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
New York, NY 10016

REPORT DATE: July 2018

TYPE OF REPORT: Annual

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 2018	Annual	1 Jul 2017 - 30 Jun 2018

4. TITLE AND SUBTITLE

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

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-17-1-0346

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Ann Marie Schmidt, MD

E-Mail: AnnMarie.Schmidt@nyumc.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

(1) We tested expression patterns of RAGE in the SOD^{G93A} vs. wild-type mouse spinal cord. Active phagocytic CD68-positive $SOD1^{G93A}$ microglia express RAGE in the ventral horn of the spinal cord by the end-stages of the disease while microglia residing in the less affected white matter have less overlap with RAGE labeling. Only a few P2Y12 positive microglia (homeostasis marker) overlap with RAGE labeling and this remains relatively unchanged until P2Y12 positive cells disappear, suggesting that it is the active phagocytic microglia that display increased levels of RAGE. RAGE labels many neurons within the spinal cord throughout the disease, however, the overlap of total RAGE and MAP2 decreases by the end-stages, in parallel with increasing neuronal death. Initially, few astrocytes labeled by GFAP overlap with RAGE labeling, however, as astrocytes become increasingly prevalent in the ventral horn in the late stages of disease, there is increased overlap with RAGE labeling. (2) We generated the microglia RAGE modulated mice and their controls in the $SOD1^{G93A}$ background and are completing studies to understand the impact on life span and pathology. (3) We showed that the small molecule antagonist of RAGE is blood-brain barrier permeable; this will be tested in $SOD1^{G93A}$ mice.

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	23	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 endproducts (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 endproducts (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 (ALS)
DIAPH1
Microglia
Neurodegeneration
Neuroimaging
Receptor for Advanced Glycation Endproducts (RAGE)
Small molecule antagonists
Spinal Cord

3). **ACCOMPLISHMENTS**

- **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.

Major Task 1: **Generation of ALS RAGE modified mice and deletion of microglia *Ager* (*Ager* is the gene encoding RAGE)**

The breeding scheme as outlined in the proposal is generating the male and female mice needed for study. Tamoxifen is being administered, as noted in the grant, 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). In order to optimize rigor and reproducibility, the study team is naïve to the genotypes of the mice. All studies as outlined in the application are underway, 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.

Major Task 2: **PET Imaging**

Dr. Ding is performing the imaging of the mice under study as indicated in the application. Although she is naïve to the mouse genotypes, she did perform pilot imaging in these $SOD1^{G93A}$ mice and her data reveal increased glial inflammation in these mice vs. their controls (see the data below under the Progress section).

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.

Major Task 3: Treating mice with RAGE antagonist vs vehicle

Mice for study are actively been bred at this time; we have tested and confirmed that the RAGE/DIAPH1 small molecule antagonist is CNS permeable; and we have synthesized the first batch of the small molecule antagonist (13 gms is already prepared). Note that these studies are to begin in Year 2; hence, the preparation is, as noted, well underway for study commencement.

- **What was accomplished under these goals?**

1). *Major activities*

Aim 1: For Aim 1, 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)

As mice are bred, genotyped and placed into study, Tamoxifen is being administered, as noted in the application, to all mice in the study on day 90. Studies underway include: analysis of survival, establishment of humane endpoint, functional tests as outlined (motor function tests including hanging wire test and grip strength), isolation of microglia, and pathological analyses.

Aim 1 imaging studies are also being performed with [^{11}C]PBR28 in the SOD1^{G93A} mice.

Aim 2: Studies in Aim 2 begin in Year 2 but we are poised to begin based on: ongoing breeding and aging of needed mice, demonstration that RAGE229 is CNS permeable and the production / synthesis of RAGE229.

2). *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.

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.

3). *Significant results or key outcomes*

Aim 1:

We began by performing a careful time course of cell- and RAGE-specific staining patterns in the spinal cord tissue of SOD1^{G93A} mice vs. controls.

General Cellular Patterns: We began by assessing the pattern of CD68, a lysosomal protein and a marker of activated phagocytic microglia. In contrast, quiescent microglia do not typically express appreciable degrees of CD68. We found that microglia residing in the most affected region of the spinal cord, the ventral horn, express CD68 by the end-stages of the disease but at the ages of 60, 90, and 120 days, there is minimal CD68 staining. At the ages of 60, 90 and 120 days, microglia throughout the spinal cord display high expression of purinergic receptor P2Y12, a marker of homeostatic surveying microglia. However, at the end-stages of the disease, ventral horn microglia have low P2Y12 expression. Altogether, there is decreased P2Y12 positive microglia in the ventral horn as CD68 positive microglia become detectable at the later to end-stages of the disease. In contrast to the ventral horn, dorsal horn homeostatic/patrolling P2Y12 positive microglia are still present at the end-stages of the disease and do not appear to change substantially over the course of the disease, thereby underscoring a region- and time-specific response.

