AWARD NUMBER: W81XWH-17-1-0346

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

PRINCIPAL INVESTIGATOR: Ann Marie Schmidt, MD

CONTRACTING ORGANIZATION: New York University School of Medicine
New York, NY 10016

REPORT DATE: JULY 2019

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
JULY 2019	Annual Report	01 Jul 2018 – 30 Jun 2019

4. TITLE AND SUBTITLE

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

5a. CONTRACT NUMBER
W81XWH-17-1-0346

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Ann Marie Schmidt, MD

E-Mail: AnnMarie.Schmidt@nyulangone.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
New York University School of Medicine
550 First Avenue
New York, NY 10016-6402

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

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 combined male and female *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 mouse groups (male and female) so that ample power is achieved for each line in order to finalize conclusions. (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. 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 (ALS), DIAPH1, Microglia, Neurodegeneration, Neuroimaging, Receptor for Advanced Glycation Endproducts (RAGE), Small molecule antagonists, Spinal Cord

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	22	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

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 nearly 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 are being generated to fully 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 breeding SOD1^{G93A} mice into the *Ager* flox/flox background and then intercrossing these mice into the *Cx3cr1* ERT2 cre recombinase background in order to generate 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)

Below are the numbers we have achieved for each sex (entered into study and either still alive as of 7/22/19 or already humanely sacrificed (Per power calculation, we are working to complete 12 mice/group.)

MALE

SOD1^{G93A} / *Ager*$^{flox/flox}$ / *Cx3cr1*$^{CreERT2\ +/wt}$ (ALS+, Microglia specific *Ager* deletion): N=13

SOD1^{G93A} / *Ager*$^{flox/flox}$ / *Cx3cr1*$^{CreERT2\ wt/wt}$ (ALS + *Ager* expressed in all cells): N=5

SOD1^{G93A} / *Cx3cr1*$^{CreERT2\ +/wt}$ (ALS + *Ager* expressed in all cells; controls for CRE mice): N=10

FEMALE

SOD1^{G93A} / *Ager*$^{flox/flox}$ / *Cx3cr1*$^{CreERT2\ +/wt}$ (ALS+, Microglia specific *Ager* deletion) : N=13

SOD1^{G93A} / *Ager*$^{flox/flox}$ / *Cx3cr1*$^{CreERT2\ wt/wt}$ (ALS + *Ager* expressed in all cells): N=13

SOD1^{G93A} / *Cx3cr1*$^{CreERT2\ +/wt}$ (ALS + *Ager* expressed in all cells; controls for CRE mice): N=5

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 in year 2 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). RAGE expression in Spinal Cord microglia: 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:

Based on the mouse groups available to us per the breeding scheme, it is evident that most of the lines / sex are completed. Although we have had an aggressive breeding protocol with interim re-introduction of NEW ALS mice breeders (responsible for the fact that copy number has been stable with no reductions to influence interpretation of our results), the "correct" genotypes are still being accrued to final numbers per the above (aggressively being finalized in our no cost extension period). The interim results are very promising however and are as follows:

With our biostatistician colleagues, first we evaluated the data cominbed (male and female mice). This revealed a significant benefit of microglia *Ager* deletion on survival in the combined group compared to ALS-Cre-expressing controls (Figure). However, we noted an independent effect of the *Cx3cr1*-Cre^{ERT2}, that is, there is a deterimental effect of the ALS-*Cx3cr1*-Cre^{ERT2} when comparing to ALS mice with the *Ager* floxed alleles (the latter is essentially a control ALS mouse)**.** When we subset the data, we see similar trends for improved survival in male mice but no differences in female mice; we currently continue to add female mice to complete the groups (see above). Again, we carefully assessed copy number and it is not diminished in an of the mice. Altogether, the results suggest that microglia *Ager* deletion may be protective in ALS independent of the deleterious impact of the *Cx3cr1*-Cre^{ERT2} or that microglia *Ager* deletion reduces some of the deleterious consequences of the *Cx3cr1*-Cre^{ERT2}-mediated partial deletion of *Cx3cr1*. For these reasons, it was critical and correct that we added the "cre-control." Our plan through the no-cost extension period is to finalize the "N" per group and to make final conclusions. Tissues are also stored and preserved for analyses.

Additional interim analyses:

Male Mice:

Weight loss: Deletion of microglia *Ager* in ALS mice reduces the rate of weight loss compared to the ALS-cre mice.

Motor function: Based on the hanging wire test, the deletion of microglia *Ager* in ALS mice reduces the rate of weight loss compared to the ALS-cre mice.

