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PRINCIPAL INVESTIGATOR: Maureen A. Su, MD

CONTRACTING ORGANIZATION: University of California, Los Angeles, CA

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Maureen Su
Partnering PI: Jason K. Whitmire, PhD

E-Mail: masu@mednet.ucla.edu

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University of California, Los Angeles
Office of Research
Administration
10889 Wilshire Blvd STE 700
Los Angeles, CA 90024-4201

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14. ABSTRACT

The human disease, Guillain Barre Syndrome (GBS), can be modeled in NOD.Aire$^{GW/+}$ mice that acquire an autoimmune peripheral neuropathy (APN). We have learned that APN in these mice requires UTX expression in CD4+ T cells. The goal of this project is to understand how UTX in T cells promotes APN, focusing on UTX's effects on gene expression in CD4+ T cells and the formation of pathogenic subsets of CD4+ T cells. In year 1, we worked toward understanding the role of UTX's demethylase domain in APN. In one series of experiments, NOD.Aire$^{GW/+}$ mice were treated with a compound that inhibits the UTX demethylase activity. We observed that this treatment failed to prevent APN, suggesting that UTX function is demethylase-independent. We also worked toward establishing a genetic model where APN-prone mice will express a mutant form of UTX that lacks demethylase activity.

15. SUBJECT TERMS

Guillain Barre Syndrome; peripheral neuropathy; Autoimmune Regulator; UTX; histone demethylase; CD4+ T cells

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1. INTRODUCTION:

The human disease, Guillain Barre Syndrome (GBS), can be modeled in NOD.Aire$^{GW/+}$ mice that acquire an autoimmune peripheral neuropathy (APN). We have learned that APN in these mice requires UTX expression in CD4+ T cells. The goal of this project is to understand how UTX in T cells promotes APN, focusing on UTX's effects on gene expression in CD4+ T cells and the formation of pathogenic subsets of CD4+ T cells. In Aim1, we will test approaches to treat APN by interfering with UTX's demethylase activity using either a genetic approach or treating mice with a chemical inhibitor of UTX. Aim2 will test approaches that interfere with either the formation of pathogenic T cells or the cytokines they make. Aim3 will examine human T cells and examine how UTX affects gene expression and CD4 T cell subset formation, including studies of UTX in T cells from GBS patients. In year 1, we worked toward understanding the role of UTX's demethylase domain in APN. In one series of experiments, NOD.Aire$^{GW/+}$ mice were treated with a compound that inhibits the UTX demethylase activity. We observed that this treatment failed to prevent APN, suggesting that UTX function is demethylase-independent. We also worked toward establishing a genetic model where APN-prone mice will express a mutant form of UTX that lacks demethylase activity.

2. KEYWORDS:

Guillain Barre Syndrome; peripheral neuropathy; Autoimmune Regulator; UTX; histone demethylase; CD4+ T cells

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Tasks	Timeline (months)	Completion?
Specific Aim 1. Determine the role of the UTX H3K27me3 demethylase function in PNS autoimmunity.		
Major Task 1.0. Establish UTX^{DMD} mice on the NOD.Aire$^{GW/+}$ background		
Submit and obtain ACURO approval	0-3	100%
Subtask 1. Backcross B6.129-UTX^{DMD} mice onto the NOD genetic background (speed congenics).	3-24	50%
Major Task 1.2. Can PNS autoimmunity be prevented by GSKJ4?		

Subtask 1. Administer GSKJ4 or vehicle to NOD.*Aire*$^{GW/+}$ mice; monitor over time for development of APN, T cell subsets, histology.	0-24	100%
Specific Aim 3. Establish role of UTX on T cells from GBS patients		
Major Task 3.0 Compare UTX and H3K27me3 levels in T cells from GBS vs non-GBS controls		
Obtain HRPO approval	1-3	100%
Subtask 1. Recruit 15 female subjects with no history of autoimmune disease and 15 female subjects with GBS; collect blood	3- 48	10%

What was accomplished under these goals?

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1. Major Activities

Our major activities centered on obtaining ACURO approval (at UCLA and UNC), HRPO approval, and local IRB approval (at both UT Houston and UCLA). As outlined in the statement of work, we have made progress toward establishing an NOD mouse strain with the UTX^{DMD} mutation. We have completed experiments to test the effects of the GSKJ4 inhibitor on neuropathy development in our mouse model. Finally, we have established a pipeline with UT Houston for shipping samples to UCLA. 2 subjects without a history of autoimmune disease have been recruited for the study. PBMCs were prepared at UT Houston and shipped to UCLA, where they could be processed viably for experiments.

