290 A NOVEL IPSC-DERIVED CAR-INVARIANT NATURAL KILLER T (INKT) CELL THERAPY PLATFORM FOR HEMATOLOGIC MALIGNANCIES AND SOLID TUMORS

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Background Following a success of autologous chimeric antigen receptor (CAR)-T cells in hematologic malignancies, allogeneic CAR-transduced cells have been developed with various immune cells including induced pluripotent stem cell (iPSC)-derived NK cells and T cells. We have developed a novel platform of first-in-class iPSC-derived CAR-invariant natural killer T (iNKT) cells. iNKT cells are a rare subset of innate lymphocytes that bridge innate and adaptive immune response. iNKT cells can recognize and kill tumor cells, and further enhance antitumor activities of host endogenous immune cells by; cross-talking with dendritic cells; cross-priming tumor-specific $CD8^+$T cells; transactivating NK cells; and reprograming protumor myeloid cells. These intrinsic functions of iNKT cells possibly lead to high persistence and durability of allogeneic CAR-T cell therapy especially for solid tumors in the immunosuppressive tumor microenvironment. Usage of iPSC derived from iNKT cells is an ideal strategy to realize clinical scale production of functional iNKT cells from such a rare cell population. A Phase 1 study of the iPSC-derived non-transduced iNKT cells is currently ongoing in patients with head and neck squamous cell carcinoma. To demonstrate that CAR-transduced iPSC-derived iNKT cells provide a novel platform for effective cancer immunotherapy, the killing activities of CD19-CAR or HER2-CAR-transduced iPSC-derived iNKT cells were investigated in this first set of study.

Methods iNKT-derived iPSCs were engineered to express CD19-CAR or HER2-CAR by targeting the adeno-associated virus integration site-1 locus using genome editing method. CAR-iNKT cells were obtained by differentiation from CAR-introduced iPSCs under feeder cells free culture condition. The expressions of CAR and iNKT cell surface markers were examined by flow cytometry analysis. *In vitro* cytotoxicity of CAR-iNKT cells against cancer cell lines was examined by xCELLigence® real-time cell analyzer.

Results CD19-CAR or HER2-CAR molecules were continuously expressed on engineered CAR-iPSCs. Both CAR-iPSCs were successfully differentiated into iNKT cells in feeder cell-free culture system without losing CAR expression. CAR-iNKT cells showed similar phenotypic properties to iPSC-derived non-transduced iNKT cells. Cytotoxic assay revealed that CAR-iNKT cells possessed enhanced antigen-specific killing activities against target molecule expressing cancer cell lines, such as CD19 positive NALM6 (B cell leukemia) or HER2 positive U-2 OS (Osteosarcoma), compared with iPSC-derived non-transduced iNKT cells.

Conclusions This study showed the first successful delivery of a CAR construct into iPS cells that differentiate precisely into iNKT cells with enhanced cytotoxicity. iPSC-derived CAR-iNKT cells is demonstrated to become a novel allogeneic cell therapy platform.

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