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CONTRACTING ORGANIZATION: Wake Forest University Health Sciences

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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

NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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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, 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.

Aim 1: Perform a systematic examination of IL6 transsignaling in both initiation and progression of ALS. We completed this aim early in the award when we initially characterized IL6 expression in the SOD1 mouse model. Our initial survey of IL6 expression demonstrated expression in muscle, spinal cord and lung that correlated with pathological events in the mouse model (Figure 1). Interestingly, we showed similar results in a patient cohort (1).

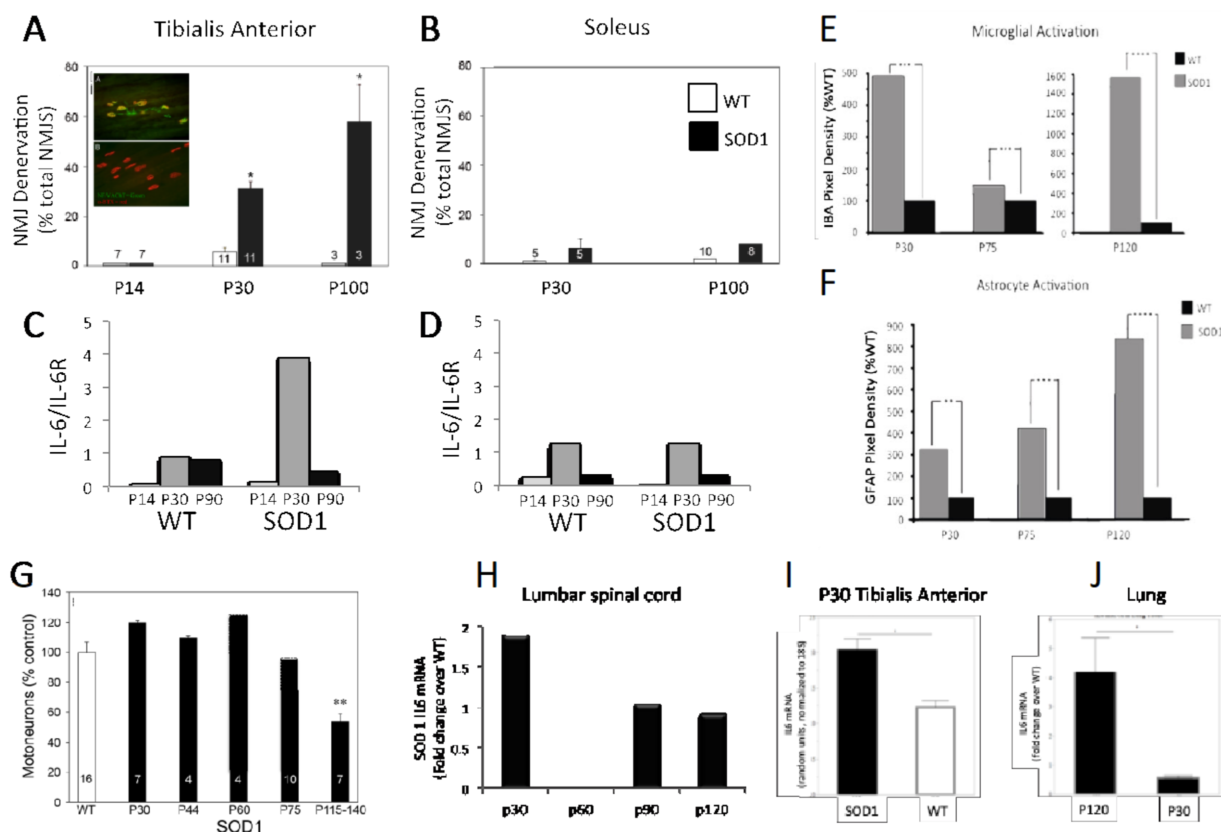

Figure 1. A, B) Muscle innervation was examined in the P14, P30 and P100 TA and soleus in *SOD1*^{G93A} and *WT* mice. While significant denervation occurs in the TA at P30, there is no denervation at P14, but by P100, 70% of TA NMJs were denervated. Soleus NMJs remain innervated even late in disease. The results are presented as % denervated of total NMJs/muscle (mean $\pm$ SEM). NMJ denervation was performed as previously described (2-5). 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. **C,D)** 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. 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. **E,F)** 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 5.* ***G)*** *Physical degeneration of MNs, loss of cell numbers occurs at endstage.* **p ≤ 0.01; statistical significance determined by t-test with Bonferroni correction *(see 49-50 for details).* ***H-J)*** IL6 mRNA levels in tissues involved in ALS pathology. **H**. 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. **I**. 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). **J**. 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**.**

