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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.

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TABLE OF CONTENTS

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.

SUMMARY
In this study we investigated the influence of enhanced IL6 trans-signaling, as occurs in individual who inherit the common *IL6R* coding variant (rs2228145; Asp^{358}>Ala^{358}; referred to as IL6R Ala^{358} variant or C allele here) on ALS disease processes in a newly developed mouse model of IL6 trans-signaling crossed with the $SOD1^{G93A}$ mouse model of ALS. We previously reported that the presence of this allele variant modifies disease progression in ALS patients (1-3). While we are completing final experiments and analyzing data for publication, our results suggest the following:

- As originally hypothesized, our initial data suggest IL6 trans-signaling provides beneficial effects at the neuromuscular junction that appears to correspond with delays in initial denervation, but later in disease increases susceptibility in traditionally resistant motor pools.
- There is a substantial increase in glial activation and while the consequences of this are not yet clear, it may contribute to the increased susceptibility of traditionally resistant motor pools.
- The RNA profiles of motor neurons and glial cells are distinct between SOD1 animals and SOD1 animals crossed with the model of enhanced trans-signaling.
- In SOD1 animals with enhanced IL6 trans-signaling, there is a small, but statistically significant earlier onset of the overt motor deficit of leg extension.
- Treatment with an IL6R function blocking antibody failed to alter onset of overt motor deficit in leg extension or overall survival. This result is comparable to that of the Tocilizumab clinical trial in ALS suggesting that while IL6 trans-signaling may play distinct roles in different aspects of disease pathology, overall blockade is not effective.

Together our results reinforce that ALS is a complex disease where individual cytokines such as IL6 play distinct and different roles at different locations and times throughout disease progression. Our results suggest that while IL6 signaling clearly plays a role in ALS disease progression, an overall blockade is not effective at altering disease course. We must continue to investigate Interdisciplinary and multi-cellular paradigms to better understand the specific mechanisms involved to develop more targeted approaches.

Below we provide: 1) specific objectives/aims; 2) major activities; and 3) significant results or key outcomes.

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

We have initially characterized IL6 expression in the SOD1 mouse model. Our initial survey of IL6 expression suggests expression in muscle, spinal cord and lung correlates with pathological events- similarly to our results in patients as discussed above (Figure 1). We realized that continuing these experiments would waste resources because we would have to include SOD1 littermate controls in experiments with the SOD1 X IL6R^{TMD} mice. We therefore now focused on generating the crosses between the SOD1 mice and the IL6 transsignaling models.

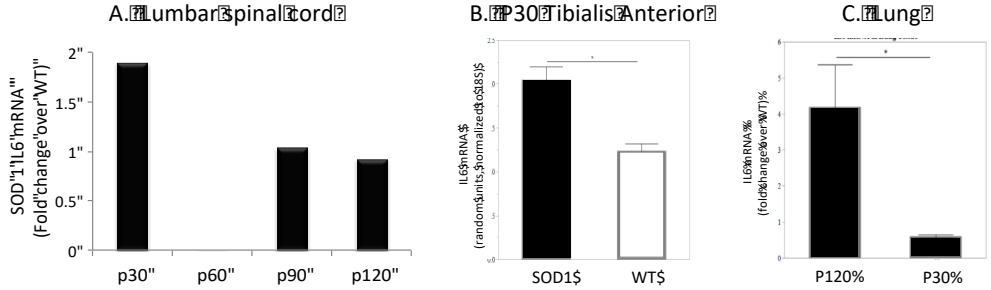

Figure 1. IL6 mRNA levels in tissues involved in ALS pathology. A. To determine if IL6 expression levels change in ventral lumbar spinal cord with disease progression, we performed a preliminary rtPCR experiment. Message levels for cytokine are increased in SOD1 mice as compared to WT at P30, 90 and 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).

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

While the progress of this Aim was greatly affected by the COVID-19 pandemic, we were fortunate to be granted funding and an extension of the project to continue this work. Further, in 2018, right after we received notice of the DoD award, we were notified that we were also receiving a NIH R03 to generate a second mouse model of IL6 trans-signaling generated by a single nucleotide polymorphism that would mirror the single nucleotide polymorphism IL6R Ala358 that occurs in humans (see Figure 2). As both grants were awarded at the same time, we worked in parallel to generate both models of IL6 trans-signaling. This was fortuitous because if one model failed to produce the desired genotype and functional phenotype, we would have the second model in hand. Importantly, we have been quite successful. Following initial characterization, both models behave as expected in terms of levels of soluble receptor. This provided two valuable experimental approaches to investigate IL6 trans-signaling. The TMD model that was proposed and generated with the AL170130 award allows us to determine specific pathological changes that are due solely to IL6 trans-signaling because in the homozygous animal there is no membrane bound receptor. We performed our studies using both mouse models and report the results below, and while both models show alterations in SOD1 pathology, *we believe the TMD model supported by this award is the better experimental model for investigators.*

