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CONTRACTING ORGANIZATION: Washington University, St. Louis, MO

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14. ABSTRACT

The APOBEC3 family of enzymes are encoded by the cellular genome and function in the innate immune response to viral infection. By catalyzing the conversion of cytidine to uracil in DNA substrates, APOBEC3 enzymes mutate viral genomes and limit replication, progeny production, and infection. However, the APOBEC3A family member is a potent deaminase that is capable of mutating the cellular genome when acting aberrantly. Through mutagenic activity, APOBEC3A causes widespread base changes and DNA damage throughout the genome implicating the enzyme in genome instability. APOBEC3A is expressed in healthy hematopoietic cells, and his overexpressed in a subset of hematologic malignancies. The overall goal of the project is to determine how APOBEC3 mutagenesis impacts hematologic malignancies. Using models of APOBEC3A in hematopoietic progenitor cells, we will evaluate the hypothesis that mutagenesis by APOBEC3A contributes to malignant transformation and cancer progression. The objectives of this project are to determine the cellular factors that affect somatic mutagenesis by APOBEC3A deamination and to determine the impact of APOBEC3A on cellular transformation.

15. SUBJECT TERMS
Hematologic malignancy, leukemia, mutagenesis, APOBEC, deamination, DNA damage, replication stress, clonal heterogeneity, mutational signatures

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

The overall goal of the project is to determine how APOBEC3 mutagenesis impacts hematologic malignancies. Using models of APOBEC3A in hematopoietic progenitor cells, we will evaluate the hypothesis that mutagenesis by APOBEC3A contributes to malignant transformation and cancer progression. The objectives of this project are to determine the cellular factors that affect somatic mutagenesis by APOBEC3A deamination and to determine the impact of APOBEC3A on cellular transformation.

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

Hematologic malignancy, leukemia, mutagenesis, APOBEC, deamination, DNA damage, replication stress, clonal heterogeneity, mutational signatures

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. Define genomic features that promote clustered mutagenesis by APOBEC3A.
Milestones: (a) determine how availability of ssDNA templates alters APOBEC3A mutational signatures – goal completion by April 2023, currently 50% complete, (b) Gain experience in computational analysis of WGS – goal completion by April 2023, currently 100% complete
2. Determine how replicative and repair DNA polymerases impact the APOBEC3 mutational spectrum.
Milestones: define the alteration in APOBEC3A mutational signatures that result from DNA polymerase activity – goal completion by Jan 2024, currently 20% complete
3. Determine how APOBEC3A contributes to malignant transformation of progenitor B cells.
Milestones: (a) Develop and characterize a mouse model of human APOBEC3A expression in hematopoetic cells – goal completion by Oct 2024, currently 60% complete, (b) Learn techniques for designing mouse models of cancer – goal completion by Oct 2024, currently 50% complete
4. Determine the effect of APOBEC3A activity on genome integrity in vivo.
Milestones: determine how APOBEC3A expression in B cells results in genome instability –goal completion by April 2025, currently 0% complete.
5. Determine how APOBEC3A activity impacts clonal diversity.
Milestones: determine clonal variability resulting from APOBEC3A expression – goal completion by Sept 2025, currently 20% complete

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.

This project includes two Specific Aims which are stated below, along with method and model development and significant results associated with both Aims.

Aim 1. Determine the cellular factors that influence APOBEC3A somatic mutational patterns. Towards this aim, we used a prior dataset from a functional genomics screen in leukemia cells which showed that the SMC5/6 complex is essential for cell viability when APOBEC3A is active (Fig 1). We used a panel of leukemia cell lines with inducible APOBEC3A expression to confirm that viability is dependent on SMC5/6 and further, that the mechanism of cell death is dependent on DNA breaks arising during DNA replication (Fig 2). In related work that is not funded by this award, we found that the mutational signatures of APOBEC3A activity are absent in human cancers with defects in SMC5/6, consistent with a synthetic lethal interaction. Given these findings, the next steps for this Aim are to define how replication forks are damaged by APOBEC3A and the facets of SMC5/6 complex that enact genome protection from deaminase activity. For example, we want to understand whether uracils resulting from cytidine deamination trigger SMC5/6 requirement, or whether uracil excision, which leaves an abasic site, is the initiator of SMC5/6 requirement. A manuscript detailing these findings is in preparation. In the long-term, we would like to target the SMC5/6 function that protects genome integrity upon APOBEC3A activity as a potential therapeutic opportunity.