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

Aim 2a: *Generation of Mouse Models of IL6 Transsignaling*

IL6 transsignaling is documented in both human and mouse (1, 8); however, mice do not naturally possess enhanced shedding of the IL6 receptor as occurs in human subjects who inherit the Il6raAla358 allele. 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) We created a unique IL6R transmembrane deletion (TMD) mouse model that exhibits tremendous shedding of the receptor (see Figures 6C and 7C). In the TMD model, there is no membrane bound receptor and all responses to IL6 are through transsignaling. This was the mouse model created with DoD funds.

We also generated a knock-in mouse model C57BL/6 Il6ra^{E357A} model of IL6 transsignaling. The **mouse *Il6ra* gene** was altered by incorporating a two base pair change (AA>CT) at the codon for amino acid 357, thus converting the Glu^{357} (GAA) to Ala^{357} (GCT) funded by R03 AI137866-01 (See Figure 6A, B). Incorporating this codon change also produced a novel Hind III site that allows us to identify mice heterozygous (HT) or homozygous (HM for the E357A allele (Figure 6B). This change targeted the Glu^{357} amino acid located at the equivalent position as Asp^{358} in the human peptide, and for which accounts for >50% of the variability of sIL6R in serum. In this mouse model, sIL6R levels in blood are elevated according to allelic dose of Il6ra^{E357A} and reflect profiles similar to those observed in humans (Figure 7A, B).

While both models exhibit increased levels of soluble IL6R, we determined that there were no differences in levels of sIL6R between serum or plasma in either model (not shown). Importantly, there are no significant differences in sIL6R levels between male and female mice and across ages (P30 to 1year: data not shown). Further, levels of sIL6R are consistent across generations of these mouse models suggest stable incorporation of the changes in the genome.

We have completed characterization of the models and 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 (6-9). Reports indicate that increased levels of *Il6* mRNA in mice within 2-6 hours of i.p. LPS injection (6-7), and we determined IL6 plasma levels are increased by 8 hours and maintained for at least 24 hours (Figure 6). Stat3 is a key transcription factor that is phosphorylated during activation by IL6 signaling (10-11). STAT3 phosphorylation in the liver, a tissue with high levels of expression of IL6R, is elevated as early as two hours following LPS administration in animals of all genotypes. While this activation subsides by 24 hours, tissues with low levels of IL6R expression (e.g., brain and kidney) show sustained STAT3 activation LPS treated Il6ra^{E357A} and il6ra^{TMD} homozygous mice as compared to WT littermates (Figure 8). our characterization of the IL6ra models to date reveals no difference between heterozygous or homozygous animals and WT littermates in terms of motor ability, appearance and behavior, survival (to 1 year), overall tissue morphology, muscle innervation or spinal cord histology (not shown). These results suggest that increased levels of sIL6R alone do not affect normal physiology.

We are now using both the TMD and Il6ra^{E357A} models to cross with the SOD1 animal experiments because consistent results across the models will confirm transsignaling mediated responses. These are important preclinical data to help with design of future trials to determine if inhibition of IL6 signaling can slow disease progression in ALS patients.

