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TITLE: INHIBITION OF MICROBIAL BETA-GLUCURONIDASE AS A STARTEGY TOWARDS BREAST CANCER CHEMOPREVENTION

PRINCIPAL INVESTIGATOR: SRIDHAR MANI MD

CONTRACTING ORGANIZATION: ALBERT EINSTEIN COLLEGE OF MEDICINE, INC

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5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

SRIDHAR MANI

5d. PROJECT NUMBER

5e. TASK NUMBER

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ALBERT EINSTEIN COLLEGE OF
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13. SUPPLEMENTARY NOTES
none

14. ABSTRACT The current view of breast cancer development is that estrogen in its active form is a driver of the disease. It is now well established that the estrogens produced by our bodies are broken down and altered in our livers and intestines to what is termed as "conjugated metabolites". These conjugated metabolites are formed by the action of enzymes in our bodies that act to dispose of active estrogens from our bodies. In the intestines, however, a very important enzyme system made by bacteria called beta-glucuronidases (BGUS), converts the excreted "conjugated metabolites" back to active estrogen-like molecules. The newly formed molecules are reabsorbed by into our bloodstream. In this manner, we constantly keep a threshold of active estrogen. We hypothesize that if we block bacterial BGUS using very specific inhibitors (those that will not affect human BGUS enzymes), we could with maximum safety prevent converting excreted estrogen back to its active form. This, we believe, will prevent breast cancer by reducing the amount of active estrogen we reabsorb each day. This hypothesis while compelling has never been tested before. In 2010, our group in collaboration with the University of North Carolina (Redinbo Laboratory) was the first to report on a first-in-class *E. coli* specific BGUS inhibitor, which has low availability in the bloodstream (limited to the intestines) and not toxic to bacteria, mouse and human cells. We now have the technical ability to inhibit microbial BGUS enzymes in rodents that would allow us to test whether this strategy reduces the incidence and/or delays the occurrence of breast cancer in rodents. To test the concept, we have chosen a well-characterized mouse model of breast cancer, PyMT (Polyoma Middle T). In this model, the breast tissue progresses from normal to hyperplasia to adenoma in an estrogen-receptor-dependent manner. Our studies will conclusively (1) establish whether blocking BGUS actually reduces breast cancer incidence in a ER-dependent model of mouse cancer development (2) whether blocking BGUS actually increases survival in mice at risk for breast cancer, and, in doing so (3) establish whether BGUS has a mechanistic role in altering estrogen burden and thus preventing breast cancer.

15. SUBJECT TERMS
Breast Cancer, Polyoma Middle T, Bacterial beta-glucuronidase, microbe, inhibitors

16. SECURITY CLASSIFICATION OF:	**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
			USAMRDC

a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	29	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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The text of the report must include all sections addressed in the table of contents to include the following. **DO** include the bolded section headings, but **DO NOT** include the *italicized* descriptions of section contents in your submitted reports.

1. **INTRODUCTION:** The current view of breast cancer development is that estrogen in its active form is a driver of the disease. It is now well established that the estrogens produced by our bodies are broken down and altered in our livers and intestines to what is termed as “conjugated metabolites”. These conjugated metabolites are formed by the action of enzymes in our bodies that act to dispose of active estrogens from our bodies. In the intestines, however, a very important enzyme system made by bacteria called beta-glucuronidases (BGUS), converts the excreted “conjugated metabolites” back to active estrogen-like molecules. The newly formed molecules are reabsorbed by into our bloodstream. In this manner, we constantly keep a threshold of active estrogen. We hypothesize that if we block bacterial BGUS using very specific inhibitors

(those that will not affect human BGUS enzymes), we could with maximum safety prevent converting excreted estrogen back to its active form. This, we believe, will prevent breast cancer by reducing the amount of active estrogen we reabsorb each day. This hypothesis while compelling has never been tested before. In 2010, our group in collaboration with the University of North Carolina (Redinbo Laboratory) was the first to report on a first-in-class *E. coli* specific BGUS inhibitor, which has low availability in the bloodstream (limited to the intestines) and not toxic to bacteria, mouse and human cells. We now have the technical ability to inhibit microbial BGUS enzymes in rodents that would allow us to test whether this strategy reduces the incidence and/or delays the occurrence of breast cancer in rodents. To test the concept, we have chosen a well-characterized mouse model of breast cancer, PyMT (Polyoma Middle T). In this model, the breast tissue progresses from normal to hyperplasia to adenoma in an estrogen-receptor-dependent manner. Our studies will conclusively (1) establish whether blocking BGUS actually reduces breast cancer incidence in a ER-dependent model of mouse cancer development (2) whether blocking BGUS actually increases survival in mice at risk for breast cancer, and, in doing so (3) establish whether BGUS has a mechanistic role in altering estrogen burden and thus preventing breast cancer.

2. **KEYWORDS:** Breast Cancer, Polyoma Middle T, Bacterial beta-glucuconidase, microbe, inhibitors
3. **ACCOMPLISHMENTS:** In Year 3, accrual goals of n = 30 (9 week assessment) controls are completed. In addition, we have completed n = 130 (week assessments) for Inh9 treated mice.