2. Specific Objectives

Our objectives were to receive ACURO approval, HRPO approval, and local IRB approval (at both UT Houston and UCLA). Additionally, our objective in the first year was to begin work on establishing a NOD mouse strain with the UTX^{DMD} mutation. Our objective was also to test the effects of the GSKJ4 inhibitor on neuropathy development in our mouse model. Finally, our objective was to begin receiving samples from UT Houston. We have ensured that PBMCs prepared at UT Houston could be viably shipped to UCLA for analysis.

3. Significant results and Key Outcomes *including major findings, developments, or conclusions (both positive and negative)*

We have made substantial progress in the first 12 months of our project. We have submitted and obtained ACURO approval (Major Task 1.0) and completed studies using GSKJ4 (Major Task 1.2). For the studies using GSKJ4 inhibitor, our data show minimal effect of this inhibitor on

neuropathy development (Fig. 1). No differences were seen on neuropathy development or weight loss (Fig. 1 A and B) with GSKJ4 inhibitor vs. DMSO control treatments. Moreover, no differences were seen in T cell infiltration of nerves (Fig. 1 C and D) or in histology analysis of immune infiltration in sciatic nerves (Fig. 1 E and F). Together, these data suggest that inhibition of UTX's histone demethylase activity by GSKJ4 does not alter neuropathy development, suggesting that the effects we see with UTX-deficient mice may be due to their demethylase-independent activity.

For Major Task 1.0, Subtask 2, we have expedited the production of NOD.Utx^{DMD} mice using the Animal Models Core at UNC. The core used an "exon replacement" approach and primer-driven CrispR-Cas-based DNA cleavage to replace exon 9 of Utx with a mutant exon 9 that encodes the H1146A and E1148A mutations that inactivate the demethylase activity of UTX. We now have a NOD.Utx^{DMD} dam that has produced a female NOD.Utx^{DMD} pup that will be bred to build up this colony. We also now have at UNC the NOD.$Aire^{GW/+}$ mice that will be intercrossed with the NOD.Utx^{DMD} mice to generate NOD.$Aire^{GW/+}Utx^{DMD}$ mice that will be used for Major Task 1.1.