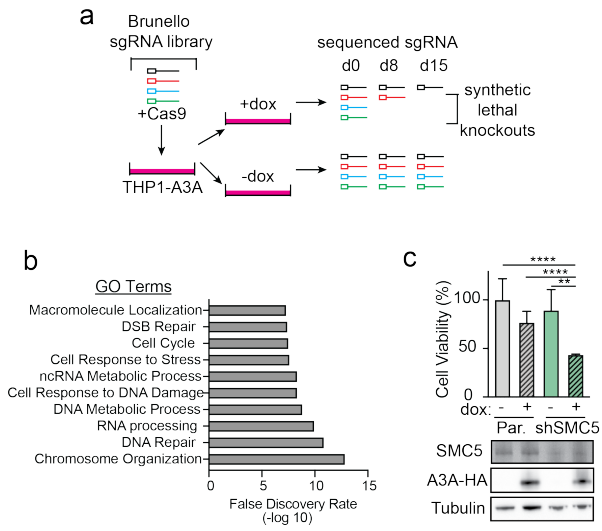

Figure 1. Functional genomics screen identifies the SMC5/6 complex as essential in APOBEC3A expressing cells. (**a**) Schematic for functional genomics screen to identify synthetic lethality with APOBEC3A. The Brunello CRISPR-Cas9 guide RNA (sgRNA) library was used in THP1 cells expressing a doxycycline-inducible, HA-tagged APOBEC3A transgene (THP1-A3A). sgRNAs were identified and quantified by sequencing at day 0 (baseline library integration), day 8, and day 15. Depletion of sgRNAs at day 15 in dox-treated cells relative to untreated controls represent potential synthetic lethal genes. The screen was performed in duplicate and results are averaged. (**b**) The top 250 genes identified as potentially synthetic lethal with APOBEC3A are grouped by Gene Ontology (GO) terms. (**c**) THP1-A3A cells were depleted of SMC5 by stable integration of shRNA. Viability of cells treated with dox or untreated was determined by FACS after staining for flurescent-labeled calcein AM (live) and DNA (dead). Cell lysates were probed with antibodies to HA and SMC5. Tubulin is used as loading control.

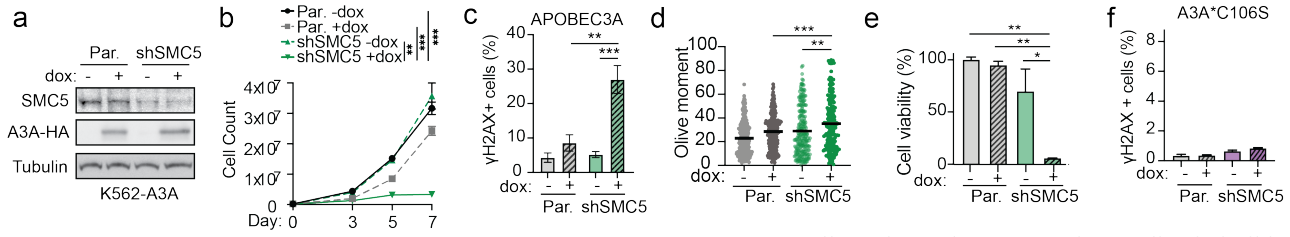

Figure 2. SMC5/6 loss potentiates APOBEC3A-mediated genotoxicity. K562 cells engineered to express doxycycline-inducible HA-tagged A3A (K562-A3A) were depleted of SMC5 by RNAi (shSMC5) and compared to parental K562-A3A cells. All results were calculated and plotted as the mean and standard deviation. All results are representative of three independent biological replicates. (**a**) Immunoblot shows A3A-HA expression and SMC5 depletion. Tubulin was used as a loading control. (**b**) Cell proliferation was measured by counting cells over the course of 7 days. (**c**) DNA damage response signaling was assessed by intracellular staining and flow cytometry analysis of the phosphorylated form of the histone variant H2AX (γH2AX). (**d**) Comet assay results plotted as dot plot and median of olive moments. (**e**) Cell viability was assessed by WST8 staining of K562 cells. (**f**) DNA damage response signaling was assessed by intracellular staining and FACS of γH2AX in cells expressing the catalytic mutant A3A*C106S.

