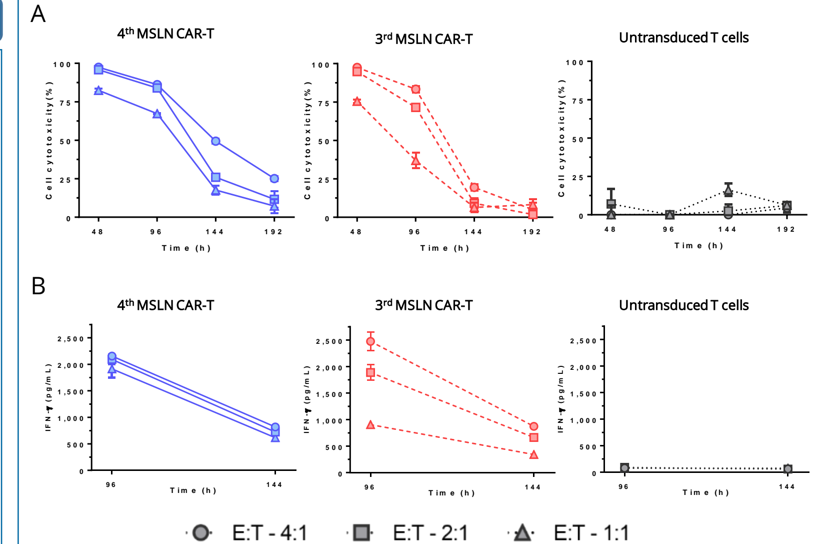

Figure 3: CAR-T armored with PD-1 blockade protein exhibited prolonged target cell cytotoxic effect. (A) Cell cytotoxicity over 192 h were measured by luciferase-based assay, with corresponding IFN-γ secretion at 96 and 144 h measured via HTRF-based assay.

Summary

The inclusion of PD-1 immune checkpoint blockade protein enhances and prolongs CAR-T cells cytotoxic killing effect in an *in vitro* re-challenge assay. This opens up the possibility of adding an immune checkpoint inhibitor transgene to existing CAR gene for enhanced CAR-T cytotoxicity.

References

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- Wu P, Wu D, Li L, Chai Y, Huang K. PD-L1 and Survival in Solid Tumors: A Meta-Analysis. PLoS ONE. 2015; 10:e0131403