We next studied the neuron populations of the ALS spinal cord using the marker, MAP2. MAP2 labeled neurons throughout the ventral horn, however, by the end-stages of the disease, reductions in MAP2 labeling become apparent, presumably due to motor neuron death that occurs by this

point. Further, astrocytes were examined by probing for GFAP positivity. Astrocytes, in contrast to microglia, reside predominantly outside or on the periphery of the ventral horn, until the end-stages of the disease, when they infiltrate and increase in number.

Summary of cell-specific changes over time in the murine SOD1^{G93A} spinal cord: Our findings suggest that microglia reside within the ventral horn throughout the disease, prior to astrocyte infiltration and/or accumulation within the ventral horn. The accumulation of active phagocytic microglia in the ventral horn of the lumbar spinal cord positions these cells such that they may contribute to pathobiology during the periods of highest disease-mediating activity.

RAGE-specific cellular patterns: We next investigated RAGE expression in microglia. Active phagocytic CD68-positive SOD1^{G93A} microglia express RAGE in the ventral horn of the spinal cord by the end-stages of the disease while microglia residing in the less affected white matter have less overlap with RAGE labeling. Only a few P2Y12 positive cells overlap with RAGE labeling and this remains relatively unchanged until P2Y12 positive cells disappear, suggesting that it is the active phagocytic microglia that display increased levels of RAGE relative to patrolling homeostatic microglia. RAGE labels many neurons within the spinal cord throughout the disease, however, the overlap of total RAGE and MAP2 decreases by the end-stages of the disease, in parallel with increasing neuronal death. Initially, few astrocytes labeled by GFAP overlap with RAGE labeling, however, as astrocytes become increasingly prevalent in the ventral horn, there is increased overlap with RAGE labeling.

Summary of RAGE-specific staining in the murine SOD1^{G93A} spinal cord: Our data place increased RAGE expression in active phagocytic microglia, especially during the most active phases of the disease, thereby supporting our hypothesis that microglial RAGE may contribute to microglial perturbation and processes that may ultimately promote neuronal injury.

Aim 1A: Testing microglial-specific deletion of *Ager*

As indicated above in this report, to ensure scientific rigor and reproducibility, the study team is naïve to the mouse genotypes under study. Based on our long-term experience working with this model, it is absolutely essential that the investigators be unaware of the mouse genotypes for assessment of any of the endpoints. Hence, our accomplishments include breeding and genotyping of the mice, placing them into study, administering tamoxifen on day 90 and monitoring survival, motor function, and then isolating microglia (ongoing) and performing tissue analyses.

Aim 1B: PET Imaging

We began by assessing spinal cord (at three vertebrae sites, L2, L3 and T13) uptake of tracer in MicroPET studies in wild type mice and in age-matched ALS mice (SOD1^{G93A}). Dr. Ding measured three parameters in each case: SUV (standardized update values); %ID/g (injected dose / gram of tissue); and voxel intensity. We found that %ID/g and voxel intensity had similar patterns as the SUV.

In spinal cord and brain, there is more update in the ALS mice than in the wild-type control mice on day 100 of life (corresponds to disease onset in the SOD1^{G93A} mice). The uptake in the ALS

mice was approximately 30% higher than that observed in the wild-type mice. This suggested higher microglial activation in the ALS vs. the wild-type mice. This was also found to be the case in matched ALS and control wild-type mice on day 130 of life (progression phase of ALS). Of note, in the ALS mice observed at day 130, in which imaging was performed in the same mice that were imaged on day 100, when compared to day 100, there did not appear to be a further increase in uptake. This is being confirmed in ongoing mice studies and, importantly, in the microglia RAGE genetically-modified mice. These data appear to suggest, at least by this imaging parameter, that microglial activation is already very high (and perhaps at peak) at disease onset in ALS $SOD1^{G93A}$ mice.

Collectively, these are most promising findings since they form the basis of tracking therapeutic agents and not solely those associated with RAGE. Given that these ALS $SOD1^{G93A}$ mice bore RAGE in all cell types, these findings may well hold promise to track spinal cord inflammation in ALS mice in general, and in the case of RAGE-specific therapies in the future.

