AWARD NUMBER: W81XWH-20-1-0703

TITLE: **Investigating Mechanisms of Leukemic Self-Renewal in Acute Myeloid Leukemia**

PRINCIPAL INVESTIGATOR: Karina Barbosa Guerra

CONTRACTING ORGANIZATION: Sanford Burnham Prebys Medical Discovery Institute

REPORT DATE: NOVEMBER 2023

TYPE OF REPORT: Final Report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
NOVEMBER 2023	Final Technical Report	1AUG2020 - 31JUL2023

4. TITLE AND SUBTITLE

Investigating Mechanisms of Leukemic Self-Renewal in Acute Myeloid Leukemia

5a. CONTRACT NUMBER
W81XWH-20-1-0703

5b. GRANT NUMBER
W81XWH-20-1-0703

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Karina Barbosa Guerra

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release, Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Leukemia accounts for ~30% of pediatric cancer diagnoses. Our lab focuses on AML with rearrangements of the AF10 gene (AF10-R), a high-risk subset associated with treatment resistance and disease relapse. A hallmark of 70% of AML cases, including AF10-R, is the dysregulated expression of the HOXA gene cluster and its co-factor MEIS1 (HOX/MEIS). Functionally, HOX/MEIS activation is a critical node in leukemogenesis. It is well established that HOX/MEIS gene expression is sustained by epigenetic regulators that are coopted in leukemogenesis. To comprehensively characterize epigenetic regulators of HOX/MEIS genes, we conducted a phenotypic pooled CRISPR using a custom, high-density domain-focused CRISPR in a MEIS1-GFP leukemia cell line. Our screen uncovered several known and novel candidate regulators of HOX/MEIS expression.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
U	U	U	UU	19	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION:

Leukemia accounts for ~30% of pediatric cancer diagnoses. The research project focuses on acute myeloid leukemia (AML) with rearrangements of the AF10 gene (AF10-R), a high-risk subset associated with treatment resistance and disease relapse. A hallmark of 70% of AML cases, including AF10-R, is the dysregulated expression of the *HOXA* gene cluster and its co-factor *MEIS1* (HOX/MEIS). Functionally, *HOX/MEIS* activation is a critical node in leukemogenesis. The purpose of this project is to evaluate two top candidate HOX/MEIS regulators in CALM-AF10 driven-leukemogenesis: the KAT7 complex and Casein Kinase enzymes. The proposed studies will provide insights into mechanisms of leukemogenesis and self-renewal in AML, which may also impact therapeutic development in additional subsets of myeloid and lymphoid malignancies.

2. KEYWORDS:

Acute myeloid leukemia, self-renewal, blood cancer, pediatric cancer, leukemia stem cells, cancer stem cells, AML, AF10-Rearranged, CALM-AF10

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Our hypothesis, based on evidence from previous studies (Deshpande et al. 2014; Rau et al. 2016; Kuhn et al. 2016, 2015; Van Vlierberghe et al. 2008), is that aberrant activation of *HOX/MEIS* genes can be reversed by targeting epigenetic regulators important for sustaining their expression. Our objective is to validate the top two candidate HOX/MEIS regulators we found in CALM-AF10 driven-leukemogenesis: the KAT7 complex and Casein Kinase 2. *HOX/MEIS* genes are causal in leukemogenesis and self-renewal in the hematopoietic system. Therefore, our findings may have an impact in a broader range of myeloid and lymphoid malignancies. The table below lists the major goals of the project for the reporting period:

Specific Aim 1. Define mechanistically how the KAT7/JADE3 regulates HOX/MEIS expression.	**Months/ %Completion**
Task 1. Assessment of the KAT7 complex as a pan-regulator of HOX/MEIS genes.	
Subtask 1. Effect of KAT7 and JADE3 loss on HOX/MEIS expression and leukemogenesis in vitro.	1-9
Subtask 2. In vivo effect of Kat7 or Jade3 deletion on leukemia maintenance.	3-12
Subtask 3. Testing self-renewal by in vivo stem cell limiting dilution.	6-18
Milestone(s) Achieved: Identified KAT7/JADE3-mediated regulation of HOX/MEIS gene expression in AML. Evaluated the genetic dependency of CALM-AF10 AMLs on JADE3 and KAT7 *in vitro* and *in vivo.*	18

