ELSEVIER

Blood 142 (2023) 953–954

The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Engineering Sequential Mutations into Human HSPCs Yields an Aggressive Myeloid Malignancy Allowing for Interrogation of Preleukemic Transformation

Cailin T Collins[1,2,3], *Yusuke Nakauchi, MD PhD*[3,1], *Thomas Koehnke, MD*[1,3], *James S Chavez, BS*[1,3], *Suyoun Choi*[3], *Rajiv Sharma, PhD*[3], *Feifei Zhao*[1,3], *Ravindra Majeti, MD PhD*[4,3,2]

[1] Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA
[2] Department of Medicine, Division of Hematology, Stanford University School of Medicine, Stanford, CA
[3] Cancer Institute, Stanford University School of Medicine, Stanford, CA
[4] Stanford Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA

Introduction: Acute Myeloid Leukemia (AML) is an aggressive myeloid malignancy that develops as the result of stepwise accumulation of mutations in hematopoietic stem and progenitor cells (HSPCs). Multiple studies of the clonal evolution of AML have determined that in general, the order of mutation acquisition follows a stereotypical fashion: mutations that occur early in development of AML involve epigenetic modifiers, whereas mutations that occur later more commonly affect proteins involved in signaling and proliferation. While multiple studies have demonstrated the ability of various combinations of mutations to promote leukemic transformation in mouse models, the majority of this work has studied concurrent alteration of driver genes, which may not accurately reflect human disease biology. To this end, we aimed to generate a model of AML by sequentially introducing mutations into human HSPCs to study driver gene dependent and independent pathways in preleukemic transformation.
Methods: Our model of sequential mutation acquisition in human AML employs CRISPR/Cas9-mediated gene disruption and AAV6-mediated homology directed repair (HDR) in human cord blood CD34+ HSPCs, allowing for high-efficiency knockout of epigenetic modifiers (*DNMT3A* or *TET2*) and expression of an activating signaling protein (*NRAS* ^{G12D}) along with the BFP fluorescent marker. Mutations are introduced sequentially through a serial xenotransplantation approach in immunocompromised mice, allowing for stepwise characterization of the preleukemic and transformed states of disease.
Results: To establish a model of preleukemic transformation using human HSPCs, we first developed a double synthetic guide RNA (sgRNA) knockout strategy that produces a ~55-65 bp deletion in either *DNMT3A* or *TET2*, leading to highly efficient INDEL (insertion or deletion) generation in cord blood CD34+ HSPCs and effective protein knockdown. Fourteen weeks after transplantation into immunocompromised mice, *DNMT3A*-KO, *TET2*-KO, and control cells demonstrate high levels of bi-lineage bone marrow engraftment (60-90%) and greater than 95% INDEL frequency at targeted loci. *DNMT3A*-KO, *TET2*-KO, and control CD34+ cells harvested from primary transplantation are subsequently edited to express *NRAS* ^{G12D}*-BFP* at efficiencies similar to freshly isolated cells (10-20%), and sorted BFP+ cells successfully engraft into secondary recipient mice. Consistent with observations from genetically engineered mouse models, we found that the combination of either *DNMT3A*-KO or *TET2*-KO with *NRAS* ^{G12D} in human cord blood CD34+ cells results in a rapidly fatal high-grade myeloid neoplasm in mice. Moribund mice develop peripheral cytopenias, decreased body weight, enlarged spleens, and have infiltration of CD45+ human cells in bone marrow, spleen, and liver. Human cells isolated from bone marrow retain near 100% BFP positivity and show persistence of >95% *DNMT3A*-KOor *TET2*-KO INDELs. BFP+ cells display a monocytic immunophenotype (CD34-, CD33+, CD14 variable, CD16 variable) and have a monocytic appearance on H&E staining. Experiments introducing *TET2*-KO/ *NRAS* ^{G12D} mutations using either a sequential or concurrent approach demonstrate mutational synergy between *TET2*-KO and *NRAS* ^{G12D}when compared with *NRAS* ^{G12D} alone. Comparisons of disease kinetics and phenotype when *TET2*-KO and *NRAS* ^{G12D} mutations are introduced sequentially vs concurrently are currently underway to investigate how temporal dynamics in epigenetic changes after *TET2*-KO affect subsequent transformation with *NRAS* ^{G12D}. In addition, genome-wide DNA methylation and gene expression characterization from cells in preleukemic (*DNMT3A*-KO and *TET2*-KO alone) and transformed states (*DNMT3A*-KO/ *NRAS* ^{G12D} and *TET2*-KO/ *NRAS* ^{G12D}) are ongoing to explore mechanisms underlying these observations.
Conclusion: Our work demonstrates that human HSPCs can be efficiently engineered to model the combination of early epigenetic modifier loss with expression of an activating signaling mutation, as is commonly seen in AML, resulting in a fully

penetrant, high-grade myeloid neoplasm. To our knowledge, this represents the first sequential model of preleukemic transformation in genetically engineered human cells. Importantly, the sequential nature of our approach allows for manipulation of the preleukemic phase of disease and subsequent interrogation of myeloid transformation.

Disclosures Koehnke: *TenSixteen Bio:* Consultancy. **Sharma:** *Graphite Bio:* Ended employment in the past 24 months. **Majeti:** *MyeloGene:* Current equity holder in private company; *Pheast Therapeutics:* Current equity holder in private company; *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; *kodikaz Therapeutic Solutions:* Membership on an entity's Board of Directors or advisory committees.

https://doi.org/10.1182/blood-2023-187422