

## Response Form for the Final Performance Summary Report\*

1. Name of Grantee: The Pennsylvania State University
2. Year of Grant: 2008 Formula Grant

***A. For the overall grant, briefly describe your grant oversight process. How will you ensure that future health research grants and projects are completed and required reports (Annual Reports, Final Progress Reports, Audit Reports, etc.) are submitted to the Department in accordance with Grant Agreements? If any of the research projects contained in the grant received an “unfavorable” rating, please describe how you will ensure the Principal Investigator is more closely monitored (or not funded) when conducting future formula funded health research.***

Sheila L. Vrana, PhD, Interim Associate Vice President for Health Science Research and Interim Vice Dean for Research and Graduate Studies, College of Medicine assumed overall responsibility for the Commonwealth Universal Research Enhancement Program at Penn State Hershey on August 1, 2014. Under her leadership, Ernest W. Johnson, PhD, Research Development Officer, has oversight responsibility for the CURE formula funds program in the College of Medicine. This includes responsibility for 1) ensuring that research proposals are peer reviewed by the College of Medicine Scientific Review Committee or other appropriate bodies prior to the submission of Strategic Plans to the Department of Health; 2) requiring the financial review of project budgets by either the Clinical Trials Office or the Office of Research Affairs to ensure that the funding requested will be sufficient to complete the proposed research projects; 3) monitoring currently funded projects to ensure that appropriate progress is being made and that any significant changes in the proposed research projects are reported to the Department in a timely manner; and 4) reviewing draft Annual and Final Progress Reports to ensure that they provide adequate detail regarding the progress that has been made, including citations of all publications resulting from each project.

At Penn State’s University Park campus, Peter Hudson, PhD, Director of the Huck Institutes of the Life Sciences provides oversight for projects supported by CURE formula funds. In addition, John Anthony, Penn State’s Tobacco Fund Manager continues to administer the University’s entire portfolio of CURE formula funded projects, including the financial management of all CURE formula fund awards.

It is anticipated that going forward, the review processes that are now in place will ensure that the projects submitted by the institution will be evaluated by the Department in the Favorable-to-Outstanding range. However, if a Final Progress Report should receive an Unfavorable evaluation, the Principal Investigator will not be eligible to receive additional CURE formula funds without fully addressing the deficiencies in their previous project to the satisfaction of the institution’s leadership.

\* Please note that grantees’ Final Performance Summary Reports, Response Forms, and Final Progress Reports *will be made publicly available on the CURE Program’s Web site.*

We would like to express our continued appreciation to the Legislature and the Department of Health for their ongoing support of the Commonwealth Universal Research Enhancement (CURE) Program. The funding that Penn State has received from the CURE Program has enabled us to develop new strategic research initiatives, attract new investigators, and advance the frontiers of knowledge in areas that will ultimately help to improve the health of people throughout the Commonwealth and beyond. With CURE Program funding, Penn State has been able to construct state of the art laboratories, establish new research core facilities, and acquire the latest research instrumentation. It has also enabled the University to recruit and support world-renowned investigators who have brought substantial new research funding to the Commonwealth. These and other initiatives are enabling Penn State investigators to make significant contributions to the development of new knowledge that will advance the promotion of good health and the improved diagnosis, treatment, cure and prevention of disease. Collectively, these investments in our research enterprise are providing Penn State with the resources that it will need to remain a leader at the cutting edge of health and biomedical research in the 21<sup>st</sup> century.

**Project Number:** 0864501

**Project Title:** Vitamin D and Crohn's Disease: From the Bench to the Bedside

**Investigator:** Cantorna, Margherita

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*B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.*

## **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

### Reviewer 1:

1. The only significant weaknesses associated with this project were the inability of the PI's to recruit the proposed number of patients into the study, and the relatively narrow scope of the data accrued.

**Recommendation:** The clinical faculty (Dr. Smith) was recruited to the study due to her experience with clinical trials relating to Crohn's disease. It is surprising that she did not anticipate the difficulties regarding recruitment of patients into the study. While this reviewer is not suggesting a particular mechanism to avoid the issue going forward, it would be prudent to ensure somehow that all co-investigators participate in their sections of grant proposal development.

**Response:** We agree that recruitment of participants was a problem and we have decided that in the future we would work with clinical faculty in other areas that have access and a track record of recruiting the numbers of patients we would need. All of the co-investigators did participate in the development of this grant proposal.

### Reviewer 2:

Recruiting subjects is a very difficult task and is time-consuming. If the investigators perform another study, it would be important to take what was learned from this pilot study with regard to recruiting so that they can reach the target subject recruitment. Not getting enough subjects hurts the statistical power of the study and lowers the impact, as one assumes that the investigators are well aware.

**Response:** We plan to collaborate in the future with other institutions/hospitals to make sure we have access to larger Crohn's patient pools.

### Reviewer 3:

1. Weakness: Small sample size. Limited number of patients participated in the trial. Changes of exclusion criteria in patient recruitment.

**Recommendation:** Increase patient numbers by collaboration with other institutions/hospitals and with more investigators and physicians who have larger IBD patient pools.

Response: We plan to collaborate as suggested in the future to increase the patient pool. However collaborating with other institutions would increase the cost of the study.

2. Weakness: Lack of a placebo control arm in the trial.

Recommendation: Increase sample size and include a placebo arm in the trial. This can be achieved through collaboration with other institutions/investigators. Perform a randomized double-blind placebo control trial.

Response: We agree that a bigger and more expensive trial should and would include a randomized double-blind placebo control design.

3. Weakness: The outcome does not support the hypothesis.

Recommendation: Modify the hypothesis and consider the impact of vitamin D on the mucosal epithelial barrier and luminal microbiota.

Response: We respectfully disagree that the outcome did not support the hypothesis. However we do think that investigating additional possible benefits (mechanisms) of vitamin D on epithelial barrier and gut microbiota would be worthwhile in future studies.

4. Weakness: Lack of mechanistic insight to support the outcome.

Recommendation: Collect colonic biopsies from the patients for studies. Perform histological evaluations. Perform studies to assess changes in colonic mucosal permeability. Instead of measuring serum inflammatory markers, evaluate colonic immune cell infiltration and immune activities.

Response: We agree that future and more expensive studies should include experiments to probe mechanism including colon biopsies and other measurements.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received a Favorable rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response:

The reviewers made some useful comments for the design of a future larger study to follow up our promising preliminary study. We are grateful to the state of Pennsylvania for making this CURE funding available.

**Project Number:** 0864502  
**Project Title:** Epigenetic Regulation of Inactive X Chromosome Expression  
**Investigator:** Carrel, Laura

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

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## **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

### Reviewer 1:

A considerable amount of public data has been produced, in large part by NIH-funded consortia, that are potentially relevant to the questions addressed in this project. It would be nice to see the PI interact with other groups that have relevant data on X-linked expression to improve her models of X-linked genes expression. At the very least, the PI could investigate the availability of relevant RNA-seq and epigenetic data in public databases such as dbGAP. This would allow the PI to bring in additional high-quality data at low or no cost to leverage the data generated here.

*Response:* We agree with the reviewers' comments and recommendations regarding evaluation of additional public datasets (reviewer 1) and analysis of an expanded number of CpG sites (reviewers 2 and 3) are on target and extremely important. Most recently we have established a collaboration to score inactive modifications. Such an approach, however, is most powerful when coupled with expression data. Unfortunately, the mosaic nature of X inactivation complicates and severely limits the analysis of RNA-seq from female samples queried in the public databases (and emphasizes the uniqueness of the lines isolated for our studies). Efforts to evaluate similar data in an allelic manner have recently been published (Cotton, et al, (2013) Genome Biology 14:R122). This elegant study did establish methods to include samples with partially skewed inactivation, but still excluded randomly inactivated samples. Nevertheless, we agree that available data may help us to extend our conclusions. Whole chromosome analysis of the lines we have isolated (as suggested by reviewers 2 and 3) is the next step that we hope to receive funding for in the near future.

### Reviewer 2:

In Specific Aim 2, the team chose to do pyrosequencing to assay CpG island methylation. This methodology is accurate, but limited with the number of CpG sites to be analyzed. In addition, the PI and her student found that most of CpGs are hypomethylated, raising a concern about how

the current methylation data are useful toward their conclusion that DNA methylation is not correlated with gene expression for those X-linked genes the investigators analyzed. A more global and comprehensive DNA methylation analysis via bisulfite sequencing would be needed to make a strong statement relevant to Aim 2.

Response: See above.

Reviewer 3:

1. The examination of alternate CpG sites would have allowed for a more comprehensive interpretation of the relationship between DNA methylation and gene expression.

Response: See above.

2. No manuscripts have been published from this project. Apparently, a methods paper was submitted and should be publishable.

Response: We have chosen to include the methods in an expanded manuscript that includes these data and will be submitted shortly.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received a Favorable rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response:

**Project Number:** 0864503  
**Project Title:** Role of UGT2B7 Genotype in Patient Response to Tamoxifen  
**Investigator:** Cream, Leah

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

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### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

The grant has ended and nothing else is planned for the future.

Response: We decided that pursuing this avenue further would not advance the field due to our negative findings.

Reviewer 2:

1. Weakness: Despite the fact that the objectives were met, the hypothesis that UGT2B7 SNPs impact upon hot flashes and tamoxifen metabolism seems to be rejected. However, it is not completely clear if the study was appropriately powered to adequately address this hypothesis.

Recommendation: Additional information could have been provided to clearly indicate the number of successful hot flash diaries that were collected and the numbers of patients falling into each of the genotype categories. This information could have been discussed in the context of suitability of fifty patients to adequately assess the objectives.

Response: The polymorphism rate was much lower than expected so we would have needed to substantially increase the number of study subjects.

2. Weakness: Future plans for this research project are on hold due to the fact that Dr. Lazarus left Penn State University and is currently at Washington State University. Dr. Cream currently does not have a basic science collaborator to continue this work.

Recommendation: Results of this study should be used as preliminary data for an NIH/NCI R21 application. If funded, it would be possible to continue the collaboration with Dr.

Lazarus to perform future genotyping studies to understand the role of UGTs in tamoxifen metabolism and patient response.

Response: Although we agree that the project could continue, we were discouraged by the negative data and didn't think that further studies would be the best use of resources.

Reviewer 3:

1. The study could have been planned based on more than one genotype of UGT and/or other target DME, considering that the co-Investigator, Dr. Lazarus, had been funded on the basic aspects of this research in the preceding 3-year period.

Response: Unfortunately, we had laboratory constraints that prohibited investigating other UGTs simultaneously. However, Dr. Lazarus still has the samples and they could be used to look at other UGTs.

2. The number of patients proposed could have been higher than 50 to arrive at a more conclusive evidence of lack of any influence of UGT<sup>268Tyr</sup> genotype.

Response: We considered expanding the cohort but decided this was not the most efficient use of resources. Obtaining 50 patients was much more time consuming and difficult than we had anticipated.

3. Although the project did not yield data consistent with the hypothesis, the patient diversity/ethnicity information planned in the study could have been generated.

Response: We did not have much ethnic diversity in the patient population.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received a Favorable rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response:

**Project Number:** 0864504  
**Project Title:** Functional Brain Imaging of Memory and  
Language for Epilepsy Surgery  
**Investigator:** Eslinger, Paul

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

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## **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

### Reviewer 1:

1. To ensure the completion and success of this project and to increase the data in patient groups, we recommend increasing the clinical co-investigators' efforts to improve the coordination between the research team and the patients before and after surgery, the clinical setting for acquiring clinical data and insurance related issues.

Response: We agree and have recruited 2 clinicians in the Epilepsy Program as co-investigators who are meeting together in multidisciplinary patient care conferences twice a month.

2. We also recommend that patient recruitment be expanded to other hospitals if there are other problems in the Epilepsy Center such as faculty member departure or not enough clinical physicians available to participate in the project.

Response: We have carefully considered this possibility. The Milton S. Hershey Medical Center remains the main surgical epilepsy center for the Central PA region and has the greatest number of patients for this research. With the new clinical faculty who have been recruited as co-investigators, we anticipate fully completing the study in a timely fashion.

3. Weakness: Ten patients were proposed to be studied in pre- and post-surgery. Only six patients have been recruited and received pre-surgical studies, two of the six patients completed the post-surgical studies. There is only one patient's post-surgical data that was reported. The data seems promising and supportive to the project goal. Unfortunately, it will be difficult to conclude that this project reached the goal with only one patient's pre-surgical and post-surgical results.

Recommendation: A better plan and coordination between the clinical team with patients is strongly needed. There are three clinical co-investigators listed in the final report who were reported to have spent only 1% effort on this project. We suggest increasing the support of the clinical co-investigators to increase their efforts and therefore to achieve the goal in patient studies.

Response: The coordination between clinical and research investigators has improved and the remaining patients are being recruited for study and completion of data analysis.

Reviewer 2:

1. As I detail at length above, I question some of the theoretical/methodological bases underpinning the specific aims of the project. I would recommend that the team discuss the plan with other researchers in the epilepsy community, who are doing similar work, to invite constructive critique and discussion.

Response: We appreciate the suggestions for expanding the research protocol and data analysis. The rationale and design of the study have been well-received by colleagues and preliminary results have been quite exciting. Based on this reviewer's suggestions, we plan to expand the data analysis to include comparison of fMRI results to anatomical measures of medial temporal sclerosis and neurocognitive testing of learning and memory functions.

2. It sounds as though recruitment of subjects was quite difficult. In addition, controls appear to have been healthcare workers; it is not clear whether they were age- and gender-matched with clinical subjects. Recruitment for projects like this can be very tricky as they depend on draw to a surgical program. The researchers themselves mention collaboration with another center and this is an excellent idea; it can be another major epilepsy program or a smaller community program, many options exist.

Response: Recruitment of research subjects has now improved with expansion of the surgical epilepsy program, clinical co-investigators, and regular patient care conference discussions.

3. Data analysis should be completed. As of the final progress report, it had not progressed significantly beyond the FY state. It is not to the point where it would hold up to peer review (e.g., the case shown where "The results of the Wada test were ambiguous" but the fMRI was concordant is not at all clear. If IAP was ambiguous, how can it also be concordant?). Also, for the case shown in Figures 9 and 10: Is there a way of showing a subtraction image so the reader can evaluate the pre-postop changes?

Response: Data analysis is ongoing and scheduled for completion when the final research subjects are followed through to post-operative status. IAP results can be ambiguous when they do not show clear lateralization of memory capacity. In the case described, the fMRI

results also did not show clear lateralization of activity as typically expected. Hence, the results of the 2 procedures were concordant, strengthening the potential validity of the fMRI results. Contrast images can be shown to display pre-post op results and will be included in final data analysis.

