Table of Contents

[redacted] - Credential Stag2-mediated chromatin looping in myelodysplastic syndrome 1
Research Proposal 3
Applicant Biosketch 6
Mentor Biosketch 10
Letter of support from mentor 15
Note from Shared Resource Director or COE 16

Application Summary

Competition Details

Competition Title:	2023 TAM Postdoctoral Fellows Pilot Award
Category:	Internal Funding Opportunity
Cycle:	2023
Submission Deadline:	12/4/2023 11:59 PM

Application Information

Submitted By:	[redacted]
Application ID:	[redacted]
Application Title:	Credential Stag2-mediated chromatin looping in myelodysplastic syndrome
Date Submitted:	12/4/2023 4:08 PM

Personal Details

Applicant First Name:	[redacted]
Applicant Last Name:	[redacted]
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Phone Number:	
School/Department:	[redacted]
Primary Appointment Title:	[redacted]
UNI:	[redacted]
Mentor's name with Rank:	[redacted]
Gender Identity:	[redacted]
Do you self-identify as an individual from underrepresented populations in the U.S. biomedical, clinical, behavioral and social sciences:	[redacted]
Do you self-identify as an individual from the following racial groups:	[redacted]
Do you self-identify as an individual from the following ethnic groups:	[redacted]
Do you self-identify as an individual with a disability:	[redacted]

Do you self-identify as an individual from a disadvantaged background: [redacted]

Application Details

Proposal Title

Credential Stag2-mediated chromatin looping in myelodysplastic syndrome

Proposal Abstract

Inside the nucleus of a cell, DNA is neatly organized to efficiently store and retrieve necessary information. During the process, genes are packed into different bags (domains) and sealed with a molecule shaped like zip ties. This molecule is cohesin. In addition to locking in particular regions of DNA, cohesin can bring the different parts of DNA into proximity and tie them together. This process leads to a change in the amount of gene information being activated. Blood stem cells use this process to selectively utilize certain genetic information to become red blood cells, white blood cells, or platelets. In blood cancer, however, this process is hijacked to stop the production of healthy blood cells, and the body's function is impacted. My proposal specifically looks at how a cohesin gene mutation called STAG2 is responsible for the abnormal genomic organization in a type of blood cancer, called myelodysplastic syndromes (MDS). The exact cause of MDS is not well understood. Plus, MDS tends to occur in older people who cannot tolerate the toxicity of current treatments well. Using a novel animal model developed in the lab, I will describe the STAG2-looping changes that are responsible for the hematopoietic transformation in MDS.

Title: Credential Stag2-mediated chromatin looping in myelodysplastic syndrome

Background: Myelodysplastic syndrome (MDS) is a pre-leukemic myeloid malignancy involving defective hematopoietic stem cells (HSC) and ineffective hematopoiesis. Ineffective hematopoiesis refers to failure in production of mature circulating blood cells necessary for homeostasis[1]. MDS usually presents in elderly populations with high comorbidity, which limits the therapeutic options. Mutations in genes involved in epigenetic regulatory processes are among the most common mutations in MDS and the premalignant state clonal hematopoiesis (CH)[2]. Genes involved in three-dimensional (3D) genome topology (i.e., the cohesin complex: STAG2, RAD21, SMC3 and the CCCTC-binding factor: CTCF) are recurrently mutated in myeloid malignancies, specifically high-risk MDS/secondary acute myeloid leukemia (20%)[3-5]. Secondary acute myeloid leukemia (sAML) refers to AML diagnosed in patients with known precedent myeloid malignancies such as MDS[6]. High-risk MDS/sAML has a dismal prognosis with high rates of induction failure[7,8]. Within the cohesin complex, STAG2 mutation, which leads to loss of function of STAG2 protein and loss of Stag2-cohesin associated loops, is linked with poor overall survival in high-risk MDS and sAML[6,9]. The role of STAG2 mutation in MDS development and progression remains unclear. However, we and others have started to uncover the effect of Stag2-loss towards HSC lineage commitment with animal models. In hematopoiesis, cohesin complex mutations result in alterations to chromatin structure and transcriptional regulation[10,11]. For example, Stag2 loss in hematopoietic tissue leads to decreased binding of master transcription factor Pu.1 on *Ebf1* gene, which specifies B lineage differentiation[11]. This results in decreased *Ebf1* expression and less mature B cell production. Given that *Stag2* mutation is linked with hematopoietic lineage commitment, it provides a compelling a compelling rationale to investigate how Stag2-specific and Stag2-independent chromatin loop formation in hematopoietic stem and progenitor cells (HSPC) contribute to development of ineffective hematopoiesis and MDS.

