

AWARD NUMBER: W81XWH-20-1-0574

TITLE: Selective Clonal Growth in Myelodysplastic Syndrome

PRINCIPAL INVESTIGATOR: Marshall S. Horwitz

CONTRACTING ORGANIZATION: University of Washington

REPORT DATE: JULY 2022

TYPE OF REPORT: Annual

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

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REPORT DOCUMENTATION PAGE			<i>Form Approved</i> <i>OMB No. 0704-0188</i>		
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1. REPORT DATE JULY 2022		2. REPORT TYPE Annual		3. DATES COVERED 1JULY2021 - 30JUN2022	
4. TITLE AND SUBTITLE Selective Clonal Growth in Myelodysplastic Syndrome			5a. CONTRACT NUMBER W81XWH-20-1-0574		
			5b. GRANT NUMBER BM190019		
			5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S) Marshall S. Horwitz E-Mail: horwitz@uw.edu			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) UNIVERSITY OF WASHINGTON 4333 BROOKLYN AVE NE SEATTLE WA 98195-0001			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 Loss of all or part of one copy of chromosome 7(7q-) is frequent in MDS and portends a poor prognosis. The recent identification of germline mutations in <i>SAMD9L</i> in individuals with ataxia-pancytopenia syndrome has helped elucidate the role of 7q- in promoting MDS. The mutations are toxic gain-of-function. Hematopoietic stem and progenitor cells undergoing somatic mutation that eliminate the mutant allele through one of three mechanisms confers a selective growth advantage. The first mechanism involves loss of all or part of the chromosome 7q region containing <i>SAMD9L</i> and is deleterious. A second mechanism involves <i>cis</i> suppressor point mutations and is better tolerated. A third and potentially beneficial mechanism involves auto-correction of the underlying germline mutation through interhomolog recombination. Our project is aimed at modeling this phenomenon in vitro and identifying drug combinations that may both promote auto-correction and confer a selective advantage to cells retaining two intact copies of chromosome 7.					
15. SUBJECT TERMS Ataxia-pancytopenia syndrome, <i>SAMD9L</i> , MDS, genetic recombination, induced pluripotent stem cell disease models					
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	17	19b. TELEPHONE NUMBER (include area code)

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1. INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

MDS is frequently accompanied by loss of all or part of chromosome 7q (7q-), typically including *SAMD9L*, toxic gain-of-function mutations which our group identified as the cause of ataxia-pancytopenia syndrome (ATXPC). Remarkably, bone marrow cells in individuals with ATXPC undergo subsequent somatic mutations that can be either deleterious or beneficial. Deleterious somatic mutations involve loss of all or part of the region of chromosome 7 containing the germline mutant-*SAMD9L* allele and confer a proliferative advantage that leads to bone marrow failure and MDS. Beneficial somatic mutations include both *SAMD9L* cis suppressor mutations and—even better—interhomolog recombination that creates uniparental disomy, the net effect of which is to completely correct the underlying germline mutation. Our proposal has three specific aims. The first is to establish in vitro cellular models of ATXPC. The second is to promote autocorrection of *SAMD9L* by suppressing DNA repair pathways that ordinarily lessen its occurrence. The third is to take advantage of the fact that genes adjacent to *SAMD9L* are involved in detoxification of xenobiotics, suggesting that a cocktail of drugs may be identified that can confer a growth advantage to cells retaining two copies of chromosome 7. The overarching goals of our research are to identify factors promoting 7q- and develop new therapies to both treat it and prevent its occurrence.

2. KEYWORDS: Provide a brief list of keywords (limit to 20 words).

Ataxia-pancytopenia syndrome, *SAMD9L*, MDS, genetic recombination, induced pluripotent stem cell disease models

3. ACCOMPLISHMENTS: The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

1. Establish in vitro models of clonal heterogeneity from patients with pathogenic *SAMD9L* mutations.
 - a. Establish EBV-transformed lymphocyte lines.
 - b. Derive iPSC and differentiate them to CD34+ hematopoietic cells.
2. Promote autocorrection of *SAMD9* and *SAMD9L* germline mutations.
3. Identify drug combinations that specifically inhibit growth of cells deficient for part or all of chromosome 7q while favoring growth of blood cells retaining two intact chromosome 7 copies.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

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

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Aim 1 was to "Establish in vitro models of clonal heterogeneity from patients with pathogenic *SAMD9L* mutations". We found that iPSCs are very resistant to the drugs we proposed to use. In addition, iPSC bearing pathogenic *SAMD9L* variants are very difficult to differentiate to committed hematopoietic lineages and we were not able to generate sufficiently robust cultures to proceed. Therefore, this year we focused on EBV transformed lymphocyte lines (LCLs) from several subjects without a revertant somatic mutation in the EBV line (germline H880Q or V1551L) and from one who was found to be mosaic for a hematopoietic clone that had lost the pathogenic H880Q variant by copy neutral loss of heterozygosity for chromosome 7q.

