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.

1. **Grantee Institution:** The Pennsylvania State University

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):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814 935 1081

5. **Grant SAP Number:** 4100050904

6. **Project Number and Title of Research Project:** 38. Chemokines and Immune Cells in Diabetic Nephropathy

7. **Start and End Date of Research Project:** 4/10/2012 - 12/31/2013

8. **Name of Principal Investigator for the Research Project:** Alaa S. Awad, MD

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:

   $ 138,418

   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
Awad	Principal Investigator	Yr 1: 4.3%	Yr 1: $9,070.90
Bronson	Co-Investigator	Yr 1: 4.0%	Yr 1: $10,760.97
You	Research Associate	Yr 1: 15.7% Yr 2: 20%	Yr 1: $18,302.69 Yr 2: $10,325.30

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
Awad	Principal Investigator	Yr 2: 0%
Bronson	Co-Investigator	Yr 2: 0%
Reeves	Co-Investigator	Yr 1: 2%; Yr 2: 2%

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
ELISA reader		31,318

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:
None	☐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 ____X__ No _____

If yes, please describe your plans:

Data generated from this Cure grant will be submitted as part of the NIH NIDDK resubmission grant R01DK093427 on March 5, 2014. Our application was favorably reviewed and scored 33rd Percentile. Grant application is ready for submission on March 5 and was approved for submission by the Penn State grant office.

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

First, we will submit the A1 application of NIH grant R01DK093427 on March 5, 2014. The major feedback by the reviewers during the first submission was to generate mouse models that overexpress CCR2 in podocytes. During the CURE grant period, we were able to successfully generate Podocin-CCR2 mice (mice that overexpress CCR2 in podocytes). Despite the fact that generating this mouse model was challenging; it will help understand the mechanism(s) of diabetic renal complications and will significantly support our NIH grant resubmission.

Second, we will further continue to examine the role of CCR2 in podocytes and endothelial cells in diabetic nephropathy and its mechanism of action. We will finally determine the

effect of CCR2 and/or MCP-1/MCP-5 inhibition on transgenic mice that overexpress CCR2 in podocytes and endothelia cells.

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 ___X____ No _____

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

The CURE grant established a new collaboration between the PI (Awad) and a Co-Investigator (Reeves). They had 2 publications together supported by the CURE grant. This collaboration will strengthen the proposed A1 application of the NIH R01DK093427 on March 5, 2014.

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 ___X__ No _____

If yes, please describe the collaborations:

We have established collaboration with Dr. M. Prinz (Germany) to examine the role of CCR2 in endothelial cells specifically in diabetic nephropathy.

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 DETAILED 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.

Diabetes mellitus (DM) is a global health problem. The prevalence of diagnosed and undiagnosed diabetes in the US is progressively increasing, from 7.8% in 2007 to 14.5% in 2010, and is expected to rise to between 24.7% and 32.8% by 2050. Diabetes is the most common cause of end stage renal disease, responsible for more than 40% of all cases in the US. Diabetic Nephropathy (DN) demonstrates infiltration of inflammatory cells such as monocytes/macrophages, and the number of kidney macrophages correlates directly with the duration and severity of renal injury in diabetes. CCR2 regulates monocyte/macrophage migration into injured tissues. However, the direct role of CCR2 in the pathogenesis of DN is not known. Our recent publication clearly shows that genetic deficiency or pharmacological blockade of CCR2 confers kidney protection in diabetic mouse models. Furthermore, our preliminary data suggest that activation of CCR2 may alter podocyte and/or glomerular endothelial cell integrity and function that may contribute to the glomerular permeability defects associated with DN. Therefore, we hypothesize that inhibition of CCR2 receptors expressed on kidney derived cells, such as endothelial cells or podocytes will ameliorate renal injury associated with DN.

Our experimental approach will use genetically altered mice (mice with complete absence of CCR2 expression and their wild type littermates, and transgenic mice that overexpress CCR2 specifically in endothelial cells (Tie2-CCR2) and podocytes (pNephrin-CCR2) to fully define the role of CCR2 in DN.

Aim 1: Test the hypothesis that CCR2 expressed on endothelial cells and podocytes directly contributes to diabetic renal injury. Our preliminary data suggest that activation of CCR2 may alter podocyte and/or glomerular endothelial cell integrity and function that may contribute to the glomerular permeability defects associated with DN. Thus we will use mice transgenic for CCR2 driven by the Tie2 promoter (endothelial specific; Tie2-CCR2) and by the pNephrin promoter (podocyte specific; pNephrin-CCR2) to test whether this overexpression of CCR2 in either of these cell types is sufficient to induce renal damage *in vivo*.

Outcome: Results of these studies will provide a basis for the development of new therapeutic modalities in the development and progression of DN.
We performed studies to examine the direct role of MCP-1/MCP-5 inhibition in diabetic renal injury. We have also examined the role of CCR2 expressed specifically in endothelial cells and podocytes. Selected data are shown:

Role of MCP-1/MCP-5 in DN
Experiments were conducted in male $Ins2^{Akita}$ and their wild type littermate mice (DBA background) starting at 8 weeks of age until 17 weeks of age. At 8 weeks of age, animals began treatment with vehicle, MCP-1/MCP-5 (0.5/0.3 mg) inhibitors biweekly or captopril (24 mg/L in drinking water) for 9 weeks (n=4-8 each group). Renal function, histology, and kidney macrophage recruitment were monitored throughout the study. Selected data are shown:

