Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Temple University – Of The Commonwealth System of Higher Education

2. **Reporting Period (start and end date of grant award period):** 1/1/2010-12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Germaine A Calicat, MA

4. **Grant Contact Person's Telephone Number:** 215.204.7655

5. **Grant SAP Number:** 4100050909

6. **Project Number and Title of Research Project:** Project 9 – Characterization of the Effects of Hypertension on Adult Brain Neurogenesis

7. **Start and End Date of Research Project:** 12/1/2010- 6/30/2012

8. **Name of Principal Investigator for the Research Project:** Tinatin Chabrashvili, M.D., Ph.D. is no longer with Temple University. Sergio Rameriz has taken over the project in her absence.

9. **Research Project Expenses**.

9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

$ 81,465.09

9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
Tinatin Chabrashvili, MD, PhD	Assistant Professor; Department of Neurology	20%
Servio H. Ramirez, PhD.,	Associate Professor	20%

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost

10. **Co-funding of Research Project during Health Research Grant Award Period.** Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

    Yes_____ No____x_____

If yes, please indicate the source and amount of other funds:

11. **Leveraging of Additional Funds**

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No____x_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes_____ No____X_____

If yes, please describe your plans:

12. **Future of Research Project.** What are the future plans for this research project?

Dr. Ramirez will continue to explore stem cell biology at vascular niches.

13. **New Investigator Training and Development**. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

    Yes_____ No____x_____

    If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				
Unknown				
Total				

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

    Yes_____ No____x_____

    If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. **Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

    Yes_____ No____x_____

    If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No____x_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____x_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____x_____

If yes, please describe involvement with community groups that resulted from the research project:

17. **Progress in Achieving Research Goals, Objectives and Aims.**
List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims <u>for the period that the project was funded (i.e., from project start date through end date).</u> Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. <u>Provide detailed results of the project.</u> Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a <u>DETAILED</u> report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16

months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Goals, Objectives and Specific Aims

Characterization of the Effects of Hypertension on Adult Brain Neurogenesis – Our underling hypothesis was that hypertension impairs neurogenesis by decreasing the number of neural stem cells (NCS) in the subventricular zone (SVZ) and the dentate gyrus leading to changes in intrinsic properties and functional characteristics. Damage to the neurogenic areas was theorized to occur as consequence of a breached BBB. Changes in intrinsic properties that affect subsequent neural progeny turn-over and diminished growth factor support to the neuronal environment. Our formulated hypothesis was based on the fact that o studies to date have directly evaluated the effect of hypertension on the neurogenic response. Clinically the susceptibility of hypertensive individuals to stroke is well known and also the correlation between hypertension and mild cognitive impairment. Although some of the project goals were accomplished, these studies have shed some light into the premise that NSC dysfunction in hypertension may explain the occurrence of both mild cognitive dysfunction and poor stroke recovery when hypertension is present.

Aim 1: Evaluation of the direct effects of chronic hypertension on the neurogenic response in-vivo.
To understand how hypertension may induce changes to the NSC, we tested whether administration of a slow pressor dose of Ang II for a given duration affected the number of proliferating NSC and viability. A comparative analysis of NSC proliferation and survival was examined at the three most neurogenic areas (forebrain SVZ, hippocampal dentate gyrus and corpus callosum) to determine whether a particular brain region is more sensitive to the hypertensive condition. Since many NSC populations can be found in "stem cell niches" with proximity to the microvasculature, we evaluated if a correlation can be established between morphological changes to the NSC and BBB compromise. The assessments in hypertensive animals was compared to normotensive animals.

Progress made on aim 1: This aim was partially fulfilled. Although the hypertensive model was well established, a better histological methodology had to be developed in order to better quantitate the status of the stem cell areas by image analysis. Therefore an important milestones was accomplished which related to not only the optimization of the

Neural stem cells in the adult mouse subgranular zone are immunoreactive for nestin and sox2.

