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TITLE: Receptor for AGE (RAGE) Signal Transduction in Amyotrophic Lateral Sclerosis: In Vivo Imaging and Novel Therapeutic Approaches

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CONTRACTING ORGANIZATION: NEW YORK UNIVERSITY GROSSMAN SCHOOL OF MEDICINE

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14. ABSTRACT

We hypothesized that the receptor for advanced glycation end products (RAGE) is implicated in the pathogenesis of ALS, at least in part through microglial perturbation. Our findings: (1) RAGE-positive Cd11b-positive cells are increased in the ventral horn of male and female *SOD1*^{G93A} mouse lumbar spinal cord and male wild-type mice displayed higher proportions of RAGE-positive Cd11b cells than female wild-type mice. (2) In male *SOD1*^{G93A} mice, microglia deletion of *Ager* in the ALS mouse background prolongs survival and slows loss of body weight and motor function. We identified an independent negative effect of the *Cre* recombinase mouse line in the ALS background (*Cx3cr1*^{ERT2} cre). At this time, we are finalizing all of the studies in the genetic model including new studies on limited numbers of male and female *SOD1*^{G93A} mice and controls sacrificed pre-humane endpoint at day 120. (3) PET imaging using tracers to mark inflammation suggests higher spinal cord inflammation at day 100 and day 130 of life in *SOD1*^{G93A} mice vs. controls. (4) Orally available (medicated chow) small molecule antagonists of RAGE/DIAPH1 are being tested in *SOD1*^{G93A} mice. To date, the high dose treatment shows promise to reduce rate of weight loss and motor decline in combined male and female mice. Additional doses are underway at this time. Collectively, our data suggest deleterious roles for RAGE in ALS and indicate that further testing of this concept is warranted in this disease.

15. SUBJECT TERMS

Amyotrophic lateral sclerosis, DIAPH1, Microglia, Neurodegeneration, Receptor for advanced glycation end products, Small molecule antagonists

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1). **INTRODUCTION**

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder that results in paralysis and death within a few years of diagnosis. Evidence indicates that in both male and female veterans, the incidence of ALS is increased compared to age-matched non-veteran persons. Because of the devastation of this disorder, urgent efforts are required to identify the causes of and new therapies for ALS. Published work from our laboratory and others has shown that the receptor for advanced glycation end products (RAGE) is highly expressed in human ALS spinal cord, particularly in microglia, and to increased degrees vs. age-matched control subject spinal cord. We previously published that RAGE and its pro-inflammatory and pro-oxidative ligands, S100/calgranulins, high mobility group box 1 (HMGB1), and advanced glycation end products (AGEs), are highly expressed in human ALS spinal cord. Our published work tested administration of a soluble form of RAGE in the mutant $SOD1^{G93A}$ mouse model of ALS. We treated male mutant $SOD1^{G93A}$ mice with either soluble RAGE (sRAGE), a recombinant protein that sequesters RAGE ligands and suppresses their engagement of the cell surface receptor RAGE, or vehicle, murine serum albumin (MSA). Treatment was begun at age 56 days (pre-symptomatic) and continued once daily until sacrifice (20% weight loss *or* the inability of the animal to right itself within 20 seconds when placed on its side). Probability of survival and life span, motor function (grip strength and performance in hanging cage test) and spinal cord neuronal counts at sacrifice were significantly higher in sRAGE- vs. MSA-treated mice. These findings formed the basis of two specific goals for our grant: (1) Identification of the specific mechanisms by which RAGE contributes to ALS; and (2) To begin to develop a more feasible strategy to target RAGE, rather than a recombinant protein, our laboratory developed and recently reported on the generation of novel small molecule inhibitors of the interaction of the RAGE cytoplasmic domain with its intracellular signaling effector, DIAPH1. These small molecules block RAGE signaling and suppress RAGE-mediated inflammation in animals and are CNS-permeable. Therefore, we hypothesize that administration of these small molecules to $SOD1^{G93A}$ mice might prolong survival and attenuate loss of motor function. Collectively, these questions form the basis of our studies.

2). **KEY WORDS**

Amyotrophic lateral sclerosis

DIAPH1

Microglia

Neurodegeneration

Receptor for advanced glycation end products

Small molecule antagonists

3). **ACCOMPLISHMENTS:**

The major goals of the project are as originally proposed:

A). **What were the major goals of the project?**

Aim 1: We will test the hypothesis that microglia RAGE, through ligand-driven upregulation of inflammatory and pro-oxidative stress and suppression of reparative processes in the ALS spinal cord, mediates neuronal death and loss of motor function.

Task 1: Generate ALS mice (Sod1^{G93A}) with microglia deletion of *Ager*

Task 2: PET Imaging (in collaboration with Dr. Ding)

Aim 2: We will test the hypothesis that small molecule inhibitors of RAGE signal transduction will significantly prolong survival and delay neurodegeneration in mutant SOD1^{G93A} mice in proof-of-concept studies.

B). **What was accomplished under these goals?**

B.i. Major Activities

TASK 1: *Generate ALS mice (Sod1*^{G93A}) with microglia deletion of *Ager*

The breeding scheme as outlined in the proposal is generating the male and female mice needed for study. We used the *Sod1*^{G93A} mouse model into which we bred the *Cx3cr1*^{ERT2} cre recombinase mice and the *Ager* flox/flox. We carefully managed this breeding such that at breeding intervals we reintroduced new *Sod1*^{G93A} breeders and, therefore, the copy number in all offspring tested as been acceptable. Tamoxifen has been administered to all mice in the study on day 90 in order to ensure deletion of microglia *Ager* and not in the periphery by approximately day 100 (disease onset). The following endpoints are completed, including: analysis of survival, establishing the humane endpoint, and functional tests as outlined (motor function tests including hanging wire test, grip strength and righting reflex), isolation of microglia, and pathological analyses). Based on the efficiency of breeding, all of the mice have now been generated to test Aim 1 hypothesis.

Aim 1: We will test the hypothesis that microglia RAGE, through ligand-driven upregulation of inflammatory and pro-oxidative stress and suppression of reparative processes in the ALS spinal cord, mediates neuronal death and loss of motor function.