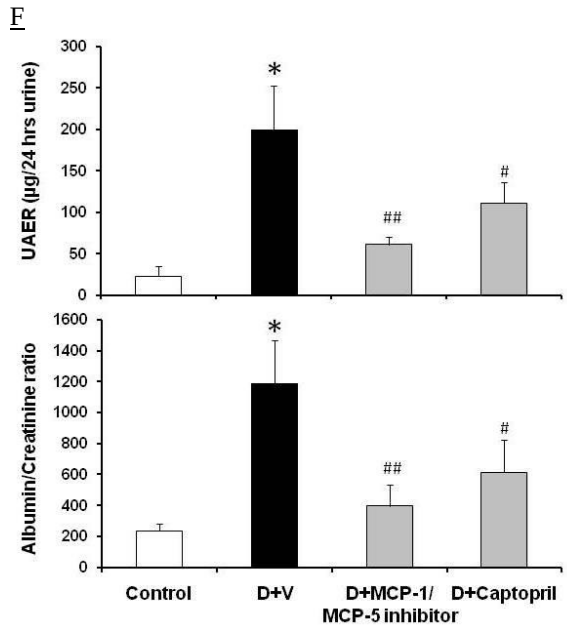

Figure 1 – Effects of a MCP-1/MCP-5 inhibitors on albuminuria in $Ins2^{Akita}$ mice. $Ins2^{Akita}$ and their wild type littermate mice were treated with MCP-1/MCP-5 inhibitors, Captopril or vehicle for 9 weeks. Urine was collected for measurement of albumin and creatinine at 17 wk of age. Data are presented as mean ± SEM. *p<0.005 compared to control; #p<0.05, ##p<0.005 compared to $Ins2^{Akita}$ vehicle-treated.

- MCP-1/MCP-5 inhibition ameliorates renal dysfunction in $Ins2^{Akita}$ mice. Vehicle-treated $Ins2^{Akita}$ mice had a significant increase in albuminuria compared to controls at 17 weeks of age. Albuminuria was significantly reduced in $Ins2^{Akita}$ mice treated with MCP-1/MCP-5 inhibitors or captopril at 17 weeks of age (*Figure 1*). Furthermore, $Ins2^{Akita}$ vehicle-treated mice show increased plasma creatinine (0.48±0.07; p<0.0001) compared to control (0.21±0.03) mice; effects

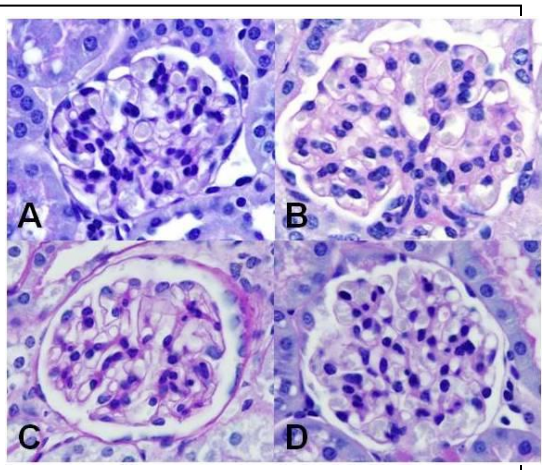

Figure 2 – Effects of a MCP-1/MCP-5 inhibitors on histological changes in $Ins2^{Akita}$ mice. Sections were stained with PAS and all glomeruli were examined at 25x. They were then graded at 40x. Images are representative of 4-8 mice in each group at 17 wk of age in control mice (A), $Ins2^{Akita}$ mice treated with vehicle (B), and $Ins2^{Akita}$ mice treated with MCP-1/MCP-5 inhibitors (C) or Captopril (D).

significantly reduced using MCP-1/MCP-5 inhibitors (0.25±0.03; $p<0.01$) or captopril (0.24±0.05; $p<0.001$) treatment despite comparable blood glucose levels, hemoglobin A1C and blood pressure levels.

- MCP-1/MCP-5 inhibition decreases renal histological changes in Ins2^{Akita} mice. PAS staining of kidney sections (*Figure 2*) showed increased glomerular cellularity and mesangial expansion (score: 0.8±0.2 vs. 0.4±0.2, $p<0.01$) at 17 weeks of age in vehicle-treated Ins2^{Akita} (Panel B) vs. control (Panel A), respectively. MCP-1/MCP-5 inhibitors (Panel C); but not Captopril (Panel D) treatment in Ins2^{Akita} mice significantly reduced glomerular changes (scores: 0.4±0.1, $p<0.05$ and 0.5±0.1); respectively compared to vehicle-treated Ins2^{Akita}.

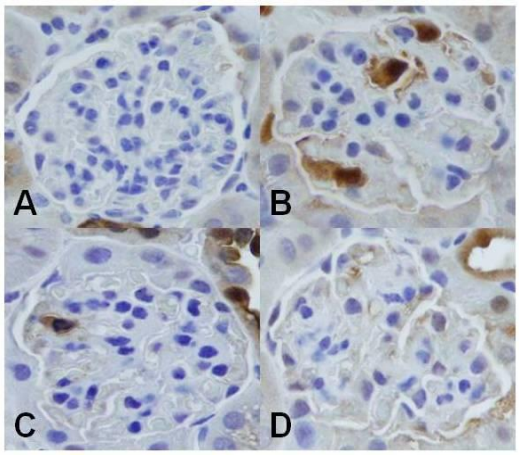

Figure 3 – Effects of a MCP-1/MCP-5 inhibitors on macrophage recruitment in Ins2^{Akita} mice. Immunohistochemical staining for Mac-2 positive macrophages in glomeruli at 17 wk of age. Images are representative of 4-8 mice in each group in control mice (A), Ins2^{Akita} mice treated with vehicle (B), and Ins2^{Akita} mice treated with MCP-1/MCP-5 inhibitors (C) or Captopril (D).

- MCP-1/MCP-5 inhibition decreases macrophage recruitment in Ins2^{Akita} mice. To determine whether MCP-1/MCP-5 is critical for kidney macrophage infiltration in DN, we show the distribution and quantitation of macrophages in kidneys by immunohistochemistry (Mac-2 positive macrophages) (*Figure 3*). The number of glomerular macrophages in control mice was low and increased significantly in vehicle-treated Ins2^{Akita} mice (0.17±0.01 and 2.4±0.25 macrophages/glomerulus; $p<0.0001$; respectively). Both MCP-1/MCP-5 inhibitors and captopril treatment in Ins2^{Akita} mice displayed significantly reduced glomerular macrophage recruitment (0.99±0.19 and 0.53±0.03 macrophages/glomerulus; $p<0.001$; respectively) compared to vehicle-treated Ins2^{Akita} mice; at 17 weeks of age.

