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ORAL ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Restoration of LAT Signaling in CD33-Directed CAR T Cells Improves Potency and Persistence Against Acute Myeloid Leukemia

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Despite the development of novel therapies, the majority of patients with acute myeloid leukemia (AML) will not achieve long-term remissions. Patients with relapsed/refractory (r/r) AML have particularly poor outcomes, as conventional chemotherapy regimens are often unable to elicit long-term remissions. Allogeneic hematopoietic stem cell transplant (HSCT) is potentially curative in eligible patients, however, bridging therapies which can induce deep remissions prior to HSCT are needed. Second generation chimeric antigen receptor (CAR) T cells targeting lineage-restricted antigens, such as CD19, have induced remissions in up to 80 - 90% of pediatric patients with r/r B-lineage acute lymphoblastic leukemia (ALL), however, post-CAR relapses are common and only ~50% of treated patients achieve durable remissions. Similar strategies targeting myeloid-restricted antigens with 2 nd generation CAR T cells in AML have not replicated the success seen in ALL. As clinical experience has highlighted the impact of CAR T cell potency, persistence, and antigen-sensitivity to long-term efficacy in ALL, strategies to boost these properties in myeloid-directed CAR T cells are necessary to extend the clinical benefit of this therapy to patients with AML.

Utilizing a global phosphoproteomics screen, we recently identified that inefficient phosphorylation of the Linker for activation of T cells (LAT) and the subsequent decrease in the downstream signaling pathways results in suboptimal CAR T cell activation. To overcome inefficient LAT activation, we developed a novel bicistronic CAR platform, consisting of a 2 nd generation CAR along with an Adjunctive LAT-Activating CAR (ALA-CART) which improved sensitivity to low-antigen leukemia cells, increased in vivo potency, and enhanced persistence in xenograft models of ALL. We hypothesized that the restoration of LAT signaling via the ALA-CART platform would also improve potency and persistence in pre-clinical models of AML. We found that while CD33 ALA-CART cells responded to CD33+ AML cells in vitro, they were less cytotoxic and produced lower levels of cytokines than CD33-28z or CD33-BBz 2 ndgeneration CAR T cells. However, CD33 ALA-CART cells demonstrated increased potency in xenograft models, eradicating AML cells at much lower doses than CD33-28z and CD33-BBz CAR T cells (Fig. 1A, $p<0.0001$). This in vivo potency correlated with an enrichment of T stem cell memory cells in the ALA-CART product, resulting in increased persistence of CD33 ALA-CART cells after leukemia clearance (Fig. 1B, $p=0.0139$). The ALA-CART platform was associated with increased mitochondrial mass and function, suggesting that the increased signaling through LAT results in an enhanced capacity for oxidative phosphorylation, potentially contributing to the increased persistence of CD33 ALA-CART cells in vivo. Thus, the ALA-CART platform, designed to restore LAT signaling in CAR T cells, demonstrates that CAR molecules can be rationally designed to enhance both in vivo potency and persistence, and represents a promising strategy for the development for more effective AML-directed CAR T cells.

Disclosures No relevant conflicts of interest to declare.

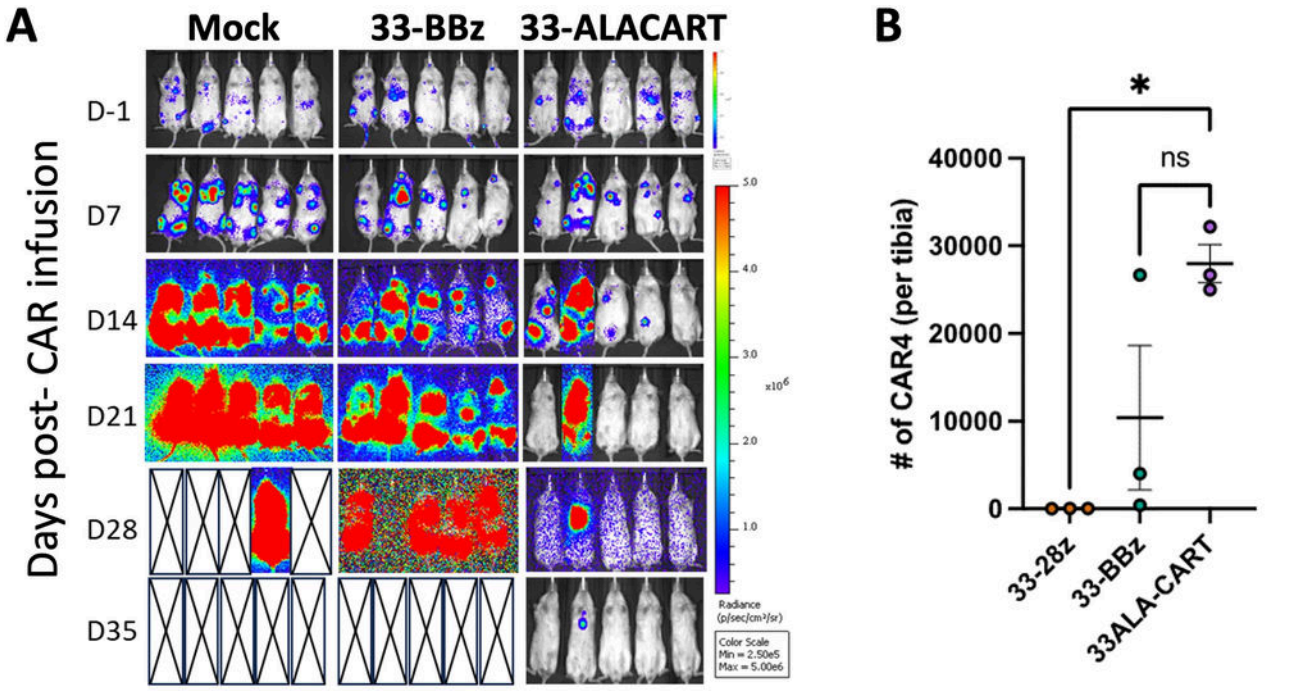

Fig 1. 33ALA-CART increases potency and persistence relative to 2nd generation 33CAR T cells A) NSG mice were engrafted with 1X10^6 parental MOLM14 and treated with 0.5X10^6 33-BBz or 33ALA-CART. B) NSG mice treated with 3X10^6 CAR T cells and were sacrificed at day 30 to evaluate persistence of CAR in the bone marrow.

Figure 1

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