PD-1 BLOCKADE PROTEIN COUPLED IN 4TH GENERATION ARMORED CAR-T CELLS ENHANCES CYTOTOXICITY EFFECT WITH IN VITRO RE-CHALLENGE SYSTEM

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**Background**
Chimeric antigen receptor (CAR)-T cells are genetically engineered T cells expressing a receptor on their surface to recognize tumor-associated antigens (TAA). CAR-T cell therapy has been coined as a ‘living drug’ and have demonstrated remarkable success with hematological malignancies. However, limited headway has been made with solid tumors due to various challenges, including recognition of tumor-specific antigens, trafficking and penetration, and survival within an immunosuppressive tumor microenvironment (TME). Some overexpression of immunosuppressive cytokines/proteins, such as TGF-β and PD-L1, downregulates cytotoxic CD8+ T cells and reduces the efficacy of CAR-T cell therapy. Immune checkpoint inhibitors (ICIs), such as anti-PD-1/PD-L1 and anti-CTLA-4 antibodies have gathered immense attention due to their efficacy across multiple solid malignancies. Therefore, a combination of CAR-T and ICIs could be a viable strategy to overcome the 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.

**Methods**
CAR-T cells were generated from primary human T cells which were isolated from human PBMC using a pan T cell isolation kit. Lentiviral particles containing the MSLN CAR gene with/without PD-1 transgene were generated in 293T cells. T cells were stimulated by CD3/CD28 beads for 2 days, followed by the addition of CAR lentiviral particles for an additional 24 hours, then further proliferated for 6 days. Cells were collected for surface marker analysis after the second stimulation or co-cultured with luciferase-transduced HCT-116 cells for re-challenge cytotoxicity assay.

**Results**
• 4th generation CAR-T cells have reduced surface PD-1 available for binding, compared to 3rd generation CAR-T cells and untransduced T cells.
• CAR-negative 4th generation T cells also have reduced available PD-1 binding sites.
• 4th generation CAR-T cell retains >70% cytotoxicity effect, while 3rd and 2nd generation CAR-T cells have markedly reduced cytotoxicity effect after two re-challenges with HCT-116-luc cells.

**Conclusions**
PD-1 immune checkpoint blockade enhances and prolongs CAR-T cells cytotoxic effect in an *in vitro* re-challenge assay. This research indicates the possibility of adding an immune checkpoint inhibitor transgene to the existing CAR gene to enhance CAR-T cytotoxicity.