Our major activities include the completion of the breeding SOD1^{G93A} mice into the *Ager* flox/flox background and then intercrossing these mice into the *Cx3cr1* ERT2 cre recombinase background and we successfully generated the following lines of mice (both males and females).

SOD1^{G93A} / *Ager*$^{flox/flox}$ / *Cx3cr1*$^{CreERT2\ +/wt}$ (ALS+, Microglia specific *Ager* deletion)

SOD1^{G93A} / *Ager*$^{flox/flox}$ / *Cx3cr1*$^{CreERT2\ wt/wt}$ (ALS + *Ager* expressed in all cells)

SOD1^{G93A} / *Cx3cr1*$^{CreERT2\ +/wt}$ (ALS + *Ager* expressed in all cells; controls for CRE mice)

TASK 2: PET Imaging

In collaboration with Dr. Ding, we performed PET imaging of the ALS and control mice on a time course to determine if CNS inflammation changed over time.

AIM 2: We will test the hypothesis that small molecule inhibitors of RAGE signal transduction will significantly prolong survival and delay neurodegeneration in mutant SOD1^{G93A} mice in proof-of-concept studies.

Our lab made significant progress to identify the optimal small molecule that met multiple criteria to go forward for in vivo testing.

B.ii. Specific Objectives

Objective in Aim 1: We will test the hypothesis that microglia RAGE, through ligand-driven upregulation of inflammatory and pro-oxidative stress and suppression of reparative processes in the ALS spinal cord, mediates neuronal death and loss of motor function.

There were two major tasks: generation of ALS mice with microglia deletion of *Ager* and PET imaging of ALS vs. control mice to discern if there were differences in neuroinflammation

Objective in Aim 2: We will test the hypothesis that small molecule inhibitors of RAGE signal transduction will significantly prolong survival and delay neurodegeneration in mutant SOD1^{G93A} mice in proof-of-concept studies.

B.iii. Significant Results or Key Outcomes

Aim 1:

TASK 1: ALS, RAGE and Microglia

a). <u>RAGE expression in Spinal Cord microglia:</u> We examined the expression pattern of RAGE and CD11b, a marker of myeloid cells including microglia, in the ventral horn of *SOD1*^{G93A} mouse lumbar spinal cord. RAGE-positive Cd11b-positive cells are markedly enhanced in the ventral horn of male and female *SOD1*^{G93A} mouse lumbar spinal cord. In line with recent publications identifying sex-specific characteristics of microglia, we found that male wild-type mice displayed higher proportions of RAGE-positive Cd11b cells than female wild-type mice. Hence our data suggest that male microglia have higher basal levels of RAGE, thus supporting our goal to include mice of both sexes in our studies to evaluate any potential sex-dependent effects imparted by these basal differences.

b). Detection of *Ager* expression in microglia: We isolated microglia from the CNS of the mice lines and verified both by RT PCR and by Western blot that *Ager*/RAGE was successfully deleted from the microglia in the *Sod1*^{G93A} *Ager flox/flox Cx3cr1*^{ERT2} mice vs the controls.

c). Analysis of testing the hypothesis that microglia RAGE contributes to perturbation in ALS spinal cord:

Consistent with the induction of *Ager* deletion just prior to disease onset (TAM @ day 90), we found that there were no differences in the point of disease onset in the microglia *Ager*-deleted *SOD1*^{G93A} mice (male or female) vs the *SOD1*^{G93A} CRE control or the FLOX control. At sacrifice (as well as the earlier work presented in these progress reports, we confirmed that by immunohistochemical analysis, RAGE was deleted in the microglia of the microglia *Ager*-deleted *SOD1*^{G93A} mice compared to the CRE control or the FLOX control (all in the *SOD1*^{G93A} mice background).

After the commencement of our studies, recent work implicated CX3CR1 in neurodegeneration processes and in ALS, mice lacking CX3CR1 in the *SOD1*^{G93A} background displayed shorter survival than the controls only in the male but not female mice. In our mouse model, any mice with the CX3CR1 cre allele are essentially hemizygous knock out of this gene (even without Tamoxifen treatment), given that the CRE mice were made with knock-in approach. Hence, we carried out full characterization of our male and female mice and ensured that we assessed the CRE controls and the FLOX controls in all cases.

When both sexes were considered together, the microglia *Ager*-deletion did not affect survival of the mice. We then analyzed the data in male vs. female mice and found that in male microglia *Ager*-deleted *SOD1*^{G93A} mice, there was significantly improved survival when compared to the CRE control mice (*SOD1*^{G93A} background). There were no differences in the female mice. These data indicated that the microglia deletion of *Ager* (containing the CRE) improved survival compared to the CRE control alone in male mice, suggesting detrimental roles of microglia RAGE on this endpoint.

As the *SOD1*^{G93A} mice display significant weight loss as the disease progresses, we examined if microglia RAGE affected this endpoint. We found that there were no significant differences after correction for multiple comparisons in body weights between the genotypes of either sex at any time point. In addition, the maximum weights did not differ among the genotypes. However, we performed a rates of change analysis using piece-wise linear modeling over discrete periods of time and separated our analysis into three phases: 90-120 days (pathology onset), 120-140 days (active pathology progression) and 140 days-death (end stage). We found that female microglia *Ager*-deleted *SOD1*^{G93A} mice exhibited an increased rate of weight loss during 120-140 days. In contrast, male microglia *Ager*-deleted *SOD1*^{G93A} mice gained weight significantly faster than the male CRE controls from day 90-120 days and then subsequently they lost weight marginally slower than the CRE control mice from day 120-140 days and 140 days-death. Male but not female microglia *Ager*-deleted *SOD1*^{G93A} mice experienced a significant one week delay to reach loss of 10% of their peak weekly average body weight than male CRE control mice. Collectively, the male microglia *Ager*-deleted *SOD1*^{G93A} mice displayed evidence of protection from weight loss vs the CRE controls but this was not observed in the female mice.