GENERATION OF THE MOUSE MODELS

To accurately investigate potential mechanisms by which IL6 transsignaling contributes to disease progression we 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) (See Figure 2A, 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 E^{357}A allele (Figure 2B). 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 3A, B). We also created a unique IL6R transmembrane deletion (TMD) mouse model that exhibits tremendous shedding of the receptor (see Figures 2C and 3C). In the TMD model, there is no membrane bound receptor and all responses to IL6 are through transsignaling. Both mouse models were generated in consultation with Dr. Dale Crowley and colleagues at the UNC Animal Models Core using CRISPR/Cas9 technology and on the C57BL/6 background. ***While we focused the work funded by this project on the TMD model, we used both in the proposed experiments as consistent results across the models will confirm transsignaling mediated responses.*** After initial crosses and characterizations, we identified one line of each model for continued study (manuscript in preparation). We have determined that

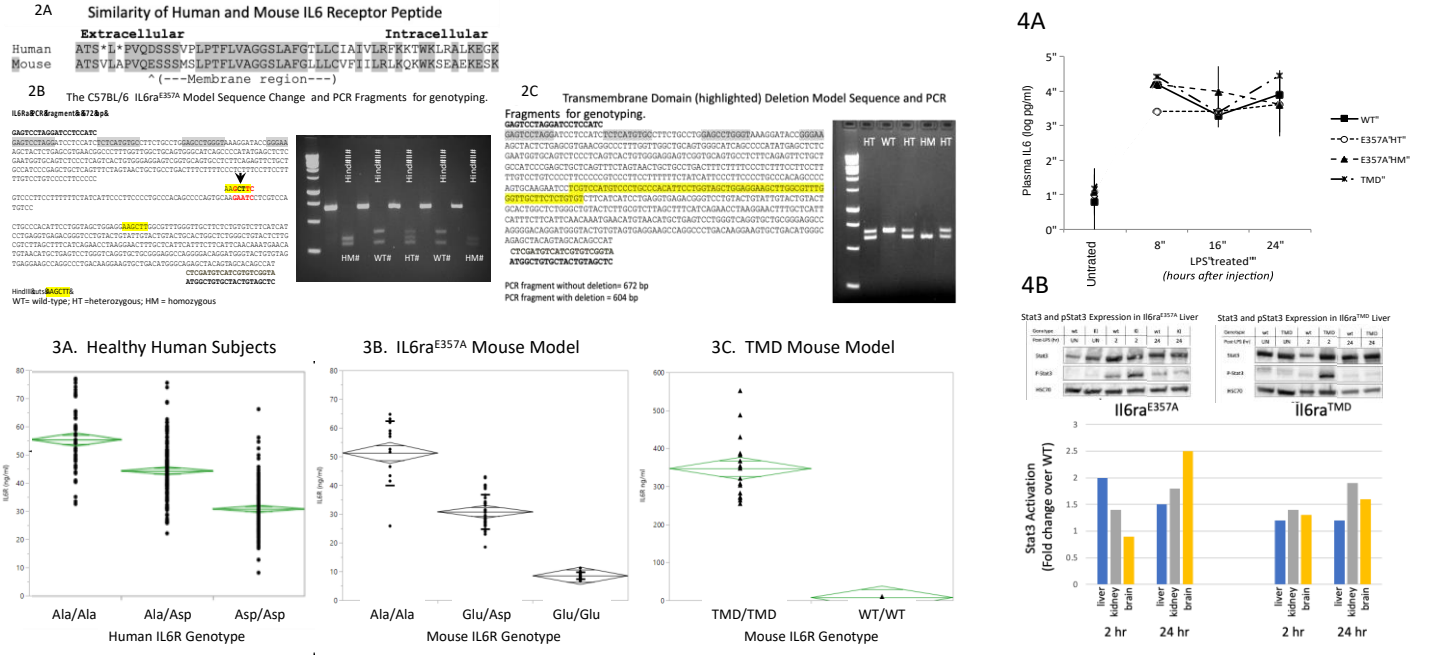

Figure 2. Generation of unique knock-in mouse model C57BL/6 *Il6ra*^{E357A} and IL6R transmembrane deletion (TMD) mouse models.

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.

there are no differences in levels of sIL6R between serum or plasma (not shown). Also, there are no significant differences in sIL6R levels between male and female mice and across ages (P30 to 1year: data not shown). 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 (4-7). Reports indicate that increased levels of *Il6ra* mRNA in mice within 2-6 hours of i.p. LPS injection (4,5), and we determined IL6 plasma levels are increased by 8 hours and maintained for at least 24 hours (Figure 2). Stat3 is a key transcription factor that is phosphorylated during activation by IL6 signaling (8,9). 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 4). 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.

SOD1 X IL6RA^{E357A} MICE EXHIBIT EARLIER SYMPTOM ONSET AND 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., 10) 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, those 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 also followed the SOD1^{G93A} X *Il6ra*^{TMD} mice that also showed earlier onset, also the difference did not reach statistical significance (not shown).

