Tackling fratricide to manufacture clinical grade NKG2D-CAR T cells for cancer therapy

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ABSTRACT

A CAR consisting of a fusion of the NKG2D protein with CD3ζ (CYAD-01) enables T cells with specificity for the NKG2D ligands providing the potential to target a broad range of tumors (Figure 1). However, T cells transiently express these target ligands during production. Consequently, CYAD-01 T cells undergo fratricide that inhibits expansion of the engineered T cell population. During manufacture, the fold expansion of CYAD-01 T cells was consistently reduced as compared to control T cells (Figure 2). This inhibition of CYAD-01 expansion was observed mainly after cryopreservation and upon maintenance at 4°C, but fails to produce high number of cells. This process has now been implemented into our on-going clinical trials testing the potency of CYAD-01 T cells against hematological and solid tumors in the THINK study (NCT03018405).

Notably, PI3K inhibition during production resulted in a dose dependent increase in CD62L + CD45RA + cells (Figure 3C and D) and the modulation of the CD4/CD8 + T cell phenotype (Figure 3A) in the resultant CYAD-01 T cells. The multi-target specificity of the NKG2D-based CAR (CYAD-01) demonstrates targeted killing of autologous T cells by CYAD-01 T cells. Together, this suggests that the transient expression of NKG2D ligands on activated T cells induces fratricide through the NKG2D-based CAR. Notably, PI3K inhibition during production resulted in a dose dependent increase in CD62L + CD45RA + cells (Figure 3C and D) and the modulation of the CD4/CD8 + T cell phenotype (Figure 3A) in the resultant CYAD-01 T cells. The multi-target specificity of the NKG2D-based CAR (CYAD-01) demonstrates targeted killing of autologous T cells by CYAD-01 T cells. Together, this suggests that the transient expression of NKG2D ligands on activated T cells induces fratricide through the NKG2D-based CAR.

REFERENCES


CONCLUSION

- The multi-target specificity of the NKG2D-based CAR CYAD-01 provides a very strong potential to treat a broad range of cancer indications. However, the transient expression of NKG2D ligands during activation leads to T cell fratricide during manufacturing and reduced cell yield. This impacts the clinical application of CYAD-01 therapy.
- Inclusion of a PI3K inhibitor provides partial protection against the effects of PI3K inhibition on cell viability. This suggests that PI3K inhibition may overcome the cell yield issue which is currently the limiting factor for the clinical application of CYAD-01.
- This process has now been implemented into our on-going clinical trials testing the potency of CYAD-01 T cells against hematological and solid tumors in the THINK study (NCT03018405).