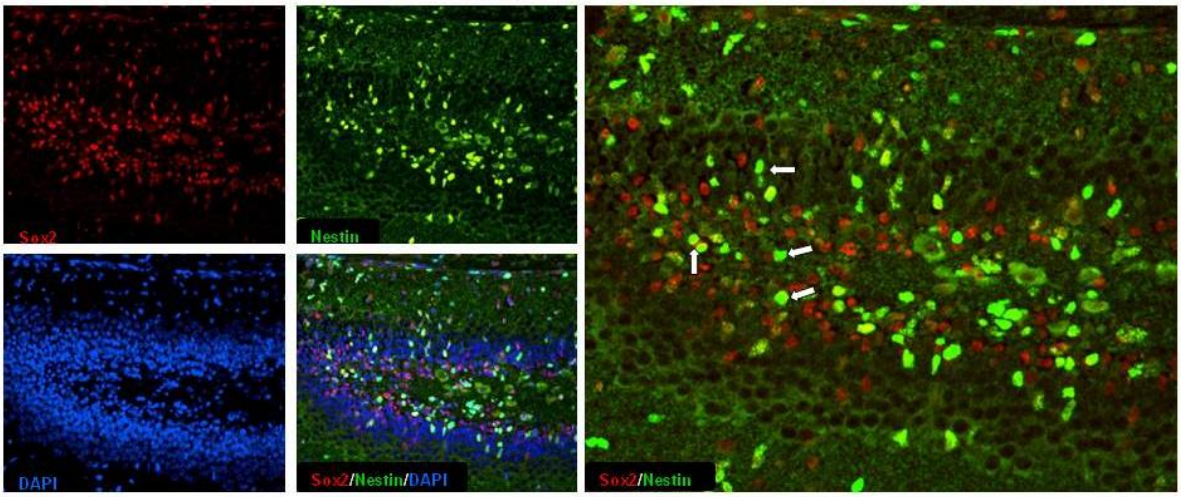

FIGURE 1: A new method using plastic embedding (JB4) has been developed in our lab for immuonlabeling of stem cells in the neurogenic areas of the mouse brain. This method provides excellent morphological preservation of the SGZ and increases immunolabeling sensitivity for neural stem cell markers.

immunostaining of neural stem cell markers but also the preservation of tissue morphology in the adult mouse brain (Figure 1). In order to better characterize/visualize the morphology of the stem cell niche we developed a novel staining procedure using the plastic resin JB4. As can be seen in figure 1 cells in the dentate gyrus are immunopositive for nestin and sox2. For this aim, establishing the right methodology was critical (which was accomplished), however the excellent progress being made under Aim 2 along with the number of animals (which required more funds) needed to comprehensively perform the image analysis precluded us from advancing further in this Aim. The hope is still process some of the material currently on hand and to publish the immunolabeling methodology in the journal of neuroscience methods.

Aim 2: <u>Effects of angiotensin II on BBB function and its impact on neural stem cell proliferation and multilineage differentiation in an in-vitro BBB model</u>.
Our preliminary experiments revealed that chronic hypertensive animals have disruptions to the blood-brain barrier (BBB), which exposes the neuronal environment to blood components. BBB permeability promotes deterioration of neuronal networks. It is therefore likely, that BBB permeability could impact NSC viability and proliferation. To understand better the mechanism for which Ang II affected the BBB and the NSC niches, we modeled this condition in-vitro. We used primary mouse BMVEC, primary mouse astrocytes and primary mouse neural stem cells. We perform co-culture experiments, using Boyden chambers, to place endothelial cells on a porous membranous surface (top chamber), astrocytes on the opposite side of the membrane and NSC on the lower chamber. We exposed the endothelial cells to recombinant Ang II and evaluated the NSC for changes in viability and proliferation. Furthermore, we evaluated how Ang II treated BMVEC altered NSC differentiation and determined the ratio and morphology of maturing astrocytes and neurons.

Progress made on aim 2: This aim was partially accomplished. The conditions suitable for co-culturing of the various primary cell types proved difficult but was overcome towards the end of

the funding period. The critical component was in formulating a compatible media that supported all the cell types in the co-culture.

Overall progress made on the project and key developments:

The findings of our project shed light on how vascular and neural stem cells communicate in health and in disease, specifically in the context of hypertension. The results of this project determined whether chronic hypertension affects the neural stem cell survival, differentiation, intrinsic properties and function in the adult brain. Targeting these negative regulators would help us design new therapeutic measures to prevent morbidity and mortality provoked by the detrimental effects of hypertension, especially stroke and dementia. In particular, the continued study of this subject could help design the therapeutic tools to promote repair and neural plasticity to improve stroke outcome and memory. One of the main outcomes of this study is that it helped us generate a framework strategy that centered on protecting the BBB during hypertension which in turn could prevent loss/damage to the neuro stem cell niches (Figure 2)