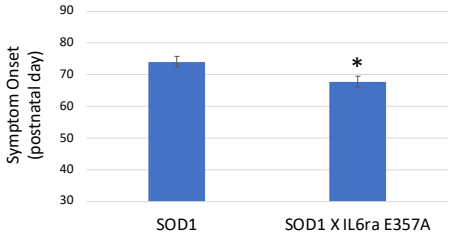

Figure 5. SOD1 mice homozygous for the *Il6ra*E^{357}A allele exhibit deficits in leg extension significantly earlier that SOD1 littermates not carrying the allele. SOD1: n=27 (20M/7F); SOD1 X E^{357}A: n=18 (8M/10F). Statistical significance determined by T-test, * p=0.025

IL6 TRANS-SIGNALING EFFECTS ON ALS DISEASE PATHOLOGY

To begin to determine differences in pathological events that may account for the earlier symptom onset, we initially focused on glial activation as increases in CSF IL6 was unique to ALS patients (vs health or disease controls; (1) and individuals inheriting the Ala^{358} variant showed decreases in CRP in response to TCZ (3). We examined "glial activation" as determined by increased expression GFAP for astrocytes. For microglial activation, we determined IBA1 expression together with CD68 (ED1; not shown) as we have found over the past few years this to remain the most reliable measure immunohistochemically of activated microglial (11-12). There is increased expression of all glial markers in SOD1X*Il6ra*$^{E357A/E357A}$ and SOD1X*Il6ra*$^{TMD/TMD}$ as compared to SOD1 at P80 (Figure 6). For these experiments, littermate controls were collected across the cohort). Microglial "activation", as determined by increased expression of IBA and CD68, is increased in SOD/transsignaling crosses (SOD1 X IL6ra^{E357A}; SOD1 X TMD) as compared to SOD1 animals. Astrocyte activation, as determined by increased expression of GFAP, is increased in the IL6 transsignaling animals as compared to WT littermates, suggesting that priming of astrocytes is occurring in individuals susceptible to enhanced IL6 transsignaling. GFAP immunohistochemistry is further increased in SOD/transsignaling crosses (SOD1 X IL6ra^{E357A}; SOD1 X TMD) as compared to SOD1 animals indicating that IL6 transsignaling is an important factor in astrocyte activation. 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, as previously shown (13) that glial cells may be primed by sIL6R transsignaling even with physiological levels of IL6.

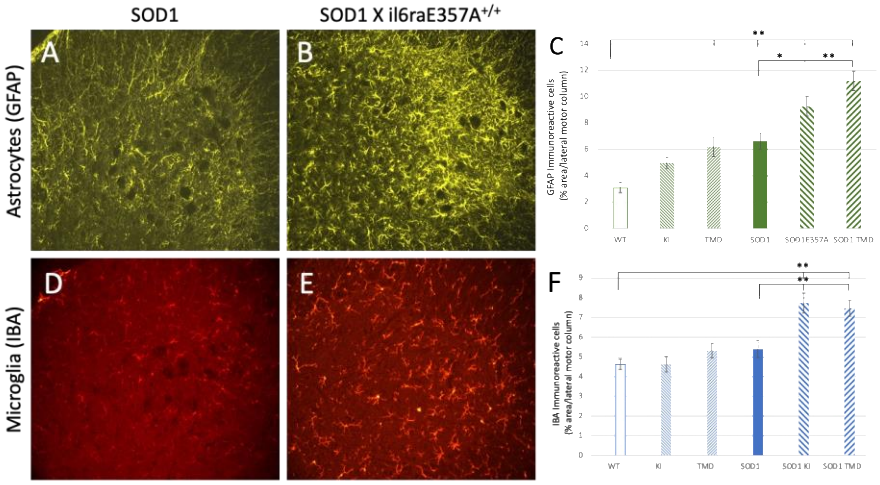

Figure 6. (A, B) Representative images immunohistochemical staining with GFAP of astrocytes in P80 L3-4 lateral motor column of SOD1 and SOD1 X Il6raE^{357}A $^{+/+}$ mice. (D, E) Representative images immunohistochemical staining with IBA of microglia in P80 L3-4 lateral motor column of SOD1 and SOD1 X Il6raE^{357}A $^{+/+}$ mice. (C, F) Quantification of percent area of IBA, CD68 or GFAP was performed using Image J software in wildtype (WT; n=8, 4F, 4M), IL6ra$^{E357A+/+}$ (n=9, 5M, 4F), IL6ra^{TMD} (n=10; 5M, 5F), SOD1 (n=11; 7M, 4F), SOD1x Il6ra$^{E357A+/+}$(n=8, 4M, 4F) and SOD1xIL6ra$^{TMD+/+}$ mice (n=8, 4M, 4F). Adjacent sections were processed for GFAP and IBA/CD68 so data shown in A/B and DE are from the same animals. * $p<0.05$;** $p<0.01$ vs. WT or SOD1 vs. SOD1 X Il6ra models as determined by one way ANOVA followed by Bonferroni and Holm post hoc tests.

