AWARD NUMBER: W81XWH-15-1-0320

TITLE: Oral Metagenomic Biomarkers in Rheumatoid Arthritis

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CONTRACTING ORGANIZATION: University of Florida
Gainesville, FL 32611

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14. ABSTRACT

There is no change in Specific Aims from the original proposal. We are at the phase of recruiting patients and collecting samples. To date, 77 subjects have been enrolled out of the expected 100 total subjects. 19 of the 77 subjects have completed the study (i.e. necessary samples collected). The delay in enrollment of subjects is primarily from the unexpected extra time (~ 1 year) taken for IRB approval for the entire project involving the VA system and University of Florida. No sequencing data has been collected to date.

15. SUBJECT TERMS

Enrollment, data collection

16. SECURITY CLASSIFICATION OF:

<table>
<thead>
<tr>
<th>a. REPORT</th>
<th>b. ABSTRACT</th>
<th>c. THIS PAGE</th>
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</table>

17. LIMITATION OF ABSTRACT

UU

18. NUMBER OF PAGES

10

19a. NAME OF RESPONSIBLE PERSON

USAMRMC

19b. TELEPHONE NUMBER

(Include area code)
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1. INTRODUCTION

The objectives of our study are to determine whether there is significant difference in the oral microbiome at the subspecies level of individuals with rheumatoid arthritis (RA). The goal is to test the hypothesis that oral microbiome and metagenomic analyses will allow us to identify new biomarkers that are useful for the diagnosis of early RA and/or biomarkers that help to predict the efficacy of specific therapeutic interventions. If a patient’s oral microbiome is causally related to RA, then this information could lead to the development of novel treatment strategies that target the microbiome or genes associated with that microbiome. The overall hypothesis is that oral microbial variation exists at both the structural and functional levels among patients that influence development and characteristics of RA. Two groups of subjects are to be enrolled in this study: 1) 25 RA adult patients “naïve to biologics” compared to 25 paired healthy controls from age-matched members of the same household, 2) 25 RA patients responsive to anti-TNFα therapy versus 25 who are resistant. Oral subgingival plaque samples are to be collected, and sequencing performed for the 16S RNA microbiome analysis as well as whole genome shotgun sequencing. Upon completion of these aims, any identified bacterial biomarkers may be developed as drug targets for disease treatment in future studies.

2. KEYWORDS

rheumatoid arthritis, microbiome, metagenomics

3. ACCOMPLISHMENTS

What were the major goals of the project?

The major goal is to test the hypothesis that oral microbiome and metagenomic analyses will allow us to identify new biomarkers that are useful for the diagnosis of early RA and/or biomarkers that help to predict the efficacy of specific therapeutic interventions.

Below is the SOW as outlined in our proposal:

<table>
<thead>
<tr>
<th>Specific Aim 1 (specified in proposal)</th>
<th>Timeline</th>
<th>Site 1</th>
<th>Site 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major Task 1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Local IRB Approval</td>
<td>1-2</td>
<td>Drs. Chan/Bubb/Nascimento</td>
<td>Dr. Bubb</td>
</tr>
<tr>
<td>Subtask 1. Recruitment of subjects</td>
<td>2-18</td>
<td>Drs. Bubb/Nascimento</td>
<td>Dr. Bubb</td>
</tr>
<tr>
<td>Subtask 2. Collection of oral DNA samples</td>
<td>2-18</td>
<td>Dr. Nascimento</td>
<td></td>
</tr>
<tr>
<td>Subtask 3. Deep sequencing and 16S RNA analyses</td>
<td>2-18</td>
<td>Dr. Wang</td>
<td></td>
</tr>
<tr>
<td>Subtask 4. 16S data correlation with Group 1 and 2</td>
<td>4-18</td>
<td>Drs. Chan/Bubb</td>
<td></td>
</tr>
<tr>
<td>Specific Aim 2 (specified in proposal)</td>
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<td></td>
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<tr>
<td>Major Task 2</td>
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<tr>
<td>Subtask 1. Metagenome analyses</td>
<td>2-18</td>
<td>Drs. Wang/Chan/ Progulske-Fox</td>
<td></td>
</tr>
<tr>
<td>Subtask 2. Metagenomic biomarkers correlation with Group 1 and 2</td>
<td>4-18</td>
<td>Drs. Chan/Bubb/ Progulske-Fox</td>
<td></td>
</tr>
</tbody>
</table>
What was accomplished under these goals?

Major task 1:

*Local IRB approval:* UF IRB approval completed 12/11/2015

DoD IRB approval completed 4/14/2016

*Subtask 1: Recruitment of subjects.*

Status is as summarized in the table below. To date, 77 subjects enrolled with 19 completed the study. Plaque samples collected have been analyzed to ensure high quality of DNA samples can be extracted for deep sequencing. We anticipate the project will run well from this point onward.

<table>
<thead>
<tr>
<th>NUMBER OF SUBJECTS</th>
<th>Group 1&gt; (Naive)</th>
<th>Group 2&gt; (Control)</th>
<th>Group 3&gt; (TNF-Responsive)</th>
<th>Group 4&gt; (TNF Non-Resp.)</th>
<th>TOTALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENROLLED</td>
<td>17</td>
<td>17</td>
<td>29</td>
<td>14</td>
<td>77</td>
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<tr>
<td>COMPLETED STUDY</td>
<td>3</td>
<td>3</td>
<td>10</td>
<td>3</td>
<td>19</td>
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<tr>
<td>SCHEDULED</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>18</td>
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<tr>
<td>WAITING TO SCHEDULE</td>
<td>7</td>
<td>7</td>
<td>8</td>
<td>7</td>
<td>29</td>
</tr>
<tr>
<td>WITHDRAWN FROM STUDY</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>1</td>
<td>11</td>
</tr>
</tbody>
</table>

*Subtask 2. Collection of oral DNA samples*

As shown in table above, 19 completed out of 100 needed.

