AWARD NUMBER: W81XWH-21-1-0283

TITLE: Interrogation of Requisite Niche Factors for Leukemia Cell Survival at Single-Cell Resolution

PRINCIPAL INVESTIGATOR: Hojun Li, MD, PhD

CONTRACTING ORGANIZATION: Massachusetts Institute of Technology, Cambridge, MA

REPORT DATE: July 2022

TYPE OF REPORT: Annual

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
July 2022	Annual	01Jun2021-31May2022

4. TITLE AND SUBTITLE

Interrogation of Requisite Niche Factors for Leukemia Cell Survival at Single-Cell Resolution

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-21-1-0283

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Hojun Li, MD, PhD

E-Mail: hojunli@mit.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

Koch Institute for Integrative
Cancer Research
Massachusetts Institute of
Technology
77 Massachusetts Avenue
Cambridge, MA 02139

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

The goal of this project is to identify the signals within the bone marrow microenvironment that allow acute myeloid leukemia (AML) cells to survive and proliferate. By identifying these signals, we can improve cell culture systems for maintaining AML cells outside of the body to study their true biology. We can also identify specific signals that are uniquely required by AML cells inside the bone marrow microenvironment, but are not needed by healthy cells in the rest of the body, and target these signals for AML-specific treatment with potentially minimal effect on normal, healthy cells. We study this question primarily in a well known mouse model of AML, with confirmation of our mouse model findings in human AML biopsy samples.

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
Unclassified	Unclassified	Unclassified	Unclassified	10	19b. TELEPHONE NUMBER *(include area code)*

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1. **INTRODUCTION:**

The goal of this project is to identify the signals within the bone marrow microenvironment that allow acute myeloid leukemia (AML) cells to survive and proliferate. By identifying these signals, we can improve cell culture systems for maintaining AML cells outside of the body to study their true biology. We can also identify specific signals that are uniquely required by AML cells inside the bone marrow microenvironment, but are not needed by healthy cells in the rest of the body, and target these signals for AML-specific treatment with potentially minimal effect on normal, healthy cells. We study this question primarily in a well known mouse model of AML, with confirmation of our mouse model findings in human AML biopsy samples.

2. **KEYWORDS:**

Acute myeloid leukemia, microenvironment, bone marrow, niche, growth factor, receptor ligand

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

  - Major goal 1-1: Identify the complete repertoire of cell surface receptors present on mouse MLL-AF9 AML cells. Goal completion month 7. 80% complete as of month 12.

  - Major goal 1-2: Identify cognate ligands expressed in bone marrow niche cells adjacent to MLL-AF9 AML cells. Goal completion month 12. 67% complete as of month 12.

  - Major goal 1-3: Identify cognate ligands present in MLL-AF9 AML bone marrow ECM. Goal completion month 12. 0% complete as of month 12.

  - Major goal 1-4: Test hypothesis that *ex vivo* culture of MLL-AF9 AML cells in the presence of highest ranked cognate ligands will preserve *in vivo* MLL-AF9 cell state as assessed by leukemia initiating capacity. Goal completion month 18. 10% complete as of month 12.

  - Major goal 2-1: Identify the repertoire of cell surface receptors present on human AML cells. Goal completion month 24. 40% complete as of month 12.

  - Major goal 2-2: Identify cognate ligands expressed in bone marrow niche cells adjacent to human AML cells. Goal completion month 24. 0% complete as of month 12.

  - Major goal 2-3: Identify cognate ligands present in bone marrow ECM of relapsed AML patients. Goal completion month 24. 0% complete as of month 12.

  - Major goal 2-4: Test hypothesis that *ex vivo* culture of human AML cells in the presence of highest ranked cognate ligands will preserve *in vivo* AML cell state as

assessed by scRNAseq and scATACseq. Goal completion month 24. 0% complete as of month 12.