We also examined neuromuscular junction innervation/denervation in animals from the cohort above. There were no differences in innervation patterns in muscles of WT or enhanced transsignaling mice. By P80, denervation of NMJs is well underway in the tibialis anterior muscle as previously reported (14-15) and we did not observe differences between SOD1 and SOD1 animals with enhanced transsignaling (Figure 7). We are currently examining an earlier time point (P40). Interestingly, while denervation is underway in the SOD1 TA (40% of NMJs are denervated), in the SOD1 X IL6raTMD animals, there is no denervation in the TA. We are evaluating additional animals and is the results hold, we believe that IL6 trans-signaling may be protective in maintaining NMJ innervation. Interestingly, in the SOD1 X TMD model of enhanced transsignaling, we observed statistically significant denervation of the soleus muscle at P80. These results are intriguing as we did not detect denervation of soleus muscle in SOD1 mice in our previous studies (14-15).

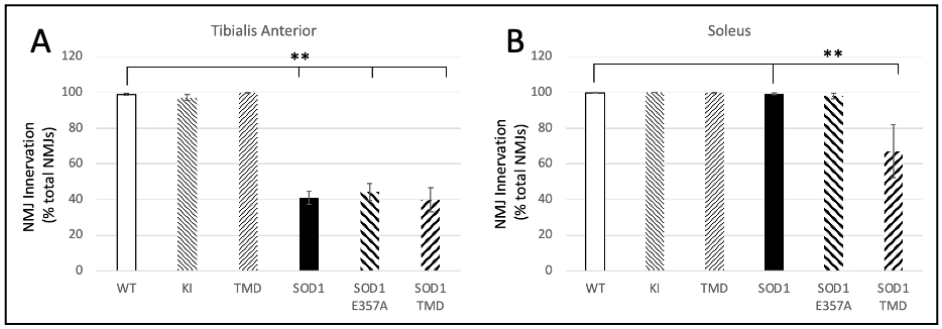

Figure 7. A, B) Muscle innervation was examined in the P80 TA and soleus in software in wildtype (WT; n=12, 6F, 6M), IL6ra$^{E357A+/+}$ (n=11, 6M, 5F), IL6ra^{TMD} (n=9; 4M, 5F), SOD1 (n=12; 7M, 5F), SOD1x Il6ra$^{E357A+/+}$(n=8, 3M, 5F) and SOD1xIL6ra$^{TMD+/+}$ mice (n=8, 4M, 4F). While significant denervation occurs in the TA in SOD1 by P80, we did not observe differences between SOD1 and SOD1 animals with enhanced IL6 transsignaling. At P80, significant denervation is occurring in the SOD1 mouse where all IL6 signaling occurs via transsignaling. NMJ innervation was performed as previously described (49-52). The results are presented as % denervated of total NMJs/muscle (mean +SEM). **p<0.01 vs. WT or SOD1 as determined by one way ANOVA followed by Bonferroni and Holm post hoc tests.

Our data suggest in the SOD1 mouse model of ALS, enhanced IL6 transsignaling promotes greater microglial and astrocyte activation (Figure 6). In the TCZ study, patients inheriting the *IL6R* Ala^{358} variant exhibited reduced CSF CRP levels following treatment with the antibody whereas those without the allele showed no change in CSF CRP (3). *Is there a specific, IL6 transsignaling mediated glial response? Do spinal MNs have unique responses in conditions of transsignaling?* To begin to identify specific cellular pathological pathways affected by IL6 transsignaling during disease progression we used a non-biased spatial RNAseq analysis of a well-characterized regions of ventral, lateral lumbar spinal cord. Pathway analysis will was performed to begin to identify IL6 transsignaling specific pathways associated with MN and glial responses throughout disease. The results of these experiments identified potential, IL6 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 powerful approach 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. We optimized tissue processing and RNA collection from SOD1 and SOD1 X IL6raTMD+/+ female littermates at P80 (Figure 8). Most importantly, we present 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 genotypes.

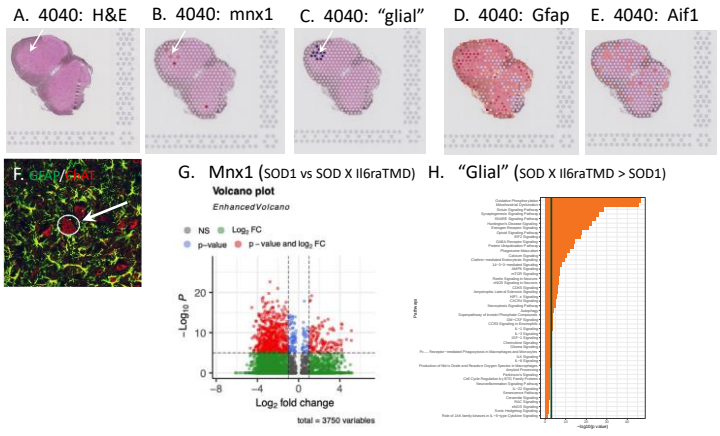

Figure 8. **Feasibility of spatial transcriptomics to determine IL6 transsignaling altered pathways that mediate disease progression in *SOD1 mouse models***. SOD1 X IL6raTMD+/+ female littermate L2-4 spinal cords at P80 were collected and processed for 10X genomics spatial transcriptomics. A-E. MNs 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 transsignaling 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 *IL6ra*TMD: animal 4040 (shown)- 164,436 reads/spot; 3224 genes/spot; 88.8 reads mapped to genome; 18, 399 total genes.

