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TITLE: Oral Metagenomic Biomarkers in Rheumatoid Arthritis

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13. SUPPLEMENTARY NOTES					
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					
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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 house hold, 2) 25 RA patients responsive to anti-TNF α 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:

Specific Aim 1(specified in proposal)	Timeline	Site 1	Site 2
Major Task 1	Months		
Local IRB Approval	1-2	Drs.Chan/Bubb/Nascimento	Dr. Bubb
Subtask 1. Recruitment of subjects	2-18	Drs. Bubb/Nascimento	Dr. Bubb
Subtask 2. Collection of oral DNA samples	2-18	Dr. Nascimento	
Subtask 3. Deep sequencing and 16S RNA analyses	2-18	Dr. Wang	
Subtask 4. 16S data correlation with Group 1 and 2	4-18	Drs. Chan/Bubb	
Specific Aim 2 (specified in proposal)			
Major Task 2			
Subtask 1. Metagenome analyses	2-18	Drs. Wang/Chan/ <u>Progulske-Fox</u>	
Subtask 2, Metagenomic biomarkers correlation with Group 1 and 2	4-18	Drs. Chan/Bubb/ <u>Progulske-Fox</u>	

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.

NUMBER OF SUBJECTS	Group 1> (Naïve)	Group 2> (Control)	Group 3> (TNF- Responsive)	Group 4> (TNF Non-Resp.)	TOTALS
ENROLLED	17	17	29	14	77
COMPLETED STUDY	3	3	10	3	19
SCHEDULED	6	6	3	3	18
WAITING TO SCHEDULE	7	7	8	7	29
WITHDRAWN FROM STUDY	1	1	8	1	11

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?

Name:	Edward K. L. Chan
Project Role:	PI
Researcher Identifier:	ORCID ID: 0000-0003-3938-9503
Nearest person month worked:	1-2
Contribution to Project:	Coordinate and oversee the entire project
Funding Support:	No other support for this project

Name:	Michael R. Bubb
Project Role:	Co-PI
Researcher Identifier:	ORCID ID: None
Nearest person month worked:	2
Contribution to Project:	Recruitment of subjects from rheumatology clinic
Funding Support:	No other support for this project

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.

e) 4/1/2015- 3/31/2018.

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