- **What was accomplished under these goals?**

  - For major goal 1-1: We have secured IACUC/USAMRDC/ACURO approval of MLL-AF9 AML cell transplantation experiments following in vitro culture. We have successfully FACS isolated c-Kit+dsRed+ MLL-AF9 AML cells from 2 male and 2 female C57BL/6J mice moribund from MLL-AF9 AML and performed scRNAseq. We have completed computational analysis of this scRNAseq data and have generated a list of expressed cell surface receptors. For those receptors with cognate ligands, we have generated a list of those cognate ligands. We have yet to perform immunofluorescence on frozen bone marrow tissue sections of 2 male and 2 female C57BL/6J mice moribund from MLL-AF9 AML as we have yet to identify a suite of antibodies suitable for immunofluorescence for every receptor.

  - For major goal 1-2: We have performed spatial transcriptomics using the Nanostring GeoMx platform on the bone marrow of C57BL/6J mice with MLL-AF9 AML infiltration at burdens of 30% and 70% of cells within the bone marrow. The Nanostring GeoMx platform is an improvement over the initial technology proposed in that it is able to assess spatial gene expression of the entire mouse transcriptome, as opposed to only a curated number of mouse transcripts. We have received the data for this spatial transcriptomic profiling, and are currently analyzing it based on the goals of major goal 1-2.

  - For major goal 1-3: We have yet to perform proteomic analysis of bone marrow ECM from mice burdened with MLL-AF9 AML. Have elected to first pursue spatial transcriptomic analysis to narrow down the potential cognate ligands to assess with proteomics, as this will significantly simplify the number of spike-in controls that we will have to develop for the proteomic analysis.

  - For major goal 1-4: We have replicated the phenotype that mouse MLL-AF9 AML cells lose leukemia initiating capacity with increased time cultured *ex vivo*. Our data indicate that tail vein injection of 100,000 freshly isolated MLL-AF9 AML cells will result in sublethally irradiated recipient mice becoming moribund within 21 days, injection of 100,000 MLL-AF9 AML cells cultured ex vivo for 24 hours will result in sublethally irradiated recipient mice becoming moribund within 30 days, and injection of 100,000 MLL-AF9 AML cells cultured ex vivo for 48 or more hours will result in no leukemia initiating capacity. We have yet to initiate experiments where cognate ligands identified in the above goals are tested in *ex vivo* culture assays determining if leukemia initiating capacity can be maintained.

  - For major goal 2-1: We have obtained IRB/USAMRDC/HRPO approval for use of de-identified patient bone marrow biopsy samples. We have identified 3

banked bone marrow samples from AML patients at relapse. We have yet to perform scRNAseq on human AML samples.

- For major goal 2-2: We will commence identification of cell surface receptor cognate ligands following scRNAseq of human AML samples.

- For major goal 2-3: We will commence identification of cognate ligands present in bone marrow ECM of relapsed AML patients following scRNAseq of human AML samples. Identify.

- For major goal 2-4: We will commence *ex vivo* culture testing of candidate cognate ligands with human AML cells after completing major goals 2-2 and 2-3.

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

  - For training and professional development of the PI, Hojun Li, MD, PhD was able to present research at the Koch Institute bi-weekly faculty meeting, and also attended the American Society of Hematology 2021 annual meeting. Dr. Li was also invited to give a seminar at the Yale Comprehensive Center for Excellence in Hematology. Dr. Li and his trainees were able to attend weekly lab meetings with both the Yaffe and Hemann labs at the Koch Institute. Dr. Li was also able to mentor and train three research technicians: Tatum Braun, Leah Hirsch, and Ngoc Hoang.

  - For profession development of trainees mentored by Dr. Li, Tatum Braun was able to present at Hemann lab meeting twice and at the Koch Institute Friday Focus seminar series once. Ngoc Hoang was able to present at the Hemann lab meeting once. Tatum Braun, Leah Hirsch, and Ngoc Hoang were able to acquire skills in flow cytometry and cell sorting, and Tatum Braun and Ngoc Hoang were able to acquire skills in mouse irradiation and tail vein injection.