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

We made a modification to this aim and used a commercially available antibody to mouse IL6R to determine if inhibition of the IL6R receptor (as occurs in patients treated with tocilizumab;TCZ) can delay symptom onset and extend survival in SOD1 and the SOD1 mice with enhanced IL6 trans-signaling. InVivoMAb anti-mouse Il6ra15A monoclonal antibody is reported to bind with the mouse IL6 receptor and inhibit IL6 from binding (manufacturers data, bxcell.com/product/mil6r/; 16). The antibody prevents both class and transsignaling and appears to be the mouse equivalent of TCZ. In these experiments we administered the antibody or the isotype control (ant-keyhole limpet hemocyanin) as recommended by manufacturer), or vehicle twice each week beginning at P30. Dose mirrored that used in the TCZ trial (8mg/kg; approximately 160ug/mouse) but will be administered intraperitoneally as i.p. in mouse has equivalent delivery as intravenous (17) but with less tissue damage at the injection site. Unfortunately, there was no change in time of symptom onset or survival in any animals treated with IL6R blocking antibody, control antibody or vehicle (Figure 9). While the number of animals treated in these experiments are below the recommended number for pre-clinical studies, we believe there is no rationale for continuing to treat additional animals and if no differences are detected, will terminate these experiments after a total of 10 animals for each group is achieved. While we cannot independently confirm function-blocking, ELISA assays determined that serum sIL6R levels confirmed drug distribution. 15 A- treated mice exhibited 2-3 fold increases in serum IL6R levels in SOD1 and SOD1 X IL6raE357A mice. We believe increases are masked by the substantially increased levels of soluble receptor in TMD model (Figure 10).

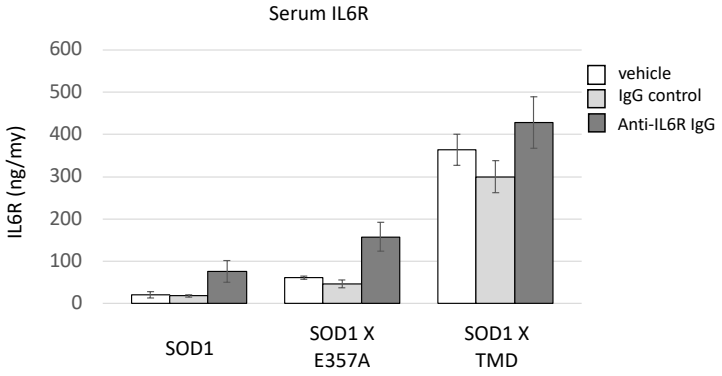

Figure 9 (left). SOD1 mice were treated as described above. SOD1: vehicle (n=6); control (n=8); 15A antibody (n=9). SOD1 X IL6ra^{E357A}: vehicle (n=7); control (n=8); 15A antibody (n=9). SOD1 X IL6ra^{TMD}: vehicle (n=3); control (n=5); 15A antibody (n=9). There were no significant differences across groups as determined by one way ANOVA followed by Bonferroni and Holm post hoc tests.

Figure 10 (right). Serum from collected from a subset of mice treated with IL6R receptor blocking antibodies shown in Figure 9. ELISAs were performed as in Figure 2. SOD1: vehicle (n=2); control (n=4); 15A antibody (n=4). SOD1 X IL6ra^{E357A}: vehicle (n=3); control (n=3); 15A antibody (n=4). SOD1 X IL6ra^{TMD}: vehicle (n=2); control (n=2); 15A antibody (n=3).

LITERATURE CITED

1. Wosiski-Kuhn M, Robinson M, Strupe J, Arounleut P, Martin M, Caress J, Cartwright M, Bowser R, Cudkowicz M, Langefeld C, Hawkins GA, Milligan C. IL6 receptor358Ala variant and trans-signaling are disease modifiers in amyotrophic lateral sclerosis. Neurol Neuroimmunol Neuroinflamm. 2019 Oct 14;6(6):e631 PMID: 31611269
2. Wosiski-Kuhn M, Caress JB, Cartwright M, Hawkins GA, Milligan C (2020). Interleukin 6 (IL6) Level is a Biomarker for Functional Disease Progression Within IL6R358Ala Variant Groups in Amyotrophic Lateral Sclerosis Patients. Amyotroph Lateral Scler Frontotemporal Degener. 2020 Sep 14:1-12. PMID: 32924606
3. Milligan C, Atassi N, Babu S, Barohn RJ, Caress JB, Cudkowicz ME, Evora A, Hawkins GA, Wosiski-Kuhn M, Macklin EA, Shefner JM, Simmons Z, Bowser RP, Ladha SS. Tocilizumab is safe and tolerable and reduces C-reactive protein concentrations in the plasma and cerebrospinal fluid of ALS patients. Muscle Nerve. 2021 Sep;64(3):309-320. PMID: 34075589 PMCID: PMC9132344.
4. Krüttgen A, Rose-John S. Interleukin-6 in sepsis and capillary leakage syndrome. J Interferon Cytokine Res. 2012 Feb;32(2):60-5 PMID: 22136372
5. Szot, P, Franklin A, Figlewicz DP, Beuca TP, Bullock K, Hansen, K, Banks WA, Raskind MA, Peskind ER. Multiple Lipospolysaccaharide (LPS) injections alter interleukin 6 (IL6), IL-17, IL-10 and IL6 and IL-7 receptor mRNA in CNS and spleen. 2017. Neuroscience 355:9-21. PMID: 28456715