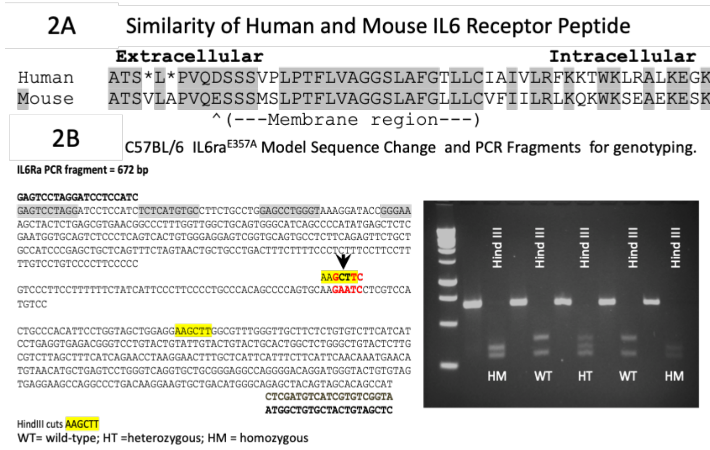
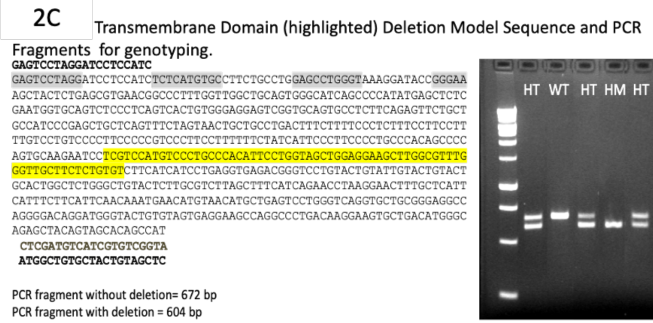
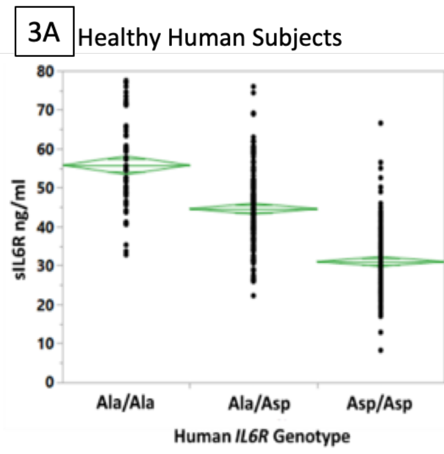
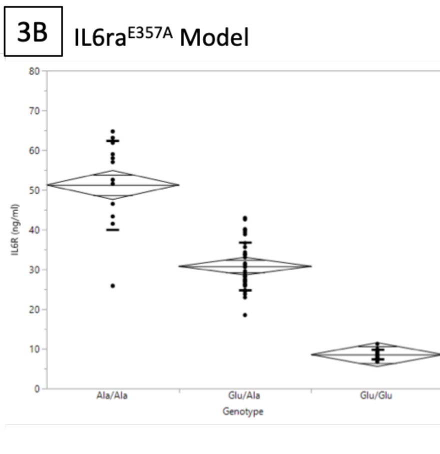
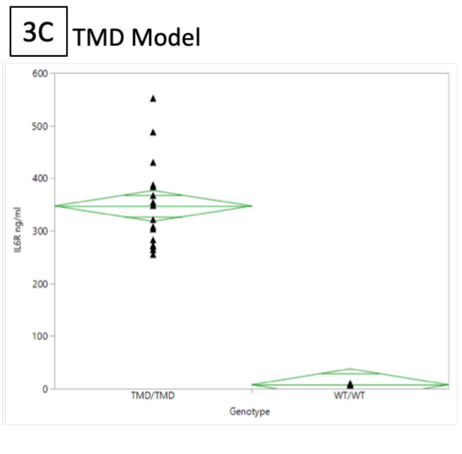
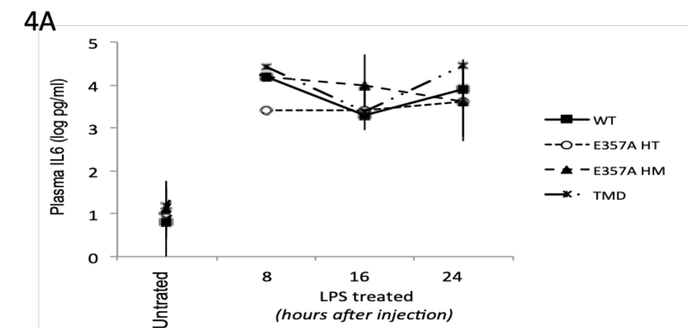
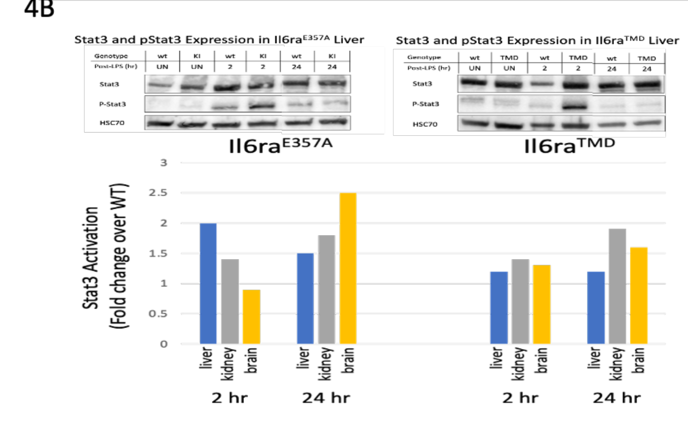

Figure 2. Generation of unique knock-in mouse model C57BL/6 Il6ra^{E357A} and IL6R transmembrane deletion (TMD) mouse models.