Local IACUC Approval	Completed 3/23/20
Task 2. Evaluation of the enzymatic activity of KAT7 and the reader activity of JADE3 for HOX/MEIS expression. We will perform rescue experiments to elucidate which activities of the KAT7 complex are involved in HOX/MEIS regulation and we will functionally corroborate results.	
Subtask 1. Identify KAT7/JADE3 activities involved in HOX/MEIS regulation.	12-24
Subtask 2. Functional validation of enzymatic activities of JADE3 and KAT7 in HOX/MEIS regulation.	12-18
Milestone(s) achieved: identified a functional role for the enzymatic regulation of *HOX/MEIS* genes by KAT7/JADE3.	24
Task 3. Evaluation of the role of KAT7/JADE3 regulation of epigenomic lesions at the HOX/MEIS loci.	
Subtask 1. Mapping of KAT7 acetylation mark deposition.	12-18
Subtask 2. Determining JADE3-dependent localization of KAT7 to the HOX/MEIS locus.	12-18
Milestone(s) achieved: correlative evidence that JADE3-mediated KAT7 acetylation at *HOX/MEIS* loci is required for sustaining their expression in CALM-AF10 AML.	24
Specific Aim 2: Investigate the regulation of HOX/MEIS genes by Casein Kinases (CKs) in AML cells.	
Task 1. Evaluation of CK regulation of HOX/MEIS genes in KMT2A-germline AML.	
Subtask 1. Effect of CSNK2A and CSNK2B loss on HOX/MEIS expression and leukemogenesis in vitro.	1-9
Task 2. Investigation of the mechanistic basis of CK-mediated HOX MEIS regulation in CALM-AF10 AML. We will address CK regulation of HOX/MEIS via perturbation of KMT2A stability.	
Subtask 1. Test CK-mediated KMT2A stability in CALM-AF10 AML.	12-18
Subtask 2. Evaluate the role for KMT2A stability in sustaining HOX/MEIS gene expression in KMT2A-germline AMLs.	18-24
Task 3. Evaluation of CK pharmacologic inhibition in CALM-AF10 driven leukemogenesis. We will address a rationale for targeting CKs in KMT2A germline AML, whereby KMT2A stabilization downregulates HOX/MEIS genes	
Subtask 1. Assess whether CK pharmacological treatment reverses HOX/MEIS expression and leukemogenesis CALM-AF10 AML.	
Milestone(s) Achieved: potential therapeutic rationale for targeting CKs in KMT2A germline AML.	24
IACUC Approval	Completed 3/23/21

What was accomplished under these goals?

Specific Aim 1. Define mechanistically how the KAT7/JADE3 regulates HOX/MEIS expression.

Task 1. ***Assessment of the KAT7 complex as a pan-regulator of HOX/MEIS genes.*** We will functionally evaluate whether there is a causal relationship between loss of KAT7 and HOX/MEIS expression in the context of leukemia disease self-renewal, initiation, and maintenance, in both *in vivo* and *in vitro* settings.

Subtask 1. Effect of KAT7 and JADE3 loss on HOX/MEIS expression and leukemogenesis in vitro.

In the previous reporting period, we described the recent literature regarding the roles of KAT7 and JADE3 in AML and the significant overlap with our statement of work items in Aim 1. We thus considered the gaps in knowledge regarding the redundancy of H3K4me3-reading pathways and focused on SGF29, a validated hit from our Epigenetics CRISPR screen.

SGF29 is a Tudor-domain containing H3K4me3 reader protein. In the previous reporting period, we showed that it is required for the proliferation and cell cycle progression of AML cells. In addition, our results showed that SGF29 is a transcriptional regulator of AML self-renewal-associated genes and differentiation in mouse and human AML cells.

In this reporting period, we completed this Subtask by performing staining assays to assess apoptosis upon loss of SGF29. In MOLM13 cells, AnnexinV staining showed a small but significant change in the percentage of AnnexinV +ve cells upon SGF29 knockout, compared to non-targeting control (NTC; Fig. 1A). We also observed an increase in apoptosis by immunoblotting for cleaved PARP in murine MLL-AF9-transformed AML cells (Fig. 1B).

Subtask 2. In vivo effect of Kat7 or Jade3 deletion on leukemia maintenance.

The experiments in this Subtask have been completed and described in the last reporting period.

Subtask 3. Testing self-renewal by in vivo stem cell limiting dilution

Please see note in Aim 1, Task 1, Subtask 1. Self-renewal effects of Hbo1/Kat7 loss on stem cell self-renewal using transgenic mice have been recently reported (Yang et al. 2022; Najm and van Galen 2022).

We are performing the *in vivo* limiting dilution experiments to assess the frequency of leukemia stem cells upon *Sgf29*-deletion in murine AML. We anticipate including these results in our peer-reviewed manuscript, which is currently under review.

Task 2. Evaluation of the enzymatic activity of KAT7 and the reader activity of JADE3 for HOX/MEIS expression. We will perform rescue experiments to elucidate which activities of the KAT7 complex are involved in HOX/MEIS regulation and we will functionally corroborate the results.

Subtask 1. Identify KAT7/JADE3 activities involved in HOX/MEIS regulation.

The experiments in this Subtask have been completed and described in the last reporting period.

Subtask 2. Functional validation of enzymatic activities of JADE3 and KAT7 in HOX/MEIS regulation.

The experiments in this Subtask have been completed and described in the last reporting period.

Task 3. Evaluation of the role of KAT7/JADE3 regulation of epigenomic lesions at the HOX/MEIS loci. If our studies show that the acetyltransferase activity of KAT7 is important for HOX/MEIS expression, we will determine which of the acetylation marks deposited by this enzyme play a role in this process.

Subtask 1. Mapping of KAT7 acetylation mark deposition.

