MODELING NKG2D-BASED CAR-T CELL THERAPY IN ANIMALS WITH ACUTE MYELOID LEUKEMIA

Fontaine M1, Breman E1, Demoulin B1, Bornschein S1, Bolsée J1, Sotiropoulou PA1, Gilham DE1

BACKGROUND
The Natural killer group 2D (NKG2D) receptor binds to 8 stress-induced ligands (NKG2DLs), the MHC I chain-related A and B (MICA, MICB) and the UL16 binding protein family (ULBPs) (Figure 1A). These ligands are absent or very low expressed in normal tissues, but frequently expressed in a large variety of tumors, rendering NKG2D a promising tool for cancer immunotherapy1,2. Celyad’s lead CAR-T product, CYAD-01, is based on the full length human NKG2D fused to the intracellular domain of CD3 (Figure 1B). To broaden our understanding of CYAD-01 activity, in this study we examined in depth CYAD-01 efficacy and activity in an aggressive AML model. Specifically, we investigated distinct dosing and injection schemes and monitored the CYAD-01 persistence and biodistribution in and without the context of cancer.

METHODS
To assess the anti-tumor efficacy of CYAD-01 CAR-T cells against AML, we used an aggressive preclinical AML model, where THP-1-luc-GFP cell line (AML subtype M5) are injected in NOD SCID Gamma/γ-/- mice (NSG). In this model, mouse survival is barely over 2 weeks without treatment. CYAD-01 cells, Mock T cells or vehicle were administered IV 1 week after the establishment of THP-1 xenografts. One or three weekly injections of one (10 million) or four different doses (0.5, 1, 3, and 10 million) of CYAD-01 cells were used, as indicated in the distinct experiments. Tumor burden was evaluated by in vivo bioluminescence imaging and persistence of CYAD-01 cells by flow cytometry on mouse blood detecting human CD45 positive cells.

RESULTS

Different doses of CYAD-01 CAR-T cells exhibit effective anti-tumor activity in an aggressive AML mouse model

To assess the effect of CYAD-01 dose in an aggressive AML model, THP-1 bearing mice received 3 weekly injections of 0.5, 1, 3 and 10 million T cells. All doses showed a trend to increase mouse survival and to reduce tumor burden, but this was significant for the 2 higher doses (Figure 2A and B). While the kinetics of persistence of the CAR-T cells were similar in all doses, with a peak after the second injection, dose dependent levels were observed (Figure 2C). The peak after the second injection indicates that a combination of the number of cells injected and the activation via the tumor load is important for T cell engraftment and persistence.

Multiple injections of CYAD-01 CAR-T cells induce a longer survival and better tumor control in a murine model of AML

Using the most effective dose of 10 million CYAD-01 cells, a comparison of the anti-tumor efficacy of a single or multiple injections was performed. After 3 injections, the tumor regression was maintained longer compared to mice that received a single injection (Figure 3A, representative of the Injections schedule). The tumor burden was maintained significantly lower for 2 more weeks and mouse survival was higher in the multiple injection scheme (Figure 3B and C). Moreover, a higher percentage of the CYAD-01 CAR-T cells was observed indicating that the persistence of the CAR-T cells can be improved by multiple injections (Figure 3D).

Biodistribution of the CYAD-01 CAR-T cells differs between tumor-bearing and healthy mice

As CYAD-01 cells can be detected in the blood of THP-1-bearing mice only for 1 week after the last injection, we studied the biodistribution of the CAR-T cells. CYAD-01 cells quickly disappeared from the bloodstream and the spleen of tumor-bearing mice (Figure 4A and B), suggesting potential homing where the cancer cells are located, such as the bone marrow. Interestingly, the percentage of the CYAD-01 cells remained low in the bone marrow of survivors and bias (Figure 4C). As illustrated in Figure 4D, in a representative bioluminescence image of mice bearing THP-1 tumors, the bioluminescence is clearly observed in the bones around the spinal cord of the mice, potentially suggesting those as the main areas of CYAD-01 homing. This is currently under investigation.

Increased persistence of CYAD-01 CAR-T cells with different NKG2D truncations in mice

Our previous work has shown that T cell activation during manufacturing induces transient up-regulation of NKG2D, (mainly MICA and MICB), resulting in self-killing or fratricide, resulting in low cell yields (Figure 5A and B). While fratricide has been successfully tackled during manufacturing, in vivo fratricide could underlie the short persistence of CYAD-01 cells in vivo. To this end, we studied the persistence of CYAD-01 cells without functional CAR, bearing intracellular or extracellular truncation, thus unable to induce cell killing (Figure 5C). CYAD-01 cells bearing intracellular or extracellular truncation, as well as control T cells, were detected with similar frequency until the end of the study, while CYAD-01 cells were undetectable after 2 weeks (Figure 5D). Moreover, the persistence of control cells when injected at 1:1 ratio with CYAD-01 cells was comparable to CYAD-01 T cells injected alone, suggesting CYAD-01-mediated killing of the control T cells (Figure 5E). These results unambiguously show that in vivo fratricide mediates short persistence of CYAD-01 cells and that inhibiting NKG2D1 expression on CYAD-01 cells would be a means to increase persistence and thus efficacy.

CONCLUSION

The multi-target specificity of the NKG2D-based CAR (CYAD-01) provides a strong potential to treat a broad range of cancer indications.

Our study provides proof of principle that multiple injections of CYAD-01 cells exhibit effective anti-tumor activity in an aggressive animal model of AML. This has been confirmed by promising preliminary results from the Phase I THNK trial (NCT05318405) testing the potency of CYAD-01 T cells against hematological and solid tumors.

Importantly, the biodistribution of CAR-T cells in the peripheral blood differs between healthy mice and AML models, probably due to CYAD-01 T cell homing to the areas where the cancer cells are located.

In vivo experiments using truncated forms of the NKG2D CAR verified that fratricide drives the short persistence of CYAD-01 cells, suggesting that downregulating NKG2D ligand expression in primary cells would be a means to increase CAR-T cell persistence and efficacy. This is currently investigated by the implementation of shRNAs targeting NKG2D ligands in the CYAD-01 vector.