Figure 3. ELISA measurement of soluble IL6 receptor in (A) humans (left; n=471), (B)*Il6ra^{E357A}* mice at P90 (Ala/Ala n=13; Ala/Glu/n=35; Glu/Glu n=19; p<0.001 across genotypes; one way ANOVA), and (C) TMD mice at P90 (TMD/TMD n=17; WT/WT n=16; p<0.001 across genotypes; one way ANOVA) plotted. NOTE: Y axis scales the same for A and B, different scale for C.

Figure 4. A) ELISA measurements of plasma IL6 in untreated or LPS-treated (3 ug/g, i.p) WT, E357A homozygous, and TMD homozygous mice at indicated time points. For each treatment group P90 sex-matched, littermate WT and il6ra littermates were used. littermate,gender matched animals were used (n=2-3 groups/treatment group/time point). **B)** Shown are representative Western blots of untreated or LPS-treated WT, E357A homozygous (HM), and TMD homozygous liver protein extracts 2 and 24 hours after LPS administration. Phosphorylated STAT3 and total Stat3 levels were normalized to Hsc70 used as a loading control and the ration of P-Stat3/total Stat3 determined for each tissue sample (liver, spleen, kidney and brain). Results are expressed as fold-change of il6ra over WT littermate. Phosphorylated Stat3 expression increased by 2 hours in all animals, but was greater in the il6ra models, a pattern observed at 24 hours.

Aim 2b: Influence of enhanced transsignaling on disease progression in the SOD1G93A

mouse model of ALS

SOD1 animals crossed with the IL6raE357A mice have earlier symptom onset and in both Il6ra models exhibit enhanced glial activation.

We crossed both mouse models with the SOD1^{G93A} mouse model (high expresser on C57BL/6 background). Serum sIL6R levels corresponded to levels observed in non- SOD1^{G93A} Il6ra genotypes. We routinely begin monitoring the SOD1^{G93A} mice for overt disease deficits and welfare beginning at P50. Using failure to exhibit full leg-extension as symptom onset (e.g., 5) the SOD1^{G93A} X Il6ra^{E357A}+/+ mice had significantly earlier onset as compared to SOD1^{G93A} littermates (Figure 5). Interestingly, when heterozygous and homozygous mice (total =85 animals) were compared with SOD1^{G93A} mice carrying the allele showed earlier onset ($p<0.01$), mirroring the results observed in our human subjects (1). There were no differences in onset between the sexes in any group (not shown). We currently following the SOD1^{G93A} X Il6ra^{TMD} mice, and do not yet have sufficient animals generated for analysis.

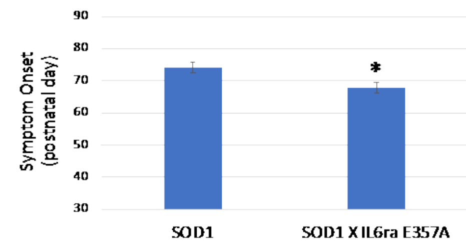

Figure 5. SOD1 mice homozygous for the Il6raE357A allele exhibit deficits in leg extension significantly earlier that SOD1 littermates not carrying the allele. SOD1: n=27 (20M/7F); SOD1 X E357A: n=18 (8M/10F). Statistical significance determined by T-test, * p=0.025

To determine differences in pathological events that may account for the earlier symptom onset, we examined NMJ denervation in the TA and soleus muscles of SOD1 and SOD1 littermates homozygous for E357A allele or IL6raTMD at P80, a time when NMJ denervation is advanced. There was no significant difference in the extent of NMJ denervation in any group at this age (not shown).