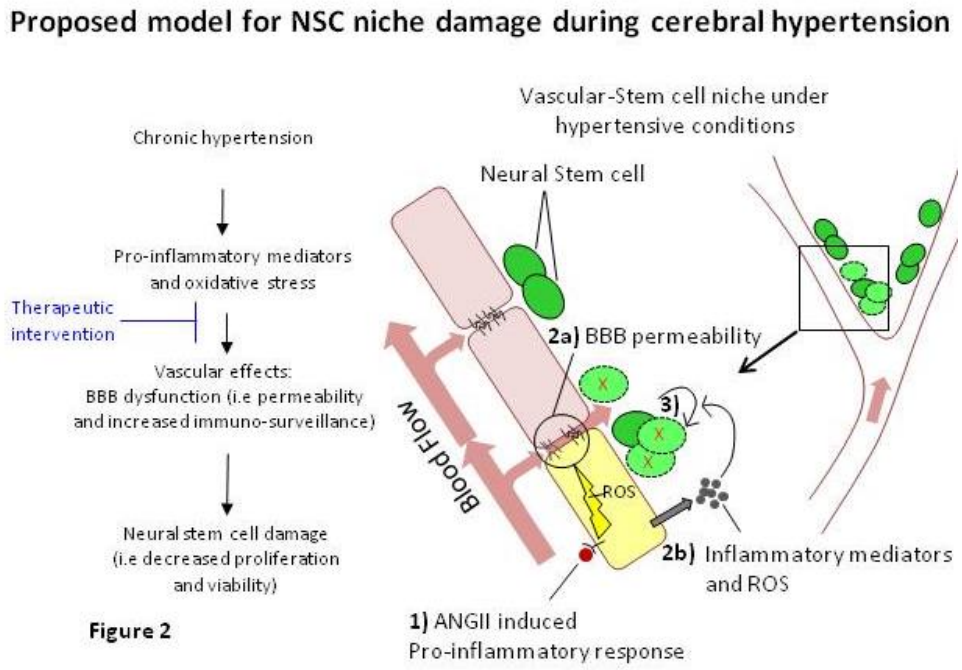

Figure 2

In regards to the animal model over this period of time we further developed the experimental model of hypertension. The selection of the appropriate animal model to study the disease process is crucial for the translational research in order to successfully transition the knowledge obtained from animal studies into clinical setting. Such models frequently require multiple steps of validation. This is especially important since hypertension is a multifactorial disease and has underlying genetic and environmental etiology. Therefore, we explored the different options for measuring the degree of hypertension in our model at different stages of the experiments.

As our blood pressure measurement system, we selected Mouse Non-Invasive Blood Pressure System (Kent Scientific, CT) – High Throughput Tail Cuff Method through which we can measure systolic and diastolic blood pressure in awake mice. Because of the appropriate animal

holder availability, we were able to measure the blood pressure in both adult and aged mice. This assisted us in evaluating the neurogenesis in hypertensive-aged mice and to compare it with normotensive mice neurogenesis.

We also established the methods to evaluate the role of MicroRNAs in the brain gene expression. MicroRNAs introduce a novel concept of regulatory control over gene expression and there is increasing evidence that they play a profound role in vascular and neuronal cell identity as well as multiple aspects of disease pathogenesis. We have applied these methods and the knowledge to build on these experiments to investigate the role of microRNAs in adult neurogenesis.

At this phase of the research, we are still analyzing the results from the animal experiments to thoroughly complete the assessment of neurogenesis during hypertension at baseline and during the additional pathological challenge - Ischemic Stroke and aging.

Key findings:

The following results relate to the underlying hypothesis described under Aim 2. As stated in the grant, previously reported observations have indicated that neural stem cells (NSCs) are found in close proximity to the microvessels of the brain. Therefore, in the developing and adult brain the NSCs and endothelial cell may regulate one another to affect localized blood-brain barrier (BBB) function, stem cell renewal and differentiation. Although stem cells and endothelial cells are found together in cluster-like structures termed the neurovascular niche, it remains unknown how 1) NSCs adhere together and 2) how NSCs may physically interact with the endothelium. Understanding how these cellular complexes are held together is of critical importance in determining how insult from hypertension may induce the disassembly of these specialized cellular regions. To this end we hypothesized that tight junction protein expression may offer a unified explanation as to how NSCs are clustered together and how the cells also attached to the brain endothelium. For this analysis we used commercially embryonic stem cells (mNSC, EMD Millipore) propagated as an adherent culture in Stempro neural supplement (Invitrogen) in CellStart (Invitrogen) coated Millicell chamber slides (Millipore). Once attached, the cells were maintained in the undifferentiated state for 3 days and then processed for RNA isolation and real-time qPCR. **Figure 3A** shows the expression profile for stem cell markers Nestin and SOX-2 as well as various tight junction proteins. The ΔΔCt method was applied to arrive at fold regulation using the U937 monocytic cell line as a reference which shows marginally detectable levels of the target genes under observation. Brain microvascular endothelial cells (BMVECs) were also used in order to examine how NSC gene expression compares with cells that use tight junction proteins to form the BBB. As expected, Figure 1A shows that only undifferentiated NSC's have high expression of stem cell markers Nestin and SOX2. Interestingly the NSCs not only show expression of genes for tight junction proteins but also show a profile that is slightly different to those expressed in BMVECs. NSCs show comparable expression to BMVEC (within a log difference) of genes encoding the following tight junction proteins: occludin (OCC), Zona occludens-1 (ZO-1), and Claudin -5. However, unlike BMVECs, NSCs shows expression for claudin-12 and low levels of zona occludens-2 (ZO-2). In validation of these results membranous protein expression of the above tight junctions were also confirmed by immunofluorescence analysis (data not shown). These results suggest that NSC expression of tight junction proteins may allow for the formation of the intercellular cluster like architecture

