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PRINCIPAL INVESTIGATOR: Gregory Hawkins

CONTRACTING ORGANIZATION: Wake Forest University Health Sciences

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6. AUTHOR(S)
Gregory Hawkins, PhD
Carol Milligan, PhD

E-Mail:ghawkins@wakehealth.edu; milligan@wakehealth.edu

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
1) IL6 transsignaling plays a potential protective role for motoneurons in the periphery, while later when extracellular levels of IL6 increase with increased muscle atrophy and decreased lung function, transsignaling promotes a breakdown in the blood brain barrier that fosters IL6 transsignaling in the CNS that can promote disease progressions through glial activation. 2) Individuals with increased levels of soluble receptor such as those with enhanced shedding due to IL6R polymorphism will be more susceptible to IL6 transsignaling and will have faster disease progression. 3) Blocking the effects of IL6 transsignaling will reduce disease progression rates and disease severity.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
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Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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1. INTRODUCTION: *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

We hypothesize that IL6 transsignaling plays a role in the progression of ALS by affecting the rate of NMJ denervation, glial cell activation, and MN degeneration. Given the difficulty studying humans with ALS and collecting samples critical to studying active disease, we are proposing to utilize the $SOD1^{G93A}$ mouse model of ALS to study the effects of IL6 transsignaling on disease severity and progression. Successful execution of this study will define the role of IL6 as an effector of ALS severity and progression, and will provide new information on how to target and treatment ALS using therapeutics that target and block the detrimental effects of IL6 transsignaling.

2. KEYWORDS: *Provide a brief list of keywords (limit to 20 words).*

Amyotrophic lateral sclerosis, ALS, CRISPR mouse model, IL6 trans-signaling, SOD1

3. ACCOMPLISHMENTS: *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Goals:

1. Perform a systemic examination of IL6 transsignaling in both initiation and progression of ALS
2. Create an ALS mouse model where IL6 transsignaling is increased , thus modeling those individuals that have inherited the IL6R polymorphism, and determine if disease pathology is altered.
3. Treat the ALS/transsignaling mouse model with the transsignaling inhibitor, soluble gp130, to determine if disease progression can be significantly slowed.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

SPECIAL NOTE: Due to the COVID-19 pandemic, the academic laboratories at Wake Forest School of Medicine were closed from mid-March, 2020 until late June, 2020.

Aim 1: Perform a systematic examination of IL6 transsignaling in both initiation and progression of ALS.

The SOD1G93A mouse model, while no longer the only ALS experimental model, is very well characterized. Mice exhibit progression of muscle weakness, involvement of both upper and lower MNs and many cellular, and molecular changes observed in humans (see Vinsant et al.,2013 Brain and Behavior 3 (4): 335–350 for review). To study MNs in the spinal cord, retrograde tracing was used together with thin section microscopy and electron microscopy to identify regions of tibialis anterior (TA) and soleus motor pools (See Vinsant et al., 2013 for details). The results of this study now allow us, and other researchers to carefully evaluate specific timing and pathology throughout disease. Early pathological changes, such as mega-mitochondria can be observed in MN dendrites, soma, axons and terminals as early as P7, the earliest time examined, and significant denervation of NMJs is observed between P14 and 30. Initial muscle

denervation occurs in the TA muscle (fast fatigable and fatigue resistant fibers) between P14 and 30, while little denervation occurs in soleus muscle (slow and fatigue resistant fibers) even late in disease (Figure 1 top panels). In support of our hypothesis, we also found IL6 mRNA to increase in the SOD1 TA muscle coincident with initial denervation (Figure 1 bottom panels, Figure 3B). Interestingly, while glial activation is observed shortly after initial NMJ denervation, robust microglia and astrocyte activation is not observed until later in disease, (Figure 2). Because of the differences in timing between early NMJ denervation and robust glial activation in the mouse model, we feel we will have the ability to distinguish specific roles for IL6 and the type of signaling that occurs in early denervation vs. glial activation and inflammation. We plan a systematic determination of expression of mouse IL6 and Il6ra in both early (initial muscle/neuromuscular denervation) and later (glial activation) events associated with disease progression in the SOD1^{G93A} mouse. We hypothesize that IL6 activity in the periphery may promote maintenance of neuromuscular junction (NMJ) innervation and MN survival. Lung tissue will also be examined since decreased lung function is correlated with increased Il6, which may contribute to enhanced peripheral transsignaling later in disease (Jiang et al., Neurodegener Dis. 2011;8(6):504-14). The results of these initial experiments provide us with a temporal-spatial "map" of IL6 signaling changes throughout disease. Our results suggest increased IL6 mRNA levels in ventral lumbar spinal cord, muscle and lung at time of disease pathology in those tissues (Figure 1, 3). In a longitudinal study of ALS patients, we showed that serum IL6 levels negatively correlate both with the patient's functional status as measured by the overall ALSFRS-R and subscores, and with respiratory function as measured by the percent predicted FVC (ppFVC). The correlations in the cases of ALSFRS-R limb and respiratory subscores, and ppFVC are only present in the two-thirds of patients who carry the IL6R *Ala358* variant that mediates IL6 transsignaling (Wosiski-Kuhn et al, 2020. ALSFTD, IN PRESS).