6. Radulovic K, Anyengo RM, Kaya B, Steinert A, Niess JH (2018). Injections of lipopolysaccharide into mice to mimic entrance to microbialderived products after intestinal barrier breach. JOVE 135: e57610 PMID: 29782025
7. Cai KC, van Mil S, Murray E, Mallet J-F, Matar C, Ismail N (2016). Age and sex differences in immune response following LPS treatment in mice. Brain, Behavior and Immunity 58:327-337. PMID: 27506825
8. Grivennikov S, Karin E, Terzic J, Mucida D, Yu GY, Vallabhapurapu S, Scheller J, Rose-John S, Cheroutre H, Eckmann L, Karin M. IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. Cancer Cell 2009; 15:103-13. PMID: 19185845 PMCID: PMC2667107
9. Nowell MA, Williams AS, Carty SA, Scheller J, Hayes AJ, Jones GW, Richards PJ, Slinn S, Ernst M, Jenkins BJ, Topley N, Rose-John S, Jones SA. Therapeutic targeting of IL-6 trans signaling counteracts STAT3 control of experimental inflammatory arthritis. J Immunol 2009; 182:613-22. PMID: 19109195
10. Gifondorwa DJ, Jimenz-Moreno R, Hayes CD, Rouhani H, Robinson MB, Strupe JL, Caress J, Milligan C. Administration of Recombinant Heat Shock Protein 70 Delays Peripheral Muscle Denervation in the SOD1(G93A) Mouse Model of Amyotrophic Lateral Sclerosis. Neurol Res Int. 2012;2012:170426. PMID: 22900172; PMCID: PMC3415202.
11. Milligan CE, Cunningham TJ, Levitt P. Differential immunochemical markers reveal the normal distribution of brain macrophages and microglia in the developing rat brain. J Comp Neurol. 1991 Dec 1;314(1):125-35. PMID: 1797868.
12. Milligan, C.E., P. Levitt and T.J. Cunningham (1991). Brain macrophages and microglia respond differently to lesions of the developing and adult visual system. J. Comparative Neurol. 314: 136-146. PMID: 1797869
13. Garner KM, Amin R, Johnson RW, Scarlett EJ, Burton MD. Microglia priming by interleukin-6 signaling is enhanced in aged mice. J Neuroimmunol. 2018 Nov 15;324:90-99. PMID: 30261355; PMCID: PMC6699492.
14. Vinsant S, Mansfield CM, Moreno RJ, Yoshikawa M, Moore V, Hampton TG, Prevette D, Caress, JB, Oppenheim RW, Milligan C. (2013) Characterization of early pathogenesis in the SOD1G93A mouse model of ALS, Part 1. Background and Methods. Brain and Behavior 3 (4): 335–350. PMID: 24381807 PMCID: PMC3869677
15. Vinsant S, Mansfield CM, Moreno RJ, Yoshikawa M. Moore V, Hampton TG, Prevette D, Caress, JB, Oppenheim RW, Milligan C. (2013) Characterization of early pathogenesis in the SOD1G93A mouse model of ALS, Part 2. Results and Discussion. Brain and Behavior 3 (4): 431–457. PMID: 24381813 PMCID: PMC3869693
16. Tsukamoto H, Senju S, Matsumura K, Swain SL, Nishimura Y. IL-6-mediated environmental conditioning of defective Th1 differentiation dampens antitumour immune responses in old age. Nat Commun. 2015 Apr 7;6:6702. PMID: 25850032; PMCID: PMC4396369.
17. Wong KP, Sha W, Zhang X, Huang SC. Effects of administration route, dietary condition, and blood glucose level on kinetics and uptake of 18F-FDG in mice. J Nucl Med. 2011 May;52(5):800-7. PMID: 21498533; PMCID: PMC3086987.

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.

Daniel Joshua Quillen is a MS graduate student in our Neuroscience Program. For part of his thesis project he is working with us to characterize the role of IL6 transsignaling in the ALS mouse model.

Alyson Curry was the senior research technician on this project and contributed to results presented in this report. She is currently a PhD student in the WFU Neuroscience Graduate Program.

William Stewart was a research technician on this project. His work here has inspired him to move onto PhD work and is applying for programs for fall 2024.