that holds these cells together in the vascular niche. We next investigated whether the presence of tight junction proteins decrease as a consequence of differentiation. These experiments were performed using NSCs while in presence of differentiation media which essentially lacks the leukemia inhibitory factor (LIF). The cells were then allowed to differentiate for either 7 days or 14 days and then processed for real-time qPCR. **Figure 3B** shows the results of gene expression analysis for Nestin, differentiation markers MAP-2 (neuronal) and GFAP (astrocyte), and tight junction protein, OCC. The results demonstrate that as Nestin expression goes down, MAP-2 and GFAP go up thus indicating differentiation. Also evident was the decrease in expression of OCC as a function of differentiation. These preliminary results imply that NSC differentiation induces the continuous loss of OCC. This is a very likely possibility in light of the fact that differentiation induces the migration of NSCs out the vascular niche which otherwise would be "anchored" by the presence of tight junction proteins.

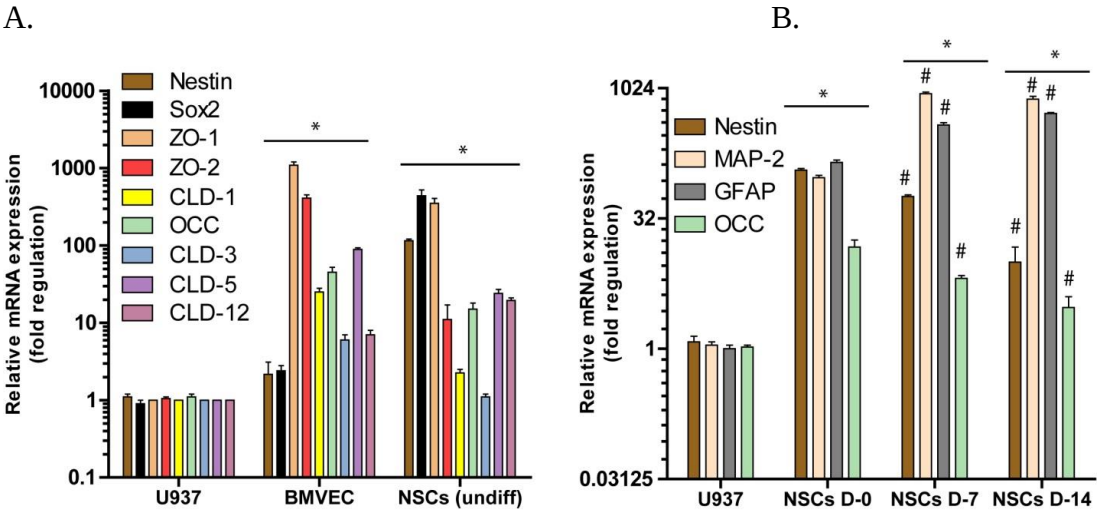

Figure 3: A gene expression profile of tight junction proteins and stem cell markers was established for undifferentiated and differentiating mNSCs, U937, and BMVEC. RNA was isolated with the RNAqueous PCR kit (Ambion) and RNA purity and concentration was determined with a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific). cDNA conversion was performed by reverse transcription using the High Capacity cDNA Reverse Transcription Kit (ABI). The cDNA (diluted 1:4) template was then mixed with both the TaqMan Universal PCR Master Mix (ABI) and with the corresponding TaqMan Gene Expression Assay (target name). TaqMan Gene Expression Assays for GAPDH and RPLPO were also used as internal controls. Quantitative Real-Time PCR was performed on an ABI StepOnePlus Real-Time PCR System. The data from ΔΔCT analysis is represented as the mean +SEM. ANOVA group comparisons to U937 gene expression is shown by the "*" and indicates statistical significance of $p<0.01$. The "#" symbolizes statistical significance of $p<0.01$ denoting the comparison between the indicated group with the corresponding one at NSC day 0.