Figures 1-3 suggest that MCP-1/MCP-5 inhibition could be a potential therapeutic lead for the management of diabetic renal complication.

- *What is the direct contribution of CCR2 expressed on endothelial cells to the progression of diabetic nephropathy (DN)?*

Our objective is to determine whether overexpression of CCR2 in endothelial cells *per se* is sufficient to induce renal damage *in vivo*. Toward this goal, we used male wild type (Mouse with intact CCR2; positive control), CCR2$^{-/-}$ (Mouse deficient of CCR2; negative control) and Tie2-CCR2/CCR2$^{-/-}$ (Transgenic mouse that overexpress CCR2 in endothelial cells in CCR2 deficient background) mice. Diabetes was induced with multiple low doses of streptozotocin (STZ; 50 mg/kg) for 5 days as recommended by the Animal Models of Diabetes Complications Consortium (AMDCC). Our data (*Figure 4*) show that diabetes increased renal injury in wild type mice (positive control) as expected by increasing the 24

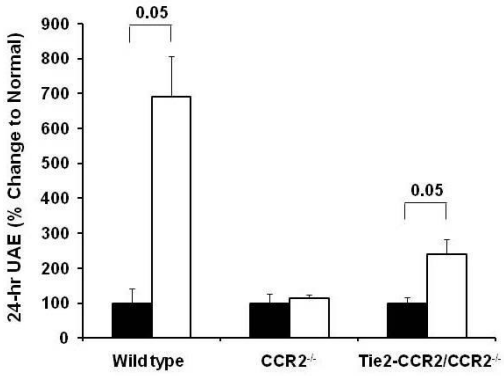

Figure 4 – CCR2 overexpression in endothelial cells deteriorates diabetic kidney injury. STZ was used to induce diabetes in wild type (positive control), CCR2$^{-/-}$ (negative control) and Tie2-CCR2/CCR2$^{-/-}$ mice. 24-hr urine was collected to determine urine albumin excretion (UAE) after 9 wks of diabetes. Data were presented as mean ± SEM. Filled bar: normal; open bar: diabetes.

hours urinary albumin excretion. Diabetic CCR2$^{-/-}$ mice (negative control) were protected as we described previously (Awad AS: Am J Physiol Renal Physiol. 2011 Dec;301(6):F1358-66). In contrast, diabetic Tie2-CCR2/CCR2$^{-/-}$ mice showed significant increase in 24 hours urinary albumin excretion as an indicator of renal injury. These data indicate that CCR2 expressed on endothelial cells might contribute to the progression of DN.

- *What is the direct contribution of CCR2 expressed on podocytes to the progression of DN?*
In this protocol, we generated Podocin-CCR2 mice (mice that overexpress CCR2 in podocytes). Our objective is to determine whether overexpression of CCR2 in podocytes per se is sufficient to induce renal damage *in vivo*.

- Generation of podocin2.5-CCR2 transgenic mice.
In order to create transgenic mice in which mouse CCR2 cDNA was controlled by human podocin promoter, mouse CCR2 full-length cDNA was cloned by PCR using a template provided by Thermo Scientific. Nco I was introduced to both ends of the cDNA. Human podocin promoter plasmid p2.5P-nlacF was a kind gift from Dr. L Holzman. The reporter gene was excised from p2.5P-nlacF and the mouse CCR2 full-length cDNA fragment was inserted. The mouse CCR2 cDNA was verified by DNA sequencing. The podocin2.5-CCR2 construct *(Figure 5)* was used for micro-injection to create podocin2.5-CCR2 transgenic mice. 6 founders were obtained from 105 pups through genotyping *(Figure 6)*.

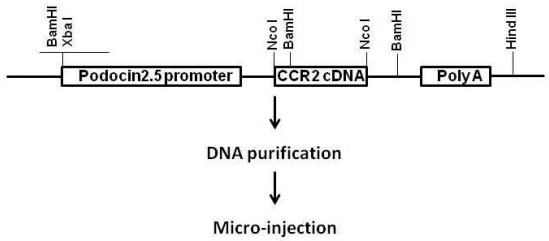

Figure 5 – Schematic of Podocin2.5-CCR2 construct and work flow for transgenic mice creation. The schematic drawing of podocin2.5-CCR2 construct was used to generate transgenic mice which contain mouse CCR2 cDNA under the control of human podocin promoter. Respective restriction sites were indicated. Purified DNA, which was released by Xba I and Hind III digestion, was used for micro-injection.

Results of these studies will provide a basis for the development of new therapeutic modalities in the development and progression of DN.

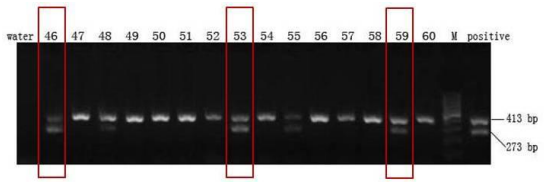

Figure 6 – Genotyping PCR. Pups genomic DNA was isolated and subjected to genotyping PCR. The 413 bp PCR product represents internal control β-actin. The 273 bp PCR product represents podocin2.5-CCR2 transgene. The podocin2.5-CCR2 construct was used as a positive control; and water was used as a negative control. Figure shows representative 3 podocin-CCR2 transgenic mice out of total 6 generated.