We next examined motor function changes in these mice. Microglia *Ager*-deleted *SOD1*^{G93A} mice did not display significantly different motor function at any time point after correction for multiple testing relative to the CRE control mice. To determine if there were discrete time point specific effects during the progression of pathology, we utilized piece-wise linear modeling of the same periods of time described above. Male microglia *Ager*-deleted *SOD1*^{G93A} mice exhibited a significantly decrease rate of motor function decline from day 90-120 days and marginally decreased rates of decline from 120-140 days. However, there were no genotype specific differences in the female microglia *Ager*-deleted *SOD1*^{G93A} mice vs. the controls. Altogether, these data indicate that male microglia *Ager*-deleted *SOD1*^{G93A} mice displayed a slower rate of disease progression vs the CRE controls by multiple measures.

On account of the fact that at sacrifice, irrespective of the genotypes, many of the immunohistochemical endpoints may not unveil underlying mechanisms, we elected to study a smaller subset of mice, all sacrificed at the same time point. Accordingly, we are now working on a subset of male and female microglia *Ager*-deleted *SOD1*^{G93A} mice (vs. the controls) that were sacrificed at Day 120. We are completing this work in the post-COVID shutdown lab re start period and intend to include these data in our manuscript in preparation.

Since our last progress report, we have now performed the bulk RNA sequencing (RNA seq) of the lumbar spinal cord tissue comparing male microglia *Ager*-deleted *SOD1*^{G93A} mice vs. the CRE controls. This was done at end stage/sacrifice point. Early but ongoing analysis of these data reveal extremely enlightening findings regarding potential RAGE-dependent mechanisms in microglia in ALS mouse model as follows: We utilized the competitive gene ranking test CAMERA, for KEGG gene sets and found that several pathway gene sets including “Complement and coagulation” were more differentially expressed relative to other pathway gene sets. We further analyzed potential causal networks that may be modulated in our data set which could explain the observed transcriptomic changes. As *Ager* was solely deleted in CX3CR1-expressing cells we limited our analysis to potential cytokines that could originate from microglia and cause transcriptomic alterations across multiple cell types. We identified several significant putative causal networks belonging to numerous cytokine families, including “IFNalpha” and “IFNbeta”.

These results indicate reduction of the complement pathway and a putative causal network involving IL1. In this context, it is known that microglia-secreted molecules such as C1q, IL1a and TNF can induce astrocyte reactivity and promote neurotoxicity. We have begun to probe this specific premise in our data set. We utilized GFAP (glial fibrillary acidic protein) as a general marker of astrocytes and found that GFAP+ rea and GFAP+/DAPI+ cell number were significantly lower in the microglia *Ager*-deleted *SOD1*^{G93A} mice vs. the CRE controls. As recent work has suggested distinct classes of reactive astrocytes and that one subset is called “A1” or reactive/pro-inflammatory astrocytes. AMIGO2 was proposed as a marker of thee reactive “A1” astrocytes, which are induced by C1q, IL1a and TNF (at least in vitro). In the lumbar spinal cord of our mice, we found that GFAP+/AMIGO2+ overlap area was significantly reduced in male microglia *Ager*-deleted *SOD1*^{G93A} mice vs the CRE controls. Altogether, these data suggest that microglia *Ager* deletion may reduce astrocyte dysfunction, at least in the end stage of disease.

In addition, our RNA-seq data suggest that microglia *Ager*-deleted *SOD1*^{G93A} mice displayed reduced IL1 and IFN signaling in the lumbar spinal cord. These key findings raise the important

possibility that RAGE contributes to the apparent and well-described transition of microglia from homeostatic to a dysfunctional phenotype. We are currently finalized the counting of microglia at the end stage of disease (using multiple markers) but we have looked at a key marker of the DAM or disease-associated microglia phenotype in our mouse model. Specifically, CLEC7A is a marker of the DAM and previously found to be highly upregulated in the spinal cord of the *SOD1*^{G93A} mice. By immunohistochemistry, we find that there is a significant reduction in CLEC7A area and a significant reduction in CLEC7A+/DAPI+ cell number in the microglia *Ager*-deleted *SOD1*^{G93A} mice vs. the CRE control. Altogether, these data from the RNA seq have already provided very interesting and impactful insights on the effects of microglia RAGE on microglia and astrocyte properties in the ALS mouse model spinal cord.

As above we are working on the Day 120 studies and we are further probing the RNA seq data for additional insights that may be tested in the mouse model.

TASK 2: PET Imaging

We performed PET imaging with the tracer to track neuroinflammation as indicated in the grant proposal and our work revealed very promising findings in ALS mice (*Sod1*^{G93A}) vs. wild-type control mice

Day 100 of Life:

On day 100, consistently higher tracer uptakes were noted in all Regions of Interest (ROI) in the ALS vs wild-type mouse (this includes L2, L3 and T13 spinal cord, brain, lung and kidney); the difference is about 30%. These data suggest higher microglia activation and higher CNS inflammation in the ALS vs. the wild-type mice.

Day 130 of Life:

On day 130, uptake in the same regions as above was higher in the ALS mice vs. the control mice. Comparing day 130 to day 100 there did not appear to be a difference between the groups (that is, no further increase in the inflammatory endpoint was observed).

AIM 2: Over the past year, we made significant progress in identification of molecule for testing. The molecule that meets these criteria, known as RAGE229, is orally bioavailable and importantly we have now shown that it crosses the blood-CNS barrier. In studies in the lab, however, we found that the administration of RAGE229 must optimally be in the food. To prepare for this study, we tested the properties of RAGE229 when given in food. First the preparation is stable – once generated, the RAGE229 is placed inside the food pellets @ different doses (to deliver 30 mg/kg per day or 10 mg/kg per day). As the food needed to be irradiated, we also checked those pellets after the irradiation for the levels of RAGE229. These were all similar to the original level detected from the original mixture before pelleting and before irradiation.

Then, we administered RAGE229 to male and female C57BL6 male and female mice at the different doses vs. the control (vehicle) and observed a dose dependent degree of RAGE229 in their blood. Based on these promising data and the finding that RAGE229 crosses the Blood CNS barrier, we are putting RAGE229 in food for male and female ALS mice study.

Dose 1: 30 mg/kg/mouse/day chow vs. control chow

Female: *SOD1^{G93A}* Mice (C57BL/6J) - underway

Male: *SOD1^{G93A}* Mice (C57BL/6J - underway

Data to date: we analyzed the effects of RAGE229 vs. vehicle on body weight and motor function after the age of 17 weeks when the most pronounced effects are noted. Early data suggest protective effects of RAGE229 vs. vehicle on weight loss and on decline of motor function by hanging wire test. At the humane endpoint, lumbar spinal cord was retrieved from RAGE229 vs. vehicle-treated mice for immunofluorescence microscopy.