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.

This work was presented at the 2022 Northeast ALS Consortium meeting. This meeting include ALS scientists, clinicians and patients and caregivers.

Curry, A, Quillen DJ, Stewart W, Rector B, Hastie A, Hawkins GA, Milligan C (2022). IL6 Transsignaling Promotes More Rapid Disease Progression in ALS. Poster presented at the 21st Annual Meeting of the Northeastern Amyotrophic Lateral Sclerosis Consortium in Clearwater, FL.

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:

- NOTHING TO REPORT- FINAL REPORT

4. IMPACT:

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 impact to other disciplines in the creation of the IL6 Trans-signaling mice. Indeed, these mice are being used in a new study to investigate the role of IL6 trans-signaling in cardiac failure with Dr. Giselle Melendez.

1 R21 AI168902-01A1
A novel, genetic model of IL-6 trans-signaling to interrogate cardiac fibrosis pathology
Major Goals: Interleukin 6 (IL-6) trans-signaling is a non-canonical IL-6 signaling paradigm that is a modifier of inflammatory disease and enhanced by the inheritance of the common mutation *IL6R* $Asp^{358}Ala$. We will use our robust, novel C57BL/6 $Il6ra^{E357A}$ mouse model of enhanced IL-6 trans-signaling to investigate whether enhanced IL-6 trans-signaling worsens cardiac function and fibrosis in a model of cardiac pressure overload, and whether IL-6R blocking therapeutic interventions may will prevent or attenuate cardiac fibrosis and HF. We will utilize spatial transcriptomics to identify the type and location of cells in cardiac tissue that are responding to IL-6 trans-signaling. The results of this study will provide insight into how IL-6 trans-signaling modifies disease pathology, cardiac fibrosis, and heart failure, and response to IL-6R blocking **.**
PI: Melendez, Hawkins
Co-I: Milligan

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.*

This work resulted in the generation of new mouse models of IL6 transsignaling. These models will be deposited to the Jackson Laboratory for use by other investigators.

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.

For Aim 3, we changed the experimental design to treat with a mouse monoclonal antibody that functionally blocked the IL6R instead of using soluble gp130 as originally proposed. The reasons for this change were two-fold. 1) The monoclonal antibody is more aligned with FDA-approved Tocilizumab that has been used tested in ALS patients. Sgp130 is not FDA approved. 2) The cost of the antibody was substantially lower (5-fold lower).

The most significant challenge we faced during this work was the COVID pandemic.

Below are the responses to Dr. Dougherty submitted in October 2020 regarding our grant AL170130. This narrative below describes the effects of the COVID-19 pandemic on the work performed.

On March 16th, 2020, Dr. Julie Freischlag, CEO and Dean, Dr. Greg Burke, Senior Associate Dean for Research and Chief Science Officer, and Chris O'Byrne, VP and Associate Dean, Research Administration notified the institution with the following to be implemented starting March 18th, 2020:

- *Pause all in-person clinical research activities not linked directly to the health of the research participant.*
- *Reduce on-site staffing for animal and mechanistic research by focusing on activities directly linked to essential functions, such as maintaining the health of animals involved in research studies.*

On March 18th, an additional notice clarifying basic science and animal research planning stated:

Basic Science and Animal Research Teams

- *Apply your lab-specific pandemic plan.*
- *Research teams should seek to reduce on-site staffing and activities to the absolute minimum possible. Please work* ***to complete ongoing experiments by close of business on March 25.***
- ***It is suggested to only pair breeder animals that will provide the number of offspring needed to continue the breeding colony. The number of offspring should not include animals for experimentation.***
- *Research teams should provide for animals as required in the approved IACUC protocol.*
- *Environmental enrichment may be provided at minimum levels, unless the specific environmental enrichment is prescribed per a special considerations plan, i.e., for some nonhuman primates.*
- ***Minimize ongoing work as much as possible now***.

Animal Resources Program

- *ARP is working to reduce staff to those essential to maintaining the health and wellbeing of the research animals. The ARP will pause on non-essential activities effective March 25, including procedures that are not mandatory for the health of the animals.*
- *Veterinary clinical support will be reduced to essential activities. Veterinarians will remain available for consultation.*
- *ARP is stopping animal procurement, effective immediately.*
- *Transport will be available on a very limited basis until close of business March 25.*

How is variance in schedule going to impact cost, and can these impacts be quantified?

Mouse Colony

In accordance with our institutional guidelines shown above and the governor mandates, faculty and staff were allowed onsite after March 25th for only timed experiments that had already been established. With this in mind, we were able to sacrifice and collect tissue from animals that had already been assigned to specific ages for collection. We were able to freeze or preserve this tissue for experiments when we could return to the labs on a regular basis. Unfortunately, we did lose some tissue when our lab refrigerator malfunctioned and we were not aware for several days because we were not to be on site daily. In addition, while considered a minimal loss, it was compounded due to the animals lost could not be quickly replaced.

