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POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

iPSC-Derived Dual Antigen Receptor T Cells Targeting GD2 and LMP2 Antigens for Extranodal NK/T-Cell Lymphoma, Nasal Type

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Epstein-Barr virus (EBV)-associated lymphomas generally exhibit poor prognosis. Given that EBV antigens, LMP1 and LMP2, are often expressed on EBV-associated lymphomas, these lymphomas should be a good target of antigen-specific cytotoxic T lymphocytes (CTL) therapy. Among these, advanced-stage ENKL is a highly aggressive disease. We previously reported that functionally rejuvenated LMP2-specific CTLs (LMP2-rejTs), generated from induced pluripotent stem cells (iPSCs), robustly suppress ENKL *in vivo* with remarkable persistence for more than seven months. To mitigate the risk of tumor antigen escape, we next generated dual-antigen receptor rejuvenated T cells from iPSCs by introduction of LMP1-directed chimeric antigen receptor (CAR) into LMP2-rejTs which have already demonstrated *in vivo* persistence. We reported that these cells recognize dual antigens via CAR and native T cell receptor, resulting in cooperative antitumor effects *in vivo*.

We have recently confirmed that ENKL cells often express disialoganglioside (GD2). GD2 has been identified as a promising target for CAR T-cell therapy aimed at GD2-expressing tumors. A number of Phase 1 clinical trials, utilizing GD2-directed CAR (GD2-CAR) T-cell therapy in neuroblastoma patients, have demonstrated not only safety but also efficient *in vivo* expansion, all while avoiding significant toxicities.

For clinical implementation, we have developed iPSC-derived dual antigen receptor T cells, where we incorporated GD2-CAR into LMP2-rejTs, resulting in GD2-CARrejTs. We reprogrammed an LMP2-CTL clone derived from a healthy donor into iPSCs using a Sendai virus vector, followed by transduction with the lentiviral GD2-CAR vector. Utilizing flow cytometry, we evaluated CAR transgene expression in the GD2-CARrejTs and found a substantial expression rate of 91.8%. Additionally, we determined a nearly perfect LMP2 antigen specificity of 99.9% for the GD2-CARrejTs, also measured by flow cytometry.

To investigate whether GD2-CARrejTs exhibited cytotoxicity against ENKL than GD2-CARTs, we performed ^{51}Cr release assays. The cytotoxicity of GD2-CARrejTs was significantly stronger against an ENKL cell line (NK-YS), than that of GD2-CARTs (79.8% vs 24.8%; $p < 0.0001$) at an effector to target ratio of 40:1. Next, to observe the *in vivo* antitumor effect of GD2-CARrejTs against NK-YS, NK-YS cells labeled with Firefly luciferase / GFP were intraperitoneally injected into NOG mice. Four days after tumor injection, mice were divided into untreated (control) and treated (GD2-CARrejTs) groups. Tumor signal was significantly suppressed in mice treated with GD2-CARrejTs compared to untreated mice on Day 35 ($p = 0.0318$).

To delineate the disparities in cytotoxicity, we undertook a single-cell RNA sequencing analysis and confirmed that GD2-CARrejTs exhibited significantly lower TIGIT expression in comparison to GD2-CAR T cells. In fact, we noted an amplification of the cytotoxic impact of GD2-CAR T cells when paired with TIGIT antibodies against GD2-expressing tumor cells. However, the enhanced cytotoxicity still did not exceed that of GD2-CARrejTs.

From these results, we conclude that GD2-CARrejTs may constitute a promising therapeutic approach against refractory ENKL. The most significant advantage of iPSC-derived T cell therapy lies in its clonality and potential for an unlimited supply of therapeutic T cells. Additional gene-editing of iPSC-derived CARrejT cells could potentially lay the groundwork for allogeneic, "off-the-shelf" GD2-CARrejT therapy, thereby introducing a promising new therapeutic avenue for patients with ENKL in the foreseeable future.