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):
1. Macrophages directly mediate diabetic renal injury	You H, Gao T, Cooper TK, Reeves WB, Awad AS.	Am J Physiol Renal Physiol.	March 2013	☐Submitted ☐Accepted X Published
2. Protective role of small pigment epithelium-derived factor (PEDF) peptide in diabetic renal injury	**Awad AS**, Gao T, Gvritishvili A, You H, Liu Y, Cooper TK, Reeves WB, Tombran-Tink J.	Am J Physiol Renal Physiol.	March 2013	☐Submitted ☐Accepted X Published
3. Arginase Inhibition Mediates Renal Tissue Protection in Diabetic Nephropathy via a Nitric Oxide Synthase 3-Dependent Mechanism	You H, Gao T, Cooper TK, Morris SM, Awad AS.	Kidney Int.	December 2012	☐Submitted ☐Accepted X Published
4. Management of Diabetic Nephropathy in Elderly: Special Considerations	Abdel-Rahman EM, Alhamad T, Reeves WB, Awad AS	J Nephrol Therapeut	October 2012	☐Submitted ☐Accepted X Published

5. Albuminuria in Diabetic Nephropathy	Tarek Alhamad, Emaad M. Abdel-Rahman and Alaa S. Awad	*Albuminuria:* Symptoms, Causes and Treatment Options. Nova Science Pub Inc; 1 edition (August 31, 2013). Book **chapter #4**	May 2013	☐Submitted ☐Accepted X Published
6. Albuminuria in the Elderly	Kevin Roe, Emaad M. Abdel-Rahman, Nasrollah Ghahramani and Alaa S. Awad	Albuminuria: Symptoms, Causes and Treatment Options. Nova Science Pub Inc; 1 edition (August 31, 2013). Book **chapter #10**	May 2013	☐Submitted ☐Accepted X 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:

We are breeding the transgenic Podocin-CCR2 mice (mice that overexpress CCR2 in podocytes). We will further determine the effect of CCR2 inhibition on transgenic mice that overexpress CCR2 in podocytes and endothelia cells. Once these studies are complete, a manuscript will be submitted. We will also submit a manuscript for the effect of MCP-1/MCP-5 inhibition on diabetic renal injury.

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.

It is too early to assess the possible clinical impact of these findings. We are encouraged by our ability to generate the transgenic Podocin-CCR2 mice (mice that overexpress CCR2 in podocytes) as well as the effect of MCP-1/MCP-5 inhibition in diabetic renal injury. Once

our remaining studies are complete, we will be able to determine the direct role of CCR2 and/or MCP-1/MCP-5 inhibition in diabetic nephropathy. A clinical trial will follow to assess the impact of our discovery for humans with diabetic renal complications.

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.

The major novel finding is our ability to generate the transgenic Podocin-CCR2 mice (mice that overexpress CCR2 in podocytes). These findings will allow us to directly determine the effect of CCR2 inhibition on podocytes expressing CCR2 receptors. Additionally, our data by using MCP-1/MCP-5 inhibition to ameliorate diabetic renal injury is novel. This data will have clinical impact and will lead to the development of new therapeutic strategies to manage diabetic renal complications.

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.")

a. Title of Invention:

b. Name of Inventor(s):

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

d. 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:

e. 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:

f. 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?_____

g. 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 ___X____ No _____

If yes, please describe your plans:

Once we fully characterize the transgenic Podocin-CCR2 mice (mice that overexpress CCR2 in podocytes), we will be able to file a patent for this discovery.

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.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Alaa S. Awad	Assistant Professor of Medicine and Cellular & Molecular Physiology
eRA COMMONS USER NAME (credential, e.g., agency login) AEA9UNIH	

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
Cairo University, Faculty of Medicine, Cairo, Egypt	M.D.	09/97	Medicine
University of Virginia, Charlottesville, VA	M.Sc.	12/08	Health Evaluation Science

A. Personal Statement

The overall goal of the proposed project is to understand the mechanisms of diabetic renal complications. Specifically, we are interested to study the role of chemokines and immune cells; specifically CCR2 in the setting of diabetic nephropathy. There is considerable evidence that CCR2 can contribute to the pathophysiologic consequences of renal tissue damage through macrophage recruitment. Currently, no information exists about the role of CCR2 or its ligands in established diabetic nephropathy or its effect on kidney derived cells, such as podocytes or glomerular endothelial cells. The applicant has over 12 years experience in rodent models of diabetes; specifically diabetic renal complications. Therefore, the proposed studies combine the applicant expertise in diabetic nephropathy with the environmental strength at the Penn State College of Medicine.

B. Positions and Honors.

March 1998 – Feb 1999	Intern in Medicine, Cairo University, Cairo, Egypt
March 1999 – Decr 2001	Resident of Pediatrics, Egypt
Jan 2002 – May 2004	Research Associate, University of Virginia, Charlottesville, VA. Department of Medicine
June 2004 – June 2005	Instructor in Medicine, University of Virginia, Charlottesville, VA. Department of Medicine, Division of Nephrology
July 2005 – August 2009	Assistant Professor of Research (non-tenure), University of Virginia, Charlottesville, VA. Department of Medicine, Division of Nephrology
Septr 2009 – June 2013	Assistant Professor of Medicine and Cellular & Molecular Physiology (Tenure-Track), Penn State University College of Medicine, Hershey, PA. Department of Medicine, Division of Nephrology
July 2013 – Present	Associate Professor of Medicine and Cellular & Molecular Physiology, Penn State University College of Medicine, Hershey, PA. Department of Medicine, Division of Nephrology

Honors:

- NIH Special Emphasis Panel/Scientific Review Group 2014/01 ZDK1 GRB-1 (J3) 1.
- Investigator (innovator) to Watch Award, Penn State College of Medicine, October 2013.
- Department of Medicine Junior Faculty Research Award, Penn State College of Medicine, June 2013.
- Northwestern University Feinberg School of Medicine Cardiovascular Junior Faculty Investigator Award, First Place Award, Chicago IL, 2009.
- American Society of Nephrology Travel Award, October 2008.
- Fellow of the American society of Nephrology (FASN), January 2006.
- Northwestern University Feinberg School of Medicine Cardiovascular Junior Faculty Investigator Award, First Place Award, Chicago IL, 2005.
- New Investigator Award, 57th Annual Fall Conference and Scientific Sessions of the Council for High Blood Pressure, American Heart Association. Washington DC, 2003.
- New Investigator Award, 56th Annual Fall Conference and Scientific Sessions of the Council for High Blood Pressure, American Heart Association. Orlando Fl, 2002.
- The United Nations International Conference on Population and Development (ICPD) award, 1994 in recognition of outstanding contribution to the organization and direction of the conference, Cairo, Egypt, 1994.