For Aims 2 "Promote autocorrection of *SAMD9* and *SAMD9L* germline mutations" and 3 "Identify drug combinations that specifically inhibit growth of cells deficient for part or all of chromosome 7q while favoring growth of blood cells retaining two intact chromosome 7 copies" we repeated studies to determine the IC50 doses for docetaxel and irinotecan. In an initial experiment, in control LCLs, the IC50 concentrations for docetaxel and irinotecan were 1.7 nM and 450 ng/ml, respectively, and they were lower in ATXPC patients, suggesting greater drug sensitivity, although the difference was not significant by t-test. To validate the IC50s, we repeated the dose-response in newly established LCLs from 4 normal controls. The results were nearly identical to those previously detected (1.5 nM for docetaxel and 536 ng/ml for irinotecan). We then grew the LCLs for up to 4 weeks in a range of drug concentrations lower than the IC50s. At the lowest tested concentrations, docetaxel at 0.6 nM and irinotecan at 20 ng/ μ l, no response to the treatment was observed by cell count and proliferation assays. At docetaxel 16 nM and irinotecan 50 ng/ μ l, untreated and treated cells from control subjects survived to the end of the testing period while treated cells showed lower proliferation compared to untreated cells, documenting a response to the drug. Therefore, we selected a concentration of 30 ng/ μ l for irinotecan to initiate the longer course experiments involving samples from affected subjects.

In this larger scale experiment with 30 ng/ μ l irinotecan, LCLs were cultured in wells and sampled periodically. The samples included the affected subject with a p.V1551L pathogenic variant who had fluctuating percentages of monosomy 7 cells and an affected subject who had not manifested copy neutral LOH for 7q but had a benign p.V266I *SAMD9L* polymorphism *in trans* with the pathogenic p.H880Q variant. LCLs from two normal controls were also included. The plates were cultured for more than four months. **Figure 1** shows that there was no significant difference by droplet digital PCR (ddPCR) in loss of the mutant allele regardless of treatment conditions. Although **Figure 1A** shows a lower mutant percentage in untreated cultures than in treated ones, it should be noted that a low mutant allele percentage in the non-treated cells was observed from the initial stage. To provide a more accurate representation of the effect of drug treatment, we have adjusted the changes in mutant allele percentage in each group during the treatment course by normalizing them with their own baseline measurements taken at the initial time point of treatment at 2 weeks. This adjusted data is depicted in **Figure 1B**. While the treated cells showed a greater decrease in mutant allele percentage at later time points, this was not significant by one-way ANOVA post-hoc test. The large error bars suggest significant variability, which may be attributed to the variable number of cells carrying the mutation from the initial stage. This variability is further demonstrated by analysis of four individual culture wells (**Figure 2**). We conclude that there is no dosage effect between 30ng/ml and 50ng/ml on selective advantage for loss of the mutant allele and retention of the wild-type one.

However, these results do not reflect the presence of any other revertant mutation. To address this issue, we have sent samples from treated cultures for sequencing the entire *SAMD9L* coding region. We plan to analyze these data when available.