Aim 2: Studies to begin in Year 2:

As we noted above in the Introduction paragraph (1), a major caveat of our previous finding that sRAGE administration to $SOD1^{G93A}$ mice decreased ALS pathology is that sRAGE is a large 32.5 kDa protein and its blood-brain-barrier permeability is unknown. It is possible that the modest effect on survival was due to peripheral effects and not direct effects within the affected regions of the spinal cord. We recently tested the CNS permeability of our highly potent and specific small molecule inhibitor of the RAGE-DIAPH1 interaction, called RAGE229, and have found that after 6 hours post intraperitoneal injection, appreciable levels of the antagonist are detectable within the brain relative to the level found in the plasma as measured by mass-spectrometry.

Preparation to date: Mice for study are actively being bred at this time; we have tested and confirmed that the RAGE/DIAPH1 small molecule antagonist is CNS permeable; and we have synthesized the first batch of the small molecule antagonist (13 gms is already prepared). Note that these studies are to begin in Year 2; hence, the preparation is, as noted, well underway for study commencement.

4). *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:

To begin to understand the expression patterns of RAGE in human ALS, we data-mined publically available RNA-Sequencing data from ALS patient induced pluripotent stem cells (iPSC)-derived motor neurons and from the frontal cortex of ALS patients for changes in the expression of components of the RAGE signaling axis. We found that the frontal cortex of patients with *C9orf72*

hexanucleotide expansions displayed increased expression of *Ager* mRNA while iPSC-derived motor neurons from other *C9orf72* ALS patients demonstrated increased expression of *DIAPH1* mRNA but not *AGER* mRNA. *C9orf72* hexanucleotide repeat expansion is the most common form of familial (f)ALS and *C9orf72* has been been directly linked to microglial function. These findings suggest that the RAGE signaling axis is altered in human patients and differentiated cells derived from patient iPSCs. Note that TargetALS is providing to us de-identified human data from >200 subjects, including those with ALS and age-matched controls, from spinal cord and various organs affected in ALS.

• 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

• 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."

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

Aim 1A: Finalize all mice studies on microglia deletion of *Ager* and then "break the code" to establish the data analysis on studies as outlined in the application

Aim 1B: Finalize all PET Imaging studies and determine if deletion of microglia *Ager* increases, attenuates or exerts no change on this marker of microglia information AND to determine how these findings are integrated with those in Aim 1A

Aim 2: Begin studies to administer RAGE229 to mice as noted in the application vs vehicle

All of the studies to be completed/performed are thoroughly described in the application.

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. This key finding means that we will be able to y 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.

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.

- **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.

- **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 a review article on RAGE and neurodegeneration:

Derk J, MacLean M, Juranek J, and Schmidt AM. *The Receptor for Advanced Glycation Endproducts (RAGE) and Mediation of Inflammatory Neurodegeneration.* Journal of Alzheimer's Disease and Parkinsonism. 2018. In Press.

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.

- **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: 10

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:

Nearest Person Month Worked: 6

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

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 0.91 calendar
NIH $541,112
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 $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 0.46 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)

Alzheimer's Association 03/01/17-02/29/20 0.84 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

(NEW)
NIH (Fisher: PI) P01 05/01/17-04/30/22 3.0 calendar
$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: Co-I

<u>THIS AWARD</u>
USAMRAA Dept. of the Army 07/01/17-06/30/19 0.91 calendar
$251,092

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

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

(NEW)
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

<u>INACTIVE</u>
(ENDED)
1R01 HL118565 06/01/13-04/30/18 0.9 calendar
NIH $248,449
RAGE, Macrophages & HDL Biology
This grant examines the molecular mechanisms by which RAGE regulates cholesterol transport.
Role: PI

(ENDED)
Harrington Discovery Institute 01/01/16-12/31/17 0.12 calendar
$50,000
Targeting RAGE/mDia1 for the Prevention and Treatment of Diabetic Complications
Natalie Haynes, Grants Officer
10900 Euclid Avenue
Cleveland, Ohio 44106
The goal of the Harrington Discovery Institute project is to develop LOCAL intraocular and transdermal treatments for diabetic retinopathy and diabetic wound healing, respectively.
Role: PI

WSQ (Sontag: PI) 12/12/12-12/31/16 0.12 calendar
$73,629
Machine Learning to Predict Undiagnosed Diabetes from Insurance Claims
Major goal of this application is to use large data sets to identify novel predictors of type 2 diabetes
The goal of this project is to use machine-learning techniques to identify novel predictors of type 2 diabetes and its complications in the Blue Cross Blue Shield insurance claims data base.
Role: Co-I

