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TITLE: Invention of a Genetic Toolkit for Immunomodulatory Gut Bacteria to Expedite the Development of New Crohn's Disease Therapeutics

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TABLE OF CONTENTS

	<u>Page</u>
1. Introduction	4
2. Keywords	4
3. Accomplishments	4
4. Impact	7
5. Changes/Problems	8
6. Products	10
7. Participants & Other Collaborating Organizations	12
8. Special Reporting Requirements	13
9. Appendices	14

1. INTRODUCTION:

Crohn’s disease (CD) is a chronic and incurable disease characterized by episodic and disabling inflammation of the gut. It is increasingly recognized the gut microbiota regulates the tenor of host intestinal mucosal immunity and the predisposition to inflammation. However, this knowledge has not yet translated into strategies that are effective in preventing or treating CD. The goal of this project is to develop genetic approaches to expedite the discovery of novel natural NF-κB suppressive bioactives from fastidious gut bacteria, and develop proof-of-principle they can suppress CD-like inflammation using in vitro and human ex vivo models.

2. KEYWORDS:

Crohn’s disease, microbiome, bioactives, NF-κB

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(specified in proposal)	Timeline	Site 1
Major Task 1: Isolate genetically tractable human gut bacteria affiliated with the Firmicutes	Months	
Use our state-of-the-art facilities for anaerobic microbiology to isolate genetically tractable fastidious gut bacteria.	21-27	Dr. Jakob Begun, Páraic Ó Cuív & Prof Mark Morrison (fecal samples already collected with UQ HREC approval)
Major Task 2: Identify human gut bacteria which produce bioactive factors that suppress NF-κB in immune (RAW 264.7 macrophage) and epithelial (Caco-2) cell lines	Months	
Apply to the DoD Office of Research Protection HRPO for approval to use the human Caco-2 epithelial cell line for secondary research. Complete: Approval Memorandum dated Feb 2, 2018	1-8	Dr. Jakob Begun & Dr. Páraic Ó Cuív
Apply to The University of Queensland Diamantina Institute to have the Caco-2 epithelial cell lines declared as exempt Complete: Approval Memorandum dated Feb 2, 2018	1-8	Dr. Jakob Begun & Dr. Páraic Ó Cuív

Use RAW 264.7 macrophage and Caco-2 gut epithelial cell based functional assays to identify bacteria secreting NF- κ B suppressive bioactives	23-29	Dr. Jakob Begun, Dr. Páraic Ó Cuív,, Prof Michael McGuckin & Prof Mark Morrison
Milestone Achieved: Complete Aim1	29	
Specific Aim 2		
Major Task 1: Assess the ability of the bioactive factors to suppress the Crohn's disease (CD) inflammatory response		
Apply to the DoD Office of Research Protection HRPO for approval to use the ileal biopsies collected to support this study for secondary research. Complete: Approval Memorandum dated Feb 2, 2018	1-8	Dr. Jakob Begun & Dr. Páraic Ó Cuív
Recruit and collect ileal biopsy samples from healthy human males (n=12) and male CD subjects (n=12, age of onset 18-30 years)	1-10	Dr. Jakob Begun (HREC approval already in place)
Determine the extent of NF- κ B suppressive activity of the bioactives in healthy and CD derived peripheral blood mononuclear cells and gut epithelial enteroids	28-32	Dr. Jakob Begun & Prof Michael McGuckin
Milestone Achieved: Complete Aim 2	32	
Specific Aim 3		
Major Task 1: Develop and apply genetic approaches to expedite the discovery of NF- κ B suppressive bioactives		
Use a combination of genomic and reverse/forward genetic approaches to identify bacterial pathways underpinning the production of NF- κ B suppressive secondary metabolites in select strains	29-38	Dr. Jakob Begun, Dr. Páraic Ó Cuív, Prof Michael McGuckin & Prof Mark Morrison
Use functional genomics to identify NF- κ B suppressive peptide bioactive factors	32-38	Dr. Jakob Begun, Dr. Páraic Ó Cuív, Prof Michael McGuckin & Prof Mark Morrison
Milestone(s) Achieved: Prepare and submit paper	34-38	Dr. Páraic Ó Cuív
Milestone(s) Achieved: Complete Aim 3	38	

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.

Approval from the HRPO and UQ-DI for the use of human Caco-2 epithelial cell line has been obtained. Recruitment and collection of ileal biopsies from healthy controls and CD patients has been completed (n=24). Bacterial supernatants have been isolated from human stool sample.

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.

Over the next reporting period the team will focus on the functional in-vitro assays to identify bacterial supernatants that secrete NF- κ B suppressive bioactives using Caco-2 epithelial cells and samples (peripheral blood mononuclear cells and gut epithelial enteroids) derived from patients with Crohn's disease and healthy controls.

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:*

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.

After delays in establishing the grant at the University of Queensland (UQ), including obtaining second tier approval of ethics from the HRPO and administrative delays at UQ, followed by the departure of original PI Dr. Paraic O Cuiv from UQ, the UQ Office of Sponsored Research requested a transfer of the grant from Dr. O Cuiv to Dr. Jake Begun which was approved by the USAMRAA on 27 March 2019. We are now awaiting HRPO continuing review approval of local human ethics approval, documentation of which was submitted to the HRPO on 3 June 2019 and will be able to re-commence human subjects research once we receive approval from the HRPO.

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”.

Name: Rabina Giri

Project Role: PhD student

Nearest person month worked: 1

Contribution to the project:

Ms Giri has collected, archived, and stored patient derived samples. Ms Giri has also isolated different bacterial strains from a human stool sample and prepared the bacterial supernatants and tested those on Caco-2 epithelial cells in vitro.

Funding Support: UQ PhD Scholarship

Name: Jakob Begun

Project Role: Principle Investigator

Nearest person month worked: 1

Contribution to the project:

Dr Begun has been responsible for patient recruitment and obtaining of biopsy samples. His other involvement included but was not limited to project supervision, data interpretation and manuscript preparation.

Funding Support: Reginald Ferguson 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.

The PI has changed from Dr. Paraic O’Cuiv to Dr. Jakob Begun. Dr. Paraic O Cuiv remains involved in the project as a consultant.

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.*

*Organization Name:*Mater Misericordiae Ltd (MML)

Location of Organization: Australia

Partner’s contribution to the project: Dr. Jakob Begun’s laboratory is supported by the Mater Research Institute, and patient samples are procured from patients at the Mater Hospital Brisbane

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: N/A

9. APPENDICES: *N/A*