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TITLE: The Impact of Germline Predisposition to Myelodysplastic Syndrome on Allogeneic Hematopoietic Stem Cell Transplant Outcomes Using Related Donors

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CONTRACTING ORGANIZATION: The University of Chicago

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Dr. Lucy Godley

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14. ABSTRACT

We will use samples from the Center for International Blood and Marrow Transplant Research (CIBMTR) to determine the frequency with which germline mutations occur in myelodysplastic syndrome (MDS) patients across the entire age spectrum. In addition to determining mutation frequency, we will associate the germline status of both matched-donor and recipient with engraftment kinetics and post-transplant outcomes. This comprehensive study will change the diagnostic and hematopoietic stem cell transplantation (HSCT) planning work-up for MDS patients by identifying age groups that are most likely to have a germline predisposition mutation, which will position the HSCT field to implement appropriate clinical testing strategies across the world to detect these types of mutations in MDS patients. This work will also shape HSCT policies regarding allogeneic stem cell donor choices and transplantation protocols for these patients with the goal of improving outcomes.

15. SUBJECT TERMS-

NONE LISTED

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

We will use samples from the Center for International Blood and Marrow Transplant Research (CIBMTR) to determine the frequency with which germline mutations occur in myelodysplastic syndrome (MDS) patients across the entire age spectrum. In addition to determining mutation frequency, we will associate the germline status of both matched-donor and recipient with engraftment kinetics and post-transplant outcomes. This comprehensive study will change the diagnostic and hematopoietic stem cell transplantation (HSCT) planning work-up for MDS patients by identifying age groups that are most likely to have a germline predisposition mutation, which will position the HSCT field to implement appropriate clinical testing strategies across the world to detect these types of mutations in MDS patients. This work will also shape HSCT policies regarding allogeneic stem cell donor choices and transplantation protocols for these patients with the goal of improving outcomes.

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

Germline predisposition; myelodysplastic syndrome

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.

Specific Aim 1: DNA extraction and next-generation sequencing

Milestones for this Aim:

- High quality DNA extraction from mononuclear cells from whole blood (n=705) using the Qiacube extraction robot- completed
- DNA quantification using a Qubit fluorometer and quantitation reagents (n=705)- completed
- Preparing next-generation sequencing libraries by fragmenting genomic DNA and ligating adapters to both fragment ends (n=705)- completed [ahead of schedule]
- Cluster amplification and sequencing/alignment of whole exome sequencing (WES) reads to a reference genome using bioinformatic software (n=705, 235 per 6 months)- completed [ahead of schedule]
- Analysis of single-nucleotide variants (SNVs) and copy number variants (CNVs) and confirmation of these variants- completed
- Confirmation of variants via Sanger sequencing/microarrays/ quantitative reverse transcription PCR- completed

Specific Aim 2: Transplant data analysis and statistics- Statistical review is completed. We have submitted this work for publication to *Blood*. The manuscript has been revised twice, and we hope that it will be accepted soon.

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.

1) Major activities: Our focus over the past year has been to finalize the statistical analysis; present the results of this project at the annual meeting of the American Society of Hematology (ASH); and to publish our findings. As noted above, we have submitted the manuscript describing this project's findings to *Blood*, and the manuscript is under its second revision. We hope that it will be accepted soon.
2) Specific objectives: We have achieved our objectives as outlined above, except for final manuscript acceptance, but we hope that this will come soon. The CIBMTR is also interested in using other sequencing data that is available to them to expand this project. We await data use agreement paperwork to be able to expand our studies to these other sequencing data.
3) Significant results include: 28 individuals out of 404 were found to have deleterious germline variants causative for their MDS = an overall rate of 7%. Importantly, these individuals were spread out over the ENTIRE age range, meaning that people of ALL ages have germline predisposition, not just people presenting at younger ages. The presence of a germline predisposition allele was correlated statistically with development of higher grade MDS.
4) Other achievements: In the process of doing this work, we developed a bioinformatic pipeline for calling CNVs from WES. This was not anticipated in the grant itself, but was a positive outcome from the work.

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."

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.

Both Simone Feurstein and Amy Trottier have learned a tremendous amount from this project in terms of calling SNVs and CNVs. Both participated in our upcoming 6th ASH Friday Scientific Workshop on Inherited Hematopoietic Malignancies and Bone Marrow Failure. Likewise, Matt Pozsgai has improved his bioinformatic skills by working on this project. Matt is now doing programming for Amazon, Inc., so he is using the skills that he developed from this project on a daily basis.