*Subtask 3. Deep sequencing and 16S RNA analysis*

This is ongoing but only limited, as not all samples collected.

*Subtask 4. 16S data correlation*

This has not begun as samples collection is incomplete to date.

Major task 2:

*Subtask 1. Metagenome analyses*

This has not begun as samples collection is incomplete to date.

*Subtask 2. Metagenomic biomarkers correlation*

This has not begun as samples collection is incomplete to date.
What opportunities for training and professional development has the project provided?

Nothing to Report.

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?

We focus on enrollment and collection of samples. Coordinators are now aware of the delays as discussed below because some of the patients are living more than 50 miles from our site. Quality controls are being taken care of to ensure the samples collected are of high quality suitable for deep sequencing. We do not anticipate additional problem in completing our objectives.

4. IMPACT

What was the impact on the development of the principal discipline 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

Change in approach and reasons for change

This is no change to our proposed study design.

The initial problem was the delay in getting IRB approval. This delay was beyond our control. The process involved first IRB approval at the VA system and then the process could move forward to IRB01
at the University of Florida (UF). Upon UF IRB approval, the process involved cycling back to VA IRB for final approval. This extend process was raised to our university administrators. As of ~6 months ago, they began a trial process to have a monthly joint IRB meeting to consider all projects that involved both UF and VA IRB. As a result of our study/complaint, future DoD projects involving both VA and UF IRB may face a shorter approval process.

**Actual or anticipate problems or delays and actions or plans to resolve them**

One unanticipated problem was the coordination in recruiting subjects. As subjects are identified as RA patients in the rheumatology clinic at UF and VA and are enrolled right there. These enrolled subjects are asked to make an appointment at our university dental clinic to get their oral health checked and samples collected at that time. As a number of patients live more than 50 miles away, they are not willing to come back to our dental facility until their next visit to the rheumatology clinic and that would be normally 2-3 months later. We have made extra efforts to ensure that the majority of enrolled patients will come back to finish up. So effectively, there is a ~2-3 month delay needed to our completion.

**Changes that had a significant impact on expenditures**

Nothing to Report.

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

Nothing to Report.

**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 biohazards, and/or select agents**

Nothing to Report.
6. **Products**

**Publications, conference papers, and presentations**

Nothing to Report.

**Journal publications**

Nothing to Report.

**Books or other non-periodical, one-time publications**

Nothing to Report.

**Other publications, conference papers, and presentations**

Nothing to Report.

**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?

<table>
<thead>
<tr>
<th>Name</th>
<th>Edward K. L. Chan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Role:</td>
<td>PI</td>
</tr>
<tr>
<td>Researcher Identifier:</td>
<td>ORCID ID: 0000-0003-3938-9503</td>
</tr>
<tr>
<td>Nearest person month</td>
<td>1-2</td>
</tr>
<tr>
<td>worked:</td>
<td></td>
</tr>
<tr>
<td>Contribution to Project:</td>
<td>Coordinate and oversee the entire project</td>
</tr>
<tr>
<td>Funding Support:</td>
<td>No other support for this project</td>
</tr>
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<table>
<thead>
<tr>
<th>Name</th>
<th>Michael R. Bubb</th>
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<tbody>
<tr>
<td>Project Role:</td>
<td>Co-PI</td>
</tr>
<tr>
<td>Researcher Identifier:</td>
<td>ORCID ID: None</td>
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<tr>
<td>Nearest person month</td>
<td>2</td>
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<tr>
<td>worked:</td>
<td></td>
</tr>
<tr>
<td>Contribution to Project:</td>
<td>Recruitment of subjects from rheumatology clinic</td>
</tr>
<tr>
<td>Funding Support:</td>
<td>No other support for this project</td>
</tr>
</tbody>
</table>

Ann Progulske-Fox, PhD, co-Investigator – work less than 1 person month per year

Marcelle Nascimento, DDS, MS, PhD, co-Investigator – work less than 1 person month per year

Gary P. Wang, MD, PhD, co-Investigator – work less than 1 person month per year

S. John Calise, OPS technician – work less than 1 person month per year

Justin Nicholas, OPS technician – work less than 1 person month per year

Jacob Burks, Study coordinator – work less than 1 person month per year

Renita Jenkins, Study coordinator – work less than 1 person month per year

Reuben Judd, Study coordinator – work less than 1 person month per year

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

We have not asked for or received any other new support related to this project.

New Active Support (Chan, Edward K.L., PI)

a) Virtual Consortium for Translational/Transdisciplinary Environmental Research (ViCTER). Subproject title: Influence of Innate Immunity on Xenobiotic-Induced Systemic Autoimmunity; R01 ES021464-02S1.

b) NIEHS, NIH.

c) Goal to examine microRNA associated with xenobiotic-induced autoimmune mouse
model.

d) Specific Aim #1 Differentiating innate immune response pathways in xenobiotic-induced autoimmunity using affiliated miRNAs; Aim #2 will define miRNA-mRNA interaction in xenobiotic-induced autoimmunity.


f) 15% effort as investigator.

g) Michael C Humble, NIEHS, NIH.

New Active Support (Bubb, Michael, co-PI)

Nothing to Report.

What other organizations were involved as partners?

Nothing to Report.

8. Special Reporting Requirements

Collaborative Awards:

Not applicable

Quad charts

Not applicable

9. Appendices

None