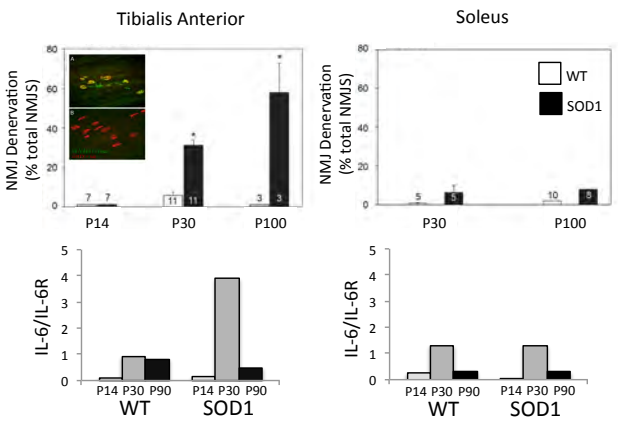


Figure 1. Muscle innervation was examined in the P14, P30 and P100 TA and soleus in *SOD1^{G93A}* and *WT* mice (top panels). While significant denervation occurs in the TA at P30, there is no denervation at P14, but by P100, 70% of TA NMJs were denervated. Soleus NMJs remain innervated even late in disease. The results are presented as % denervated of total NMJs/muscle (mean $\pm$ SEM). NMJ denervation was performed as previously described (46, 47, 64). The results are presented as % denervated of total NMJs/muscle (mean $\pm$ SEM). The number of mice for each condition is indicated in the bars of the graph; *$p \leq 0.05$ as compared to *WT* as determined by unpaired T-test. Expression levels of Il6 (Cell Signaling, #12912) and Il6ra (Santa Cruz: sc-374259) were determined by western blot of TA and soleus muscles at times indicated in the bottom panels. Levels were normalized to actin and results are present as a ratio of normalized Il6 to normalized Il6ra, and indirect measure of transsignaling. There is an increase in transsignaling in the TA coincident with early NMJ denervation. *n*=2-3 mice for each group.

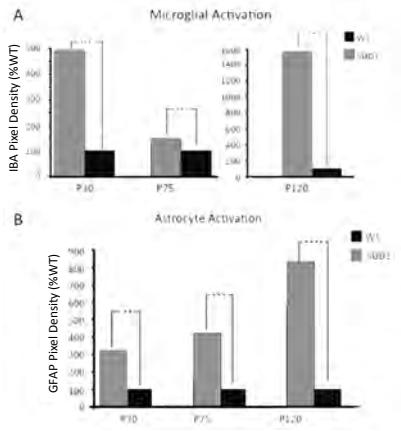


Figure 2. The fluorescent pixel density of Iba1 staining for microglia (A) and GFAP for astrocytes (B) was increased in the ventral spinal cord of SOD1 versus WT mice at P30, and P120. In microglia, there is a decrease in activation compared to P30 followed by a dramatic increase later in disease. In astrocytes, increase in activation is gradual with disease progression. Statistical significance was determined using a two-way ANOVA at P30 and a repeated measure (mixed model) ANOVA at P75 on all of the measured values above baseline (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$). $N = 4$ for each group. ***Figure modified from Gifondorwa et al., Neurol Res Int. 2012;2012:170426.***