3P01AG026467-05S1 01/15/14-01/14/16 3.3 calendar
NIA $722,948
Aging & Vulnerability to Ischemia: Pathways & Rescue (Sandy Supplement)
This grant focuses on the role of RAGE in augmenting ischemic injury in aging.
Role: PI

P01AG026467 03/01/08-02/28/15 1.2 calendar
NIA $1,001,522
Aging & Vulnerability to Ischemia: Pathways & Rescue
This grant focuses on the role of RAGE in augmenting ischemic injury in aging.
Role: PI

P01HL60901-S1 (Sandy Supplement) 12/01/13-11/30/15 3.0 calendar
NIH $668,867
RAGE and Mechanisms of Vascular Dysfunction
This grant focuses on the mechanisms by which diabetes accelerates atherosclerosis via RAGE.
Role: PI

4-2011-25 03/01/11-02/28/15 2.4 calendar
JDRF $758,638
RAGE Signal Transduction: Novel Treatments for T1D
The overall objective of this Multi-Project Grant is to explore a highly novel, logical and feasible

target for the complications of T1D.
Role: PI

OVERLAP
None

Ding, Yu-Shin

ACTIVE
1P41EB017183-01A1 (Sodickson); 09/30/14-07/31/19 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.

THIS AWARD
USAMRAA Dept. of the Army 07/01/17-06/30/19 2.4 calendar
$251,092
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

(NEW)
American Heart Association (Schmidt) 04/01/17 – 03/31/21 0.6 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

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

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

INACTIVE
(ENDED)
NA (de Leon, M. & Marmar, C.) 01/01/15 - 12/31/17 0.6 calendar
Steven & Alexandra Cohen Fndtn $2,000,000
Validation of Tau Blood Test for TBI Using PET Imaging
Role: Co-Investigator

OVERLAP
None

Li, Huilin

ACTIVE
1R01DK110014-01 (Li) 07/01/2016-06/30/2020 2.31 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

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

(NEW)
5P30CA16087 (Neel) 03/01/13 – 02/28/19 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

5U01CA18237 (Pei, Ahn) 04/01/2014 – 12/31/2018 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

1 R01 K100492-01A1 (Sevick) 09/18/2014- 07/31/2019 0.60 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

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.

(NEW)
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

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.

R01CA204113 (Chen) 04/01/2016 – 03/31/2021 1.00 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

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.

1R01HL132516 12/09/16-11/30/20 0.58 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: Statistician

<u>THIS AWARD</u>
USAMRAA Dept. of the Army (Schmidt) 02/01/17-01/31/19 0.58 calendar
$251,328

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

(NEW)
NIH (Fisher: PI) P01 05/01/17-04/30/22 1.8 calendar
$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.

(NEW)
American Heart Association 04/01/17-03/31/21 0.69 calendar
$900,663
Braking Inflammation in Obesity & Metabolic Dysfunction: Translational and Therapeutic Opportunities
Role: Co-I

INACTIVE
(ENDED)
15-A0-00-00-0039-29-01 (Blaser) 02/01/2014 – 11/30/2017 0.61 calendar
Janssen Pharmaceuticals, Inc. $421,806
Studies of Type I diabetes in NOD mice
This work is designed to test the hypothesis that specific changes in the microbiota are accelerating the progression of type1 diabetes in an animal model

(ENDED)
5P30CA16087 (Goldberg) 03/01/2013 – 02/28/2018 0.87 calendar
NIH/NCI $157,904
Cancer Center Support Grant (Biostatistics Shared Resource)
To provide biostatistics support to the NYU cancer community
Role: Co-I

(ENDED)
JDRF 2-SRA-2016-153-S-B (Blaser) 02/01/2016 – 01/31/2018 0.60 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.

(ENDED)
2015210 (Hochman) 07/01/16-06/30/2017 0.57 calendar
DORIS DUKE CHARITABLE FOUNDATION $27,000
New York University Langone Medical Center's Fund to Retain Clinical Scientists (FRCS) Major goal of this application is to understand macrophage-adipocyte interactions in high fat feeding and obesity.
Role: Statistician

OVERLAP
None

- **Other organizations involved as partners**

N/A

8). **SPECIAL REPORTING REQUIREMENTS**

N/A

9). **APPENDICES**

N/A