Patent Disclosures:

U.S. Patent #: 7,396,825 (Issue Date: 07/08/2008): Agonists of A2A Adenosine Receptors to Treat or Prevent Diabetic Nephropathy
U.S. Patent #: 13/469,731 (Issue Date: 05/13/2011): Treatment of Renal Injury

C. Selected Peer-reviewed Publications from total of 22 (in chronological order).

Most relevant to the current application

1. **Awad AS,** Webb RL, Carey RM, Siragy HM. Renal Nitric Oxide Production is Decreased in Diabetic Rats and Improved by AT1 Receptor Blockade. J Hypertens. 2004 Aug;22(8):1571-7. PMID: 15257181.
2. **Awad AS**, Webb RL, Carey RM, Siragy HM. Increased Renal Production of Angiotensin II and Thromboxane B2 in Conscious diabetic Rats. Am J Hypertens. 2005 Apr;18(4 Pt 1):544-8. PMID: 15831366.
3. **Awad AS,** Huang L, Ye H, Duong ET, Bolton WK, Linden J, Okusa MD. Adenosine A2A Receptor Activation Blocks Inflammation and Injury in Diabetic Nephropathy. Am J Physiol Renal Physiol. 2006 Apr;290(4):F828-37. PMID: 16332931.
4. **Awad AS**, Michael Rouse, Lixia Liu, Amy L. Vergis, Diane L. Rosin, Joel Linden, John Sedor and Mark D. Okusa. Activation of podocyte adenosine 2A receptors (A2ARs) preserves structural and functional integrity of podocytes. J Am Soc Nephrol. 2008 Jan;19(1):59-68. PMCID: PMC2391028.
5. **Awad AS,** Rouse M, Huang L, Vergis A, Reutershan J, Cathro HP, Linden J, Okusa MD. Compartmentalization of neutrophils in the kidney and lung following acute ischemic kidney injury. Kidney Int. 2009 Apr;75(7):689-98. PMCID: PMC2656389.
6. **Awad AS**, Rouse M, Khutsishvili K, Huang L, Bolton WK, Lynch KR, Okusa MD. Chronic Sphingosine 1-Phosphate 1 Receptor Activation Attenuates Early-Stage Diabetic Nephropathy Independent of Lymphocytes. Kidney Int. 2011 May;79(10):1090-8. PMID: 21289599; PMCID: PMC3155206.
7. Morris SM Jr, Gao T, Cooper TK, Kepka-Lenhart D, **Awad AS**. Arginase-2 Mediates Diabetic Renal Injury. Diabetes. 2011 Nov;60(11):3015-22. PMID: 21926276; PMCID: PMC3198072.

8. **Awad AS,** Kinsey GR, Khutsishvili K, Gao T, Bolton WK, Okusa MD. Monocyte/Macrophage Chemokine Receptor CCR2 Mediates Diabetic Renal Injury. Am J Physiol Renal Physiol. 2011 Dec;301(6):F1358-66. PMCID: PMC3233863.
9. Abdel-Rahman EM, Alhamad T, Reeves WB, **Awad AS**. Management of Diabetic Nephropathy in Elderly: Special Considerations. J Nephrol Therapeut 2012, 2:124. PMCID: PMC3760431.
10. You H, Gao T, Cooper TK, Morris SM, **Awad AS**. "Arginase Inhibition Mediates Renal Tissue Protection in Diabetic Nephropathy via a Nitric Oxide Synthase 3-Dependent Mechanism". Kidney Int. 2013 Dec;84(6):1189-97. PMCID: PMC3783645.
11. **Awad AS**, Gao T, Gvritishvili A, You H, Liu Y, Cooper TK, Reeves WB, Tombran-Tink J. "Protective role of small pigment epithelium-derived factor (PEDF) peptide in diabetic renal injury". Am J Physiol Renal Physiol. 2013 Sep 15;305(6):F891-900. PMCID: PMC3761290.
12. You H, Gao T, Cooper TK, Reeves WB, **Awad AS**. Macrophages directly mediate diabetic renal injury. Am J Physiol Renal Physiol. 2013 Dec;305(12):F1719-27. PMID: 24173355.

Research Support

Ongoing

Title: Role of Arginase in Diabetic Nephropathy
Principal Investigator: Alaa Awad **Period:** 07/15/2012 to 06/30/2017
Agency: National Institutes of Health
Type: R01 DK 094930
Summary: This study focuses on the role of arginases in diabetic nephropathy.

Title: Role of a Small Bioactive peptide of Pigment Epithelium-Derived Factor in Diabetic Nephropathy
Principal Investigator: Alaa Awad **Period:** 02/01/2013 to 01/31/2015
Agency: Novo Nordisk Diabetes and Obesity Science Biologics Science
Summary: The major roles of this project are to define the role of a small bioactive peptide of pigment epithelium-derived factor in diabetic nephropathy.

Pending

Title: Chemokines and Immune Cells in Diabetic Nephropathy
Principal Investigator: Alaa Awad
Agency: National Institutes of Health
Type: A1 R01DK093427
Summary: The major role of this project is to define chemokines and immune cells in diabetic nephropathy. (A0 Application was favorably reviewed and scored 33th Percentile).

Completed

Title: Podocyte Microenvironment in Diabetic Nephropathy
Principal Investigator: Alaa Awad **Period:** 09/01/2007 to 08/31/2013
Agency: National Institutes of Health
Type: K99/R00 DK077444
Summary: This study focuses on the role of macrophage and macrophage/podocyte interaction in diabetic nephropathy

Title: Evaluation of Anti-inflammatory Agents to Attribute Nephropathy Parameters in a Mouse Model of Diabetic Nephropathy
Principal Investigator: W. Brian Reeves
Co-Investigator: Alaa Awad **Period:** 09/14/2011 to 09/13/2012
Agency: Johnson & Johnson Pharmaceutical Research & Development, LLC
Summary: The major goals of this project are to define the role of inflammatory cytokines in diabetic nephropathy.