In accordance with the pandemic guidelines, we could not continue our breeding colony to generate animals for new experiments. We therefore had to sacrifice several pre-weaned litters of mice. We had to reduce our colony to only a few breeding cages to maintain the colony.

How did this impact our experiments?

We were able to finish collecting tissue from the IL6R mouse models at postnatal day 90 and 180 to characterize IL6R receptor expression in serum and tissues.

We were not able to continue to characterize the model in terms of responses to LPS stimulation because we were requested to "pause all experiments" and the litters of mice that had been generated for these experiments has to be sacrificed.

We were further set back as we were beginning to establish the crosses of the SOD1 X IL6R mice, and had to discontinue the colony expansion and reduce back to only cages that could maintain the colony

In July 2020, when we could return to the labs, we increased our number of breeding cages to expand the colony and began to resume experiments. With the COVID-19 pandemic, our research was set back by 6-9 months.

Personnel

During the shut-down, per Medical School guidelines, faculty and technical staff that were fully supported on research grants were not furloughed, but could have reduced hours. From March 25- July 1 our lab technician's time was reduced to 30 hours/week to account for reduced effort. She was primarily responsible for maintaining the colony during the "pause in research" and worked at home to maintain and update colony and lab records. Drs. Hawkins and Milligan continued to meet virtually to plan for experiments upon return to the labs as well as continue data analysis and manuscript preparation.

COVID-19 Cost Effects

The cost effects were multi-fold: 1.) cost to rebuild breeding colony, including animal per diem and reagents and supplies; 2.) unplanned cost to pay technical staff to expand animal colonies (Ms. Sabrina Lambeth). If desired, we calculate estimated losses.

Of note, during the COVID shutdown, we have transitioned into a no cost extension (NCE) on our grant. Dr. Milligan and I have significantly reduced our salary support under the NCE, however we continue to devote the required effort to the project. Because of the initial delay in hiring staff while the animals were being generated at UNC, we are able to continue with salary for our lab technician.

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

Yes. As mentioned above, we did not anticipate having to cull our colony, thus the regeneration costs of the mouse colony was a direct effect COVID 19. As a result, all of the mouse per diem, reagents, and supplies used to regenerate and genotype our colony will have to be extended, and thus can be contributed to the COVID 19 shutdown. As per our original communication, while we are in the process of restoring our research productivity, with the previous delays and concerns of another possible shutdown as we enter the fall and winter months, we thought it best to question if another NCE is possible. It is difficult for us to predict at this time anticipated remaining funds, but it is likely we may not be able to continue to support technical staff.

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?

Yes to both. Before the COVID shutdown, the project was successfully progressing towards developing the IL6 transsignaling mouse/SOD1 mouse cross. Because of previous delays obtaining the IL6R mice at Wake Forest (3 months), we knew we would have to take our project into at least a 1 year NCE and had created a timeline to finish our work during the 1 year NCE. Now with the COVID shutdown, we will have to repeat or delay multiple research steps, including regeneration of the IL6 breeding colony, delayed generation of the IL6 transsignaling/SOD1 mouse cross, and critical functional experiments that we expected to be performing at this time, but which will now be delayed at least 6-9 months.

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.

COVID-19 Cost Effects

The cost effects were multi-fold: 1.) cost to rebuild breeding colony, including animal per diem and reagents and supplies; 2.) unplanned cost to pay technical staff to expand animal colonies (Ms. Sabrina Lambeth). If desired, we calculate estimated losses.

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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

NOT APPLICABLE

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).*

  PUBLICATIONS ARE IN PREPARATION

  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.*

Curry, A, Quillen DJ, Stewart W, Rector B, Hastie A, Hawkins GA, Milligan C (2022). IL6 Transsignaling Promotes More Rapid Disease Progression in ALS. Poster presented at the 21st Annual Meeting of the Northeastern Amyotrophic Lateral Sclerosis Consortium in Clearwater, FL.

- **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.

Name: Carol Milligan, PhD
Project Role: co-I
Nearest person month worked: 2.7 months
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.

TECHNICIANS WHO WORKED ON THIS PROJECT:
Phonepasong Arounleut
Sabrina Miller
Brian Rector
Daniel Joshua Quillen
Alyson Curry
William Stewart

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.

1 R21 AI168902-01 4/1/23 – 3/31/25
PI: Hawkins, Melendez; co-I**:** Milligan
A novel, genetic mode of IL-6 trans-signaling to interrogate cardiac fibrosis pathology
This project will investigate the role of IL6 trans-signaling in congestive heart disease and cardiac failure.

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.

While work in this project focuses on the role of IL6 trans-signaling in ALS, the results have significance for other neurodegenerative diseases, including Alzheimer's Disease.

Quillen D, Hughes TM, Craft S, Howard T, Register T, Suerken C, Hawkins GA, Milligan C. Levels of Soluble Interleukin 6 Receptor and Asp358Ala Are Associated with Cognitive Performance and Alzheimer Disease Biomarkers. Neurol Neuroimmunol Neuroinflamm. 2023 Feb 21;10(3):e200095. PMID: 36810164; PMCID: PMC9944616.