The development of a CNV calling pipeline from WES data is another major accomplishment for our team.

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.

Simone Feurstein presented our results in an oral session at the annual ASH meeting; and our results have been described in a manuscript that is under its second revision at *Blood*; we hope that it will be accepted soon.

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.

During the next reporting period, we will use the bioinformatic pipelines developed in this project to analyze additional sequencing data sets that the CIBMTR has from other ongoing projects. This will advance our findings to other patients with myeloid malignancies.

4. **IMPACT:** D*escribe 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).

As stated in the opening response, we anticipated when we wrote this grant that this work would change the diagnostic and HSCT planning work-up for MDS patients by identifying age groups that are most likely to have a germline predisposition mutation. In fact, our work has shown that germline predisposition mutations are present at >5% in MDS patients of ALL ages. We anticipate that this finding will render germline genetic testing standard of care for all MDS patients. This result will position the HSCT field to implement appropriate clinical testing strategies across the world to detect these types of mutations in MDS patients. Our results challenge long-held ideas that people with germline predisposition present at much younger ages than the average.

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.

We anticipate that once our work is published, it will impact the diagnostic work-up of patients diagnosed with MDS/leukemia as well as HSCT. Our CNV calling approach will be described in this publication as well and we will distribute the computer codes freely.

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 (yet). But we hope that our findings will change diagnostic approaches for all patients diagnosed with MDS and will impact HSCT policies.

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:*

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report

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.

None; so far this project has gone extremely smoothly.
We have had no problems performing the work to date, and we are excited to advance our approaches to additional sequencing data sets from the CIBMTR.

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.

No, fortunately, we already had our WES data in hand when the covid pandemic hit and closed our laboratory to bench research—so we were able to work from home to continue the bioinformatic analysis. In fact, if anything, the covid pandemic accelerated the research, since the staff working on this project were not able to do any laboratory bench work and they focused even more on this project. This allowed us to achieve some milestones ahead of our anticipated schedule. The CIBMTR is so excited by our findings that they are opening additional sequencing data sets from other patients with myeloid malignancies to us for analysis.

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

None

Significant changes in use or care of vertebrate animals

N/A

Significant changes in use of biohazards and/or select agents

None

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 (yet). As stated above, we presented this work in an oral session at to the 2021 annual ASH meeting, and a manuscript describing these findings has been submitted to *Blood* for publication and is under its second revision now.

  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 (yet), but as noted above, a manuscript describing these findings has been submitted to *Blood* for publication and is under its second revision now.

- **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.

N/A

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

None

- **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.

None

- **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.*

None

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: *Lucy Godley*
Project Role: *Principal Investigator*
Researcher Identifier (e.g. ORCID ID): *ORCID ID: 0000-0003-1914-9158*
Nearest person month worked: *2*
Contribution to Project: *Dr. Godley has supervised the generation of DNAs from the primary samples; the expansion of whole exome sequencing to cover non-coding regions; the performance of the next-generation sequencing; and the calling of deleterious variants.*

Name: *Matt Pozsgai*
Project Role: *Godley Laboratory Research Technologist*
Researcher Identifier (e.g. ORCID ID): *none*
Nearest person month worked: *5.5*
Contribution to Project: *Mr. Pozsgai has coordinated delivery of the primary DNA samples to the sequencing center, and he has organized the retrieval of sequencing data.*

Name: *Simone Feurstein*
Project Role: *Godley Laboratory Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID): *none*
Nearest person month worked: *9*
Contribution to Project: *Dr. Feurstein has analyzed the sequencing data and called variants.*

Name: *Amy Trottier*
Project Role: *Godley Laboratory Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID): *none*
Nearest person month worked: *4*
Contribution to Project: *Dr. Trottier has analyzed the sequencing data and called variants.*

Name: *Jeremy Segal*
Project Role: *Co-Investigator*
Researcher Identifier (e.g. ORCID ID): *none*
Nearest person month worked: *1*
Contribution to Project: Dr. Segal has discussed variant curation with the Godley staff.

Name: *Aelin Kim*
Project Role: *Research Analyst*
Researcher Identifier (e.g. ORCID ID): *none*
Nearest person month worked: *8*
Contribution to Project: *Ms. Kim has assisted in preparing DNAs for the project.*

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.

Nothing to Report

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. The CIBMTR statisticians have finalized their analysis of transplant outcomes now.

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.*

N/A

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

N/A

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.*

N/A