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POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Runx2 Overexpression Enhances Potency and Counteracts Exhaustion in CD8+ CAR T Cells

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Chimeric antigen receptor (CAR) T cells are highly effective in treating B cell malignancies, but 50% of patients eventually relapse, often due to progressive dysfunction of the remaining CAR T cell population. While several others have implicated the bZIP transcription factor (TF) family in CAR T cell exhaustion (Seo et al. Nat Immunol. 2021, Lynn et al. Nature. 2019, Zhang et al. Cancer Cell. 2022), here we reveal a novel role for the TF Runx2 in mediating resistance to exhaustion and enhancing potency of CD8+ CAR T cells (CAR8). We have previously shown that CAR8 derived from naïve or memory T cell subsets maintain functional features of the T cell population from which they were derived (DeGolier et al. ASH. 2021). We predicted that an epigenomic and transcriptomic comparison of CAR8 derived from naïve or memory cells could reveal drivers of T cell differentiation and function.

To precisely control T cell antigen experience, and to study T cell persistence in the absence of xenogeneic GvHD, we chose to use a syngeneic murine model, with adoptive transfer of E2A-PBX B cell acute lymphoblastic leukemia treated by anti-murine CD19 CAR T cells (Qin et al. Blood. 2018). We used a well-characterized ovalbumin vaccination model to produce memory T cells with which to generate memory-derived CAR8, and concurrently generated naïve-derived CAR8 from naïve hosts (Ahonen et al. J Exp Med. 2004). Comparative ATACseq of naïve CD8+ T cells to memory CD8+ T cells at 28 days post-vaccination revealed differential chromatin accessibility at target loci for Runx family transcription factors. These differences were maintained upon transduction of these T cell populations with a CAR, and after reinfusion into leukemia-bearing hosts. While the Runx family contains three members, RNAseq analysis uncovered that only Runx2 transcripts were highly enriched in memory over naïve cells prior to CAR-transduction but showed that gene expression levels converged upon CAR transduction and reinfusion. Additionally, broad comparisons of chromatin accessibility and transcriptomic profiles to published datasets showed that naïve-derived CAR8 progressively became more "memory-like", while memory-derived CAR8 became more "effector-like."

We hypothesized that establishing Runx2 expression in naïve-derived CD8+ CAR T cells (CAR8 $_{ND}$) could enhance the existing "memory-like" state of these T cells and boost T cell potency and anti-leukemia response. Indeed, overexpression of Runx2 in naïve-derived CAR8 (Runx2-CAR8 $_{ND}$) strongly enhanced leukemia clearance ($p<0.05$) in mice treated with a sub-curative 1e5 CAR+ T cell dose. While there was no difference in the PD1+ proportion ($p = 0.47$), indicating similar activation and/or exposure to target antigen, mice treated with Runx2-CAR8 $_{ND}$ exhibited a lower proportion of PD1+/CD39+ cells ($p<0.001$) as well as a dramatically reduced proportion of PD1+/TOX+ cells ($p<0.001$), suggesting that Runx2 overexpression counteracts the differentiation trajectory toward terminal exhaustion. Additionally, Runx2-CAR8 $_{ND}$ showed enhanced T cell proportions in the marrow at 11 days post-CAR infusion ($p<0.01$), with >2-fold higher levels of Runx2-CAR8 $_{ND}$ compared to control CAR8 $_{ND}$. Finally, in mice treated with a curative 2e6 CAR+ T cell dose, Runx2-CAR8 $_{ND}$ demonstrated a trend toward increased persistence in the blood at 19 days post-CAR infusion, relative to control CAR8 $_{ND}$ ($p = 0.053$).