In the previous reporting period, we showed that loss of SGF29 results in KAT2A eviction to the cytoplasm. In addition, we performed ChIP-qPCR for H3K9ac, the major histone modification deposited by the SAGA complex.

We now finished this Subtask, having completed ChIP-sequencing experiments for H3K9 acetylation where we found that SGF29 deletion led to significant changes in this histone mark. We observed 10,834 peaks with reduced acetylation and 3,119 peaks showing increased acetylation in SGF29 knockout compared to NTC-transduced U937 cells (Figs. 2A-C). There was also a pronounced decrease in acetylation levels at the promoter of several AML oncogenes (Fig. 2B), including the proto-oncogene MYC. These results point to a role for SGF29 in the recruitment of the SAGA complex and its downstream transcriptional activation via H3K9ac deposition in key AML oncogene loci. A working model for our understanding of the mechanism by which SGF29 regulates leukemogenic transcription in AML is shown in Figure 3.

Subtask 2. Determining JADE3-dependent localization of KAT7 to the HOX/MEIS locus.

The experiments in this Subtask have been completed for SGF29 and KAT2A, which follow a similar mechanism of H3K4me3-reading and acetylation recruitment activity, also found in the JADE3-KAT7 interaction. The results are described in the last reporting period (Chromatin enrichment for proteomics) and in Specific Aim 1, Task 3, Subtask 1 of this reporting period (ChIP-sequencing for H3K9ac).

Specific Aim 2: Investigate the regulation of HOX/MEIS genes by Casein Kinases (CKs) in AML cells.

Task 1. ***Evaluation of CK regulation of HOX/MEIS genes in KMT2A-germline AML.*** We will assess whether CK modulates HOX/MEIS expression by regulation of wild-type KMT2A in KMT2A-germline cells and address the functional impact.

Subtask 1. Effect of CSNK2A and CSNK2B loss on HOX/MEIS expression and leukemogenesis in vitro.

The experiments in this Subtask have been completed and described in the last reporting period.

Task 2. Investigation of the mechanistic basis of CK-mediated HOX MEIS regulation in CALM-AF10 AML. We will address CK regulation of HOX/MEIS via perturbation of KMT2A stability.

Subtask 1. Test CK-mediated KMT2A stability in CALM-AF10 AML.

The experiments in this Subtask have been completed and described in the last reporting period.

Subtask 2. Evaluate the role for KMT2A stability in sustaining HOX/MEIS gene expression in KMT2A-germline AMLs.

The experiments in this Subtask have been completed and described in the last reporting period.

Task 3. Evaluation of CK pharmacologic inhibition in CALM-AF10-driven leukemogenesis

Subtask 1. Assess whether CK pharmacological treatment reverses HOX/MEIS expression and leukemogenesis CALM-AF10 AML.

In the previous reporting period, we described a potential role for the SUV39H1 and SETDB1 histone methyltransferases in a CK2-dependent inclusion of H3K9me3 repressive marks to HOX/MEIS loci. We performed additional CRISPR resistance screens with the whole-genome pooled Brunello library (Sanson et al. 2018). Our screen results agree with our previously reported chromatin enrichment for proteomics (ChEP) observations upon CSNK2A1-knockout, whereby loss

of CK2 led to an increase in SUV39H1 and SETBD1 in the chromatin fraction, compared to NTC targeting in U937 cells. Gene ontology enrichment analysis in our CRISPR screen showed that terms related to H3K9me3 are enriched in on-pathway and resistor interactions (mid-left and top-center sections of the 9-square plot in Figs. 3A-B). This observation suggests that CK2 is antagonized by H3K9me3, as we previously hypothesized. Interestingly, HOX gene activation and acetylation terms are enriched in the bottom-center section, consistent with a synthetic lethal interaction for gene-activating acetylation and CK2. These results may point to the dynamic regulation of H3K9 acetylation and methylation by CK2, a hypothesis which merits further study, beyond the scope of this Award. Our working model for the mechanism by which CK2 regulates HOX/MEIS gene expression in AML is shown in Figure 5.

