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702. CAR-T CELL THERAPIES: BASIC AND TRANSLATIONAL

Comparison of lentiviral promoters on the therapeutic efficacy of tri-specific CAR-T cells targeting CD19/CD20/CD22

Yang Liu[1], *Borwyn Wang*[1], *Jessica Morris*[1], *Olivia Wise-Dent*[1], *Yanping Xie*[1], *Gregory Sowd*[1], *JiHyun Kim*[1], *Xiaorong Wu*[1], *Maggie Yates*[1], *Beatrix Ferencz*[1], *Ibeawuchi Oparaocha*[1], *Oxana Slessareva*[1], *Zhongyu Zhu*[1], *Boro Dropulic*[1], *Ying Xiong*[1]

[1] Caring Cross, Gaithersburg, United States

Abstract Chimeric antigen receptor (CAR) -T cell therapy is a promising approach for cancer treatment, particularly for hematologic malignancies, such as B cell leukemia and lymphoma, with remarkable remission rates. Currently, the field has moved forward to targeting two or three B cell antigens simultaneously, hoping to overcome the disease relapse induced by antigen loss after single-target CAR-T treatment. However, the complexity of multi-targeting system brings challenges to CAR-T cell manufacturing, such as potentially lower lentiviral vector (LV) titer, lower transduction efficiency of primary T cells and cell surface CAR expression level due to the increased LV payload, which could potentially impact the function of CAR-T cells. Trying to overcome the obstacles mentioned above, we engineered a tri-specific CAR construct targeting CD19/CD20/CD22 with two different promotors, EF1α and MND, and compared the CAR-T performance based on LV titer, CAR expression, CAR-T cell potency and persistence.

First, research grade vectors were produced without any purification, and good titers were observed for both LVs even though the payload was at the limit of LV construct capacity. For small-scale CAR-T transduction (12-well plate), the vector with MND promoter induced higher CAR expression level with lower MOI/dose. On the other hand, similar overnight killing profile was observed between the CAR candidates while the one with EF1α promotor showed relatively higher cytokine secretion level after tumor stimulation. In the multi-stimulation assay where the CAR-T cells were co-incubated with Raji tumor line for 4 rounds, persistent tumor eradication was observed for both CARs.

Second, large-scale vectors were generated with complete purification and sterilization process mimicking GMP LV production (pre-clinical grade). G-Rex 6M system was used to test the CAR-T generation as a scale-up model in preparation of clinical level CAR-T manufacturing in G-Rex 100M. Although the vector with MND promotor showed higher titer by dPCR, similar CAR-T performances were observed as in the small-scale study from CAR expression level to the CAR-T potency and persistence in the 3-round multi-stimulation assay.

Taking all the *in vitro* results into consideration, CAR-T cells generated from both pre-clinical grade LVs were proceeded for *in vivo* study using a xenograft NSG mouse model engrafted with Raji tumor cells. While the mice received tri-specific CAR treatment with MND promotor showed slightly slower tumor control profile, both candidates managed to clear the cancer cells and all the mice stayed tumor-free until the end of the study.

Overall, both *in vitro* and *in vivo* results demonstrated the high tumor clearance efficacy of our tri-specific CAR construct. In addition, the LV with MND promotor could be produced with higher titer at the clinical scale, while the CAR-T cells with EF1α promotor eradicate Raji tumor cells faster in the mouse model. These findings give us flexibility of candidates in different disease/cost scenario, especially in low/mid income countries.

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