In conclusion, a comparison of naïve and memory-derived CD8+ CAR T cells at the epigenetic level revealed Runx2 overexpression in naïve-derived CAR T cells as a novel strategy to enhance the anti-leukemia response. Preliminary findings suggest

Runx2-CAR8 $_{ND}$ show enhanced T cell proportions and decreased leukemic burden, as well as favorable exhaustion phenotypes. Future studies will expand our characterization of Runx2-CAR8 $_{ND}$ to better understand the effects of Runx2 on CAR T cell epigenetic profile, function and persistence in the bone marrow microenvironment, as well as test the effects of Runx2 overexpression in human CAR T cells. We anticipate that Runx2 overexpression will prove to be a useful strategy in enhancing the durability of CAR-mediated leukemia remissions.

Disclosures Scott-Browne: *Lyell Immunopharma:* Patents & Royalties: Patent licensed to listed organization. . **Fry:** *Sana Biotechnology:* Current Employment, Current equity holder in publicly-traded company.

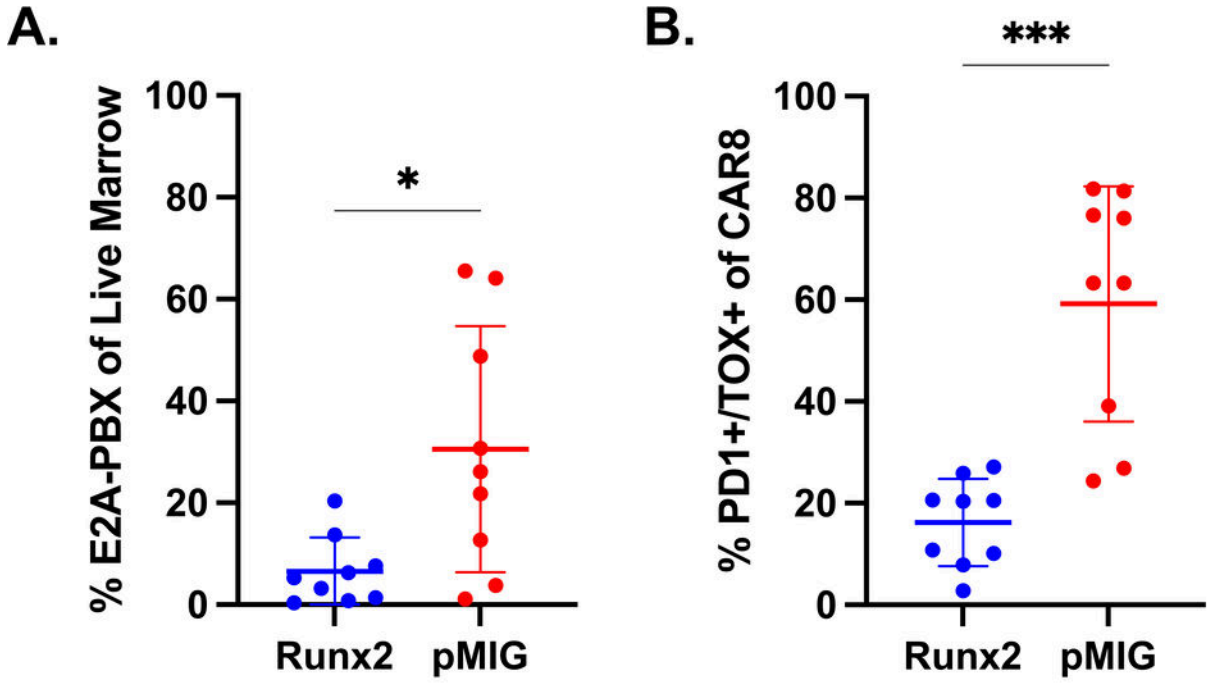

Figure 1: Rag1-/- mice were given 1e6 E2A-PBX murine leukemia intravenously on day -3, followed by 1e5 CAR+ CD8+ T cells retro-orbitally on day 0. Bone marrow was analyzed at 11 days post-CAR. **A)** Leukemia clearance, as a proportion of live bone marrow. **B)** PD1+/TOX+ proportion of CAR+ CD8+ T cells. Statistical analyses done via Mann-Whitney tests (* p<0.05, *** p<0.001).

Figure 1

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