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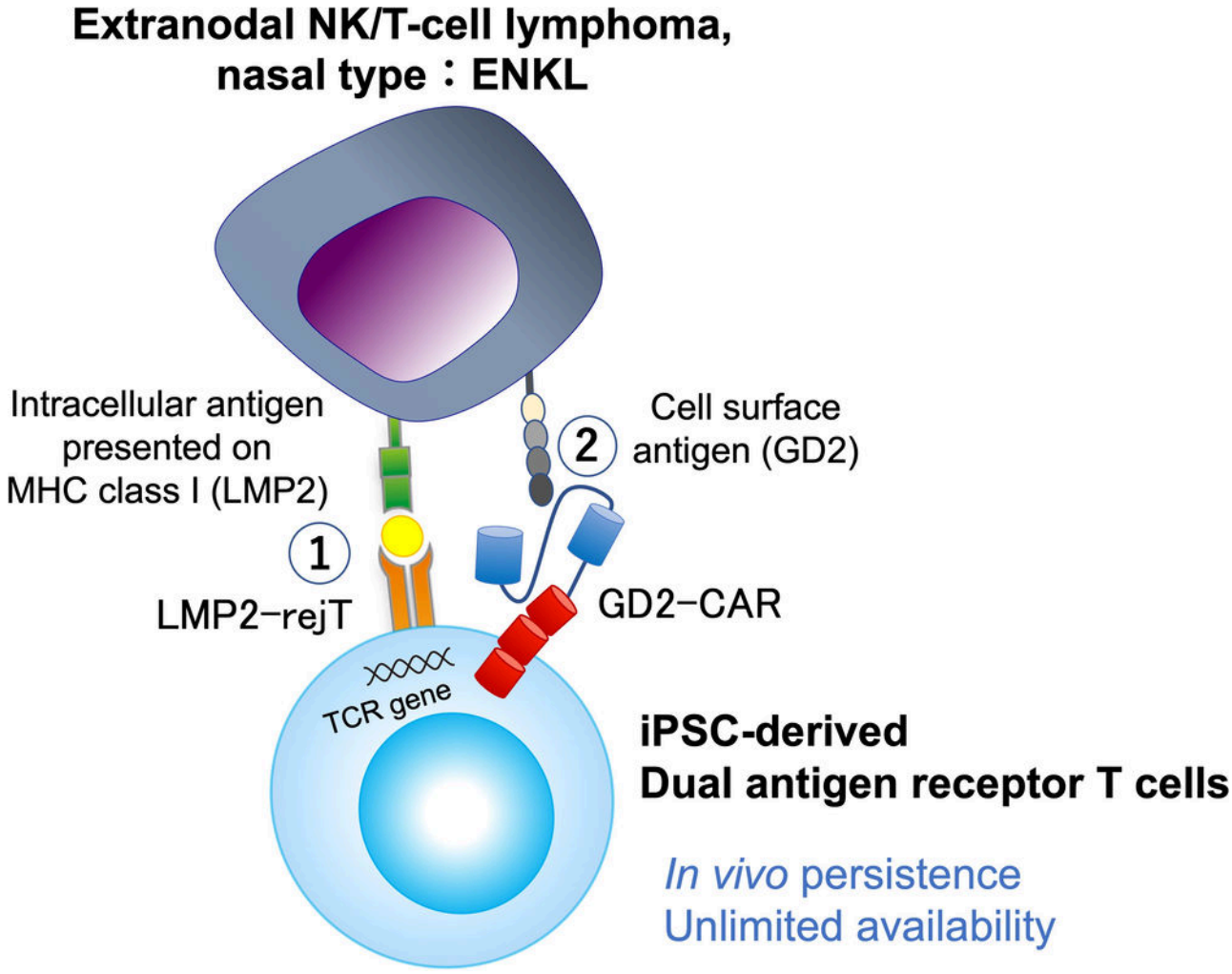

Figure 1

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