Reviewer 3:

It would be nice to see a full peer-reviewed journal article from these results, once the target enrollment number is reached and outcome data are available.

Response: We agree. That is our plan for completion and distribution of the results.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received a Favorable rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response: The reviews were very helpful in refocussing our efforts in completing this research and sharpening the data analyses. We look forward to publishing the findings and sharing with colleagues how to further improve the surgical treatment of patients with intractable epilepsy.

**Project Number:** 0864505  
**Project Title:** Molecular Targets for Preventing Loss of Skeletal Muscle Mass  
**Investigator:** Jefferson, Leonard

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

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#### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

None.

Reviewer 2:

None.

Reviewer 3:

None.

*No Responses Required.*

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received an Outstanding rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response: We would like to thank the State Legislature and the Pennsylvania Department of Health for their support of our research, and we thank the reviewers for their review of our report. We especially appreciate their positive, supportive comments and the “Outstanding” score.

**Project Number:** 0864506  
**Project Title:** Research Infrastructure - Biological Research Laboratory Construction  
**Investigator:** Kennett, Mary

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### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

None.

Reviewer 2:

Develop an outreach system for external investigators to gain appropriate access to BSL3 facility.

Response:

Due to the sensitive nature of the research and need for security, external investigators will not be granted access to the BSL-3 facility. However collaborative research with external partners is encouraged, as long as a Penn State faculty member, who is trained and cleared to use the facility is willing to oversee the project. In addition the BSL-3 facility manager is highly experienced in contract research, and external investigators may request that specific projects be performed via this route. Any external inquiries are routed through the manager of the facility, the Scientific Director of the Lab, and the Program Director to determine the most effective means of accomplishing external projects.

Reviewer 3:

The strategic plan should have more unambiguously stated the specific activities performed under this formula grant funding.

Response:

The specific activity of this project as stated in the strategic plan is as follows: "The scope of this project is to design and build an animal biosafety level three (ABSL-3) research laboratory for the study of immunology and infectious diseases requiring high level Biocontainment." The specific plan goes on to provide details of the building components and the type of research to be performed. Although the scope of the project increased due to leveraged additional funding, the

basic building components and research purpose did not change. The lab was built as planned and is operational.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received an Outstanding rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response: The investigator thanks the reviewers for their time and thoughtful reviews. The lab has recently been the recipient of two awards: The American Institute of Architecture New England 2013 Honor Award for Excellence in Architecture, Payette, and the R&D 2014 Laboratory of the Year, High Honors Award.

We would like to express our appreciation to the Pennsylvania Department of Health and the State Legislature for their support of our research through the Commonwealth Universal Research Enhancement (CURE) program

**Project Number:** 0864507  
**Project Title:** Regulation of Nutrient Sensing and Muscle Wasting by Alcohol  
**Investigator:** Lang, Charles

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

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### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

The central hypothesis was not tested.

Response: It is true that the central hypothesis was not tested. The original data generated indicated that our original hypothesis was not valid and therefore we did not extend our initial findings to the alcohol model. We believe that was an appropriate way to proceed, considering also that the funding for this project was to support a student for a period of 1 year.

Reviewer 2:

1. The lab has no weakness as far as technical expertise, hypotheses generation, and productivity are concerned. However, the effects of leucine on protein synthesis in alcoholic muscle would be of great interest to the alcohol research community. These experiments may be a part of the MERIT grant that was procured with a portion of the results from this project.

Response: We agree with the reviewer and indeed a detailed mechanistic investigation into the molecular etiology for alcohol-induced leucine resistance is part of my MERIT award. Again, the funding obtained was to support a student for a period of 1 year and it was simply not possible for him to perform all of the desired studies in this time frame.

2. The role of PRAS40 on the myoblast cell cycle should be encouraged (without the grander implications of alcohol-induced effects). Particularly, the observed effects of PRAS40 knockdown on increased myoblast diameter and blunted fusion rates in the absence of increased protein synthesis are intriguing.

Response: Based on our published data from this research, we no longer believe that

alcohol-induced changes in PRAS40 are causally related to the decrease in muscle protein synthesis. While continuing this line of research for basic knowledge is a laudable goal, we believe we have more interesting targets available for investigation which may lead to therapeutic modalities.

Reviewer 3:

1. There appears to be a disconnect between the proposal and what was accomplished. Only part of Experiment 1c appears to have been conducted on this project. It seems that the PDOH funding was used as supplemental to the NIH funding that was reduced, but the application/strategic plan does not mention this and the final report is vague on this point. If, in fact, the funding was only to be used to fund a graduate student, this should be explicitly stated in the application document and the final report. Aims/experiments are not part of the PDOH funding should not be included in the application or the results from such aims/experiments should be included in the final report.

Response: One can certainly understand the confusion on the part of the reviewers because the proposal does contain some aims/experiments that were not completed. However, it must be re-emphasized that the award was for a single year and only supported the stipend of the student for that period. To expect more from the student in that time frame would have been unrealistic.

2. As per weakness 1: No effects of nutrient (leucine) or alcohol (Experiments 1a-c) appear to have been examined and no results on these are reported in the final report. Obviously, the aims laid out in the proposal could not be accomplished with the amount of funding obtained through this grant. In the final report, a better explanation is needed as to the circumstance for awarding PDOH funds for this project and what the expected outcomes directly related to that funding were.

Response: As noted in response to question #1 above, we acknowledge that the student was not able to complete all of the proposed experiments within the one year funding period. However, we believe that the student was productive during this period and we were pleased with the results that he obtained.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received a Favorable rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response:

**Project Number:** 0864508  
**Project Title:** Murine Induced Pluripotent Stem Cells:  
Differentiation and Bone Formation  
**Investigator:** Niyibizi, Christopher

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

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### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

None.

Reviewer 2:

None.

Reviewer 3:

1. The quality of publications resulted from this project and the number of students involved in this project are considered weaknesses. The paper published in BBRC is not directly related to the project objectives.

Response: Some of the funds supported related work published in BBRC and therefore we acknowledged the support of Tobacco Cure. In addition, we published a manuscript partly resulting from the proposed studies in another journal (*“Li Feng, Bronson Sarah and Niyibizi Christopher, Derivation of murine induced pluripotent stem cells (iPS) and assessment of their differentiation toward osteogenic lineage. J. Cell Biochem. 2010*). The data also established a foundation that led to an additional publication demonstrating bone formation by iPSC- derived cells in vivo (*Li Feng, Niyibizi Christopher, Cells derived from murine induced pluripotent stem cells (iPSC) by treatment with members of TGF-beta family give rise to osteoblasts differentiation and form bone in vivo, BMC Biol. 2012*). The number of students was limited due to the limited amount of funding that was allocated to us for the project.

2. The researchers showed that they had proposed to direct iPSC derived MSCs into cartilage cells in the original research proposal, but were not successful in directing the cells to cartilage formation. There were no data or information in their progress reports or publications regarding the proposed bone formation in vivo of the original research plan.

Response: Yes, we were not successful in directing the cells to cartilage formation and because

of the limited funding, we focused on in vitro studies which laid down a foundation for future studies which were funded by NIH. From the NIH funded grant, we were able to show that iPSCs derived cells make bone in vivo (*Li Feng, Niyibizi Christopher, Cells derived from murine induced pluripotent stem cells (iPSC) by treatment with members of TGF-beta family give rise to osteoblasts differentiation and form bone in vivo, BMC Biol. 2012*).

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received an Outstanding rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response: The Tobacco CURE program funding was instrumental in obtaining preliminary data that enabled us to compete successfully for NIH funding to continue and expand the studies. We would like to thank the Commonwealth and the Department of Health for providing the CURE funds and the reviewers for their comments and the “Outstanding” evaluation.

**Project Number:** 0864509

**Project Title:** Evaluation of mTOR as a Chemoprevention Target in Skin Cancer

**Investigator:** Shantz, Lisa

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## **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

### Reviewer 1:

None.

### Reviewer 2:

1. Weakness: While understanding and elaborating the role of mTOR signaling in proliferation and apoptosis of epidermal keratinocytes may be scientifically interesting it falls short of the overall goal of exploiting mTOR signal modulation for prevention of non-melanoma skin cancers.

Recommendation: A more directed approach to quickly establish the involvement of mTOR in the development of non-melanoma skin cancer and then screening for agents that prevent UV-induced carcinogenesis will have higher significance and better impact.

Response: We have a current collaboration (not supported by CURE funds) that is designed to identify chemotherapeutic agents that act synergistically with mTOR inhibition in cancers with activated AKT signaling, including UVB-induced skin cancer.

2. Weakness: No collaborations planned with researchers either within or outside the PI's institution.

Recommendation: Continued collaboration with Dr. DiGiovanni and other researchers and clinicians (especially dermatologists) may help better focus the research in the direction of relevance to human skin cancers.

Response: We have an ongoing collaboration with Dr. DiGiovanni, and are exploring the possibility of collaborations within the Dermatology Department at Penn State Hershey.

Reviewer 3:

1. There is no current long-term NIH funding in place. Given the amount of preliminary data generated by this proposal, long-term additional funding should be attained.

Response: We currently have an R01 application pending and are also planning an R21 application using preliminary data generated by these CURE-funded studies.

2. The single manuscript is not in a high-tier journal. Additional preliminary data proposing a mechanism of mTOR action might provide more novelty to the findings and allow for a second higher-impact publication.

Response: We have a manuscript in preparation that expands on the CURE-funded work to include more mechanistic data.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received a Favorable rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response: We very much appreciate the support that we received for this project from the Commonwealth Universal Research Enhancement (CURE) program of the Pennsylvania Department of Health

**Project Number:** 0864510

**Project Title:** IRES-mediated Synthesis of Proteins Integral to Adaptation to Hyperoxia

**Investigator:** Shenberger, Jeffrey

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.)

## **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

### Reviewer 1:

1. A cell system that mimics redox stress likely to take place in clinically-relevant conditions should be chosen, and preferably using cells that typically encounter that degree of oxidative stress.

Response: Although we would agree with the reviewer's suggestion, studying translation in native type II cells or type I cells is problematic for several reasons, including the fact that type II cells in culture differentiate into type I cells very rapidly and Type I cells do not proliferate.

2. Using a system that assesses effects on native translation rather than relying on reporter constructs will be necessary to fully test the hypothesis.

Response: This was previously done by our group. In these studies, we utilized reporter constructs because they allow the differentiation of translational mechanisms that cannot be ascertained in native cells.

### Reviewer 2:

1. Failure to significantly pursue the described objectives: the study of GADD45 $\alpha$  and p53 mRNAs in human lung epithelial A549 cells was ultimately replaced with different target sequences and different cell types. The rationale for these changes for p53 and A549 cells was appropriate and adequately explained (although the fate of GADD45 $\alpha$  was not addressed), and the flexibility of this project can be viewed as a strength. However, these shortcomings could have been more productively identified in preliminary experiments, which would have allowed the project to focus earlier on proteins such as BiP that display the expected oxygen-dependent responses. Thus, a general recommendation would be to require

additional justification, rationale, and preliminary data for specific proposed experimental protocols.

Response: We agree with the reviewer's comments.

2. These findings should be submitted for publication, without delay, to support successful application for further funding.

Response: We are currently in the process of doing that.

3. Rationale for the suitability and relevance (physiological or disease) of non-lung cell lines and BiP should be clarified.

Response: Non-lung cell types were not the primary objective of these studies. The use of those cells was necessitated by poor transfection efficiency of the lung cell lines. BiP is a translationally regulated protein that contains a putative IRES, though this has recently been challenged. It is part of the integrative stress response and unfolded stress response and has been documented to have increased expression at the protein level in the hyperoxia-exposed lung.

Reviewer 3:

1. Cap-independent GADD45A translation was reported in As (+3) treated HEAS-2B by Fei Chen's group (Ref #7 in the original proposal), who had already provided the related dicistronic reporter constructs to the PI. Therefore, studies of IRES-mediated protein translation of GADD45A in HEAS-2B cells in response to hyperoxia using dicistronic reporter assay may be promising.

Response: We agree and believe that this would be an important avenue to pursue.

2. Studies on BiP cap-independent translation in A549 and BEAS-2B cells are preferred to HeLa and HEK293 cells, since the originally proposed project was aiming to study hyperoxia-induced protein synthesis in respiratory epithelial cells.

Response: Non-lung cell types were not the primary objective of these studies. The use of those cells was necessitated by poor transfection efficiency of the lung cell lines. BiP is a translationally regulated protein that contains a putative IRES, though this has recently been challenged. It is part of the integrative stress response and unfolded stress response and has been documented to have increased expression at the protein level in the hyperoxia-exposed lung.

3. Measurements for changes of endogenous p53, GADD45A, and even BiP transcription and translation upon hyperoxia challenge in respiratory cells are important. Genes with reduced mRNA and increased protein syntheses are then selected for further 5-UTR analyses. The PI should perform these experiments first as described in their original proposal.

Response: We agree with the reviewer's comment.

4. It appeared that the major concern to switch different cell lines was so called “low transfection efficiency (20-30%) of A549”. What was the efficiency for HEK293 or HeLa cell transfection? Does the difference between these cell transfection efficiencies significantly affect the sensitivity of the Luc-reporter assay? The PI should perform experiments using a positive control construct (pRL-HCV-FL) to verify this before switching to other cells.

Response: The transfection efficiency of HEK293 and HeLa cells was > 80%. With low transfection efficiency, the readout is often below detection. Ramping up the cell number can offset this aspect, but the use of increased reagents becomes very expensive. Positive control constructs were used for each transfection experiment.

5. Data from the dicistronic reporter assay for p53 in A549 may just suggest no changes in IRES-mediated p53 translation upon O<sub>2</sub> treatment. It will be worthy to study 5'-UTR of GADD45A or BiP in A549 cells if their endogenous protein translation is increased upon high concentration O<sub>2</sub> exposure.

Response: This is a valid point which deserves exploration.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received a Favorable rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response : We appreciated the support that this project received from the Commonwealth Universal Research Enhancement (CURE) program sponsored by the Pennsylvania Department of Health.

**Project Number:** 0864511  
**Project Title:** Stroke Recovery in Type II Diabetes  
**Investigator:** Simpson, Ian

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.)

## **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

### Reviewer 1:

The unexpected results should be published/reported so that other investigators can benefit from this experience. There are no plans to do so.