Significance: Chromatin looping is an emerging mechanism of leukemogenesis. In normal HSC, regulation of gene expression relies on coordination between cis-regulatory elements, transcription factors and 3D genome organization, including the cohesin complex. Cohesin complex mutations, specifically STAG2, result in alterations to chromatin structure and transcriptional regulation in cancer. *STAG2* is the most commonly mutated cohesin complex member in human cancer including and beyond myeloid malignancies, including Ewing's Sarcoma (40-60%)[12], bladder cancer (20-35%)[13,14], glioblastoma (4-5%)[12,15], and endometrial cancer (6-20%)[16]. So far, our conditional *Stag2* deletion murine model has provided great insights towards cohesin biology in the context of hematopoietic development. In this proposal, we will mechanistically characterize **a 'first-of-its-kind' reversible murine model that can delete and restore copy neutral *Stag2* expression**. Our novel murine model system uniquely positions us to determine how looping is altered and how Stag2 contributes to transformation across different lineages.

Application of HICCC Shared Resources: The proposed research will utilize high-throughput next-generation sequencing provided by the JP Sulzberger Columbia Genome Center. This includes bulk RNAseq, bulk ATACseq and Hi-C to profile gene expression, chromatin accessibility and chromatin looping changes in HSPC population when *Stag2* expression is lost and then after its restoration. I will conduct cell purification and construction of each genomic library before submitting to the Genome Center to sequence on the Illumina NovaSeq platform.

Hypothesis: Stag2-specific looping targets are relevant to normal HSPC differentiation and to HSPC dysfunction in myelodysplasia.

Aim: Aim 1: Determine the gene expression and chromatin accessibility alterations in Stag2-deleted and Stag2-restored HSPC. Approaches: We will collect lineage negative HSPCs from Stag2-deleted (refer to as Stag2^{GL} Dre) and Stag2-restored (refer to as Stag2^{GL} DreCre) at 16 weeks post transplantation. We will also collect control cells: WT Dre and WT DreCre. Cells will be processed for bulk RNAseq for gene expression and bulk ATACseq for chromatin accessibility. We will intersect the bulk RNAseq and ATACseq results to determine the regulatory relationship and identify most up/down-regulated hematopoietic target genes for looping analysis in aim 2.

Aim 2: Map the lineage specific 3D chromatin loop structure in Stag2-deleted and Stag2-restored HSPC. Approaches: We will collect lineage negative HSPCs as described in aim 1 and generated high resolution Hi-C datasets. Using established in house pipelines, we will compare loop alterations on the targeted genes from aim 1 between Stag2^{GL} DreCre vs Dre. Any

loop structure disappeared and then recovered during the Stag2 'off-on' will be deemed as Stag2-specific, whereas looping changes not associated with Stag2 deletion or recovery will be deemed as Stag2-independent.