We examined "glial activation" as determined by increased expression GFAP for astrocytes. For microglial activation determined IBA1 expression together with CD68 (ED1) as we have found over the past few years this to remain the most reliable measure immunohistochemically of activated microglial (12-13). There is increased expression of these glial markers in SOD1XIl6ra$^{E357A/E357A}$ and SOD1XIl6ra$^{TMD/TMD}$ as compared to SOD1 at P80 (Figure 6). Interestingly, although reduced when compared to SOD1 counterparts, the Il6ra$^{E357A/E357A}$ and Il6ra$^{TMD/TMD}$ mice showed increase immunohistochemistry for GFAP (astrocytes) as compared to WT animals, suggesting that glial cells may be primed by soluble IL6R transsignaling even with physiological levels of IL6 (not shown).

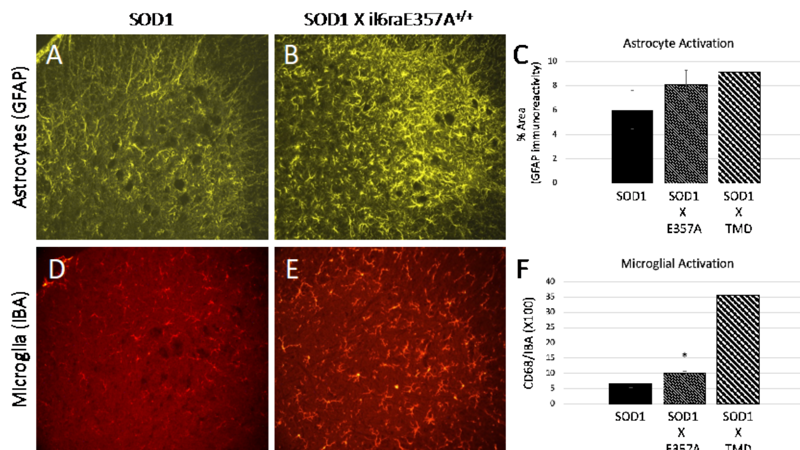

Figure 6. (A, B) Representative images immunohistochemical staining with GFAP of astrocytes in P80 L3-4 lateral motor column of SOD1 and SOD1 X il6raE357A $^{+/+}$ mice. (C) Quantification of GFAP staining (percent area using Image J) in SOD1(n=3), SOD1x il6raE357A $^{+/+}$(n=6) and SOD1xTMD$^{+/+}$ mice (n=2). (D, E) Representative images immunohistochemical staining with IBA of microglia in P80 L3-4 lateral motor column of SOD1 and SOD1 X il6raE357A $^{+/+}$ mice. (F) Microglial activation was determined by quantification of percent area for CD68 (not shown)/percent area of IBA in SOD1(n=3), SOD1x il6raE357A $^{+/+}$(n=6) and SOD1xTMD$^{+/+}$ mice (n=2). * $p<0.05$ SOD1 vs SOD1 X il6raE357A $^{+/+}$ mice as determined by Student's T-test.

Our preliminary data suggest in the SOD1 mouse model of ALS, enhanced IL6 transsignalling promotes greater microglial and astrocyte activation (Figure 6). In the Actemra study, patients inheriting the IL6raAla^{358} allele exhibited reduced CSF CRP levels following treatment with the antibody whereas those with this allele showed no change in CSF CRP (14). ***We began to identify specific cellular pathological pathways affected by IL6 transsignaling during disease progression***

using a non-biased spatial RNAseq analysis of a well-characterized regions of ventral, lateral lumbar spinal cord. Pathway analysis was initially performed to identify IL6-transsignaling specific pathways associated with MN and glial responses throughout disease. The results of this initial experiment suggest that we will be able to identify IL-6 transsignaling specific CNS responses to MN pathology that may identify novel pathways of glial activation or MN responses and provide targets for effective, therapeutic interventions.

Spatial transcriptomics can reveal gene expression architecture in native tissue to 1-10 cell resolution. This is a powerful approach because it allows pathway analysis within individual cells within the spatial confines of the tissue architecture. We have carefully mapped the L2-4 region of the spinal cord when we performed single cell RNAseq on TA and soleus motor pools in a previous study (R21NS091953). We have *optimized tissue processing and RNA collection from SOD1 and SOD1 X IL6raTMD+/+ female littermates at P80 (Figure 7). Most importantly, we present preliminary data regarding pathway analysis within MNs, and surrounding glia cells in SOD1 and SOD1 X Il6raTMD+/+ mice demonstrating differences in gene expression and pathway activation between the genotypes.*

