Humanized mouse model: A preclinical platform feasible for CAR-T therapy

Yirong Kong[1], Nan Mou[2], Yan Wang[1], Hongwu Li[1], Siying Peng[1], Jijun Yuan[2]
[1]Beijing IDMO Co., Ltd [2]Shanghai Genechem Co., Ltd

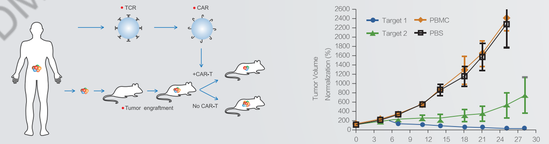
INTRODUCTION

Chimeric antigen receptor (CAR) T cells form part of a broad wave of immunotherapies that are showing promise in clinical trials. This clinical delivery has been based upon preclinical efficacy testing that confirmed the proof of principle of the therapy. However, with recent reports of adverse events associated with CAR T-cell therapy, there is now concern that current preclinical models may not be fit for purpose with respect to CAR T-cell toxicity profiling. CAR T-cell therapy does not exist alone as T cells are generally given in combination with patient preconditioning, and may also include systemic cytokine support, both of which are associated with toxicity. Here, we explore the preclinical humanized mouse models used to validate CAR T-cell function and examine their potential to predict CAR T-cell driven toxicities for the future.

RESULTS

Fig. 1 Study design using Patient-derived xenograft (PDX) model:

NPI (NOD. Prkdc^{sc} Il2rg$^{-/-}$) mice were engrafted with patient tumor tissues. Meanwhile, T cells from the same patient were activated and transfected with CAR genes by either lentivirus (LV) or electroporation. In vivo efficacy study was carried out using engineered CAR T-cells infused into PDX mice.



(A)

Fig. 2 Case study:

The PDX model was established with subcu implantation of tumor tissues from a colon cancer patient and individual mice assigned to four treatment groups: PBS control, PBMC control, engineered CAR-T cells of target 1 and CAR-T cells of target 2.T cells were injected intratumorally. (A)Tumor volume was measured twice a week; (B) IFN-Y release was measured by ELISA on day 7 post-infusion.

(B)

Fig. 3 Study design using Hu-CD34 models:

NPI (NOD. Prkdc^{sc} Il2rg$^{-/-}$) mice were preconditioned and engrafted with human CD34 positive hematopoietic stem cells (HSCs) from cord blood. Human T cells were isolated from spleens of 16~20 weeks reconstituted NPI mice and activated in a certain environment in which they can actively proliferate. The T Cells were then transfected with CAR genes by either an integrating gammaretrovirus (RV) or by lentivirus (LV) vectors. Engineered CAR T-Cells were infused into the NPI mice or tumor-bearing NPI mice reconstituted with HSCs from the same donor (HuNPI).

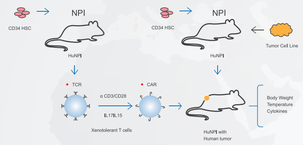


Fig. 4 In vitro validation:

T cells were isolated from spleens of HuNPI mice, stimulated for 24hr and collected for lentiCD19T transduction. (A) T cells proliferated after lentiviral transduction (B) Engineered CD19CAR-T cells showed specific killing when co-cultured with target-positive tumor cell line K562-CD19, determined by IFNY release.

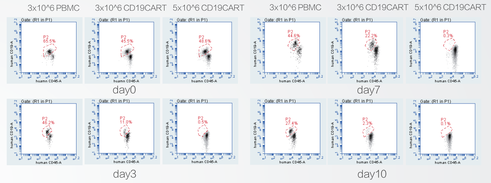
Mouse No.	CD3 cell isolation	Cell number before Transduction	Cell number (Expansion D12)
#1	Yes	1,4E6	7,2E6
#2	No	1,2E6	2,8E7

(A)

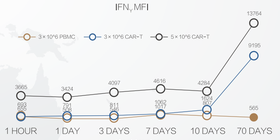
(B)

Fig. 5 In vivo validation:

Engineered CAR T-Cells were infused into the HuNPI mice reconstituted with CD34+HSCs from the same donor.(A) Percentage of CD19+ B cells were monitored by FACS analysis day 0,3,7and10 post-treatment. (B) Long-term IFN-Y release in peripheral blood was measured by ELISA.



(A)



(B)

Fig. 6 On target, off tumor toxicity:

Engineered CAR T-Cells were infused into the HuNPI mice reconstituted with CD34+HSCs from the same donor. Percentage of CD19+ B cells in peripheral blood were monitored by FACS analysis day 0, 3, 7, 10 and 150 post-treatment. There was no CD19+ B cells detected even after 150 days.

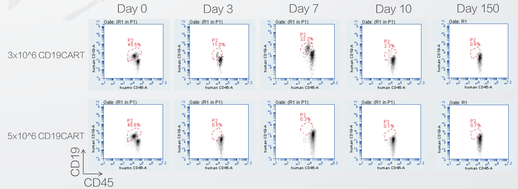
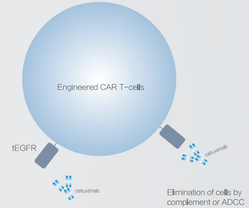
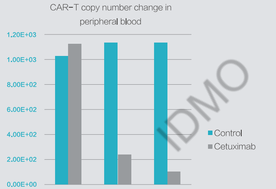


Fig. 7 Toxicity-management safety switch:

(A) The mechanism of safety switch is mediated by the clinically approved therapeutic antibody when the transduced cells are engineered to express the antibody targeted cell surface antigen such as truncated EGFR (tEGFR), a human EGFR polypeptide retaining the intact cetuximab binding site in extracellular domain III. The engineered CAR T-cells are eliminated through antibody-dependent cellular cytotoxicity. (B) Engineered tEGFR+ CD19 CAR T-Cells were infused into the HuNPI mice reconstituted with CB from the same donor. When all the CD19+ B cells were depleted in peripheral blood, cetuximab was administrated and copy number of CAR T cells was determined by QPCR in 30ng genomic DNA.



(A)



(B)

CONCLUSIONS

1. Patient-derived xenograft (PDX) model, more clinically relevant, is a better preclinical in vivo efficacy model for CAR T-cell therapy.
2. Xeno-tolerant T cells from HuNPI mice are functional in vitro assay and in vivo efficacy study.
3. Long-term cytokine release and toxicity of on target/off tumor can be further investigated using HuNPI mice.
4. HuNPI models can be used to study selective depletion mediated by the clinically approved therapeutic antibody when the transduced cells are engineered to express the antibody targeted cell surface antigen.