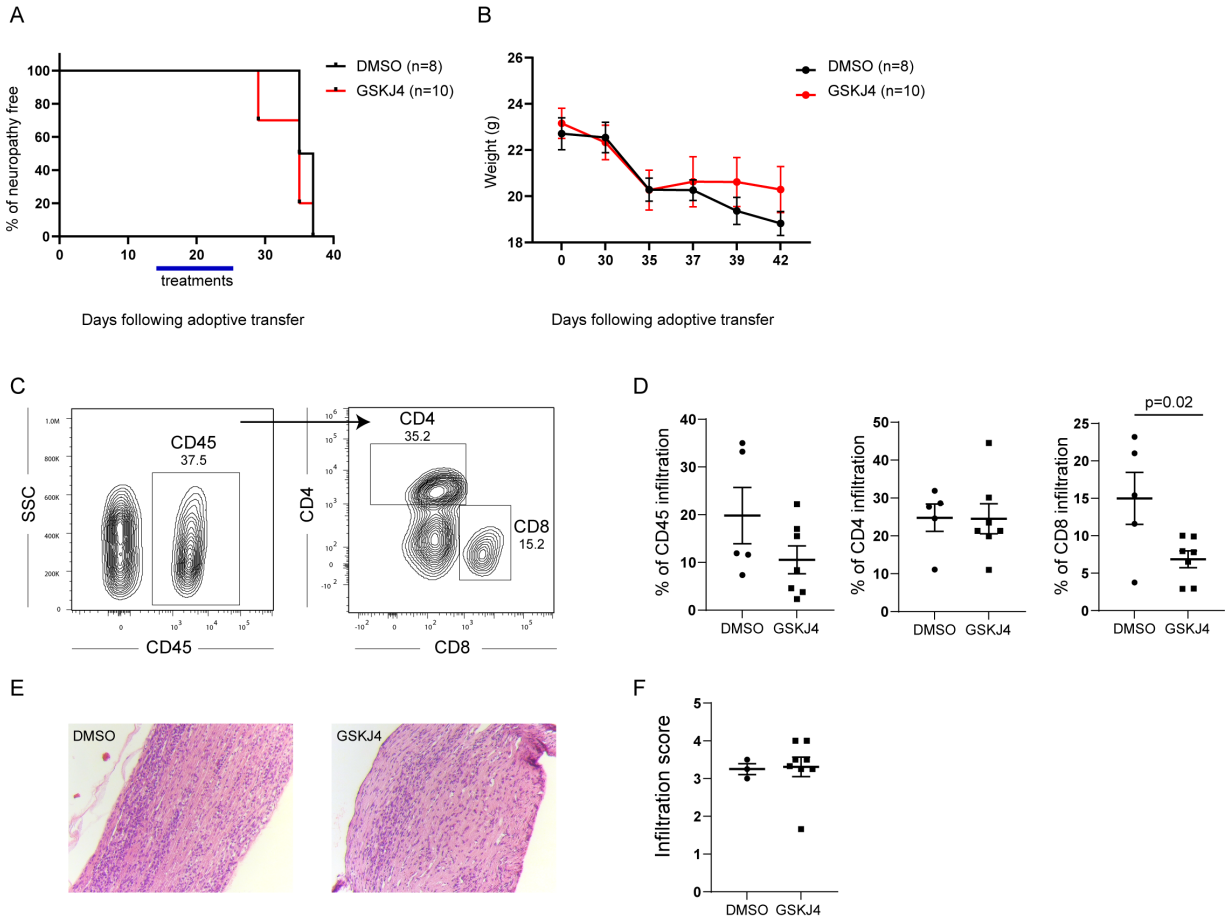

Figure 1. GSKJ4 treatment does not protect against neuropathy. A and B) Neuropathy free curve (A) or weight curve (B) for mice transferred with NOD.AireGW/+ splenocytes treated with either GSKJ4 or DMSO vehicle control. Log rank test p>0.05. C and D) Flow cytometric analysis of T cell subsets after treatment with DMSO or GSKJ4. Representative plots are shown in (C) and cumulative data are shown in (D). p=0.02 Student's t-test as indicated. E and F) Histology analysis of sciatic nerves from mice treated with GSKJ4 or DMSO vehicle control. Representative samples (E) and infiltration scores (F) are shown. P>0.05 Mann Whitney Test.

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

Maryam Seyedsadr, PhD, is a postdoctoral fellow and has been provided the opportunity to work closely with the PI to increase her knowledge and skills. She has been acquiring the skillset to analyze autoimmune peripheral neuropathy phenotype in our *NOD.Aire*$^{GW/+}$mouse model. She will be an active participant in our immunology seminars, molecular biology seminars, and the annual PNS society meeting, where she will present a poster on this work.

Ashlyn Buzzelli, PhD has now joined the Whitmire lab at UNC as a postdoctoral fellow. Her thesis research at the University of Alabama, Birmingham concerned STAT signaling and CD4+ T cell differentiation in the context of experimental autoimmune encephalomyelitis. As a post-doc, she is studying how gene expression in T cells is regulated by UTX, and she is involved in the production of the *NOD.Aire*$^{GW/+}$*Utx*^{DMD} mice. She is learning other techniques related to adoptive transfers of cells, gene expression quantification, and will soon learn CHIP-based approaches to track UTX activity in the genome of T cells. Her career goals are to run a research lab at a teaching-heavy institution, and this project will allow to follow that path.

How were the results disseminated to communities of interest?

Dr. Su will present this work at the GBS/CIDP Foundation international meeting in October 2022 and the PNS Society meeting in June 2023.

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

Goals	Timeline (months from start)	Site
Specific Aim 1. Determine the role of the UTX H3K27me3 demethylase function in PNS autoimmunity.		
Major Task 1.0. Establish UTX^{DMD} mice on the NOD.$Aire^{GW/+}$ background		
Subtask 1. Backcross B6.129-UTX^{DMD} mice onto the NOD genetic background (speed congenics).	3-24	Site 2, 3, Whitmire
Specific Aim 3. Establish role of UTX on T cells from GBS patients		
Major Task 3.1 Compare UTX and H3K27me3 levels in T cells from GBS vs non-GBS controls		
Subtask 1. Purify naïve T cells from blood samples; quantify UTX message and protein levels before and after stimulation; quantify H3K27me3 levels by FACS.	12-48	Site 1 Su
Subtask 2. Measurement of changes in gene expression and H3K27me3 positioning in normal control versus GBS T cells.	12-48	Site 1, 2, 5 (Whitmire, Su)
Subtask 3. Bioinformatic analysis of RNA-seq and ChIP-Seq data	12-48	Site 1, 2, 5 (Whitmire, Su)
Major Task 3.2 CRISPR-Cas9 disruption of UTX in human T cells		
Subtask 1. Design and implement genome editing to delete UTX in T cells from healthy control or GBS subjects	0-18	Site 1 Su
Subtask 2. Compare human T cell differentiation into Th17 subsets when UTX is mutated or not.	18-48	Site 1 Su
Subtask 3. Compare human T cell differentiation into Tfh cells when UTX is mutated or not.	18-48	Site 1 Su
Subtask 4. Measurement of changes in gene expression and H3K27me3 positioning in control or UTX-deleted T cells.	18-48	Site 1, 2, 5 (Whitmire, Su)
Subtask 5. Bioinformatic analysis of RNA-seq and ChIP-Seq data, comparing in control or UTX-deleted T cells.	18-48	Site 1, 2, 5 (Whitmire, Su)
Major Task 3.3 Assess effects of GSKJ4 demethylase inhibition on human CD4+ T cells		
Subtask 1. In vitro culture of GBS or normal T cells into Th17 or Tfh subsets in the presence or absence of GSKJ4; measurement of cytokines and surface marker expression.	18-42	Site 1, 5 Su
Subtask 2. In vitro culture of GBS or normal T cells into Th17 or Tfh subsets in the presence or absence of GSKJ4; measurement of changes in transcript expression assessed by RNA-seq.	24-48	Site 1, 5 Su
Subtask 3. In vitro culture of GBS or normal T cells into Th17 or Tfh subsets in the presence or absence of GSKJ4.	24-48	Site 1, 2, 5 (Whitmire, Su)