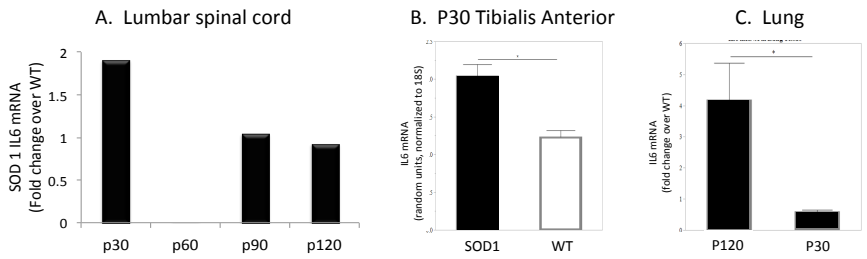


Figure 3. IL6 mRNA levels in tissues involved in ALS pathology. A. To determine if IL6 expression levels change in ventral lumbar spinal cord with disease progression, we performed a preliminary rtPCR experiment. Message levels for cytokine are increased in SOD1 mice as compared to WT at P30, 90 and 120. Interestingly, the pattern mirrors microglial activation shown above. B. IL6 mRNA is expressed at higher levels in the SOD1 mouse as compared to wild-type age-matched controls ($p<0.001$; student's t-test; n=6 per group). C. Relative to age-matched wild type controls, IL6 mRNA is expressed at higher levels in the SOD1 at end stage (p120) than at p30 ($p=0.028$; student's t-test; n=4 per group). Proposed ELISA and immunohistochemistry experiments will provide more information regarding protein expression and cellular localization.

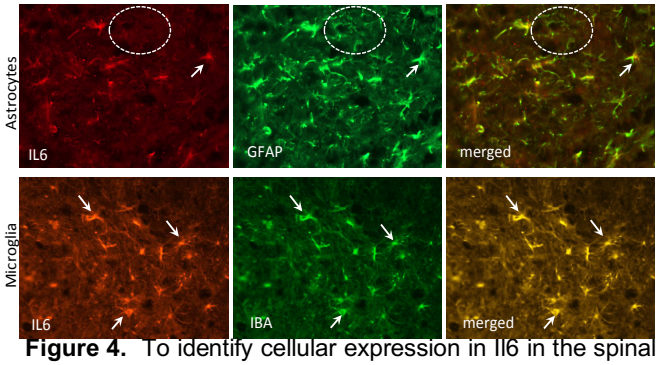


Figure 4. To identify cellular expression in Il6 in the spinal cord of end stage SOD1 mice, immunohistochemistry was performed to measure GFAP (astrocytes) and Il6 (top panels), or IBA (microglia) and Il6. While some astrocytes express Il6 (white arrows), this is not consistent for all GFAP positive cells and processes (circled area). In situ analysis for Il6 will reveal if this staining reflects astrocyte production of Il6 or the cytokine's binding to receptor (IL6R or gp130). All IBA positive microglia at this stage express Il6 at this stage (e.g., white arrows). We did not detect Il6 in oligodendrocytes or neurons (not shown).

Cellular expression of IL6 signaling. While the experiments above will provide definitive levels of cytokine and receptor expression in tissues, it is critical to determine which cells express the cytokine and its receptors (Il6ra and gp130). Using tissue from WT, SOD1^{G93A}, and the SOD1XIl6ra crosses at time points indicated in the original proposal, immunohistochemical analysis and/or in situ hybridization (FISH) will also be performed to determine if Il6ra and/or Il6 is expressed on mouse muscle, postsynaptic terminal (αBTX), presynaptic nerve terminal (anti-VAChT), or Schwann cells (anti-s100b) in muscle and on MN (anti-ChAT), microglial (anti-IBA), astrocytes (anti-GFAP), vascular endothelial cells (anti-CD31) in the spinal cord (see Figure 12). While we expect gp130 to be expressed on all cells, we will also confirm its expression with immunohistochemistry. Lung tissue will also be examined since decreased lung function is correlated with increased Il6 that may contribute to enhanced peripheral transsignaling later in disease.

Further characterization of transsignaling in disease pathology will occur as we continue to evaluate these processes in the SOD1 X Il6Ra sec/TMD crosses ice as compared to SOD1X IL6Rs WT.

Aim 2. Create an ALS model where IL6 transsignaling is increased and determine if disease pathology is altered.