Response: Initially there was no vehicle that provided an opportunity to publish negative data. However, we recently became aware that the Journal of Cerebral Blood Flow and Metabolism does provide such an opportunity and we will inquire whether such data fits their criteria.

### Reviewer 2:

1. In the future, the investigators need to think more carefully about the underlying pathophysiology of animal models of diabetes and how these correlate with human disease. Both the ob and db models in mice mimic exceedingly rare human diseases (leptin deficiency and leptin receptor absence respectively) and are not reflective of the usual type 2 diabetes patient. Better models include diet-induced insulin resistance in the NON model, which the authors will now be using.

Response: Up until very recently, the only models for type II diabetes were the *ob/ob* and *db/db* mice and we were acutely aware of the many deficiencies in these mouse strains. However, despite these limitations, the vast majority of publications reporting outcomes associated with type II diabetes have employed these mice. The NON mice are much better and we hope to submit a paper shortly in which we will describe their use in the study of Stroke. Interestingly, Metformin will normalize blood glucose but it did not improve stroke outcome.

2. Darglitazone was a strange choice for a TZD given that it is not clinically available. The cardiac toxicity seen with this agent may have been a general TZD effect or it may have been specific to this molecule. Pioglitazone would have been a better choice.

Response: Our colleagues at Pfizer had recommended using Darglitazone as it has a much greater sensitivity compared to other TZD. Our initial studies in the *ob/ob* mice were extremely successful (see Kumari et al Journal of Cerebral Blood Flow & Metabolism (2010), 30(2), 352–360). As was explained in the introduction to the proposal, we were forced to switch from the *ob/ob* to the more insulin resistant *db/db* mouse due to the alteration in the *ob/ob* phenotype. The inability to normalize glucose levels has been indirectly reported by Tureyen et al (2011) J. Neurochem. 116(4), 499–507.

Reviewer 3:

It would be useful to conduct a pilot study to test the usefulness of the animal models prior to the start of the proposed experiment.

Response: We have several publications using *db/db* mice prior to the current study which itself represented a pilot. Please see above for further comments.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received a Favorable rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response: We would like to thank the reviewers for their comments and understanding as to the limitations of conducting animal research. We would also like to thank the Pennsylvania Department of Health and the Legislature for their support of our research.

**Project Number:** 0864512  
**Project Title:** Modulation of Basal Ganglia Electrophysiology by  
Dopaminergic Cell Transplant  
**Investigator:** Subramanian, Thyagarajan

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

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## **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

### Reviewer 1:

In future work, the investigator should consider using other rodent models of Parkinson disease to reproduce the main findings of this project, to strengthen the relevance of dopaminergic cell grafts.

Response: We thank the reviewer for the comprehensive review and suggestions. We agree that these studies need to be broadened to other models of Parkinson's disease and we have begun such studies in collaboration with Dr. Anders Bjorklund from Sweden who has published a model that over expresses alpha synuclein in the substantia nigra in the rat. Our specific limitations to expand these studies readily to other rodent models is the lack of well described dyskinesias and motor fluctuations in other rodent models (e.g. transgenic mice and rotenone exposed rats) and the limited scalp space to install hardware for electrophysiological recordings. Our studies have focused primarily on the notion that levodopa-induced dyskinesias and motor complications stem from the inherent disadvantage of the current modality of pharmacotherapy using levodopa in Parkinson's disease patients that has remained the mainstay of treatment for 50 years despite many attempts to come up with a better form of pharmacotherapy. Our experiments with *Mucuna pruriens* are placed in this context. Nevertheless, we recognize the limitations of our current rodent model and will continue to pursue alternate rodent models besides the 6-OHDA rat model that meet the criteria to test this hypothesis.

### Reviewer 2:

1. As noted above, the investigators may wish to consider how their methodologies could be used to study effects of transplantation of iPSC-derived or fibroblast-derived pure neuronal populations at different differentiation states, including those which may be autologous (self-derived).

Response: We thank the reviewer for the excellent review and suggestions. We are indeed pursuing the suggestions that the reviewer has made. Collaborating with Dr. Niyibizi at Penn State University and Dr. Freed at the NIDA Campus in Baltimore, we have examined both allogenic, xenogenic, and autologous stem cell transplants and its effects on basal ganglia function. The implications of such studies are vast and we are pursuing the electrophysiology, immunology, neuronal plasticity and biochemistry outcomes from such studies. These studies form the key areas proposed in our next NIH renewal submission.

2. The investigators may also wish to consider expanding their model to evaluate activity patterns in the cerebral cortex and basal ganglia after implantation of DBS electrodes.

Response: This is also an excellent suggestion. There are a variety of technical limitations for such studies given that DBS is high frequency stimulation and creates stimulation artifacts that need to be eliminated to get good neurophysiological recordings. These are easier to undertake in humans and in large animal models like the primate, but much more challenging in the rat. However, newer techniques described in the literature recently may help us with these challenges and we plan to pursue these as a pilot project in the near future.

Reviewer 3:

None.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received an Outstanding rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response: We thank all the reviewers for their excellent review and suggestions. We would also like to thank the State and the Department of Health for providing the CURE funds that supported these studies.

**Project Number:** 0864513  
**Project Title:** Identification and Analysis of Arterial Blood Pressure  
Noise in Baroreceptor Denervated Rats  
**Investigator:** Tang, Xiaorui

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.)

## **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

### Reviewer 1:

The PI should seek development of new areas of investigation, using new techniques or approaches. This will be useful for identification of a research niche the PI can carve out for herself. This can be accomplished by actively pursuing new collaboration with researchers with different views and approaches on the greater research area of cardiovascular regulation.

Response: We appreciate the reviewer's specific and valuable suggestions. They are very helpful.

### Reviewer 2:

1. Lack of publications.

Response: We would agree that publishing is essential to document research progress, even from a small study like this that was of limited scope.

2. Lack of engagements of colleagues.

Response: We would agree that virtually any investigator-initiated study would benefit from the engagement of colleagues who are knowledgeable in related areas of research.

3. Broader understanding of how the APV regulators fit together. The current data is rather limited.

Response: We agree.

4. More aggressive attempts at funding.

Response: With the benefit of hindsight, we agree that more aggressive attempts to acquire external funding would have been desirable.

Reviewer 3:

1. The study design will not allow one to determine mechanisms. Suggest a conscious preparation subjected to various denervation protocols. Use of telemetry procedures.

Response: These are excellent recommendations that were beyond the scope of the resources available for this project.

2. Lack of central studies. Need to record from rVLM neurons and correlate discharge to peripheral sympathetic nerve variability. Even this will be difficult due to multiple cell types and projection to various areas of the CNS as well as to the spinal cord.

Response: We agree that central studies would be both very desirable and very challenging.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received a Favorable rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response:

**Project Number:** 0864514  
**Project Title:** Myocardial Protein Synthesis After Alcohol Intoxication  
**Investigator:** Vary, Thomas

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

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#### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

No Response is provided for this project; the Principal Investigator is deceased.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received an Outstanding rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response:

***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

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### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

#### Reviewer 1:

1. Although effective in K562 cells, the PI was not successful in developing an effective shRNA to knockdown bcr-abl in 32D cells or in the in vivo system. Thus, evaluating the in vivo activity of RNAi was not completed. Can K562 cells engraft recipient mice and induce a tumor that could have been treated with the siRNA? If so, then the tumor model should be established and the exciting potential of bcr-abl siRNA could be evaluated. If not, other models of bcr-abl leukemia can be developed as a platform for assessing the activity of the siRNAs.

Response: Xenograft models exist which likely would engraft with K562 cells. Currently NSG mice are in use and might be engrafted with this line. Thus the use of liposomal shRNA to treat such animals in-vivo is interesting and might be feasible.

2. The PI mentions that the original Aim 3 could not be pursued due to the lack of transfectability of the 32D cells. An alternate means of introducing the siRNA should have been attempted.

Response: We think it is likely that a retroviral vector would need to be constructed to successfully transduce these cells. Even there the efficiency of transduction might not allow the successful appreciation of a therapeutic effect given the "negative selection" of the process.

3. The degree of bcr-abl knockdown in K562 cells is not shown. The relationship between knock-down and the decreased MTS signal needs to be confirmed.

Response: We agree with the reviewers that we would need to demonstrate reduced bcr-abl RNA or protein expression to fully clarify this data.

Reviewer 2:

1. PI should try to publish their data.

Response: We may try to return to the issue of abl knockdown as this approach is interesting. Further data would likely be needed for publication.

2. The ceramide experiments are somewhat weak. Would be better to pursue more molecular-focused experiments in the context of the program project grant going forward.

Response: As part of the PO1 activity, we are pursuing optimization of ceramide based nanoliposomal therapy. Interesting data are available showing potentiation of C6 ceramide cytotoxicity by inhibitors of autophagy. These data seem likely to yield reagents which may move towards the clinic in coming years.

Reviewer 3:

None.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received a Favorable rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response: We would like to thank the Pennsylvania Department of Health and the Legislature for their support of our research, and we thank the reviewers for their comments and recommendations. Without this support, the PO1 application might not have been funded by NIH.

**Project Number:** 0864516  
**Project Title:** P16 Alteration and BRAF Mutation and  
Patient Outcomes in Papillary Thyroid Cancer  
**Investigator:** Goldenberg, David

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

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### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

1. The investigators' major problem was not studying enough patients. The numbers seriously hampered their ability to draw conclusions from the work.

Response: We agree but were unable to accrue a larger group.

2. The analysis of the data was rudimentary. Multivariate statistics should be used for this data and this was not done. Even simple comparisons were omitted that would have been of interest. The investigators appeared uncomfortable with data analysis.

Response: We will reassess plans for data analysis if we are able to accrue more subjects.

Reviewer 2:

Even given the limited funding provided, analysis of a larger patient cohort should have been possible under the aegis of this proposal.

Response: We agree but were unable to accrue a larger group.

Reviewer 3:

1. Weakness: Small number of specimens analyzed and lack of consideration of statistical limitations in interpreting the data.

Recommendation: The investigators should accrue and analyze more specimens, perhaps through multi-institutional effort, provide power calculations and consider statistical limitations in interpreting their findings.

Response: We agree and will further consider options for increasing patient recruitment.

2. Weakness: The investigators have used semi-quantitative and often subjective IHC method to evaluate p16.

Recommendation: Quantitative immunohistochemical methods, such as AQUA and Vectra, are available that provide more reliable estimates of protein expression. These methods should be considered.

Response: We will consider this if we are able to accrue more subjects

3. Weakness: Mutation in BRAF was assessed by ASPCR.

Recommendation: Although ASPCR is a valid method, availability of antibodies to mutant BRAF (at least to the V600E mutant), makes evaluation of the expression of the mutant BRAF protein feasible and allows correlation between p16 staining and mutant BRAF expression.

Response: We agree.

4. Weakness: Both BRAF mutation and p16 levels were present in similar (78%) and much larger proportion of cases than reported in the literature.

Recommendation: The investigators should provide an explanation for this unusual occurrence (may be due to small sample size?).

Response: As the reviewer suggested, we have attributed this to the small sample size.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received a Favorable rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response:

**Project Number:** 0864517  
**Project Title:** The Interaction of Environmental Agents and  
LDL-Cholesterol in Parkinson's Disease  
**Investigator:** Mailman, Richard

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

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## **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

### Reviewer 1:

This project has been adequately accomplished and human results obtained from this project have a high impact on Parkinson disease research. The grantees need to put more efforts on animal studies, particularly on developing and validating their animal models before performing the study.

Response: We thank the reviewers for their positive comments about our work, and we would like to clarify our view of the suggestions made by Reviewer 1. Our studies were based on two sets of clinical findings. The first (pioneered by Co-PI Huang and since confirmed by numerous groups) was the clinical association of high circulating cholesterol with lower incidence and slower progression of Parkinson's disease. The second was that a variety of environmental contaminants were linked to occurrence of sporadic PD. Our study was the first attempt to test a specific hypothesis in animals (high circulating cholesterol is protective against brain toxicity of dopamine neurotoxicants) that could provide a mechanism integrating these clinical findings.

We gave a great deal of thought to the animal model. Our original idea was to use mice with "humanized ApoE", but it became clear that there were no advantages to this versus simple dietary manipulation. We validated our model carefully (indeed we expected stable high cholesterol within weeks, but it took months before this occurred, dramatically impacting the number of experiments we could do and the cost of each). Unfortunately, the resulting data supported the null hypothesis, but this is valuable as it directs us to other, and more complex, mechanisms. [Please see additional comments in Section D].

Hundreds of investigators in the PD field have sought valid animal models for a half-century, yet even today, after many millions of research funding, the field relies upon chemical (e.g., 6-OHDA, MPTP, rotenone) or genetic (e.g.,  $\alpha$ -synuclein over-expression) models, none of which recapitulates anything resembling idiopathic PD. Although our model was simple, it was

validated and provided a direct test of our hypothesis. If there was a mouse model that showed continued progression of damage and other neurodegenerative changes similar to PD, we would of course seize upon it.

On the other hand, the “kick start” provided by the CURE funding has facilitated our continued work in this area; indeed, two additional publications triggered by this grant are listed below. We shall continue to generate data that will lead to a successful NIH R01 application.

Du G, Lewis MM, Sterling N, Kong L, Chen H, Mailman RB, Huang X. (2014) Microstructural changes in the substantia nigra of asymptomatic agricultural workers. *Neurotoxicol. Teratol.* Jan-Feb;41:60-4. doi: 10.1016/j.ntt.2013.12.001. Epub 2013 Dec 12. PMID: 24334261

Jones BC, Huang X, Mailman RB, Lu L, Williams RW. (2014) The perplexing paradox of paraquat: the case for host-based susceptibility and postulated neurodegenerative effects. *J Biochem Molec. Toxicol.* 2014 Mar 5. doi: 10.1002/jbt.21552. [Epub ahead of print] PMID: 24599642.

Reviewer 2:

None.

Reviewer 3:

None.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received an Outstanding rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response: We would like to thank the Pennsylvania Department of Health and the Legislature for their support of our research, and we thank the reviewers for their comments and the “Outstanding” evaluation.

**Project Number:** 0864518  
**Project Title:** Moving Experimental Cancer Therapeutics from the  
Research Bench to the Clinic  
**Investigator:** Robertson, Gavin

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

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## **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

### Reviewer 1:

Published papers in high-impact journals are limited.

Response: Manuscripts are in the process of being published.

### Reviewer 2:

This is an outstanding proposal that achieved all its goals and has potentially high impact in the development of novel therapeutic strategies to treat melanoma patients.