Female Mice:

Weight loss: No significant differences noted among groups; mice still being accrued to study to fill in genotypes

Motor function: No significant differences noted among groups; mice still being accrued to study to fill in genotypes

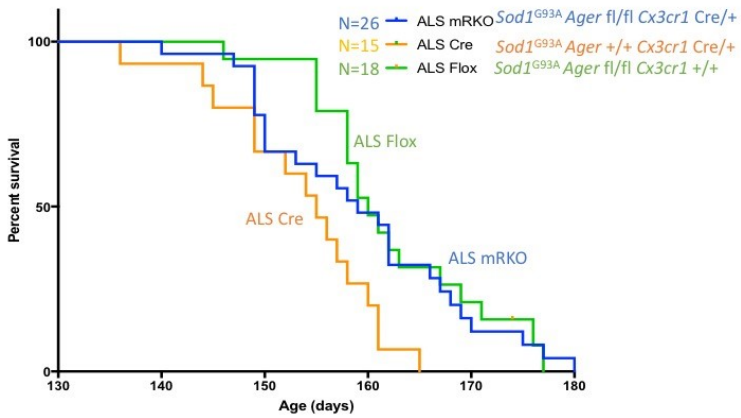

Figure: Kaplan-Meir survival curve of ALS mice (male and female) treated administered tamoxifen at the age of 90 days to induce microglia *Ager* deletion in ALS mRKO mice. ALS Cre mice have a marked decrease survival relative to ALS flox controls ($p<0.01$) and ALS mRKO mice ($p<0.05$). ALS flox and ALS mRKO have similar survival ($p>0.5$) but the ALS flox LACKS the Cre, which inherently is reducing survival in this model.

d). Immunohistochemical analyses:

- At the sacrifice time point, in male mice, deletion of microglia *Ager* does not affect microglia number or expression of Cd68 or P2ry12 /microglia relative to the ALS-cre control mice.

-At the sacrifice time point, in male mice, deletion of microglia *Ager* significantly reduces astrocytosis (measured by GFAP+ cells) relative to the ALS-cre control mice.

-At the sacrifice time point, in male mice, NeuN+ cells (neuron) do not differ between mice devoid of microglia *Ager* vs. the ALS-cre controls. As this is the terminal point this result is not surprising, however, it will be necessary to examine mice at an earlier time point prior to terminal to finalize these results (underway).

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 3 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. This will be performed in no cost extension period to completion.

B.iv. Other Achievements:

As part of our laboratory's efforts in ALS, we have begun working with TargetALS (http://www.targetals.org/), which is an initiative to treat and cure ALS with the key premise being that only if ALS researchers share their data and findings may we come to a more rapid means to understand the cause of ALS and to identify effective treatments. From TargetALS and from publicly-available databases, we have already learned the following from databases in which de-identified human subject data were deposited:

We have obtained RNA-Sequencing data of CNS tissues through our ongoing collaboration with **TargetALS** (http://www.targetals.org) from a total of 147 (s)ALS patients and 9 "control" subject tissue from subjects 18-95 years of age. As new patient samples undergo RNA-Sequencing, these data are made available to us we include these new data in ongoing analyses. Preliminary analyses indicate that (s)ALS patients display a broad range of *AGER* (the gene encoding RAGE) expression in cervical spinal cord tissue whereas the range of *AGER* expression is much more limited in the control subjects. Further, the patients with the highest *AGER* expression display differential gene expression in several pathways, including multiple pathways relevant to "inflammation" when compared to those patients with the lowest *AGER* expression within cervical spinal cord tissues. Spatial transcriptomic analyses of lumbar spinal cord tissue of *SOD1*^{G93A} mice and human sALS patients unveiled distinct population of cells that display differential genes in the KEGG pathway "AGE-RAGE signaling pathway in diabetic complication. Strikingly, these populations of murine and human cells were enriched in glia signature transcripts. Altogether, these findings strongly implicate a link between the degree of *AGER* mRNA expression and alterations in microglia and/or astrocyte function. For this reason, these studies in HUMAN subjects provide strong support for our hypotheses and experimental approach.

• 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
- Understanding premise of RNA sequencing and data analysis
- Interaction with TargetALS to obtain de-identified human ALS deposited RNA seq data
- Preparation of abstract and presentation

Conferences: Members of our study team will attend the Cold Spring Harbor meeting this July 2018 on "Glia in health and disease" - Abstract (to be presented by Michael MacLean) has been prepared and accepted for presenation (see below for details)

Furthermore, our study team attended the NY Academy of Science meeting as follows: Transformative Research in Neurodegenerative Disease and Neuropsychiatric Disorders: 2017 Innovators in Science Award Symposium" on Wednesday, November 29, 2017

In 2019, the graduate student, Michael MacLean attended a Keystone conference on Microglia and presented a poster of his work in progress on RAGE in ALS.