As proposed originally, to accurately investigate potential mechanisms by which IL6 transsignaling contributes to disease progression (ALS, other neurological and non-neurological disorders such as the etiology of obstructive airway disease and cancer) it was necessary to generate a unique IL6R transmembrane deletion (TMD) mouse model, *Il6ra*^{Sec} mouse that exhibits tremendous shedding of the receptor. We have successfully created the *Il6ra*^{Sec} mouse at UNC and had established two breeding lines. The mouse model was generated in consultation with Dr. Dale Crowley and colleague at the UNC Animal Models Core using CRISPR/Cas9 technology and on the C57BL/6 background. After initial crosses and characterizations, we identified one line for continued study (see Figures 5B and 6B; manuscript in preparation). There are no significant differences in sIL6R levels between male and female mice and across ages (P30 to 1year: data not shown). We have confirmed that there are no differences in levels of sIL6R between serum and plasma (not shown).

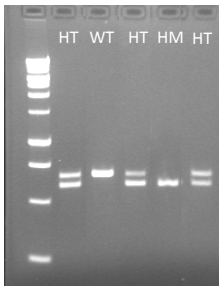
Figure 4A. Similarity of Human and Mouse IL6 Receptor Peptide

```
          Extracellular                                    Intracellular
Human     ATS*L*PVQDSSSVPLPTFLVAGGSLAFGTLLCIAIVLRFKKTWKLRALKEGK
Mouse     ATSVLAPVQESSSMSLPTFLVAGGSLAFGLLLCVFIILRLKQKWKSEAEKESK
                    ^(---Membrane region---)
```

Figure 5B. Transmembrane Domain (highlighted) Deletion Model sequence and PCR fragments for genotyping.

GAGTCCTAGGATCCTCCATC
GAGTCCTAGGATCCTCCATCTCTCATGTGCCTTCTGCCTGGAGCCTGGGTAAAGGATACCGGGAA
AGCTACTCTGAGCGTGAACGGCCCTTTGGTTGGCTGCAGTGGGCATCAGCCCCATATGAGCTCTC
GAATGGTGCAGTCTCCCTCAGTCACTGTGGGAGGAGTCGGTGCAGTGCCTCTTCAGAGTTCTGCT
GCCATCCCGAGCTGCTCAGTTTCTAGTAACTGCTGCCTGACTTTCTTTTCCCTCTTTCCTTCCTT
TTGTCCTGTCCCCTTCCCCCGTCCCTTCCTTTTTTCTATCATTCCCTTCCCCTGCCCACAGCCCC
AGTGCAAGAATCCTCGTCCATGTCCCTGCCCACATTCCTGGTAGCTGGAGGAAGCTTGGCGTTTG
GGTTGCTTCTCTGTGTCTTCATCATCCTGAGGTGAGACGGGTCCTGTACTGTATTGTACTGTACT
GCACTGGCTCTGGGCTGTACTCTTGCGTCTTAGCTTTCATCAGAACCTAAGGAACTTTGCTCATT
CATTTCTTCATTCAACAAATGAACATGTAACATGCTGAGTCCTGGGTCAGGTGCTGCGGGAGGCC
AGGGGACAGGATGGGTACTGTGTAGTGAGGAAGCCAGGCCCTGACAAGGAAGTGCTGACATGGGC
AGAGCTACAGTAGCACAGCCAT
CTCGATGTCATCGTGTCGGTA
ATGGCTGTGCTACTGTAGCTC

PCR fragment without deletion= 672 bp
PCR fragment with deletion = 604 bp



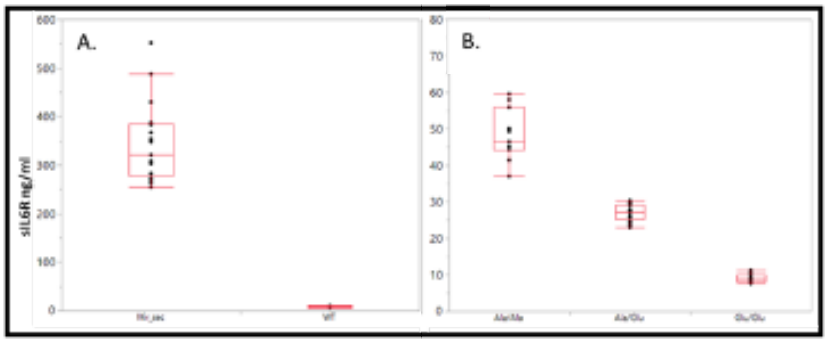


Figure 6. ELISA measurement of soluble IL6 receptor in (A) TMD mice at P90 (TMD/TMD n=17; WT/WT n=16; p<0.001 across genotypes; one way ANOVA), and (B) human IL6R Asp^{358}Ala (rs2228145) (n=471) plotted. NOTE: Y axis scales are different for A and B.