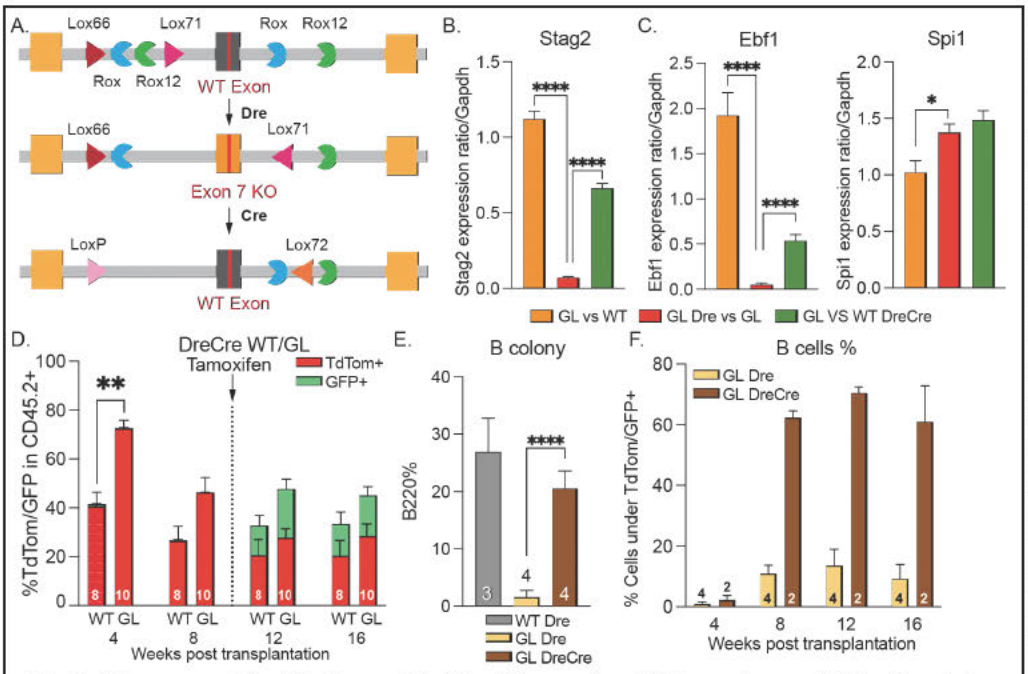

Fig 1. The reversible Stag2 model. The GL construct (A) can turn off *Stag2* and its associated *Ebf1* expression and then back on, without affecting much on PU.1 encoding gene *Spi1* (B-C). TdTom is detected in Stag2^{GL} Dre recipients' blood, while GFP is detected post tamoxifen treatment (D). Functionally, the reversibility is seen in B lineage regeneration *in vitro* and *in vivo* (E-F). Unpaired t-test.

Preliminary data: To manipulate *Stag2* expression, we construct a "on-off-on" dual recombinase Stag2 allele (Stag2^{GL}) using the GOLD-lox construct[17,18]. In this construct, we employed both Dre-Rox and Cre-Lox recombinases systems (**Fig 1A**). To identify 'Off' and 'On' population, the Stag2^{GL} strain is crossed with a Rox-flanked TdTom-Stop-Lox-flanked eGFP reporter (RLTG) and a tamoxifen inducible Cre recombinase (Ubc-CreER^{T2}) to generate *Ubc*-CreER^{T2} Stag2^{GL} RLTG. In this model, I can inactivate *Stag2* using Dre-rox (TdTom+), then re-express with Cre-lox system (GFP+) The RT-qPCR analysis showed Stag2^{GL} cells had significant reduction of *Stag2* mRNA level, which is then recovered after Cre activation (**Fig 1B**). Since Stag2 regulates *Ebf1* expression, I performed qPCR assay on *Ebf1* and *Spi1*, which encodes transcription factor PU.1 and is not directly associated with *Stag2* expression. The results showed that *Ebf1* expression declined then recovered following the *Stag2* manipulation, whereas *Spi1* level is largely unaffected (**Fig 1C**).

To initiate the *Stag2* 'Off' in hematopoietic tissue, I collected HSCs from *Ubc*-CreER^{T2} *Stag2*^{GL} RLTG mice and control (*Ubc*-CreER^{T2} RLTG) mice and performed Dre mRNA electroporation using an optimized protocol: the Dre electroporated HSCs are cultured for 4 days in StemSpan media with 10ng/mL rmSCF and 100ng/mL rmTPO prior to transplant into lethally irradiated recipients. Transplant recipients were followed for 16 weeks with half of the cohorts received tamoxifen treatment at 8 weeks post transplantation. TdTomato+ population can be detected in the Dre electroporated HSCs prior to transplantation and in the peripheral blood of recipients with little GFP level). Post tamoxifen treatment, a proportion of GFP+ population can be detected in the peripheral blood, suggesting successful *in vivo* Cre-mediated reversion (**Fig 1D**). Finally, I performed B methylcellulose assay and secondary transplantation to functional compare the B cell generating capacity in Stag2^{GL} Dre and Stag2^{GL} DreCre population. The *in vitro* and *in vivo* assay demonstrated that the DreCre population successfully regain the ability to produce mature B cells (B220+), indicating recuperating of Stag2-cohein mediated loops (**Fig 1E-F**). The preliminary data demonstrated that we have a validated reversible Stag2 model to mechanistically investigate the Stag2-specific and independent looping events in HSPC.