Figures

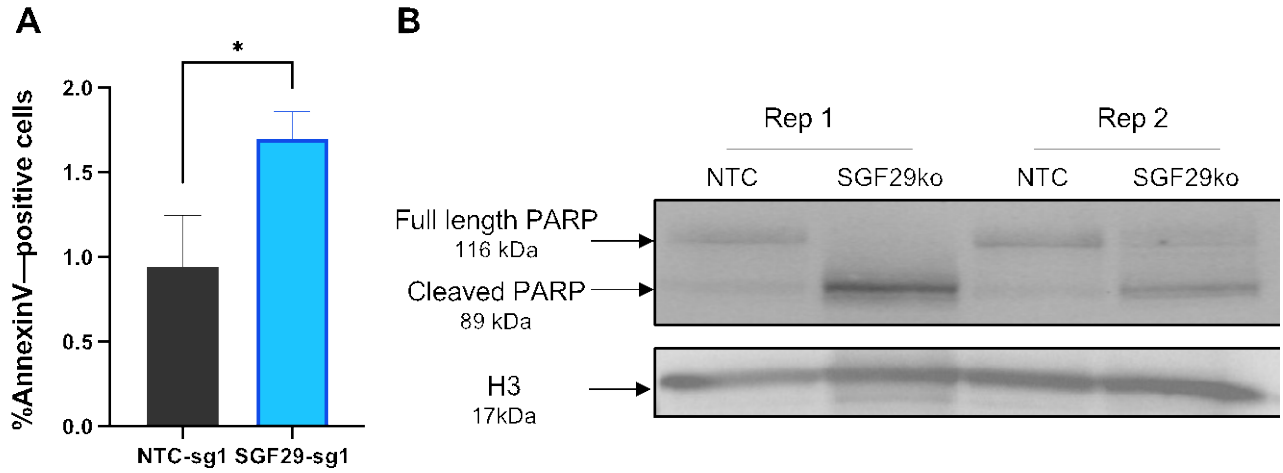

Figure 1. Effect of SGF29 deletion on apoptosis in human and murine AML cells. (A) AnnexinV-staining in MOLM13 cells transduced with NTC or SGF29 sgRNAs (n=3, *p<0.05). (B): Immunoblots for PARP protein cleavage in MLL-AF9 murine leukemia cells expressing NTC- or Sgf29- targeting sgRNAs. Two representative replicates are shown.