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

Specific Aim 1(specified in proposal) **<u>Aim 1:</u> To characterize the effect of Inh1 on the incidence, pathology and time of onset of mammary hyperplasia-adenoma and survival in PyMT mice.**	**Timeline** 1-36 Months	**Site 1** Dr. Mani 75 mice/year
Major Task 1: To characterize the effect of Inh1 on the incidence, pathology and time of onset of mammary hyperplasia-adenoma and survival in PyMT mice :	Months	
Subtask 1: Approval and amendments of animal use protocol for the DOD grant; ACURO approvals included REPORT: Done	1-3	N/A

Subtask 1: Exploratory fecal BGUS activity in mice exposed to different doses of Inh1 in drinking water REPORT: Exploratory data obtained **SEE APPENDED DATA AND FIGURES**	1-2	10 or 15 mice used
Subtask 2 – (a): Analysis of mice exposed to control and Inh1 for ductal hyperplasia at end-point 2 weeks. All studies pertaining to pathology included in this window for analysis. Mice studies are done in a staggered fashion as they are bred. REPORT: Final report on Bioarchives & JBC (see publication below)	2-4	30 of 30 mice used
Subtask 3 - (a): Analysis of mice exposed to control and Inh1 for ductal hyperplasia at end-point 4 weeks. REPORT: Final report on Bioarchives & JBC (see publication below)	4-8	30 of 30 mice used
Subtask 4 – (a): Analysis of mice exposed to control and Inh1 for ductal hyperplasia at end-point 6 weeks. REPORT: Final report on Bioarchives & JBC (see publication below)	8-12	30 of 30 mice used
Subtask 5 – (b): Analysis of mice exposed to control and Inh1 for ductal adenoma at end-point 2 weeks. All studies pertaining to pathology included in this window for analysis. Mice studies are done in a staggered fashion as they are bred. REPORT: Final report on Bioarchives & JBC (see publication below)	1-3	30 of 30 mice used
Subtask 6 – (b): Analysis of mice exposed to control and Inh1 for ductal adenoma at end-point 8 weeks. REPORT: Final report on Bioarchives & JBC (see publication below)	3-6	30 of 30 mice used

- **What was accomplished under these goals?**
  - PLEASE SEE THE REPORT SECTION AND RESPONSE TO THE SOW
- **What opportunities for training and professional development has the project provided?**
  - NOTHING TO REPORT
  - Describe opportunities for training and professional development provided to anyone POSTDOCTORAL FELLOW LEARNED GAVAGE EXPERIMENTS, IACUC TRAINING, PREPARATION FO CARCASS FOR PATHOLOGY
- **How were the results disseminated to communities of interest?**
  - NOTHING TO REPORT
- **What do you plan to do during the next reporting period to accomplish the goals?**
  - NOTHING TO REPORT

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?**
  - NOTHING TO REPORT
- **What was the impact on other disciplines?**
  - NOTHING TO REPORT
- **What was the impact on technology transfer?**
  - NOTHING TO REPORT
- **What was the impact on society beyond science and technology?**
  - NOTHING TO REPORT

5. **CHANGES/PROBLEMS:** *The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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**
  - There are no significant changes in scope or objectives.
- **Actual or anticipated problems or delays and actions or plans to resolve them**
  - PyMT mice are very environment sensitive and breeding can be erratic. Thus synchornizing mice for proximal time-points as noted in the SOW is unpredictable. Thus,

we completed the distal time point of 9 weeks first as that could be done and are on our way to completing the 2-8 week time point assessments. While the order of the SOW subtasks were changed due to this reason, each subtask as noted will be completed and the overall direction of the project has not changed.

- **Changes that had a significant impact on expenditures**
  - There was a delay in the hire of a partial technical help which is now resolved.
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  - Grant awarded start date 4/ 2017. Final ACURO approval obtained 12/21/2016. Inh9 amendment approved by Einstein 3/3/2017. ACURO approved amendment on 3/6/2017. Animal breeding started after 3/6/2017 in April after index mice were transferred from Breast Cancer Core to our mouse space.
- **Significant changes in use or care of human subjects N/A**
- **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:** Nothing to Report

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

  Report only the major publication(s) resulting from the work under this award.
  - **Journal publications.** Nothing to Report
  - **Books or other non-periodical, one-time publications.** Nothing to Report
  - **Other publications, conference papers, and presentations.**

    (Beck et al. 2019). This is currently in review in Scientific Reports

    (Ervin et al. 2019)

- **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?**

- *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."*

Name:	Sridhar Mani
Project Role:	Postdoctoral fellow
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2.4
Contribution to Project:	Dr. Mani is the PI and has planned all experiments and did troubleshooting guidance with Dr. Hao
Funding Support:	DOD
Name:	Hao Li
Project Role:	Postdoctoral fellow
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Dr. Hao has performed work involving animal breeding and carcass preparation
Funding Support:	DOD

- **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
    - **Organization Name:**
    - **Location of Organization:** *(if foreign location list country)*
    - **Partner's contribution to the projec**t *(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.**

8. **SPECIAL REPORTING REQUIREMENTS** N/A

- **COLLABORATIVE AWARDS:** *For collaborative awards, independent reports are required from **BOTH** the Initiating 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://ers.amedd.army.mil for each unique award.*
- **QUAD CHARTS:** *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) 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. Reminder: Pages shall be consecutively numbered throughout the report.* ***DO NOT RENUMBER PAGES IN THE APPENDICES.***

References:

Beck, Amanda P., Hao Li, Samantha M. Ervin, Matthew R. Redinbo, and Sridhar Mani. 2019. 'Inhibition of Microbial Beta-Glucuronidase Does Not Prevent Breast Carcinogenesis in the Polyoma Middle T Mouse', *bioRxiv*: 746602.

Ervin, S. M., H. Li, L. Lim, L. R. Roberts, X. Liang, S. Mani, and M. R. Redinbo. 2019. 'Gut microbial β-glucuronidases reactivate estrogens as components of the estrobolome that reactivate estrogens', *J Biol Chem*, 294: 18586-99.