### Reviewer 3:

1. This reviewer believes additional publications should have resulted from the data obtained. This is particularly true considering that post-docs were trained and contributed to the project as described.

Response: Manuscripts are in the process of being published.

2. It is unfortunate that SBIR funding was not pursued further. This is an outstanding source of funds which would help with IND efforts and eventual studies designed for the clinic.

Response: Thank you for this suggestion.

3. This reviewer does feel that additional cell lines should have been used in the analysis of the agent. In addition, fresh tumor samples in melanoma are not difficult to obtain, especially early disease and nevi from local dermatologists as well as metastatic disease obtained from pathology departments at the Medical Center. In addition, fresh tumor can be purchased from outlets such as the NCI supported CHTN. These studies will have

greatly enhanced the results obtained and the potential for translational studies in the future.

Response: This is a great suggestion to further explore in the future.

4. It is unfortunate that the reagent was not investigated in combination with immune response. There are many areas of potential interaction. The agent induces apoptotic bodies, an ideal source of antigen for dendritic cells. The process of antigen presentation leads to both cell mediated immune responses (CD4 helper and CD8 cytolytic) as well as humoral responses (antibodies) In addition, the positive or negative effect on immune suppressive mechanisms could have been explored. These include Tregs, tissue macrophages and myeloid derived suppressor cells (MDSC).

Response: This is a great suggestion to further explore in the future.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received an Outstanding rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response: Thank you for the very useful comments.

**Project Number:** 0864519  
**Project Title:** Changes in Oxygen-induced Proliferative Retinopathy in  
4E-BP1/2 Knockout Mice  
**Investigator:** Shenberger, Jeffrey

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.)

## **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

### Reviewer 1:

1. A major weakness is that investigators examined changes in protein expression at p17, a time point beyond detecting significant changes in VEGF levels and other protein phosphorylation, so they might have missed critical changes and were misled that there is no effect between WT and KO mice. A suggestion of collaborating with biochemist or cell signaling scientist can help greatly to guide investigations.

Response: The responses were investigated at various time points from P8-P21, but in the context of regulation of vascular development. The number of mice needed for protein studies is quite large and the poor breeding of the mice makes this an extremely-time consuming endeavor. That said, we totally agree with the reviewer's comments.

2. Investigators should have pursued seeking extramural funding from foundations as well as the NIH.

Response: This was done but the proposals were not funded.

### Reviewer 2:

1. It was not clear to this reviewer whether the research team has tried to compare the phenotypes of their 4E-BP1/2 double knockout mice in another genetic background, such as C56BL6. If not, this approach may be useful.

Response: Yes, the phenotype will be compared to C57BL/6 mice.

2. The data related to the use of BalB/c mice in OIR model should be published, which will be beneficial to some researchers in the field.

Response: We agree. It will be part of the manuscript.

3. To publish the results related to mTOR pathway ASAP, which will allow the PI to apply for additional grants.

Response: Publishing the manuscript is the primary focus of our current efforts.

4. The strategy that Aim 2 was dependent of the success of Aim 1 should be avoided in future grant preparation.

Response: We agree – a domino effect is never a good strategy.

Reviewer 3:

1. More quantitative analysis of the data would have better supported the conclusions.

Recommendation: when possible, utilize quantitative analysis in a blinded fashion vs. conclusions based on gross observations. Adequate replicates and data with proper statistical analysis is always beneficial to support conclusions.

Response: We agree with the reviewer's recommendation.

2. The assumption is made that genotyping of animals was carried out based on the fact that heterozygous animals were crossed (support comes from the western blot showing lack of expression of 4E-BP1/2).

Recommendation: Always err on the side of providing more information in progress reports (i.e., state activities explicitly, and don't assume that a reviewer is intimately knowledgeable about all of the details of the methodology in the project).

Response: The genotyping was done as expected and it should have been reported as recommended by the reviewer.

3. The strategic plan indicated that confocal microscopy would be utilized to quantify vascular density in the retinas of the animals, but this was never initiated and no explanation was provided for its abandonment. Also, there was no clear explanation of why real-time PCR experiments were not carried out, was it lack of animals again? This could have been stated.

Recommendation: Again, provide additional information concerning experimental design/protocol changes rather than simply omitting any reference to these changes. There are presumably reasonable explanations for not being able to carry through on a proposed procedure. Confocal microscopy could have provided more quantitative data than gross examination.

Response: The reviewer is correct that quantification would be ideal (and perhaps essential). However, the breeding of these animals is complex and the reproductive

capacity is extremely low. As a result, we struggled getting enough mice/retinas to perform the work and proceeded with the highest yield at the time.

4. Need to more clearly address changes in research focus.

Recommendation: Acknowledge failure to support a hypothesis as this is new knowledge and then provide findings that suggest an alternative mechanism for testing. Don't let inconsistencies in progress reports suggest you lost focus of your objectives and don't expect different data to address failure to explain unexpected results by omission.

Response: We agree and appreciate the insight.

5. No major discoveries resulted from this work.

Recommendation: While negative data is never as exciting as proving your hypothesis, it is also important to publish negative outcomes to inform other investigators contemplating the same experiments. This also has implications for the "measures of impact and effectiveness of the research being conducted," if it hasn't been published, then it essentially has not been done. While it is difficult to publish negative data, it can/should be included as background information for why alternative pathways were studied (subsequent to the funded work) and this may be more easily published.

Response: We agree and are pursuing publication presently.

6. Future plans unclear – final progress report states in Question 12: "We plan to finish the BiP studies within the next six months once additional funds are available. This should complete the project in terms of a publishable study." What is "BiP" and how does it lead to a publishable study? Plans to apply for additional funding were abandoned after 10/2010 NIH application, which was well before this final progress report stating no plans to apply for additional funding (final progress report Question 11B).

Recommendation: Always plan for additional funding, acknowledging how the results of the previously funded work has directed new lines of research for the grant proposal to be submitted.

Response: We agree.

7. No major discoveries/outcomes from this research. The PI was unsuccessful in leveraging additional funds to expand this work. The PI lists no plans for additional grant proposals based on this negative outcome, and yet is proposing a different strategy for study which presumably will require research funding.

Recommendation: As stated above, always plan for additional funding, acknowledging how the results of the previously funded work - even if negative - has directed new lines of research for the grant proposal to be submitted.

Response: We agree.

8. This work resulted in no publications. The PI states plans to submit following the end of this work in 2011, but a search of the literature at the end of 2013 shows no published work attributable to this funding. Continuation of collaboration with the co-PI – No new collaborations or extension of work outside of the institution.

Recommendation: Find new collaborators to help with expanding the laboratory's technical capabilities so performance measures can be attained, e.g., the confocal imaging would have been a nice addition to this study, and Dr. Barber was listed as being available to assist with this portion of the experimental design.

Response: A manuscript is currently in preparation. For the record, the PI is now pursuing a different line of research, at another institution, and functioning as a clinical director.

9. Several inconsistencies were apparent when reviewing the annual progress reports vs. the final progress report.

Recommendation: Always ensure that new data is provided to back up changes in conclusions. As noted above, the conclusions should be based on more quantitative data and not simple gross observations. There is nothing wrong with changing research direction (which inevitably is driven by funding), but an adequate explanation for the abandonment of an original hypothesis should be provided. In this case, new data provided in the final progress report and carried out after the end of the funded project (with alternate funding) supported a new hypothesis pointing to a new potential means of intervening in retinopathy of prematurity.

Response: We appreciate the reviewer's constructive and insightful comments and think the reviewer is the type that is needed more often in funding agencies. His/her comments are insightful and aim to get the investigator on the right track. Thanks.

***C. If the research project received an "unfavorable" rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive "unfavorable" ratings.***

Response: This project received a Favorable rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response:

**Project Number:** 0864520  
**Project Title:** Molecular Mechanisms of Uninfected Red Cell  
Phagocytosis in Severe Malarial Anemia  
**Investigator:** Stoute, José

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.)

## **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

### Reviewer 1:

1. The investigator's experimental model was not suitable to pursue the stated aims as the model did not support increased phagocytosis of uninfected red blood cells in malaria-infected mice. The investigator needs to define an experimental plan that will generate meaningful data from his SMA rodent malaria model.

*Response:* In our view, the fact that the model did not support our original hypothesis does not mean that the model is not worth pursuing as there may be alternative mechanisms that we have not considered. In addition, the fact that we did not observe a difference between iRBCs and uRBC in this model does not mean that the latter are not damaged or do not have increased susceptibility. We need to further compare these RBCs to baseline RBCs. However, and discussed below, we do plan to expand our investigation of the SMA model by identifying the mechanism of expansion of F4/80 macrophages in the liver.

2. The investigator found some evidence of increased phagocytosis in the Plasmodium chabaudi-infected mice compared to uninfected mice. These data would have been worthwhile to pursue and the PI could develop a research plan that pursues this work. Unfortunately, this model does not have features of severe malaria anemia as he observed in his P. chabaudi / P. berghei model.

*Response:* We agree with the reviewer that the P. chabaudi model unfortunately does not have features characteristic of SMA. With limited resources, we have chosen to concentrate on the P. chabaudi/ P. berghei model but we will continue to make comparisons to the P. chabaudi model as appropriate.

### Reviewer 2:

1. Novel approaches may be necessary to successfully answer these questions. Is it possible to

develop unbiased interaction events where both infected and uninfected cells are applied to a monolayer of monocytes / macrophages, and infected vs uninfected cells are identified by imaging?

Response: Yes, this is feasible. We are planning to use transgenic fluorescent *P. berghei* parasites in the future. When combined with labeling of red cells, this should allow for the identification of macrophages that contain infected vs. uninfected RBCs.

2. Are there clues to the molecular components of these processes provided by studies of "innocent bystander" hemolysis in humans with sickle cell anemia receiving blood transfusions?

Response: Yes, in humans the sickle cell studies have shown increased translocation of phosphatidyl serine (PS) is an important mechanism of increased phagocytosis. Unfortunately, our studies did not show convincingly that PS is increased in the *P. chabaudi*/*P. berghei* model. In mouse models of autoimmune hemolysis, complement activation is an important mechanism

3. Investigators published erythrophagocytosis in the sequential *P. chabaudi* and *P. berghei* infection model, and anemia in C3 <sup>-/-</sup> mice. Further investigation of phagocytosis of infected red cells in this system would be useful, since clinically available complement blockers are being proposed as potential therapies for hematological disorders with "bystander" hemolysis which may have similar mechanisms.

Response: We do plan to continue to pursue the role of complement in this model. We would like to test the effect in other knockout models such as C4 and C1q which could also be involved in phagocytosis.

#### Reviewer 3:

1. Improve statistical analysis and presentation of data.

Response: We would welcome any specific recommendations that would improve the presentation of our data and the statistical analysis.

2. Investigate other etiologies of SMA in the murine model, such as dyserythropoiesis.

Response: We do plan to expand our investigation of the mechanisms of SMA in the *P. chabaudi*/*P. berghei* model. One line of investigation that we plan to pursue as noted above is the mechanism of expansion of F4/80 macrophages in the liver. We will test the effect of CCR2, a receptor for the chemokine CCL2 which is critical in migration of monophagocytic cells from the bone marrow. Our results suggest that accelerated destruction and not decreased production is the critical factor in our model.

3. Do parallel experiments on culture-derived *P. falciparum*.

Response: Given the fact that the in vivo model is a lot more complex, the in vitro studies

with *P. falciparum* in the absence of in vivo studies are difficult to interpret. However, we are very interested in testing the effect of *P. falciparum* hemozoin on macrophages and the effect that this may have on erythrophagocytosis.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received a Favorable rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response:

**Project Number:** 0864521  
**Project Title:** In Vivo Anti Tumoral Properties of Ceramide Nano  
Liposomes in a Murine Hepatocellular Cancer  
**Investigator:** Tagaram, Hephzibah

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.)

## **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

### Reviewer 1:

This is a highly-focused research project in liver cancer field, with limited novelty and significance. The results are very much expected, which diminishes enthusiasm to low or medium level.

The applicant is encouraged to interact more with clinical doctors and develop a research project or direction, which can provide a fresh view on cancer therapy.

Response: The growth inhibition and pro-apoptotic properties of nanoliposomal C6-ceramide (LipC6) were demonstrated previously in human HCC as described in my first author GUT manuscript. We now demonstrate the efficacy of LipC6 in a novel orthotopic murine HCC model. The model was developed by seeding the tumorigenic hepatocytes from SV40 T antigen transgenic MTD2 mice into the livers of syngeneic C57BL/6 mice. This resulted in SV40 T antigen specific tumors in the immune competent C57BL/6 liver and is a source to evaluate the efficacy of combinatorial effects of LipC6 and antigen directed immunotherapy on HCC progression. We believe that this does represent a novel approach with potential for application in cancer therapeutics.

### Reviewer 2:

None.

### Reviewer 3:

1. It is unclear why no attempts were made to obtain additional funding, and even more curious, why no future grant applications are planned. Furthermore, the future plans for the research are cursory and vague. If no attempts are made for leveraging these funds, then a justification should be provided.

Response: A manuscript describing the combinatorial effects of lip-C6 and immunotherapy is in preparation and will be submitted in the near future. We have evaluated the efficacy of the LipC6 in combination with immunotherapeutic strategy to control HCC and investigated the underlying mechanism that increased the antitumor immune response. LipC6 treatment of tumor bearing mice followed by adoptive transfer of TCR-1 cells and Immunization reverses immune tolerance to tumor antigens in an orthotopic murine model developed in the laboratory. We appreciate the reviewer's comments regarding the importance of leveraging these funds and will take them under advisement in developing future plans for our research.

2. There is little justification given for why such a large portion of the budget was spent on an item that appears to have modest value to the project. While this item may improve the research infrastructure at the institution, the details regarding this are not provided. More details regarding how this item impacts research quality and capacity should be given.

Response: We developed a novel orthotopic mouse model of HCC through seeding of tumorigenic hepatocytes from SV40 T antigen (Tag) transgenic MTD2 mice into the livers of syngeneic C57BL/6 mice. These MTD2- derived hepatocytes form Tag expressing HCC tumors specifically within the liver. This approach provides a platform to test therapeutic strategies and antigen specific immune-directed therapy in an immunocompetent murine model. The synergistic effects of Lip-C6 combined with immunotherapy studies were performed in this model. The combination of Lip-C6 with adoptive transfer of tumor antigen- specific CD8+ T cells prolongs survival and leads to the regression of established tumors.

