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CRISPR-Cas9 Knock out of CD5 Enhances the Anti-Tumor Activity of Chimeric Antigen Receptor T Cells

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Chimeric Antigen Receptor T cells (CART) have led to unprecedented clinical responses in relapsed or refractory (r/r) B-cell acute lymphoblastic leukemia (B-ALL), non-Hodgkin lymphomas (NHL), and multiple myeloma. However, despite these exciting results, most patients treated with CART therapy either do not respond or eventually relapse. Moreover, CART therapy has not yet been proven effective in several hematological malignancies, such as T cell lymphoma and leukemia (T-NHL/T-ALL) and acute myeloid leukemia (AML). Thus, there is a need to enhance currently available CART products and also to develop next-generation CART therapies to successfully treat additional neoplasms like T-NHL /T-ALL and AML.

To this goal, we studied the cysteine-rich scavenger receptor CD5, an attractive target for CART immunotherapy because of its dual role in malignant cells and normal T cells. In *malignant cells*, CD5 is expressed by ~90% of TCL cells, by ~15-20% of AML cells, and also by most cases of chronic lymphocytic leukemia and mantle cell lymphoma (MCL). Of note, promising results using a CD28-based anti-CD5 CART against T-NHL and T-ALL were reported at this meeting in 2019 (LaQuisa C. Hill #199). In *T cells*, CD5 is highly expressed and inhibits T cell receptor (TCR)-mediated activation through several mediators including SHP-1, CBL, CBL-B, and GRB2. Therefore, we hypothesized that the genetic deletion of CD5 in engineered T cells could potentially enhance their effector functions.

First, we designed and screened six 4-1BB-costimulated anti-CD5 lentiviral CAR constructs designed to have high, medium, and low affinity for CD5. We then selected the lead CAR5 construct (high affinity, heavy to light light chain orientation) based on its superior anti-tumor function *in vivo* in NOD-SCID IL2Rg^{null} (NSG) mice engrafted with T-cell leukemia (Jurkat). Then, to further improve CART5 activity, we optimized a CD5 short-guide RNA and deleted CD5 in CART5 cells using CRISPR-Cas9. CD5 gene deletion was reproducibly efficient (95-100% by flow cytometry and TIDE) during manufacturing (6 donors). Interestingly, the growth rate of wild type (WT) CART5 was comparable to CD5 KO CART5 and the expression of CD5 in WT CART5 was reduced. However, at the end of manufacturing, CD5 KO CART5 had increased central memory T cells (33.0% vs. 18.4%) and reduced expression of activation/exhaustion markers (PD-1 4.4% vs. 14.8%, LAG3 13.1% vs. 55.9%) compared to WT CART5, potentially indicating that CD5 KO reduces CART5-CART5 fratricide during manufacturing.