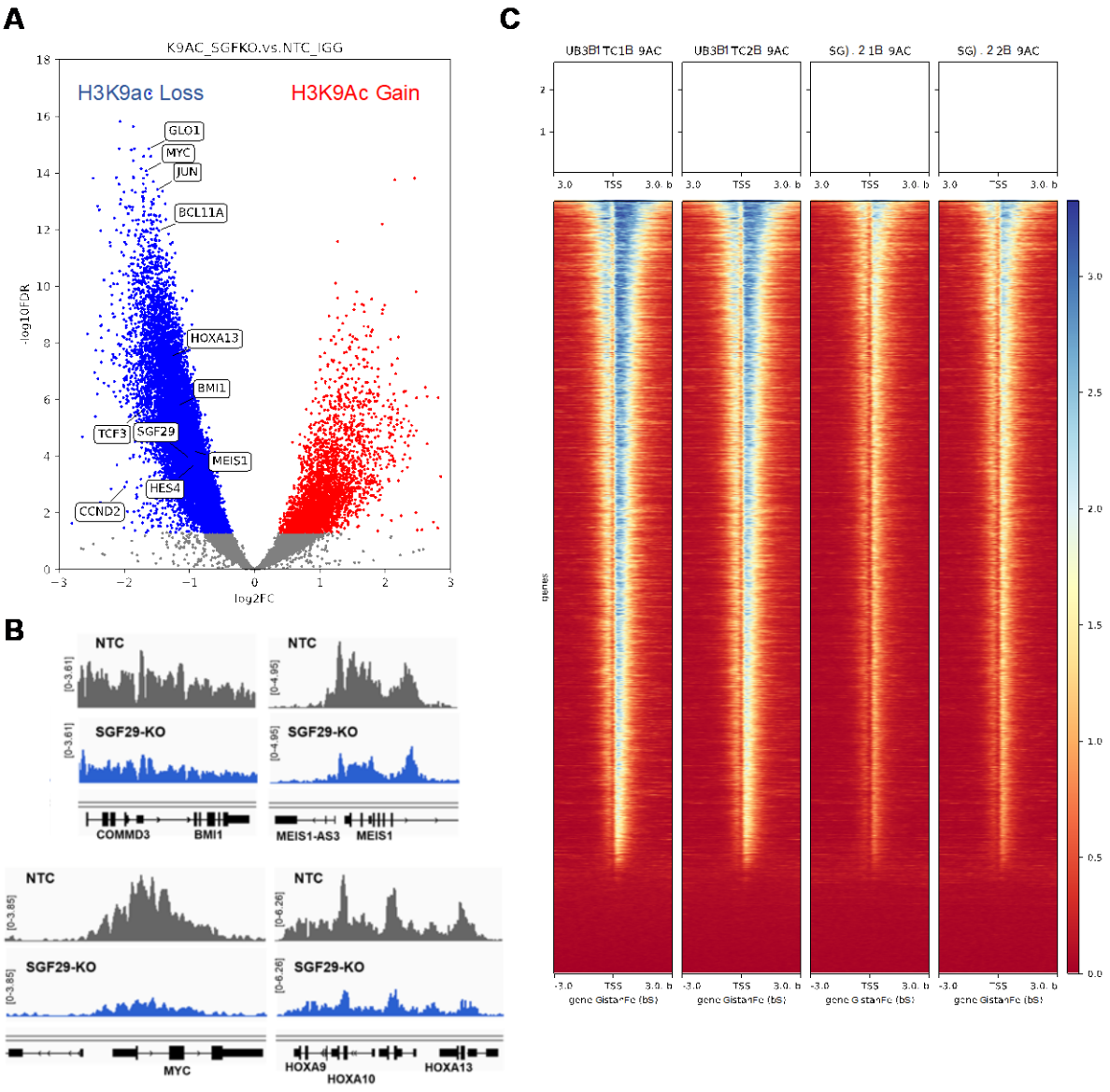

Figure 2. Effect of SGF29 deletion on H3K9 acetylation and antileukemia activity in human AML cells. (A) Volcano plot indicating changes in H3K9 acetylation peaks in U937 cells upon SGF29 deletion. Dots represent loci with significantly decreased (blue), increased (red) or unchanged (grey) acetylation peaks. Key SGF29-dependent AML oncogenes are labeled and (B) IGV tracks are shown for a subset of loci. (C) Heatmaps showing histone H3K9 acetylation (H3K9Ac) peaks centered around transcription start sites (TSS) in UB3 cells transduced with non-targeting control (NTC) or SGF29 targeting sgRNA (n=2).

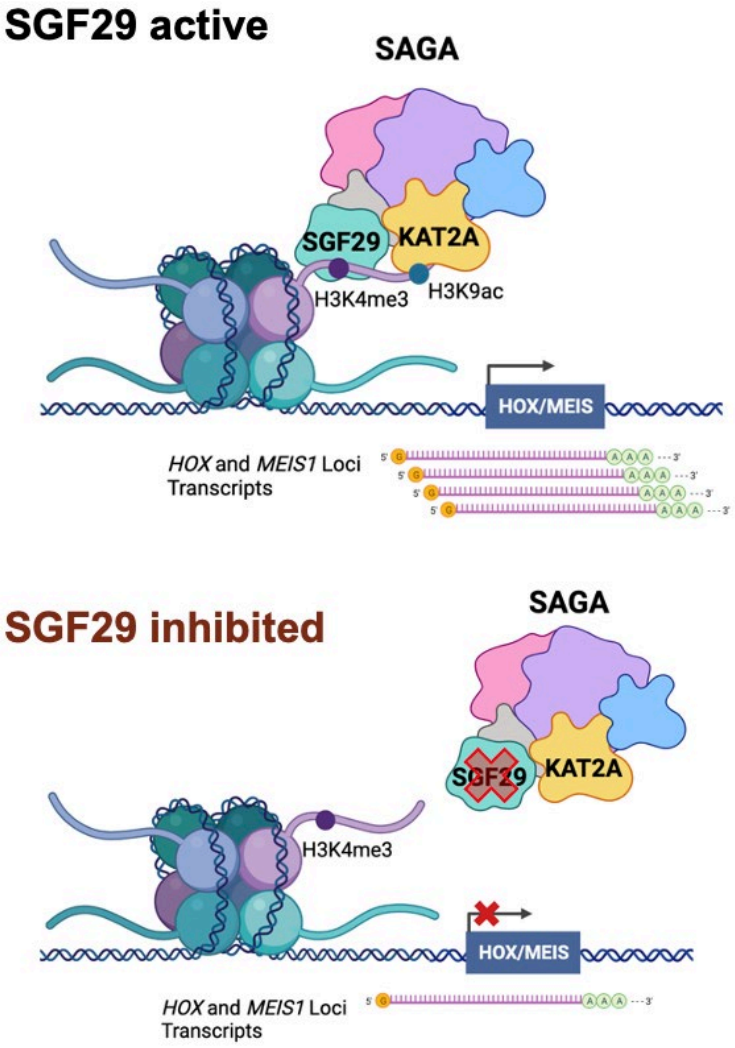

Figure 3. Working model for the transcriptional control of SGF29 leukemogenesis in AML.