In summary, these results suggest that tight junction proteins in NSCs and brain endothelial cells may provide a means for cell to cell interaction. Furthermore, identification of this tight junction protein expression strongly suggests an explanation for how NSCs may be held together in the neurosphere configuration. Our study also indicated that the differentiation process can change the TJ protein expression profile. Taken together the dynamic tight junction protein expression in NSCs offers the possibility of explaining how these precursor cells in the adult and developing brain may physically interact with the brain microvasculature.

Implications that extend beyond the immediate project
The methodologies established from this project involving in-vivo and in-vitro models to study the neurogenesis, not only can be applicable for hypertension, but possibly to other disease conditions in which neuroinflammation is also present.

18. **Extent of Clinical Activities Initiated and Completed**. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

    18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?
    _____Yes
    ___X___ No

    18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?
    _____Yes
    ___X___No

    If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

    18(C) How many hospital and health care professionals were involved in the research project?
    _____Number of hospital and health care professionals involved in the research project

    18(D) How many subjects were included in the study compared to targeted goals?

    _____Number of subjects originally targeted to be included in the study
    _____Number of subjects enrolled in the study

    Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

    18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

    Gender:
    _____Males
    _____Females
    _____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. **Human Embryonic Stem Cell Research.** Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
____x__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. **Articles Submitted to Peer-Reviewed Publications**.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For

example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:
Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care
If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X_____ No_____

If yes, please describe your plans:

The following article is planned for submission for the end of this month. This article employs methodology developed under the active period of the grant.

Journal: Stem Cells
Section: Tissue Specific Stem Cells

Dynamic regulation of tight junction protein expression in human neural stem cells
Andrea K. Watters‡, Marie K Dematatis‡, Yu Zhou‡, Jonathan Cena‡, Raghava Potula‡§, Andrew Skuba#, Young-Jin Son#, Yuri Persidsky‡§ and Servio H Ramirez‡§#
Department of ‡Pathology & Laboratory Medicine, the §Center for Substance Abuse Research and the #Shriners Hospitals Pediatric Research Center, Temple University School of Medicine, Philadelphia, PA, USA.

21. **Changes in Outcome, Impact and Effectiveness Attributable to the Research Project.** Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If

there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. **Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment**. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. **Inventions, Patents and Commercial Development Opportunities.**

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___x_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

Title of Invention:

Name of Inventor(s):

Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No___x_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

Former PI - Tinatin Chabrashvili, M.D., Ph.D. is no longer with Temple University and therefore her Biosketch is not available at this time. Servio Ramirez has assumed the role of Principal Investigator on this Project and has attached his Biosketch as such.

NAME	POSITION TITLE
Servio H. Ramirez	Associate Professor Department of Pathology & Laboratory Medicine Temple University School of Medicin
eRA COMMONS USER NAME (credential, e.g., agency login) SRAMIREZ	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Rochester Institute of Technology, Rochester, NY	B.S M.S	05/98	Biotechnology
University of Rochester School of Medicine and Dentistry, Rochester, NY	Ph.D.	05/01	Microbiology & Immunology
University of Rochester School of Medicine and Dentistry, Rochester, NY	Postdoctoral	05/04	Immunology, Microbiology and Virology
University of Rochester School of Medicine and Dentistry, Rochester, NY	Postdoctoral	06/05	Pharmacology and Physiology
University of Nebraska Medical Center Omaha, NE		06/08	Pharmacology & Experimental Neuroscience

A. Personal Statement

Our ongoing research interest is in the study of how brain pathologies affect the neurovascular unit and how these changes lead to the disruption of the Blood-Brain Barrier (BBB). Our research in this field is conducted with two key observations in mind. The first is that the brain endothelium generates the BBB which is responsible for maintaining an optimal neuronal environment. The second is that BBB dysfunction is observed in virtually all aspects of neurological impairment arising from neurodegerative disorders, cerebrovascular disease (including stroke), traumatic brain injury (TBI) and infectious diseases of the brain. The study of the BBB in conditions of neuroinflammation is pursued by a constant commitment to the development of new methodologies in cell isolation (specifically primary human brain microvascular endothelial cells), in-vitro BBB modeling, and in-vivo imaging modalities for (real-time) evaluation of BBB function. Understanding BBB remodeling during inflammation also offers unique opportunities for the discovery of BBB derived biomarkers. Overall, it is our hope that these studies will lead to the discovery of therapeutic agents that are both anti-inflammatory and barrier protective in order to attenuate the deleterious outcomes of brain pathologies. In addition to BBB biology, our expertise also includes neurobiology, immunology, and molecular biology. My laboratory is also proficient in using animal models of neuroinflammation. In particular, as it relates to drug abuse, sepsis, HIV-1 neuropathogenesis and TBI. Lastly we employ a translational approach to our research by using relevant clinical samples (tissue, serum and CSF) to validate and further extend our findings.

