Uncovering the phenotype, functional and homing properties of NKG2D CAR T cells

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RESULTS

To validate the relevance of those data in CYAD-01 cells from patients, we performed phenotypic analysis of CYAD-01 cells produced from AML patients. As illustrated in Figure 6, CYAD-01 cells from patients exhibit more variable phenotype compared to healthy donors. Despite this variability, the CD4/CD8 ratio is also in favor of the CD8+ T-cells in AML CYAD-01 (Figure 6A). In addition, AML CYAD-01 cells are less positive for CD25+, while they do not express LAG-3, similarly to the healthy donor-derived CYAD-01 cells (Figure 6B and C respectively).

Interestingly, the AML CYAD-01 memory phenotype is less differentiated. Compared to healthy donors, a reduction of effector memory proportion (CD62L-CD45RA+) with a concomitant increase of the central memory proportion (CD62L+CD45RA-) is observed (Figure 6D and E respectively). Concerning homing abilities and probably linked to the memory phenotype, the chemokine receptor CXCR3 is almost absent from AML CYAD-01. However, the CYAD-01 T-cell population positive for CXCR4 is enhanced. This is crucial for the AML indication, as it drives homing to the bone marrow, where the leukemia blasts and stem cells have been described to be located [6].

Finally, upon co-culture with K562 cells, both healthy donor and AML-derived CYAD-01 cells secrete high level of IFN-γ. Importantly, this secretion was hindered using a NKG2D specific antibody, supporting a CAR-dependent secretion of IFN-γ (Figure 6H).

CONCLUSIONS

CYAD-01 cells generated using healthy donor or AML patient primary material, consist of an activated, non-exhausted, predominantly effector memory T cell population. Interestingly, the strong expression of CXCR4 may support the ability of CYAD-01 cells to home to the bone marrow, an important property to facilitate targeting of AML leukemic stem cells and blasts. The breadth of cytokines produced by CYAD-01 cells confirms the ability of the NKG2D CAR to provide strong activation and co-stimulatory signaling. These results support the encouraging preliminary results from the THINK clinical trial.

REFERENCES:

MATERIALS AND METHODS

CYAD-01 T cell manufacturing process: CYAD-01 T cells were transduced with NKG2D CAR followed by 48 hours. Transduced T cell were then cultured for 6 days in X-vivo FCS in presence of NKG2D-stimulating antibody and IL-2. Transduced T cells were assessed for their functional profile. Generation CYAD-01 T cells were ex vivo expanded and analyzed on the Attune NxT flow cytometer.

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