We combined male/female mice in the analysis and found that there were no differences in CD11B+ area. In addition to general markers of microglia, we assessed levels of CLEC7A, an established marker of the **pro-damage** DAM phenotype in microglia and found that there were trends to reduced CLEC7A (pro-damage DAM marker) (area and intensity) in RAGE229- vs. vehicle-treated mice. Furthermore, there were trends to increased NeuN/ChAT (motor neuron) area in RAGE229- vs. vehicle. Finally, we found that there were no differences in GFAP+ cells between the two groups (combined male and female mice). Collectively, these data suggest that RAGE229 may reduce weight loss and delay loss of motor function in *SOD1^{G93A}* mice, in parallel with reducing pro-damage DAM microglia markers and sparing motor neurons (at humane sacrifice), without effects on astrocyte content (GFAP). Pending is completion of the high dose study and the initiation of the second dose (10 mg/kg/day in medicated chow) vs. vehicle.

B.iv. Other Achievements: As above

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

The project was not intended to provide training and professional development opportunities, hence, based on this type of grant mechanism, there is "nothing to report."

However, there were extensive opportunities for training and professional development:

One of the PI's graduate students, Michael MacLean, has been exposed to extensive opportunities for training in the following areas:

- ALS: understanding of epidemiology, pathogenesis and history of therapeutic appraoches
- ALS: understanding of epidemiology with respect to veterans
- Breeding of SOD1^{G93A} mice and serial assessment of copy number
- Functional testing of SOD^{G93A} mice (hanging cage wire, grip strength)
- Monitoring of SOD1^{G93A} mice
- Establishing the humane endpoint (serial body weights and righting reflex)
- Using Automacs to isolate microglia
- Immunofluroesence microscopy to detect RAGE and cell types
- Performing RNA sequencing and data analysis
- Preparation of abstract and presentation

Conferences: Members of our study team attended the recent Cold Spring Harbor meeting on glia. Michael MacLean presented his work there as follows (abstract):

MacLean M, Juranek J, Cuddapah S, Hu J, Gugger P, Li H, Schmidt AM. Microglia RAGE exacerbates the procession of amyotrophic lateral sclerosis in male but not female SOD1G93A mice. Glia in Health & Disease; 2020 July 16; Cold Spring Harbor, NY, United States.

- **How were results disseminated to communities of interest?**

Nothing to report.

For the scientific community, we recently disseminated findings (as detailed above and cited below) at the Cold Spring Harbor meeting on "Glia in health and disease."

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

Aim 1: We are finalizing all studies based on continued aggressive breeding to obtain the final genotypes needed for full analysis. The interim analysis data are very promising and set the stage for beginning to understand how RAGE expression in microglia is deleterious in ALS mouse models.

In progress: Day 120 staining studies and then the final experiments to be performed based on the analysis of the completed RNA seq work

Aim 2: Complete studies to administer RAGE229 to mice as noted in the application vs vehicle. Based on extensive progress to identify an optimal reagent (RAGE229) and an optimal and feasible means for delivery of RAGE229 to the mice these studies are set through the no cost extension period. Dose 1 (30 mg/kg/mouse/day) is nearly completed and dose 2 will be begun shortly (10 mg/kg/mouse/day).

4). **IMPACT**

- **What was the impact on the development of the principal disciplines of the project?**

To date we have established the following:

- We have identified that in the ALS mouse (called $SOD1^{G93A}$) spinal cord, that the molecule called receptor for AGE or RAGE is highly expressed and particularly it is expressed in activated microglia, and not the unstimulated microglia in the spinal cord. This finding, based on the known biology of RAGE, strongly implicates this molecule in the pathogenesis of ALS and loss of neurons in the spinal cord, which causes, ultimately, paralysis and death.
- We have found that a lead molecule that blocks RAGE actions, which is a small molecule compound, is able to enter the central nervous system, of which the spinal cord, is a part. We have now found the optimal means to deliver this molecule in the food of the mice. This key finding means that we will be able to treat the mouse model of ALS, the $SOD1^{G93A}$ mouse, with this agent to test if it improves survival and motor

function. Our first data suggest benefit of the high dose of this molecule in the *SOD1*^{G93A} on motor function and body weight.
- We have found that by using noninvasive imaging of the ALS mouse spinal cord we can discern activated microglia (in the *SOD1*^{G93A} mice) from no glial activation in a normal mouse that does not have ALS.
- Our very promising data reveal that deletion of microglia RAGE is protective in combined male mice when compared to the cre control (ALS). In male mice, to date, we see similar results and we continue to add the female mice to completion. These key data suggest that RAGE in microglia may exert negative effects and further strenthens the relevance of this target for ALS. The basis of the sex dependent effects will be the subject of future investigations.

Taken together these findings hold great promise to:

- Identify an important pathway in the pathogenesis of ALS
- Identify a non invasive way to track glial inflammation in ALS (as a part of future therapeutic programs using the imaging as a way to indicate if agents might be effective, or not)
- Identify a new treatment for ALS

- **What was the impact on other disciplines?**

Nothing to report

- **What was the impact on technology transfer?**

Nothing to report

- **What was the impact on society beyond science and technology?**

Nothing to report

5). **CHANGES/PROBLEMS**

- **Changes in approach and reasons for change**

There were no significant changes in objectives and scope.

Studies are progressing as outlined to completion through the no cost extension period

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

The COVID-19 shut down (March 20 to June 12 2020) caused significant setback in that we needed to stop breeding and ongoing studies. The lab is now back up and running toward completion of this work.

As above, studies are progressing as outlined.

Breeding of mice is optimized; copy number is stable but when males and females are born, it is clear that the exact needed genotypes are not always obtained. For this reason, we continuously refresh the breeding with new ALS mice (to control copy number) until all the Ns are minimum of 12 (see above).

- **Changes that had a significant impact on expenditures**

Nothing to report

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards and /or select agents**

There are no significant changes in any of these areas.