The Imaris Confocal Analysis software package was purchased with supplemental funds expressly designated for acquisition of the equipment. This software provides crucial 3D and 4D image analysis capabilities that were not previously available and will also benefit other projects in multiple areas of clinical and basic science research at the PSU College of Medicine.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received a Favorable rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response:

**Project Number:** 0864522  
**Project Title:** The Use of Biomarkers to Predict the Onset of  
Vasospasm in Aneurysmal Subarachnoid Hemorrhage  
**Investigator:** Cockroft, Kevin

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

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## **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

### Reviewer 1:

Complete enrollment. Perform linear combination analysis to improve predictability. Define a positive or negative definition of poor outcome, and do scattergrams to complete definition of poor versus not poor outcomes. Perform receiving operator curves (ROC) analysis to determine sensitivity and specificity of each definition of cytokine level or linear combination to predict poor outcome.

Response: We are seeking funding to continue this project and enroll more patients. If we are able to achieve this, perhaps with a more limited array of cytokines, we will proceed with a more detailed statistical analysis. The reviewer's suggestions for such analysis are appreciated.

### Reviewer 2:

Statistical power requires a larger sample size. Partner with other institutions in order to achieve statistical power for future studies to include biomarkers that appeared interesting from the current data and the rate of change of the biomarkers.

Response: We agree and hope to use the preliminary data to support a proposal to study additional patients, potentially in collaboration with other institutions.

### Reviewer 3:

1. Relatively low number of patients in the study (20) was lower than expected, significantly decreasing the statistical power. A recommendation would be to use current data to estimate standard deviations and determine the number of patients needed to secure clinically-reasonable biomarkers.

Response: We agree and should be able to do this with available resources and without the need for additional funding.

2. As investigators indicate, the data on CSF, as originally proposed may have been a better source than serum for isolation of biomarkers but done, ostensibly due to lack of funds. These would greatly strengthen the results and should be collected.

Response: Funding was not the only issue with regard to CSF. Many patients did not have CSF collected as they did not have external ventricular drains placed, thus the sample size for CSF samples is very low.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received a Favorable rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response: We thank the reviewers for their thoughtful comments. We hope to use the preliminary data in support of future studies. We would also like to thank the State Legislature and the Pennsylvania Department of Health for their support of our research.

**Project Number:** 0864523

**Project Title:** Tim2 Expression on Oligodendrocytes: A new Immune System Target

**Investigator:** Connor, James

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

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## **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

### Reviewer 1:

1. As outlined in Section A. there were several suggestions provided that are reiterated here: As stated in the strategy for Aim 1 provided by the investigators, they had initially intended to examine the post-mortem materials from 12 MS cases (all female) with a classification of plaque stages. In Table 1 of the progress report, the investigators indicated that they had examined 5 samples (4 Female/ 1 Male), all with active plaques. The original stated plan would have yielded greater insight into the association between Sema4A and white matter lesions in MS by providing additional information on its expression across lesion types.

*Response:* The reviewer is correct that our original plan would have yielded greater insight. Unfortunately we experienced technical problems with non-specific staining and autofluorescence. We have addressed these technical concerns by obtaining homogenates of MS lesion samples for immunoblot analysis (the antibody does not show evidence of non-specific staining in this modality) and by obtaining CSF from MS patients in different stages of disease. We demonstrated in Figure 4 of the progress report that Sema4A could be identified in white matter of homogenates of MS tissue with 3 different stages of plaques. However, we also obtained similar levels of Sema4A in normal appearing white matter (NAWM) from the MS patients. The levels of Sema4A in the NAWM could be a harbinger of oligodendrocyte damage and future demyelination and thus could be a very exciting observation. We are in the process of obtaining white matter from non-MS patients to serve as a control before such an interpretation can be evaluated. These analyses are on-going and important for future funding and impact of this project.

2. An analysis of Sema4A expression in lesion types was provided in Figure 4 of the progress report (which was mislabeled on Page 9 of the report as referring to Figure 3) which showed an analysis of Western blotting results without providing the primary data. The investigators also alluded to a potential issue of antibody cross-reactivity. It was

unclear why the authors did not repeat these experiments with additional sources of commercially-available antisera.

Response: We regret having mislabeled the figure. Since the submission of the final report, we have repeated the western blotting results. There is some confusion in the way we presented the data. The 55kDa band detected on the western blot from CSF samples was smaller than that previously reported for Sema4A. We found that this band did not represent a cleaved product of Sema4A. Thus we concluded that Sema4A was not detectable in CSF of MS patients. We did not mean to imply that the antibody was non-specific.

3. Results from Aim 2 were stated by the investigators to have been negative or inconclusive; they could not detect Sema4A in the CSF of clinical samples. Validation of the samples using a standard, such as oligoclonal banding could have benefitted the interpretation of this experiment. It might have been recommended to use an ELISA approach for detection of this protein from the CSF samples.

Response: An ELISA approach would be more sensitive. There were insufficient funds and time to develop an ELISA and there is no commercially available ELISA. It is unclear what an oligoclonal banding pattern would provide in this analysis.

4. The investigators indicated that their studies now indicate a potential role for Tim2 and Sema4A in cerebral malaria. Inclusion of additional information on these related findings, which may have stemmed from the investigations supported by this project, would have enhanced the impact of the project progress report.

Response: We did not include those results because they were preliminary and not part of the proposal. However, we mentioned them because they were relevant as both a future direction and potential for clinical impact. We have found that in an animal model of cerebral malaria that Sema4A levels are increased in the brain. At this point we cannot make a direct connection between the presence of Sema4A in the brain and death of oligodendrocytes and demyelination. This is an area that we hope to be able to pursue in the future.

Reviewer 2:

The data generated by this proposal have, thus far, been quite sparse. The rationale of this research is not necessarily convincing.

Response: It appears that we have identified a link between Sema4A and oligodendrocyte cell death. These data are convincing. We are striving to demonstrate the clinical relevance of our finding.

Reviewer 3:

1. As the role of Sema4A has been defined in the immune cells in MS patients, it will be critical to evaluate the effect of this molecule on OL survival and its expression from OLs. This can be done by cryosectioning and double immunofluorescence or, if fresh tissue is available, by

cell sorting OLs from immune cells and determining the level of expression.

Response: We appreciate this suggestion and will continue to explore the relationship between Sema4A and MS. Our focus has been to demonstrate the presence of the receptor for Sema4A on human oligodendrocytes.

2. Also, revisiting the concentration of Sema4A in CSF compared to sera in these patients should be compared using ELISA and other antibody-based systems.

Response: We agree and hope that a commercial ELISA becomes available.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received a Favorable rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response:

**Project Number:** 0864524  
**Project Title:** Mechanisms of Microsatellite Mutagenesis in Human Cells  
**Investigator:** Eckert, Kristin

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

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### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

#### Reviewer 1:

The main weakness of this project was that Aim 1 was not accomplished because of difficulties in the primary technique planned for this aim. This is a considerable weakness to the overall project, but the investigators remained quite productive for the second aim.

Response: The reason we could not accomplish Aim 1 was biological, and not a consequence of our lack of experimentation. We discovered that a major molecular genetics technique used not only by us but by many investigators (at the time this project was funded) caused an increase in the level of mutations in human cells. Because the goal of Aim 1 was to measure changes in mutation levels associated with a specific gene, the interference due to the technique itself precluded our performing the experiments proposed. We did perform alternative, more indirect experiments, but the results we obtained were not conclusive. We were unable to carry out additional avenues of research towards this aim, due to the limited funding and time period supported for this project.

#### Reviewer 2:

1. Would have been nice to have publications in more impactful journals.

Response: The open-access G3: Genes, Genomes, Genetics journal was only launched in June, 2011; therefore, the impact factor of this journal cannot be fully evaluated at this time. As of July 2014, our paper was downloaded a total of 1,218 times (according to the G3 journal website), demonstrating that our study has had a strong impact, world-wide. We originally submitted our study to the journal Genetics, which is one of the leading and most established journals in the Genetics field. Although we received a favorable response, the reviewers asked us to perform additional experiments prior to publication. This was impossible, because of the limited funding (amount and time) we received for this project. The Editors of Genetics therefore offered to publish our study, as submitted and with no additional data, in their new sister journal, G3: Genes, Genomes, Genetics.

2. While not every project is successful, there is no clear path forward to clinical translation to a diagnostic test that would have medical utility.

Response: Although we wrote this proposal as a basic science research project, the paper we published in G3 has, in fact, provided a foundation for several new grant applications in which we proposed using microsatellites as biomarkers for cancer progression in Inflammatory Bowel Disease patients. It also appears that Reviewer #3 appreciated this potential for clinical translation, writing “a strength of this project is a series of new findings that have distinct health-related implications”.

3. No new techniques developed.

Response: The development of new techniques was not a goal of this project. The project was hypothesis-driven research, with the goal of deducing the roles of specific enzymes in maintaining human genome stability.

#### Reviewer 3:

The weaknesses of the project are they did not use controls in the experiments with pol  $\kappa$ . It would have been nice to have a xeroderma pigmentosum deletion control to show the role of pol  $\eta$ , but this may have been previously published. Also, it would have been nice to have different types of microsatellite sequences studied in this manner. However, presumably this funding was clearly only a tokenism to get them started for an NIH grant.

Response: We have previously examined a xeroderma pigmentosum deletion human lymphoblastoid cell line (as suggested by the reviewer), but we found that this cell line cannot replicate the shuttle vectors we use in our mutagenesis assay. Therefore, the experimental approach used in Aim 2 was not possible at the time of this study. We have recently (in 2013) acquired different xeroderma pigmentosum deficient human cell lines, and will be performing these experiments if adequate funding is available.

Regarding the second point about the types of microsatellites, we previously published studies that examined several distinct tetranucleotide microsatellites, which is why Aim 2 was focused on two types of dinucleotide microsatellites. We have a variety of shuttle vectors available in our lab to test additional microsatellite sequences. However, because our experimental approach is costly and both time and labor intensive, we would require additional funding to carry out such studies.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received a Favorable rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response: We are very proud of the research we accomplished with the \$46,000 investment for this one year research project. As a result of this funding, we:

- obtained a four-year, interdisciplinary R01 grant from the National Institutes of Health, totaling \$1,437,288;
- increased the quality and capacity for research at the Pennsylvania State University by extending our research project to include Drs. Makova and Krasilnikova at Penn State, University Park;
- published our research results in an Open Access, peer-reviewed journal with world-wide readership; and
- supported a Ph.D. graduate student, thereby contributing to the overall learning environment of Penn State University.

We thank the Pennsylvania Department of Health and the Legislature for their support of our research, and we thank the reviewers for their review.

**Project Number:** 0864525  
**Project Title:** Epigenetic Therapy of Human B Cell Malignancies  
**Investigator:** Epner, Elliot

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

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### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

The Principal Investigator for this project is no longer at Penn State University.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received a Favorable rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response:

**Project Number:** 0864526  
**Project Title:** Novel Multielectrode Recording Techniques for Assessment of  
Taste Functions in the Brain  
**Investigator:** Hajnal, Andras

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

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#### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

None.

Reviewer 2:

The relationship between the project and the publications were not clear. The grant was titled "Novel Multielectrode recording Techniques for Assessment of Taste Functions in Rats" yet the techniques reported in the publication and the two conference papers all used recording techniques that pre-date the grant. Why was the grant acknowledged? Also, although the reporting period was from 1/1/2009 through 12/31/2012, the research was completed by 6/30/2010. In the progress report, the data reported in Figures 1 and 2 is all behavioral observations and the electrophysiological data reported in Figure 4 is from 17 neurons. Why were the recordings stopped at 6/30/2010 (the end of the research project)? A more full description of the relationship of the grant to the other significant work of the investigators would have been appreciated.

Response: The publications included data obtained by using the new multielectrode methods, although not exclusively. This 'combined' analysis proved to be useful in as much as it confirmed consistency between methods with respect to critical variables (e.g., delay in sucrose-responses, time-locked responses in neighboring neurons, etc.). Because only a few assemblies (N=17 neurons) were collected using multielectrode arrays, more complex network analysis was impractical and therefore was not included in the published paper. Nevertheless, the posters for the conference presentations included some of the initial data and provided details on the microdrive design.

Regarding the reviewer's comment on the end date of the project, this specific project was funded for a period that started 7/8/2009 and ended on 6/30/2010.

Reviewer 3:

In the absence of any text describing the development of the multi-electrode recording technique, I do not understand why this project was framed in that way. Essentially the funds were used to conduct two experiments, only one of which involved electrophysiological recordings. So, as previously noted, this work seems more about gaining experience with, rather than the development of, a new recording technique. All the other experimental procedures and treatments are standard. Nonetheless, the work appears well-conducted and NIH funding was obtained.

Response: In addition to gaining experience with chronic recordings as the reviewer noted, we attempted to develop various microdrives and adapt analytical methods specifically to hindbrain (pontine) taste recordings. With the benefit of hindsight, we regret that our report focused on the experiments we conducted and the results that we obtained and did not include notes and details on those technical developments. However, as mentioned above in our response to Reviewer 2, we had the opportunity to share some of our technical developments with peers at two conferences.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received an Outstanding rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response: We thank the reviewers for their encouragement and Outstanding rating and we express our appreciation to the Pennsylvania Department of Health for supporting this study through the Commonwealth Universal Research Enhancement program.

**Project Number:** 0864527  
**Project Title:** Glycosphingolipids and Diabetic Retinopathy  
**Investigator:** Kester, Mark

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.)

## **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

### Reviewer 1:

As stated above, the project has achieved the majority of the initially stated specific aims. The outstanding performance of the project is well-documented by 3 peer-reviewed publications, training student, post-doc fellow and obtaining extramural funding.

The only part left that was not fully executed was studies of inhibiting or knocking down glucosylceramide synthase. Initial studies were done in control rats that demonstrated that inhibitor worked without altered retina toxicity. Later, studies using same inhibitor in diabetic rat retina showed degree of toxicity that was thought to be attributed to diabetes state. However, these studies were halted although they had great potential to develop "a drug" related to the findings generated by research team.

Also, it was mentioned that knocking down expression of that particular enzyme using siRNA did not produce same protective effect on lipid metabolite similar to pharmacological inhibitor. Authors propose to do genetic deletion of the enzyme using general or tissue specific knock out mice. It is not clear how deletion of the enzyme in vivo can provide more detailed or useful information taking into consideration the negative results of siRNA in vitro. It might be highly possible that the pharmacological inhibitor has target effects beyond inhibition of GCS. Extended studies are warranted to further explore and develop that particular area of research.