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

In the next reporting period (months 12-24), we will focus on completing tasks in Specific Aims 1 and 3. We will complete Major task 1.0, Subtask 2, and begin tasks outlined for Specific Aim 3. These tasks involve comparison of GBS and non-GBS control samples by FACS and RNAseq/ChIPseq. We will also commence CRISPR-Cas9 studies of UTX in human T cells.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Based on our preliminary findings to date, the use of GSKJ4 for neuropathy may not be beneficial for patient populations with GBS. However, it remains possible that our future studies using inhibitors of Bcl6, Il21R, and IL17A may provide pre-clinical evidence that they benefit patients with GBS.

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:

Changes in approach and reasons for change

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report.

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

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 of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

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**

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Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	***Maureen Su, MD***
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-3680-9982*
Nearest person month worked:	*2*
Contribution to Project:	*Dr. Su oversees the project and works closely with Dr. Whitmire to ensure milestones are being met.*
Funding Support:	*N/A*
Name:	***Jason Whitmire, PhD***
Project Role:	*Partnering PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0003-2578-6073*
Nearest person month worked:	*2*
Contribution to Project:	*Dr. Whitmire submitted and obtained ACURO approval at UNC. He worked with the Animal Models Core to generate the mice described in Aim1, Major Task 1.0, Subtask 1. He recruited Dr. Buzzelli to join the project team. He works with Dr. Su to ensure we have regular ZOOM meetings that include Dr. Shpargel and other collaborators.*
Funding Support:	*N/A*
Name:	***Kazim Sheikh, PhD***
Project Role:	*Subaward PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0003-2263-1500*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Sheikh is involved in IRB protocol submission, reviewing patient records for confirmation of GBS diagnosis, consent and recruitment, and supervision of PBMCs isloation from periphral blood for shipping to UCLA*
Funding Support:	*N/A*
Name:	***Aline Hoang***
Project Role:	*Staff Research Associate*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*4*
Contribution to Project:	*Aline Hoang provides technical support for mouse husbandry and in vivo experiments.*
Funding Support:	*N/A*
Name:	***Maryam Seyedsadr***
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID)	*0000-0001-9315-9544*
Nearest person month worked:	*8*
Contribution to Project:	*Dr. Seyedsadr plans and performs in vivo and in vitro experiments.*
Funding Support:	*N/A*
Name:	***Karl Shpargel, PhD***
Project Role:	*Collaborator*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-9658-456X*

Nearest person month worked:	*2*
Contribution to Project:	*Dr. Shpargel provides regular advice regarding UTX biology. He has provided us retroviral reagents related to UTX, he has provided us antibodies and protocols for staining for UTX or for ChIPing with UTX.*
Funding Support:	*N/A*
Name:	***Ashlyn Buzzelli, PhD***
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-1704-3619*
Nearest person month worked:	*3*
Contribution to Project:	*Dr. Buzzelli studies how UTX affects gene expression in T cells. She works with mice NOD.Aire GW/+ mice that are prone to developing peripheral neuropathy. She is responsible for ensuring we generate the NOD.UTX DMD;Aire GW/+ mice.*
Funding Support:	*N/A*
Name:	***Maggie DeMonia***
Project Role:	*Technician*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*6*
Contribution to Project:	*Ms. DeMonia is involved in genotyping and otherwise maintaining lines of mice for UTX-related studies. She assists with experiments involving mice, including cell preparations.*
Funding Support:	*N/A*
Name:	***Gang Zhang***
Project Role:	*Research Coordinator*
Researcher Identifier (e.g. ORCID ID):	*0000-0001-5944-5459*
Nearest person month worked:	*1*
Contribution to Project:	*Gang assists Dr. Sheikh in submitting the IRB, identifying patients admitted to our facility with the diagnosis of GBS, initial screen, and blood draws.*

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

Dr. Su's support from National Organization for Rare Diseases (NORD) ended January 2022 per project completion. Dr. Whitmire's support from R01AI131685 expired in February, 2022, as that was the end date for the award. His support from R01AI103083 expired in August, 2022 at the end of that award.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: