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TITLE: Post-Transcriptional Gene Regulation of Inflammatory Genes in Steroid-Resistant COPD

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CONTRACTING ORGANIZATION: University of Michigan

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<b>13. SUPPLEMENTARY NOTES</b>					
<b>14. ABSTRACT</b>  Due to the COVID pandemic, all research was paused at the University of Michigan in March 2020. It was re-started in stages in December 2020, at which time we applied for IRB approval. This delayed receiving IRB approval. As of June 4, 2021, we have received IRB approval from both the University of Michigan and Ann Arbor VA Healthcare System. To date, we have identified 440 participants (116 males and 323 females) who are interested in participating in the present study. The demographics of these individuals are listed in an attachment. We will start recruiting appropriate subjects from the different demographic groups and start sample collection. We started recruiting subjects after final IRB approval but had to stop in fall 2021 because of the COVID surge. All clinical studies were placed on hold at Ann Arbor VA Healthcare System. Clinical studies were re-started in spring 2022. These delays have resulted in significant delays in the project. We were granted a six month no cost extension to continue the project.					
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## 1. INTRODUCTION

The diagnosis of steroid-unresponsive COPD is essentially a slow death sentence since there are few therapeutic options left. Incidence of pulmonary diseases in Veteran populations such as COPD is increasing and these patients have higher rates of health care utilization and have poor responses and outcomes. Prognosis and survival of steroid-unresponsive COPD is *even worse* than other subtypes of COPD, but its' molecular mechanisms are not well understood. A recent publication by Christensen et al identified an IL-17A gene signature in steroid-unresponsive COPD derived airway epithelium. Though this is a significant advance, the source of IL-17A in COPD patients remains unknown. There is poor correlation between mRNA levels and protein. However, many inflammatory genes, including IL-17A, are regulated at this level. **R**NA **i**mmunop**r**ecipitation (RIP)-Seq combined with RNA-Seq is a powerful way of better understanding posttranscriptional gene expression. Many pro-inflammatory cytokines are regulated by RNA binding proteins (RBPs) at levels of mRNA stability and translation. The RBP HuR (*elavl1*) regulates IL-17A gene expression and signaling cascades. However, if IL-17A is driving steroid-unresponsive inflammation in COPD, identification of its' source is critical since interfering in its expression may ameliorate or prevent lung inflammation in this form of COPD. Our *long term goal* is to understand posttranscriptional gene regulation in airway inflammation. The *objective of this application*, which is our next step in pursuit of that goal, is to determine whether RBP HuR is regulating key pro-inflammatory molecules elaborated by CD4<sup>+</sup> T cells in steroid-unresponsive COPD. The *central hypothesis* is that *HuR is controlling IL-17A expression by CD4<sup>+</sup> T cells in steroid-unresponsive COPD patients*. The *rationale* for this proposal is that previous work has demonstrated that HuR controls differentiation of CD4<sup>+</sup> Th17, as well as playing a key role in IL-17 mediated signaling cascades. Therefore, we predict HuR may play an important role in allowing IL-17A driven inflammation in COPD and thus may potentially serve as both as a novel biomarker and mechanistic regulator. We will test the central hypothesis and accomplish the objectives by the following three *specific aims*: Aim 1: Identify molecular regulation of IL-17A in human CD4<sup>+</sup> T cells; Aim 2: Determine if HuR interference may be a novel method of preventing IL-17A production in COPD; and Aim 3: Identify HuR-regulated genes in COPD by RNA-Seq and RIP-Seq.

## 2. KEYWORDS

COPD: chronic obstructive pulmonary disease

RBPs: RNA binding proteins

*Elavl1*: embryonic lethal abnormal vision gene 1

RIP: RNA immunoprecipitation

IL-17A: interleukin 17A

CPRS: computerized patient record system

FEV<sub>1</sub>: forced expiratory volume

FVC: forced vital capacity

PBLs: peripheral blood lymphocytes

RT-qPCR: reverse transcription quantitative PCR

ELISA: enzyme-linked immunosorbent assay

ICS: intracellular cytokine staining

mRNA: messenger RNA

ILC3: type 3 innate lymphoid cells

shRNA: short hairpin RNA

AICAR: 5-Aminoimidazole-4-carboxamide ribonucleotide, aka acadesine

DMSO: dimethylsulfoxide

## 3. ACCOMPLISHMENTS

### Major Task 1:

Approval of IRB  
Recruitment of human subjects

Renewed 2/23/23  
in progress; >400 subjects identified

**Major Task 2:**

Measure HuR expression in COPD patient T cells	to be done	0% completed
Measure kinetics of IL-17A gene expression	to be done	0% completed
Identify whether HuR directly interacts with IL-17A mRNA	to be done	0% completed