Response: We appreciate that the reviewer noted the grant had achieved "outstanding performance" and we agree that "extended studies are warranted to further explore and expand that particular area of research" (inhibition of glucosylceramide synthase (GCS) in models of diabetic retinopathy). In fact, since submitting the final report, we have continued these studies, obtained a proprietary GCS inhibitor from Lilly, and have demonstrated that it is nontoxic after IP administration in diabetic models. The retinas from these diabetic animals are now being analyzed for lipidomics as well as for inflammatory and neurodegenerative markers of retinopathy. We will acknowledge support from the PA CURE Program in a subsequent

publication.

Reviewer 2:

1. Since studies in animals do not necessarily mimic human studies it is important that studies in humans are taken into consideration to avoid performing studies that may be irrelevant for human pathology.

*Response:* We agree, and have chosen animal models that exhibit inflammatory and vascular changes associated with human diabetic retinopathy.

2. Sphingolipids play a role in cell survival and cell apoptosis and that fact needs to be considered when programming future studies. Over simplification may lead to conclusions that, although interesting, are not relevant for human physiology or pathology.

*Response:* We agree, and for that reason all pharmacological and molecular therapeutic approaches are validated by mass spectrometry-based lipidomic approaches that quantify the 100s of different sphingolipid species from diabetic retinas.

3. Validation of the studies performed in Aim 2 by determining whether or not GCS inhibitors have an anti-inflammatory effect independent of their ability to inhibit GCS is essential to further pursue this area of research. If silencing the GCS gene has no effect in reducing retinopathy, other avenues to explore alternative effects of GCS inhibitors need to be launched.

*Response:* We agree, and as noted above, we are presently investigating a GCS inhibitor that has less off-target effects.

Reviewer 3:

Since Aim 2 had potential therapeutic value, it is a shame this aim was not completed. The PI should be encouraged to continue on Aim 2.

*Response:* We agree, and as stated above, we are actively pursuing this line of investigation. In addition to animal experiments investigating the new Lilly GCS inhibitor, we are also investigating a genetically engineered animal model that does not express GCS (obtained from the Proia lab of NIDDK). With the model, we hope to confirm that GCS is a novel target for diabetic retinopathy. We are also re-investigating the siRNA experiments for GCS knockdown using more specific constructs that are delivered in less toxic, less cationic, formulations.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received an Outstanding rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response: Drs. Fox and Kester would like to thank the PA CURE Program as well as the State of Pennsylvania for providing much needed funding for these therapeutically-driven translational studies. We believe that this is an exceptionally important funding mechanism and hope that it will continue to fund other clinically significant studies in the Commonwealth.

**Project Number:** 0864528  
**Project Title:** Epithelial/Dendritic Cell Cross-Talk in Acute Kidney Injury  
**Investigator:** Reeves, William

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.)

### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

None.

Reviewer 2:

A more clear discussion of A) how the results of Specific Aim 1 are interpreted by the PI and B) how does this finding relate to AKI models in mice and AKI in humans would be helpful.

Response: We found in Aim 1 that direct activation of TLR4 by LPS in cultured mouse or human proximal tubular cells results in little production of TNF. However, pretreatment of cells with cisplatin renders them exquisitely sensitive to LPS (*Am. J. Physiol: Renal Physiology*. 292:F812-819, 2007). The implication of these findings is that renal epithelial cells possess an intact TLR4 signaling pathway but that portions of the pathway are inhibited under resting conditions. Our previous work showed that cisplatin, but not LPS, activates p38 MAPK in proximal tubule cells and that inhibition of p38 MAPK reduces cisplatin nephrotoxicity in vivo (*Am. J. Physiol: Renal Physiology*. 289:F166-F174, 2005). Thus, we envision cytokine production, and AKI, to be a 'two-hit' phenomenon wherein activation of TLR4 is a necessary, but not sufficient, stimulus to elicit proinflammatory cytokine production by renal epithelial cells and that cell injury provides a second signal for cytokine production (perhaps through p38 MAPK).

Reviewer 3:

None.

***C. If the research project received an "unfavorable" rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive "unfavorable" ratings.***

Response: This project received an Outstanding rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response: We appreciate the support of the CURE program and the helpful comments of the reviewers.

**Project Number:** 0864529

**Project Title:** Synergistically Acting Targeted Therapeutics for Melanoma

**Investigator:** Robertson, G.P.

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.)

### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

None.

Reviewer 2:

The only recommendation would be to explore siRNA screen data more rigorously to identify additional kinase targets other than Wee1, which could be leveraged as a future therapeutic approach for melanoma.

Response: This is a very useful suggestion, thank you.

Reviewer 3:

1. The PI is strongly encouraged to publish the results of the described studies.

Response: They are in the process of being published.

2. The absence of any data obtained from the grant project made the success of this proposal rather difficult to assess.

Response: They are in the process of being published.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received an Outstanding rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response: We thank the reviewers for their excellent suggestions and Outstanding rating and we express our appreciation to the Pennsylvania Department of Health for supporting this study through the Commonwealth Universal Research Enhancement program.

**Project Number:** 0864530  
**Project Title:** Diabetic Changes in Contractile Proteins and  
Contractility in Arterial versus Venous Grafts  
**Investigator:** Segar, Lakshman

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.)

## **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

### Reviewer 1:

1. The sample size is too small to draw any meaningful conclusions. A power analysis was not performed to determine the number of grafts necessary to show significant differences amongst the groups. The investigators failed to realize that LIMA specimens available for the study would be small and might not allow for analysis of contractility and expression of contractile proteins, further undermining the number of specimens that would be needed to show meaningful results.

*Response:* Since the submission of the Final Progress Report, we have increased the sample size substantially. The data obtained from several specimens have now enabled us to perform statistical analysis to show meaningful results. In particular, the data collected from nondiabetic human saphenous vein specimens were recently presented at the Basic and Cardiovascular Sciences meeting held in Las Vegas, NV (BCVS/AHA July 14-17, 2014; Abstract # 273; page 183).

To address concerns regarding the analysis of contractility and expression of contractile proteins with the small amount of available LIMA specimens, we adopted an alternative approach. Instead of measuring contractile response and protein expression in two different segments from the same specimen, we now measure these two parameters in the same segment (ring preparation). We have optimized this procedure using human saphenous vein and the same procedure is being employed for LIMA specimens. Specifically, we perform isometric tension measurements using ring preparations to measure the changes in contractile response. After the completion of contractility studies, we snap-freeze these ring preparations and store them at -80°C for subsequent analysis of proteins. This procedure provides a realistic approach to obtain meaningful results with regard to the changes in contractile response and the associated changes in protein expression and phosphorylation.

2. The selection of patients for the study was based on age alone and the presence or absence of diabetes. However, the investigators should have also matched for other factors which could affect graft function and patency including ejection fraction, presence of renal abnormalities, hyperlipidemia and the use of statins, hypertension and the use of ACE inhibitors, smoking, the use of vasodilators such as IV nitrite and nitroglycerin, and also the diabetic status, insulin vs oral agents and the level of glucose control at the time the specimens were harvested.

*Response:* We thank the reviewer for these thoughtful comments regarding confounding variables. In addition to age and diabetes, we are taking into consideration several variables including hypertension, dyslipidemia, and smoking for the ongoing studies that assess changes in contractility and protein expression/phosphorylation. In the future, we will also consider the other variables noted by the reviewer.

3. The investigators failed to account for surgical trauma in harvesting the conduits. No mention is made as to whether the veins were harvested open vs endoscopically, and whether they were distended or stored in cold blood. No mention is made as to whether the IMA was harvested as a skeletonized graft or whether it was dilated or received a topical vasodilating agent. These factors will influence graft contractile function and vascular reactivity. In addition, the investigators have not commented on how they will assess the quality of the veins they are studying i.e., varicosities, thin and thick walled, etc., this is important in determining vein graft function. Not all harvested veins are perfect.

*Response:* We will carefully address the suggested points regarding the procedures for vessel harvest while reporting the results in our publication. As pointed out, it is important to assess the quality of the vein. We will address these concerns by performing histological studies using hematoxylin and eosin staining. In addition, we routinely assess the viability of the veins by stimulation with 80 mM KCl while performing contractility studies.

4. The investigators have failed to take into account that not all contractile proteins may be responsible for altered vasoreactivity and that a larger sample size will be needed to characterize the role of contractile proteins, if any, in these conduits.

*Response:* As advised, we will characterize the role of different contractile proteins (in addition to smooth muscle  $\alpha$ -actin and calponin) using a larger sample size.

5. The investigators have failed to appreciate the clinical relevance of their study. It is well known that saphenous vein graft patency is reduced in both diabetic and non-diabetic patients and the mechanism for this failure is multifactorial. This includes the size of the vessels, the underlying lipid levels and adequacy of glycemic control. Contractile protein expression may have no significant role in altering these processes and, at best, may only have a small role in determining graft patency. Even more important is the knowledge that total arterial revascularization may be best in these patients and the role of saphenous veins may be of limited importance in the diabetic patient undergoing CABG surgery.

Response: We will address the suggested points regarding the clinical relevance of our study while reporting the results in our publication.

Reviewer 2:

1. Inadequate subject recruitment - better infrastructure for approaching/informing/consenting potential research subjects. Need apparently better interaction and collaboration between basic science principal investigator and clinician investigators.

Response: Since the submission of the Final Progress Report, we have performed contractility studies using specimens obtained from 28 subjects. There is an active collaboration between the basic science investigator and the clinical investigators as evidenced by the recent presentation at the Basic and Cardiovascular Sciences meeting held in Las Vegas, NV (BCVS/AHA July 14-17, 2014; Abstract # 273; page 183).

2. Inadequate amount of tissue from each patient to allow all analyses to be performed on each patient-better protocol design and communication with clinician investigator to ensure that adequate material for the investigation is obtained.

Response: To address the concerns regarding the analysis of contractility and expression of contractile proteins with the small amount of available LIMA specimens, we have adopted an alternative approach. Instead of measuring contractile response and protein expression in two different segments from the same specimen, we now measure these two parameters in the same segment (ring preparation). We have optimized this procedure using human saphenous vein and the same procedure is being employed for LIMA specimens. Specifically, we perform isometric tension measurements using ring preparations to measure the changes in contractile response. After the completion of contractility studies, we snap-freeze these ring preparations and store them at  $-80^{\circ}\text{C}$  for subsequent analysis of proteins. This procedure provides a realistic approach to obtain meaningful results with regard to the changes in contractile response and the associated changes in protein expression and phosphorylation. Notably, the principal investigator discusses with the clinician investigator on a routine basis to ensure that adequate tissue can be obtained for the planned studies.

3. Incomplete presentation of experimental data - Figure 1 has apparently erroneous interpretation of the limited data shown: no loading control for protein gels; the internal mammary artery limited data is stated as no differences although a difference in band intensity is visually apparent. No PCR data is shown, though it is stated samples were collected and techniques optimized. No quantitative vessel contractile physiology is shown, though again it is stated that it was obtained.

Response: As pointed out by the reviewer, there will be a limitation in the interpretation of data with low 'n' values. Our earlier presentation of immunoblot data was very preliminary. Since we have collected several specimens and performed a number of immunoblot studies to date, we carefully analyze these data using appropriate loading controls. While we use  $\beta$ -actin as a loading control for proteins, we do not use this as a control for qPCR analysis. Instead of using  $\beta$ -actin or GAPDH, we utilize  $\beta$ 2-microglobulin as an internal control for mRNA analysis as reported recently (Pyla *et al.*, *American Journal of Physiology* 304:

C574–C589, 2013). In our recent presentation, we have shown quantitative data on saphenous vein contractile response (BCVS/AHA July 14-17, 2014; Abstract # 273; page 183).

4. Immunohistochemistry - the addition of this approach to the original protocol is a plus. However, no data on patients is ever shown; only method verification initial results; and these data are not quantified.

Response: We now perform immunohistochemical analysis including quantification of smooth muscle  $\alpha$ -actin expression.

Reviewer 3:

1. Continue the study only pending a realistic patient recruitment plan.

Response: Since the submission of the Final Progress Report, we have performed contractility studies using specimens obtained from 28 subjects.

2. Perform more molecular analyses on an extended number of samples, in order to establish statistical significance.

Response: In addition to contractile proteins, we have been determining the expression and phosphorylation state of different proteins including AMP-activated protein kinase (AMPK), ERK, and Akt. Since we have performed studies utilizing several specimens, we are now able to analyze the data for statistical significance.

3. Team up with other clinician/researchers in the field to recruit sufficient number of patients and perform more extensive analyses.

Response: There is an active collaboration between the basic science investigator and the clinical investigators as evidenced by the recent presentation at the Basic and Cardiovascular Sciences meeting held in Las Vegas, NV (BCVS/AHA July 14-17, 2014; Abstract # 273; page 183). We have performed extensive studies using saphenous vein to analyze the changes in contractile response and molecular signaling.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project was funded with CURE funds in the amount of \$30,000 direct costs. The internal program that funded this project was designed to support the acquisition of preliminary data that would contribute to the resubmission of an NIH grant application. The project resulted in an award from NIH in the amount of \$1,034,387. Although the publications occurred after the final report was submitted, the project did contribute to several publications.

For these reasons, we believe that this project was successful and we regret that it received an unfavorable rating. At least in part, we attribute this to the award of resources that were not sufficient to enable the PI to successfully pursue all the experiments that were initially proposed. To prevent this problem from occurring in the future, we have instituted a peer review process to ensure that the aims described in Strategic Plans proposed for support from the CURE program can be completed within limits of the time and funding provided.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response: When I submitted the NIH R01 grant, I had very strong preliminary data for Specific Aim 1 and Specific Aim 2 and a modest amount of preliminary data for Specific Aim 3.

Specific Aim 1 is focused on studies with human aortic smooth muscle cells in culture. We have done a significant amount of work for this Aim and published the following two articles:

- 1) Zhao et al. *American Journal of Physiology Cell Physiology* 300, C1375-1385, 2011; and
- 2) Pyla et al. *American Journal of Physiology Cell Physiology* 304, C574-C589, 2013.

Two more articles are in preparation for submission to suitable journals (for Specific Aim 1).

Specific Aim 2 is focused on studies with rodent model. We have also done a significant amount of work for this Aim and published the following two articles:

- 1) Jun et al. *American Journal of Physiology Endocrinology and Metabolism* 301, E145-E154, 2011; and
- 2) Jun et al. *Atherosclerosis* 225, 341-347, 2012.

In addition, we have another manuscript submitted to *Biochemical Pharmacology* (Pyla et al. 2014); under revision.

Furthermore, we have a fourth manuscript in preparation for submission to a suitable journal (for Specific Aim 2).