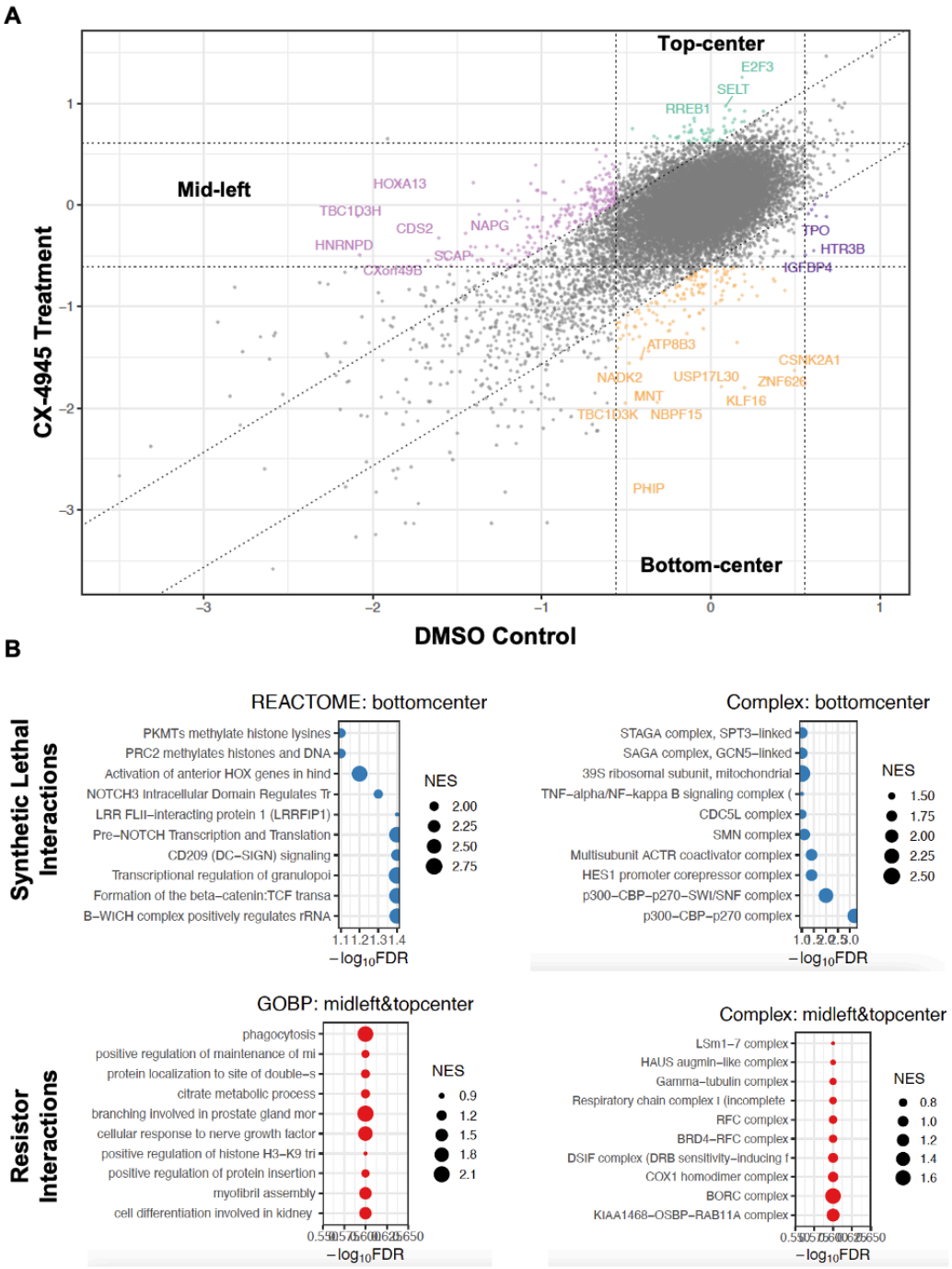

Figure 4. Whole Genome CRISPR resistance screen to the CX-4945 CK2 inhibitor in AML. (A) A 9-square plot of Normalized Beta scores (computed with MAGeCK-VISPR) for treatment of Brunello library-expressing U937 cells with CX-4945 vs DMSO (n=2 per arm). (B) Gene ontology enrichment terms for gene categories indicated in the 9-square plot.

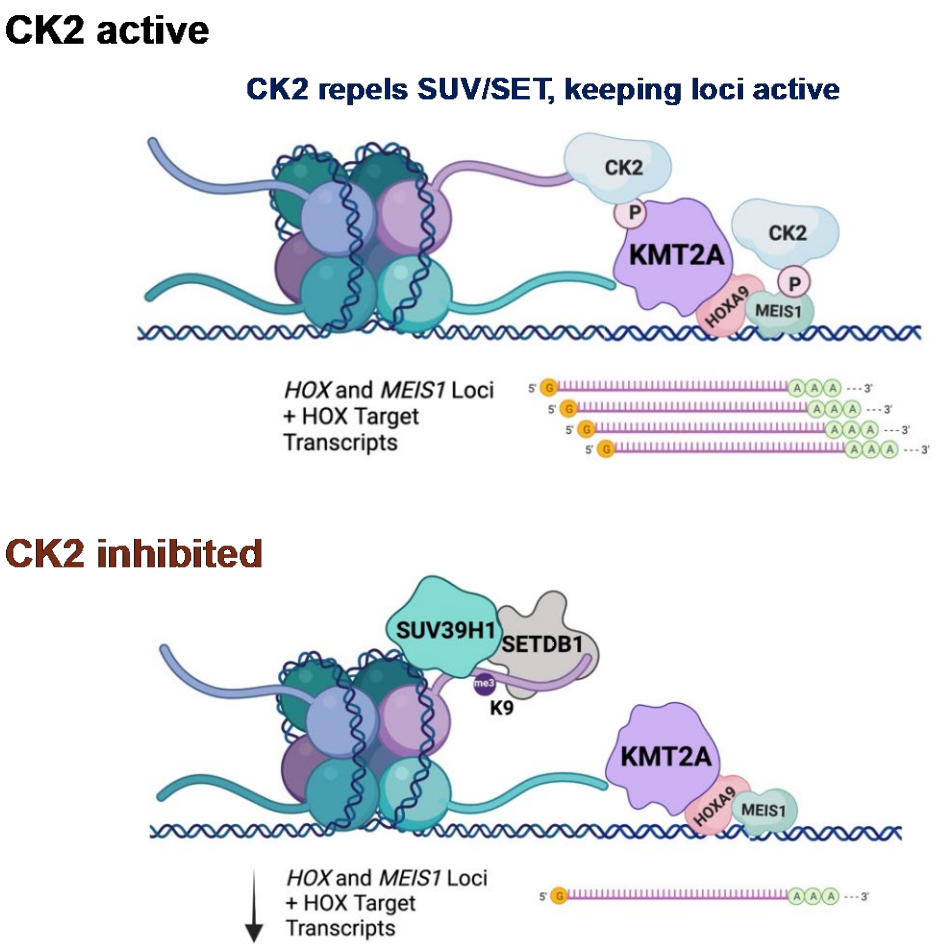

Figure 5. Working model for the control of HOX/MEIS expression by CK2 in AML.