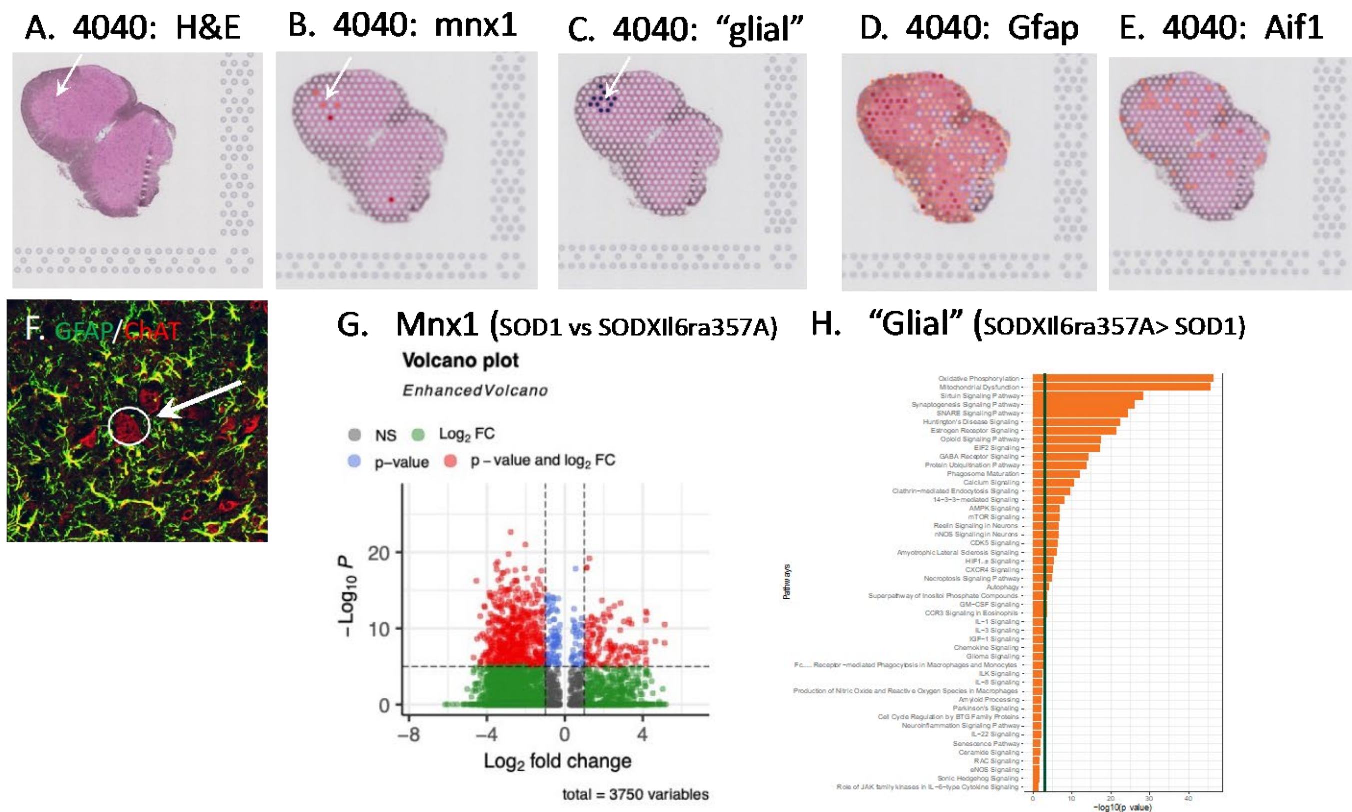

Figure 7. **Feasibility of performing spatial transcriptomics to determine IL6 transsignaling gene and pathways that mediate disease progression in *SOD1 mouse models*.** SOD1 X IL6raTMD+/+ female littermate L2-4 spinal cords at P80 were collected and processed for 10X genomics spatial transcriptomics. A-E. MNs were identified by location and morphology in H&E staining (A) and overlaying "spots" that also expressed the MN gene mnx1 (B) were identified in 6 spinal cord sections from each mouse. 30 MN spots were isolated from each animal and gene expression differences identified (G). Surrounding "glial" spots (C) that also expressed GFAP and/or IBA (aif1) were also selected, pooled/animals and pathways that showed enhanced expression in the transsingaling animals were identified (H). SOD1: animal 4039- 142,321 reads/spot; 2642 genes/spot; 88.7 reads mapped to genome; 18,378 total genes. SOD1 X IL6raTMD: animal 4040 (shown)- 164,436 reads/spot; 3224 genes/spot; 88.8 reads mapped to genome; 18, 399 total genes.