As shown in Figure 6, the mean serum soluble IL6 receptor (sIL6R) concentration is ~350 ng/ml for homozygous *Il6ra*^{Sec} mice compared to WT mice (mean =7.6 ng/ml). The mean sIL6R levels for the homozygous *Il6ra*^{Sec} mice is ~7-fold higher that sIL6R levels found in humans homozygous for the *IL6R* Asp^{358}Ala mutation and mice homozygous for the *Il6ra* Glu^{357}Ala mutation (*Il6ra*^{E357A} constructed using NIH Grant AI37866-01; data not shown). Thus, our construct was highly effective in producing sIL6R available for IL6 transsignaling.

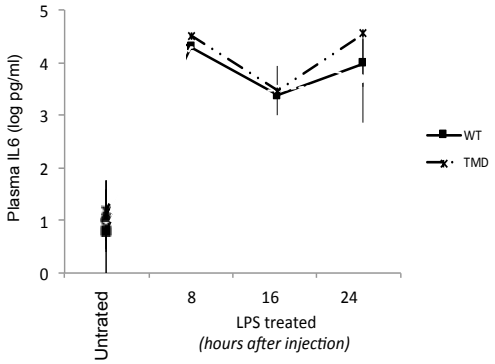


Figure 7 (above). ELISA measurements of plasma IL6 in untreated or LPS-treated (3 ug/mouse, i.p) WT, and TMD homozygous mice at indicated time points. For each treatment group littermate,gender matched animals were used (n=2-3 groups/treatment group/time point).

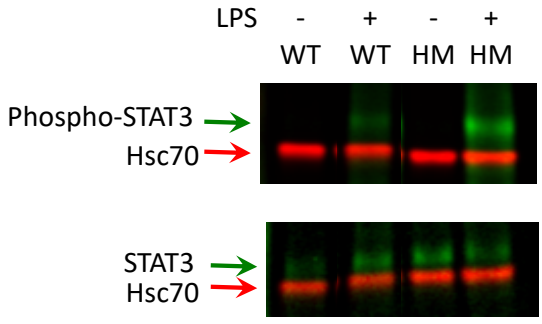


Figure 8. Shown are representative Western blots of liver protein extracts from untreated or LPS-treated WT, or homozygous (HM) TMD mice. Phosphorylated STAT3 expression increased by 8 hours (not shown) and is sustained at 24 hrs at much higher levels in TMD mice as compared to WT. Pan-STAT3 levels were consistent across treatment. The constitutive heat shock protein 70 was used as a loading control as this protein did not change expression across genotypes or treatment.

We have characterized the models to further demonstrate functionality of the shed receptor as reflected by enhanced and sustained liver activation/phosphorylation of STAT3 following lipopolysaccharide (LPS) treatments. LPS is a well-studied and potent systemic inducer of inflammation and immune responses that includes IL6 (Krüttgen A, Rose-John, J Interferon Cytokine Res. 2012 Feb;32(2):60-; Szot et al., 2017. Neuroscience 355:9-21). Reports indicate that increased levels of *Il6* mRNA in mice within 2-6 hours of i.p. LPS injection (Radulovic et al., 2018. JOVE 135: e576; Cai et al., 2016, Brain, Behavior and Immunity 58:327-337), and we determined IL6 plasma levels are increased by 8 hours (not shown) and maintained for at least 24 hours following LPS administration (Figure 7). Stat3 is a key transcription factor that is phosphorylated during activation by IL6 signaling (Robinson et al., 2015, Am J Physiol Lung Cell Mol Physiol. 2015 May 22:ajplung.00288.2014). STAT3 phosphorylation in the liver is elevated 8 hours following LPS administration in animals of all genotypes (not shown) but sustained at much higher levels in in the liver of LPS treated TMD homozygous mice as compared to WT animals (Figure 8).