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Deshpande, Aniruddha J., Anagha Deshpande, Amit U. Sinha, Liying Chen, Jenny Chang, Ali Cihan, Maurizio Fazio, et al. 2014. "AF10 Regulates Progressive H3K79 Methylation and HOX Gene Expression in Diverse AML Subtypes." *Cancer Cell*. https://doi.org/10.1016/j.ccell.2014.10.009.

Kuhn, M. W., M. J. Hadler, S. R. Daigle, R. P. Koche, A. V. Krivtsov, E. J. Olhava, M. A. Caligiuri, et al. 2015. "MLL Partial Tandem Duplication Leukemia Cells Are Sensitive to Small Molecule DOT1L Inhibition." In *Haematologica*, 100:e190-3. Italy.

Kuhn, M. W., E. Song, Z. Feng, A. Sinha, C. W. Chen, A. J. Deshpande, M. Cusan, et al. 2016. "Targeting Chromatin Regulators Inhibits Leukemogenic Gene Expression in NPM1 Mutant Leukemia." *Cancer Discovery* 6 (10): 1166–81.

Najm, Fadi J., and Peter van Galen. 2022. "Renewing Your HBO1 Subscription." *Blood*. American Society of Hematology.

Rau, Rachel E., Benjamin A. Rodriguez, Min Luo, Mira Jeong, Allison Rosen, Jason H. Rogers, Carly T. Campbell, et al. 2016. "DOT1L as a Therapeutic Target for the Treatment of DNMT3A-Mutant Acute Myeloid Leukemia." *Blood* 128 (7): 971–81.

Sanson, Kendall R., Ruth E. Hanna, Mudra Hegde, Katherine F. Donovan, Christine Strand, Meagan E. Sullender, Emma W. Vaimberg, et al. 2018. "Optimized Libraries for CRISPR-Cas9 Genetic Screens with Multiple Modalities." *Nature Communications* 9 (1): 1–15.

Van Vlierberghe, Pieter, Martine van Grotel, Joëlle Tchinda, Charles Lee, H. Berna Beverloo, Peter J. van der Spek, Andrew Stubbs, et al. 2008. "The Recurrent SET-NUP214 Fusion as a New HOXA Activation Mechanism in Pediatric T-Cell Acute Lymphoblastic Leukemia." *Blood* 111 (9): 4668–80.

Yang, Yuqing, Andrew J. Kueh, Zoe L. Grant, Waruni Abeysekera, Alexandra L. Garnham, Stephen Wilcox, Craig D. Hyland, et al. 2022. "The Histone Lysine Acetyltransferase HBO1 (KAT7) Regulates Hematopoietic Stem Cell Quiescence and Self-Renewal." *Blood* 139 (6): 845–58.

What opportunities for training and professional development has the project provided?

The PI attended and presented their work at the Conferences outlined in section 6.: Products.

How were the results disseminated to communities of interest?

The PI took part in two community Open House events hosted by the Sanford Burnham Prebys NCI Designated Cancer Center during the reporting period. The techniques, conceptual approaches, and/or results of this project have served to illustrate the impact of biomedical science in solving health challenges.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Acute Myeloid Leukemia (AML) is an aggressive blood cancer. While the disease prognosis and survival have improved over the past decades and there are encouraging recent treatment approvals, many patients still relapse or do not respond to therapy. Our research builds on the understanding of the molecular mechanisms that sustain leukemia cells. We focused on the "stemness" gene programs – the activation of genes that render leukemia cells virtually immortal, with an indefinite self-renewal ability. We conducted experiments to identify, validate and characterize a number of genes critically involved in the stemness pathways of leukemia cells. Importantly, our findings may inform current clinical trials related to these pathways, such as those for Menin inhibitor drugs. Our studies also propose that the genes we identified: SGF29, CSNK2A1, KAT7, and JADE3 could serve to repurpose existing drugs or guide the development of new ones to treat AML and potentially other cancers.

What was the impact on other disciplines?
Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

A no-cost extension was requested and subsequently approved to complete the mouse experiments for testing the effects of in vivo stem cell self-renewal upon loss of Jade3 and Kat7, outlined in Aim 1 of the proposal and the oral CX-4945 in vivo experiments outlined in Aim 2. General delays in

reagent and personnel availability due to the Coronavirus pandemic affected the completion of the tasks outlined.

Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to Report.

