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POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Stag2-Cohesin Loss Attenuates Flt3 ^{ITD} Myeloid Blast Expansion Yet Preserves Mutant HSC

Jane J Xu, PhD[1], Yi Chen, MD PhD[2], John Pantazi[2], Sebastian Fernando[3], Besmira Alija[2], Varun Sudunagunta, BA[4], Govind Bhagat, MD[5], Robert L. Bowman, PhD[6], Aaron D. Viny, MDMS[7]

[1]Columbia University Irving Medical Center, Department of Medicine, Division of Hematology / Oncology, New York, NY
[2]Columbia Stem Cell Initiative, Columbia Irving Medical Centre, New York, NY
[3]Columbia Stem Cell Initiative, Columbia Irving Medical Centre, New York
[4]Columbia University Vagelos College of Physicians and Surgeons, NEW YORK, NY
[5]Department of Pathology, Columbia University Irving Medical Center, New York, NY
[6]Department of Cancer Biology, University of Pennsylvania, Philadelphia, PA
[7]Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY

FLT3 is mutated through an internal tandem duplication (ITD) in 20-25% of acute myeloid leukemia (AML), driving aberrant STAT5/AKT signaling and leukemogenesis. Murine modelling of ITD showed marked myeloid progenitor expansion and hematopoietic stem cell (HSC) exhaustion in various co-mutant settings. Here we present a Stag2 $^{\Delta}$ Flt3 ^{ITD} model with stark reversal of these two aforementioned features. STAG2, a cohesin complex member, maintains the integrity of the 3D genome partitioning structure known as topologically structural domains. Loss of Stag2 impairs the access and engagement of key hematopoietic transcription factors such as PU.1 to their target genes.

sAML is diagnosed when patients present leukemia with known history of hematological malignancies or chemotherapy/radiotherapy treatment. Comparing to *de novo* AML, sAML often arises in older patients and harbors a poor prognosis with a 5-year overall survival rate of <30%. Advances in genomic studies found various epigenetic mutations, such as STAG2, are often associated with sAML and AML-myelodysplastic related changes (MRC) subtype. STAG2 mutations are found in 14-20% of sAML cases and is suggested to reside within a dominant clone during the pre-leukemia phase, MDS to sAML transformation, such as with FLT3 ^{ITD} acquisition and persists during remission.

To understand the mechanistic contribution of STAG2-cohesin loss with FLT3 ^{ITD}, we generated sequential mutagenesis murine models where the order of Stag2 and Flt3 ^{ITD} mutation is set as either ITD ^{1st} Stag2 ^{2nd} (*de novo* like) or Stag2 ^{1st}ITD ^{2nd} (sAML like) using tamoxifen-inducible Cre/Flpo recombinase or pIpC-inducible *Mx1*Cre. In the *de novo* like model, ITD is constitutively active then Stag2 is deleted when mice reach 6-8 weeks of age. Surprisingly, loss of Stag2 attenuates LSK to MP transition at 4 weeks post deletion, while MPP3 remains elevated, suggesting aberrant remodeling of myeloid differentiation. In the sAML like model, Stag2 is deleted via *Mx1*Cre and waited for 4 months to mimic the MDS phase, which is then followed by activation of ITD mutation via Flpo, which represents the MDS to sAML transformation. After activating both mutations, mice were followed for another 4 months before analyzing the hematopoietic stem and progenitor compartment.

In contrast to the *de novo* like model, sequential Stag2 ^{1st}ITD ^{2nd} preserves the HSC population defined by either immunophenotyping or transcriptome via scRNAseq (**Figure 1**). The mutant HSC is more quiescent but retains the capacity to reconstitute lethally irradiated recipients in the short term. Similar to the *de novo* like model, sequential Stag2 ^{1st}ITD ^{2nd} mice also exhibits a blocked myeloid differentiation. Comparing to ITD mutant, Stag2 ^{1st}ITD ^{2nd} LSK cells have decreased expression of *Socs2* and *Cish*. While functionally determining the role of mutant HSC, we are performing RNA-seq during at early timepoints post ITD activation to determine how preceding Stag2 mutation could have altered the stem cell fate decision. Targeted therapy with inhibitors of FLT3 have had an overall survival benefit in FLT3-mutant AML, though the magnitude of effect has been modest. STAG2 mutations are more likely to be identified in poor responders to FLT3 inhibition as both reported by us with Pexidartinib treatment, as well as in the setting of Crenolanib treatment where expansion of the STAG2-mutant clone was observed during treatment. Thus, remodeling of the chromatin landscape though altered CTCF binding or cohesin function might impact leukemia identity in FLT3-mutant AML. Our data highlights an important regulatory role of Stag2-cohesin in Flt3 ^{ITD} mediated leukemogenesis, while generating a model that mimics the genetic evolution of sAML. This model will not only shed light on the sAML pathogenesis but also with creates a pre-clinical testing platform with potential therapeutic relevance.