B. Positions and Honors

Positions and Employment

2014 -present Associate Professor, Dept of Pathology & Laboratory Medicine
Temple University School of Medicine, Philadelphia, PA
2008-*2014* Assistant Professor, Dept of Pathology & Laboratory Medicine
Temple University School of Medicine, Philadelphia, PA
2011-*present* Director, Histopathology Research Core
Dept of Pathology & Laboratory Medicine
Temple University School of Medicine, Philadelphia, PA

Secondary Appointments:
2011- *present* Center for Substance Abuse Research (CSAR)
Temple University School of Medicine, Philadelphia, PA
2011- *present* Center for Neural Repair and Rehabilitation
Shriners Hospitals Pediatric Research Center
Temple University School of Medicine, Philadelphia, PA
2011- *present* Concussion and Athletic Neurotrauma program
Temple University School of Medicine, Philadelphia, PA

Professional Memberships
2002 – present: Society For Neuroscience, member.
2006 – *present:* International Brain Barriers Society, (Founding Member).
2006 – *present:* International Society of Neuroimmunology, member.
2008 – *present:* Society on NeuroImmune Pharmacology, member
2010 – *present:* National Neurotrauma Society, member

Noteworthy Awards and Honors
2006: Travel award for The 8th International Congress of Neuroimmunology, Nagoya, Japan
2006: The Carl Storm travel award for Gordon Research Conference: Barriers of the CNS, Tilton, NH
2008: Recipient of the 2008 Research Innovation Award from the UNeMed Corporation, Omaha NE
2012 NIH Early Career Reviewer (ECR) program. Brain Injury and Neurovascular Pathologies (BINP) study section. (June 2012, Chicago, IL)
2012: External reviewer for the Portuguese Foundation for Science and Technology (FCT). (September 2012)
2013: External reviewer for the Israel Science Foundation (ISF). (March 2013)
2013: External reviewer for the United Kingdom Medical Research Council (MRC). (August 2013)
2013: Invited speaker to the "Fox Chase Cancer Center inflammation seminar series"
(May 29th 2013, Philadelphia, PA)
2013: Invited speaker to the "Harvard Medical School/MGH Neuroscience Center Seminar Series"
(June 6th, 2013, Boston, MA)
2014: Invited speaker to the "UPENN –Pathology Seminar Series"
(March 20th, 2014, Philadelphia, PA)
2014: Ad hoc reviewer for the NIH, Brain Injury and Neurovascular Pathologies (BINP) study section. February 2014, Washington, DC; June 2014 (scheduled), San Francisco, CA.
2014: Standing member reviewer for the American Heart Association: Vascular Endothelial Biology and Function - Basic Science V study section

Patent Applications Filed:
1. "Method for detecting injury to the Brain". The assay is based on rapid detection of brain endothelium derived microvesicles containing tight junction proteins as biomarkers for traumatic brain injury". *Patent filed to the USPTO U.S. (Provisional Application No. 61/664,352 filed June 2012, International Patent Application PCT/US2013/047470 (Filed on 6/25/13); WO-2014004424-A1.* Inventors: Servio H. Ramirez, Yuri Persidsky and Slava Rom.
2. "Antibodies capable of specifically recognizing phosphorylated forms of occludin and claudin-5 and uses thereof". *New Invention Notification filed with UneMed Oct 2007.* Inventors: Servio H. Ramirez, Yuri Persidsky, Tsuneya Ikezu.
3. "Novel adjunctive therapies for neuroAIDS, based on pharmacologic manipulation of adenosine 2A receptor signaling". International Patent Application PCT/US2006/009390 *(Filed on 3/18/2005); WO-2006101920-A3* (based on U.S application No. 60/663,059 filed 3/18/2005). Inventors: Servio H Ramirez, Shongshan Fan, Harris A. Gelbard, Sanjay B. Maggirwar, Stephen Dewhurst.