IL6 transsignaling is documented in both human and mouse (Garbers et al., J Biol Chem. 2011 Apr 29;286(17):14804-11); however, mice do not naturally possess enhanced shedding of the IL6 receptor and subsequent enhanced transsignaling that would occur with

increased levels of IL6 such as those that occur with initial NMJ denervation in the SOD1^{G93A} mouse (see above). To accurately assess the potential mechanism by which enhanced IL6 transsignaling contributes to disease progression we have created an IL6R mouse model where the transmembrane portion of IL6R is deleted (IL6R^{TMD}). The TMD model will now allow us to identify pathological events significantly mediated by transsignaling because in homozygous IL6R^{TMD} mice, there is no membrane bound receptor to mediate classical signaling. With these models now in hand, we have also started to cross the *Il6ra*^{Sec} mouse with the SOD1^{G93A} mouse model of ALS (C57BL/6 background). We had only begun to collect the offspring from the initial crosses (SOD1 males X heterozygous *Il6ra*^{Sec} females) when our institution asked us to "pause" ongoing research because of SARS-CoV-2 and COVID-19. We maintained the crosses, but at requested minimum. Nonetheless, our preliminary data are very encouraging with 25% of SOD1 mice (n=8; 6M 25% with deficits; 2F) begin to exhibit deficits in leg extension at P60-65 whereas 100% of the SOD1/ *Il6ra*^{Sec} (n=1F; 2M). All SOD1/ *Il6ra*^{Sec} mice resulting from these initial crosses are heterozygous for the IL6R genotype.

Once we were able to return to our labs in June, we ramped up breeding of the animals and will soon begin to characterize the influence of IL6 transsignaling in ALS pathogenesis.

Aim 3. Treat the mouse models with transsignaling inhibitor, soluble gp130 to determine if disease progression can be significantly slowed

We have not yet progressed to this aim.

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

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

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

Our plans for the next reporting period are:

1. **Finish validation of the *Il6ra*^{Sec} mouse model**
2. **Breed the *Il6ra*^{Sec} mouse model with SOD1 mutant mice to establish the ALS/enhanced IL6 transsignaling model. By examining SOD1X IL6Ra compared to SOD1 littermates, wew will be able characterize the influence of IL6 transsignaling in ALS pathogenesis.**
3. **Treat the ALS/transsignaling mouse model with the transsignaling inhibitor, soluble gp130, to determine if disease progression can be significantly slowed.**

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).

By determining if IL6 transsignaling is critical in promoting ALS progression and severity, we will have identified a critical pathway for ALS treatment. The success of this study will also give us new insights into how the inflammation, and most specifically IL6 signaling, may contribute to ALS initiation.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

The developed mouse model will be used to investigate the role of IL6 transsignaling in exacerbating Cytokine Release Syndrome (CRS) in the lung following infections (e.g., such as those caused by SARS-CoV-2) or injury.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*

- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

NOTHING TO REPORT

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

NOTHING TO REPORT

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

NOTHING TO REPORT

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Due to the COVID-19 pandemic, the academic laboratories at Wake Forest School of Medicine were closed from mid-March, 2020 until late June, 2020. *We had to pair-down our mouse colony to a maintenance level. Once we were allowed back in the labs in June, we increased the breeding colony and now obtaining necessary offspring to complete the study.*

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

NOTHING TO REPORT

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

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:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  NOTHING TO REPORT

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g.,*

book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

NOTHING TO REPORT

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

NOTHING TO REPORT

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

NOTHING TO REPORT

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

We have developed a mouse model where the transmembrane domain or the IL6 receptor has been removed.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

NOTHING TO REPORT

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*

- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

NOTHING TO REPORT

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name: Gregory Hawkins
Project Role: PI
Nearest person month worked: 2.7 months
Contribution to Project: Dr. Hawkins is a Professor of Biochemistry with experience in IL6 transsignaling research. Dr. Hawkins is working closely with Dr. Milligan in designing and testing the mouse model produced in this proposal and will be involved in data interpretation and manuscript preparation.

