IPSC-DERIVED CAR-INKT CELLS TARGETING HER2 SHOW PROLONGED TUMOR CONTROL AND PROMOTE DURABLE SURVIVAL IN A TUMOR XENOGRAFT MODEL

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Background Invariant natural killer T (iNKT) cells are a rare subset of T lymphocytes that express a semi-invariant TCR, which recognizes glycolipids presented by the monomorphic MHC like molecule CD1d. iNKT cells can directly kill tumor cells through TCR, while also indirectly exert antitumor activities by prompting dendritic cell maturation, priming tumor-specific CD8+ T cells, and reprogramming pro-tumor myeloid cells. iNKT cells do not induce graft-versus-host disease (GVHD), which makes them an ideal cell source for ‘off-the-shelf’ cell product. However, the rarity of iNKT cells in human blood poses a challenge in manufacturing large quantities of iNKT cells from a single donor for multiple doses. Induced pluripotent stem cells (iPSCs) offer a promising strategy to overcome this hurdle by their potential to generate thousands of doses of iNKT cells from a single manufacturing campaign. In the present study, taking advantage of gene editing technology, we established a CAR-iPSC cell line and differentiated it into CAR-iNKT cells with in vitro and in vivo anti-tumor activities.

Methods The HER2-CAR construct was inserted into the AAVS locus of iPSC by the CRISPR/Cas9 system. Following cloning and screening, a CAR-iPSC cell line was obtained. CAR-iPSCs were then differentiated into CAR-iNKT cells under a feeder cell-free culture condition. The expression levels of CAR and iNKT cell markers were analyzed by flow cytometry. In vitro cytotoxicity of CAR-iNKT cells against cancer cell lines was examined using xCELLigence® real-time cell analyzer, and in vivo efficacy was evaluated using a SK-OV-3 (Ovarian cancer cell line) xenograft model.

Results The HER2-CAR was stably expressed on the surface of engineered CAR-iPSCs throughout multiple passages. CAR-iPSCs were successfully differentiated into iNKT cells without compromising the expression of HER2-CAR under the feeder cell-free condition. In vitro cytotoxicity assay demonstrated that HER2 CAR-iNKT cells exerted potent killing activity against HER2-positive cancer cell lines. In the SK-OV-3 xenograft model, the mice in the HER2 CAR-iNKT treatment group survived longer than those in the control or non-CAR introduced iNKT cell treatment groups.

Conclusions This study demonstrated that a CAR construct can be effectively integrated into iPSCs, and the matured CAR-iNKT cells can be successfully differentiated from CAR-iPSC while maintaining stable CAR expression. Furthermore, the differentiated CAR-iNKT cells derived from CAR-iPSC showed potent anti-tumor effects both in vitro and in vivo. These findings suggest that iPSC-derived CAR-iNKT cells would be a novel allogeneic cell therapy platform.

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All human materials were handled following approval (#BP20180419) by the human genome ethics committee of BrightPath biotherapeutics Co.,Ltd. (Tokyo, Japan).

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