**Major Task 3:**

Determine mechanisms of HuR control of IL-17A expression	to be done	0% completed
Identify whether acadesine will decrease IL-17A expression	to be done	0% completed

**Major Task 4:**

Quantitate steady-state mRNA levels in COPD CD4 <sup>+</sup> T cells	to be done	0% completed
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**Major Task 5:**

Determine transcriptomic landscape of COPD CD4 <sup>+</sup> T cells	to be done	0% completed
Determine HuR direct target genes in COPD CD4 <sup>+</sup> T cells	to be done	0% completed

**Training:** Nothing to Report

**Dissemination to communities of interest:** Nothing to Report

**4. IMPACT**

Nothing to Report

**5. CHANGES/PROBLEMS**

Due to the COVID pandemic, all research was paused at the University of Michigan in March 2020. It was re-started in stages in December 2020, at which time we applied for IRB approval. During the COVID shutdown, the IRB ceased to process applications. This delayed receiving IRB approval, which also had to be done at two different institutions: University of Michigan and Ann Arbor VA Healthcare System. Final IRB approval was obtained February 2021, however, there were a series of amendments which had to be filed. All the amendments were approved as of June 4, 2021. There were no significant changes in recruitment of subjects for this study.

We started recruiting subjects after final IRB approval but had to stop in fall 2021 because of the COVID surge. All clinical studies were placed on hold twice at Ann Arbor VA Healthcare System. Clinical studies were re-started in spring 2022. These delays have resulted in significant delays in the project. We were granted a six month no cost extension to continue the project. Unfortunately, the COVID shutdowns resulted in loss of critical staff, including our study coordinator, Ms. Lisa McCloskey (left Feb. 2023) and Dr. Jason Ellis (left March 2022). We have recruited a new lab manager, Ms. Laura Vallance (October 2022) and have arranged for a part-time coordinator at the VA to assist with the grant application. Due to these problems, DOD granted an additional six month no cost extension. Additionally, we were awarded a new VA Merit grant (funding starts July 1, 2023) with areas of overlap (it focuses on asthma) so we expect to be able to continue with this present DOD project.

**6. PRODUCTS**

Nothing to report

## 7. PARTICIPANTS

Name:	<i>Ulus Atasoy</i>
Project Role:	<i>PI</i>
Research Identifier:	-
Nearest Person month worked:	<i>12</i>
Contribution to Project:	<i>Dr. Atasoy has been the project leader and supervising personnel</i>
Funding Support:	

Name:	<i>Jeff Curtis</i>
Project Role:	<i>Co-PI</i>
Research Identifier:	-
Nearest Person month worked:	<i>12</i>
Contribution to Project:	<i>Dr. Curtis has helped coordinate the project at Ann Arbor VA Healthcare System (AAVA)</i>
Funding Support:	

Name:	<i>Jason Ellis</i>
Project Role:	<i>Research Assistant Professor</i>
Research Identifier:	-
Nearest Person month worked:	<i>12</i>
Contribution to Project:	<i>Dr. Ellis helped prepare IRB application at University of Michigan. He left for another position in March 2022.</i>
Funding Support:	

Name:	<i>Kristin Bahleda</i>
Project Role:	<i>Lab Manager, Atasoy Lab</i>
Research Identifier:	-
Nearest Person month worked:	<i>6; Ms. Bahleda accepted a new position September 2022 and left the laboratory</i>
Contribution to Project:	<i>Ms. Bahleda analyzed the subject enrollment and helped with advertisements</i>
Funding Support:	

Name:	<i>Lisa McCloskey</i>
Project Role:	<i>Study Coordinator</i>
Research Identifier:	-
Nearest Person month worked:	<i>12</i>
Contribution to Project:	<i>Ms. McCloskey has assisted with interfacing with IRB at the University of Michigan and AAVA. Ms. McCloskey accepted a new position Feb. 2023</i>
Funding Support:	

Name:	<i>Laura Vallance</i>
Project Role:	<i>Lab manager</i>
Research Identifier:	-
Nearest Person month worked:	<i>6</i>
Contribution to Project:	<i>Ms. Vallance is the lab manager for the PI and started Oct. 2022 and has completed all of the required training to participate in the project</i>
Funding Support:	

## 8. SPECIAL REPORTING REQUIREMENTS

Nothing to report