Conclusions: We have built and performed the initial validation on the feasibility of Stag2 reversible murine model. We demonstrated that the *Stag2* expression can be turned off and then restored *in vivo*. HSPCs with recovered Stag2 cohesin regain B lineage differentiation.

Future directions: With this model, we will provide the essential evidence needed to detail the dynamic role of cohesin-mediated chromatin structure throughout hematopoiesis and establish the role of Stag2 loss for MDS maintenance. I will describe gene expression changes and chromatin accessibility mostly affected by Stag2 loss and gain via bulk RNAseq and ATACseq. Then, I will describe the looping changes that is responsible for the transcription and chromatin alterations. I will validate the looping changes by comparing to known MDS/AML associated looping changes[5,10,11] prior to functional tests.

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BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: [redacted]

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: [redacted]

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
[redacted]				

A. Personal Statement

My long-term research interests focus on understanding chromatin organization alterations and epigenetic dysregulation in leukemia. Through a fundamental understanding of the mechanism of epigenetic drivers, I aim to leverage these insights towards disease monitoring and targeted for therapeutic intervention.

Prior to starting my research career, I undertook an internship at [redacted] as part of the Laboratory Medicine degree. During the internship, I observed how devastating cancer is to patients and their families, as well as how research benefits clinical care. This experience led to pursue a research direction that will have direct translational impact for leukemia patients.

During my Honors and PhD training, I developed a novel murine model of RNA splicing mutation for exploring its effect on hematopoiesis *in vivo*. During my graduate career, I gained extensive technical skills in murine modelling, disease characterization, genomic assays and CRISPR-Cas9 editing. I was first author of an RNA splicing mutation murine model review paper published in [redacted] For my PhD work, I have a first-author publication in [redacted] describing a CRISPR-Cas9 screen that explored new therapeutic targets and genetic vulnerability of splicing mutation. My third first-author publication involving the novel murine leukemia model of chronic myelomonocytic leukemia.

My academic training and research experience have provided me with solid foundation in myeloid malignancies, molecular biology, and cancer biology. For my postdoctoral training, with the goal of adding technical and conceptual cancer epigenomics to my skillsets, I have pursued my postdoctoral training in the [redacted] Lab. Dr. [redacted] is an exceptional pioneer in cohesin dysfunction in leukemia. With his postdoctoral training at [redacted] lab, he was the first to describe the cohesin abnormalities and 3D genome organization rearrangement in leukemia. Under his mentoring, I have begun to explore the mechanisms which cohesin factor loss facilitates leukemia initiation and maintenance. Here I have expanded upon my strengths in leukemia biology through the lens of cohesin biology and epigenetic alterations in leukemia cells. In addition, my career development will involve. Combined with a professional development plan that includes grant writing, public engagement, and

workshops, I will ensure a strong base for my future research career in cancer & chromatin biology as an independent principal investigator.

B. Positions and Honors

Position and Employment

2021 -
2020 - 2021 [redacted]

Other Experience and Professional Memberships

2017 - 2017
2017 - [redacted]

Honors

2023 [redacted]
2023
2022
2019
2017 - 2020
2016
2011 - 2013

Professional training

2022 [redacted]

C. Contributions to Science

1. The role of the cohesin complex in leukemia initiation and maintenance. In my current research, I have generated co-mutation murine models of Stag2 with Npm1c or Flt3-ITD mutation to determine the how Stag2-cohesin loss contributes to generation of acute leukemia (*de novo* and secondary). I characterized the phenotypic, transcriptomic and chromatin alterations post mutation acquisition, especially in the stem and progenitor compartment. I have discovered key changes in either myeloid-biased MPP compartment in Stag2Npm1c model or hematopoietic stem cell preservation in the Stag2Flt3ITD model. This ongoing research has been selected for various oral and poster presentations at top-tier hematological malignancy conferences.

