NKG2D-BASED MULTI-SPECIFIC CAR T-CELLS TO OVERCOME ANTIGEN ESCAPE AND IMPROVE ANTI-TUMOR EFFICACY

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Background CAR T-cell therapies have shown remarkable success in treating hematological malignancies. However, fewer than 50% of patients treated with commercial CAR T-cells for B-cell malignancies, have shown durable disease control. Linked largely to antigen modulation or inadequate T-cell function, it is likely to pose an even greater challenge in solid tumors. NKG2D ligands (NKG2DL), are a group of 8 stress-induced ligands expressed by virtually all cancer types and absent from normal healthy cells. These represent an attractive approach for multi-specific CAR T-cells.

Methods We designed different NKG2D-based multi-specific CAR T-cells, utilizing both tandem and dual-CAR approaches. These receptors all encompass the extracellular domain of the natural NKG2D receptor fused to or co-expressed with an anti-CD19, an anti-BCMA or an anti-PSMA scFv. Functionality of the different NKG2D-based multi-specific CAR T-cells was evaluated in cocultures with cells expressing the main antigen (i.e., CD19/BCMA/PSMA and NKG2DL) or not (only NKG2DL) and was compared to the functionality of corresponding single-targeting CAR T-cells. In addition, we evaluated the in vivo anti-tumor activity of selected CD19/NKG2DL tandem CAR T-cells in a B-ALL relapse model.

Results We show that the majority of CD19/NKG2DL multi-specific CAR T candidates were able to secrete cytokines, proliferate and eliminate CD19 KO Nalm-6 cells in vitro. Some of these dual-CAR T-cells displayed an even higher level of in vitro functionality compared to CD19 single CAR T-cells when co-cultured with WT Nalm-6 cells, indicating the potential for this approach to target CD19 positive cancer cells as well. Interestingly, upon chronic antigen stimulation, several dual-CAR T-cell candidates showed a significantly higher proliferative capacity. In vivo, the selected CD19/NKG2DL tandem candidate showed a very good anti-tumor activity against Nalm-6 cells and exerted some tumor control against CD19 KO Nalm-6 cells.

Preliminary results obtained during the in vitro screening of BCMA/NKG2DL and PSMA/NKG2DL candidates showed that several multi-specific CAR T-cells were able to secrete cytokines, exert a cytotoxic activity and proliferate in response to cancer cells expressing the main antigen or not.

Conclusions In vitro data, as well as preliminary in vivo data, indicate that the NKG2D-based multi-specific CAR platform is an attractive approach to overcome antigen escape and improve anti-tumor efficacy against both hematological and solid indications. By targeting stress-induced targets rather than lineage specific targets, the issues with antigenic modulation may be overcome. Additional in vivo studies will be performed to validate in vitro results obtained for all the selected candidates.

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