Disclosures Viny: *Arima Genomics:* Membership on an entity's Board of Directors or advisory committees.

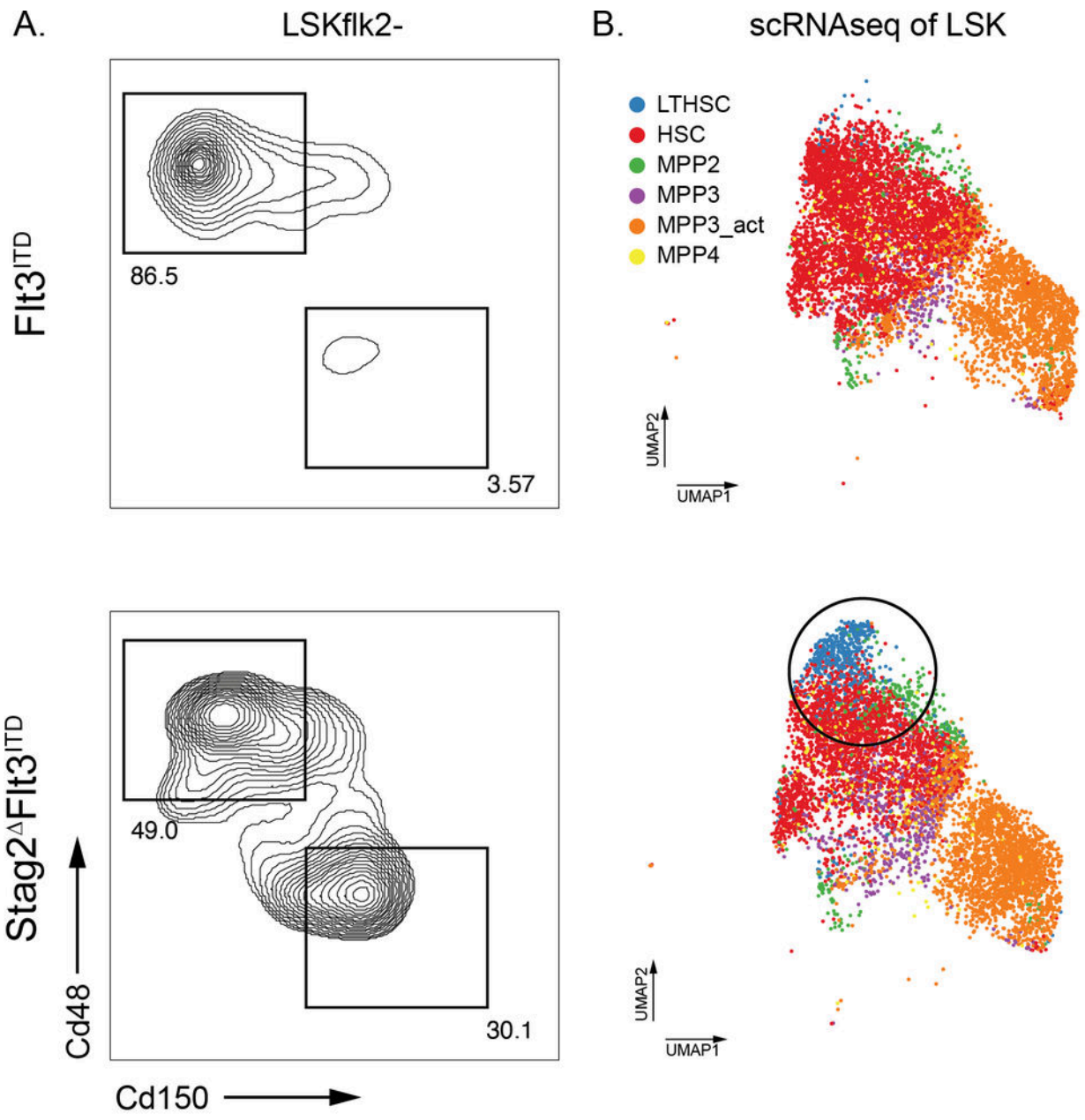

Figure 1 Sequential Stag2-Flt3^{ITD} mutation leads to preservation of HSC population. (A) Representative FACS plot of HSC or MPP3 population of Flt3^{ITD} and Stag2^{1st}Flt3^{ITD}. (B) scRNAseq of LSK population with LTHSC population showing with a circle in the Stag2^{1st}-Flt3^{ITD}.

Figure 1

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