Next generation NKG2D-based CAR-T cells (CYAD-02): Co-expression of a single shRNA targeting MICA and MICB improves cell persistence and anti-tumor efficacy in vivo

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RESULTS - 1

• The rapid approval of two anti-CD19 chimeric antigen receptor (CAR) T-cell therapies and the advanced development of anti-BCMA CAR T-cell therapies demonstrate the potential of the approach in B-cell malignancies. However, targets with a similar profile for CAR-T cell therapy in other diseases including acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) are lacking.

• The Natural Killer group 2D (NKG2D) receptor binds to 8 stress-induced ligands (NKG2DL). The MHC Class I chain-related A and B (MICA, MICB) and the UL16 binding a strain family (ULBP) 1-6 (Figure 1A). These ligands are absent or expressed at very low levels in normal tissues but found at high frequencies across a wide range of tumors, rendering NKG2D a promising tool for cancer immunotherapy.

• CYAD-01 is an autologous CAR-T cell therapy based on the full-length human NKG2D receptor fused to the intracellular domain of CD3ζ (Figure 1B). CYAD-01 is currently evaluated in relapsed/refractory r/r AML/MDS patients in Phase I clinical trials (THNK: poster 3826 and DEPLETHINK: poster 3844), displaying promising results in hematological malignancies.

• Activated T-cells transiently upregulate NKG2DL, leading to recognition by the CAR and thus limiting the in vitro cell expansion. We have previously shown that robust cell yields are generated with the addition of a blocking antibody and a PI3K inhibitor during manufacturing (in vivo cell expansion). In this work, we investigated the ability of an optimized short hairpin RNA (shRNA) technology to modulate NKG2DL expression on NKG2D CAR-T cells (CYAD-02) and to determine whether this confers an increase in anti-tumor activity compared to CYAD-01.

RESULTS - 2

• CYAD-02T cells produced with the OptimAb manufacturing process display an enriched early memory phenotype (Figure 5A) and increased in vitro cytotoxic activity upon culture with PANIC-1 cancer cells, compared to CYAD-01 T-cells cultured in standard conditions (Mab manufacturing process) (Figure 5B).

• To develop a stress-test animal model titrated for minimal activity of CYAD-01, mice were injected with an aggressive AML cell line (THP-1), followed by 3 weekly injections of 0.3, 1, or 10 x 10^6 CYAD-01 cells. The doses of 0.3 and 1 x 10^6 were excluded only a slight trend to increase mouse survival, while injection of 10 x 10^6 CYAD-01 cells led to reduction in tumor burden and significantly increased mouse survival (Figure 6A and B). Based on these results, the intermediate dose of 3 x 10^6 CYAD-01 cells was used in the subsequent experiments.

CONCLUSIONS

• The NKG2DL, MICA and MICB are upregulated on T-cells upon activation, limiting in vitro expansion of NKGD2 CAR-T cells.

• The shRNA-mediated knockdown of MICA and MICB expression on NKG2D CAR-T cells enhances in vitro expansion.

• CYAD-02 T-cells expressing the NKG2D CAR with a shRNA targeting MICA and MICB show an enrichment in early memory phenotype and better anti-tumor activity in vitro compared to CYAD-01 manufactured with the Mab process.

• CYAD-02 T-cells display increased in vivo engraftment, persistence and antitumor activity, which suggests that CYAD-02 could generate a stronger and durable anti-tumor activity in patients compared to CYAD-01 manufactured with the Mab process.

• The IND application for CYAD-02 has been accepted by the US FDA and the CYCLE-4 Phase I study (NCT04167696) is scheduled to be initiated in early 2020.

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