2749. Preclinical assessment of chimeric antigen receptor (CAR) T persistence and functionality in the disseminated NALM6-Luc human B cell acute lymphoblastic leukemia (ALL) model

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Introduction

- Establishing long-term CAR T persistence and efficacy remains a barrier to broader application of CAR T therapies in the clinic, thus development of robust platforms that can provide longitudinal assessments of CAR T persistence and functionality is paramount.
- Using the NALM6-Luc ALL model, Labcorp Drug Development has developed a flow cytometry platform that provides quantitative analysis of CAR T cells over time as well as surface markers that are documented to correlate with sustained T cell persistence, activation and exhaustion in vivo.

Methods

- Human peripheral blood mononuclear cells (PBMCs) were transduced with lentivirus, expanded in culture and cryopreserved for future use.
- Flow cytometry featuring a CAR-specific monoclonal antibody (mAb) or anti-CD3 mAb was used to determine transduction efficiency and persistence, respectively.
- To assess in vitro activity, co-cultures of T cells and CD19-expressing NALM6-Luc-mCh-Puro cells were used to measure cytotoxicity. Human MV-4-11-Luc-mCh-Puro AML cells were used as a negative control.
- For evaluation of in vivo efficacy of anti-CD19 CAR T cells, activity against the disseminated NALM6-Luc-mCh-Puro-ALL model in female NOD.Cg-Prkd×SzJ (NSG) mice was determined.
- All animal work was performed in an AAALAC-accredited facility, in alignment with applicable animal welfare regulations and with predetermined humane euthanasia criteria in all studies.
- For flow cytometric analysis, blood was collected 24h after CAR T cell infusion and weekly thereafter. After RBC lysis, single cell suspensions were labeled with fluorescent antibodies. Cells of blood were quantified using Precision Count Beads® (Biolegend). Data was acquired on an Attune NxT flow cytometer (Thermo Fisher Scientific) and analyzed using FlowJo® software (BD). Where indicated, statistical analysis was performed using a Student's T-test (* p<0.05).

Results

Conclusions

- After transduction of PBMCs with lentivirus, approximately 25% of T cells were CD3+/CAR+.
- Anti-CD19 CAR T cells show specificity in a cell killing assay towards CD19 expressing NALM6 cells compared to CD19-null MV-4-11 cells.
- Anti-CD19 CAR T cell delayed tumor growth in the systemic NALM6-Luc ALL model but mice eventually succumbed to disease.
- Uncontrolled disease progression coincided with increased exhaustion marker expression (PD-1 and ICOS LAG-3).
- Treatment-induced effects observed are likely independent of graft versus host disease because the CAR T cell phenotype was not recapitulated in non-tumor bearing mice.