Human subjects: not applicable

Biohazards (PET Imaging agent): no changes/no problems

Select Agents: not applicable

Vertebrate animals: There were no significant changes to the use of vertebrate animals. All amendments to the protocol were reported to and approved by ACURO IACUC (adding personnel).

Approval Date of the Institutional Animal Care and Use Committee protocol:

Original Approval Date: 12/23/16

Updated Approval after triennial review: 6/17/2020

Expiration Date: 12/23/2022

6). **PRODUCTS**

- **Publications, conference papers and presentations**

Journal publications

We published the following review articles on RAGE and neurodegeneration:

YEAR 1

Derk J, MacLean M, Juranek J, and Schmidt AM. *The Receptor for Advanced Glycation End products (RAGE) and Mediation of Inflammatory Neurodegeneration.* Journal of Alzheimer's

Disease and Parkinsonism 2018;8(1). pii: 421. doi: 10.4172/2161-0460.1000421. Epub 2018 Jan 24.

YEAR 2

In the past year, we published an additional article:

MacLean M, Derk J, Ruiz HH, Juranek JK, Ramasamy R, Schmidt AM. The Receptor for Advanced Glycation End Products (RAGE) and DIAPH1: Implications for vascular and neuroinflammatory dysfunction in disorders of the central nervous system. Neurochem Intl 2019 Jun;126:154-164. doi: 10.1016/j.neuint.2019.03.012. Epub 2019 Mar 20.

YEAR 3 NCE

MacLean M, Juranek J, Cuddapah S, Hu J, Gugger P, Li H, Schmidt AM. Microglia RAGE exacerbates the procession of amyotrophic lateral sclerosis in male but not female SOD1G93A mice. Glia in Health & Disease; 2020 July 16; Cold Spring Harbor, NY, United States.

Our manuscript based on this work on RAGE and ALS is now in final stages of preparation now that the RNA seq is completed.

Books or other non-periodical one time publications

Nothing to report

Other publications, conference papers and presentations

Our work was presented at Cold Spring Harbor meeting this July on Glia in Health and Disease (abstract on our findings, as detailed above). Title of the poster/authors is:

MacLean M, Juranek J, Cuddapah S, Hu J, Gugger P, Li H, Schmidt AM. Microglia RAGE exacerbates the procession of amyotrophic lateral sclerosis in male but not female SOD1G93A mice. Glia in Health & Disease; 2020 July 16; Cold Spring Harbor, NY, United States.

- **Websites or other internet sites**

Nothing to report

- **Technologies or techniques**

Nothing to report

- **Inventions, patent applications and / or licenses**

Nothing to report

- **Other products**

Nothing to report

7). **PARTICIPANTS AND OTHER COLLABORATING INSTITUITONS**

- **What individuals have worked on the project?**

Ann Marie Schmidt

Project Role: Principal Investigator

Researcher Identifier: SCHMIDTAM (eRA Commons ID)

Nearest Person Month Worked: 1.0

Contribution to Project: Dr. Schmidt oversees all aspect of the project, project team, mouse care and use, data analyses and all interactions with co-investigators.

Funding Support: N/A

Yu-Shin Ding

Project Role: Co-investigator

Researcher Identifier: YU_SHIN_DING (eRA Commons ID)

Nearest Person Month Worked: 2

Contribution to Project: Dr. Ding has overseen the implementation and performance of the imaging studies on the mice using [^{11}C]PBR28 as outlined in the protocol.

Funding Support: N/A

Judyta Juranek

Project Role: Associate Research Scientist

Researcher Identifier: JKJ2110CU (eRA Commons ID)

Nearest Person Month Worked: 12

Contribution to Project: Dr. Juranek's role has been to monitor the mouse behavioral endpoints, humane endpoint determinations and she has overseen and organized the mice allocated to imaging studies. She performs the biochemical and molecular analyses on the mouse tissues.

Funding Support: N/A

Huilin Li

Project Role: Co-investigator

Research Identifier: LIHUILIN09 (eRA Commons ID)

Nearest Person Month Worked: 1

Contribution to Project: Dr. Li oversees all aspects of power calculations and statistical analysis of the data.

Funding Support: N/A

Jiyuan Hu

Project Role: Post-doctoral research scientist

Researcher Identifier: HUJI010 (eRA Commons ID)

Nearest Person Month Worked: 1

Contribution to Project: Dr. Hu works with Dr. Li; she is a biostatistician who has performed all aspects of power calculations and statistical analysis.

Funding Support: N/A

Michael MacLean

Project Role: Graduate Student

Researcher Identifier: mm8848 (eRA Commons ID)

Nearest Person Month Worked: 8

Contribution to Project: Mr. MacLean breeds and genotypes the mice and works together with Dr. Juranek to perform the behavioral analyses, humane endpoint determinations and the indicated biochemical and molecular analyses.

Funding Support: Mr. MacLean is funded by the Sackler graduate school at NYU School of Medicine.

• **Change in other or active support of the PD/PIs or senior/key personnel**

Schmidt, Ann Marie

ACTIVE

1R01DK109675 04/01/16-03/31/21 0.91 calendar
NIH $313,163
RAGE/mDia1, Macrophage Trafficking and Inflammation in High Fat Feeding
Major goal of this application is to understand macrophage-adipocyte interactions in high fat feeding and obesity.
Role: PI

1R01HL132516 12/09/16-11/30/20 0.45 calendar
NIH $375,975
RAGE/mDia1, Macrophage Trafficking and Inflammation in Regression of Diabetic Atherosclerosis
The major goal of this grant is to probe the mechanisms by which macrophage (Mø) RAGE impairs regression of atherosclerosis in diabetic or IR mice.
Role: Multi-PIs (Schmidt & Ramasamy-Contact-PI)

P01HL131481 05/01/17-04/30/22 2.85 calendar
NIH (Fisher: PI) P01 $1,434,208
Macrophage Dysfunction in Obesity, Diabetes and Atherosclerosis
Major goal of this application is to determine mechanisms of macrophage trafficking, metabolism and inflammation in the context of RAGE/DIAPH1 in obesity
Role: Project 3 and Core C Leader

(THIS AWARD)

