Abstract: S179

Title: DISCOVERY AND DEVELOPMENT OF CLL-1 AS A CELLULAR IMMUNOTHERAPY TARGET IN JUVENILE MYELOMONOCYTIC LEUKEMIA

Abstract Type: Oral Presentation

Session Title: Translational studies in MDS

Background:

Juvenile myelomonocytic leukemia (JMML) is a myeloproliferative disease of infants and toddlers that is treated with hematopoietic stem cell transplantation (HSCT). However, long-term survival after HSCT is only ~50%, so additional therapeutic options are needed. Adoptive immunotherapy with chimeric antigen receptor (CAR) T cells has been shown to be effective in advanced lymphoid malignancies but is not currently available to patients with JMML.

Aims:

We hypothesized that identifying proteins overexpressed on the cell surface of JMML mononuclear cells would facilitate the development of CAR T for JMML. Additionally, we sought to identify potential targets that are specifically found on JMML leukemic stem cells (LSCs) that drive disease progression and relapse.

Methods:

Bulk and scRNA-Seq data were generated from 85 and 22 samples, respectively, and were analyzed to identify genes that encode for cell surface proteins and compared to healthy controls. Flow cytometry for individual targets was performed on 52 samples. Unbiased mass spectrometry using wheat germ agglutinin-horseradish peroxidase labeled surface proteins was performed on 7 samples enriched for CD34+ cells. CAR T cells were generated by lentiviral transduction. CAR T cell efficacy using *in vitro* cytotoxicity assays was assessed by flow cytometry. T cell receptor alpha chain constant (TRAC) knockout for *in vivo* studies was performed using CRISPR Cas9. Patient derived xenograft (PDX) studies involved injection of OKT3-treated primary JMML cells into NSG mice.

Results:

Hierarchical clustering of surface protein encoding genes from bulk RNA-Seq demonstrated that JMML patients cluster separately from other pediatric hematologic malignancies. Our selection pipeline indicated that 14 genes including *CLEC12A* (encoding CLL-1) were highly expressed in JMML but minimally expressed in pediatric healthy controls or in healthy tissue as per the GTEx database (Fig. 1A). To further identify targets on JMML LSCs, we analyzed our scRNA-Seq and mass spectrometry data. There were three upregulated genes/proteins enriched in CD34+ cells that also had a median TPM <20 in normal tissue: CD48, ADGRE2 and *CLEC12A* (CLL-1) (Fig. 1B). As CLL-1 was overexpressed on JMML mononuclear and hematopoietic stem cells, we therefore focused our remaining studies on this target. Flow cytometry confirmed increased median fluorescence intensity and CLL-1+ percentages in JMML peripheral blood and bone marrow mononuclear cells compared to healthy controls. A CLL-1 CAR (CD8α hinge and transmembrane domain, 4-1BB costimulatory domain) was efficacious *in vitro* reducing myelomonocytic CD11b+ or CD14+ primary JMML cells. To reduce alloreactivity *in vivo*, we used a TRAC knockout. CLL-1 CAR reduced tumor burden in cardiac blood, bone marrow and spleen compared to untransduced T cells or PBS (Fig. 1C). Notably, the stem cell compartment was also significantly reduced in the CLL-1 CAR treated mice (Fig. 1D).

Summary/Conclusion:

Targeting CLL-1 is a promising cellular therapeutic strategy in JMML, capable of reducing bulk cells and the LSC population. To our knowledge, this is the first report of efficacy of any CAR T cell therapy in a JMML-specific PDX model. Of note, phase 1 anti-CLL-1 CAR T clinical trials in pediatric and adult AML (NCT04219163,

NCT04789408) are ongoing and might facilitate development of CAR T cell therapy for this orphan disease. CLL-1 CAR T cells could potentially be used as salvage therapy in patients with relapsed JMML.

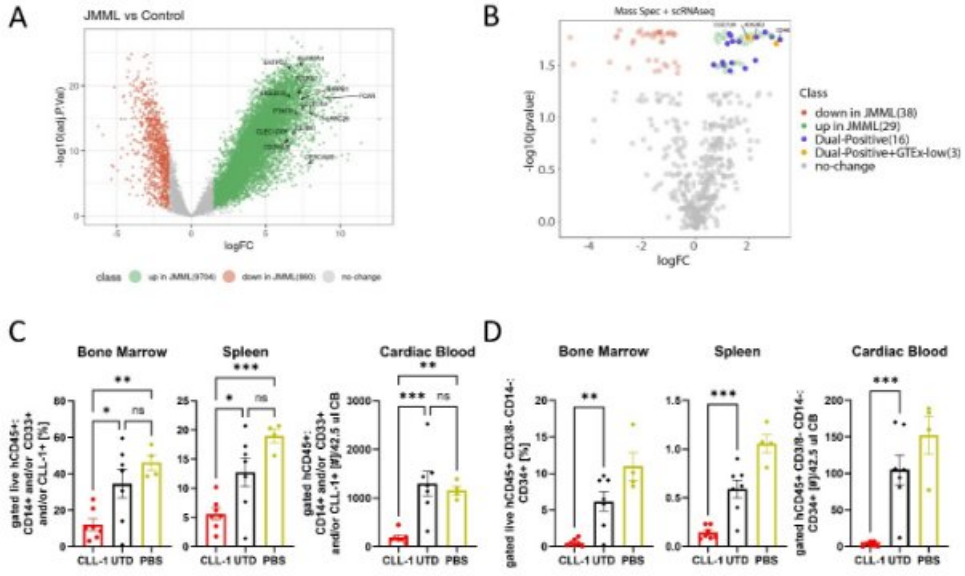

Figure 1. A) Volcano plot displaying upregulated cell surface proteins in JMML. The log2-fold change comparing JMML and healthy control is shown on the x-axis, while the -log10(adjusted p-value) is shown on the y-axis. Proteins with log2-fold change >6.56 and adjusted p-value <0.05 were considered significantly upregulated, with selected proteins labeled. B) Volcano plot showing mass spectrometry of JMML peripheral blood and bone marrow CD34+ cells vs adult healthy control apheresis CD34+ cells. Differentially expressed surface proteins based on Wilcoxon rank sum test are displayed. "Dual-Positive" denotes surface proteins also upregulated by scRNA-Seq. C) In a JMML PDX model, 6 weeks post JMML injection and 3 weeks post CAR T injection, JMML burden was assessed upon mice termination by staining for CD14, CD33 and CLL-1 in different tissues. UTD=untransduced T cells. D) Same study as in C). LSC burden was assessed by staining for CD34. Statistical analysis in C) and D) was performed by one-way ANOVA with Tukey's correction. (*=P≤0.05, **=P≤0.01, ***=P≤0.001, ****=P≤0.0001)

Keywords: CAR-T, Cancer immunotherapy, Adoptive immunotherapy, JMML