Educational contributions at Temple University School of Medicine (TUSM)
Teaching experience -selected lectures for TUSM:
Dept. of Biochemistry (course #9220 –Fall semester): Special topics in biophysical chemistry
Dept. of Pathology (course #9603 –Spring semester): Principles of organ pathology

Dept. of Neuroscience (course #8703 –Spring semester): Translational science of nervous system disease

Committees:

2008 – *present*: Member, Graduate Studies Committee, Dept. of Pathology & Laboratory Medicine
2008 – *present*: Member, The MD/PhD Program Admissions Committee
2012 – *present*: Member of the Biomedical Sciences Graduate Program Faculty:
Primary: Neuroscience Cluster,
Secondary: Organ Systems and Translational Medicine Cluster
2013 – *present*: Elected Member, Temple University Invention and Patent Committee
Temple University School of Medicine
2010: PhD Thesis External Examiner for Brian Teets (Department of Biochemistry, TUSM) on "*SF-1 but not DAX-1 prevents P19 cells from differentiating to either TROMA-1 or TUJ1 positive cells upon RA treatment".*
2011: PhD Thesis External Examiner for Ke Yuan (Department of Biology, TUSM) on *miR-148a in hepatitis B associated Hepatocellular Carcinoma".*
2013: PhD Thesis External Examiner for Valentina Lucchesi (Department of Pharmacology, University of Pisa, Italy) "*Rational Design, Synthesis and Pharmacological Evaluation of New Cannabinoid CB2 Receptor Ligands".*
2011 - *present*: Doctoral candidate committee member for: Larisa Gofman (PhD program), Yu Zhou (PhD program), Jeremy Hill (PhD program) and Kaitlin Collura (MD/PhD program).

Mentoring:
2009– *present*: Mentor for the College of Science and Technology summer undergraduate research program (CST-SURP) and neuroscience independent study at Temple University (main campus). Trainees include: Marie Dematatis (Summer of 2010), Andrea Watters (2010-present), Kevin Chavarria (2011-present)
2010– *present*: Mentor for TUSM, summer research opportunity for medical students.
Kerry Lydon (Summer of 2011)
2012 -*present*: PhD Thesis Advisor for Steven Merkel (Department of Pathology and Laboratory Medicine)
2013 -*present*: PhD Thesis Advisor for Evan Lutton (MD/PhD program)
2014 -*present*: Mentor for postdoctoral fellow Allison Andrews PhD

Other
Ad hoc reviewer for the following journals: Antioxidant and Redox Signaling, the Journal of Psychopharmacology, Cellular and Molecular Life Sciences, PLoS ONE (Editorial Board Member), FASEB journal, Brain Research, The British Journal of Pharmacology and Clinical Research in HIV/AIDS (Editorial Board Member).

C. Selected Peer-reviewed Publications

Book Chapters
1. Yuri. Persidsky and Servio H. Ramirez, 2011. "The blood-brain barrier in the setting of neuroinflammation" . *The Neurology of AIDS 3rd Edition*. Editors: Howard E. Gendelman, Igor Grant, Ian Paul Everall, Stuart A. Lipton and Susan Swindells. *Oxford University Press, New York*
2. Servio H. Ramirez, Steven Merkel and Yuri Persidsky. "The Blood-Brain Barrier in HIV Infection". *The Blood-Brain Barrier in Health and Disease 1st Edition.* Editor: Katerina Dorovini-Zis. CRC Press, Taylor and Francis Group. (*expected publication, 2014*)

Peer-reviewed Publications (10 out of 33), h-index = 22:
1. M.V. Singh, D.C. Davidson, J.W. Jackson, J. Silva, **S.H. Ramirez**, S.B Magirwar. Characterization of platelet-monocyte complexes in HIV-1 infected individuals. *J Immunol 1302318; published ahead of print April 11, 2014,*
2. V.V.Shuvaev, J. Han, S. Tliba, E. Arguiri, M. Christofidou-Solomidou, **S.H. Ramirez**, Holly Dykstra, Yuri Persidsky, Dmitriy Atochin, Paul Huang, and Vladimir R. Muzykantov. Anti-inflammatory effect of targeted delivery of SOD to endothelium: mechanism, synergism with NO donors and protective effects in vitro to in vivo. PLoS One. 2013;8(10): e77002.
3. **Ramirez SH**, Reichenbach NL, Fan S, Rom S, Merkel SF, Wang X, Ho WZ, Persidsky Y. Attenuation of HIV-1 replication in macrophages by cannabinoid receptor 2 agonists. *Journal of Leukocyte Biology*. 2013 May;93(5):801-10