USAMRAA Dept. of the Army 07/01/17-06/30/21 No Cost Extension
Receptor for AGE (RAGE) Signal Transduction in Amyotrophic Lateral Sclerosis: In Vivo Imaging and Novel Therapeutic Approaches
Major goals of this grant includes testing the hypothesis that microglia RAGE, through ligand-driven upregulation of inflammatory and pro-oxidative stress and suppression of reparative processes in the ALS spinal cord, mediates neuronal death and loss of motor function and probing the hypothesis that PBMM-specific deletion of Ager attenuates neuronal stress, accumulation of Aβ and amyloid plaques, synaptic dysfunction and cognitive impairment in APPswe/PS1 mice.
Role: PI

USAMRAA Dept. of the Army 09/30/17-09/29/21 No Cost Extension
$489,612

RAGE/Diaph1, Diabetes, and Kidney Disease: Mechanisms and Novel Therapeutic Strategies
Major goals for this grant involves (a) testing the hypothesis that RAGE and DIAPH1 mediate podocyte dysfunction in DN through disengagement of homeostatic actin cytoskeleton dynamics and upregulation of pro-inflammatory and pro-fibrotic molecules (b) testing the hypothesis that RAGE and DIAPH1-expressing macrophages contribute to structural and functional derangements in DN through upregulation of tissue-destructive and profibrotic mediators and (c) determining if administration of novel small molecule antagonists of RAGE-DIAPH1 interaction in diabetic mice protects against DN.
Role: PI (Ramasamy-Partnering PI)

American Heart Association 04/01/17-03/31/21 3.6 calendar
$900,663
Braking Inflammation in Obesity & Metabolic Dysfunction: Translational and Therapeutic Opportunities
The major goal of this grant is to investigate the novel hypothesis that impaired adipocyte, macrophage and other inflammatory cell signal transduction thwarts weight loss and its anti-inflammatory and metabolic benefits, at least in part through the activation of the receptor for advanced glycation endproducts, or RAGE pathway, which has been shown to regulate a unique repertoire of inflammatory and metabolic processes.
Role: Center Director, Project 1 Leader

1P01HL146367-01 08/01/19-06/30/24 3.0 calendar
NHLBI $1,629,629
Macrophages, Cell-Cell Communication, Ischemic Injury in Diabetes and the RAGE/DIAPH1 Signaling Axis
The major goal of this grant is to probe the mechanisms and identify new therapies for untoward monocyte and macrophage responses in ischemia, which, together with cellular perturbation in the microenvironment, amplify damage in myocardial infarction and peripheral arterial disease, especially in diabetes.
Role: PI

1R01DK122456-01A1 07/01/20-03/31/25 0.91 calendar
NIDDK $672,000
Targeting RAGE/DIAPH1: Novel Therapeutic Strategy for Diabetic Complications
The major goal of this grant is to develop small molecule antagonists to treat diabetic complications.
Role: MPI (Schmidt (contact PI)/Ramasamy)

<u>INACTIVE</u>
(ENDED)
P01HL60901 07/15/11-11/30/18 0.12 calendar
NIH $897,537 No Cost Extension
RAGE and Mechanisms of Vascular Dysfunction
This grant focuses on the mechanisms by which diabetes accelerates atherosclerosis via RAGE.
Role: Project 1 and Core A Leader, Core C Co-Leader

1R24DK103032 08/01/14-07/31/19 0.06 calendar
NIH No Cost Extension
Targeting RAGE-mDia1 in Diabetic Complications: Mechanisms & Therapeutics
Major goal of this application is to develop small molecule inhibitors of the interaction of the RAGE cytoplasmic domain with DIAPH1.
Role: PI

Alzheimer's Association 03/01/17-02/29/20 0.24 calendar
$134,612
RAGE, Diaph1, Microglia and Alzheimer's disease
Major goal of this grant is to probe the hypothesis that microglial-specific Ager deletion modulates neuronal stress, accumulation of Aβ and amyloid plaques, synaptic and cognitive dysfunction in APPswe/PS1 mice.
Role: PI

OVERLAP
None

Ding, Yu-Shin

ACTIVE
(NEW)
1R21AT010771-01 (Ding, Y.-S.) 09/15/19 - 08/31/21 3.51 calendar
NIH/NCCIH $150,000
Identifying the Mechanisms of Action for CBD on Chronic Arthritis Pain
The proposed study will be the first PET imaging study to determine the key targets of cannabidiol (CBD) that are related to its mechanisms of action on pain treatment in osteoarthritis (OA).
Role: PD/PI

(THIS AWARD)
USAMRAA Dept. of the Army 07/01/17-06/30/21 No Cost Extension
Receptor for AGE (RAGE) Signal Transduction in Amyotrophic Lateral Sclerosis: In Vivo Imaging and Novel Therapeutic Approaches
Major goals of this grant includes testing the hypothesis that microglia RAGE, through ligand-driven upregulation of inflammatory and pro-oxidative stress and suppression of reparative processes in the ALS spinal cord, mediates neuronal death and loss of motor function and probing the hypothesis that PBMM-specific deletion of Ager attenuates neuronal stress, accumulation of Aβ and amyloid plaques, synaptic dysfunction and cognitive impairment in APPswe/PS1 mice.
Role: Co-I

American Heart Association (Schmidt) 04/01/17 – 03/31/21 0.57 calendar
AHA/ Obesity Center $900,663
Braking Inflammation in Obesity and Metabolic Dysfunction: Translational and Therapeutic

Opportunities
The major goal of this grant is to investigate the novel hypothesis that impaired adipocyte, macrophage and other inflammatory cell signal transduction thwarts weight loss and its anti-inflammatory and metabolic benefits, at least in part through the activation of the receptor for advanced glycation endproducts, or RAGE pathway, which has been shown to regulate a unique repertoire of inflammatory and metabolic processes.
Role: Project 1 Co-Investigator

(NEW)
3R21AG064474-02S1 (Ding, Y.-S.) 06/01/20 - 05/31/21 1.50 calendar
NIH/NIA $233,619
Bispecific Antibody-Based PET Ligands for Imaging Tauopathies (Admin Suppl)
John Hsiao
In this project, we will build upon our experience to create, for the first time, bispecific antibody-based PET ligands with high specificity/selectivity and the capability to cross the bloodbrain barrier (BBB) for in vivo imaging of tauopathies.
Role: PD/PI