Abstract: Oral presentations (Presenting author underlined)

[redacted]

Abstract: Poster presentations (Presenting author underlined)

[redacted]

2. Molecular pathology of RNA splicing mutation in leukemia. Using our novel *Srsf2* mutation model, I generated an *in vitro* CRISPR-Cas9 screen to determine the genetic vulnerability of RNA splicing mutation. I reported that *Srsf2*-mutant cells are sensitive to disruption of DNA repair and Cell cycle pathways. I then validated this finding by demonstrating the efficacy of CDK6 inhibitor, Palbociclib in targeting the splicing mutant cells. This paper provides additional insight into the genetic dependance and therapeutic options for splicing mutant leukemia.

Research papers

[redacted]

Abstract: Poster presentations (Presenting author underlined)

[redacted]

3. Synergy between cooperating mutation in chronic myelomonocytic leukemia. Using a double mutant murine model, I explored the mutational co-operativity of *Srsf2*, an RNA splicing factor and *Tet2*, a DNA de-methylation protein *in vivo*. I discovered that two mutations do cooperate and result in CMML after long latency. The CMML phenotype high resembles human disease with morphologically monocytic blasts and high monocytes identified through immunophenotyping. This work is the first model to describe a non-transplant murine model of chronic myelomonocytic leukemia.

Research papers

[redacted]

Abstract: Poster presentations (Presenting author underlined)

[redacted]

Complete List of Published Work:

[redacted]

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: [redacted]

[redacted] COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
[redacted]			

A. Personal Statement

My career goal is to better understand the functional role of chromatin structure in transcriptional regulation of both normal and malignant hematopoiesis. This work is focused primarily through the lens of cohesin complex alterations but likely represents a convergent mechanism of transformation with other epigenetic drivers in cancer, particularly acute myeloid leukemia. My independent laboratory program interrogates the effects of epigenetic disease alleles on 3-dimensional DNA structure in normal and malignant tissue. These structural aberrations will be leveraged for epigenetic reprogramming and synthetic lethal modifications. The work in my laboratory will extend the observed dynamic structural events that occur during hematopoietic lineage commitment and which are altered in leukemic transformation. Using mechanistic studies in mouse models and primary patient samples, my work will enhance our understanding of normal and malignant stem cell function and will aim identify a new class of targets for therapeutic intervention. Moreover, the implications of my work may extend beyond cancer and uncover previously unappreciated aspects of developmental biology as well as cellular reprogramming and elucidate how studies of high-resolution DNA topology can be used to study critical features of normal development and malignant transformation.

As a tenure-track assistant professor and junior physician scientist, I am fully committed to mentorship and trainee career development. I have been the recipient of outstanding mentorship and in addition to developing my scientific acumen, I have also enjoyed the benefits of learning to emulate the mentorship style of [redacted]. In fact, formative mentorship training was his guidance in taking on a young undergraduate student who had no lab experience. Under my mentorship, with [redacted] as an advisor, she has developed into a wonderful young physician scientist. She joined as my first technician in the lab and is now a 1st year medical student at [redacted] Medical School with a full scholarship. Although it has only been [redacted] years since my lab

opened at CUIMC, I am confident that I can train exceptional young scientists. I already have had success in facilitating the career goals of four other direct mentees. A former pediatrics resident with whom I investigated healthcare disparities in hematological malignancies is now faculty at CUIMC. Together we published our findings ([redacted]). One former technician is currently at Columbia University, one as a graduate student in the lab of [redacted] and another graduated from Columbia's Vagelos College of Physicians & Surgeons. One former international trainee that I directly mentored for 3 years has now gone on to become an international leader in COVID-19 at [redacted] Medical Center in [redacted] with several first author publications.