Changes that had a significant impact on expenditures
Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to Report.

Significant changes in use or care of human subjects
Nothing to Report.

Significant changes in use or care of vertebrate animals
Nothing to Report.

Significant changes in use of biohazards and/or select agents
Nothing to Report.

6. PRODUCTS:

- **Journal publications.**

  Barbosa, K. & Deshpande, A. J. (2023). Therapeutic Targeting of Leukemia Stem Cells in Acute Myeloid Leukemia*. Manuscript under review.*

  Barbosa, K.*, Deshpande, A.*, Xiang, P., Chen, B. R., Brown, A., Robertson, N., Schischlik, F., Lei, X., Sun, Y., Brown, A., Doench, J. G., Humphries, R. K., Ruppin, E., Shendure, J., Mali, P., Adams, P. D., & Deshpande, A. J. (2022). High-Density Domain-Focused CRISPR Screens Reveal Novel Epigenetic Regulators of HOX/MEIS Activation in Acute Myeloid Leukemia. *Biorxiv preprint manuscript available and currently under review*.

  Books or other non-periodical, one-time publications.
  Nothing to Report.

Other publications, conference papers and presentations.

Barbosa, K. Transcriptional Control of Leukemogenesis by the Chromatin Reader SGF29. Oral presentation at: Janssen Mini Symposium with the Sanford Burnham Prebys Graduate School.; April 19th, 2023. La Jolla, CA, United States.

Barbosa, K. High-density CRISPR screens reveal chromatin regulation mechanisms of stemness networks in acute myeloid leukemia. Poster and Oral presentations at: The California Institute for Regenerative Medicine Sanford Stem Cell Symposium; October 20th, 2022; La Jolla, CA, United States.

Barbosa, K. Identifying Novel Epigenetic Regulators of The HOX/MEIS Gene Cluster in Acute Myeloid Leukemia. Poster and Oral presentations at: The American Association for Cancer Research Special Conference on Cancer Epigenomics; October 7th-8th, 2022; Washington, DC, United States.

Barbosa, K. The Epigenetic Regulator Landscape of Stemness Networks in AML. Poster presented at: The International Society for Experimental Hematology Annual Meeting 2022; September 3rd, 2022; Virtual presentation.

- **Website(s) or other Internet site(s)**
  Nothing to Report.

- **Technologies or techniques**
  Nothing to Report.

- **Inventions, patent applications, and/or licenses**
  Nothing to Report.

- **Other Products**
  Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Karina Barbosa Guerra

Project Role: Graduate Student (until 12/04/2022), Postdoctoral Associate (since 12/05/2022)
Researcher Identifier (e.g. ORCID ID): 0000-0002-6233-3332
Nearest person month worked: 12
Contribution to Project: Ms. Barbosa Guerra is responsible for the experimental design and performance of all experimental procedures in this project. She leads all communication endeavors related to the project, under the mentorship of Drs. Deshpande and Reya.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to Report.

What other organizations were involved as partners?
Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

Award Chart submitted as an appendix to the Annual Report.

COLLABORATIVE AWARDS:
Nothing to Report.

QUAD CHARTS:
Nothing to Report.

9. APPENDICES:

Award Chart.

W81XWH-20-1-0703: Investigating Mechanisms of Leukemic Self-Renewal in Acute Myeloid Leukemia

PI: Karina Barbosa Guerra, Sanford Burnham Prebys Medical Research Institution, California

Budget: $273,279.00

Topic Area: Cancer Research Program **Mechanism:** FY19, PRCRP, Horizon Award

Research Area(s): Blood cancers, Cancer in children, adolescents and young adults **Award Status:** 08/01/2022-03/15/2023

Study Goals:

The purpose of this project is to evaluate two top candidate HOX/MEIS regulators in CALM-AF10 driven-leukemogenesis: the KAT7 complex and Casein Kinase (CKs) enzymes. The proposed studies will provide insights into mechanisms of leukemogenesis and self-renewal in AML, which may also impact therapeutic development in additional subsets of myeloid and lymphoid malignancies.

Specific Aims:

Specific Aims: Aim 1: Investigate the function of the KAT7 complex in CALM-AF10 leukemia.
Aim 2: Investigate the regulation of *HOX/MEIS* genes by casein kinases (CKs) in AML cells.

Key Accomplishments and Outcomes:

a) **Loss of SGF29 results in the SAGA complex member KAT2A eviction from the nucleus and the subsequent loss of H3K9ac in AML oncogene loci.**
b) **CK2 antagonizes H3K9 repressive methyltransferases to maintain AML oncogene activation.**

Publications.

Barbosa, K. & Deshpande, A. J. (2023). Therapeutic Targeting of Leukemia Stem Cells in Acute Myeloid Leukemia. *Manuscript under review*.
Barbosa, K.*, et al. (2022). High-Density Domain-Focused CRISPR Screens Reveal Novel Epigenetic Regulators of HOX/MEIS Activation in Acute Myeloid Leukemia. *Biorxiv preprint manuscript available and currently under review*.

Patents: none to date
Funding Obtained: