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**TITLE:** A Multidisciplinary Approach to Study the Role of the Gut Microbiome in Relapsing and Progressive MS

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# REPORT DOCUMENTATION PAGE

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<b>13. SUPPLEMENTARY NOTES</b>						
<b>14. ABSTRACT</b> <i>While this contract was awarded in January 2015, it was finally approved in April 2016. Thus, all activities described in this Annual Report correspond to activities performed between April and September 2016.</i> Despite these challenges, the project is now well underway and on track. A summary of accomplishments to date include: HRPO approval, Chart reviews to identify eligible subjects for this study at UCSF and Mt Sinai, Clinical evaluation and invitations to participate, mailing kits have been designed, prepared and started to being distributed to eligible participants, bacterial DNA is being purified from stool samples, quality control is being performed and Tob1/2D2 mice are currently being derived germ-free. These activities encompass most of the items described in the original SOW. Finally, while expenses have been posted according to the delayed start, the project is financially on track.						
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## 1. INTRODUCTION

We hypothesize that specific human GI microbiota can alter the balance of inflammatory and regulatory immune cell populations thus leading to disease in genetically susceptible hosts. Furthermore, we hypothesize that gut microbiota from subjects with RMS and PPMS is fundamentally different and can elicit distinguishable effects when transferred into susceptible animal models of the disease.

## 2. KEYWORDS

Microbiome  
Multiple sclerosis  
Primary Progressive  
Relapsing Remitting  
Knockout mice  
Bioinformatics  
Immunology

## 3. ACCOMPLISHMENTS

**3a. Major goals of the project:** This project has two major goals or aims. In the SOW, each Aim was subdivided into Major Tasks and subtasks:

**Aim#1: To compare the gut microbiome of subjects with RMS and PPMS.**

Major Task 1: To seek and obtain HRPO approval

Major Task 2: Identification and recruitment of research subjects

Subtask 1: Perform Chart reviews to identify eligible patients from MS clinic at UCSF and Mt Sinai.

Subtask 2: Clinical evaluation and invitation to participate in the study

Major Task 3: Sample collection and initial processing

Subtask 1: Preparation of collection mailing kits

Subtask 2: Bacterial DNA extraction from stool material

Subtask 3: Genotyping and HLA characterization of host DNA.

*Milestone #1: Recruitment and processing samples from 150 RMS, 150 PPMS and 150 healthy controls.*

Major Task 4: 16S ribosomal gene sequencing and initial bioinformatics analysis.

Subtask 1: sequencing of 16S ribosomal RNA gene in all DNA samples from MS patients and controls

*Milestone #2: Sequencing of the MS microbiome.*

Major Task 5: Data integration and advanced bioinformatics analysis.

Subtask 1: Integration of microbiome and genomic data

**Aim#2: To test the effect of human MS microbiota in spontaneous and induced experimental models.**

Major Task 1: microbiota transfer into germ-free mice and EAE induction

Subtask 1: re-derivation of Tob1/2D2 mice into a GF line  
Subtask 2: Transfer of live microbiota from select patients into germ-free mice, EAE induction and follow-up

Major Task 2: Immuno-pathological characterization of experimental mice

Subtask 1: tissue dissection, harvesting and pathological analysis

Subtask 2: Flow cytometry

Subtask 3: Immunohistochemistry

Subtask 4: Molecular characterization

*Milestone #3: Co-authored manuscript*

### **3b. Accomplishments to date:**

Subject recruitment began at both Mt. Sinai and UCSF. Mt. Sinai recruited a total of 17 subjects: 8 with relapsing remitting MS, 2 with primary progressive MS and 7 unaffected controls. Blood samples were collected for all 17 subjects and these subjects completed their clinical visits and questionnaires. Stool samples were received for 5/8 of the RRMS, 2/2 of the PPMS, and 4/7 controls. The Mt. Sinai research coordinator is following up on receiving the rest. Additional subjects are in the being screened.

UCSF recruited a total of 64 subjects: 25 with relapsing remitting MS, 7 with primary progressive MS and 32 controls. Blood and stool samples were collected from all subjects and all subjects completed their clinic visits. Case report questionnaires are either completed or in progress for all subjects.

Some reasons for recruitment being somewhat slower than anticipated have to do with the initial delays in contracting, the study starting during the summer months, and essential study staff being unavailable due to planned leave. We anticipate that the recruitment rate will improve now that the study is up and running and will readdress potential recruitment challenges if recruitment is less than expected at the time of the next progress report.