Specific Aim 3 is focused on studies with human tissue specimens. The success of Specific Aim 3 is dependent on the completion of the studies proposed in Specific Aims 1 and 2. Since we have made significant progress in Specific Aims 1 and 2, we are now in a position to interpret the findings from Specific Aim 3 with confidence. In my personal view, it is very important that we handle and analyze the human tissue samples with adequate care. Since we followed our studies in the following order – Specific Aim 1, Specific Aim 2, and Specific Aim 3 – there was a delay in obtaining meaningful data for human tissue specimens. When we submit the manuscript on ‘human tissue specimens’ to a suitable journal within the next two months, we will have the utmost confidence of reporting the findings that have been analyzed adequately. More importantly, we will submit this manuscript to a high-impact journal (as before) to obtain critical comments from expert reviewers.

I really appreciate the comments made by all three reviewers for the Tobacco CURE fund. Specifically Reviewer #2 appreciates the technical capabilities of our lab (page 7, paragraph 2, last sentence). However, all three reviewers feel that we do not have sufficient data because of: i) inadequate subject recruitment; and ii) lack of interaction with clinical collaborators. We have made significant progress on these points since the submission of the Final Progress Report in

2012. Importantly, we have been performing numerous studies with human tissue specimens last year and this year (2013 and 2014).

We thank the reviewers for their constructive comments and helpful suggestions to improve the quality of our research work using human tissue specimens.

**Project Number:** 0864531  
**Project Title:** Autism Indicators: Erythrocyte Membrane  
Fluidity and/or Lipid Composition  
**Investigator:** Schengrund, Cara-Lynne

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.)

### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

1. Study was underpowered. No power calculation was performed in planning the study, at least as reported. No assessment of the signal-to-noise of the assays and the expected range of variation of the assay values between case and controls was discussed.

Response: This study was planned as a preliminary set of experiments done to ascertain whether the hypothesis might be worth pursuing using a larger cohort. It was also based on the number of samples that could be realistically obtained within a year (the time frame for which funding was provided).

2. Groups were not sex matched. Male controls could have been selected to match the expected excess of males among the autistic group. Lack of sexual dimorphism stated (but not shown) among different controls (number unknown) is necessary but not sufficient to justify lack of sex-matching in the study.

Response: The controls used were siblings of affected children and, as stated in the original proposal, this was done to reduce potential environmental effects.

3. The GM3 data were not emphasized as potentially the most capable of serving to discriminate between cases and controls. This is a disappointment. If they were excluded from further consideration due to some known non-specific regulation of red cell GM3 levels, this should have been explained and discussed.

Response: Originally GM3 expression was to be investigated and Western blots were done but something went wrong with the replicates (the antibody did not stain any of the bands) and hence the data was not used. We couldn't repeat the experiment as we had used material from some of the samples for other assays and could not do additional blots. The focus on

GM1 reflects the fact that there is accumulating evidence for the fact that alterations in membrane cholesterol can affect the ability of cholera toxin to bind to cell surface GM1 [Lingwood et al (2011) Nat. Chem. Biol. 7, 260-262] and by extrapolation may affect its normal receptor function(s). There is also accumulating evidence that alterations in expression of GM1 may affect accumulation of A $\beta$  and contribute to the onset of Alzheimer disease. For a recent paper on this topic see: Yamamoto, N. et al. (in press) J. Neurochem. DOI: 10.1111/jnc.12828.

4. The explanation of the failure of NMR to detect the missing phospholipids known to be present in the samples was inadequate.

*Response:* We were unable to reproducibly separate phosphatidylserine and phosphatidylethanolamine so that no quantitative information regarding the relative amounts of specific phospholipids could be obtained. One problem was that the actual lipid composition of the sample appeared to affect chemical shifts of the specific lipids and the cause of this problem was not identified. Therefore we did not pursue this approach further.

5. The lipidomics experiments appear not to have been state-of-the-art. No discussion of the fatty acyl constituents was provided. No investigation of fatty acyl constituents was proposed or performed.

*Response:* The first five lipidomic analyses were done by Dr. Xianlin Han (currently at Sanford-Burnham Medical Research Institute, Orlando, FL). He is a well-known expert in the field. The data for the first five did not show a significant difference in the fatty acid composition of the extracted phospholipids. Subsequent samples eventually were analyzed by a colleague who had gone to Dr. Han's lab to learn the technique but the data were not readily interpretable.

#### Reviewer 2:

1. Auto-oxidation of membrane lipids and/or proteins after collection of blood is a concern since oxidation can affect both membrane fluidity as well as lipid composition. Any future studies along these lines should incorporate tests to ensure that lipid/protein oxidation does not occur after collection of the samples.

*Response:* As a lipid chemist, I am well aware of this possibility. We did obtain blood as soon as possible after it was drawn from a child and isolated the RBC membranes as soon as we obtained the cells and then kept them at -80°C until analyzed. Gangliosides tend to keep well as they have predominantly saturated fatty acids. When isolating phospholipids for fatty acid analysis, they were isolated as soon as possible after samples were obtained, dried under N<sub>2</sub> and stored dry at <-20°C.

2. Although not proposed, lateral diffusion of membrane lipids and proteins may be strongly influenced by changes in cholesterol composition as well as changes in the composition of lipid raft domains, and would be worth studying in light of the project's findings regarding changes in cholesterol and GM1 in erythrocyte membranes from individuals with ASD.

Response: Both are excellent suggestions.

3. Although a significant decrease in GM3 was reported in the Year 2 progress report, this result was neither included in the final progress report nor in the published manuscript, leading one to question whether or not this result was reproducible. This issue should be addressed by the PI.

Response: Please see response to point 3 above raised by the first reviewer. The statement in year 2 reflected data from one set of experiments. When we went to repeat it, something went wrong and the antibody did not bind to anything on the blot. We could not do it again as we had used up several of our samples and therefore we excluded it from the manuscript.

Reviewer 3:

1. The relationship between cholesterol content and autism is extremely weak and may only represent 19% of individuals with autism. As such, a much larger cohort of samples must be tested to validate these preliminary findings (on the order of 150-200 samples). These investigators should perform power analysis to predict just how many samples would be required given preliminary data on 20 (not so well matched) samples.

Response: This comment is absolutely correct. However, that would require more time and manpower than was available. With respect to how well matched the samples were, the use of siblings of the affected individuals was deliberately done with the thought that it would minimize environmental variability.

2. The slot blot method used in Figure 2 is not really state-of-the-art for quantification. Several options exist including IR secondary antibodies read on a fluorescent scanner to quantify these results. Alternatively, some sort of NMR methodology or quantitative Mass Spec method to evaluate GM1 levels would be preferable for quantitative correlations.

Response: The cholera toxin used was conjugated with horseradish peroxidase and the bands were visualized using Super Signal West Femto Maximum Sensitivity Substrate<sup>TM</sup> followed by exposure in a Fuji Film LAS 3000. Multigauge software was used to calculate intensities. This eliminates the need for a secondary antibody and is considered a standard method for looking at cell surface GM1.

3. It is clear that the impact of these studies, even if they had succeeded in producing a test using blood samples, would only be effective in approximately 20% of autism cases (which they knew prior to engaging in these studies). This is really an incremental contribution to the field and will not significantly impact autism diagnosis and treatment.

Response: We agree. However, if a larger study indicates that availability of GM1 to act as a binding site is reduced, it could affect signal transduction. As indicated in response to point 3 made by Reviewer 1, changes in cell surface GM1 can affect neuronal function as well as that of the oxytocin receptor which can affect behavior. This is a novel observation and if

larger studies are done, they could provide important insights regarding the mechanism of autism.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received a Favorable rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response:

**Project Number:** 0864532  
**Project Title:** Efficacy of Gemcitabine for Pancreatic Cancer:  
Role of DNA Polymerases  
**Investigator:** Spratt, Thomas

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.)

## **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

### Reviewer 1:

1. The main weakness of this proposal is the failure to recruit enough patients for this interesting and even provocative project focused on assessing the roles of DNA polymerases as suppressors of gemcitabine activity in pancreatic cancer. Although alternative approaches were implemented, the most aggressive means to increase enrollment would have been to recruit the support of surrounding high-volume centers.

*Response:* The involvement of surrounding high volume centers would undoubtedly increase enrollment. In designing the project, we were able to convince a colleague at Penn State Hershey to donate the time of a clinical coordinator to help our pilot project which allowed us to stay within the budget ceiling. Going to outside institutions to get samples would have been outside our budget, and thus we did not consider this option.

2. A second but somewhat less weak point is that another avenue of pursuit could have been done using archived pancreatic cancer tissue as well as genomic samples and TMAs to determine the level of these six DNA polymerases in a variety of pancreatic cancer patients. Likewise, samples from mouse models of pancreatic cancer (e.g. p48-Cre/LSL-Kras/mtp53 and others) could have been proposed for a similar type of study. Indeed, the levels, though correlative in nature, could have then been associated with outcome measures (prognosis, survival, etc.) to help strengthen the rationale of the initial proposal. Plus, it would also have laid the groundwork for targeting these DNA polymerases (either individually or in combination) in combination with gemcitabine using cell culture and then preclinical models. This too would have strengthened the notion of testing DNA polymerase activity in the plasma of pancreatic cancer patients before and after gemcitabine therapy.

Response: These suggestions are extremely good, and we will consider pursuing them in the future.

3. Another major weakness of this work is that an alternative project, which in this case included the evaluation of base analog incorporation in several cell lines with several DNA polymerases, was not pursued quickly enough or even during the initial recruitment phase. Had investigators chosen to begin these assessments sooner, the publication would have been in press sooner while additional experiments for future publications and/or preliminary data for grant submissions would have been further along.

Response: In retrospect, beginning these side-projects sooner would have been a good idea. At the time, we did not pursue these side projects because we were conserving the money for the analysis of the patient samples. We began these side projects after realizing that the number of patient samples would be less than the target number that we budgeted for.

4. A final but more minor weak point includes the use of various cancer cells (liver, colon, and lung) for the *in vitro* evaluation of DNA polymerase incorporation of base analogs (the alternative project). Though the data is interesting, even compelling, it is difficult to know why these cancer cells were selected over pancreatic cancer cell lines. Since the focus of the primary study was pancreatic cancer, it is not known why these cancer cell lines were not used for these cell culture experiments.

Response: The objective of the experiment was to see if cells with different polymerase content would incorporate different amounts of gemcitabine. As such, the identities of the cell were not so important. However, since the objective of the project was pancreatic cancer, we should have included a pancreatic cancer cell line.

5. The final weakness is also more minor in that trends observed and reported in the progress reports should have been extended out for a second triplicate study to determine if any trends became more statistically significant. Though some of these did turn out to move in that direction, as reported in the published manuscript, it was not demonstrated in any annual progress report.

Response: Yes, additional experiments may have led to statistical significance. These experiments were performed late in the study and failure in the primary objectives led us to discontinue the research.

Reviewer 2:

1. With respect to design and conduct of correlative studies on patients, the applicants should consider the feasibility of the proposed studies with respect to patient accrual before proposing such studies in the future. Specifically, the difficulties imparted to the patients with respect to obtaining blood and other specimens is a major impediment to translational research of this nature and should be taken into consideration.

Response: Yes, the difficulties in patient accrual were not adequately considered in planning the experiments. Most of the funding was slated for analytical experimentation and not for patient accrual.

2. With respect to the identification and recruitment of patients for clinical correlative studies, the level of effort provided at this center was modest. In the future, these investigators should consider reaching out to other high-volume pancreatic cancer centers to help with patient accrual and also to learn how to accrue patients in this disease.

Response: As a laboratory scientist, the difficulties in patient accrual were unanticipated. Future studies that I undertake will account for this and in addition, the institution is developing mechanisms through the Clinical and Translational Science Institute to assist laboratory investigators in designing clinical studies.

3. The investigators should develop a better long range view of their research in mechanism of action of gemcitabine. The subject area is important and interesting, and yet the potential significance of the research was not evident in plans to carry it forward.

Response: The proposed research was to have tested a very specific aspect of our overall hypothesis. Therefore in the proposal and reports we did not delve into aspects of our hypothesis that did not pertain to the specific experiments.

Reviewer 3:

1. Failed to test its primary hypothesis adequately.

Response: We would agree that the lack of patient accrual made it impossible for us to test our hypothesis.

2. Failed to result in new grants.

Response: I had hoped that we would obtain exciting preliminary clinical data that would lead to a competitive grant proposal for a larger and more definitive study. Without this preliminary data, a grant proposal would be hampered by our lack of expertise in pancreatic cancer and would not be competitive for support.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received a Favorable rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response:

**Project Number:** 0864533  
**Project Title:** Embedded Rural Clinical Research Infrastructure:  
Utilization of Community-Based Nurses and Paramedics  
**Investigator:** Terndrup, Thomas

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.)

#### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

The Principal Investigator for this project is no longer at Penn State University.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received a Favorable rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response:

**Project Number:** 0864534  
**Project Title:** Cytoadherence in Maternal Malaria  
**Investigator:** Gowda, Channe

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.)

## **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

### Reviewer 1:

1. This is a very challenging, high-risk project, and it is unclear that the line of research will result in a practical solution to the problem of malaria in pregnancy. Only a partial picture of the adhesion-receptor binding phenomenon will be obtained from the approach outlined, and it is not clear whether the data obtained will be helpful or misleading in screening for a small molecule inhibitor of binding. The recommendation is to complement the current approach of only using structural analyses of dissected binding components with a more biologically relevant binding assay. This would require development of an assay where the native VAR2CSA is expressed on the surface of the infected erythrocyte and is studied in the context of its natural milieu and interacting with an appropriate placental tissue binding partner or synthetic particle coated with C4S. This cell adhesion assay could be used to test small molecules and antibodies for their ability to prevent binding or affect a release of the infected red cell.