Funding support:

R03 AI37866-01
Generation of a mouse model to study IL6 transsignaling
This project will develop a transgenic mouse model to study IL6 transsignaling in multiple diseases.
Role: Co-Principal Investigator

P30 CA012197-43
Wake Forest Baptist Comprehensive Cancer Center - Cancer Center Support Grant
The Wake Forest Baptist Comprehensive Cancer Center is a multidisciplinary interdepartmental research center, organized into four divisions: Two Basic Science Programs, Clinical Research, and Cancer Prevention and Control.
Role: Co-Investigator

1R01HL122393-01A1
Social Stress, Diet, and Primate Monocyte Programing in Cardiovascular Risk
The project seeks to determine the effects of psychosocial stress on monocytes (cells that are important in the process of cardiovascular disease), and to evaluate whether a more healthy diet can improve the hypothesized adverse effects of psychosocial stress on monocytic characteristics.
Role: Co-Investigator

R01 NS036695-15A1
Genetic Environmental Risk Factors for Hemorrhagic Stroke
To determine the gene expression and epigenetic factors that contribute to hemorrhagic stroke.
Role: Co-investigator

DOD
Synergistic targeting of the JAK2-STAT3 and SMO-GLI1 pathways in triple-negative and HER2-enriched breast cancers The primary objective of our application is to provide preclinical results as the basis for future clinical development of novel targeted combination therapies for triple-negative and trastuzumab-resistantHER2-positive breast cancers. The secondary objective is to move quickly towards clinical trials and thus maximize the translational impact of our proposal by concentrating our efforts on drugs that have received FDA approval for other diseases and/or are currently tested in clinical trials for breast cancer.
Role: Co-Investigator

1R01HL142992-01
Effects of Rare Variants and Ancestry on Beta Agonist Response in Asthma and COPD
Surveillance trials suggest that the risk for life-threatening asthma exacerbations and asthma-related deaths are increased with long-acting beta$_2$-adrenergic receptor (β2AR) agonist (LABA) therapy; however, large clinical safety trials have not confirmed these observations despite studies showing that African Americans with asthma are more likely to respond adversely to LABA therapy. We have shown that ancestry-specific rare variants in the β2AR gene are associated with worse asthma control in people using LABA and that African genetic ancestry associates strongly with lung function in African Americans with severe asthma and COPD suggesting that genetic variants could play a role in drug response and disease severity. We propose genetic studies based on β2AR pathway gene variants and whole-genome studies of rare variants and genetic ancestry to identify novel mechanisms for inter-ethnic differences in drug response and disease severity.
Role: Co-Investigator

Lung Cancer Retreat Pilot (Zhao)
WFBCCC
A Novel Nano-Immunotherapy System to Enhance Anti-Lung Cancer Immunity
To study a novel nanoparticle-immunotherapy system to synergize with immune checkpoint inhibitors against lung cancer in mouse models
Role: Co-Investigator

1R15 NS098405 (Hegde)
NINDS
Nuclear Role of the Proteasome in Synaptic Plasticity
This project will study how the gene-regulating function of the proteasome contributes to change in the synapses. Proteasome is important for normal function in the brain. Proteasome function is compromised in many diseases and disorders of the brain. This research could help explain how memory forms in the normal brain and how memory loss occurs in brain diseases.
Role: Co-Investigator

1P30 DK124723-01 (McClain)
NIH/NIDDK
North Carolina Diabetes Research Center
The prevalence of diabetes mellitus in the United States is reaching epidemic proportions and accounts for a huge national burden of morbidity, mortality, and health care expenditures. The mission of the Diabetes Research Centers is to serve as a key component of the NIDDK-supported research effort to develop new therapies and improve the health of Americans with, or at risk for, diabetes and related endocrine and metabolic disorders.
Role: Co-Investigator

Name: Carol Milligan, PhD
Project Role: co-I
Nearest person month worked: 2.7 months on AL170130
Contribution to Project: Dr. Milligan is a Professor in Neurobiology and Anatomy with experience and expertise in neurodegenerative processes, notably those that occur in ALS. She is working with Dr. Hawkins in designing and testing the mouse model produced in this proposal, evaluating the role of IL6 transsignaling in the ALS mouse model and will be involved in data interpretation and manuscript preparation.

