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TITLE: The Role of the Gut Microbiome in Colorectal Cancer

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CONTRACTING ORGANIZATION: Temple University-Of the Commonwealth System of Higher Education

Philadelphia, PA 19140

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14. ABSTRACT

Inflammatory bowel disease (IBD) is a chronic condition of the gastrointestinal tract that predisposes individuals to develop CRC. Chronic inflammation is one of the key hallmarks of cancer and dysbiosis of the gut microbiome is proposed to promote CRC. The prevalence of IBD has increased 2- to 3-fold among veterans. The objective of this proposal is to utilize Il10-/- mice, a model of human IBD, together with Stat2-/- mice, which are more resistant to CRC to: 1) identify unique microbial communities in the gut and 2) metabolites of bacterial and host origin that mediate anti-inflammatory and antitumor effects to control inflammation and drastically reduce the risk of CRC development.

15. SUBJECT TERMS- NOTHING LISTED

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a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
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1. INTRODUCTION:

Inflammatory bowel disease (IBD) is a chronic condition of the gastrointestinal tract that predisposes individuals to develop CRC. Chronic inflammation is one of the key hallmarks of cancer and dysbiosis of the gut microbiome is proposed to promote CRC. The prevalence of IBD has increased 2- to 3-fold among veterans. The objective of this proposal is to utilize Il10-/- mice, a model of human IBD, together with Stat2-/- mice, which are more resistant to CRC to: 1) identify unique microbial communities in the gut and 2) metabolites of bacterial and host origin that mediate anti-inflammatory and antitumor effects to control inflammation and drastically reduce the risk of CRC development.

2. KEYWORDS:

Colorectal, cancer, microbiota, dysbiosis, colitis, chronic, IBD, STAT2 and Il10

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.

In Year 1, the major goals for Specific Aims 1 and 2; Major Task 1 (Subtask 1), were to establish a breeding colony of specific-pathogen free (SPF) mice of the following genotypes: wild type, Il10-/- , Stat2-/- and *Il10;Stat2* double knockout (dKO). In Aim 1, Major Task 1 (Subtask2), the goal was to start monitoring colitis and collection of fecal pellets starting at 4-weeks of age. This activity has just begun with wild type and *Il10-/-* mice. Establishment of SPF *Stat2-/-* and *Il10;Stat2* dKO mouse colonies, however, has not been initiated as Stat2-/- mice are being rederived at Fox Chase Cancer Center. This task is 50% completed. Major goals for Aims 2 and 3; Major Tasks 2 and 3 are not to start until year 2.

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.

The major activities of our project in Year 1 were to establish specific-pathogen free (SPF) mouse breeding colonies consisting of wild type, single and double *Il10* and *Stat2 KO* mice to be used for Specific Aims 1-2 (Major Task1/Subtask 1 in both aims). This Major Task is in progress. While breeding colonies of wild type and Il10-/- mice have been successfully established in our SPF animal room, we are still waiting for rederivation of *Stat2-/-* mice to be completed, which will allow us to expand this mouse colony and cross these mice to *Il10-/-* mice. The next activity for Year1 extends only to specific Aim 1 (Major Task1/Subtask2-3). We recently weaned mice wild type and Il10-/- mice and these mice are being monitored for colitis and fecal samples and tissues are to be collected starting at 4-weeks of age. No results to report at this moment. Other major tasks of this project are to be started in Year 2.

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.

Nothing to Report

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 adhere to the SOW and as we continue developing the project as outlined in the proposal.

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).

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

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.

We have encountered a delay in generating SPF Stat2-/- mice and consequently *Il10;Stat2* dKO mice. Stat2-/- mice were, at first, slow to reproduce for re-derivation purposes at the transgenic animal facility of Fox Chase Cancer Center. This problem has now been resolved and we are confident that these mice will become available in the coming months to continue with the breeding plan. In the unlikely event SPF Stat2-/- mice are not produced, we have devised two strategies. The first approach is to generate heterozygote Stat2 mice for rederivation, which is already in progress. The second approach is to use our existing Stat2-/- mouse colony housed in our conventional animal room. Note that these mice carry helicobacter and norovirus. Our preliminary studies show that conventional, non-SPF Stat2-/- mice are less prone to chemically-induced acute colitis thus making continuation of the proposed study entirely feasible.

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

Nothing to Report

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 or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

No significant changes 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

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.)*

Ana Gamero (PI): No change

Cagla Tukel (Collaborating PI): No change

Tess Cremers
Role: Lab Technician
Nearest person month worked: 10
Contribution to Project: Ms. Cremers has helped establish mouse breeding colonies and instituted protocols for verifying mouse genotypes.

Dorret Lynch
Role: Senior Lab Manager
Nearest person month worked: 4
Contribution to Project: Ms. Lynch handles the general maintenance of the lab and animal colonies. She brings her experience in molecular cell biology to 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.

Completed:
NIH R03CA215929-01 awarded to Dr. Gamero (PI) has closed in March.

New Active Grant:
NIH R21 A1137541-A1 was awarded to Dr. Tukel (PI) in July 2018 with Dr. Gamero is Co-I.
Title: Epithelial type I interferon signaling in Salmonella typhimurium infection
Grant provides 10% of salary support to Dr. Gamero and no additional funds.
No Overlap

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:
The tasks to be executed at the site of Collaborating PI do not take place until Year 2 of the grant. However, she has been consulted throughout Year 1 in preparation for completing Major Tasks in grant.

QUAD CHARTS:
Not Applicable

9. APPENDICES: *NOTHING TO REPORT*

CA170751: The role of the gut microbiome in colorectal cancer

PI: Ana Gamero, Temple University

Budget: $634,000 **Topic Area:** Colon cancer **Mechanism:** FY17, PRCRP, Idea Award with Special Focus

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Research Area: Refer to Scientific Classification System (0412, 0400) **Award Status:** July 15, 2018– July 14, 2020

Study Goals:

The **objective** of this proposal is to utilize *Il10-/- mice*, a proven model of human IBD, together with *Stat2-/-* mice, which are more resistant to CRC to *1) Identify genetics factors that play a role in modulating the composition of the intestinal microbiome with the intent to 2) identify microbial communities that can attenuate inflammation, lower the incidence of CRC and reduce the mortality associated with metastatic disease.*

Specific Aims:

Aim 1: Determine *in vivo* the role of STAT2 as a genetic factor in shaping the intestinal microbial composition before and during inflammation.

Aim 2: Conduct *in vivo* studies to determine the contribution of STAT2 in modulating the composition of the gut microbiome in early and after the onset of CRC.

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Key Accomplishments:

Publications: None to date
Patents: None to date
Funding Obtained: None to date