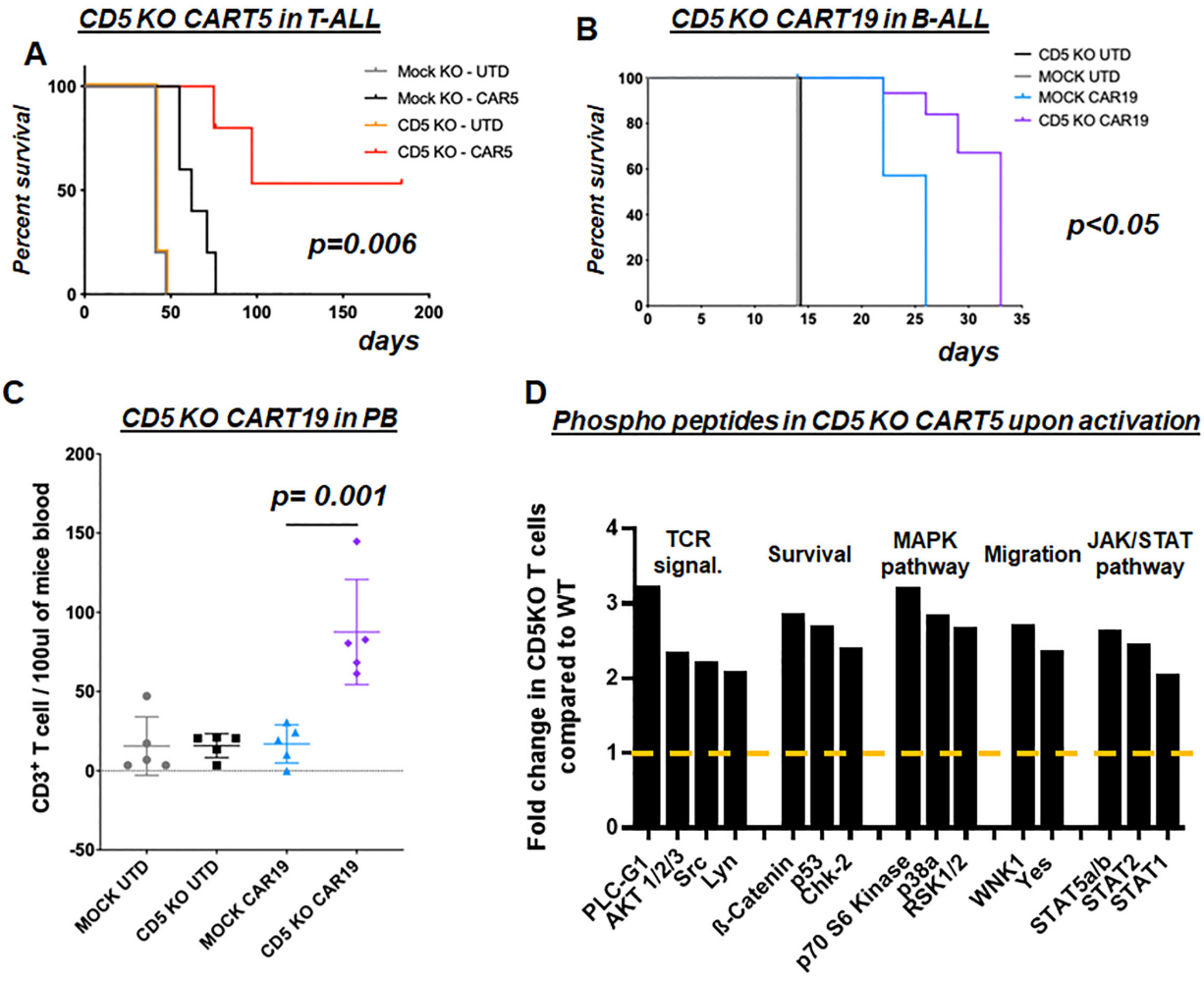
We then compared wild type (WT) CART5 to CD5 KO CART5 *in vitro* using several T-NHL/T-ALL, MCL, and AML models, including primary samples (Sezary cells, primary MCL cells, and CD5+ AML cells). Both WT and CD5 KO CART5 were highly effective in killing CD5+ malignant cells, but CD5 KO CART5 showed enhanced proliferation upon activation. In two xenograft models of T-cell leukemia (primary T-ALL and Jurkat), CD5 KO CART5 showed dramatically increased tumor control compared to WT (**Fig.1A**, median overall survival for WT= 62 days vs. CD5 KO=not reached, p = 0.006, Mantel-Cox). This enhanced anti-tumor effect was associated with increased expansion of CD5 KO CART5 in the peripheral blood (PB) compared to WT CART5.

To test the hypothesis that deletion of CD5 could increase the anti-tumor effect of CART targeting antigens other than CD5, we knocked out CD5 in anti-CD19 CART cells and tested their function in a CD19+ B-ALL xenograft model (NALM6). Remarkably, CD5 KO CART19 displayed significantly enhanced anti-leukemia activity and PB expansion compared to WT (**Fig.1B,C**, $p<0.05$, $p = 0.001$, Mantel-Cox).

Finally, we aimed to define the mechanisms by which CD5 KO enhances CART anti-tumor efficacy. We analyzed the phosphorylation of multiple targets in T cells after 15 minutes of CAR stimulation. Remarkably, CD5 KO CART5 cells had higher (>2fold) phosphorylation of several signaling proteins, including key regulators of T cell activation, migration, and survival compared to WT CART5 (**Fig. 1D**). To confirm that the CD5 pathway was indeed the mediator of this effect, we knocked out SHP-1 in CART19 cells using CRISPR/Cas9 and observed increased leukemia killing.

In conclusion, we demonstrate that CRISPR-Cas9 KO of CD5 enhances the anti-tumor activity of CAR T cells by enhancement of CAR-mediated activation and proliferation. These findings support the development of CD5 KO CART products in early-phase clinical trials.

Figure 1



Disclosures:

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