Funding support:
1 R03 AI137866-01
Development of IL6 Trans-signaling Mouse Model as a Shared Resource
This project is to develop a mouse model that incorporates a single nucleotide mutation in the protease cleavage site for the IL6 receptor that should mimic the human polymorphism that results in enhanced shedding of the receptor from the cell membrane. If successful, the model will be used in research related to neurodegenerative diseases, asthma, cancer, and cardiovascular diseases.
Role: co-PI
Overlap: None

NIH 5R25NS089458
Training in Health Disparity Research for a Diverse Neuroscience Workforce
This is a new Master's Program specifically developed to broaden educational opportunities for individuals interested in pursuing a career in Health Disparities in Neuroscience-related Disorders (HDND). The overall goal is to train individuals who will contribute to expanding diversity in the Neuroscience work-force. The program takes advantage of a strong Neuroscience Graduate Program and the Maya Angelou Center for Health Equity (MACHE) at WFU. Drs. Milligan, Director of the Neuroscience Program and Ronny Bell, Director of the MACHE are co-directors/co-PIs of the HDND MS program. Dr Bertoni took over for Dr. Bell when Dr. Bell left the institution.
Role: co-PI
Overlap: None

Hope for Tomorrow ALS Foundation (Milligan)
The funds provided support for preliminary studies of IL-6 signaling in ALS patients.
Role: PI (effort as needed)
Overlap: None

Brian White ALS Foundation Funds
These funds are a contribution by the Department of Neurology for the Project "Hsp70 as a Potential Treatment for ALS"
Role: PI (effort as needed)
Overlap: None

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Hawkins

R01 DK120667-01A1 (Solberg Woods) 09/20/2019 – 06/30/2024 0.24 CM
NIH/NIDDK $439,353
Systems Genetics to Identify Neuronal Genes for Diet-induced Obesity
The goal of this project is to identify genetic loci and underlying genes that regulate adiposity in response to a high fat diet. In addition to genetic mapping, we will use RNAsez to determine transcript abundance of genes in the hippocampus and hypothalamus and integrate these data with the physiological mapping.
Overlap: NONE

1P30 DK124723-01 (McClain) 04/07/2020 – 03/31/2025 1.2CM
NIH/NIDDK $874,592
North Carolina Diabetes Research Center
The prevalence of diabetes mellitus in the United States is reaching epidemic proportions and accounts for a huge national burden of morbidity, mortality, and health care expenditures. The mission of the Diabetes Research Centers is to serve as a key component of the NIDDK-supported research effort to develop new therapies and improve the health of Americans with, or at risk for, diabetes and related endocrine and metabolic disorders.
Overlap: NONE

P30 AG049638 **(Hawkins, Milligan, Hughes) 07/01/2020 to 06/30/2021.**
Effort as needed
WF Alzheimer's Center Pilot Award $20,000
Genetic and Biomarkers of IL6 Transsignaling in Alzheimer's Disease
We propose to investigate if increases in CSF IL6 and soluble IL6 receptor are exhibited by AD patients, and if patients who inherit the IL6R variant allele Asp358Ala demonstrate faster conversion from MCI to dementia. Data from this pilot award will serve as preliminary data for RO1 application.
Role: Co-PI
Overlap: NONE

Lung Cancer Retreat Pilot (Zhao) 09/24/2019 – 09/23/2020 0.12 CM
WFBCCC $100,000
A Novel Nano-Immunotherapy System to Enhance Anti-Lung Cancer Immunity
To study a novel nanoparticle-immunotherapy system to synergize with immune checkpoint inhibitors against lung cancer in mouse models
Role: Co-Investigator
Overlap: NONE

Milligan
DOD W81XWH2010265 (Lu PI) 08/01/2020 – 07/31/2022 1.8 pm
Novel Cas9/gRNA Ribonucleoprotein Bionanoparticles for Safe and Efficient Inactivation of ALS Disease-Causing Mutations
We propose to engineer an AAV capsid-based bionanoparticle to achieve efficient CRISPR/Cas9 RNA delivery to the CNS leading to only transient expression of Cas9. We will use these novel bionanoparticle to deliver *Cas9* mRNA and gRNAs to remove expanded G4C2 repeats from the *C9ORF72* gene in a mouse model of ALS.
Role: Co-Investigator
Overlap: NONE

P30 AG049638 (Hawkins, Milligan, Hughes) 07/01/2020 to 06/30/2021.
Effort as needed
WF Alzheimer's Center Pilot Award $20,000
Genetic and Biomarkers of IL6 Transsignaling in Alzheimer's Disease
We propose to investigate if increases in CSF IL6 and soluble IL6 receptor are exhibited by AD patients, and if patients who inherit the IL6R variant allele Asp358Ala demonstrate faster conversion from MCI to dementia. Data from this pilot award will serve as preliminary data for RO1 application.
Role: Co-PI
Overlap: NONE

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)
- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

NOTHING TO REPORT

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

NOTHING TO REPORT