1R21 AG064474-02 (Ding) 09/01/19 – 05/31/21 3.84 calendar
NIH/NIA $152,000
Bispecific Antibody-Based PET Ligands for Imaging Tauopathies
The research goal is to create bispecific antibody-based PET ligands with high specificity/selectivity and the capability to cross the blood-brain barrier (BBB) for *in vivo* imaging of tauopathies.
Role: PI

(NEW)
R21AG067549 (Osorio, RS, Ding, YS) 06/01/2020-03/31/2021 1.44 calendar
NIH/NIA $136,892
Locus-coeruleus function in normal elderly and AD risk (LEAD)
Miroslaw Mackiewicz, Grants Officer
Growing evidence suggests that Alzheimer's disease (AD) pathological changes begin decades before clinical symptoms and tau abnormalities in the locus coeruleus (LC) can be observed since midlife. We have previously demonstrated functional vulnerability of the LC to aging and stress, as well as an association between higher CSF tau and impaired sleep phenomena influenced by the LC.
Role: MPI

INACTIVE
(ENDED)
Department Seed Grant (Ding) 08/01/18 – 07/31/20
NYU Department of Radiology $30,000
Development and Validation of in vivo PET Imaging Ligands to Facilitate Discovery of LRRK2 Inhibitor Drugs for Parkinson's Disease.
Role: PI

(ENDED)
R21 (Osorio) 12/01/16-11/31/18 0.6 calendar
NIH/NIA $322,512
Orexin (hypocretin) and tau pathology in normal elderly: a new prevention strategy for Alzheimer's disease
Role: Co-Investigator

(ENDED)
5T35DK007421-35 (Blazer & Munger) 06/01/14 - 05/31/19
NIH/NIDDK
SHORT TERM RESEARCH TRAINING GRANT FOR MEDICAL STUDENTS
The grant provides fellowships for students doing basic research with an NYU faculty mentor with a focus in diabetes, digestive disorders, and kidney diseases.
Role: Mentor

(ENDED)
1P41EB017183-01A1 (Sodickson); 09/30/14-08/31/19 0.96 calendar
NIH/NIBIB $4,642,333
Center for Advanced Imaging Innovation and Research (CAI^2R)
Sub ID 8315, #3: Advancing MR and PET through Synergistic Simultaneous Acquisition and Joint
The proposed BTRC combines three areas of novel and high-impact imaging technology development with a unique new model for interdepartmental and academic-industrial collaboration aimed at translating that technology rapidly and effectively into clinical practice. Technology Research and Development (TR&D) project #3 is addressed at new uses of simultaneity, advancing the fundamental capabilities of MR and PET through synergistic simultaneous acquisition and joint reconstruction.
Role: Co-Project Lead of TR&D #3, and Co-Investigator for Collaborative Projects and Service Projects.

(ENDED)
1 R01 DK112289-01 (Ding) 12/01/16-11/30/19 2.4 calendar
NIH/NIDDK $1,969,711
Brown Adipose Tissue in Sleep/Wake Homeostasis
Role: PD/PI

OVERLAP
None

Li, Huilin

ACTIVE
1R01HS026522-01 (Schoenthaler) 09/01/2018-6/30/2023 0.60 calendar
AGENCY FOR HEALTHCARE RESEARCH AND QUALITY $400,000

i-Matter: Investigating an mHealth texting tool for embedding patient-reported data into diabetes management
We will use the Technology Acceptance Model and Capability-Opportunity-Motivation Model of Behavior to evaluate the efficacy of a technology-based patient-reported outcome system, the Modern Journal System, for management of T2D.
Role: Co-I

1R01MD012243-01A1 (Danil Makarov) 8/22/2018-3/31/2023 0.36 calendar
NIH/NIMHD $809,622
Randomized trial of community health worker-led decision coaching to promote shared decision making for prostate cancer screening among Black male patients and their providers
We will use the Technology We propose testing the efficacy of a CHW-led decision coaching program to facilitate SDM for PSA screening among Black men at a primary care Federally Qualified Health Center (FQHC). Role: Co-I

5P30CA16087 (Neel) 03/01/13 – 02/28/24 0.24 calendar
NIH/NCI $160,799
Cancer Center Support Grant (Biostatistics Shared Resource)
To provide biostatistics support to the NYU cancer community
Role: Co-I

R01CA204113 (Chen) 04/01/2016 – 03/31/2021 0.35 calendar
NIH/NCI $649,625
The Foregut Microbiome and Risk of Gastric Intestinal Metaplasia, and Gastric Cancer Risk
This project aims to evaluate the role of oral and gastric microbiome in the development of gastric cancer. Since bacterial profiles are modifiable, identification of bacterial factors that influence gastric cancer risk may lead to clinical applications and improvements in more cost-effective cancer screening and risk stratification.

1R01DK109675 (Schmidt) 04/01/2016-03/31/2021 0.90 calendar
NIH/NIDDK $313,163
RAGE/mDia1, Macrophage Trafficking and Inflammation in High Fat Feeding
Major goal of this application is to understand macrophage-adipocyte interactions in high fat feeding and obesity.
Role: Statistician

1R01HL132516 12/09/16-11/30/20 0.45 calendar
NIH $375,975
(Multi PIs: Schmidt and Ramasamy (contact PI))
RAGE/mDia1, Macrophage Trafficking and Inflammation in Regression of Diabetic Atherosclerosis
The major goal of this grant is to probe the mechanisms by which macrophage (Mø) RAGE impairs regression of atherosclerosis in diabetic or IR mice.
Role: Co-I

(THIS AWARD)

USAMRAA Dept. of the Army (Schmidt) 07/01/17-06/30/21 0.58 calendar
No-Cost Extension
Receptor for AGE (RAGE) Signal Transduction in Amyotrophic Lateral Sclerosis: In Vivo Imaging and Novel Therapeutic Approaches
Major goals of this grant includes testing the hypothesis that microglia RAGE, through ligand-driven upregulation of inflammatory and pro-oxidative stress and suppression of reparative processes in the ALS spinal cord, mediates neuronal death and loss of motor function and probing the hypothesis that PBMM-specific deletion of Ager attenuates neuronal stress, accumulation of Aβ and amyloid plaques, synaptic dysfunction and cognitive impairment in APPswe/PS1 mice.
Role: Co-I

NIH (Fisher: PI) P01 05/01/17-04/30/22 0.30 calendar
$144,770
Macrophage Dysfunction in Obesity, Diabetes and Atherosclerosis
Major goal of this application is to determine mechanisms of macrophage trafficking, metabolism and inflammation in the context of RAGE/DIAPH1 in obesity.