- **How were the results disseminated to communities of interest?**

  Nothing to report yet

- **What do you plan to do during the next reporting period to accomplish the goals?**

  - For major goal 1-1: Following identification of the most promising cell surface receptors from scRNAseq data using orthogonal spatial transcriptomic data, we will begin rigorous testing of antibodies for the remaining immunofluorescence experiments.

  - For major goal 1-2: We will complete image analysis of the raw spatial transcriptomic data to identify cognate ligands expressed adjacent to MLL-AF9 AML cells.

- For major goal 1-3: Based on the most promising cognate ligands identified in major goal 1-2, we will initiate proteomic experiments of mouse bone marrow ECM.

- For major goal 1-4: We will initiate experiments where cognate ligands identified in the above goals are tested in *ex vivo* culture assays determining if leukemia initiating capacity can be maintained.

- For major goal 2-1: We will identify 2 additional banked bone marrow samples from AML patients at relapse, and perform scRNAseq on all samples, performing library preparation at the same time to avoid batch effects.

- For major goal 2-2: We will identify cell surface receptor cognate ligands following scRNAseq of human AML samples.

- For major goal 2-3: We will identify cognate ligands present in bone marrow ECM of relapsed AML patients following scRNAseq of human AML samples. Identify.

- For major goal 2-4: We will begin *ex vivo* culture testing of candidate cognate ligands with human AML cells after completing major goals 2-2 and 2-3.

4. **IMPACT:**

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

     Nothing to report yet

   - **What was the impact on other disciplines?**

     Nothing to report yet

   - **What was the impact on technology transfer?**

     Nothing to report yet

   - **What was the impact on society beyond science and technology?**

     Nothing to report yet

5. **CHANGES/PROBLEMS:**

   - **Changes in approach and reasons for change**

     Nothing to report

   - **Actual or anticipated problems or delays and actions or plans to resolve them**

Our original proposed platform for spatial transcriptomics unfortunately could not be performed, as the company we had planned to work with subsequently informed us they could not profile as many genes for surface receptors and cognate ligands as we had originally agreed with them upon. Thus, we spent a significant amount of time identifying a different vendor, GeoMx platform from Nanostring Technologies, to perform spatial transcriptomic studies for this project. We have now been able to perform the spatial transcriptomic studies through this alternative vendor, and are proceeding with the project

- **Changes that had a significant impact on expenditures**

  Due to the scarcity of available postdoctoral associates with the COVID-19 pandemic related personnel shortages, we were unable to hire a postdoctoral fellow to work on this project. Thus, we had to hire a second research technician, and also supplement additional effort from other research technicians in the lab.

- **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:**

- **Publications, conference papers, and presentations**

  - **Journal publications.** Nothing to report

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

  - **Other publications, conference papers, and presentations.** Nothing to report

- **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:	Hojun Li, MD, PhD
Project Role:	Principal Investigator
Researcher Identifier:	0000-0002-8129-0390 (ORCID)
Nearest person month worked:	3
Contribution to Project:	Dr. Li has overseen all aspects of work on this project, and arranged obtaining human AML samples
Funding Support:	Charles W.(1955) and Jennifer C. Johnson Cancer Research Fund

Name:	Tatum Braun
Project Role:	Research Technician
Researcher Identifier (e.g. ORCID ID):	n/a
Nearest person month worked:	10
Contribution to Project:	Ms. Braun has performed mouse MLL-AF9 transplantation and isolation experiments, and tissue isolation for single cell RNA sequencing and spatial transcriptomics.
Funding Support:	n/a

Name:	Leah Hirsch
Project Role:	Research Technician
Researcher Identifier:	n/a
Nearest person month worked:	8
Contribution to Project:	Ms. Hirsch has performed cell sorting, cell isolation, and molecular biology related to this project.
Funding Support:	n/a

Name:	Ngoc Hoang

Project Role:	Research Technician
Researcher Identifier:	n/a
Nearest person month worked:	4
Contribution to Project:	Ms. Hoang has performed mouse MLL-AF9 transplantation and isolation experiments.
Funding Support:	Charles W.(1955) and Jennifer C. Johnson Cancer Research Fund

- **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