Title: Chemokines and Immune Cells in Diabetic Nephropathy
Principal Investigator: Alaa Awad **Period:** 04/10/2012 to 06/30/2014
Agency: PA Tobacco Settlement Fund (TSF).
Summary: The major goals of this project are to define the role of podocytes and endothelial cells expressing CCR2 in diabetic nephropathy.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
W. Brian Reeves, M.D.	Professor of Medicine
eRA COMMONS USER NAME WREEVES	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Pennsylvania State University, University Park, PA	B.S.	1977	Pre-Med
Jefferson Medical College, Philadelphia, PA	M.D., cum laude	1979	Medicine
University of Texas Medical School, Houston, TX	Resident	1979-1982	Internal Medicine
University of Texas Medical School, Houston, TX	Chief Resident	1982-1983	Internal Medicine
University of Texas Medical School, Houston, TX	Fellow	1983-1986	Nephrology

A. Personal Statement

I am a clinical nephrologist and scientist with a research interest in kidney injury. We have made significant contributions regarding the role of the innate immune system, including inflammatory and anti-inflammatory cytokines, TLR receptors and dendritic cells, to the pathogenesis of kidney disease. I am an active clinician and treat patients with kidney disease on an almost daily basis. Although most of my research pertains to acute kidney injury, many of the tools and concepts which we use are also relevant to chronic kidney disease such as diabetic nephropathy as described in Dr. Awad's proposal..

B. Academic Positions and Honors

Professional Experience:

1986-1988 Assistant Professor, Division of Nephrology, University of Texas Medical School at Houston

1988-1994 Assistant Professor, Division of Nephrology, University of Arkansas for Medical Sciences

1994-2000 Associate Professor, Division of Nephrology, University of Arkansas for Medical Sciences

1998-2000 Associate Professor, Department of Physiology and Biophysics, Univ. Arkansas for Med Sciences

2000-present Professor of Medicine, Chief, Division of Nephrology, Penn State College of Medicine

2005-present Vice Chair for Research, Department of Medicine, Penn State College of Medicine

Honors: Alpha Omega Alpha, Squibb-American Heart Association Clinician Scientist Award, 1985-1990, American Heart Association Established Investigator Award 1995-2000. Editorial Boards: Kidney International (1995-2003); Am. J. Physiology: Renal Physiology (1997-2001); ASN Grant Review Committee (2003-present); Ad hoc member PBKD study section 2007,2008,2011; Member NIH ZRG1 DKUS-K Study Section 2009, Member, NIH ZDK1 GRB-S Study Section 2010. Standing member PBKD study section (2012-present). Associate Editor, Kidney International (2012-present)

C. Selected Publications:

Most relevant to the current proposal

1. Ramesh, G., Reeves, W.B. TNF-α mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. *J. Clin. Invest*. 110: 835-842, 2002. PMID: 12235115
2. Zhang, B., Ramesh, G., Uematsu, S., Akira,S., Reeves, W. B. TLR4 signaling mediates inflammation and tissue injury in nephrotoxicity. *J Am Soc Nephrol*, 19:923-932, 2008. PMID: 18256356
3. Tadagavadi, R, Reeves, WB**.** Dendritic cells ameliorate nephrotoxic acute kidney injury. *J Am Soc Nephrol*. 21: 53-63, 2010. PMID: 19875815
4. Tadagavadi, RK, Reeves, WB. Endogenous IL-10 Attenuates Cisplatin Nephrotoxicity: Role of Dendritic Cells. *J Immunol* 185:4904-4911, 2010. PMID: 20844196
5. Lin, L, Bu, G, Mars, WM, Reeves, WB, Tanaka, S, Hu, K. tPA Activates LDL Receptor-Related Protein 1-Mediated Mitogenic Signaling Involving the p90RSK and GSK3{beta} Pathway. *Am J Pathol* 177: 1687-1696, 2010. PMID: 20724593
6. Gao, G., B. Zhang, G. Ramesh, D. Betterly, R. K. Tadagavadi, W. Wang and W. B. Reeves. TNF-alpha mediates increased susceptibility to ischemic AKI in diabetes. *Am J Physiol Renal Physi*ol 304(5): F515-521, 2013.
7. Awad, AS, T Gao, A Gvritishvili, H You, Y Liu, TK Cooper, WB Reeves and J Tombran-Tink. Protective Role of Small Pigment Epithelium Derived Factor (PEDF) Peptide in Diabetic Renal Injury *Am J Physiol Renal Physiol*, 2013, in press.

Additional recent papers of importance

1. Ramesh, G., Reeves, W. B. p38 MAPK inhibition ameliorates cisplatin nephrotoxicity in mice. *Am. J. Physiol: Renal Physiology*. 289:F166-F174, 2005. PMID: 15701814
2. Ramesh, G. Kimball, SR, Jefferson, LS, Reeves, WB. Endotoxin and cisplatin synergistically stimulate TNF-α production by renal epithelial cells. *Am. J. Physiol: Renal Physiology.* 292:F812-819, 2007. PMID: 17032936
3. Ramesh G, Zhang B, Uematsu S, Akira S, and Reeves WB. Endotoxin and cisplatin synergistically induce renal dysfunction and cytokine production in mice. *Am J Physiol: Renal Physiol* 293:F325-332, 2007. PMID: 17494092
4. Zhang, B., Ramesh, G., Norbury, C. C., Reeves, W. B. TNFα Produced By Renal Parenchymal Cells Mediates Cisplatin-Induced Nephrotoxicity, *Kidney International*, 72:37-44, 2007. PMID: 17396112
5. Bylander J, Li Q, Ramesh G, Zhang B, Reeves WB, and Bond, JS. Targeted disruption of the meprin metalloproteinase beta gene protects against renal ischemia/reperfusion injury in mice. Am. J Physiol: Renal Physiol. 294:F480-490, 2008. PMID: 18172000
6. Reeves WB, Kwon O, Ramesh G. Netrin-1 is an early biomarker of acute kidney injury. Am. J. Physiol: Renal Physiol. 294:F731-738, 2008. PMID: 18234954
7. Yura, RE, Bradley GS, Ramesh G. Antonetti, DA, Reeves WB and Bond JS. Meprin A Metalloprotease Modulates the Host Response to Lipopolysaccharide. Am. J. Physiol: Renal Physiol. 296:F135-F144, 2009. PMID:18971209
8. Wang, W, Reeves, WB, Pays, L, Mehlen, P, Ramesh, G: Netrin-1 overexpression protects kidney from ischemia reperfusion injury by suppressing apoptosis. *Am J Pathol,* 175:1010-8, 2009**.** PMID:19700747
9. Adalsteinsson V, Parajuli O, Kepics S, Gupta A, Reeves WB and Hahm J-i. Ultrasensitive Detection of Cytokines Enabled by Nanoscale ZnO Arrays. *Anal. Chem.* 80:6594-6601, 2008. PMID: 18681455
10. Wang, H, Malvadkar, N, Koytek, S, Bylander, J, Reeves, WB & Demirel, MC: Quantitative analysis of creatinine in urine by metalized nanostructured parylene. *Journal of Biomedical Optics* 15**:**027004-5, 2010.