Aim 3. Blockade of IL6R delays disease progression in SOD1 animals with enhanced IL6 transsignaling.

Using a commercially available inhibitors of IL6R, we will determine how and if inhibition of the receptor (as occurs in patients treated with tocilizumab) affects disease progression and extend survival. InVivoMAb anti-mouse IL6R 15A monoclonal antibody is reported to bind with the mouse IL6 receptor and inhibit IL6 from binding (manufacturers data, bxcell.com/product/m-il-6r/; Tsukamoto et al., 2015). The antibody prevents both class and transsignaling and appears to be the mouse equivalent of tociluzumab. In these experiments we are administering the antibody or placebo (InVivoMAb rat IgG2b isotype control, anti-keyhole limpet hemocyanin as recommended by manufacturer) to SOD1, SOD1XIl6ra^{E358A}, and SOD1 X IL6Rra^{TMD} mice beginning at P30 and twice weekly thereafter. Dose and frequency will mirror that used in the tociluzumab trial (8mg/kg; approximately 160ug/mouse) but will be administered intraperitoneally (ip) as ip injections in mouse has equivalent delivery as intravenous but with less tissue damage that occurs with frequent injections at the tail vein injection site. ELISA assays to determine serum and CSF sIL6R levels will be used to confirm drug distribution. 15A treated mice are expected to exhibit 2-3 fold increased in serum sIL6R and 0.5 fold increase in CSF sIL6R.

We are pleased to report we have recently started injections in our first cohort of animals. Initially, we planned to perform these experiments with sgp130 as this agent originally was reported to block specifically IL6 transsignaling. We moved forward with the 15A antibody because this approach mirrors the clinical trial with Tocilizumab that is already FDA approved and has been shown to be safe and well-tolerated in ALS patients. Additionally, 15A and its placebo control are significantly less expensive and allow us to move forward with these experiments in the most cost-efficient manner.

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.

Presenting abstract of result at the 2022 Northeast ALS (NEALS) Consortium meeting (Nov. 1-3, 2022, Clearwater, Florida.

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:

- Our goal for the next reporting period will be to complete the therapeutic Arm of Aim 3.

- Preparation of a manuscript that describes the IL6 mouse model(s)

- Preparation of a manuscript that reports the result of IL6 therapy in the SOD1/IL6 transsignaling mouse models

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).

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.

Nothing to Report

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

Have there been research-associated costs that were unanticipated due to COVID-19?

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.

Are there impacts to performance that will affect cost and/or schedule?

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.

Have there been research-associated costs that were unanticipated due to COVID-19?

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:

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

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

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

1R21 CA253362-01
Tumor Microenvironment at Single Cell Level in Black and White NSCLC Patients
Major Goals: To test our hypothesis that distinct tumor immune cell ecosystems exist between AA and CA lung cancer patients, which may be caused by differential tumor genetic mutation patterns, and which may explain observed patterns of disparate lung cancer outcomes that may be partially overcome in the era of immunotherapy.
Role: Co-Investigator

1P30 DK124723-01
North Carolina Diabetes Research Center
Major Goals: 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

R01 MD015395
Social Factors, Epigenomics, and Lupus in African American Women (SELA
Major Goals: We will specifically seek to identify and characterize the epigenetic mechanisms by which positive and negative social experiences affect gene function and thereby influence the risk of lupus in African American women.
Role: Co-Investigator

P30 AG049638
WF Alzheimer's Center Pilot Award
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: PI

Overlap: None

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 R21 NS125171-01
Cell Senescence and Death in Neurodegenerative Diseases
This project will investigate cell senescene in the SOD1 mouse model of ALS and further determine if administration of senolytics can improve outcome.
Role: co-PI
Overlap: None

DOD W81XWH2010265
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-I
Overlap: None

P30 AG049638
WF Alzheimer's Center Pilot Award
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

1T32NS115704-01A1
Neuroscience Training at Wake Forest
The funds will support the broad-based, interdisciplinary training of our PhD students in the Wake Forest Neuroscience Program.
Role: 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.

Nothing to report

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