In addition to my academic history, I also am a survivor of [redacted]. The passion and dedication that I bring to the bench, influenced by the time I spent at bedside, give me a unique perspective that will undoubtedly increase the likelihood of my success.

Current Research Support I would like to highlight:

[redacted]

[redacted]

B. Position and Honors

Positions and Employment

[redacted]

[redacted]

Board Certifications

[redacted]

Other Experience and Professional Memberships

[redacted]

Honors

[redacted]

[redacted]

C. Contribution to Science

1. **Molecular Pathogenesis of Cohesin Mutant Leukemia.** Using primary patient samples, *in vitro* systems, and our novel conditional knockout mouse models of cohesin member loss of function we have described the frequency and clinical phenotype of cohesin mutations in myeloid malignancies. We reported in the largest patient cohort to date that cohesin mutations are mutually exclusive, co-associate with epigenetic disease alleles, and do not contribute to karyotypic abnormalities. Subsequent work in our mouse models of *Smc3* and *Stag2* losses of function illustrate enhanced self-renewal and impaired lineage determination and differentiation. My work identified that while both Stag1 and Stag2-cohesin can both support genome neighborhood integrity through insulation of topologically associating domains (TAD), only Stag2-cohesin can establish sub-TAD promoter-enhancer interactions essential for lineage priming. This function of cohesin is essential for pioneer function in a hematopoietic context, with the pioneer factor PU.1 insufficient for DNA accessibility in the absence of Stag2. Together this highlights key physiology of nucleosome remodeling as a function of cell fate commitment as requiring Stag2-cohesin for dynamic changes in DNA insulation and accessibility. This paper highlights my laboratory program's extension of hematopoietic functional characterization towards a refined assessment of higher order chromatin structure.

[redacted]

* Co-first author

2. Role of Mutations in Epigenetic Modifiers in Myeloid malignancies and clonal evolution: We have investigated the role of novel mutations in epigenetic modifiers, including in TET2, ASXL1, and IDH1/2, using epigenomic studies in patient samples and model systems and mechanistic studies, which have allowed us to delineate clonal hierarchy in acute leukemias that arise from antecedent clonal hematopoiesis, MDS, or MPN and characterize the mechanisms of transformation by these mutations in epigenetic modifiers.

[redacted]

[redacted]

3. Lymphoproliferation in Large Granular Lymphocyte Leukemia is semi-autonomous, driven by antigenic stimulation of MICA and has frequent co-occurrence with both hematopoietic and solid tumors. As a medical student at a large bone marrow failure referral center, we analyzed and annotated a large cohort of LGL leukemia patients. We characterized two major clinical findings: splenectomy is not an effective treatment strategy and there is a very high incidence of other malignant conditions that co-occur with LGL leukemia. To better understand the lymphoproliferative drive in this disease, using a bioinformatics approach with SNP arrays, we found that LGL patients have enrichment for SNP in linkage disequilibrium with the *MICA* gene. Sequencing revealed a frequent trinucleotide repeat leading to truncation of the cytoplasmic tail. This allele led to increase cell-surface expression and was targeted in an antigen-independent fashion from NKG2D expressing lymphocytes.

[redacted]

4. Genetic and non-genetic determinants of racial inequality in outcomes of hematological malignancies. During my personal experience as a [redacted] patient at age [redacted] I was frustrated with the lack of data in the adolescent and young adult (AYA) populations. This led me to find a clinical research mentor in Dr. [redacted] and the work that I led was subsequently published in an NIH monograph on AYA cancer. Subsequent work describing our findings led to a mentorship role for a Columbia University Irving Medical Center [redacted] fellow, [redacted]. Together, we leveraged the SEER database, identified key barriers to care for AYA patients with hematologic malignancies, and identified a distinct racial disparity in the AYA population. These findings were published with me as senior author.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

December 1st, 2023

To Trainee Associate Membership Program Postdoctoral Fellows Pilot committee:

I am delighted to provide my highest level of support for [redacted]'s application for the Trainee Associate Membership Program Postdoctoral Fellows Pilot Award titled '**Credential Stag2-mediated chromatin looping in myelodysplastic syndrome**' as her primary mentor. [redacted] is currently a [redacted] postdoctoral scientist in my laboratory in the [redacted], at Columbia University Irving Medical Center (CUIMC). [redacted] is a leading intellect in my developing laboratory and her intelligence, work ethic, and potential as a research scientist compare favorably to the best postdoctoral fellow, I worked with during my six years in [redacted] lab at [redacted]. She is an outstanding scientist and a superb candidate for support.

