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ORAL ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Gene Correction of DNMT3A:R882H in Primary Human AML Demonstrates That This Mutation Is Not Required for Disease Maintenance, but Is Associated with Increased Leukemia Stem Cell Frequency

Thomas Koehnke, MD[1,2], Daiki Karigane, MD PhD[1,2], Eleanor Hilgart[3], Kensuke Kayamori, MD PhD[1,2], Amy C Fan, PhD[4,1], Cailin T Collins[1,2], Fabian P Suchy, PhD[1,2], Athreya S Rangavajhula[1,4], Yang Feng, PhD[1,2], Yusuke Nakauchi, MD PhD[2,1], Eduardo Martinez-Montes[3], Michael Koldobskiy, MD PhD[3], Andy Feinberg, MD MPH[3], Ravindra Majeti, MD PhD[5,2]

[1] Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA
[2] Cancer Institute, Stanford University School of Medicine, Stanford, CA
[3] Center for Epigenetics, Johns Hopkins University School of Medicine, Baltimore, MD
[4] Stanford Cancer Institute, Stanford University School of Medicine, Stanford, CA
[5] Stanford Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA

Introduction: The genetic landscape of AML comprises numerous genetic alterations contributing to disease initiation and progression. Among these lesions, frequently occurring missense variants in DNMT3A:R882 are typically found as pre-leukemic events. An important and understudied question in leukemia pathogenesis is whether individual mutations are required for disease initiation, maintenance of established disease, or both. This is particularly the case with pre-leukemic mutations that affect the epigenome, as such mutations might be required to provide the epigenetic background necessary for disease initiation, but may no longer be required for disease maintenance. Here, we dissect the contribution of DNMT3A mutations to leukemic initiation and maintenance by gene correction in primary patient AML specimens.
Methods: To functionally interrogate the contribution of DNMT3A mutations in human AML pathogenesis, we generated a novel CRISPR/Cas9-mediated toolset to selectively and specifically revert DNMT3A:R882 missense variants back to wildtype, or re-introduce the original mutation as a control, directly in both primary pre-leukemic HSCs (pHSCs) and primary leukemic blasts from 7 patient samples, leaving the remaining mutations and underlying germline background intact. Gene-corrected or re-mutated cells were subjected to functional assays both *in vitro* and *in vivo*.
Results: We demonstrate that correction of DNMT3A mutations is feasible, achieving 5-40% gene editing both in purified pHSCs and leukemic blasts (n=7 patient samples). Using this approach, we first establish that DNMT3A mutations confer increased stemness in human pHSCs, with increasing serial replating capacity (mean increase re-mutated vs. corrected 5.53-fold, $p<0.05$). Surprisingly however, correction of DNMT3A mutations in leukemic blasts led to no immediate functional consequences. Specifically, primary patient leukemic blasts corrected for DNMT3A mutations retained the ability to engraft leukemia in NSGS mice and showed no difference in human chimerism or immunophenotype compared to re-mutated control from 5/5 primary patient samples. Importantly, limiting dilution transplantation assays (LDA) with two cases revealed no impact of DNMT3A mutations on leukemia-initiating cell (LIC) frequency in primary recipients, highlighting that DNMT3A mutations have no immediate effect on disease maintenance. We then performed whole-genome bisulfite sequencing and RNA-sequencing on leukemic blasts isolated from engrafted mice 4 months after DNMT3A correction or re-mutation. Global methylation levels between corrected and re-mutated cells were within the variance in either of these groups, and gene expression profiles were similarly driven by patient. However, paired analysis of each patient sample revealed that DNMT3A correction led to a consistent loss of stem cell expression signatures, suggesting influence of DNMT3A mutations on leukemia stem cell programs. Thus, we interrogated the effect of DNMT3A mutations on LSCs by performing secondary transplantations with two cases. In LDA assays, we observed a significant decrease in LIC frequency in the corrected leukemic blasts compared to re-mutated controls, indicating that correction of DNMT3A mutations induces a reduction in leukemia stem cell frequency, but does not completely abrogate leukemogenic potential.
Conclusion: In summary, our work demonstrates a novel CRISPR/Cas9 approach to dissect the contribution of individual somatic lesions in human leukemia. The precise correction of single lesions while leaving co-occurring mutations intact allows us to perform controlled, functional genetic experiments on primary human AML. Here, we employ this approach to dissect the contribution of DNMT3A mutations to leukemia initiation and maintenance. We find that DNMT3A mutations impart increased stemness on human pHSCs, consistent with a key role in leukemia initiation. However, in primary patient blasts,

we find that DNMT3A mutations are not required for leukemia maintenance. Strikingly, DNMT3A mutations in established leukemia were found to increase leukemia stem cell frequency. This approach will now allow for a detailed evaluation of differential methylation in mean and entropy and can be extended to other mutations of interest, extending our toolkit to unravel human AML biology directly in primary cancer specimens.

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

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