Response: VAR2CSA is a very large (~350 kDa) protein, consisting of six adhesive (DBL) domains. Based on the results of previous studies, we have hypothesized that the chondroitin 4-sulfate (C4S)-binding site of VAR2CSA comprise amino acid residues of several DBL domains (mainly those in the N-terminal DBL domains), coming to close proximity to each other in a folded structure and forming a conformational C4S-binding pocket. Knowledge on VAR2CSA structure will help characterizing the C4S binding site. However, because of the high molecular weight, intact VAR2CSA is not easily amenable to various physical technical to understand its VAR2CSA structure. Therefore, we produced recombinant proteins of individual domains and studied preliminarily interactions between domain pairs by using various physical techniques. The results indicated that DBL2x and DBL3x interact with each other. Since DBL2x and DBL3x are the two key domains that account for the majority of interactions involved in C4S binding by VAR2CSA, the data will be useful in dissecting the structural interactions involved in C4S-binding to VAR2CSA. Although in this project we proposed to develop sensitive C4S 12 mer-based assay using DBL domain proteins to screen

the inhibitors of C4S binding to VAR2CSA, in subsequent studies we have produced larger VAR2CSA constructs (see response to Reviewer 3). These data will be used as preliminary results in support of an R01 grant application, which we are planning to submit to NIH in October/November 2014. We agree that a cell-based assay is more relevant to the *in vivo* situation seen in *P. falciparum*-infected pregnant women. As such, in the planned grant application, we will propose to use the cell-based assay that has been standard in our lab to validate the inhibitors identified by the initial screening of drug libraries using the VAR2CSA constructs that we have produced. For these reasons, we believe that the results we have obtained in this project are valuable.

2. Publications have been very few along this line of research over the past 4 years, other than a review article. The data currently available should be packaged and published soon to bolster chances for major funding.

Response: Recently, we expressed the N- and C-terminal portions of VAR2CSA in mammalian cells and standardized protein purification procedures. Currently, we are planning to assess these proteins for C4S binding using a fluorescent-labeled C4S 12-mer probe and develop an assay for screening small molecule libraries to identify the inhibitors. We are planning to publish these data and those already obtained from this project.

Reviewer 2:

Generate and study VAR2CSA constructs other than the 3D7 variant in the future.

Response: The current knowledge on VAR2CSA structural features indicates that the C4S binding site is highly conserved although the other regions of VAR2CSA are extremely polymorphic. Since our project is focused to characterize the C4S-binding site and identify small molecule inhibitors of C4S binding, we are not currently planning to generate constructs of other strains.

Reviewer 3:

1. It appears that little to no effort was placed on making multiple tandem DBL constructs or in characterizing such proteins. As these are likely to be key to understanding interaction with the C4S target, it would be important to focus on this aspect.

Response: This is a valuable suggestion. In the continuation of this project, we have recently expressed the N- and C-terminal portions of VAR2CSA that comprise multiple DBL domains. Specifically, we cloned the constructs encompassing DBL1x-DBL3x, DBL1x-DBL4ε and DBL4ε-DBL6ε, expressed proteins in HEK 293F cells, and standardized the protein purification procedures. We are planning to use these data as preliminary results for the submission of an R01 grant application to NIH.

2. It is very worrying that ~75% of the total allocated funds were diverted to support a departmental resource (microscope) that had only peripheral relevance to this project. This was a disservice to the original overall goals of the project that would have been advanced to

a much greater extent had the resources not been used for other causes.

Response: Funds were not diverted from this project to support the purchase of the microscope. Rather, funds were added to my budget to purchase the microscope for use in this project and as an investment to enable future studies by other investigators in the institution.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received a Favorable rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response: In studies performed after submission of this performance report, we have produced multi-domain constructs of VAR2CSA and performed C4S-binding studies using these larger constructs. These results and those produced in earlier studies should strongly support the R01 grant application, which we are planning to submit to NIH soon. We are grateful to the State for making this CURE funding available.

**Project Number:** 0864535  
**Project Title:** Impact of iPS Cell-derived Highly Reactive Immune Cells on Cancer  
**Investigator:** Song, Jianxun

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.)

### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

None.

Reviewer 2:

1. Experiments should be implemented in mouse models to evaluate whether tumor-specific CTL generated from iPS have superior "stemness" as compared to those generated from peripheral T cells. These experiments are crucial to convince the scientific community that the proposed approach may have real advantages as compared to peripheral T cells.

Response: These experiments have been done and three related manuscripts are in preparation: 1. Regulation of c-Myc from Costimulatory Signals Modulates the Generation of CD8<sup>+</sup> Memory T Cells during viral infection. Revised in *PLoS Pathogens*. 2. Utilizing Regulatory T Cells Against Rheumatoid Arthritis. Accepted for publication in *Frontiers in Oncology*. 3. Melanoma immunotherapy in mice using Ag-specific iPSC-CTL. We plan to submit these manuscripts to *Cancer Research* in August 2014.

2. Additional studies must be performed as discussed in criterion 6 to move this project to a future clinical translation. This reviewer suggests including these experiments in a revised version of the proposed RO1 if a revised version is needed.

Response: Yes, we agree and have included these experiments in a revised R01 application that was submitted on July 05, 2014.

Reviewer 3:

1. The researchers tested the iPS induced CTL only for preventive effect for melanoma. It will be more meaningful to test if those iPS-induced CTL could have therapeutic effect, i.e., to establish the tumor before injecting iPS-induced CTL for tests of therapeutic efficacy. In

addition, the clinical potential of this approach could be limited to one subtype of melanoma since it has to be antigen-specific.

Response: We have evaluated a compound (Vemurafenib) and iPSC-CTL, which is likely to be used for therapeutic application. Some melanoma-associated antigens (MAA, such as MART, Tyrosinase, Tyrosinase-related Ag 1 or 2) have been used for immunotherapy and we may test other types of MAA as well.

2. It would be helpful if the researchers could clearly describe the name and source of human iPS cells used in this study.

Response: The iPS cells used in this study were from the Vector-free human iPSC line: iPS DF6-9 9T.B (SLA#12-W0253) obtained from the WISC Bank.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received an Outstanding rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response: We would like to thank the Pennsylvania Department of Health and the Legislature for their support of our research. We are also thankful for the reviewer’s comments and the “Outstanding” evaluation.

**Project Number:** 0864536  
**Project Title:** Cannula Development and In Vivo Testing for  
Pediatric VAD Development  
**Investigator:** Weiss, William

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.)

## **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

### Reviewer 1:

1. The investigators have yet to meet the second objective of their aims which is to extend the survival of the animals to 8 weeks and study the degree of thromboembolic deposits, coagulation parameters, biomarkers of the inflammatory response, and end organ damage associated with the use of these cannulas and connectors in these VADs.

***Response:*** Since our last report in December 2012, we have performed 2 surgical shams (control group) and 7 animal studies with the implanted Pediatric VAD (PVAD) using the cannulae/connector system (Table 1). These results were presented at the 60<sup>th</sup> Annual Conference of the American Society for Artificial Internal Organs (ASAIO) in Washington DC in June 2014. The two most recent studies (#4754 and #4755) exceeded the 8 week duration. There were 3 intra-operative terminations not related to the device function.

*Table 1. Summary of animal studies performed since the last report.*

Group	Anticoagulation Target	N	ID #	Duration (days)
Surgical Shams	TEG R time 2x normal	2	1272	54
			1274	56
PVAD 2x normal	TEG R time 2x normal	6	1318	27
			1319	50
			1347	0
			1348	0
			1227	0
			4754	77
PVAD normal	TEG Coag Index (CI) normal	1	4755	63

The anticoagulation targets are described below. We have moved from aPTT to TEG (thromboelastography) measurements to adjust heparin dose (Table 2). TEG-based heparin anticoagulation protocols based on whole blood coagulability may provide a more accurate animal model for predicting thrombogenicity in humans than aPTT alone.

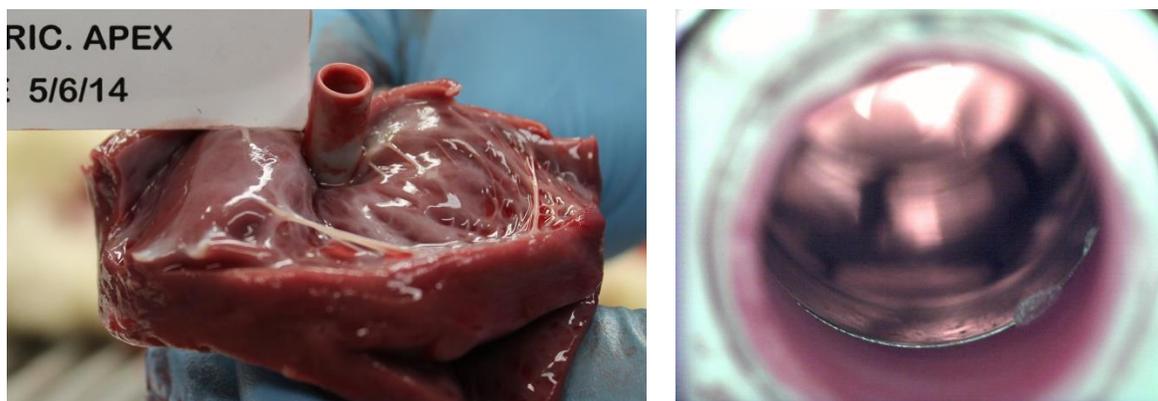
*Table 2. Anticoagulation target and corresponding heparin range.*

	Anticoagulation Target	Anti-Xa > 3 wks (U/ml)	Heparin > 3 wks (U/kg/hr)
Clinical	aPTT 1.5-2.0 x normal	0.3 - 0.7	
2005-2008	aPTT 1.5-2.0 x normal (40-54 s)	0.39 – 0.56	25-45
2008-2014	TEG R time 2x normal	< 0.05	15-20
2014 >	TEG Coag Index (CI) normal	< 0.05	0

- The authors still must determine the biocompatibility of the cannulas and the connectors by the device explant analysis parameters they have listed in the grant to assure that they will be clinically biocompatible and be capable of use for an extended period of time in clinical practice. More long term animal data is needed to complete this part of the grant.

*Response:* As shown in Table 2, we have reduced the heparin anticoagulation levels as we have gained confidence in the non-thrombogenic properties of the pump and cannulae. To briefly summarize our findings, even with extremely low levels of heparin, the Penn State Pediatric VAD has demonstrated

- No evidence of thromboembolism affecting end organ function
- Infrequent chronic renal infarcts consistent with levels found in surgical shams
- Infrequent and minor surface depositions, well-adhered



*Fig 1. Apical cannula from Animal # 4755 duration 63 days showing (left) excellent incorporation and no macroscopic thrombi and (right) connector/graft junction showing a small fibrin deposit.*

The animal results will be published after a larger number of studies have been completed under the current R01 grant, and will include clinical chemistry, hematology data, and complete necropsy and histopathology reports. We continue to measure urinary biomarkers, focusing on GSTP1, HSP70, and HAVCR1, since these have been shown to be the most sensitive of the candidate biomarkers.

Reviewer 2:

Despite the fact that this grant project has been given an overall rating of “outstanding,” and that the project has been completed, there is one recommendation that could enhance the impact of future research projects. As initially addressed under the heading of Criterion 2, assessment of the effects of the PVAD on clot formation, blood coagulation and ischemic injury in their sheep model was only conducted in two animals, and completed in just one. Thus, conclusions that the investigators could draw concerning the biocompatibility of their devices were minimized. Results from one animal cannot account for biological variability in the magnitude of the clotting and coagulation responses, potential variations in the extent of renal ischemia and possible discrepancies in surgical implantation of the PVAD. Thus, in spite of the additional time, effort, and cost of conducting more animal studies, it is suggested that, in future investigations, a greater number of animals should be studied (perhaps 4 or 5) to determine biocompatibility.

Response: The reviewer is absolutely correct that variability in animals and surgical procedures require a larger number of animals in order to draw statistically significant conclusions regarding biocompatibility. The major objective of the current R01 grant is to perform a minimum of 8 additional studies of 8 week duration with a focus on biocompatibility assessment.

Reviewer 3:

1. Minor: As previously mentioned, a more thorough explanation about whether the cannulae design will require any significant modifications or adjustments for successful clinical implementation in patients and the impact, if any, that these adjustments might have.

Response: We have made minor improvements in the cannulae/connector system, but fortunately the animal testing results have confirmed that the all-ePTFE approach is non-thrombogenic, kink resistant, and can be cut to length. Some of the minor improvements have been: more secure bonding of the graft to the titanium shell supporting the apical connector, extending the silicone overcoating all the way to the aortic anastomosis, and increasing the collet clearances to allow for easier connection to the pump. We are also currently modifying the apical nut to further reduce the size. The only expected change for clinical use will be the addition of a velour covering where the percutaneous cannula exits the skin, to reduce infection risk. This is not expected to affect the blood-contacting surface.

2. A true chronic animal study was never achieved; perhaps, additional attempts will be successful and yield useful data in the overall development of the PVAD.

Response: Please see the response to reviewer #1.

3. Minor: A learning activity to stimulate community engagement and the inclusion of a pre-doctoral or post-doctoral student from an underrepresented or minority group in the STEM disciplines would add value to the project.

Response: We agree. We plan to pursue supplemental funding from NIH under PAR-12-149 Research “Supplements to Promote Diversity in Health-Related Research”.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received an Outstanding rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response: We wish to thank the reviewers for their thorough review and supportive comments. We would also like to thank the Pennsylvania Department of Health and the Legislature for their support of our research,

**Project Number:** 0864537  
**Project Title:** Role of Leucine Metabolism in Leucine Signaling  
**Investigator:** Lynch, Christopher

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***B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.***

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### **SPECIFIC WEAKNESSES AND RECOMMENDATIONS**

Reviewer 1:

There could have been more of an effort to involve undergraduate and graduate students in the project in order to provide specific training in laboratory techniques, animal handling experience and data analysis and interpretation.

Response: That is a great idea. However, our understanding was that these funds could not be used for educational purposes or for training and so we did not use them for that. However, we did involve graduate students in conducting some of the animal studies.

Reviewer 2:

None.

Reviewer 3:

The original objective of determining whether the "energy wasting" phenotype of BCATm KO mice is due to elevated BCAA or BCKA depletion (or both) has not been met. While there is significant progress towards meeting the replacement objective of generating and studying tissue-specific knockouts, this replacement objective augments but does not replace the value of the original objective.

Recommendation: The originally-proposed studies should still be performed BCATm KO mice.

Response: We agree that this objective was not fully met during the project period. However, we subsequently have shown that knocking out the next step in BCAA metabolism on the BCATm background intermediately reduces the weight loss of the BCAT KO. This supports the idea that both elevations in BCAAs and loss of BCKA signaling are involved. Another unpredicted problem was that in the BCATm KO, mTOR signaling from insulin and other nutrients were intact but Leu activation of mTOR signaling was severely impaired in every system we studied.

***C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.***

Response: This project received an Outstanding rating.

***D. Additional comments in response to the Final Performance Review Report (OPTIONAL):***

Response: We would like to thank the State Legislature and the Pennsylvania Department of Health for their support of our research, and we thank the reviewers for their review of our report. We especially appreciate their positive, supportive comments and the “Outstanding” score.