[redacted] has an outstanding track record that makes her well qualified to pursue her career goals in academic medicine and hematologic malignancies research. In the short time she has been in the [redacted] lab, she has already demonstrated the insight of a scientist well beyond that of a typical postdoctoral scientist at her level, as evident by the proposal submitted for this award. I am fully committed to providing her the mentorship and support needed to allow her to reach her full potential. With these goals in mind, we have identified several aspects of her training that are critical during the next year, including:

- Improve her technical repertoire: Although [redacted] is well trained in [redacted], she will need to acquire additional technical skills and to broaden her knowledge base during the next few years. This will include training in cancer epigenetics and genomics, bulk and single cell bioinformatics, and development/assessment of murine models. She has attended the [redacted] Course and will continue to gain further bioinformatic skills.
- Expand her knowledge base in hematologic malignancies and translational potential of her work: As a faculty member on the [redacted] service at CUIMC, I attend on the inpatient service 6 weeks per year. [redacted] has and will continue to join me on rounds to serve as a conceptual framework for the clinical relevance of her work and inform her hypothesis driven work.

In summary, [redacted] is a bright, passionate, and highly motivated postdoctoral scientist with unlimited potential. Her insight, knowledge, and technical skills are quite advanced for someone at her stage of training. I am deeply committed to providing her with the environment, mentorship, and support needed for her to achieve her stated career goal of becoming an independent cancer scientist working on the molecular mechanisms of hematopoietic malignancies. **I simply cannot think of someone more deserving of the TAM Postdoctoral Fellow Pilot Award.** [redacted] is an outstanding candidate for support, and I recommend her with utmost enthusiasm.

[redacted]

[redacted]

COLUMBIA UNIVERSITY
Department of Systems Biology

JP SULZBERGER COLUMBIA GENOME CENTER

November 27th, 2023

To Trainee Associate Membership Program Postdoctoral Pilot Award committee,

I am the Director of the Single Cell Analysis Core and Columbia Genome Center, located at the JP Sulzeberger Columbia Genome Center. [redacted] has consulted with me on her bulk RNAseq, ATACseq and Hi-C sequencing experiments proposed in this grant "**Credential Stag2-mediated chromatin looping in myelodysplastic syndrome**". I confirm that we will provide the library sequencing, and data preprocessing at the Columbia Genome Centre.

The Genome Center is part of the Genomics and High Throughput Screening Shared Resource and was launched in 2017 with generous support from the Columbia Precision Medicine Initiative, Irving Institute for Clinical and Translational Research, Herbert Irving Comprehensive Cancer Center, and Department of Medicine. We provide multiple services using the 10x Genomics Platform: 3' Single Cell Gene Expression, 5' Single Cell Gene Expression (plus V/D/J sequencing), Single Cell ATAC, and Single Cell Multiome ATAC + Gene Expression. We utilize the sequencing capabilities and high-performance computing (HPC) environment of the Sulzberger Columbia Genome Center. We have processed over 2500 single cell samples using the 10x platform since inception from over 80 different laboratories. The Genome Center currently hosts two NextSeq 500/550s, one MiSeq, one NovaSeq 6000, and one Element Aviti sequencer.

We are supported by multiple grants, including the P30 Cancer Center Support Grant. Cancer-related publications involving research performed by our core can be found online under NIH/NCI Cancer Center Support Grant P30CA013696. We are also supported by the National Center for Advancing Translational Sciences in the National Institutes of Health, through Grant Number UL1TR001873.

I look forward to working with her on this project.

Sincerely,

DocuSigned by:
Erin C. Bush
ECFA8A1ABD4149C...

Erin C. Bush, MA, MHA
Director, Single Cell Core
Director, Columbia Genome Center