C. Research Support

Ongoing Research Support

PHS RO1 DK081876 09/01/09-08/31/16
NIH/NIDDK
Epithelial/Dendritic Cell Cross-talk in Acute Kidney Injury
The main objective of this proposal is to determine the interactions between epithelial cells and dendritic cells which modulate the response to acute nephrotoxic insults.
Role: PI

PHS RO1 DK0880116 (Hahm) 09/01/10-6/30/15
NIH/NIDDK
ZnO Nanorod-based early biomarker screening for acute kidney injury
The main objective of this proposal is to develop ultra-sensitive assays for biomarkers of acute kidney injury and to validate certain novel biomarkers in a population of ICU-AKI patients.
Role: PI of subcontract from Georgetown University

PHS R25 DK078381 04/01/12-06/31/17
NIH/NIDDK
Preparing for research careers related to diabetes, digestive & kidney diseases (Step-up)
This proposal provides short-term experience and training in biomedical research for undergraduate students from underrepresented, and disadvantaged groups. The objectives of the proposal are that the students will (1) learn, through hand-on experience, how discovery research is conducted and the results are disseminated to others, (2) explore careers in biomedical research and become familiar with the requirements to pursue selected career options, (3) become proficient in using the Internet to acquire reliable information and to exchange ideas and information, and (4) learn about the prevention, cause and treatment of obesity, diabetes, and kidney/urologic diseases
Role: PI

PHS U01 DK082183 (Chinchilli) 09/01/08-06/30/18
NIH/NIDDK
Acute kidney injury natural history consortium
This project is to serve as the data coordinating center for the ASSESS-AKI consortium, a multicenter study of the natural history of acute kidney injury.
Role: Co-I

PHS U54 RR026071 (Sinoway) 06/01/11-5/31/16
NIH/NCCR
Penn State Clinical and Translational Science Institute
This CTSA proposal is to establish and support a Clinical and Translational Science Institute at Penn State.
Role: Co-leader of the Novel Methodologies Section.

Completed research support

Genzyme Renal Innovations Program (Miller) 07/01/09-6/30/12
Genzyme
TRPM2 channels in acute kidney injury
The main objective of this project is to determine the role of TRPM2 ion channels in the pathogenesis of ischemic acute kidney injury.
Role: Co-PI

No number 12/1/2011-11/30/2012
Johnson and Johnson
Evaluation of anti-inflammatory agents to attenuate nephropathy parameters in a mouse model of diabetic nephropathy
The purpose of this study is to evaluate the efficacy of anti-inflammatory agents (monoclonal antibodies) in a rodent model of diabetic nephropathy. This study will utilize aged D2.B6-Ins2^{Akita}/MatbJ mice, a recently characterized model of type 1 diabetes with impaired renal function.
Role: PI

PHS R01 DK063120 (Reeves) 4/01/04-3/31/11
NIH/NIDDK
Tumor necrosis factor in cisplatin nephrotoxicity
The main objective of this proposal is to determine the mechanisms whereby TNF production is increased by cisplatin and the mechanism whereby TNF produces acute renal failure.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Sarah K. Bronson	Associate Professor
eRA COMMONS USER NAME (credential, e.g., agency login) SBronson	

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
St. Olaf College, Northfield, MN	B.A.	1986	Biology
Washington University, St. Louis, MO	Ph.D.	1991	Immunology

A. Personal Statement

Dr. Bronson's research program has had a long-standing interest in manipulating the mouse genome via genetic alteration of mouse embryonic stem cells to study diseases of hematologic, immunologic, developmental, and metabolic importance. The program's most recent activity has been in using genetic models of diabetes to study diabetic retinopathy, specifically to query the role that epigenetic regulation of gene expression may play in persistent changes in retinal function in the context of diabetes, as well as to study interventions for diabetic stroke and nephropathy. Dr. Bronson has over 10 years of experience in rodent models of diabetes and 20 years of experience in generating and characterizing genetically altered rodents. Dr. Bronson has been the Director of the Penn State Hershey Diabetic Complications Animal Models Core since 2002. The Core supports streptozocin induced models of type I diabetes in rats and mice, the $Ins2^{Akita}$ spontaneous genetic model of type I diabetes, as well as the breeding and genotyping of a number of genetically altered lines of mice.

B. Positions and Appointments

1987- 1991 Ph.D. Thesis, Cloning of the Human Major Histocompatibility Complex in Yeast Artificial Chromosomes
1991- 1996 Postdoctoral Fellow with Oliver Smithies D.Phil., Excellence Professor of Pathology, University of North Carolina at Chapel Hill
1996 Instructor, Carolina Workshops, Gene Targeting in ES Cells & Transgenic Mice
1996-2005 Assistant Professor, Department of Cellular and Molecular Physiology, The Pennsylvania State University College of Medicine
2001-present Member, Executive Committee for the IGDP in Genetics, Hershey Campus
2005-present Associate Professor, Department of Cellular and Molecular Physiology, The Pennsylvania State University College of Medicine
2005-2009 Director, Transgenic Core, Section of Research Resources, The Pennsylvania State University College of Medicine
2006-2008 Co-Director (Hershey Campus), Huck Institute for Life Sciences IGDP in Genetics
2004-2013 Director, Summer Undergraduate Research Internship Program, The Pennsylvania State University College of Medicine
2010-present Co-Director Junior Faculty Development Program PSU-Hershey
2010-present Biomedical Sciences Graduate Program, Non-Option
2011-present Director, Cell & Molecular Biology Graduate Program
2012-present Director, Graduate Core Curriculum, Penn State College of Medicine
2013-present Director of Interdisciplinary Research, Penn State College of Medicine

