232 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 multi-specific 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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