Additional Accomplishments:

- HRPO approval has been obtained (Specific Aim 1. Major Task 1)
- Chart reviews are being conducted to identify eligible subjects for this study at UCSF and Mt Sinai (Specific Aim 1. Major Task 2. Subtask 1).
- Clinical evaluation and invitations to participate are being conducted (Specific Aim 1. Major Task 2. Subtask 2).
- Mailing kits have been designed, prepared and started to being distributed to eligible participants (Specific Aim 1. Major Task 3. Subtask 1).
- Bacterial DNA is being purified from stool samples. Quality control is being performed (Specific Aim 1. Major Task 3. Subtask 2)
- Tob1/2D2 mice are currently being derived germ-free (Specific Aim 2. Major Task 1. Subtask 1)

### **3c. Opportunities for training and professional development**

While it is expected that participating lab members engage in exchange of ideas, methodologies and training, the short period covered by this report did not allow these

activities to fully take place. We anticipate more training activities to take place in the second period.

**3d. Dissemination of results to communities of interest**

Nothing to report. The short period covered by this report did not allow these activities to fully take place. We anticipate more dissemination of results to take place in the second period.

**3e. Plans for accomplishing project goals during the next reporting period**

Despite a slow start, the project is well underway. We do not expect significant hurdles for the upcoming period.

**4. IMPACT**

**4a. Impact on the development of the principal discipline(s) of the project**

Nothing to report

**4b. impact on other disciplines**

Nothing to report

**4c. impact on technology transfer**

Nothing to report

**4d. impact on society beyond science and technology**

Nothing to report

**5. CHANGES/PROBLEMS**

**5a. Changes in approach and reasons for change**

Nothing to report

**5b. Actual or anticipated problems or delays and actions or plans to resolve them**

Aside from the late start, no other significant problems/issues have been identified. An anticipated major challenge will be recruitment of the 150 primary progressive MS subjects. Already it is apparent that due to the relative scarcity of this phenotype, recruitment for the relapsing remitting subjects will complete prior to ascertainment of the primary progressive subjects. Strategies for identifying and targeting primary progressive MS patients for recruitment based on medical record review are being implemented.

**5c. Changes that had a significant impact on expenditures**

Nothing to report

**5d. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to report

**6. PRODUCTS**

**6a. Publications, conference papers, and presentations**

Nothing to report

**6b. Website(s) or other Internet site(s)**

Nothing to report

**6c. Technologies or techniques**

Nothing to report

**6d. Inventions, patent applications, and/or licenses**

Nothing to report

**6e. Other Products (Reportable outcomes)**

Aim 1 is in its recruiting phase. No reportable outcome at this time.

Aim 2 is in progress (animals are being re-derived). No reportable outcome at this time.

**7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

**7a. Individuals working on the project**

<b>Personnel</b>	<b>Project PI</b>	<b>Role</b>	<b>Nearest person Month</b>	<b>Contribution</b>
Sergio Baranzini	Baranzini	PI	1	Project overview
Bruce Cree	Cree	PI	1	Clinical PI
Sneha Singh	Cree	Coordinator	6	Clinical coordinator
Adam Santaniello	Cree	Database manager	1	Set up project database
Rob Knight	Knight	PI	1	Technical development PI
Evguenia Kopylova	Knight	Post Doc	4	Development of fast algorithms for OTU picking

				that will be used in the 16S analysis and fast matching for separating human reads from microbial that will be used in the shotgun metagenomics.
Tomasz Piotr Kosciolk	Knight	Post Doc	3	Development of methods to identify ncRNAs in the genomic and metagenomic data.
Luke Thompson	Knight	Post Doc	6	Development of metagenomic processing pipeline.
Zhenjiang "Zech" Xu	Knight	Post Doc	1	Development of genome annotation pipeline.
Gail Lesley Ackermann	Knight	Staff Research Assoc, Metadata	2	Metadata curation.
Jeffrey E Dereus	Knight	Programmer Analyst	3	Management of the software team, setup of project tracking and LIMS for this project.
Antonio Gonzalez Pena	Knight	Programmer Analyst	5	Development of Qiita for multi-omics integration, including setup and integration of the metagenomics and metabolomics components in Qiita.
Karenina F Sanders	Knight	Lab Asst	3	Benchmarking of laboratory protocols for efficient DNA extraction for this project
Joshua Shorenstein	Knight	Jr Specialist	2	User interface development to make algorithms more accessible in Qiita.

**7b. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

**7c. What other organizations were involved as partners?**

**Partner (sub-contract) 1**

**Organization Name:** California Institute of Technology

**Location of Organization:** Pasadena, CA

**Partner's contribution to the project** (identify one or more): Germ-free mouse experiments



**Partner (sub-contract) 2**

**Organization Name:** Icahn School of Medicine at Mount Sinai

**Location of Organization:** New York, NY

**Partner's contribution to the project** (identify one or more): Patient recruitment

**8. SPECIAL REPORTING REQUIREMENTS:**

This is a Collaborative award (3 Principal Investigators).

As approved by the Program official assigned to this project (Amie Bunker) the same report is being submitted under each PI's account.