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ORAL ABSTRACTS

509.BONE MARROW FAILURE AND CANCER PREDISPOSITION SYNDROMES: CONGENITAL

BCOR Loss Confers Increased Stemness and Partially Rescues RUNX1-Deficient Phenotypes in Human Hematopoietic Stem and Progenitor Cells

Kendra K Jackson[1,2,3,4], *Amy C Fan, PhD*[5,4,2,6], *Daiki Karigane, MD PhD*[4,2,3], *Feifei Zhao*[4,2,3], *Cailin T Collins*[4,7,3], *Yusuke Nakauchi, MD PhD*[3,2,4], *Kensuke Kayamori, MD PhD*[3,2,4], *Athreya S Rangavajhula*[5,2,4], *Thomas Koehnke, MD*[2,4,3], *Ravindra Majeti, MD PhD*[2,3,4]

[1] Physician Scientist Training Program, Stanford University School of Medicine, Stanford, CA
[2] Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA
[3] Cancer Institute, Stanford University School of Medicine, Stanford, CA
[4] Department of Medicine, Division of Hematology, Stanford University School of Medicine, Stanford, CA
[5] Stanford Cancer Institute, Stanford University School of Medicine, Stanford, CA
[6] Immunology Graduate Program, Stanford University School of Medicine, Stanford, CA
[7] Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Ann Arbor, MI

Introduction: Loss of function mutations in *BCL-6 corepressor (BCOR)* commonly occur in de novo acute myeloid leukemia (AML) and germline hematopoietic malignancies, most notably in those arising from RUNX1 familial platelet disorder (RUNX1-FPD). Second only to loss of heterozygosity of *RUNX1*, mutations in *BCOR* are the most commonly acquired mutations upon progression to myeloid malignancy in RUNX1-FPD. Additionally, *RUNX1* and *BCOR* mutations commonly co-occur in de novo AML, and both are classified as "adverse risk" by the European LeukemiaNet if found at AML diagnosis. However, the role of BCOR in leukemogenesis is poorly understood, and there are limited studies investigating co-loss of BCOR and RUNX1 in hematopoietic stem and progenitor cells (HSPCs).

Methods: We developed a model of BCOR loss in primary human CD34+ HSPCs using CRISPR/Cas9 and AAV6-mediated homology directed repair to knock-in a fluorescent reporter expression cassette into the *BCOR* locus. We used this system to investigate how BCOR knockout (KO) perturbs normal HSPC function. We combined this approach with a previously developed analogous system to disrupt the *RUNX1* locus to investigatehow BCOR loss in the setting of RUNX1 KO modulates the effects of RUNX1 loss on HSPC function.

Results: Here, we found that BCOR loss increased capacity for serial replating and conferred a competitive advantage over control HSPCs. In serial replating assays, BCOR-deficient HSPCs had a 5-fold increase in colony formation after one passage with a striking majority of colonies being erythroid. In liquid differentiation assays, BCOR-deficient HSPCs consistently had a 2-fold increase in CD34+ cells over a 21 day time course. When co-cultured with control HSPCs in a 1:1 ratio in stem expansion media (base media + SCF, FLT3-L, TPO, IL-6, UM-171), BCOR-deficient HSPCs expanded to comprise 70% of the culture at 14 days. This competitive advantage was recapitulated *in vivo*. At the 12-week timepoint of a competitive transplantation assay in NSG mice using HSPCs from 3 different donors, BCOR KO cells (marked by BFP) on average made up 75% of the engrafted human CD45+ population, while control cells (expressing GFP from the AAVS1 safe harbor locus) made up only 21%.

Additionally, BCOR loss partially rescued RUNX1-induced stem cell dysfunction *in vitro*. Compared to RUNX1 deficiency alone, HSPCs deficient in both RUNX1 and BCOR had a 4-fold increase in colony formation after two passages in serial replating assays. Our lab has previously shown that RUNX1 loss ablates erythroid colony formation and skews towards monocytic colonies. Despite BCOR loss alone having a strong skew towards erythroid colonies, BCOR loss in RUNX1-deficient HSPCs did not induce erythroid colony formation. This suggests that in double mutant HSPCs, RUNX1 loss drives lineage decisions while BCOR loss drives the increase in serial replating potential. Ongoing efforts are aimed at characterizing combinatorial effects of BCOR and RUNX1 loss on hematopoiesis and stem cell function through *in vitro* liquid differentiation assays and *in vivo* transplantations, as well as investigating the molecular underpinnings of these phenotypes through RNA-seq.

Conclusion: In summary, we have established a model of BCOR loss in primary human HSPCs to evaluate not only the role of BCOR in hematopoiesis, but also to characterize how BCOR is involved in leukemic transformation when combined with RUNX1. We show that BCOR deficiency enhances several attributes associated with stem cell function, including increased potential for serial replating, increased CD34 positive population, and increased engraftment compared to control HSPCs.

When combined with RUNX1 loss, BCOR loss increases the serial replating potential of RUNX1-deficient cells, suggesting some rescue of RUNX1 KO-induced stem cell dysfunction. Ultimately, this model will enable us to elucidate the molecular mechanisms underlying increased stemness and competitive advantage in BCOR-deficient HSPCs, interrogate RUNX1-FPD disease progression associated with BCOR mutation, and validate potential therapeutics.

Disclosures Koehnke: *TenSixteen Bio:* Consultancy. **Majeti:** *858 Therapeutics:* Membership on an entity's Board of Directors or advisory committees; *Orbital Therapeutics:* Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees; *MyeloGene:* Current equity holder in private company; *Pheast Therapeutics:* Current equity holder in private company; *kodikaz Therapeutic Solutions:* Membership on an entity's Board of Directors or advisory committees.

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