American Heart Association 04/01/17-03/31/21 1.0 calendar
$204,574
Braking Inflammation in Obesity & Metabolic Dysfunction: Translational and Therapeutic Opportunities
Role: Co-I

1R01DK116845 (Mueller) 12/17/18 - 11/30/22 0.30 calendar
NIH/NIDDK $256,479
The zinc finger protein ZNF638 is a novel transcriptional regulator of thermogenesis
We expect that the studies outlined will shed novel light into the mechanisms regulating energy balance and will ultimately permit the definition of new strategies to modulate energy metabolism with possible impact on the development of anti-obesity therapies. Role: Co-I

(NEW)
1U24NS113844 (Petkova) 09/30/19 – 08/31/24 1.0 calendar
NIH/NIAID $7,910,977.00
EPPIC-NET DCC
The Data Coordinating Center (DCC) of the Early Phase Pain Investigation Clinical Network (EPPIC-Net) will be the data and biospecimen manager for pain research within the HEAL Partnership.
Role: Co-I

(NEW)
1P01HL146367-01 (Schmidt) 08/01/19 – 06/30/24 1.0 calendar
NIH $1,629,632
Macrophages, Cell-Cell Communication, Ischemic Injury in Diabetes and the RAGE/DIAPH1 Signaling Axis
This Program Project will probe the mechanisms and identify new therapies for untoward monocyte and macrophage responses in ischemia, which, together with cellular perturbation in the

microenvironment, amplify damage in myocardial infarction and peripheral arterial disease, especially in diabetes.
Role: Co-I

(NEW)
1R01CA159036 (Hayes/Ahn) 09/01/9- 08/31/24 0.45 calendar
NIH/NCI $251,686
The Oral Microbiome and Upper Aerodigestive Squamous Cell Cancer
In this large prospective study, we will test the hypothesis that oral (fungal) mycobiome influences squamous cell head and neck cancer development, potentially related to alcohol and tobacco carcinogen metabolism.
Role: Co-I

(NEW)
1U01AI122285-01 (Blaser) 05/01/2019 – 4/30/2022 0.57 calendar
NIH/NIAID $37,434
NIAID Extension of Investigator - Initiated Clinical Trials (Microbial, immune, metabolic perturbations by antibiotics)
This proposal is to examine the effects of a single antibiotic course in young adults on their microbiota, and on immune and metabolic parameters.
Role: Co-I

INACTIVE
(ENDED)
1R01DK110014-01 (Li) 07/01/2016-06/30/2020 2.10 calendar
NIH/NIDDK $225,000
Novel Statistical Methods in Analyzing Microbiome Data for Longitudinal Study
This proposal will develop and implement novel statistical methods to study the temporal change of microbiome composition between groups defined by treatment or interested phenotype, probe the causal relationships between disruption of the microbiome and human disease, and identify key bacteria taxa that affect susceptibility to complex traits.
Role: PI

(ENDED)
2P01HL060901 (Schmidt) 07/01/2011-11/30/2018 0.88 calendar
NIH/NHLABI $897,537 No Cost Extension
RAGE and Mechanisms of Vascular Dysfunction
This grant focuses on the mechanisms by which diabetes accelerates atherosclerosis via RAGE.
Role: Co-I

(ENDED)
5U01CA18237 (Pei, Ahn) 04/01/2014 – 03/31/2019 0.21 calendar
NIH/NCI $318,145
Role of oral microbiome in the etiology of esophageal adenocarcinoma
To examine whether indigenous oral microbes contribute to the development of esophageal

adenocarcinoma.
Role: Co-I

(ENDED)
R01CA188353 (Gold) 04/01/2015- 03/31/2019 0.24 calendar
NIH/NCI $293,181
Treatment and outcomes in diabetic breast cancer patients
Conduct an empirical assessment of the nuanced treatment adoption process for low-risk prostate cancer and shed light on modifiable factors that can influence future technology adoption and diffusion.

(ENDED)
JDRF 2-SRA-2016-153-S-B (Blaser) 02/01/16 – 07/31/18 0.04 calendar
Juvenile Diabetes Research Foundation $181,818
Effect of early life antibiotic exposure on type 1 diabetes in NOD mice
This project is aimed to determine whether early life antibiotic exposure (STAT) accelerates the athophysiology and onset of type 1 DM in NOD mice.
Role: Co-I

(ENDED)
1U01AI122285-01 (Blaser) 04/01/2016 – 03/31/2019 1.20 calendar
NIH/NIAID $250,000
Microbial, immune, metabolic perturbations by antibiotics (MIME study)
This proposal is to examine the effects of a single antibiotic course in young adults on their microbiota, and on immune and metabolic parameters.

(ENDED)
1R21DK100492-02 (Sevick) 08/20/2016-05/31/2018 0.60 calendar
NIH/NIDDK $150,000
Behavioral Management of Phosphorus in Hemodialysis
The purpose of this 2-phase study is to provide proof of concept and describe feasibility and acceptability of a behavioral intervention to engage hemodialysis patients in multiple behavior changes to properly manage hyperphosphatemia, including adherence to phosphate binders and reduction of dietary phosphorus while assuring adequate protein intake.

(ENDED)
1 R01 K100492-01A1 (Sevick) 09/18/2014- 07/31/2019 0.59 calendar
NIH/NIDDK $427,513
Lifestyle Management of CKD in Obese Diabetic Patients
To evaluate, when compared to usual care, the efficacy of 3 different technology-supported approaches to engaging 300 individuals with diabetes and concurrent chronic kidney disease in weight loss, physical activity, dietary sodium restriction, and dietary restriction of inorganic phosphates.
Role: Co-I

OVERLAP

None

- **Other organizations involved as partners**

N/A

8). **SPECIAL REPORTING REQUIREMENTS**

N/A

9). **APPENDICES**

N/A