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POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES OTHER THAN CAR-T CELLS: BASIC AND TRANSLATIONAL

Development of a Novel Allogeneic *Super*-Γδ T Cell Therapy for Acute Myeloid Leukemia and Multiple Tumors

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Introduction: Relapsed or refractory (R/R) acute myeloid leukemia (AML) has a dismal prognosis. Due to the highly heterogeneous nature of AML blasts, Chimeric Antigen Receptor (CAR) -T cells targeting specific antigens, including CD123, CLL-1 and CD33, have demonstrated limited efficacy across leukemic cells and certain off-target toxicity to normal cells. On the other hand, $\gamma\delta$ T cells are immune cells with inherent anti-tumor activity through multiple receptors such as $\gamma\delta$ TCRs, NKRs, DNAM-1 and they can be developed into allogeneic cell therapy. Unmodified $\gamma\delta$ T cells can be efficiently expanded *in vitro* and show killing of AML cell lines. In clinical trials, they show certain activity toward AML yet efficacy would still need to be improved. It is likely that $\gamma\delta$ T based cell therapy may offer solution to address the heterogenic nature of AML if its natural killing mechanism can be further enhanced. To this end, we have designed various constructs with different targeting moieties and tested them for in vitro killing. Our top candidate *Super*-$\gamma\delta$ T is armed with an NKG2D based CAR and is manufactured by non-viral site-specific integration technology that we have developed in-house (PrecisionGENE, PrecisionGENetic Engineering). This *Super*-$\gamma\delta$ T showed enhanced killing toward AML and several tumor cell lines from various blood and solid tumors, with no obvious toxicity toward normal human cells.

Methods: In the current study, a novel NKG2D based CAR was inserted into a target gene locus by the non-viral site-specific integration technology. Specific killing activities of *Super*-$\gamma\delta$ T cells to AML cell lines (HL60, THP1) and several tumor cell lines (including NSCLCs, HCCs, RCCs and OCs) were measured by a luciferase-based method. To investigate the killing activity for CLL1 negative tumor cells, a CLL1 knocked out tumor cell line-THP1-CLL1-KO was generated, and the specific killing of unmodified $\gamma\delta$ T, CLL1 CAR-$\alpha\beta$ T, and *Super*-$\gamma\delta$ T to THP1- CLL1- KO were studied in vitro. To evaluate the toxicity of *Super*-$\gamma\delta$ T cells to PBMCs, unmodified $\gamma\delta$ T, CLL1 CAR-$\alpha\beta$ T, and *Super*-$\gamma\delta$ T were co-cultured with human PBMC, respectively, and PBMC composition changes were measured by a flow-based assay.

Results: To minimize off-target editing, we used site-specific integration technology PrecisionGENE for the manufacturing of *Super*-$\gamma\delta$ T cells and have achieved over 70% integration efficiency. Cytotoxicity of the newly developed *Super* -$\gamma\delta$ T cells to AML cell lines was tested *in vitro*. Notably, *Super*-$\gamma\delta$ T cells showed almost 100% killing to AML cell lines (HL60 cells) *in vitro* at effector-to-target ratio of 3:1, which is comparable to CLL1 CAR-$\gamma\delta$ T, but over 5-fold more potent than unmodified $\gamma\delta$ T with 20%-30% killing. In addition, there was significantly increased cytokine secretion from *Super* -$\gamma\delta$ T cells when co-cultured with AML tumor cells compared to $\gamma\delta$ T. To evaluate whether *Super* -$\gamma\delta$ T cells could overcome cancer cell heterogenicity, a CLL1 negative target cell THP1-CLL1-KO cell line was used for comparison. Importantly, *Super*-$\gamma\delta$ T cells showed almost 80% killing efficiency against the THP1-CLL1-KO at a E:T ratio of 1:3, while CLL1 CAR- $\alpha\beta$ T's killing was minimal. Moreover, normal PBMCs and fibroblast cells were used to study for the off-target cytotoxicity of *Super*-$\gamma\delta$ T cells. It is worth mentioning that while there was no obvious off-target toxicity by *Super*-$\gamma\delta$ T for monocyte when co-culturing with PBMC, CLL1 CAR-$\alpha\beta$ T showed killing toward monocytes. To further investigate the killing potential toward different tumor cell lines, cytotoxicity of *Super*-$\gamma\delta$ T cells to various blood and solid tumor cell lines was studied *in vitro*. *Super*-$\gamma\delta$ T showed enhanced cytotoxicity to a broad-spectrum of tumor cell lines including NSCLC, HCC, RCC and OC cell lines, independent of their mutation background. In vivo efficacy for *Super*-$\gamma\delta$ T is under-going and the result will be presented.

Conclusions:

Taken together, *Super*-$\gamma\delta$T showed much enhanced anti-tumor functionality and superior safety profile *in vitro*. *Super*-$\gamma\delta$T demonstrated anti-tumor activity against both blood and solid tumor cell lines independent of their mutation background. Therefore, *Super*-$\gamma\delta$ T has the potential to be used as a novel cellular therapeutic agent for treating R/R AML and multiple solid tumors, and it could be efficacious toward cancers of various types, stages and forms.

Disclosures No relevant conflicts of interest to declare.

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