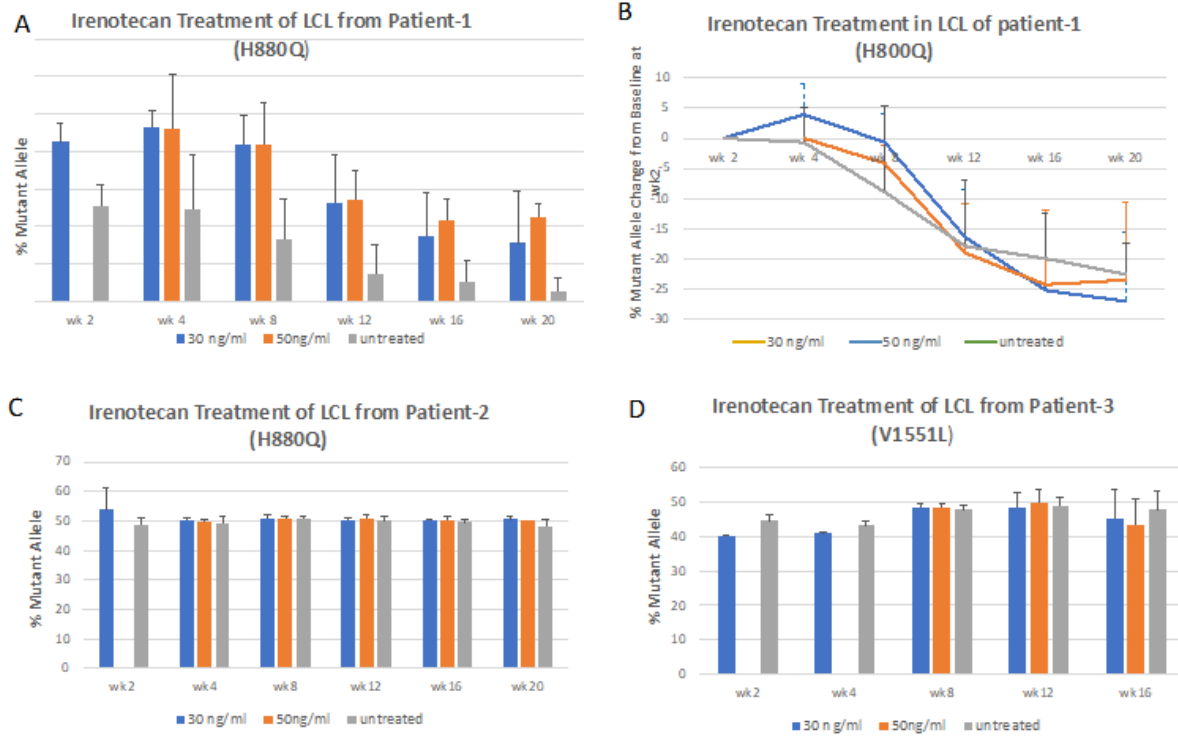


Figure 1. Mutant allele percentage detected by droplet digital PCR (ddPCR) in LCLs grown with and without irinotecan for 5 months. **A** and **B**: Patient-1 with H880Q, who demonstrated copy neutral LOH for 7q in a previous study; **B**: Adjusted changes in mutant allele percentage in each group by normalizing with their own baseline measurements taken at 2 weeks; **C**: Patient-2, a relative with H880Q who did not show cnLOH; and **D**: Patient-3, with V1551L who had fluctuating mosaic monosomy 7. Error bars represent the standard deviation of the mutant allele percentage measured in four culture wells. There is no significant difference among treatment conditions and non-treatment by one-way ANOVA post-hoc method.

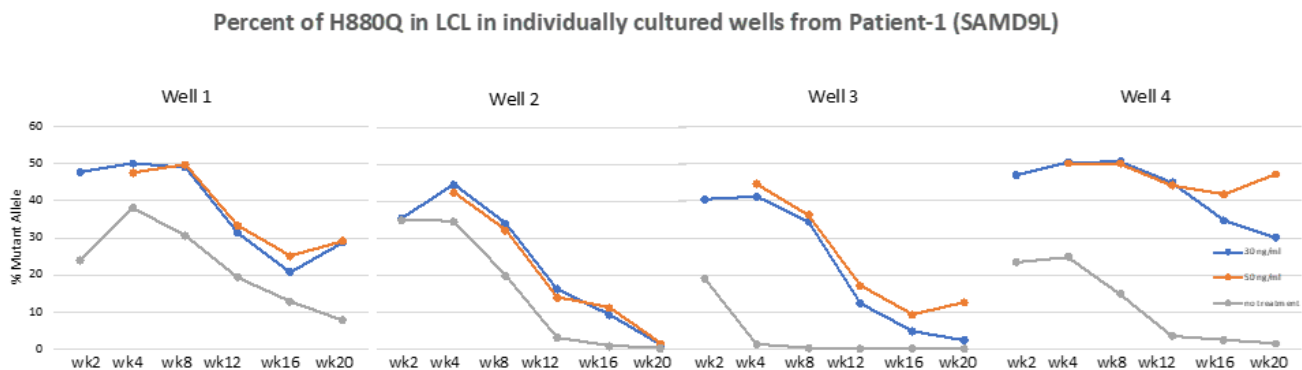


Figure 2. Significant variability of the proportion of the mutant allele is independent of culture conditions but may be related to the fraction of cells with LOH 7q at initiation of the culture.

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Co-I Dr. Dong-Hui Chen mentored an undergraduate student in laboratory research. The student took required courses in bloodborne pathogens, human subjects research and was taught basic tissue culture techniques. The student then assisted with cell culture and harvesting.

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report.

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."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We will continue culturing the EBV lines with and without the toxin and use ddPCR to monitor for loss of the pathogenic variant over longer periods of time. At the end of the culture process, we will send selected samples for medium depth genome or exome sequencing to detect acquisition of a revertant mutation.