Aim 2. Evaluate the impact of APOBEC3A deaminase activity on oncogenesis and cancer progression. Towards this aim, we are developing a mouse model of APOBEC3A expression in cancer. We started by that expresses the ETV6-RUNX1 translocation only in hematopoietic cells (through Vav-Cre cross breeding). We have demonstrated that ETV6-RUNX1 is expressed in bone marrow cells from these mice. We extracted bone marrow from these mice and transduced with lentivirus that expresses APOBEC3A (or empty vector). While this is an ideal model for ex vivo and syngeneic experiments, we found that ETV6-RUNX1 expression impacts proliferation of B cells and the breeding efficiency of these mice is very low. We have initiated a second genetically engineered a mouse model of APOBEC3A expression to circumvent these problems. In short, the second model uses a human APOBEC3A trasnsgene knocked in to the Rosa26 safe harbor locus. Given the substantial toxicity of aberrant APOBEC3A activity, we put several "safeguards" on transgene expression including a Lox-STOP-Lox preceding the APOBEC3A start site, and a dox-inducible promoter. The breeding strategy for three necessary alleles is detailed in Fig 3. The F2 generation is now breeding. Once these mice are ready for experimentation, we will transduce bone marrow ex vivo with an ETV6-RUNX1 retrovirus and transplant into syngeneic mice. We will examine leukemia development in the following cohorts: (1) APOBEC3A expression alone (+dox, transduction of empty vector), (2) ETV-RUNX1 expression alone (-dox, transduction of ETV6-RUNX1 vector), (3) combination of APOBEC3A and ETV6-RUNX1 expression, (4) control mice (-dox, transduction of empty vector). While developing an adequate mouse model for these studies has been challenging, we look forward to results from both models and believe that orthogonal approaches will strengthen our findings.

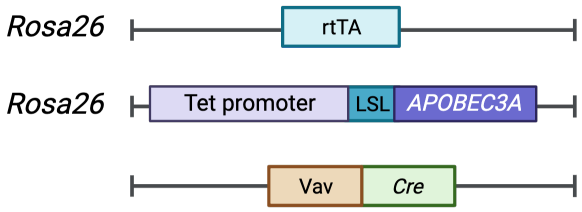

Figure 3. Genetically engineered mouse model of inducible APOBEC3A. Mice will be bred to harbor three alleles: (1) the tetracycline transactivator (rtTA), necessary for activation of tetracycline promoters, (2) the APOBEC3A transgene downstream of a tetracycline inducible promoter and a lox-STOP-lox cassette, which requires Cre recombinase to remove the STOP codon prior to transcription of APOBEC3A, and (3) Cre recombinase transgene downstream of a tissue-specific promotor, in this case the Vav promoter is pictured which is expressed in all hematopoietic progenitors. Mice with transgenes in the Rosa26 locus will be bred as homozygotes and then crossed independently to mice with a Vav-Cre transgene. Secondary crosses will be between F2 progeny to produce mice with one of each Rosa26 allele and at least one Vav-Cre allele. Homozygous mice will be maintained for breeding. Alternative options for evaluating APOBEC3A mutagenesis exist by using different tissue-specific Cre promoters (i.e. CD19 specific to B cell progenitors).

We have made substantial experimental progress though have had difficulty generating BRCA1 and BRCA2 knockout clones in DT40 cells. To troubleshoot, we are working with Dr. David Szuts's lab who has previously generated these cells.

Collaborative work on DNA damage in B cells was published this year (Johnston R, Mathias B, Crowley S, Schmidt H, White L, Mosammaparast N, **Green AM**, Bednarski JJ (2022). Distinct DNA damage responses in B cells directed by nuclease-independent functions of RAG1. *EMBO Reports*, e55429). Our data were presented at international meetings – Brazilian Society of Genetics (Sept 2023, Sao Paulo, Brazil) and Deaminet Conference on Base Editing (Jan 2023). Additional training and professional achievements are outlined below.

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.

Technical skills: I have gained skills in computational analysis of genome sequencing. Our lab recently hired a computational biologist who is enhancing our ability to analyze sequencing. I additional have designed, generated, and executed novel mouse models of cancer with assistance from the Genome Engineering and iPSC Core at Wash U and our collaborator Dr. Jeff Bednarski.

