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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 still at the phase of recruiting patients and collecting samples. To date, 111 subjects were enrolled. 44 of the subjects completed the study with all the 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. Further delay is primary from patient dropout from enrollment. Sequencing data is being collected and analysis will be performed in the next few months.

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-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:

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/ Progulske-Fox	
Subtask 2, Metagenomic biomarkers correlation with Group 1 and 2	4-18	Drs. Chan/Bubb/ Progulske-Fox	

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

Most recent UF IRB re-approval (IRB201500286) completed 10/9/2017 and will expire 10/5/2018 pending further renewal.

Subtask 1: Recruitment of subjects.

Status is as summarized in the table below. To date, 111 subjects were enrolled and that is above the initial anticipated 100 subjects (25 for each of the 4 groups). The main problem is that the dropout rate is high with only 44 completed the study to date. Plaque samples collected are being analyzed to ensure high quality of DNA samples can be extracted for deep sequencing. The majority of deep sequencing has been scheduled for the last week of Jan.

NUMBER OF SUBJECTS	Group 1> (Naïve)	Group 2> (Control)	Group 3> (TNF-Responsive)	Group 4> (TNF Non-Resp.)	TOTALS
ENROLLED	28	28	37	18	**111**
COMPLETED STUDY	10	10	16	8	**44**
SCHEDULED	6	6	3	3	**18**
Patients not responded	10	10	11	4	**35**
WITHDRAWN FROM STUDY	8	8	10	6	**11**

Subtask 2. Collection of oral DNA samples

As shown in table above, 44 completed out of 100 initially anticipated.

Subtask 3. Deep sequencing and 16S RNA analysis

This is ongoing and all data expected to be collected in the next 3-6 months, as not all samples collected.

Subtask 4. 16S data correlation

This is ongoing and all data expected to be collected in the next 3-6 months, as not all samples collected.

Major task 2:

Subtask 1. Metagenome analyses

This is ongoing and all data expected to be collected in the next 3-6 months, as not all samples collected.

Subtask 2, Metagenomic biomarkers correlation

This is ongoing and all data expected to be collected in the next 3-6 months, as not all samples collected.

What opportunities for training and professional development has the project provided?

One DDS/PhD student was working on this project which was offered as a potential thesis project. A MD fellow in rheumatology has been assigned currently to work on this work to enhance the ability to obtain samples from enrolled patients.

How were the results disseminated to communities of interest?

Nothing to Report to date.

What do you plan to do during the next reporting period to accomplish the goals?

We have focused 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.

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. Based on the current issue with high rate of dropout, we have recruited a MD rheumatology fellow Dr. Susanne Anderson to help participate in the enrollment of patients. We anticipate this issue will be overcome and reaching a higher total number of individuals in each of the four planned groups.

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

Susanne Anderson, MD. – will begin working Jan 2018 ~ 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 xenobioticinduced 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