Honors and Awards

1986	Departmental Distinction, Biology, St. Olaf College
1991	Spencer T. and Ann W. Olin Fellow, Washington University
1992-1995	Cancer Research Fund Damon, Runyon-Walter Winchell Foundation, Fellow
2005-2008	PSU College of Medicine GSA Excellence in Teaching
2009	Dean's Award for Excellence in Teaching PSU College of Medicine
2010	ASM/NIGMS Learning Interventions Institute
2010	Distinguished Educator Award, PSU College of Medicine
2011-2013	Judge, Developmental Biology and Genetics, ABRCMS

C. Selected Peer-Reviewed Publications (From a total of 37)

1. **Bronson, S.K.** and Smithies, O. (1994). Altering Mice by Homologous Recombination Using Embryonic Stem Cells. *J. Biol. Chem.* 269:27155-27158. **PMID: 7961620**
2. **Bronson, S.K.**, Reiter, C.E.N., and Gardner, T.W. (2003) An Eye on Insulin *J. Clin. Invest.* 111(12):1817-1819 **PMC161434**
3. Barber, A.J., Antonetti, D.A., Kern, T.S., Reiter, C.E.N., Soans, R.S., Krady, J.K., Levison, S.W., Gardner, T.W., and **Bronson, S.K.**, 2005, The Ins2^{Akita} mouse as a model of early retinal complications in diabetes, *Investigative Opthamology and Visual Science*, 46(6):2210-2218 **PMID: 15914643**
4. Antonetti, D.A. Barber, A.J., **Bronson, S.K.**, Freeman, W.M., Gardner, T.W., Jefferson, L.S., Kester, M., Kimball, S.R., Krady, J.K., LaNoue, K.F., Norbury, C.C., Quinn, P.G., Sandirasegarane, L., and Simpson, I.A., 2006, Diabetic Retinopathy: Seeing beyond glucose-induce microvascular disease *Diabetes*, 55:2401-2411 **PMID: 16936187**
5. She, P., Reid, T. M., **Bronson, S.K.**, Vary, T.C., Hajnal, A., Lynch, C.J., and Hutson, S.M. 2007 Disruption of BCATm in mice leads to increased energy expenditure associated with the activation of a futile protein turnover cycle, *Cell Metabolism*, 6(3):181-94 **NIHMS30078**
6. Hong, E-G.; Jung, D. Y.; Ko, H.J.; Zhang, Z.; Ma, Z.; Jun, J.; Kim, J.H.; Sumner, A.; Vary, T.; Gardner, T. W.; **Bronson, S**. ; Kim, J., 2007, Nonobese, Insulin-Deficient Ins2^{Akita} Mice Develop Type 2 Diabetes Phenotypes Including Insulin Resistance and Cardiac Remodeling, *Am. J. Physiol.,* 293(6):E1687-E1696 **PMID: 17911348**
7. Gastinger MJ, Kunselman AR, Conboy E, Bronson SK, Barber AJ. Dendrite remodeling and other abnormalities in the retinal ganglion cells of Ins2Akita diabetic mice. *Invest Ophthalmol Vis Sci*. 2008 Jun;49(6):2635-42 **PMID: 18515593**
8. Brucklacher, R. M., Patel, K.M., VanGuilder, H.D., Bixler, G.V, Barber, A.J., Antonetti, D.A., Lin, C.-M., LaNoue, K.F., Gardner, T.W., **Bronson, S.K**, and Freeman, W.M.. Whole genome assessment of the retinal response to diabetes reveals a progressive neurovascular inflammatory response. *BMC Med Genomics*. 2008 Jun 13;1(1):26 **PMC2442612**
9. Freeman, W.M., Bixler, G.V., Bruckacher, R.M., Lin, C.-M., Patel, K.M., VanGuilder, H.D., LaNoue, K., Barber, A.J., Antonetti, D. A., Gardner, T.W. and **Bronson, S. K.** 2010 A Multi-Step Validation Process of Biomarkers for Preclinical Drug Development. *Pharmacogenomics Journal* 10(5):385-95 **PMID: 19997081**
10. Freeman, W.M., Bixler, G.V., Brucklacher, R.M., Walsh, E., Kimball, S., Jefferson, L.S., and **Bronson, S.K.**, Transcriptomic 2009 Comparison in the Retina of Two Mouse Models of Diabetes *J. Ocular Biology, Diseases and Informatics* 2(4):202-213 **PMC2816812**
11. Fox TE, Bewley MC, Unrath KA, Pedersen MM, Anderson RE, Jung DY, Jefferson LS, Kim JK, **Bronson S.K.**, Flanagan JM, Kester M., 2010 Circulating sphingolipid biomarkers in models of type 1 diabetes, *J Lipid Res.* 52:509-517 **PMC3035687**

12. VanGuilder,H.D., Bixler, G., Kutzler, L., Brucklacher, R.M., **Bronson, S.K.**, Kimball, S.R., Freeman, W.F. 2011 Multi-modal proteomic analysis of retinal protein expression alterations in a rat model of diabetic retinopathy. *PLoS ONE*, 6(1):16271 **PMC3020973**
13. Bixler, G.V., VanGuilder, H.D., Brucklacher, R.M., Kimball, S.R., **Bronson, S.K**., Freeman, W.F. 2011 Chronic insulin treatment of diabetes does not fully normalize alterations in the retinal transcriptome. *BMC Med Genomics*, 4(1):40 **PMC3113923**
14. Dennis, M.D., Schrufer, T.L., **Bronson, S.K**., Kimball, S.R., and Jefferson, L.S. 2011 Hyperglycemia-induced O-GlcNAcylation and truncation of 4E-BP1 in the liver of a mouse model of type 1 diabetes. *J Biol Chem*, 286(39):34286-97 **PMC3190771**
15. **Bronson, S.K.**, Verderame, M.V., Keil, R.L. 2011 Graduate Education: A Case Study, *Cell* 147(6):1207-8 **PMID:22153063**