• How were results disseminated to communities of interest?

Nothing to report.

For the scientific community, we are disseminating first findings (as detailed above and cited below) at the Cold Spring Harbor meeting on "Glia in health and disease." Further, Michael MacLean presented his findings at a recent Keystone conference on Glia.

• 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.

Aim 2: Begin 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.

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.
- 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/female 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.

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**

No significant problems arose that were not surmountable.

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

Effective Date: 5/31/18

Expiration Date: 12/23/19

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.

Books or other non-periodical one time publications

Nothing to report

Other publications, conference papers and presentations

Our work is to be 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, Derk J, and Schmidt AM. RAGE Signaling in Microglia: a potential contributor to neuroinflammation in Amyotrophic Lateral Sclerosis.

In 2019, our work was presented at conference (Keystone) on glia by M MacLean on RAGE and microglia in ALS.

- **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

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

1R01DK109675 04/01/16-03/31/21 0.91 calendar
NIH
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 0.46 calendar
NIH
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)

Alzheimer's Association 03/01/17-02/29/20 0.24 calendar

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

NIH (Fisher: PI) P01 05/01/17-04/30/22 2.86 calendar

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 07/01/17-06/30/19 0.91 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: PI

USAMRAA Dept. of the Army 09/30/17-08/31/20 0.73 calendar

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

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

INACTIVE
(ENDED)
P01HL60901 07/15/11-11/30/18 NIH 0.12 calendar
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

OVERLAP
None

Ding, Yu-Shin

ACTIVE
1P41EB017183-01A1 (Sodickson); 09/30/14-08/31/19 0.96 calendar
NIH/NIBIB
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.

(THIS AWARD)
USAMRAA Dept. of the Army 07/01/17-06/30/19 2.4 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

American Heart Association (Schmidt) 04/01/17 – 03/31/21 0.6 calendar AHA/ Obesity Center
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)
Department Seed Grant (Ding) 08/01/18 – 07/31/20
NYU Department of Radiology
Development and Validation of in vivo PET Imaging Ligands to Facilitate Discovery of LRRK2 Inhibitor Drugs for Parkinson's Disease.
Role: PI

(NEW)
1 R21 AG064474-01 (Ding) 09/01/19 – 08/31/21 3.6 calendar
NIH/NIA
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

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

INACTIVE

(ENDED)
R21 (Osorio) 12/01/16-11/31/18 0.6 calendar
NIH/NIA
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

OVERLAP
None

Li, Huilin

ACTIVE
1R01DK110014-01 (Li) 07/01/2016-06/30/2020 2.10 calendar
NIH/NIDDK
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

(NEW)
1R01HS026522-01 (Schoenthaler) 09/01/2018-6/30/2023 0.60 calendar
AGENCY FOR HEALTHCARE RESEARCH AND QUALITY
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

(NEW)
1R01MD012243-01A1 (Danil Makarov) 8/22/2018-3/31/2023 0.4 calendar *****

NIH/NIMHD
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
***Dr. Li's effort is temporarily reduced from 1.2 CM to 0.4 CM during Year 1**

1 R01 K100492-01A1 (Sevick) 09/18/2014- 07/31/2019 0.59 calendar
NIH/NIDDK
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

R01CA204113 (Chen) 04/01/2016 – 03/31/2021 0.96 calendar
NIH/NCI
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
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 0.58 calendar
NIH
(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: Statistician

<u>(THIS AWARD)</u>
USAMRAA Dept. of the Army (Schmidt) 07/01/17-06/30/20 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

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 0.69 calendar

Braking Inflammation in Obesity & Metabolic Dysfunction: Translational and Therapeutic Opportunities
Role: Co-I

(NEW)
1R01DK116845 (Mueller) 12/17/18 - 11/30/22 0.30 calendar
NIH/NIDDK
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

INACTIVE
(ENDED)
2P01HL060901 (Schmidt) 0.88 calendar
07/01/2011-11/30/2018 NIH/NHLABI 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)
5P30CA16087 (Neel) 03/01/13 – 02/28/19 0.24 calendar
NIH/NCI
Cancer Center Support Grant (Biostatistics Shared Resource)
To provide biostatistics support to the NYU cancer community
Role: Co-I

(ENDED)
5U01CA18237 (Pei, Ahn) 04/01/2014 – 03/31/2019 0.21 calendar

NIH/NCI
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
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
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
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
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.

OVERLAP

None

- **Other organizations involved as partners**

N/A

8). **SPECIAL REPORTING REQUIREMENTS**

N/A

9). **APPENDICES**

N/A