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ORAL ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

In Vivo Generation of Functional CD19 CAR-T Cells Using a Serum-Stable CD3-Targeted Lentiviral Vector

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The high cost and complexity of CAR-T therapy has spurred efforts to generate CAR-T cells directly within patients via systemic infusion of serum-stable lentiviral vector particles that target T cells in situ for CAR transgene delivery. To ensure effective in vivo delivery, these vectors need to show resistance to neutralization by human serum, demonstrate transduction specificity for T cells, and support subsequent expansion of CAR-T cells.

Lentiviral vectors (LV) pseudotyped with VSV G-glycoprotein (G-WT) transduce target cells via the LDL receptor (LDLR), which is ubiquitously expressed at variable levels and is upregulated on activated T cells. G-WT LVs are widely used to generate therapeutic CAR-T cells ex vivo, following purification and ex vivo activation of the T cells.

To generate LVs capable of targeting circulating T cells in vivo, we utilized rational engineering of the VSV-G glycoprotein to design a G-CD3. This modified G protein avoids LDLR recognition, incorporates a CD3-specific single-chain antibody for targeted recognition of T cells, and minimizes LV particle inactivation upon exposure to human serum. G-CD3 LVs were shown to target and transduce resting T cells in peripheral blood mononuclear cell (PBMC) cultures with very high efficiency. The G-CD3 LVs powerfully activated T cells, evident from their increased CD25 expression, and the infection was not hindered in the presence of fresh, complement-active human serum (25%). Additionally, G-CD3 LVs efficiently transduced T cells in fresh human whole blood without apparent off-target transduction of non-T cell lineages.

In vivo evaluation of G-CD3 LV efficiency was performed in humanized NSG mice by intravenous (IV) or intraperitoneal (IP) delivery of G-CD3 LVs encoding anti-CD19 CAR and GFP transgenes. Both IV and IP delivery of G-CD3 LV led to in vivo CAR T cell generation with peak levels of 17% of total circulating human CD45+ cells on day 21 post vector administration. The in vivo generated CD19-CAR-T cells were fully functional as evidenced by the disappearance of endogenous human CD19+ B cells from peripheral blood. Tissue analysis of spleen, bone marrow, and liver at the time of sacrifice (day 39 post LV injection), showed anti-CD19 CAR-T in all three tissues accompanied by an absence or dramatic reduction in tissue-resident human CD19+ B cells. In contrast, CAR-T cells were not detected at any time point in humanized NSG mice injected with G-WT LV, regardless of whether the virus was administered IV or IP.

In summary, these data highlight the potential applicability of fully retargeted, serum stable LVs for in vivo gene delivery or genome editing, offering promising prospects for future human gene therapy applications.

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