4. IMPACT: Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

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

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report

What was the impact on other disciplines?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions;*
or
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

- 5. CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:*

Nothing to Report.

that

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

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

The COVID pandemic continued to have a negative impact on research progress. People with any manifestation suggestive of COVID were required to isolate. Compliance with the University of Washington’s rules for social distancing hampered efficient use of tissue culture facilities and access to our lab’s DNA sequencing equipment and the ddPCR apparatus in another department. The rules have been relaxed and we anticipate improved progress for the remaining 6 months.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

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

Report only the major publication(s) resulting from the work under this award.

Journal publications. List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to Report

Books or other non-periodical, one-time publications. Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to Report

Other publications, conference papers and presentations. Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.

Nothing to Report

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*

- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change”.

Example:

Name: Mary Smith
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): 1234567
Nearest person month worked: 5

Contribution to Project: Ms. Smith has performed work in the area of combined error-control and constrained coding.

Funding Support: The Ford Foundation (Complete only if the funding support is provided from other than this award.)

Name: Marshall Horwitz
Project Role: PI
Researcher Identifier ORCID ID 0000-0002-1683-1680
Nearest person month worked .6
Contribution to Project Overall design of experiments, lab management, and interpretation of data.
Funding Support N/A

Name: Wendy Raskind
Project Role: co-PI
Researcher Identifier ORCID ID 0000-0001-8141-9054
Nearest person month worked .6
Contribution to Project Shared responsibility for overall design of experiments, lab management, and interpretation of data.
Funding Support N/A

Name: Dong-Hui Chen
 Project Role: co-I
 Researcher Identifier ORCID ID 0000-0001-8141-9054
 Nearest person month worked 2
 Contribution to Project Dr. Chen supervised the technical staff in culturing the LCLs, determining the ID50s and harvesting the samples, performed the ddPCR assays, mentored the undergraduate student
 Funding Support N/A

Name: Sudeshna Seal
 Project Role: Research Scientist
 Researcher Identifier none
 Nearest person month worked 6
 Contribution to Project Established iPSC hematological differentiation assays.
 Funding Support N/A

Name John Wolff
 Project Role Research Scientist
 Researcher Identifier None
 Nearest person month worked 1
 Contribution to Project Processed samples and established LCLs
 Funding Support N/A

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Horwitz, Marshall
 Competing renewal awarded:
 *Title: Allen Discovery Center for Cell Lineage Tracing

Major Goals: Our proposed Allen Discovery Center for Cell Lineage Tracing has three aims. First, we will improve these technologies to realize their potential to deeply reconstruct cell lineage histories throughout development while also recovering the spatial context and functional type of each cell in the resulting trees. Second, we will generate global maps of cell lineage in two vertebrate model organisms, the zebrafish and the mouse, across multiple developmental timepoints and biological replicates. Third, we will integrate the recording of molecular histories with cell lineage to study the signaling and transcriptional events that drive cell fate decisions

Project Number: 12357
 Name of PD/PI: Shendure, Elowitz, Schier
 *Source of Support: The Paul G. Allen Family Foundation
 *Primary Place of Performance: University of Washington, Seattle
 Project/Proposal Start and End Date: 11/2022 – 10/2025

* Total Award Amount (including Indirect Costs): (Horwitz)

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY) Person Months (##.##)

5. 2022-23 1.2 calendar

6. 2023-24 1.2 calendar

7. 2024-25 1.2 calendar

Overlap: none

New

*Title: Life History of MDS in Females: From Embryogenesis to Adult

Major Goals: The goal of this project is to understand the origins, classification, and treatment of MDS. To do so, we will analyze patient samples, using a new approach for extracting information about the history of cell divisions.

Project Number: DRG2022

Name of PD/PI: Horwitz

*Source of Support: Edward P. Evans Foundation

*Primary Place of Performance: University of Washington, Seattle

Project/Proposal Start and End Date: 9/2022 – 8/2025

*Total Award Amount (including Indirect Costs):

*Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY) Person Months (##.##)

1. 2022-23 1.2 calendar

2. 2023-24 1.2 calendar

2. 2024-25 1.2 calendar

Overlap: none

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner’s contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner’s facilities for project activities);*
- *Collaboration (e.g., partner’s staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner’s staff use each other’s facilities, work at each other’s site); and*
- *Other.*

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ebrap.org/eBRAP/public/index.htm> for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on <https://www.usamraa.army.mil/Pages/Resources.aspx>) should be updated and submitted with attachments.*

9. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*