Endogenous DAP10 provides optimal co-stimulation for NKG2D-based CARs

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BACKGROUND

NKG2D is a transmembrane receptor expressed by Natural Killer cells and some subsets of T cells, and binds to 8 stress ligands frequently expressed on tumor cells: MICA, MICB and ULBP1-6 [1,2]. The surface adaptor DAP10, which is endogenously expressed on T cells, associates with and stabilizes NKG2D expression.

NKR-2 is a chimeric antigen receptor (CAR) composed of the full-length human NKG2D fused to the CD3ζ cytoplasmic signaling domain. Ligand binding to NKR-2 triggers a primary signal via CD3ζ and a secondary signal via DAP-10, resulting in T-cell co-stimulation and cytotoxicity (Figure 1).

CYAD-01 is an autologous T-cell therapy that has shown promising results in multiple preclinical models and in the clinic. CYAD-01 may be an effective treatment for solid and hematological tumor types that express NKG2D ligands and is currently being investigated in multiple clinical trials [3-5 and Poster P235].

RESULTS

In this study, different NKG2D-based CAR constructs were created and compared side by side to our current lead construct. Two types of modifications were performed: DAP10 overexpression, and addition of CD28 or 4-1BB co-stimulatory domains to generate “classical” 2nd generation CAR designs. All constructs also included a truncated form of CD19 (tCD19) as a marker gene to allow the evaluation of transduction efficiency and purification of the transduced cells.

METHODS

In vitro, overexpression of DAP10 or addition of CD28 or 4-1BB co-stimulatory domains did not improve the cytotoxicity or the level of secreted cytokines. The pattern of cytokines was not modified either.

In vitro and in vivo experiments show that endogenous adaptor protein DAP10 provides optimal co-stimulation to the unconventional NKR-2 CAR complex.

• In vitro, overexpression of DAP10 or addition of CD28 or 4-1BB co-stimulatory domains did not improve the cytotoxicity or the level of secreted cytokines. The pattern of cytokines was not modified either.

• In vivo, overall survival was significantly improved by the addition of co-stimulatory domains. Although increasing the level of receptor at the cell surface, overexpression of DAP10 did not improve survival either (Figure 4).

ENDOGENOUS DAP10 PROVIDES OPTIMAL CO-STIMULATION FOR NKG2D-BASED CAR T CELLS

T cells transduced with the different NKG2D-based constructs were co-cultured for 100 hours with the pancreatic cancer cell line PANC-1. Cytotoxicity was followed in real-time with the Incucyte live-cell imaging system and was similar between the different groups (Figure 3B).

Cytokine secretion is not dramatically altered by the addition of co-stimulatory domain

To compare the ability of the different NKG2D-based CAR T cells to produce cytokines in response to tumor cells, CAR T cells were co-cultured for 48 hours with K562, a CML cell line. As illustrated in Figure 3C, the levels of IFNγ were slightly but significantly lower for CYAD-01 + DAP10 CAR T cells that secreted slightly though significant lower levels of IFNγ. The levels of other cytokines secreted by T cells transduced with the different constructs in presence of K562 cells were measured by multiplex assay. Neither the pattern nor the levels of these cytokines were different between the constructs (Figure 3D-3H).

CONCLUSIONS

Assessment of the in vivo potency of CYAD-01 and CYAD-01 derived constructs

To assess whether functional differences between the distinct NKG2D-based constructs could be observed in vivo despite the similar in vitro responses, as has been indicated in the literature [6], we tested the in vivo efficacy of T cells bearing the different constructs in an AML model.

Seven days after injection of THP-1 cancer cells, mice (n=5 per group) were injected once with 10 million control or NKG2D-based CAR T cells produced from 2 donors presenting different CD4/CD8 ratios (ratio donor #1 >1, ratio donor #2 <1). Compared to CYAD-01, overall survival was not significantly improved by the addition of co-stimulatory domains. Although increasing the level of receptor at the cell surface, overexpression of DAP10 did not improve survival either (Figure 4).

REFERENCES:

[5]. Williams R et al. Nature Biotechnology 2018;36:847-856