4. Ramirez, S.H., S. Fan, H. Dykstra, A. Mercer, N. Reichenbach, L. Gofman, Y. Persidsky. Inhibition of glycogen-synthase-kinase 3β promotes tight junction stability in brain endothelial cells by half-life extension of occludin and claudin-5. *PLoS One. 2013;8(2):e55972*

5. S. Rom, S. Fan, N. Reichenbach, H. Dykstra, **S.H. Ramirez**, Persidsky Y. Glycogen Synthase Kinase 3β Inhibition Prevents Monocyte Migration Across the Brain Endothelial Cells via Rac1-GTPase Suppression and Down-Regulation of Active Integrin Conformation. *American Journal of Pathology.* 2012 Oct;181(4):1414-25.

6. Ramirez, S.H., J. Hasko, A. Skuba, S. Fan, R. McCormick, H. Dykstra, N. Reichenbach, I. Krizbai, A. Mahadevan, M. Zhang, R. Tuma, Y. Son, Y. Persidsky. Activation of Cannabioid Receptor 2 Attenuates Leukocyte-Endothelial Interactions and Blood-Brain Barrier Dysfunction under Inflammatory Conditions. *Journal of Neuroscience*. 2012 Mar 21;32(12):4004-16.

7. M. Zhang, Y. Mao, **S.H. Ramirez**, R. Tuma, T. Chabrashvili. Angiotensin II Induced Cerebral Microvascular Inflammation and Increased Blood-Brain Barrier Permeability via Oxidative Stress. *Neuroscience*. 2010 Dec 15;171(3):852-8.

8. Li Ye, X. Wang, J. Li, J. Liu, **S. H. Ramirez**, W. Ho. Lack of Evidence for Restricting HCV release from Hepatocytes by Tetherin. *Journal of innate immunity.* 2012 Jun;18(3):398-405

9. R. Potula, B. J. Hawkins, J. M. Cenna, S. Fan, H. Dykstra, **S. H. Ramirez**, B. Morsey, M. Brodie, and Y. Persidsky. Methamphetamine causes mitochondrial oxidative damage in human T-lymphocytes leading to functional impairment. *Journal of Immunology. 2010 Sep 1;185(5):2867-76.*

10. M. J. Bernas, F. L. Cardoso2, S. K. Daley, M. E. Weinand, A. R. Campos, A. J. Ferreira, J. B Hoying, M. H. Witte, D. Brites, Y. Persidsky, ***S.H. Ramirez, ***M. A Brito. Establishment and characterization of primary cultures of human brain microvascular endothelial cells: a new and simplified method to obtain an in vitro model of the blood-brain barrier. ***-** shared corresponding authorship. *Nature Protocols. 2010 Jul;5(7):1265-72. (Journal Cover)*

D. Research Support

Ongoing Research Support

1 R01 NS086570-01 Ramirez SH (PI) 10/01/2013 – 11/30/2018
NIH-NINDS
Project title: Novel serological biomarkers for BBB dysfunction during HIV-1 infection
The major goals of this project are: We have developed unique approaches for discovery of novel brain endothelial derived biomarkers that could indicate the status of the BBB during HIV-associated neuroinflammation.
Role: PI

Shriner's Hospitals Research Grant, Ramirez SH (PI) 01/01/2014 – 12/31/2016
Shriner's Hospitals for Children
Project title: Tight junction protein (TJP) release from the blood-brain barrier as biomarkers for TBI
The major goals of this project are: Traumatic brain injury (TBI) is the leading cause of death and disability in children in the United States. Analysis of TJP in TBI could contribute to our understating of brain trauma by revealing underlying mechanisms of BBB injury.
Role: PI

GRANT 11003189 Wolfson M. (PI) 05/01/2012 – 04/30/2016
Office of Naval Research
Project title: Far-Forward Perfluorochemical Based Treatments for Environmental Stress-Induced Multi-Organ Injury
The central goal of the project is to optimize NP-PFC cooling to maximally attenuate TBI related neuropathology.
Role: Co-I

Shriner's Hospitals Research Grant Ferguson T. (PI) 01/01/2014 – 12/31/2016
Shriner's Hospitals for Children
Project title: The Role of Glutamate and astrocytes in Cerebral Palsy (CP) Pathogenesis
The major goal of this project is: We have formed a multi-disciplinary team to refine a novel screening test to accurately identify infants with CP at birth with a simple blood sample. mples to identify fetal injury.
Role: Co-I