Collaborations: in addition to those listed above, we have established collaborations with additional investigators including those developing inhibitors of APOBEC enzymes (Dr. Rahul Kohli, UPenn and Dr. Min Xue, UC Irvine). These collaborations have yielded publications and additional projects.

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.

I participate as a faculty speaker in several student groups including the Wash U undergraduate cancer research club and the Wash U Summer Research Program in Pediatric Research. I am a faculty mentor in the American Cancer Society Diversity in Cancer Research program which recruits students underrepresented in science to experiences in cancer research. Through these forums, I interact with undergraduate and high school students interested in research careers.

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

I will continue to interact with interested students. I will also discuss my research when possible with patients, families, and pediatric cancer advocacy groups. For example, I will participate in Pedal the Cause, a local fundraiser aimed at generating awareness and support for cancer research.

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).

APOBEC3A is an enzyme that acts abnormally in cancer cells to cause mutations and damage to DNA. We found that a cellular protein complex, SMC5/6, is essential for the survival of cancer cells that have active APOBEC3A. This finding has generated two interesting lines of research: (1) how can SMC5/6 be targeted as cancer therapy, and (2) what is the mechanism by which SMC5/6 protects the genome from APOBEC3A-mediated damage. Our long-term goal is to use APOBEC3A activity as a biomarker to define patients whose tumors may be susceptible to targeting of SMC5/6.

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.

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.

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.

As described above, we have had technical problems generating mouse models of ETV6-RUNX1 expression and cell lines with BRCA1 and BRCA2 depletion. We have established a new mouse model using multiple engineered alleles, and are working with genome engineering experts to troubleshoot BRCA knockout.

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

None.

Significant changes in use or care of vertebrate animals

None.

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).*

Green AM, Rubenstein JD, Grimley M, Pfeiffer T (2023). Virus specific T cells for the treatment of systemic infections following allogeneic hematopoietic cell and solid organ transplantation. *J Ped Infect Dis Soc, In press*

Johnston R, Mathias B, Crowley S, Schmidt H, White L, Mosammaparast N, **Green AM**, Bednarski JJ (2022). Distinct DNA damage responses in B cells directed by nuclease-independent functions of RAG1. *EMBO Reports*, e55429.

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.*

Brazilian Society of Genetics 68th Annual Meeting, Ouro Preto, Brazil, September, 2023
Fundamental Aspects of DNA Repair and Mutagenesis, University of Sao Paulo, Brazil September, 2023
Environmental Toxicology Seminar Series, University of California Riverside, April, 2023
Genetic Medicine Colloquium, New York University, New York, NY, February, 2023
Deaminet – International Conference on Base Editing, Palm Springs, CA, January, 2023
Microbiology and Molecular Genetics Department Seminar Series, University of Vermont School of Medicine, Burlington, VT, November, 2022

- **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.

Our lab website is kept up to date regarding research projects, publications, and lab member activities: www.abbygreenlab.org

- **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

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.*

Cell lines and animal models as described above.

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:	*Abby Green*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-6436-2217*
Nearest person month worked:	*3.6*
Contribution to Project:	*Dr. Green conceives experimental plans, analyzes data, manages collaborations, presents the data, and writes manuscripts, grants, and abstracts.*
Funding Support:	*This award.*

Name:	*Jessica Devenport*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*6*
Contribution to Project:	*Ms. Devenport has developed mouse models of APOBEC3A-expression in hematopoietic cells.*
Funding Support:	*This award.*

Name:	*Rachel DeWeerd*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	0000-0001-9309-113X
Nearest person month worked:	*6*
Contribution to Project:	*Ms. DeWeerd performed all DT40-APOBEC3A experiments and analyzed sequencing. She is developing knockout cell lines.*
Funding Support:	*National Science Foundation Graduate Student Research Fellowship*

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.

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:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (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.*

Research Centre for Natural Sciences, Budapest, Hungary. Dr. David Szuts and his research team are collaborators on our research project. They provide computational analysis and advice regarding our genome sequencing.

Washington University, St. Louis, MO. Dr. Jeffrey Bednarski and his research team are collaborators and provide guidance and technical assistance for generation and evaluation of animal models.

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.